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Getting started

Welcome to CytoVision™ 3.6

Overview
Welcome to the CytoVision family of workstations. CytoVision fully integrates the capture and analysis of brightfield, fluorescent, FISH, CGH, RxFISH and M-FISH samples.

Getting started
The Introductory topics contain basic information about using the Genus or CytoVision workstation and the Windows interface. The topic Using the help files provides information about using these help files.

Key features
- User definable karyotype templates and ideograms.
- Automatic karyotyping of plant, animal and human chromosomes.
- Ideogram Editor for creating sets of ideograms.
- Capture of brightfield, fluorescent, FISH, RxFISH, CGH and M-FISH samples.
- User definable classifiers for automatic karyotyping.
- Image enhancement filters and contrasting tools.
- Printing images.

What's new in CytoVision 3.6

Image Gallery

Select Gallery at the top of the navigator
Convert images obtained with earlier versions of CytoVision by clicking on the Generate thumbnails button.
You can select to view only the type of image you want to see. Example, check only the Karyotype box to see only Karyotype images in the Gallery.
Double click on a thumbnail image to drop an image into one of CytoVision's display windows.
In the navigator it is now possible to batch delete raw images
See topics in online help under CytoVision Basics - About the Gallery and in Working with images - Working with the Gallery for more details in how to use the Gallery window.
Intelligent Karyotyping and Extended layout

- Dynamic scaling automatically accommodates different types of metaphases (chromosome number and/or length).
- In the Karyotype layout window, select between Short or Extended format for the Karyotype template to accommodate longer chromosomes.
- This new format can be selected for existing Karyotypes or for new Karyotypes. As chromosomes are moved layout is extended both horizontally and vertically to accommodate chromosomes. It is no longer necessary to use the layout function.
- Restoring chromosomes will no longer undo scaling
- Chromosomes can be manually scaled at the end if desired.
  - The image quality of chromosomes that are reflected, trimmed, or straightened will not be degraded by the dynamic scaling feature
- Species templates with less than a height of 800 will not have scroll bars when the Classic layout is selected.

- A new Tidy Karyogram button on the analysis operations toolbar can be used to automatically space and align the chromosomes in the karyotype window after manual scaling if Auto Layout is not selected.
NOTES:

- In the Large Navigator or Fullscreen layouts, the extended Template will appear with scroll bars. In the classic template the main window will be stretched downward. It will be necessary to design new Print templates or formats to accommodate these extended Karyotypes.
- The Dynamic scaling is a option. To take advantage of this new feature select Always Auto Layout in the Karyotype Layout window.
- See topic in online help under Analysis - Karyotype cells - Using the Karyotype Layout dialog for more details.
MultiCell

- It is now possible to view chromosomes from different slides at the same time in the MultiCell viewer window.
- The slide and cell label for each pair are clearly visible.
- You can use the new scroll bars to advance through the chromosomes or use the drop down menu to select a specific pair.
- Double clicking on a pair of chromosomes in the viewer will automatically load the associated karyotype in the main window.
- You now have full interaction with the karyotype while the MultiCell window is on display. The karyotype can be annotated, rearranged, saved, printed etc. and any changes made can be updated to the MultiCell window.
- With the MultiCell window open, fresh metaphases can be loaded from the gallery, karyotyped and updated to the MultiCell window.
- Chromosomes appear in MultiCell in the same orientation as they are in the karyotype.
- There are new scaling options and the ability to display different band levels for ideograms.
- Horizontal reference lines can be drawn across the MultiCell window to mark specific bands.
- Scaling features - select Autoscale off for no scaling, Autoscale to scale all chromosomes in the cell to the ideogram size, or Autoscale by cell to scale the left chromosome to the ideogram and maintain the ratio for the right chromosome.

- See topic in online help under Analysis - Composites, profiles and annotations - MultiCell viewer for more details.

Segmentation - New Custom Analysis features
Getting started

- Draw Axes now has an option to highlight each chromosome in a color as they are drawn.

- Freehand drawing has a new hold to draw style. In this mode you simply hold down the left button as you draw the lines, and complete all drawing with a single right click.
- The backup step size for freehand drawing styles is now selectable.
- See topic in online help under Analysis - Options - Customize Analysis for more details.

**New Annotation Features**

- Larger selection of font styles and sizes in the Text Style window.
- Italic and bold options for fonts in the Text Style window.
- Different line thickness as well as outline or fill are available for arrows and other drawing objects.

- Allows for a longer annotation user test string by allowing the field to scroll left/right.
CytoVision 3.6

- Text phrases can be saved when typed in the Text box. The drop down selector allows for easy access to select previously saved phrases. Phrases can be selected and deleted using the Delete button.

- See topics under Analysis - Composite, profiles and annotation - Adding text, shapes or arrows - to add text to an image and to select the line thickness or fill type for more detail.

High Resolution CGH Improvements - HRCGH-2

CytoVision 3.6 includes some major improvements to the Hi Resolution CGH module. These improvements are designed to improve the accuracy of CytoVision CGH analysis even further.

Image processing and measurement changes (HRCGH-2 features)

- Improved background subtraction during capture processing
  This results in slightly less overall background subtraction applied for the test and reference images, but with more accurate subtraction on the metaphase chromosomes when compared to earlier CGH versions.
- Improved chromosome axis determination
  HRCGH2 has an improved object trimming which generates slightly longer axes on the chromosomes and improves CGH detection towards the telomeres.
- Improved image sampling for more accurate ratio profiles.
  More regular and frequent ratio measurements increase the accuracy of the profiles for all chromosomes in the metaphase regardless of shape, size or orientation.

Profile Display and analysis features

- Improved display of Profiles for easier identification of unreliable profile regions.
- Display all chromosome ratio profiles for a slide
- Additional 99.99% confidence interval
  - To eliminate or reduce false positives if the SRI being used is not representative of the case being analyzed.
  - To improve identification of false positives if the SRI being used is not representative of the case being analyzed (i.e. if the Dynamic SRI values (Fit Automatically) are < -2 or > 3)
- Faster drawing of Standard Reference Intervals
- Improved CGH Expanded Profile window
  More chromosomes are visible when opened and a new Confidence option to quickly identify which chromosomes profiles lie outside the slide confidence intervals, to identify "stray chromosomes" which can be deleted from the karyotype.

New User options

- Manual image registration tool
  To allow for manual adjustment of the DAPI, Test and Reference images to a
specific area of the metaphase or chromosome for detailed investigation of ambiguous or suspected profile regions.

- **Able to apply HRCGH-2 features to old cases**
  Can switch to HRCGH-2 for previously analyzed cases and also back to previous analysis mode for comparison purposes.

  **Note:** Be sure to have an archived copy of the original analysis, because all user corrections to chromosomes axes are lost when applying HRCGH-2 to old cases (or back again).

Existing CGH cases are unaffected by CytoVision 3.6 installation. **The new HRCGH-2 features are only applied if you choose to use them.** Any cases analyzed with the new features can be reverted by switching off HRCGH-2 features in the profile display dialog window and reprocessing. Alternatively you can archive a case before analyzing with HRCGH-2 switched on and restore if you do not want to keep with the changes.

The HRCGH-2 features are enabled via toggle buttons in the CGH Profiles Display window. The following notes provide a tour of the new features - for more details please consult the HRCGH-2 sections in the CytoVision online help.

**Figure 1 - Controlling HRCGH-2 Features**

The **HRCGH-2 This Case** toggle identifies if a case is currently in HRCGH-2 or using the previous CGH mode. When used in conjunction with Change Options it allows the user to convert between the two. This provides a quick mechanism to confirm the
effects of the new changes on old cases. (Change Options does not affect chromosome classification or centromere location - only chromosome axis and ratio profile). If used on a previously analyzed case the axes will be recalculated and may need to be corrected before profile interpretation.

The HRCGH-2 New Cases toggle instructs CytoVision to apply HRCGH-2 processing modifications to all new cases captured and processed on the system as default. If this toggle is switched off all cases will continue to be analyzed using the previous CGH mode.

A new 99.99% confidence interval is provided.

All chroms toggle button is provided to display profiles for all chromosomes in the current slide (Fig. 2)

Figure 2 - Display all chromosome profiles for a slide and the two profiles of the current cell

Manual registration of CGH fluorochrome images is enabled whenever a CGH metaphase is placed in the main display window. Clicking the Registration button opens the control shown in Fig 3. and displays the metaphase with the Reference colour and the DAPI object boundaries

Register raw CGH images

Figure 3 - Manual image registration tool

This control allows you to modify alignment of the fluorochrome images in relation to each other and the DAPI objects to correct for any automatic registration errors or to
specifically allow exact registration on one metaphase area or chromosome. Note: You can use keyboard shortcut controls - Alt+R and Alt+T.

When **Register Test** is switched on it is possible to also see the Ratio highlight for easier determination of shift values.

When you click **OK**, the chromosome ratio profiles are recalculated and updated in the profile diagram.

**New Standard Reference Intervals**

- HRCGH-2 uses a new set of default Standard Reference Intervals, which are based on the slightly longer chromosome axes generated by the new software.
- You will only see one set of "Default" SRIs depending on which mode you are in. When you switch on HRCGH-2 CytoVision automatically uses these new files instead of the original shorter versions. If you turn off HRCGH-2 CytoVision automatically reverts to using the original versions.

**Important**

If you have built your own Standard Reference Interval (SRI) files and wish to make use of HRCGH-2 you will need to re-generate your SRI files. This can be done by the following simple procedure:

1. Load the case(s) used for the original SRI files
2. Switch on HRCGH-2 This Case in the CGH Profile Display dialog. and Apply Options
3. Reload each cell and correct any axes if necessary.
4. Add each cell to a new SRI file - use a new name to distinguish from your old file.

**Printing - New printing features include:**

- The ability to add and save a logo to the print or print template. Only BMP files may be used and only one logo can be selected (i.e. not different logos for each print layout).

- Panel reporting
  - Users can insert items from a save phrase list to an image.
CytoVision 3.6

- Templates can be saved with phrases already attached EX. CLL panel report.
- 1 to 6 images per page may now be selected for printing.
- The patient name has now been removed form the Slide/cell label.
- These new features will require the creation of new print templates.
- Extended karyotypes require creation of new print templates.

**Capture Improvements**

**Fluorochrome Selection**

<table>
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</tr>
</tbody>
</table>

- The Auto sequence feature has been moved to the Fluorochrome selection panel
  - It is now possible to turn this feature on or off without opening the customize window
  - The check mark indicates the feature is activated
- Retresholding and ROI capture have been improved.
  - When Auto Setup is selected the computer will quickly adjust the settings and automatically turn off

**HINTS:**

- For fully automatic image acquisition, turn Auto Camera Setup on in customize and then select capture, the computer will automatically adjust the settings and capture the cell.
- In Brightfield mode combine with Streamlined Capture for one step capture
- In Probe mode combine with Auto Sequence for one step capture

**Scanning Improvements**

- Improved auto focus
- In the Prescan Wizard it is now possible to ignore bad focus maps
  - When all focus maps have finished, the system will start scanning and just skip any bays where the focus map failed
  - The failed bays can be manually focused later and then scanned
- Faster upload of image fragments, meaning that there is less delay at the end of scanning a slide while a large number of objects to upload to the review screen.

**Capture Count**
• If auto camera setup is turned on in the capture customize dialog then auto camera setup will happen automatically for every capture
• Fused chromosomes are now marked with an 'F'
• Spurious left clicks when fusing caused multiple tiny fragments to be cut - this has been resolved
• Saving RAW images now works correctly in the capture count dialog

**Capture Wizard**

• The Wizard now shows the case name as well as the slide name
• In Chromoscan it is now possible to skip the focus map step
  • Select autofocus every frame and autocapture for seamless scanning

**Exporting Images**

• Allows you to scale the image up or down to particular dimension in pixels or percentage of the original image scale.

![Export Image Window](image)

• For more details GO TO HELP > INDEX > Export > Export Images in TIFF Format

**Software corrections related to:**

• **Chromoscan / SPOT: digital capture**
  • Chromosomes fused into images using the Count feature are now marked with the "F" annotation in the metaphase

• **Probe Capture:**
  • Intermittent crashes during probe, MFISH and CGH capture modes have been resolved

For additional information on what's new in 3.6, please refer to the CytoVision 3.6 Release Notes you received with the system or software disk.

**New in the CytoVision 3.5 release**
Streamlined Capture

- Streamlined Capture enables one-button capture in brightfield and fluorescent mode. This feature means fewer mouse clicks and faster image acquisition.

Consolidation of Contrast and Enhance windows

- The Contrast and Enhance dialogs have been combined into one window for easy access to both sharpen and contract at the same time.
- Selecting opens this window.
- Operation in slightly different depending on whether you have switched Preview Enhancements on in the Customize Analysis window.
- Important: Macros involving enhance and contrast will need to be reprogrammed!

Fuse Field Chromosome Flag

- The fused field flag, F, attached to a chromosome in a metaphase, is now visible after the metaphase has been karyotyped (and saved), and can be toggled on and off via the Customize Analysis dialog.

Toolbar Display Buttons

- It is no longer necessary to open the Customize Analysis dialog to change the Probe, CGH, M-FISH or RxFISH display appearance. New icons have been added to the main vertical operations toolbar for Fluorochrome selection, CGH Ratio Image, Pseudo Class and Tint Class

New M-FISH features

Many new features have been added to improve the utility of the M-FISH module.

- Manual Thresholding of Counterstain
- Expected Class Color Bars
- Tint Class View
- Pseudo Class View
- M-FISH Composite Viewer
- M-FISH Cleanup
- Show chromosomes by M-FISH class

Customer Feature Requests

- Annotation - allows user to see entire typed text, even in large text mode. See topic in Analysis > Composites, profile and annotation > Adding text, shapes or arrows > To add text to an image
- Change Filter Message - See topic in Probes > Capturing cells > Probe capture process

Digital Camera Improvements

See topic in Capture > Using a Digital camera

- Capture
- Analysis
Scanning System Improvements

- Slide naming - The same name for different slides attached to different cases can be used when using the Wizard in the scan screen.
- AutoFocus - Autocapture with the high power objectives produces usable metaphases. Note: If capture is done at 100x, the image will need to be minimized to karyotype and fit in the display.
- Tip: Remember to set the customize features in capture for the options you want during autocapture. Try using autostop, predict threshold and autothreshold with autocapture for metaphase images.

New in the CytoVision 3.0 release

General Use

Third party file import

Third party file import allows you to incorporate test requisitions, count sheets, and other related documents into a case. Just drag and drop document and image files onto the case in the Navigator. All files remain editable in their native software. They can be stored in any level; case, slide or cell.

Third party file import supports documents in Microsoft Word (.doc), Adobe Acrobat (.pdf), plain text (.txt) and rich text format (.rtf). Spreadsheets in Microsoft Excel (.xls). Images in bitmap (.bmp), Graphics Interchange Format (.gif), Jpeg (.jpg), Portable Network Graphics (.png), Paint Shop Pro (.psp) and Tagged Image File Format (.tif). Zipped archives (.zip)

Sources for files can be paper documents or images scanned using a standard flat bed scanner. Files created in Microsoft Word, Excel, or other supported software. Images from disks, the web or from email. Include documents from the Lab Information System.

Image import (Capture From File)

Capture images from a file instead of the camera. Version 3.0 provides full import of common file types for use in CytoVision. Work with them just as you would any captured image. Supported formats are Bitmap (.bmp), Graphics Interchange Format (.gif), Jpeg (.jpg), Portable Network Graphics (.png), Paint Shop Pro (.psp) and Tagged Image File Format (.tif).

Single images can be captured in brightfield or fluorescent mode. Multiple layer composites can be captured in probe. The image can be thresholded, segmented, karyotyped or enhanced just like a captured image.

Capture from File is an option in Capture Customize.

Expanded range of file formats for image export

The range of file types the CytoVision exports in has been expanded to include: Bitmap (.bmp), Graphics Interchange Format (.gif), Jpeg (.jpg), Portable Network Graphics (.png), Paint Shop Pro (.psp) and Tagged Image File Format (.tif).

Windows XP operating system

CytoVision is switching to the Windows® XP® operating system. Windows XP gives the same solid performance and networking capabilities of NT, but with a more user-friendly interface. One advantage of Windows XP over Windows® NT® is its built in support for hardware such as printers and USB devices.
Analysis

Modal analysis tools
Modal analysis tools provide a way to separate groups with a single click. Once a tool is selected it remains active until turned off or swapped for a different tool. This works with split, overlap, draw axes, delete, join and chromosome. It works in manual or automatic mode.

Modal operation is an option in Analysis Customize.

Enhancement preview
Enhancement and Contrast now have real-time live previews of the image. As adjustments are made you can see the effects on the image.

Live Image Preview is an option in Analysis Customize.

Metaphase Labeling
Show chromosome class labels on the metaphase with new 'Metaphase Annotation' option in Customize. Attach labels to individual chromosomes or to an entire cell.

Attach and show labels from Customize in Analysis.

Automatic chromosome arrangement
If you spend time arranging chromosomes to face away from each other in a karyotype you’ll love the new automatic arrangement. The curve of a chromosome is measured to determine the best position in the class.

Left curves are placed on the left and right curves to the right. If two chromosomes curve the same direction the most bent chromosome is placed on the outside. This only takes place when the cell is first classified or when a chromosome is moved into a class so you can override the feature if needed. Automatic arrangement is a real time saver for perfectionists.

Scanning and Spot counting

SPOT Assay Selector
The assay and scripting tools for interphase FISH analysis have been given a makeover. An assay panel lets you associate FISH assays with diseases and the appropriate script. This makes it easier to find the right settings and quick to set-up.

Better scripting
This Script Configuration dialog has been rearranged for better workflow. Additional fields and settings give greater control. Z-Stack settings are now saved within the script so additional Fluorochrome files do not need to be created.

Scripts have more control over analysis and scanning. There are options to end scans based on number of cells found. This number can be based on a total or by class. Results are continuously monitored to let you know when you have enough cells and can move to the next scan. A progress panel shows the results as cells are analyzed. Stopping a scan when sufficient cells have been analyzed saves time.

Boolean operators have been included to allow for better classification of assays such as UroVysion™. Advanced options let you customize the way the script looks for cells and signals.

Faster capture algorithms
The capture process for scanning has been altered to improve the speed of scanning.
**Scan wizard**

The steps needed to set up for a scan have been consolidated and automated. A wizard has been created to walk you through the couple of steps that are needed. A new system of saving scan settings in a SuperScan is an integral part of this.

These SuperScans contain all the settings needed to scan a slide in one file. This includes finder application; metaphase scanning or interphase FISH analysis. The camera capture settings. Assay names for interphase FISH applications and classifiers for metaphase applications. Filter names and positions for fluorescent applications. And finally the scanning parameters for prescans such as the focus frequency and number of cells to stop after. Also the scan area for each slide. Multiple SuperScans can be saved so commonly run samples such as bone marrows or UroVysion can be started in a click.

**Capture wizard**

Automatic capture of both metaphases and interphase FISH is more automated and easier to use. A wizard takes you through the steps and automates some of the tasks. All that is needed is a single focus point and camera settings, then you’re ready to begin capturing. Focus maps are created and auto capture starts immediately without any need for further user interaction.

**Ways to get help**

**Applied Imaging Help Contents and Index**

Help can be obtained in the usual way through clicking on Contents in the Help menu. This launches the HTML Help Viewer. Use the Contents tab to browse through subjects, use the Index to quickly find specific subjects or words. You can search through all of the topics from the Search tab and add topics you want to bookmark to the Favorites tab.

**Windows Help Contents and Index**

Microsoft® Windows help files may be accessed by clicking on Help from the Start menu. Use these files for any questions related on how to use Windows.

**Conventions**

Menu items are listed as Menu > Submenu. For example instructions say choose File > Open, click on the File menu and choose Open.

Context menus are opened by clicking on an item with the right or secondary mouse button.

**Service and Support**

There are call centers in the United States and in the United Kingdom. You can get help with hardware, software or sales related questions from these offices or through your local distributor.

[Contacting Applied Imaging](#)

**Contacting Applied Imaging**

Customer support call centers and sales offices are at the following locations or from your local distributor. A current list of distributors can be obtained from the corporate website using the link below.

**North and South America**

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Corporation
120 Baytech Drive
San Jose, CA 95134
USA
Phone +1 (800) 634-3622
or +1 (408) 719-6400
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(GMT)
Applied Imaging
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BioScience Centre,
Times Square
Newcastle upon Tyne
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sales@aii.co.uk

Websites
Corporate http://www.aicorp.com
CytoNet http://www.cytonet.com

Using dialogs and controls

Dialog boxes and controls are used to send commands to the software. Dialog boxes are separate panels that open on the screen. Controls are contained within these boxes and are the means to communicate with the software.

Radio button

O Brightfield
O Fluorescent
O Probe
O CGH
O MFISH

Radio buttons are used when only one option can be selected. Click on the circle by the option to select it.

Check Box

✓ Auto Threshold
✓ Predict Threshold
O Zero Threshold

Check boxes are used when more than one option can be selected. Place a check in the box to turn an option on.

Check boxes are used when more than one option can be selected. Place a check in the box to turn an option on.
Sliders

Select a value from the range by sliding the pointer. Most sliders can be nudged by using the arrow keys.

Preset values

Select a value by clicking on its button.

Combo Box

Combo boxes contain a list of possible choices. Click the arrow to expand the list, then select one of the choices by clicking on it.

List Box

Click on an item in the list to select it. To select more than one, hold the Ctrl button down for each additional selection. To select a continuous block, click on the first entry, then while holding the Shift button click on the last.

Edit Boxes

Edit boxes are text fields that allow text to be entered directly.

Non-editable text fields
Text fields that are background colored cannot be changed.

**Spin controls**

Click on the upper arrow to increase the value by 1 and the lower to decrease it by 1. Click and hold the arrows to rapidly scroll through the values.

Click on the upper arrow to increase the value by 1 and the lower to decrease it by 1. Click and hold the arrows to rapidly scroll through the values.
CytoVision Basics

CytoVision basics

The system runs on a Microsoft® Windows operating system. If you have used Windows, the user interface will be familiar to work with. If you are not familiar with Windows we recommend reading the Windows Help topics. To start Windows Help click on Start > Help.

The images are saved in a proprietary format. All images are saved in cases that are organized into subfolders.

About the workarea

The CytoVision workarea has a main toolbar at the top of the screen, image windows and a panel for the navigator. There are one to four views depending on the capabilities of the system. Each view is used to perform specific tasks. The main view, Analysis contains the tools for working with captured images. The Capture view contains all functions related to capturing live images. Scanning systems also have Scan, used to scan a slide for cells or metaphases, and Review for sorting and viewing the objects found. The buttons on the left side of the main toolbar are used to select views.

There are three layouts available, Large Navigator, Classic and Fullscreen, that can be selected from the Main Menu - Layout.

- The **Classic** layout moves the smaller image windows to the right side of the screen, shortening the navigator panel.
- The **Large Navigator** layout increases the size of the Navigator window to provide access to more items, such as if you have cases with numerous slides and cells or if you like to have several cases open at once.
- The **Fullscreen** layout doubles the size of the main work window and the other image windows are reduced to thumbnails. The Navigator can be accessed through a button - that will appear active on the toolbar when using the Fullscreen layout.

The Gallery button is on the Large Navigator and Classic layout above the Navigator (not seen in images below). See topics 'About the Gallery' and 'Working with the Gallery' for more details.
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**Large Navigator view of Analysis**

**Full screen view of Analysis**

**About the Gallery**

When a case is displayed in the navigator, click on the Gallery button to display the thumbnail views of all the associated images in the case in the navigator. The gallery button location will
be dependent upon the selected layout view and is found at the top of the navigator window in the Classic and Large Navigator layouts.

The Gallery displays thumbnails images of the captured or analyzed images associated to the slide/cells/ in the navigator.

**Converting images for gallery viewing**

If a case was captured with earlier versions of CytoVision, the gallery thumbnail view are not immediately displayed. Instead, the display shows a series of squares with question marks. These case images must be converted to thumbnail images by clicking on the Generate thumbs button.

**Note:** This conversion needs to be done only once. When the case is reopened, the gallery images will always be displayed when the Gallery button is selected.

Click on the generate thumbs button to convert the display to the thumbnail images.

Generate thumbs
Related topics
Working with the Gallery

About image windows

There are six image windows in the workarea. The largest window is the main work window used for capturing or editing images. The smaller windows provide storage for additional loaded images.

The image in the main window can be moved into a blank window, or swapped with a loaded image, by clicking on the smaller window. Swap images in smaller windows by clicking on one with the middle mouse button, then click on the second image with the left mouse button.

There are three layouts available, Large Navigator, Classic and Fullscreen, that can be selected from the Main Menu - Layout.

About the Navigator

The Navigator shows the contents of a case in a file tree format. It is used to load images and organize the slides and cells in cases. Slides and cells are automatically numbered as they are created, but can be renamed. When an object in the Navigator is selected, it becomes the active object and is highlighted. If a slide or cell contains images, they will also be selected. In the Fullscreen layout, the Navigator is displayed or hidden by clicking on the Navigator button in the toolbar.

Different icons are used to show the types of images contained in a cell. The example below is of a brightfield cell with a metaphase, fuse image and karyotype. The active image is the metaphase that is highlighted. Additional icons are shown below.
The command buttons under the navigator are used to load, clear and save images. **Load** and **Load Cell** load the active image into a work window. See the topic **Loading images** for details. **Clear** removes a selected image from the work windows. **Clear All** removes all loaded images. **Save** stores changes made to an image. When Load, Clear and Save are clicked the cursor will change to a question prompt.

Select the work window to apply the action to by clicking on it.

**Additional Navigator icons**

- **Flex** Flex image
- **CGH** CGH profile
- **Met** Fluorescent metaphase
- **Raw** Raw image
- **Kar** Fluorescent karyotype
- **Probe** Probe image
- **Spot** Spot counting list

**Third party files** (see the topic **Importing third party files** for details)

- **Microsoft Word, Rich text format (.doc, .rtf)**
- **Microsoft Excel spreadsheets (.xls)**
- **Adobe Acrobat (.pdf)**
- **Plain text (.txt)**
- **Supported image files (.bmp, .tif, .jpg, .gif, .psp)**
- **Compressed (zipped) folders (.zip)**

**Tip** - Click on an icon with the right mouse button to access the most common commands for that image type.

**Common toolbar buttons**

These buttons are used on the toolbar in every view.
**Analysis** switches to the view used to edit images.

**Capture** switches to the view used to capture images.

**Scan** is found on scanning systems only. Scan view has the tools used to scan a slide for cells or metaphases.

**Review** is found on scanning systems only. This view has the tools used to view and edit the results of a scan.

**Library** contains the history of a case including where it has been archived and any searchable keywords that have been saved with the case.

**CytoNet** sends and retrieves cases to other users via the web.

**New Case** creates a new case.

**Open Case** opens an existing case.

**Case Details** are the files that contain patient or case information.

**Close Case** closes the current case.

**Import Images** import image files from other image formats.

**Archive and Restore** contains the backup tools for cases and other system data.
**Navigator Toggle** lists the contents of open cases in a file tree format, and is used to load, clear and work with images.

Tip - Buttons on the toolbar can be hidden if they are not used. See the topic Hiding or displaying toolbar buttons.

### Hiding or displaying toolbar buttons

The toolbars shown change for each view. Analysis view also has a toolbox next to the main work window in addition to the toolbar. Each toolbar can be customized by adding or removing icons. To open the Customize Toolbar dialog choose View > Toolbars > Customize and select a toolbar.

The list on the right shows the buttons that are currently displayed. The list on the left shows hidden buttons that are available for the toolbar. Click on Default to return the toolbar to its default configuration.

**To hide a button:**
Select the button in the list on the right and click on Delete.

**To display a button:**
Select the button in the list on the left and click on Add.

![Customize Toolbar](image)

**Note** - Hiding and displaying buttons can cause the position of buttons to shift. Macros that involve the toolbar may need to be re-recorded.

### Shortcuts for working faster
There are three ways to get your work done faster, shortcut menus, hotkeys and macros.

**Shortcut menus**
Shortcut menus are opened by clicking on a window or navigator item with the right mouse button. They contain the most common commands used for those items. The menu changes depending on the object. When using shortcut menus in images, make sure that the cursor is not over an object in the image. If you see the blue rectangle, move the cursor slightly until it disappears. For raw or unthresholded images move to the far right edge of the image to the border area.

**Hotkeys**
Hotkeys replace a mouse click with a keyboard button. This is a good way to perform repetitive tasks such as trimming. The mouse pointer can remain in the area of interest while the keyboard is used to send a command. Use a hotkey by pressing the key instead of using the mouse to press a button. You can view a list of what keys are programmed by pressing F12 then Hotkeys.

**To program a hot key:**
Click on a command button with the middle mouse button. When the cursor changes to a press any of the letters on the keyboard.

**Note** - Only letter keys can be used for hotkeys, not numbers or symbols.

**Macros**
A macro automates a series of commands and instructions by grouping them together as a single command. Instead of manually performing a series of repetitive actions, they are replaced with a single keystroke. The macro keys are F1-F10. Use a macro by pressing the F key the macro has been recorded to. View the list of recorded macros by pressing F12 on the keyboard. Stop any macro while it is running by pressing F11.

Macros record the position of the mouse when a button is clicked, not the button name. For this reason separate macros are recorded for each layout within a view due to the shift in button positions. A macro recorded in the Large Navigator layout of Analysis must also be recorded in the Fullscreen layout if it is to be used there. There are three sets for each view, one for the Classic view, one for the Large Navigator view, and another one for the Fullscreen view. Each set can use all of the macro keys. For example the F1 key can be used for a sharpen macro in both the Large Navigator and Classic layout of Analysis. F1 can also be used for a completely different function in Capture.

**To record a macro:**
1. Press F12 on the keyboard. The Macros and Hotkeys dialog will appear.
2. Place a dot in the circle by the desired F key and enter a name to identify the macro.
3. Click on the Record button and the dialog will disappear.
4. Perform the tasks as you would normally. The mouse movements and clicks will be recorded.
5. When finished press F11 on the keyboard to stop recording.

**Tips on using macros**
- Macros are best used to replace tasks that require more than one mouse click. If the task only requires a single click on a command button use a Hotkey instead.
- Before recording a macro, plan the steps needed to complete the task. Include all steps like Select All and Deselect All.
• Some steps cannot be included in a macro and must be done manually before running the macro. These include selecting individual objects or images, and the Load, Clear or Save commands.
• When recording macros for contrasting images, click on Reset before moving the sliders. That way the contrast values will be the same every time.
• When recording several tasks into a single macro, remember that Undo only reverses the last action. When using macros to replace image enhancement tasks it is best to limit a macro to a single enhancement.

Drawing techniques and styles

The same basic method is used to draw lines when using most tools. The left mouse button starts drawing. The right mouse button will stop the current line and the middle button will reverse or undo the line. If you are using a tool that allows multiple lines to be drawn, pressing the right button a second time will apply all lines and exit from drawing mode.

There are two different methods or styles used to draw lines, Rubberband and Freehand. The Rubberband method uses straight lines that are tacked down at points to change direction. The Freehand method does not require tack points, the line will follow the mouse until drawing is stopped.

Drawing styles are selected from the customize dialogs in Capture or Analysis screens.

To use Rubberband style:
1. Position the mouse where you want to begin drawing and click the left mouse button once.
2. Move the mouse cursor along the cutting path until the direction needs to change.
3. Change directions by clicking the left button once to tack the line down.
4. If you make a mistake, click the middle button to remove the last tack point and back up.
5. When the line is finished click the right mouse button once.
6. To draw another line repeat steps 1-5.
7. When finished, click the right button a second time to exit cutting mode.

To draw with Freehand style:
1. Position the mouse where you want to begin drawing and click the left button once.
2. Move the cursor along the cutting path. The line will follow the mouse without having to tack it down to change directions.
3. If you make a mistake move the cursor back to where you want to restart and click the middle mouse button until the line is removed.
4. When the line is finished click the right mouse button once.
5. To draw another line repeat steps 1-5.
6. When finished, click the right button a second time to exit cutting mode.

*Line drawn using Freehand style*

**To draw using the optional Freehand hold to draw style**
In Customize Analysis, after a Freehand style is selected, check Freehand hold to draw style box.

1. Position the mouse where you want to begin drawing and click and hold down the left button.
2. While holding the left mouse button down, move the cursor along the cutting path. The line will follow the mouse without having to tack it down to change directions.
3. If you make a mistake, while still holding the mouse button down, use the middle mouse button to back up.
4. Continue drawing line to complete action. Release left mouse button to finish line.
5. To draw another line repeat steps 1-4.
6. When finished, click the right button to exit cutting mode.

**Disk space**
For optimum performance, the hard disk should have at least 40% of space free. As the disk fills it will become harder to defragment the files, and finding space for paging files will slow the system down. It is also advisable to regularly and frequently archive important case data to removable disks as insurance against hard disk failure. See the section titled *Archive and restore overview* for details.

**To check how much space is available on a disk:**

1. Double click on the My Computer icon on the desktop.
2. Select a drive, C contains the operating system files and D contains the case data.
Working with cases and the library

Working with cases and the library overview

Each case is a separate directory on the hard drive. Slides and cells are subdirectories contained within the cases and are used to organize the images. The slides and cells are created as needed using the Navigator. There is no limit on the number of slides and cells in a case. They can be renamed or deleted but cannot be moved between cases.

Case Details stores information about the patient or case. It has commonly used fields such as patient name and date of birth, and an area for creating custom field titles. Case Details also contains a Completed flag used to mark cases as finished.

A history file known as the Library lists every case that has been created on the system. The Library serves three purposes: to prevent duplicate cases from being created, to provide a means of searching details about cases that are no longer on the system and to provide a list of disks each case has been archived onto. A separate utility called the Case and Library Manager is used to edit case names contained in the Library.

Every dialog box that is used to access cases contains a filter section. The filter searches for specific cases or limits the list of cases visible in the dialog box. The filter can search through current cases or the information in the Library.

Archiving provides a way to safely back up data on removable storage disks as well as automatically delete completed cases from the hard drive. See the topic Archive and restore overview for more information.

Opening and creating cases

Create a case by clicking on the New Case button on the toolbar.

The Create New Case dialog will open with blank fields. Enter a unique name in the Case field. This will be the filename of the case. Case names must be between 2-14 characters long and may contain any letter, number and the following characters: _ - + .

The other fields can be filled in now or later from Case Details. Click on OK to create the case and close the dialog.

There is a security check to prevent duplicated cases. A name can only used once and is stored in the Library even after it has been deleted from the hard drive. To re-use a case name delete the library entry using the Case and Library Manager program.

Open a case by clicking on the Open Case button on the toolbar. Select the case you want from the list and click Open Case or double-click the name to open the case. If there is a long list of names to choose from, you can type the name in the box directly or use the Filter to find the case for you.

Close a case by clicking on the Close Case button on the toolbar or choose Close Case in the right-click shortcut menu in the Navigator.
**Note** - If you are unable to open a case and see one of the following error messages, use Clear Locks in the Case and Library Manager.
- "Case is being archived" – but you know the case is not being archived.
- "This case is already open" – but the case is not open on any systems.

**Filtering case lists**

The filter can be used to limit displayed cases or to find a specific case. It searches for strings of characters in case lists, case details currently on the hard disk or Keywords in the Library.

To use the filter, enter the string of characters in the **For** field and click on the **Apply Filter** button. Partial strings can be used. For example "demo" would find a case named demo orDemonstration. The **Include** option shows all cases that match the criteria, **Exclude** shows all cases that do not contain the criteria.

The check boxes modify the search. **Case Sensitive** differentiates between upper and lower case. By default only the case names are used for searching. **Search Details** looks for the string within the case details files of cases currently on the system. **Search Keywords** searches through the Keywords in the library. The **Completed Cases** option limits the list to cases that have been marked as completed in the Case Details file.

Click on the **All Items** button to remove the filter.

**Marking a case as complete**

The main benefit of marking a case as completed is to filter case lists. Completed cases can be excluded from the list when opening a case. Case lists can be filtered to show only completed cases for reviewing. Completed cases can be quickly selected for archiving.

**To mark a case as completed:**

1. Open the case and click on Details.
2. Place a check in Complete.
3. Click on OK to save the change.

**Tip** - You can also mark a case as completed by clicking on its name in the Navigator with the right mouse button and choosing Complete.

**Create or rename slides and cells**
Slides must be created in an open case. Cells must be created in a slide. Slides and cells are automatically numbered but can be renamed after they are created.

**To create a slide:**
Right click on the case name in the Navigator and choose New slide.

**To create a cell:**
Select a slide in the Navigator and click on the New Cell button under the main image window in Capture. Alternatively choose New Cell from the context menu in the Navigator.

**To rename slides or cells:**
1. Select the slide or cell in the Navigator.
2. Click the selected object with the right mouse button and choose Rename.
3. A text entry field will open. Enter the new name and click anywhere outside of the text field with the mouse to save the new name.

**Deleting items from the Navigator**
Cases, slides, cells and images can all be deleted from the navigator. This removes the object and all of its contents from the hard disk. Images must be cleared from the image windows before they can be deleted.

**Note** - Cases deleted in this method will remain in the Library. To remove all references to a case use the Case and Library manager. See the topic Rename or delete a case.

**To delete an item:**
1. Select the item in the Navigator.
2. Click on it with the right mouse button and choose Delete.
3. Answer Yes to the warning box if the object listed is correct.

No–Cancel will exit without deleting.

**Deleting a raw image**

**About Case Details**
Information about a case such as the patient name and results, are stored in Case Details. 
Open Case Details by selecting an open case in the Navigator and clicking on the Case Details button on the toolbar.

There is a set of common fields with fixed titles and an area where custom titles can be created. These are called Variable Fields. See the topic Creating custom fields for details on how to use Variable Fields. All fields are optional.

The information can be edited at any time. To save changes close the dialog using the OK button. Click on Cancel to close without saving changes. There are two fields that cannot be edited. Case name and Archived On. The case name can only be changed from the Case and Library Manager. See the topic Rename or delete a case. Archived On shows the list of disks the case has been archived to. This information is saved in the Library as part of the archiving process.
Keywords is a special field that is saved in the Library. Their purpose is to provide a way to search for cases that have been archived and are no longer on the system.

Most fields in Case Details can be printed with the images to create reports. See the topic Selecting case details fields.

<table>
<thead>
<tr>
<th>Case</th>
<th>This is the unique name used for the case. You cannot change the case name from within the program. If you want to change the name of an existing case you must use the Case and Library Manager program.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Archived On</td>
<td>Any disks that the case have been archived to will be listed. You cannot edit this field.</td>
</tr>
<tr>
<td>Complete</td>
<td>Mark a case as completed by placing a check in this box. This is a convenient way to mark that work has been done on the case and it is ready to be archived.</td>
</tr>
<tr>
<td>Keywords</td>
<td>Enter any words you would like to associate with the case for searches in the library. You do not need to separate entries but you can use commas or colons to make them easier to read.</td>
</tr>
</tbody>
</table>
**Variable fields**  Create custom data files with your own titles.

**Save titles**  Used to save the current Variable Fields as a new template.

**Load titles**  Used to load Variable field templates.

**Reset**  Clears all Titles from the Variable fields.

**Field lengths - Maximum characters allowed in the data fields**

<table>
<thead>
<tr>
<th>Field</th>
<th>Maximum number of characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case</td>
<td>14</td>
</tr>
<tr>
<td>Patient Name</td>
<td>39</td>
</tr>
<tr>
<td>Date of Birth</td>
<td>11</td>
</tr>
<tr>
<td>Specimen</td>
<td>49</td>
</tr>
<tr>
<td>Reason for referral</td>
<td>257</td>
</tr>
<tr>
<td>Technologist</td>
<td>39</td>
</tr>
<tr>
<td>Date</td>
<td>11</td>
</tr>
<tr>
<td>Comment</td>
<td>257</td>
</tr>
<tr>
<td>Keywords</td>
<td>63</td>
</tr>
<tr>
<td>Result</td>
<td>257</td>
</tr>
</tbody>
</table>

**Creating custom fields**

Custom or Variable fields and templates can be created for the Case Details files. This allows you to label fields to meet your needs. **Save titles** saves the fields as templates. **Load titles** is used to load these templates. **Reset** clears all entries from the Titles column.

**To create a template:**

1. Click once below the Title column to highlight the field. Then click once more to open the text entry fields.
2. Enter the text for the title into the column on the left and press the Enter key on the keyboard.
3. Repeat for all additional titles.
4. Click on Save titles. The Variable title format dialog will open.
5. Enter the name you want to use for your template in the Selection field and click on OK.

**To enter data into variable fields:**

1. Click once on the title of the field you want to enter data into to highlight it. Then click once more to open the text entry fields.
2. Enter the data into the column on the right and press the Enter key on the keyboard.
To load a Variable field template:

1. Click on Load titles in the Case Details dialog and the Variable title format dialog will open.
2. Select the name of the template that you want to load from the Available formats list and click on OK. The titles will be loaded into Case Details.

About the Library

The Library keeps a record of every case that has been created on the system. On networks, the Library contains references to all cases created on networked systems as well. The main function of the Library is to prevent the creation of cases with duplicate names. Information stored in the Library is the case name, the system a case was created on, archive disk labels and Keywords.

Open the Library by clicking on the Library button on the toolbar.

To view the information about a case click on its name in the list. Machine lists the name of the system the case was created on. This is useful on networks as renaming or deleting of cases must be done on the system where the case was created. See the topic Rename or delete a case for more information about this.

Keywords are the only field in Case Details that are saved in the Library. This allows you to search for certain details of a case after it has been archived and removed from the hard disk.

Archived on shows a list of all disks or tapes the case has been archived to. This list can be recreated should the Library ever be lost. See the topic Restoring a library.
About the Case and Library Manager

The Case and Library Manager program serves three functions. It is used to edit library entries, synchronize data between libraries on a network, and repair any network errors regarding cases. To run Case and Library Manager choose Start > Programs > Applied Imaging CytoVision > Case and Library Manager.

Rename Case renames a current case in both the Library and case list. Delete Case removes a case and its contents from the hard disk and the Library. Sync Library is used whenever a new system is added to a network or if the libraries have become unsynchronized.

Clear Locks is used to clear any case locking files left as a result of network failure. There are security features which prevent a case from being opened on more than one machine, or from being opened while it is being archived. In the event of a network failure, these locking files can be left on the machine. Clear Locks removes these files so the cases can be accessed.

To clear case locks:
1. Make sure that none of the systems on the network are running CytoVision.
2. Click on Start > Programs > Applied Imaging CytoVision > Case and Library Manager.
3. Click on Clear Locks.

⚠️ Warning Before running Case and Library Manager, make sure that CytoVision is not running. On networks make sure CytoVision is closed on all systems.

## Rename or delete a case
Using the Case and Library manager is the only way to edit the contents of both the cases directory and the Library. Cases can be deleted from within the navigator but their name will remain in the Library and that case name cannot be used again.

**To rename a case:**
1. Click on Start then point to Applied Imaging in the Programs menu and select. Case and Library Manager.
2. Select the case from the list and click on Re-Name on system and in libraries.
3. Enter the new name for the case in the pop-up dialog.
4. You will be asked to confirm that you want to change the name. Click on OK and the name will be changed along with the library entry for that case.

**To delete a case from Library:**
1. Click on Start > Programs > Applied Imaging CytoVision > Case and Library Manager.
2. Select the case name in the list.
3. Click on Delete from system and libraries.

**Notes**
- You must be logged on as administrator to run Case and Library manager.
- Only cases that have not been archived can be renamed.
- On peer-to-peer networks cases must be renamed at the station they were created on. This information can be found by clicking on the case name in the Library and checking the Machine field.

**Synchronizing libraries**

Synch Library checks all libraries on a network to make sure they contain the same information. Any missing data will be appended to the Library file.

The following are situations that require libraries to be synchronized:

- Whenever one or more systems are added to a network for the first time Synch Library must be run on the new systems.
- When a system that was removed temporarily from a peer-to-peer network is returned, run Synch Library on the returned system. Networks that are configured using AutoNC and are Windows only operating systems are an exception. It is not necessary to synch the Library of the temporarily removed system in this case.
- Peer-to-peer networks that contain some UNIX systems should synchronize the libraries after reconnection even if AutoNC is used.

**Note** - It is advisable to wait a few minutes after connecting a system to a network before performing any network configuration tasks. This gives time for Windows to adapt to the change, which makes further configuration more reliable.

**To synchronize system libraries:**
1. Click on Start > Programs > Applied Imaging CytoVision > Case and Library Manager.
2. Click on Sync Library with other libraries on network.
3. Answer Yes when asked to continue and the library of that system will be updated.
4. Repeat for all systems that need updating.

**Restoring libraries**

Libraries that have been lost due to hard disk failure can be partially restored. A list of cases along with the disks they have been archived to can be created using archive disks. Keywords cannot be recovered.
To recreate a library from archive disks:

1. Place an archive disk the drive.
2. Open Archive and Restore.
3. Select the Restore tab.
4. Click on the Restore Library button.
5. Repeat the procedure for all archive disks.

⚠️ Note - On networks, if the Library is only damaged or lost for one system use Synch Libraries in the Case and Library Manager to restore the Library.
Working with images

Images captured on the system are stored in cases. Cases are directories that contain slide and cell sub-directories. Images are kept within the cell sub-directories. During capture, images are saved directly into an open cell sub-directory. This eliminates the need to save individual images during capture. Images are accessed and loaded into windows using the Navigator.

Objects within metaphase and probe images can be separated using the cutting tools but they cannot be moved, rotated or resized within the image. Objects from other cells cannot be copied into metaphases unless they are fuse images from the same cell. To copy objects from more than one cell into the same image use flex images. Flex images allow you to combine objects from different image types and cases. They also allow objects to be moved, rotated and resized.

Change the viewable size of an image by using zoom or Fullscreen. Automatically decrease the size of a large image using Scale to Fit.

Save changes made to images by clicking on the Save button below the Navigator, then on the image window.

Related topics

- Load and clear images using the Navigator
- Working with the Gallery
- Select objects in a cell
- Delete, copy and paste objects
- Move, rotate or invert objects
- Delete images from a case

Working with the Gallery

The Gallery image shows thumbnail images of the cells associated to the opened case(s) in the Navigator. The Gallery button toggles the display between the main working window and the gallery view. The Gallery button is found above the navigator when the Large Navigator or Classic main layout view is selected. To access the Gallery button when the Fullscreen layout is selected, click on the Navigator button in the analysis toolbar to open the navigator window.

If cases were collected with previous CytoVision software versions, the gallery images will have to be converted. See the topic 'About the Gallery' for details on converting gallery images.

Note: The Gallery will display all open cases in the Navigator. If you have three cases open, all will be displayed in the gallery view.
**Generate thumbs button**

Generate Thumbs converts images collected with previous CytoVision software versions to gallery images.

**Check boxes**

The type of images in the gallery display will depend on the images associated to the case in the navigator. When the image type is checked, all of the selected image types in the case(s) displayed in the navigator are in seen in the gallery display. For example, to display only the metaphase images in the gallery, uncheck all other image boxes and only the metaphase images will be displayed in the gallery window.

**Load a gallery image in the main working window**

To load gallery images in the main working window, open the case in the navigator and select the Gallery button. The display changes to the gallery display.

Single click on a gallery image - a red box will appear around the image and the associated image number is highlighted in the navigator window.
Each gallery image has an image label associated to the case, slide / cell / image number and type in the navigator. In the above example, the second gallery image is labeled 1/1/8 (Probe) – and is associated in the navigator to slide 1, cell 1, image 8 - Probe. The case name is displayed vertically to the left of the image.

**To load a gallery image**

Double click on the gallery image - the cursor changes to a question mark and the display changes to the main working window. Left click on any main working image window to place the selected gallery image in the working window. If the image is a metaphase with a karyotype (or vice versa), the selected image loads and the question mark returns to automatically load the associated image.

- **Reappear** When the Reappear box is checked, the window will automatically return to the Gallery display after the image is loaded.
- **Reappear** When the Reappear box is unchecked, the display will remain the main working window.
- **Gallery** The Gallery and main working window displays can also be toggled by clicking on the Gallery button.
Related topics
About the Gallery

Loading images

Images are loaded from the Navigator in the Analysis screen. Some image types are loaded as sets. These are metaphase/karyotype pairs and CGH metaphases/karyotypes/profiles.

To load an image from the Navigator:
1. Double-click on the image in the Navigator, or select it and click on the Load button, to open the load icon.
2. Move the load icon over an image window and click on the window to load the image. The icon will remain if there are additional images to load. Click a new image window to load next image. Repeat until load icon disappears.

The Load Cell button loads every image type contained in a cell without prompts. If there are not enough empty windows some loaded images will be cleared.

The image in the main window can be swapped by clicking on another image window. Images in smaller windows can be swapped by clicking on the first image with the middle mouse button, then on the second image with the left mouse button.

Clear images by clicking on the Clear button below the Navigator, then select the image window. Clear All removes all loaded images.

To load an image from the Gallery view:
1. To load images using the Gallery view, double click on a Gallery image, the view will change to the Analysis view.
2. Place the load icon on the image window to load the selected image.
If the Reappear box is checked, the screen will revert to the Gallery view after loading the image.

If the Reappear box is unchecked, the screen will stay in the Analysis view.

See topic - Working with the Gallery for more detail on the use of the Gallery window.

**Image magnification**

**Zoom**
Images can be temporarily magnified using the zoom function. Click on an image in the main window with the middle mouse button to magnify it. Clicking a second time will return it to normal size. There is a choice between zoom levels of 2 or 4 times actual size. This setting is remembered and is selected in Customize.

**Fullscreen**
The main window itself can be enlarged by clicking on the Fullscreen button in the toolbar.

This switches the screen to the Fullscreen layout and displays the image at a slightly larger size, about 1.5 times larger. In the Fullscreen layout the Navigator is opened by clicking on the Navigator button on the main toolbar. Click on Fullscreen again to return to the previous layout.

**Scale to Fit**
Used mainly to work with large images from a digital camera, this command automatically scales an image to fit the main work window. Click on Scale to Fit in the Analysis toolbox.

Use the middle button to return back to the original larger size.

**Selecting objects**

Objects must be selected before some commands can be used. Objects are selected individually or in groups.

**To select an object:**
1. Move the mouse pointer over the object.
2. When the blue box appears click the left mouse button once.
3. When selected, the box will turn magenta.
4. Repeat these steps to deselected the object.

*Active object*  *Selected object*
CytoVision 3.6

The following selection tools work with groups of objects. These buttons are found on the main toolbar.

Select All selects all objects that have not been deleted.

Deselect All deselects everything.

Select Group uses lines drawn around or through objects to select them. Any object touched by the drawing line, or completely encircled by is selected.

Edit commands

Copy objects by selecting them, then hold the Ctrl key down (the cursor changes to ) while dragging them to a new window. Copy dialog objects like profiles and Fluomaps the same way, except rather than selecting them, just place the cursor over the object. Then drag it into a new window while holding the Ctrl key.

Copy an image to the Windows clipboard by loading it into the main window. The click on it with the right mouse button and select Copy, or choose Edit > Copy to the clipboard. If nothing is selected, the entire image will be copied. Copy parts of an image by selecting them first, then use the Copy command.

Remove objects from an image using the Delete button in the toolbar.

If not using modal operations, first select the objects, then and click the delete button to delete them.

If using modal operations, click the delete button to turn delete mode on, then click objects to delete them.

If the object is in a metaphase it will be removed from the karyotype and excluded from image enhancements, but will not be removed from the image. Deleted items are outlined in red if Object highlights is turned on in Customize.

Reverse the last action by clicking on the Undo button on the toolbar.

Undo buttons are also found in many of the dialogs that are used to change image appearance.

Move, rotate and invert objects

An object or chromosome is ready to be moved, exchanged, rotated or inverted when it is active. Place the cursor over the object, the blue rectangle is seen when it is active.
Active image

**Move and Drag**
Move an object to a new location by clicking and holding the left mouse button. Move the object, then let go of the mouse button. This will also be referred to as dragging an object throughout the help files.

![Moving an object](image)

**Exchange**
Two chromosomes in a karyotype can be exchanged. Drag one chromosome over the top of the other. When the red outline appears let go of the mouse button and the two will change places.

![Exchanging two objects](image)

**Rotate**
Rotate an object by clicking and holding the right mouse button over an object, the cursor will change to rotating arrows. Move the mouse from right to left as if you were using a slider bar, the outline of the object will rotate. When the rotation angle is correct let go of the mouse button and the object will rotate.
Invert

Move an object to a new location by clicking and holding the left mouse button. Move the object, then let go of the mouse button. This will also be referred to as dragging an object throughout the help files.

Pseudocolor

Pseudocolor creates a color histogram based on the gray levels in the image. It is mainly used to check for uneven illumination in probe capture. Right click on a probe image and select Pseudocolor. The background should be one solid color. Any hotspots or uneven areas will be a different color. Pseudocolor is also available for raw images in the Analysis screen.

💡 Tip - Use Pseudocolor on a live image to center fluorescent lamps.
Archive and restore data

Archiving and restoring data

Archiving copies cases and system data to removable disks for backup protection and storage. Regular archiving is an important part of system maintenance for two reasons; it protects against data loss if the hard disk becomes corrupted, it provides a safe means for removing finished cases from the hard disk. As hard disks fill with data the system performance will decline. Removing finished cases to free up disk space will help keep the system running at peak performance.

The Archive and Restore dialog has six tabbed pages. The Settings tab selects the archive disk. The cases tabs copy case data. The classifier tabs copy classifier and species template data. User profiles saves personal settings created for each login. The dialog is opened by clicking on the Archive and Restore button on the toolbar.

Disks must be formatted before they can be used for archiving. See the topic Formatting disks.

Selecting a drive

Drives are selected from the Settings tab. Any removable disk installed on the system, any network drive or any shared drive may be chosen. Archive devices may be shared with other systems on a network by the CytoVision network config utility. See the topic Sharing drives for archiving. Local drives are listed by drive letter, shared drives show the share name given in network config.

Selecting cases

Select a single case by clicking on its name in the list. Select additional cases by holding the Ctrl key while clicking on the names. Select contiguous cases by selecting the first case, then while holding the Shift key, click on the last case.

Isolate groups or types of cases by using the Filter to limit the list. Then use Select All to choose all of the filtered cases. Archive all cases that have been marked as complete by choosing the Completed Cases option in the Filter.

Note If you want to archive cases from networked machines, make sure you select All machines in the Machine list before selecting cases for archiving.

Archiving cases

Archiving provides a safe, efficient method of removing cases that are no longer needed. Cases can be deleted after they have been archived and verified on the disk. Use the Complete flag in Case Details to mark when a case is finished. Then quickly isolate these cases using the filter.
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Before opening the dialog insert the archive disk in the drive. The label is read and free space on the archive disk is calculated when the Archive Case tab is opened. As cases are selected, the total amount of disk space needed is calculated. The space Available must be more than that of Selected.

On networks, any system can archive cases stored anywhere on the network. The **Machine** combo-box selects a system and displays its case list. Select All Machines to select cases from the entire network.

The **Label** button is used to label the archive disk. See the topic [Label a disk](#).

The **Filter** section contains standard case filtering tools for limiting lists and selecting cases. See the topic [Filtering case lists](#) for information about using these tools. **Select All** selects every case in the list. When used in conjunction with the filter, selecting cases is done quickly. For example, turn Completed Cases on, click on Apply Filter then Select All. Every completed case on the system is selected for archiving.

Place a check in **Delete after archiving** to remove the cases after they have been copied onto the storage disk. The system performs checks to verify the archive before the cases are deleted.

Click on **Archive** to start copying the cases. The Archive dialog will close and a small status box will appear instead. When archiving is finished, a message box lists successfully.

**To archive cases:**

1. Place a disk in the drive.
2. Click on the Archive and Restore button on the toolbar.
3. If the archive disk is not read click on the Settings tab and choose the correct drive. Then return to the Archive Cases tab.
4. Select cases for archiving.
5. Turn Delete after archiving on (Note - leave this option off to keep the cases on the drive).
6. Click on Archive.
**Archive and restore data**

![Archive and Restore dialog box](image)

**Tip** - Archive cases frequently to keep your system running quickly and efficiently. Full hard drives can cause a system to run slowly. It is also good insurance against data loss.

### Restoring cases

Any case stored on a removable disk can be copied back onto the hard drive using Restore. The case is left on the archive disk, only a copy is placed on the hard disk. Restore performs some security checks as a part of case retrieval. If a case already exists there is an option to overwrite it or to cancel. Overwriting a case loses any changes made since the case was archived.

When the Restore Cases dialog is opened the drive selected in the Settings tab is read and a list of cases on the disk is displayed. The limited set of filtering tools can be used to locate a specific case. If you are unsure which disk the case is on and you archive onto labeled disks, the Library will list the disks that contain the case.

This dialog can also be used to restore archive labels to a damaged or missing Library. Restore Library reads the cases stored on an archive disk along with the disk's label. Then the Library is updated if any of this information is missing. On networks, this only needs to be done at one system. The other system libraries will be updated as part of the normal networking process.

**To restore cases:**

1. Insert the archive disk in the drive.
2. Click on the Archive and Restore button on the toolbar.
3. Click on the Restore Cases tab.
4. Select the case(s) and click on the Restore button.
Note - Cases from UNIX CytoVision archives can be only be restored from the Administrator login.

Archiving classifiers

Creating custom classifiers can be time consuming. We strongly recommend archiving new classifiers when they are completed. The Archive Classifier tab is used to backup both classifiers and CGH standard reference intervals.

To archive a classifier:

1. Click on the Archive and Restore button on the toolbar.
2. Click on the Settings tab and select a drive for archiving.
3. Click on Archive Classifiers tab.
4. Choose the Classifier option in the Display section.
5. Select the item in the list.
6. Click on Archive Classifier.

To archive a CGH standard reference interval:

1. Click on the Archive and Restore button on the toolbar.
2. Click on the Settings tab and select a drive for archiving.
3. Click on Archive Classifiers tab.
4. Select the CGH Standard Reference option.
5. Select the interval you want to archive.
6. Click on Archive Classifier/std. ref.

Restoring classifiers

Restoring classifiers is used to replace missing or corrupted classifiers or to share classifiers with other sites. Classifiers with the same name are overwritten.

1. Click on the Archive and Restore button on the toolbar.
2. Click on the Settings tab and choose the drive that contains the data.
3. Click on the Restore Classifiers tab.
4. Select the classifiers you want to restore.
5. Click on Restore.

Archive species data

Creating templates, classifiers and ideograms for new species can be time consuming. We recommended archiving new data for a species when it is completed. Each folder contains the templates and ideograms created. The archive buttons enable when their data type is selected in the list. To archive classifiers and CGH reference intervals, select the template they are associated with first. The will appear in the list to the right. Choose the Classifier or CGH Standard Reference options depending on the item to archive.

All data for a species can be selected and archived by clicking on the Archive Species button. Archiving an entire species will save all of the templates, classifiers, ideograms and CGH standard reference intervals associated with that species.

To archive a species:
1. Open the archive and restore dialog by clicking on the tool button.
2. Click on the Settings tab and select a drive for archiving.
3. Click on Archive Species Data tab.
4. Select the species you want to archive.
5. Click on Archive Species.

**To archive a classifier:**

1. Open the archive and restore dialog.
2. Click on the Settings tab and select a drive for archiving.
3. Click on Archive Species Data tab.
4. Select the species template that is compatible with the classifier you want to archive.
5. Select the Classifiers option in Display list of.
6. Select the classifier you want to archive.
7. Click on Archive Classifier/std.ref.

**To archive an ideogram set:**

1. Open the archive and restore dialog.
2. Click on the Settings tab and select a drive for archiving.
3. Click on Archive Species Data tab.
4. Select the ideogram set you want to archive.
5. Click on Archive Ideogram Set.

**To archive CGH standard reference intervals:**

1. Open the archive and restore dialog.
2. Click on the Settings tab and select a drive for archiving.
3. Click on Archive Species Data tab.
4. Select the CGH Standard Reference option.
5. Select the interval you want to archive.
6. Click on Archive Classifier/std. ref.
## Restoring species data

Restoring data provides the best way to share species data with other sites. Data is placed in an existing species folder if one with the same name exists. If not a new species folder is created to retain the directory structure.

**To restore data:**

1. Open the Archive and Restore dialog.
2. Click on the Settings tab and choose the drive that contains the data.
3. Click on the Restore Species Data tab.
4. Select the data you want to restore.
5. Click on Restore.

💡 **Tip** - The CytoVision software installation disk contains some species templates, ideogram sets and classifiers. With help from our users this list should grow so check it out if you are going to work with a new species. To restore from the software disk, insert the disk and select the CD-ROM drive from the Settings tab.

## Archive and restore User Profiles

User profiles are saved for each login name. Once you have recorded all of your preferences at one machine, archive the data to keep as a backup or use it to copy the settings to other systems on the network.

The following items are saved in User Profiles:

- Macros
- Hotkeys
• Fluorochrome lists
• Fluorochrome Selection panel settings
• dialog positions
• Customize options for all screens
• Fluomaps

To archive a User Profiles:
1. Login as the user you want to archive.
2. Insert the disk in the drive.
3. Open the Archive and Restore dialog.
4. Select the correct drive from the Settings tab.
5. Select the User Profile tab.
6. Click on Save.

To restore a User Profile:
1. Login as the user you want to restore the settings for.
2. Insert the disk in the drive.
3. Open the Archive and Restore dialog.
4. Select the correct drive from the Settings tab.
5. Select the User Profile tab.
6. Click on Restore.
7. Log off and login back on again to apply the restored settings.

Drives and disks

Sharing drives for archiving

There are two types of sharing for archive devices. Sharing under the CytoVision network using Network config makes the drive available for archiving within CytoVision. To have access to a network drive from Windows Explorer as well it must also be shared in the drive properties. See the Windows help files for information about drive sharing.

To share a drive for archiving:
1. Click on Start > Programs > Applied Imaging CytoVision > Network config.
2. Click on Share Archives and all shareable drives are listed.
3. Select the drive for sharing.
4. Choose the Shared As: option and enter a share name for the drive.

5. Click on OK.
6. Click on OK in Network Config.

Formatting disks
The normal cyto login only has permission to format floppy disks. You must login as
Administrator to format any other type of media. Most disks are already partitioned and can
be formatted from My Computer, however some media like the mini optical disks must first be
partitioned using Disk Administrator.

There are different procedures for formatting DVD Ram disks depending on the hardware
installed on your system. Panasonic drives will usually be in systems installed in 2001 or
earlier. IBM/Hitachi drives are found in the midnight gray systems from late 2001 onward. If
you are unsure about which type is installed, check the software installed by clicking on Start
> Programs. Either Panasonic DVD Ram or VBO InstantWrite (used for the IBM/Hitachi drive).

Format from My Computer
Format mini optical disks or new media using Disk Administrator
Format a DVD disk
Format a Panasonic DVD disk
Format an IBM DVD disk

Format from My Computer

This method only works for disks that have already been partitioned like ZIPs and floppies, or
for reformatting disks.

To format a disk using My Computer:
1. Double click on the My Computer icon on the desktop.
2. Insert the disk in the drive.
3. Select the drive that contains the disk and choose File > Format.
4. Click on Start to begin formatting.

Formatting DVD disks

Disks must be formatted before they can be used for archiving. The preferred format is UDF
2.0 though FAT formatted disks can be read and used. A small utility is used for formatting.

To format a disk:
1. Disable Norton Anti-Virus. Click on Start > All Programs > Norton Antivirus > Norton
Antivirus.
2. Choose Configure > File system realtime protection.
3. Remove the check from Enable file system realtime protection.
4. Click on OK.
5. Click on Start > All Programs > Write DVD > Format UDF.
6. The adapter should read Secondary.
7. Choose Option 0. The message area should show the DVD drive.
8. Choose UDF 2.0.
9. Click on Format.
10. Answer OK to the warning.
11. The progress bar will close when formatting is finished. Close the dialog.
12. Re-enable the virus protection when finished by turning Enable file system realtime
   protection back on.
Format a DVD in a Panasonic drive

1. Double-click on My Computer on the desktop.
2. Right click on the DVD drive and select Format.
3. Select format type *Universal disk format (UDF).
4. Click on Start.
5. Answer Yes to continue with formatting. When finished a message will appear stating that formatting is done.
6. Click on OK and Close.

* UDF is compatible with Windows 95/98 or MacOS as long as the drivers are installed. The FDISK FAT - 16 is compatible with Windows 95/98 but is less efficient than UDF. You can choose any of the format options and the disk will work for archiving.

Format a DVD disk in an IBM/Hitachi drive.

1. Insert the disk in the drive.
2. Click on Start > Programs > VBO InstantWrite > InstantWrite Format.
3. Click on Next.
4. Click on Next to Hitachi DVD-RAM.
5. Choose Fast Format.
6. Click on Next.
7. Choose UDF 1.5*.
8. Enter a volume label for the disk.
9. Click on Next.
10. Click on Next to begin formatting. All data will be erased from the disk. It usually takes under a minute to format a 5G disk.
11. Click on Close when formatting is complete.
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*UDF 1.5 format is specified because it is readable by older DVD drives and is compatible with other CytoVision systems.

**Format mini optical disks**

Optical disks are seen as removable drives by the operating system and must be formatted and partitioned before they can be used. The 3½" optical disks are usually sold unformatted and unpartitioned. Disk Administrator is supplied as part of Windows NT and is used to create and manage partitions on disks.

**Before beginning:**

- You must be logged in as system Administrator.
- The disk must be in the drive before running Disk Administrator or it will not be listed.
- The Optical disk is usually in the Disk 2 section (the F drive).

**To partition and format a disk:**

1. Login as Administrator.
2. Insert an unformatted cartridge in the drive. **The disk must be in the drive before running Disk Administrator.**
3. Click on Start > Programs > Administrative Tools (common) > Disk Administrator.
   You may see a message that states that your unformatted disk has no signature. Click Yes to confirm.
4. Right click in the hashed field to the right of the disk and click Create.
5. Click OK.
6. Right click the field again and select Commit changes now.
7. Click Yes.
8. Click OK. A repair disk is not needed.
9. Right click on the disk and click Format.
10. Use the default settings of FAT and choose long format.
11. Click Start.
12. Click OK to start the formatting process.

**Note** - Once a disk has been partitioned it can be reformatted using My Computer.

If you have trouble formatting a disk using Disk Administrator the following is a more detailed explanation of the process, as well as some errors and their causes.

In Disk administrator there are four main commands that are used for formatting Optical disks. They may not all be necessary depending on what level of formatting the disk already has. The commands are:

- **Delete**, that removes all formatting and partition information on a disk. It is used to remove partitions that are formatted with the wrong file structure. An example would be old UNIX disks. After deleting a partition a new one must be created.
- **Create** makes a new partition on the disk. Archive disks should only have one partition so when creating a new partition, accept the default size which should be the maximum available. Formatting new disks usually starts with this command.
- **Commit Changes Now** must be used whenever partitions are created. Immediately after creating a partition choose Commit Changes Now from the context menu. This disk cannot be formatted until the changes to its partitioning are saved.
- **Format** is the last part of the procedure. If the Format command is listed in the right-click menu then a partition already exists and the disk can just be formatted.

There are 4 states a disk can be in:
1. The disk is formatted for a different file system, either UNIX, Mac or something other than Windows. Disks of this type must have their partition deleted first. Then follow the instructions for creating a partition and formatting it.

2. The disk is not partitioned and unformatted. This will appear in the Disk Administrator window as a White Box with Diagonal black lines (hatching). This is how new disks are normally purchased. Just follow the instructions on how to partition and format a disk listed above.

3. The disk is partitioned but not formatted. This will be displayed as a white box with a label of "Unknown" or "Unformatted". These disks are already partitioned with an appropriate file format for Windows NT. Start at step 9 in the instructions above.

4. The disk is formatted for Windows. In this case the box should say FAT (File Allocation Table) and a number (the size in Mb) or NTFS. This is the only situation where the disk can be used for archiving.

In summary

For an optical disk a new partition must be created on the disk before it is ready for formatting. When creating a partition accept the default settings which are 508MB for a mini-OD. You must then Commit changes now to save this new disk partition. Where the disk has previously been formatted for the wrong file type (e.g. an old UNIX disk from a previous CytoVision system) the existing partition must be deleted first, then create a new one. **Note** that this will delete all of cases on the disk.

Once the partitioning is saved the Format option will appear in the right-click shortcut menu for the first time. FAT is the default but NTFS can also be used. Choose long format (short format is only for pre-formatted disks and is the equivalent of an erase). This takes approximately 5 minutes to work and progress is shown using a Windows style status bar. Remember to check that disk being formatted is the mini-OD, the drive letter is F for standard CytoVisions.

Troubleshooting

If there are problems try logging out then login again and try again. If that doesn't work try rebooting the system.

**Can't read disk signature:** Sometimes starting disk administrator gives this message when you start, select yes and it should display the OD drive as above, if you do not give the disk a signature it will not try to read it.

**Formatting failed (couldn't complete):** Sometimes this occurs for no apparent reason. In this case the best thing to do is restart Disk administrator and start from the beginning (Delete, Create, Commit & Format).

**Commit changes now doesn't work, or format doesn't appear in the menu, or formatting fails.** This has been seen to occur when the "Commit changes now" option has been used from the right-mouse. Try using the option from the toolbar menu instead.

**The disk "loses" it's FAT formatting.** This can sometimes occur if the disk has been formatted but then stored without writing information to it. Copy a simple file (a CytoVision label etc.) onto it and it should be OK. If the format is lost or not recognized you must repeat the above procedure.

**Label a disk**

One of the items stored in the Library is the name, or label of every disk a case has been archived to. This can make it easier to locate a case if it needs to be restored. Disk labeling is optional; cases can be archived onto unlabeled disks.
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The label is a small text file saved on the disk specifically for CytoVision and is not the same as the volume label seen in Windows Explorer.

**To label a disk:**

1. Insert a formatted disk into the drive.
2. Click on Archive.
3. Type the name you want to use in the Archive Name field.
4. Click on Label. A message will tell you when the disk is labeled.
Networks

Overview of networks

There are two types of networks, peer-to-peer and client-server. On peer-to-peer networks, each system is an equal part of a workgroup, and is used to store cases and system data. Client-server networks have two types of systems, servers and clients. The server stores all case data and shared system data such as classifiers and templates. It is generally only used to administrate and archive cases. The client systems are used for capture and analysis. Unless you have more than ten systems networked you will probably have a peer-to-peer network.

In addition to Windows networking there is the CytoVision network configuration. This information is used by the software to update the Library and case lists across the network. Special networking processes run every few minutes to update these files.

The CytoVision network can be configured by manually adding systems or by automatic detection. The automatic method has the advantage of being more flexible. See the topic Automatic versus manual configuration for a comparison.

Networks with UNIX OS CytoVisions have some limitations involving file compatibility and network setup. See the topic Networking to UNIX CytoVisions for details.

A general note about Windows networking: Whenever a network cable is first connected there will be a delay before Windows finds the new systems and adds them to its network list. You will not be able to add new systems in the CytoVision network configuration until Windows can see them, so you may need to wait a few minutes after connecting to a network before getting the new network list. This applies to manual and AutoNC networking. Similarly, when a cable is disconnected, wait a few minutes before configuring the network, as Windows may not immediately realize that systems are no longer present and AutoNC may time-out as it tries to connect to each disconnected system.

About the Network config utility

Network Config is a separate utility for setting up the CytoVision network. In order to properly share cases and update Library information, all systems must be added to the CytoVision network. The utility is also used to share archive devices, set-up a Lab Management connection and start the NFS printer service.

Run Network config by choosing Start > Programs > Applied Imaging CytoVision > Network Config. The dialog has two lists. The left column contains a list of all systems on the Windows network. The right column lists all of the systems on the CytoVision network; only CytoVision systems should be added to this list.

Add is used when the network is manually configured. Select a system and click on the Add button to include it in the CytoVision network. Select a system in the list on the right and the buttons changes to Remove. Click on Remove to take a system off of the network.

Share Archives is used to make local drives available to networked systems for archiving. LMS is used to designate and connect to a Lab Management System. Print Service makes shared printers available to networked, UNIX based CytoVisions.

Auto toggles between automatic and manual configuration. See the topic Automatic versus manual configuration for the differences between these methods. Refresh is only enabled in auto mode, and starts a new AutoNC scan. Exclude Domains is only enabled in auto mode. If
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A network domain is selected in the left hand pane, this button can be used to exclude all systems within it from AutoNC scans.

**Show All Domains** when checked, shows all available network domains rather than just the local domain. This can be used in both auto and manual modes.

**Server** is used to designate a system as the file server. It is only present if Client Server Network is turned on in CytoVision Capabilities.

![Network Configuration Page](image)

**Automatic versus manual configuration**

The CytoVision network can be configured by manually adding systems or by automatic detection. The automatic method has the advantage of being more flexible. It allows you to run some systems while others are turned off or removed without causing hang-ups during network updates. If systems are added to the network list manually then all systems must be turned on in order for the software to function normally and without hang-ups.

Notes about automatic configuration are:

- When a change is detected in the network, a message box will alert you to this. However, CytoVision will not use the new network configuration until the software is closed and restarted, though this does not need to be done immediately unless networking problems are encountered. The system does not need to be rebooted in any case.
- On peer-to-peer networks, the cases on systems that are turned off will not be accessible. If you have a station that is seldom used, such as a FISH station, do not create the cases on that system. Create them on the systems that are on constantly. This will ensure access to them at all times.

While CytoVision is running, AutoNC runs in the background continuously checking for changes to the network. When using AutoNC on a large network with many domains, excluding all but the CytoVision domain will decrease the amount of time it takes for the AutoNC scan to complete.

Manual network configuration was the original method of networking and will be found on earlier systems. This method should be used if UNIX systems are on the network. If the network is manually configured, all systems must be turned on when the CytoVision software
is run on any system. If a system is rarely used in this scenario, for example it is in another part of the building and not conveniently located, it can be left turned on for long periods. A system does not need to be running CytoVision or even logged in to be recognized by the network.

**About peer-to-peer networks**

The maximum number of systems on a peer-to-peer network is ten. All systems are equal partners that store cases and shared data such as classifiers and templates. All data used by CytoVision is stored in shared folders that can be used by any system on the network.

When a case is created it is saved to the hard drive of the system it is created on. Dialogs with case lists have a Machine combo list that selects the source of the cases. Display all cases on the network by choosing *All machines*, filter the list to one system by selecting its name in the list. The Library on each system will be periodically updated as part of the networking process. This ensures that each system on the network has a complete case history file.

All cases on the network can be archived from one workstation, eliminating the need to perform multiple archives.

Printers can be shared by all machines on a network. They will appear in the list of available printers as if they were local.

⚠️ **Warning** - Because cases can be opened from other machines it is important to check that no-one has a case open, or is archiving cases off of your machine, before shutting it down or rebooting.

**Related topics**

- Synchronize networked libraries
- Share a printer

**Configuring peer-to-peer networks**

How a system is added will depend on whether the network is using automatic (AutoNC) or manual configuration. In either case, when a network is set up for the first time, or a system is added to a network for the first time, it is essential to synchronize the libraries on all of the systems. See the topic *Synchronizing libraries* for information on this.

⚠️ **Note:** Due to the way Windows works, it may be necessary to wait for a few minutes after physically connecting a system before it can be added to the network.

**To add a system using AutoNC:**

The system must be configured with the correct workgroup, IP address and subnet mask. Physically connect the system to the network.
Choose on Start > Programs > Applied Imaging CytoVision > Network config. Turn the Auto toggle on. CytoVision systems will automatically be added to the network list. If the systems do not appear even after you have waited a few minutes try clicking on the Refresh button or rebooting the system.

If there is more than one domain physically connected to the network, all but the CytoVision domain should be excluded. This will decrease the time it takes for AutoNC to scan the network for changes. To exclude domains, turn Show All Domains on. Then select the non-CytoVision domains and click on the Exclude Domain button.
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Reconnecting a system using AutoNC:
To reconnect a system boot the system with the network cable attached. If other systems have CytoVision running, close the software and restart it when asked to do so.

To add a system to the network manually:
2. Select the system you want to add from the column on the left and click on Add.

A note for Notebook users:
If you are planning on using a Notebook as a portable system then AutoNC should be used. This allows the Notebook to be easily connected and removed without having to reconfigure the network each time.

Removing a system from the network

There are occasions when a system should be removed from the network configuration. If a system on a manually configured network is going to be physically removed or turned off for a period of time, the remaining systems may freeze or hang while trying to communicate with it. To prevent this freezing, remove the system from the network configuration.
2. Selecting the name in the right column and click on Remove.
3. Click on OK.

Notes:
• This will permanently remove a system. If you are only temporarily removing a system then you can just disconnect it provided you are using the automatic networking facility. If you are not using AutoNC then you must remove the system as described above.
• To permanently remove a system under AutoNC, the system must be listed as disconnected first. Physically remove the network cable so it is listed as Disconnected in the network list. Then select the disconnected system and click on the Remove button.
• If UNIX systems are on the network, AutoNC is not advised as UNIX systems do not have this option. Any system that is not present or turned on, must be removed from the network list of all systems.

About client-server networks

Client-server networks use one system to store all information that is shared across the network. This is the server. The workstations are used to capture and analyze cells that are saved on the server. The Machine combo list found in any dialog box that lists case names will only have the name of the server. The exception to this is if the network has UNIX stations in which case the names of the UNIX stations will also appear.

If the systems are networked using AutoNC, then a system that is disconnected from the network will automatically begin to use its own Cases directory to store cases. When the system is reconnected to the server the AutoNC will automatically switch back to using the Cases directory on the server. Any cases that were worked on locally while the client was disconnected will not be accessible once the system is reconnected to the server. To access these local cases, archive them or upload them to CytoNet before reconnecting the system to the network. If you do not need to access the local cases you do not need to do anything. They will remain on the local drive and will be accessible again the next time the system is removed from the network. This type of activity will normally only apply to Notebook users.
Cases can be archived from any system on the network though using the server is recommended.

Network printers can be shared by all machines on a network when they are installed on the server. They will appear in the list of available printers as if they were local. Printers can be connected to client systems or the server.

**To setup a client-server network:**

1. The Client Server toggle must be switched to on in the capabilities file. You will need a code and assistance from Applied Imaging for this.
2. Choose on Start > Programs > Applied Imaging CytoVision > Network config.
3. Select the name of the server in the left column.
4. Click on the Server button.
5. Click on Done.

**Notes**
- If there are UNIX systems on the network they must **added as workstations**.
- If you are connecting a notebook that you plan to use as a portable system turn **Auto** on after step 4 above, then click on **Done**.

**Related topics**

- Configure a client-server
- Install a printer

**Installing network printers**

The installation procedure will depend on the printer manufacturer and the drivers used. Have your network administrator install and setup your printer for you.

**About networking with UNIX CytoVisions**

**About networking to UNIX based CytoVisions**

Mixed networks contain both UNIX CytoVisions and Windows NT based systems. Third party NFS software from Sun Solstice is used to transfer data between the two operating systems.

AutoNC is not recommended for mixed networks with one exception; if you have a notebook on a client-server network AutoNC may be used. The Solstice NFS software is not compatible with Windows 2000 so should not be installed on the notebook.

There are some restrictions on what can be done on a mixed network. There are also some differences in the capabilities of the CytoVision software for Windows NT system versus the UNIX version. There is a UNIX compatibility toggle that restricts the Windows NT system so that all files are universal. This toggle is in the **Capabilities** utility.

**Opening and creating cases on a mixed network**

Most cases that were created at a Windows™ NT station can be opened on a UNIX station and visa versa. Every system on the network will appear in the Machine combo list. The same rules will apply regarding case locking and libraries. Only one user at a time can open a case, and the library will prevent duplicate cases from being created.
There are some cell types that the UNIX CytoVision will not be able to handle. This includes HiResCGH images and cells captured or karyotyped into flexible templates on a Genus. Probe karyotypes and cells with more than 250 objects* cannot be loaded on a UNIX system. Images captured with a digital camera at full resolution will not be viewable in their entirety.

*Note - If the UNIX compatible flag is turned on in capabilities, then you will not be permitted to capture images with >250 objects on the Windows NT system as well.

**About the UNIX compatibility toggle**

Under normal use there is no limit as to the number of objects you can have in a cell. The UNIX systems have a limit of 250 objects. If you try to load an image with more than 250 objects on the UNIX CytoVision it will fail.

Turn the UNIX Compatible flag on to ensure that all images captured and analyzed on the Windows NT CytoVision will be also be readable by the UNIX systems.

The flag is turned on and off from a utility in the Applied Imaging Program group.

**To change the status of the flag:**

1. Login as Administrator.
2. Click on Start Programs Applied Imaging CytoVision Capabilities.
3. Click in the check box next to UNIX Compatible. A check in the box means that the flag is turned on.
4. Click on Apply.
5. Click on Done.

**Archiving and restoring**

**Archiving and restoring between UNIX and Windows NT systems**

Mixed networks have certain restrictions placed on the archiving and restoring of cases. The basic rule for archiving is that disks that have been formatted at the UNIX station can only be used at UNIX stations. Likewise, NT formatted disks can only be used at NT stations. Cases on a UNIX formatted disk can be restored on a Windows NT system from the Administrator login.

UNIX systems can use devices on the Windows NT system. Windows NT systems cannot use any device that is attached to UNIX systems. If your archive device is on your UNIX system all archiving will need to be done from the UNIX station.

**Summary**

<table>
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<th>Media Formatted in</th>
<th>Operating System</th>
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<td>Windows NT</td>
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<td>UNIX</td>
<td>Local drive if logged as Administrator (restoring only)</td>
</tr>
</tbody>
</table>

**Related topics**
Archiving cases between UNIX and Windows NT

For optical disk drives, a UNIX system can use a local disk, a disk in another UNIX workstation or a disk in an NT workstation. NT systems can use a local drive or a drive from another NT workstation. They cannot use an archive device on a UNIX system.

Cases can be selected from any system on the network. Use the Machine list to select cases from specific systems, or from all machines.

What you can do:
- From a UNIX workstation, insert a UNIX disk into a UNIX workstation and archive cases selected from UNIX and/or NT workstations.
- From a UNIX workstation, insert an NT disk into an NT workstation and archive cases selected from UNIX and/or NT workstations.
- From an NT workstation, insert an NT disk into an NT workstation and archive cases selected from NT and/or UNIX workstations from the NT workstation.

What you cannot do:
- Archive from an NT workstation to a UNIX disk placed in a UNIX workstation.
- Archive from an NT workstation to a UNIX disk placed in an NT workstation.
- Archive from a UNIX workstation to an NT disk placed in a UNIX workstation.

Setup UNIX to archive on NT

UNIX systems can use archive devices on Windows NT.

To setup a UNIX workstation to use an NT optical disk:

1. On the UNIX workstation Login as admin.
2. At the top, right side of the screen, enter the name of the NT workstation in the On machine field.
3. Turn on the MS-Windows toggle.
4. Select the Remote OD option.
5. On the Windows NT workstation, login as Administrator.
6. Double-click the My Computer icon on the desktop.
7. Right-click the drive you want to use for UNIX archiving and select Sharing.
8. Select the NFS Sharing tab.
9. Click on Shared As.
10. Enter usr3 as the Share Name
11. Click on OK and reboot the system.

Restoring files between UNIX and NT

Any case from any disk can be restored from the NT workstation. If the disk is from a UNIX workstation, you must login as Administrator before the disk can be read. UNIX workstations can only read cases that were archived to UNIX formatted disks.

What you can do:
CytoVision 3.6

- Insert an NT disk into an NT station and restore cases.
- Insert a UNIX disk into an NT workstation and restore cases (login as Administrator).
- Insert a UNIX disk into a UNIX workstation and restore cases.

**What you cannot do:**
- Insert an NT disk into a UNIX workstation and restore cases.
- Insert a UNIX disk into an NT workstation and restore from a different NT workstation.

**Printing from UNIX systems**

**Printing between Windows and UNIX systems**

UNIX systems can print to a local printer, a printer on another UNIX CytoVision, or to a printer attached to the Windows NT system. Windows NT systems can only print to local printers or printers attached to other Windows NT systems. The UNIX systems use the NFS Print Service on the Windows NT system to send images to the printer. When the Print Service is running its icon is shown in the system tray.

**What you can do:**
- Print from a UNIX workstation to a printer attached to another UNIX workstation.
- Print from a UNIX workstation to an Windows NT workstation.
- Print from an NT workstation to another Windows NT workstation.

**What you cannot do:**
- Print from an Windows NT workstation to a UNIX workstation.
- Print from a UNIX workstation to more than one Windows NT workstation.

**Note** Whenever a printer is added or removed from the Windows NT workstation, the Print Service will need to be refreshed. Make sure the printer is installed or removed from the Administrator login then reboot the system. This will refresh the list of printers the UNIX workstation reads. Or you can use the Print Service button in Network config.

**Related topics**
- To setup printing from UNIX to Windows NT
- About Windows NT and UNIX networks

**Setup printing between UNIX and NT**

The NFS print service must be running on the Windows station before UNIX stations can use the printer. If it is not running the printer list will be empty on the UNIX station. The print service is refreshed when the Windows systems is rebooted but it can be forced to refresh if necessary, for example is the UNIX printer list is empty.

**To setup UNIX printing to an NT station:**
1. Login as admin.
2. Turn on the toggle labeled Enable NT printing in the printer area.
3. Enter the name of the NT system with the printer in the text field. Do not enter the NT workstation name in the list above. This area is for UNIX printers only.
4. Click on Configure in the print area.

**At the NT station**
5. Login as Administrator.
6. Share the printer. (see Sharing files and printers in the help files)
7. Click on Start > Programs > Applied Imaging CytoVision > Network config.
8. Click on the Print Service button.
9. Click on the Start button; the AII print service icon will appear in the taskbar.

**To stop the print service:**

1. Login as Administrator.
2. Share the printer. (see Sharing files and printers in the help files)
3. Click on Start > Programs > Applied Imaging CytoVision > Network config.
4. Click on the Print Service button.
5. Click on the Start button; the AII print service icon will appear in the taskbar.

Note The list of NFS printers will be refreshed whenever the Windows NT system is rebooted.

**To refresh the NFS print service:**

Click on Start > Programs > Applied Imaging CytoVision > Network config.
Click on Print Service.

[2]Note: The list of NFS printers will be refreshed whenever the Windows NT system is rebooted
Capture

Capture overview

There are six modes used to capture different sample types. Some basic principles and commands are common to all six modes. The available tools and Customize options will change slightly depending on the mode chosen.

Thresholding is an important part of the capture process. It is how chromosomes or nuclei are separated from the background.

The type of camera used will also affect the way the tools work and the options available. You will either have an analog or a digital camera interface.

Basics of capture

Basics of capture

The central toolbar will change to include only those tools specific to the Capture screen. You must open a case and a slide before you can capture an image. This is because cells are saved automatically as they are captured. Opening a case and choosing a slide and cell is the same as choosing a save location.

Each cell folder should contain a single image type, that is only brightfield or only FISH. Brightfield, fluorescent (Q-banded) and probe images can be captured into the same slide. Images that require analysis must be captured into their own slide. This applies to CGH, M-FISH, RxFISH and images for spot counting. Fuse images are special image types used to capture any stray chromosomes that do not fit in a single frame. There is no limit on the number of fuse images that can be saved in a cell. Fuses can only be captured for brightfield and fluorescent cells.

The live image is always displayed in the main window. Below this a contrast bar that shows the range of gray levels present in the image. The Capture Setup tools are used to adjust the appearance of the live image in brightfield and fluorescent capture. Capture and Fluorochrome Setup is used to work with images stained with probes.

Related topics

- Use the capture toolbar
- Use the capture command bar
- Use the live contrast bar
- Select the capture mode
- Capture fuse images

Saving captured images

Images are saved automatically during capture and placed in the active cell of the Navigator. When saved a new icon will appear in the Navigator.

Capture commands
Basic Capture

These three commands are used to create a cell, display a live image from the microscope and to grab, or capture that image. They are located below the main image window of the Capture screen.

New Cell + Live (are now one button) - pressing this button creates a new cell in the navigator, displays a live image and pops up the capture set-up controls.

Capture - Captures and automatically thresholds the image.

Fuse - displays a live image for capturing additional chromosomes from a large spread metaphase that does not fit in one capture window.

The text under the window instructs you to use the camera setup sliders or the microscope lamp to improve the image.

NOTE: When you select Streamlined Capture in the customize window Save Raw Image, Auto Threshold and Predict Threshold are automatically turned on. You can deselect any of these options if desired. For example, if you want to manually threshold the image each time you capture just deselect Auto Threshold.

Streamlined Capture

Streamlined Capture enables one-button capture in brightfield and fluorescent mode. This feature means fewer mouse clicks and faster image acquisition.

1. Go to the capture screen and select brightfield capture mode. Open the customize window.

2. Select the streamlined capture checkbox

The buttons below the main capture window change:

Selecting Capture Mode

Capture modes tell the system what kind of sample is being captured. The
image adjustment tools, thresholding and image processing will change depending on the selection. Typically slides will only have one type of sample however, you can have a mix of Brightfield, Fluorescent and Probe cells in a single slide. CGH, M-FISH, RxFISH and Spot Counting samples can only have one sample type in the slide. This is due to the image processing performed on the images.

The mode is selected in the Capture Mode dialog. Open this dialog by clicking on the Capture Mode button on the toolbar.

Some modes may be disabled. Only capture modes that are included in the software package purchased will be enabled.

Recapturing an image

Cells can be recaptured if the image is not acceptable. The original cell will be overwritten. Only images that have not been karyotyped can be recaptured. To recapture a karyotyped cell, delete the cell first.

To recapture an image:
1. Click on the image you want to recapture in the Navigator.
2. Click on Live. A warning box will appear. Choose Recapture Metaphase.
3. Proceed with capture as normal.

Capturing stray chromosomes (fuse images)

Fuse images are used to capture cells that do not fit into a single frame. Capture a fuse by clicking on Live without clicking on New cell first. A fuse can be captured at any time, even after a karyotype has been produced. Just select the metaphase in the Navigator and click on Live. There is no limit on the number of fuse images you can capture.

To capture a fuse:
1. Capture the main metaphase first. It should contain most of the chromosomes. If you are adding the stray chromosomes after the initial capture, select the cell in the Navigator before hitting Live.
2. Click on Live.
3. Choose Join(fuse) when the warning box appears.
4. Proceed with capture as normal.

**Note** - If possible use the same settings for Bright, Black and thresholding. That way the fuse chromosomes will match those in the metaphase in appearance. To do this make sure Predict threshold is turned off in Customize.

Stray chromosomes that have been added to the metaphase can be flagged in the metaphase image with a Fuse Field Chromosome tag. See topic in Analysis > Options > fused field chromosome flag on displaying the flag.

**Related topics**

- Adding fuse images to a karyotype
- Flagging a fused chromosome

**Setting up the microscope**

Proper preparation of the microscope is very important for good image quality. Check the following to ensure that your microscope is optimized for capturing images.

- Optics should be clean and free of specks.
- A Green Interference Filter (GIF) will improve contrast of brightfield images and should be used.
- Center and focus the bulb.
- Center and focus the condenser.
- Use the field, lens and condenser diaphragms to maximize contrast according to the individual microscope and objectives.

**About thresholding**

**About thresholding**

Thresholding is the process that separates individual objects in an image by removing the background. After an image is captured, the thresholding dialog will appear with a histogram showing the gray levels in the captured image. A slider controls the mask that separates objects from background. As it is adjusted, a colored overlay shows how the mask shrinks and expands around the objects. Anything in the image that is covered by the mask will be treated as background. Background is all white in brightfield and black in fluorescent or probe modes. Any area not covered by the mask will be treated as an object.

There are additional functions that thresholding can perform. The contrast of the image can be stretched by a simple contrast stretch or by isolating ranges of gray levels in the image.

There are different ways to eliminate background noise. Region of Interest (ROI) allows segments of the image to be eliminated before thresholding. Background subtraction removes noise based on size. Probe images also have Counterstain Mask and isolation tools to remove noise.

Thresholding can be manually set for each image or automatically applied. These options are selected in Customize.
Basics of thresholding

An analog (COHU camera) captured image captured at 100% contrast contains 256 gray levels ranging from black (0) to white (255). Usually images have less gray levels than this. As the slider is dragged to the right, the cutoff gray level for background is set. Any object that is to the left of the slider will become white in brightfield images and black in fluorescent or probe images. In the following example we can see that any gray level that is 171 or more will become white.

To threshold an image, drag the slider to the right to shrink the mask as tightly as you can around objects or chromosomes, without losing parts of the chromosomes or objects. If too much background is left around the chromosomes the image will look fuzzier and banding will not be as clear. Tighter thresholds also mean less cutting in Analysis. If there is colored mask between two close objects, they will be seen as separate chromosomes in the metaphase and will not need to be split apart.

Threshold mask on a G-banded metaphase

Related topics

- Use the threshold dialog
- Threshold with a single slider
Using the threshold dialog

The thresholding dialog opens after the capture button is pressed. The basic dialog shows a histogram of the gray levels present in the image and has one slider to set the cutoff for the background. In brightfield images this starts with 255, or pure white. In fluorescent images the slider starts at 0 or black. This switching means that the same movement is made to set the background mask regardless of image type.

Other controls can be shown or hidden by clicking on the Customize button and selecting the options used. The second slider is enabled if the Dual Gated Sliders option is turned on in Customize. It is only used for stretching the contrast of the image. The remaining tools provide ways to isolate areas of the image and remove background.

Customizing the threshold dialog

Customize in the threshold dialog is used to select the options you want displayed. When you click on Customize the controls are replaced by check boxes. Display an option by placing a check in the box.

Customize threshold options

Note - Counterstain Mask is also used to hide or display the ROI (Region of Interest) button.
Tip - If Contrast Stretch is turned on in threshold, it will remain on even if the display is turned off.

To stretch contrast while thresholding

Place a check in the Contrast Stretch box of the threshold dialog to perform a contrast stretch on the image while eliminating background. This will stretch the range of gray values present in the image to cover the full range between absolute black and white. The contrast can be stretched using one slider or gate, or using the dual gated method.

Single gate

When only one gate is used then moving the slider does two things. Any object that is to the left of the slider will become background and, it stretches the remaining gray levels from 0-255. In brightfield this means that the gray level of the slider and everything to the left will be changed to white and the remainder of the image will be stretched. In fluorescent and probe capture the gray level of the slider and anything to the left of it will be changed to black.

Dual gate

Dual gated thresholding only works when Contrast Stretch is turned on. In all modes of capture, the blue slider is used to set the background level while the red slider is used to stretch the contrast at the other end of the gray-scale. This means that when thresholding fluorescent or probe images the values of the sliders are inverted compared to brightfield. This is done for consistency; the blue slider is always moved to the right to isolate objects of interest from background.

Brightfield

In brightfield, the blue or top slider is used to set the cutoff level for background. The red slider is used to set the cutoff for dark grays. As the blue slider is moved to the right, the blue-green mask is wrapped around the objects in the cell eliminating the background and setting the cutoff level for white. Any gray levels that are to the left of the cutoff line will be set to white. Move the red slider left, and the image will start to look darker overall. This is because the slider is setting any gray level that is outside of the cutoff line to black. Use the histogram to see how much of the image is outside of the cutoff bars.

Fluorescent and probe

The blue slider is still used to set the background level but in fluorescence this is black. Any grays to the left of the slider will be turned black. The red slider sets the cutoff for white. Moving it left will brighten the image and any gray level to the right of the slider will be changed to white.

Notes

- Turn Contrast on Capture and Normalize off if you do not want the image to be contrasted any further after the threshold is set. These options are found in Capture Customize.

About Background Subtraction

Background Subtraction is used to remove background noise and objects based on the size of their banding profiles. To use it, estimate the size of the objects you want to keep in pixels. Then use half of that value as the subtraction level. As a guideline, try 10-15 for chromosomes, 5-8 for medium sized objects like alpha probes and 3-5 for small objects like cosmid probes (this assumes a 100X objective, lower the values when using a lower power.
objective). For a detailed description of how Background Subtraction see topic Background Subtraction in depth.

**Notes**

- Often nuclei are broken up by background subtraction levels set for chromosomes or only the edges remain. This will happen if there is any banding detail within the nuclei that is approximately the same as the chromosomes or signals.
- It is better to err on the large side, so if you are unsure choose a larger number.
- If you want to use Background Subtraction to capture nuclei use values of 50-100.
- Separate Background Subtract levels will be remembered for brightfield/fluorescent and probe capture.
- Use can save background subtraction levels in fluorochrome defaults. This allows you to create fluorochromes for specific types of capture. e.g. FITCpaint might have 14 saved to its default while FITCcosmid might have 3 saved to its default file.
- Automatically apply Background Subtraction during capture by turning Auto Background Subtract on in Customize.

**How Background Subtraction works**

If a line is drawn across an image to measure the contents of each pixel you will get a profile of the gray levels. This can be seen in the example below; each chromosome encountered creates a narrow peak and the nucleus creates a wide band. Notice that the background noise is a relatively flat line between the peaks at the base of the profile.

Now imagine sliding a box along the bottom of the profile. If the object is very small it will fit into even the narrow peaks. The larger it is, the wider the peak has to be to accommodate it. The box would also fit along the flat sections of noise between the peaks. Setting the Background Subtraction level is actually setting the size of the box used to travel along the profile.
Using the histogram functions

The histogram functions isolate and expand a segment of the histogram making it easier to work with. Stretch works by zooming in on the histogram. By stretching a small area out over the distance of the slider you have finer control over thresholding. This is particularly useful when thresholding 12bit images.

Adjust the cutoff bars to include only the desired area of the histogram. Click on Stretch and the cutoff values will become the new minimum and maximum values in the image. Stretch can be applied multiple times.

Optimize performs a contrast stretch by taking the minimum value and setting it to 0 or black, and the maximum value to white. The sliders used to set minimum and maximum switch between brightfield and fluorescent images. The minimum and maximum text fields will show the gray values present in the image after Stretch or Optimize has been applied. When Histogram is turned on the image displayed for thresholding will automatically be optimized first.

The Histogram buttons are displayed or hidden using the Customize settings in the threshold dialog.

Processing a saved, raw image

Raw images can be thresholded to produce fuse images or to overwrite the metaphase. There is no limit as to the number of fuses that can created from a raw image.

To threshold a raw image:

1. Go to the Capture screen.
2. Load the raw image in the main window.
3. Click on Threshold.
4. Adjust the slider and click on Accept.
5. Choose Recapture metaphase if you want to overwrite the existing metaphase. Choose Join(fuse) to save it as a fuse image. Choose Cancel to exit without saving.
Tip - Thresholding raw images as fuses can be a good way to work with uneven or hard to threshold images. Threshold the metaphase correctly for most of the cell, then threshold the raw image for the hard to capture areas and save it as a fuse.

Notes - If see an error stating that the image you are thresholding is too large, see the topic Rethresholding on a Cohu capture station for instructions on how to change your settings temporarily.

Tools and options

Tools and options

The Capture Setup dialog is used to adjust the contrast of live brightfield and fluorescent images. Capture and Fluorochrome Setup is used to work with all types of probe images. Contrast can be adjusted automatically or manually in any of image using Auto Setup.

If the system is capable of motorized focus control a small dialog with a slider is used to adjust focus.

There are several levels of automation and image enhancement that you can use when capturing cells. Set these capture preferences in Customize.

Related topics

- Work with Capture Setup
- Work with Capture and Fluorochrome Setup
- Customize capture
- Use the Focus controls

Using the Capture toolbar

This toolbar is only visible in the Capture screen.

<table>
<thead>
<tr>
<th>Button</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Count and Analyze</td>
<td>For scanning system users. Opens the dialog used to count and analyze cells from a scan.</td>
</tr>
<tr>
<td>Customize</td>
<td>Select which options you want to use for capturing images.</td>
</tr>
<tr>
<td>Capture Mode</td>
<td>Select different modes of capture for different types of chromosome stains.</td>
</tr>
<tr>
<td>Fullscreen</td>
<td>Used to increase the capture window when digital cameras are used.</td>
</tr>
<tr>
<td>Capture Setup</td>
<td>The tools used to adjust the contrast of a live image.</td>
</tr>
<tr>
<td>Auto Capture</td>
<td>Opens the control panel used to automatically capture cells found by the metaphase finder. This feature is disabled if you do not have the scanning system package.</td>
</tr>
<tr>
<td>Threshold</td>
<td>Threshold raw images to overwrite the metaphase or create fuse images.</td>
</tr>
</tbody>
</table>
Use Auto setup to adjust camera settings once

The Auto Setup box is below the main window next to the Contrast bar.

- Placing a check in Auto Setup will adjust the Bright, Black and if present, the Exposure sliders to achieve the best contrast.
- The process will stop automatically when the system determines the best contrast.

Auto Setup is only applied once. If you want the contrast to be adjusted for every capture use Auto Camera Setup.

**Note** - If you are using a digital camera then Auto Setup cannot adjust the Bright slider.

Using the live contrast bar

When a live image is in the main window the Contrast bar will reflect the range of gray levels present in the image. The range is shown as a white bar. Use the position as an indication of the shift of gray levels. The left end of the bar is black or 0, the right side is white or 255. The percentage of gray levels present is shown to the left of the bar.

Contrast bar showing a live image with 87% contrast

Using the focus control slider

If you have a system with a focus, or Z-axis motor, you can control microscope focus from the Capture screen.

Move the slider bar to adjust the focus by variable amounts. Click above or below the slider bar, in the slider track with the right mouse button to move the focus by the amount specified in Step size. Step size can be set between 0.1-1.0 microns. Click in the slider track with the middle mouse button to move 10X the Step size. Push the 0 button to set the current focal plane as position 0.
About Capture setup

Capture setup contains the tools used to adjust live images before capture. There are two ways the dialog appears depending on the type of cells you are capturing. The brightfield and fluorescent dialogs are the same except for a slider used to control exposure time. Shading and Gamma are tools used to improve hard to capture images. The dialog changes to Capture and Fluorochrome Setup for probe capture modes to include settings specific to fluorochromes.

Note - If you use a digital camera different rules apply to capture.

Related topics

- Use the Capture Setup dialog box
- Use the Bright and Black sliders
- Use Exposure
- Use Shading
- Use Gamma

Using the Capture setup dialog box
The Capture setup dialog always opens with the default settings. If you change the slider positions and want to make these the new default settings press **Apply**. **Done** will close the dialog without saving the changes made.

<table>
<thead>
<tr>
<th>Bright</th>
<th>Controls gain, or the sensitivity of the camera to light.</th>
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<tbody>
<tr>
<td>Black</td>
<td>Controls offset, or where black starts in your image.</td>
</tr>
<tr>
<td>Exposure</td>
<td>Used for fluorescence, Exposure determines how long the camera will scan an image before it captures it.</td>
</tr>
<tr>
<td>Apply</td>
<td>Saves current settings as the new default.</td>
</tr>
<tr>
<td>Gamma</td>
<td>Gamma is used to lighten or darken the live image overall. It can help compensate for very darkly or lightly stained cells.</td>
</tr>
<tr>
<td>Shading</td>
<td>Opens the Shade Correction window used to load or capture shade images. Shade images are pictures of debris or shaded areas that are subtracted from the image before thresholding. Use Shading to minimize cytoplasm, uneven staining or light, and debris.</td>
</tr>
</tbody>
</table>

**Note:** For systems that use a digital camera, see Auto setup under Capture - Using a Digital camera - Capture Setup

About probe Capture Setup

**Using the Bright and Black sliders**

The **Bright** slider controls the sensitivity of the capture board to light. As you move this slider to the right, the image will become brighter. If you move it too far you will saturate, or lose the paler parts of your image. Red will start appearing in saturated areas.

The **Black** slider controls the offset, or where black starts in the image. Moving this slider to the left will effectively darken the image. If you move it too far you will start losing information in the darker areas of your cell. Areas that are saturated for black are seen as blue in the live image.

The **Contrast bar** will reflect how many gray levels you are getting in your live image. Higher contrast values mean you will see more detail in your image.
See Bright and Black sliders in depth for a detailed description of the sliders

The Bright and Black sliders in depth

The **Bright** slider controls gain, or the sensitivity of the capture board to light. As you move the slider to the right, gain is increased. The image looks brighter. The **Black** controls the offset value. As you move this slider to the left the image becomes darker. These sliders work by moving the limits of the gray levels received from the camera. Gray levels can be thought of as colors of gray and go from 0 (Black) to 255 (White). Here is an example of the camera input from a live image.

If we move the top limit down to where the camera input is, we essentially re-set white to the upper limit of our camera input. In other words we’ve set the lightest value coming from the camera as 255 or white. This is done by moving the **Bright** slider to the right. If we move the **Black** slider to the left, we move the black limit up or give the darkest value coming from the camera the new value of 0.
If we move the top limit too far down, (move the **Bright** slider too far to the right), we start clipping off the upper part of the curves and we lose the paler parts of the image. These areas become saturated and are represented by red in the live image. Likewise, if the lower limit is moved too far up into the range we lose all of the darker values as they become saturated black. **Black** saturation is represented by blue in the live image. We've taken our range of 57-217 and spread it into the available range of 0-255. This is called stretching the contrast of an image. Stretching the contrast makes it easier to see small variations in gray levels as they are spread out over a larger range.

**Using Exposure**

The **Exposure** slider controls the length of time the camera chip is exposed to the image before the information is read.

- This is similar to the shutter speed on a regular camera.
- The increments are in 1/1000 of a second.
- The longer the time, the brighter the image will be. This is known as integration on an imaging system. Because of the delay in reading the data, the image is no longer "live". Adjustments to the image will not be visible until the next reading.

For example if exposure is set at 1000 and the **Bright** slider is moved, the effects will not be noticeable for 1 second.

**Use Gamma to capture light or dark images**

Using gamma during capture can compensate for faintly or darkly stained metaphases. It can be difficult to achieve a good contrast stretch in poorly stained cells. Adjust gamma with a live image in the window. Use values >1 for faint cells, and <1 for darkly stained ones.

In most cases, you will only want to apply a gamma correction to one cell. If you want to apply a gamma correction to all of your captures, click on **Apply** to save the new setting as the default before clicking on **Done**.

**Default** resets the value to 1.0.

**To use Gamma:**

1. Click on **Capture setup**.
2. Click on **Gamma**.
3. Move the slider up or down until you have the amount of correction needed.
4. Adjust the contrast of the image.
5. Click on **Capture**.
6. Click on **Done** in the Gamma dialog after the image has been captured.

### About Shading

**Shading** correction compensates for variations across an image caused by uneven illumination or staining. Essentially, you capture a picture of what you want to remove. It is also useful for subtracting cytoplasm or debris from cells. Shade images are captured and loaded from the **Capture Setup** dialog.

Turn **Shading** correction on or off from **Customize**.

### To capture a shade image:
1. Click on **Capture setup**.
2. Click on **Shading**.
3. Click on **Live Shade Image**. If you are already live you can skip this step.
4. Adjust the focus depending on what type of noise to want to reduce.
5. Click on **Capture Shade Image**.
6. Type a name you want to save the shade image under in the **Save Shade Image** field and click **Save Shade Image**.

### Load a shade image:
1. Select the mode you want from **Capture Mode**.
2. Click on **Capture setup**.
3. Click on **Shading**.
4. Select the name of the shade image you want to use from the **Save Shade Image** field.
5. Click on **Done**.

**Note** - Different shade images are saved for brightfield and fluorescence. Make sure you choose the mode of capture before opening the Shading window.

### Common uses for shading correction
Minimize the appearance of cytoplasm

Eliminate debris

Minimizing cytoplasm appearance

1. Adjust the light, position and contrast of your cell.
2. Without changing any of the settings, de-focus the cell until only the cytoplasm remains visible as a faint gray smudge. The more in focus the shade image is the more gray is subtracted from your cell. If you subtract too much, you will lose pale areas of chromosomes.
3. Click on Capture setup. If they are already open you can skip this step.
4. Click on Shading.
5. Click on Live Shade Image. If you are already live you can skip this step.
6. Adjust the focus depending on what type of noise to want to reduce.
7. Click on Capture Shade Image.
8. Type a name you want to save the shade image under in the Save Shade Image field and click Save Shade Image.
9. Make sure that Shade correction is turned on in Customize.
10. Bring the image back into focus and click on Capture.

About Shading

Eliminating debris

1. Adjust the light position and contrast of your cell.
2. Without changing any of the settings de-focus the cell. You want the debris to be as clear as it can without being able to see any of the chromosomes themselves.
3. Click on Capture setup. If they are already open you can skip this step.
4. Click on Shading.
5. Click on Live Shade Image. If you are already live you can skip this step.
6. Adjust the focus depending on what type of noise to want to reduce.
7. Click on Capture Shade Image.
8. Type a name you want to save the shade image under in the Save Shade Image field and click Save Shade Image.
9. Make sure that Shade correction is turned on in Customize.
10. Bring the image back into focus and click on Capture.

About Capture

Customize capture

About Customizing capture

Customize is used to select your options for capture. These options determine the level of user interaction during capture and if any image enhancements are applied to cells as part of capture. Your choices will be remembered and saved as the new default settings. If Mode settings is turned on, a different set of preferences will be saved and loaded automatically for each capture mode.
### Customize Capture

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Automate Camera Setup</strong></td>
<td>Place a check in the box if you want the Bright, Black and Exposure sliders adjusted automatically. <strong>Auto Camera Setup</strong> will activate when the Capture button is pressed and will continue to adjust the sliders until a contrast of at least 85% is achieved. See topic under Capture - Tools and options - Customize capture - Auto Camera Setup.</td>
</tr>
<tr>
<td><strong>Auto Sequence</strong></td>
<td>Eliminates the need to click on Live between fluorochromes. If a filterwheel is attached to the microscope, or the system uses an automated dichroic turret, <strong>Auto Sequence</strong> will automatically change filters between fluorochromes. Auto Sequence is selected in the Fluorochrome Selection dialog window. See topic under Capture - Tools and options - Customize capture - Auto Sequence.</td>
</tr>
<tr>
<td><strong>Split Chroms</strong></td>
<td>Place a check in the box to separate any chromosomes that are seen as touching. See topic under Capture - Tools and options - Customize capture - Auto Split Chroms.</td>
</tr>
<tr>
<td><strong>Auto Threshold</strong></td>
<td>Thresholds the image automatically. The threshold value can be the last one used, predicted or zero depending on the other options chosen. See topic under Capture - Tools and options - Customize capture - Auto Threshold.</td>
</tr>
<tr>
<td><strong>Predict Threshold</strong></td>
<td>Place a check in the box to have the threshold level estimated for each cell. Manually adjust the threshold if necessary and click Accept to proceed with capture. See topic under Capture - Tools and options - Customize capture - Predict Threshold.</td>
</tr>
<tr>
<td><strong>Zero Threshold</strong></td>
<td>Automatically sets the threshold to zero cutoff level or no thresholding. This allows for automatic capture of unthresholded cells. See topic under Capture - Tools and options - Customize capture - Zero Threshold.</td>
</tr>
<tr>
<td><strong>Auto Register Images</strong></td>
<td>Used only for RxFISH, CGH and M-FISH capture. If you use different cubes for capture and have problems with registration errors, place a check in the box to try to resolve these errors during capture. If you use a single cube then leave this option turned off. Image processing is faster when the option is off. See topic under Capture - Tools and options - Customize capture - Auto Register Images.</td>
</tr>
<tr>
<td><strong>Auto Focus Offset</strong></td>
<td>Used only for probe capture on systems with a focus motor installed. Place a check in the box to automatically adjust focus between fluorochromes. These offsets will be saved with the fluorochrome.</td>
</tr>
<tr>
<td>Setting</td>
<td>Description</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Use Last Capture</strong></td>
<td><em>For digital capture only.</em> When it is turned on, pressing the <strong>Capture</strong> button will use the last image displayed to create the cell. This helps prevent fluorochromes from bleaching during long exposure times. See topic under Capture - Tools and options - Customize capture - Auto Focus Offset.</td>
</tr>
<tr>
<td><strong>Shading Correction</strong></td>
<td>Place a check in the box to use the loaded shade image for capture. Shade images are pictures of dirt, noise or uneven backgrounds that are subtracted from the image before thresholding. See topic under Capture - Tools and options - About Capture setup - About Shading - Using Shading to correct illumination.</td>
</tr>
<tr>
<td><strong>Probe Background Cut</strong></td>
<td>Determines the minimum value on the edge of an object, and subtracts this from every pixel. See topic under Capture - Tools and options - About Capture setup - About Shading - Probe Background Cut.</td>
</tr>
<tr>
<td><strong>Auto Background Subtract</strong></td>
<td>Automatically applies the currently set background subtraction value. Background subtraction removes objects that are larger than the value set. See topic under Capture - Tools and options - About Capture setup - About Shading - Auto Background Subtract.</td>
</tr>
<tr>
<td><strong>Normalize Image</strong></td>
<td>Performs a contrast stretch based on all objects in the image. The relative intensities or darkness of objects will not change. See topic under Capture - Tools and options - About Capture setup - About Shading - Normalize Image.</td>
</tr>
<tr>
<td><strong>Contrast on Capture</strong></td>
<td>Maximizes contrast within each object individually. The relative intensity of objects will be changed. Use this option if you want the maximum number of bands within each object, but do not need to maintain relative intensities. See topic under Capture - Tools and options - About Capture setup - About Shading - Contrast on Capture.</td>
</tr>
<tr>
<td><strong>Save Raw Image</strong></td>
<td>Place a check in the box to save a raw image with each capture.</td>
</tr>
<tr>
<td><strong>Region of Interest drawing style</strong></td>
<td>This is only used in probe thresholding. Choose the drawing method used to circle regions of interest during thresholding.</td>
</tr>
<tr>
<td><strong>Upload images to database</strong></td>
<td>For SPOT users only. Sends the raw images to the database. If you want to reprocess the scans in the <strong>Review</strong> application this option must be turned on.</td>
</tr>
<tr>
<td><strong>Mode settings</strong></td>
<td>Turn this option on if you want to save a different set of options for each mode of capture. They will be remembered automatically and loaded whenever you switch to that mode.</td>
</tr>
<tr>
<td><strong>Camera</strong></td>
<td>If you have more than one camera attached a numbered list will appear here. Switch between cameras by selecting their numbers from the drop-down list.</td>
</tr>
<tr>
<td><strong>Capture from file</strong></td>
<td>Place a check in the box to capture an image from a file rather than a camera.</td>
</tr>
<tr>
<td><strong>Streamlined Capture</strong></td>
<td>Enables one-button capture in brightfield and fluorescent mode. This feature means fewer mouse clicks and faster image acquisition.</td>
</tr>
</tbody>
</table>

**Auto Camera Setup**
**Auto Camera Setup** will automatically adjust the live contrast of a cell. It replaces the manual operations of adjusting the **Bright, Black** and **Exposure** sliders. When the target contrast level has been reached the image will be grabbed and the thresholding screen will appear. The default target value for brightfield, fluorescence and probes is 85%. The contrast target value for probes can be changed in **Capture and Fluorochrome Setup**. **Auto Camera Setup** is activated by the **Capture** button, and is applied to every capture while it is turned on.

**To use Auto Camera Setup:**

1. Click on **Live** and adjust the focus, placement of the image and microscope light level.
2. Click on **Capture**.

Turn **Auto Camera Setup** on or off from **Customize**.

**Note** - If you are using a digital camera then **Auto Setup** will adjust the settings to achieve near 100% contrast. In some situations autosettings may cause high exposure times which may need reducing manually. With **Auto Setup** ON the gain and offset sliders will try and adjust to compensate for the lower exposure time.

**Capture successive fluorochromes automatically**

Used in probe modes, **Auto Sequence** replaces the **Live** command between fluorochromes of the same cell. The capture process will automatically move to the next fluorochrome after an image is thresholded. If you have a filterwheel or motorized microscope, they will automatically turn to the next filter. If you change the cubes manually, you will be prompted to switch to the next filter after a fluorochrome has been captured.

Turn **Auto Sequence** on or off in the **Fluorochrome Selection** dialog window.

**Automatically separate chromosomes during capture**

When **Auto Split Choms** is turned on, any objects seen as touching will be split at the end of the capture process. As this feature is very conservative in its splits, it is very likely that some touching groups will remain.

Turn **Auto Split Choms** on or off from **Customize**.

**Automatically threshold**

**Auto Threshold** will perform the thresholding part of the capture process. Clicking on the **Capture** button will activate the thresholding process. When finished the final image will be displayed in the main window.

There are three ways to determine the level of thresholding used. If you want the best threshold level estimated for each cell, turn **Predict Threshold** on as well. If you want to use the same level for each cell then leave **Predict Threshold** turned off. Capture one image manually to set the level you want to use, then turn **Auto Threshold** on. To capture cells with no threshold turn **Zero Threshold** on.

**Note**: For M-FISH assays, to ensure that terminal ends of chromosomes are not removed, the user can manually threshold the DAPI counterstain image by unchecking the Auto Threshold box in the Customize Capture window.
Automatically estimate threshold level

Forces the system to estimate where the best threshold level is. The thresholding dialog still appears allowing you to adjust the threshold further before capture. If turned off, the last threshold value used will be the level the dialog opens with.

Turn **Predict Threshold** on or off from **Customize**.

💡 **Tip** - When capturing cells with stray chromosomes it is advised to turn this feature off. That way the fuse will match the metaphase in appearance.

Zero Threshold

Sets the threshold level to 0. Use it with **Auto Threshold** automatically capture unthreshholded cells without having to manually set the threshold.

Automatically correct registration

This is only used for RxFISH, M-FISH or CGH capture. If you are using different blocks for capturing probe signals and have problems with registration errors use **Auto Register Images** to correct the errors as part of the capture process. If you use a single cube for capturing, leave this feature off to speed up the image processing time.

Turn **Auto Register Images** on or off from **Customize**.

Automatically apply focus offset between fluorochromes

**Auto Focus Offset** is only used on systems with a focus motor attached to the microscope or with motorized microscopes. When turned on, focus movements you make with the **focus slider bar** are monitored. The focus offset of each fluorochrome relative to the counterstain is automatically remembered. After initial setup the focus position for each fluorochrome is automatically adjusted whenever you click on the **Live** button.

**To use Auto Focus Offset:**

1. Place a check in the box marked **Auto Focus Offset** in **Customize**.
2. Focus the first fluorochrome using the microscope controls or the slider. Then focus each additional image using only the control slider in the capture screen. Once the first fluorochrome is captured do not use the microscope knobs.
3. After the first cell is completed, the focus will be adjusted automatically whenever the **Live** button is clicked.

If you find a focus offset needs changing just use the control slider to focus the image and the new settings will be saved automatically. Disable **Auto Focus Offset** by removing the check in **Customize**.

Use Last Capture

The image displayed while adjusting the contrast is created with a temporary captured file. When **Use Last Capture** is turned on the display image will be used to create the cell. This is particularly useful when capturing faint fluorochromes with long exposure times. If you turn
this option off then when the **Capture** button is pressed the current exposure will finish and the image will be created from a new capture file.

**Note** - This option is only available for users with a digital camera.

**Probe Background Cut**

**Probe Background Cut** is intended for use in quantitative or ratio work. As you adjust the contrast, you adjust the signal level as well as the background noise level. If you want to measure the actual value of a probe signal or compare the intensities of two probe signals, the background should be set at black. **Probe Background Cut** takes the minimum value on the edge of an object, and subtracts it from every pixel in the image. With the background fluorescence removed, the probe signal will always be above a black background.

Turn **Probe Background Cut** on or off from **Customize**.

**Related topics**

- Use Background Subtraction during thresholding

**Auto Background Subtract**

Automatically applies the **Background Subtraction** level selected in the threshold dialog box. One value is saved for all brightfield and fluorescent capture. Just threshold a cell manually and set the subtraction level.

Separate values for each fluorochrome are saved for probe capture. Save these values by manually setting the subtraction level for each fluorochrome. Then save the fluorochrome list or if you want the subtraction to be part of the default settings for a fluorochrome, use Save as Default.

**Related topics**

- About Background Subtraction

**Applying a global contrast during capture**

**Normalize Image** performs a contrast stretch at the end of the thresholding process. It takes the darkest part of the whole image and makes it black, the lightest becomes white. The remaining gray levels are stretched across the rest of the range. Using **Normalize Image** retains the relative intensity of the objects within an image.

Turn **Normalize Image** on or off from **Customize**.

**Related topics**

- Stretching the contrast during thresholding
- What is a contrast stretch
- Apply object based contrast during capture

**Applying an object based contrast during capture**
**Contrast on Capture** performs a contrast stretch on each object individually at the end of the thresholding process. The darkest part of the image will be stretched to black, the palest to white. This maximizes contrast within objects but does not maintain their relative intensities to each other. Debris and other background material can be emphasized. **Contrast on Capture** is not recommended for FISH, NOR, C-banding, small G-banded markers or any other image where relative intensity is important. **Normalize Image** is a better option for these types of images.

The number in the text box limits how far the pale regions will be stretched. If set at 0, then the lightest gray value will be white or 255. If at 5 then the lightest gray level of each object will be set to 250. Higher numbers will prevent pale regions from disappearing into the background. The standard settings used are between 0-20.

Turn **Normalize Image** on or off from **Customize**.

**Related topics**
- Stretching the contrast during thresholding
- What is a contrast stretch

**Save Raw Image**

Saves a raw image along with the metaphase. Anything in the background that would be removed from the image by thresholding will be seen in the raw image. No contrast stretches will be applied. Turn **Save Raw Image** on or off from **Customize**.

**Tip** - If you have a cell that is hard to threshold turn **Save Raw Image** on. Then you can threshold the raw image differently from the metaphase and save it as a fuse.

**Region of Interest drawing style**

This is only used in FISH capture modes. You can choose either the Rubberband or Freehand method to circle regions of interest during probe image thresholding.

Choose **Region of Interest** drawing style from **Customize**.

**Capturing cells**

**Capturing cells**

To capture brightfield and fluorescent cells you adjust the live image using **Capture Setup** and capture the image. The thresholding dialog is limited to the cutoff sliders only.

Probe capture uses **Capture and Fluorochrome Setup** to adjust the live image and has additional thresholding tools used to isolate signals from the background.

CGH, RxFISH and M-FISH use the same methods as probe capture but involve image analysis as part of the capture process.

**Related topics**
- Capture a brightfield image
Capture a fluorescent image
Capture a probe image
Capture CGH images
Capture RxFISH images
Capture M-FISH images
Capture from file

Brightfield and fluorescent

Capture a brightfield image

When capturing banded brightfield images you want to try and get at least 70% contrast ranges in the live image before capturing them. This is done by adjusting the light on the microscope and the Black and Bright sliders.

It is best to start with Bright set about 10 and Black set about 160, then set the light level on the microscope. Make the light as bright as you can without losing pale regions on the chromosomes. Then move the Black slider down to shift the image towards black and move the Bright slider up to brighten the image. As you move the sliders you should see the effects on the Contrast bar under the main window.

When the image looks good click on Capture and the thresholding dialog will appear. Use the cutoff sliders to eliminate the background and if you have Contrast Stretch on, to further stretch the contrast of the image. Read About thresholding for details. Click on Accept to complete the capture process.

Related topics
Capture stray chromosomes
Using Capture Setup
Setting up the microscope
Troubleshooting brightfield capture

Troubleshooting brightfield capture

What's wrong?

<table>
<thead>
<tr>
<th>Observation</th>
<th>Troubleshooting Tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>I can't get a good contrast value</td>
<td>• Use Gamma correction if the cell is stained poorly before adjusting the contrast.</td>
</tr>
<tr>
<td></td>
<td>• Use a Green Interference filter.</td>
</tr>
<tr>
<td></td>
<td>• Is the microscope properly adjusted?</td>
</tr>
<tr>
<td></td>
<td>• If a cell looks well contrasted and banded in the microscope but very flat gray in</td>
</tr>
<tr>
<td></td>
<td>the live image, you may have some Infrared interference (IR) from the microscope.</td>
</tr>
<tr>
<td></td>
<td>There are filters that can be placed in the camera to eliminate IR. Contact Applied</td>
</tr>
<tr>
<td></td>
<td>Imaging if you think you have this problem.</td>
</tr>
<tr>
<td>I keep getting errors about too much noise</td>
<td>This error is seen when there are too many objects in an image. Usually this is</td>
</tr>
<tr>
<td>when thresholding</td>
<td>caused by large amount of small specks in the background. Try to tighten the</td>
</tr>
<tr>
<td></td>
<td>threshold so the number of specks is reduced. If you can't tighten the threshold</td>
</tr>
<tr>
<td></td>
<td>enough</td>
</tr>
</tbody>
</table>

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without losing your areas of interest try one of the following:

- Use Shading Correction or Background Subtraction to eliminate the debris or cytoplasm.
- Capture the cell with a looser threshold so that the cytoplasm is not broken up into specks. Then capture a fuse image and adjust the threshold for just for the chromosomes covered in cytoplasm. Use them for the karyotype.
- Unless you want to use the cells on a UNIX CytoVision, make sure that the UNIX Compatible toggle is turned off in capabilities.
- Capture the cell with Save Raw Image on. Then you can use the raw image to create fuse images that are thresholded for the troublesome areas. Several fuses can be generated from the same raw image. Show me how

<table>
<thead>
<tr>
<th>I can't threshold without losing information</th>
<th>Use Shading Correction or Background Subtraction to eliminate the debris.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capture the cell with Save Raw Image on. Then you can use the raw image to create fuse images that are thresholded for the troublesome areas. Several fuses can be generated from the same raw image.</td>
</tr>
</tbody>
</table>

| My captured cells look darker than my live image | **Contrast on Capture** will produce darker cells after capture. Either use the contrasting tools to brighten the image, or capture with **Normalize Image** instead. Both of these options are found in **Customize**. |

---

**Capture fluorescent images**

When capturing Q-banded fluorescent images you want to try and get at least 70% contrast ranges in the live image before capturing them. This is done by adjusting the Exposure and the Black and Bright sliders.

It is best to start with **Bright** set about 70 and Black set about 160, then set the Exposure level until the image is bright and visible. You want a flat, dark background with no vertical lines; (lines indicate too little light). If you set the exposure too high the image will be very flat and gray with little contrast.

Now move the **Black** slider down to shift the image down towards black and darken the background. Then move the **Bright** slider up to brighten the image. As you move the sliders you should see the effects on the Contrast bar under the main window.

When the image looks good click on **Capture** and the thresholding dialog will appear. Use the cutoff sliders to eliminate the background and if you have **Contrast Stretch** on, to further stretch the contrast of the image. Read About thresholding for details. Click on **Accept** to complete the capture process.

**Related topics**

- Capture stray chromosomes
- Using Capture Setup
- Setting up the microscope

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Troubleshooting fluorescent capture

When I go live there is no image
If you have a filterwheel attached to the microscope, it is probably in the blank position. Set the position to clear to use a different filter block, or to the filter position that you use for fluorescent capture and turn the filterwheel off in the software.

<table>
<thead>
<tr>
<th>Observation</th>
<th>Troubleshooting Tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>When I go live there is no image</td>
<td>If you have a filterwheel attached to the microscope, it is probably in the blank position. Set the position to clear to use a different filter block, or to the filter position that you use for fluorescent capture and turn the filterwheel off in the software.</td>
</tr>
</tbody>
</table>

FISH modes

FISH modes

Probe capture
FISH images are captured as black and white images which are then rendered with the color chosen for each fluorochrome. Each fluorochrome is captured as a separate layer. These layers are combined to form the final probe image. There are additional tools used to adjust the live image contrast and for controlling filterwheels if you have them. See the section About probe capture for detailed information.

CGH, RxFISH and M-FISH capture
These three modes of capture share some common features that differ from standard probe capture. All three take raw images and process them to extract the data needed to generate metaphases. Because the system needs to process these cells there are quality control checks that determine whether data can be generated accurately or not. The results of these tests are shown in the Image Capture and Cell Measurements dialog boxes. After reviewing the results the metaphase and probe images are built from the raw image data.

If you want to use a species template for these sample types you must select the template as part of configuring the Fluorochrome Selection panel for capture.

Related topics

- Use the Image Capture dialog
- Work with Cell Measurements
- Reprocess a slide

Using the Image Capture dialog box

After all of the fluorochromes in the first cell are captured, the Image Capture dialog box will open. The system will begin performing quality control tests on the images in the background
while the remaining cells are captured. An hourglass icon (⌛) is displayed next to cells that are being processed. These cells cannot be viewed, and the capture screen cannot be exited until all of the evaluations are completed.

When processing is finished, a tick (✔️) is shown by cells that have passed all of the tests and a cross (❌) marks those that have failed one or more tests. Click on a cell name in the list and it will be displayed in the main window. This also brings up the Cell Measurements dialog where you can see the results of the quality control tests. Change the pass or fail status of a cell from Cell Measurements. The Image Capture cell list is also used to select an image for re-thresholding manually.

When all of the cells have been processed click on Batch Complete to build the metaphase and probe images. If any of the cells are marked as failed when Batch complete is clicked you will be given the option to keep or discard them. Click on Discard to delete any failed cells.

Reprocess allows you to delete all but the raw images in a cell and reprocess the images. You can click on Reprocess at any time during the background processing.

**Related topics**
- To reprocess a slide after capture
- CGH Image Capture
- RxFISH Image Capture
- M-FISH Image Capture

### Cell Measurements and reasons for failure

The tests will vary slightly depending on the mode of capture. Some tests are shown for both test and reference DNA in CGH mode.

<table>
<thead>
<tr>
<th>Test</th>
<th>Description</th>
<th>Reasons for failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banding</td>
<td>Measures how visible the banding is within each of the fluorochromes and</td>
<td></td>
</tr>
<tr>
<td>Strength</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Metric</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic of Fluorescence</td>
<td>Measures the intensities of the fluorochromes inside the chromosome compared to that of the background. A low value suggests one of the fluoros is very faint. A value of 0.00 is usually caused by the Black level being set to low.</td>
<td>• Faint fluorochrome&lt;br&gt;• Poor capture of fluorochrome</td>
</tr>
<tr>
<td>Fluor Contrast</td>
<td>Measures the range of intensities inside the chromosomes for each fluorochrome. Low values indicate faint fluorochromes which will contain little data. Click on the ? to see what the values are for each of the fluorochromes.</td>
<td></td>
</tr>
<tr>
<td>Automatic Registration</td>
<td>Measures the ease with which the raw images can be layered and aligned to get an accurate probe image and metaphase.</td>
<td>• Vibration of microscope&lt;br&gt;• Misalignment of microscope optics&lt;br&gt;• Too many touching chromosomes&lt;br&gt;• Very faint fluorochromes&lt;br&gt;Registration errors from using manual cube slides</td>
</tr>
<tr>
<td>Separate Chromosomes</td>
<td>Counts the number of chromosomes that are touching or overlapped. Ratio fluorescence values can be influenced by touching chromosomes.</td>
<td>• Choosing cells that are not well spread or have too many overlaps</td>
</tr>
<tr>
<td>Variation of Painting</td>
<td>Measures how even the intensities of the fluorochromes are across the cell. The higher the value the more variation there is. Click on the ? to see what the values are for each of the fluorochromes.</td>
<td>• Uneven hybridization&lt;br&gt;• Off-center lamp</td>
</tr>
<tr>
<td>Chromosome Length/Width</td>
<td>Measures chromosome compression or the ratio of length to width. If the chromosomes are too compressed it is difficult to visualize small bands and abnormalities may be missed or results may be incorrect.</td>
<td>• Poor slide making or culture technique&lt;br&gt;• Choosing cells that are too short when capturing.</td>
</tr>
<tr>
<td>Test Variation of Painting</td>
<td>Measures how even the intensity of the test fluorochrome is across the cell. The higher the value the more variation there is.</td>
<td>• Uneven hybridization&lt;br&gt;• Off-center lamp</td>
</tr>
<tr>
<td>Granulation</td>
<td>Measures the quality of fluorochrome hybridization to the cell. Poor hybridization results in a grainy appearance.</td>
<td>• Bands are visible in the reference fluorochromes due to poor denaturation of target DNA.</td>
</tr>
<tr>
<td>Median Intensity</td>
<td>Measures the brightness of the test fluorochrome. A low value indicates that the fluorochrome is very faint and that little quantitative information can be derived from the image.</td>
<td></td>
</tr>
<tr>
<td>Counterstain Banding</td>
<td>Measures how visible the banding is within the counterstain. The lower the</td>
<td></td>
</tr>
</tbody>
</table>
To reprocess a slide after capture

1. Open the case in Analysis.
2. Click on the slide in the Navigator.
3. Switch to the Capture screen.
4. Click on Reprocess in the Image Capture dialog box.

If you are still in Capture you can click on Reprocess anytime during processing.

**Note** - Everything in the cells except the raw images will be deleted.

Using the Cell Measurements dialog box

When a cell is selected in the Image Capture dialog the Cell Measurement dialog box will open with the list of quality control tests and how the captured cells’ parameters compare with the preset thresholds. To display the definition of a test from within the program click on the ? to the left of its name. The tests performed will vary somewhat depending on the type of cells you are capturing.

The preset thresholds of the tests can be changed by typing over the existing value. To restore the original values click on Defaults then Continue.

If a cell has failed but in your opinion it is good enough to analyze, click on the Keep button. A ✔ will appear by the cell in the Image Capture dialog. Likewise if a cell has passed but you do not want to keep it, click on Discard and a ✗ will be displayed. This only applies to CGH and RxFISH images. The status of M-FISH cells cannot be changed.
### CGH Cell Measurements

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Value</th>
<th>Threshold</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automatic Registration</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Test Granulation</td>
<td>0.76</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Reference Granulation</td>
<td>0.85</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Test Variation of painting</td>
<td>40.61</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Reference Variation of painting</td>
<td>40.97</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Test Dynamic of Fluorescence</td>
<td>4.77</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Reference Dynamic of Fluorescence</td>
<td>4.42</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Test Median Intensity</td>
<td>98</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Reference Median Intensity</td>
<td>89</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Counterstain Banding Strength</td>
<td>11.01</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Common genomic Length / Width</td>
<td>3.10</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Separate Chromosomes</td>
<td>38</td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

**Value**  
The results of the test are displayed in the Value column.

**Threshold**  
The default threshold values were taken from good quality cells. You can change these thresholds by entering an new value in the text field.

**Status**  
Each test is marked as passed or failed to make it easier to find which tests failed.

**? boxes**  
Click on a ? box to see a description of each test. For some tests possible reasons for failure are also listed.

**Discard**  
Click on Discard if you want to delete the cell from the slide. A X will be placed by the cell name in the CGH Image Capture window and will be deleted when Batch Complete is clicked.

**Keep**  
Press to keep a cell that has failed the quality tests. The status will change to passed in the Image Capture dialog.

**Defaults**  
Click to return the thresholds to the default values that came with the system.

**Note**  
Marking a cell as passed does not guarantee that the system can create a metaphase from it. If the quality is too poor the cell will be deleted.

### Related topics
Listing of quality control checks and reasons for failure

Manually threshold color banded chromosomes

CGH, RxFISH and M-FISH cells are automatically thresholded during capture. You can load and rethreshold the raw counterstain image by doing the following:

1. Click on the cell name in the Image Capture dialog.
2. Click on Threshold.
3. Enter a value in Background Subtraction that is equal to half of the chromosomes width in pixels (usually 15-20).
4. Click on Subtract.
5. Adjust the sliders with or without Contrast Stretch turned on.
6. Click on Accept.

Tip - To bring up the Image Capture dialog if you are not currently capturing the cells click on the slide in Analysis then switch to the Capture screen.

Note - It is not mandatory that you use background subtraction when thresholding, however it is recommended. If you do not use it, the fluorescence ratios and CGH profiles will be affected.

Using a Digital camera

Using a Digital camera

If you have a digital capture system, specific tools are used to adjust the live image. Capture Setup is used for brightfield and fluorescent capture. Capture and Fluorochrome Setup is used to capture all types of probe images. The Histogram tools in the threshold dialog will help give finer control over the thresholding.

There are a few ways to select the captured image size and content. You can use the screen layout options to select the size and area captured. Binning affects image size as well as frame rate. Or use Region of Interest to define specific areas of an image.

Capture with a Digital camera

- When an image is captured with or without the stretch option checked in the threshold dialog, the image automatically scales to fit the display window. All chromosomes for thresholding are visible in the window as well as in the saved image. There are no scroll bars.
- CGH, M-FISH and RxFISH live and captured raw images are contrast stretched to maximize their dynamic range.

Analysis with a Digital camera

- Metaphases automatically scale to fit in the analysis window. A red box around the image denotes that the image display is scaled. There are no scroll bars.
- When copying a chromosome from a large image format to a flex window, the chromosomes automatically scale to fit in the window. Objects will be immediately visible and objects rotated in the image will not disappear.
- Dragging something from a 'scale to fit' window in a flex window causes all images to be scaled to fit. A red border appears in the flex window.
Related topics

- Use Capture Setup
- Use Capture and Fluorochrome Setup
- Use the screen layout options
- Use Binning
- Define a Region of Interest
- Rethreshold digital images on a Cohu capture station

Image size

The standard capture window is designed to accommodate resolutions of 768X575. Since most digital cameras are capable of higher resolutions than this there are a couple of ways to compensate. If your image is larger than the window scroll bars will allow you to pan around the image.

There are two ways to eliminate the use of scroll bars in the capture window:

- Check the **Stretch** button to scale the whole image into the capture screen in the Capture Setup dialog window.

![Capture Setup - Fluorescent](image)

- Capture in Fullscreen layout mode.

Digital Capture Setup

The minimum and maximum values for the gain and offset sliders is 128. Using high gains above 100 can cause grainy images and it is recommended that the exposure time is increased in such situations.

- Use **Region of Interest (ROI)** to capture portions of the image.
- **Gamma** and **Shading** are used when cells are difficult to capture.
- Check the **Stretch** button to scale the whole image into the capture screen.

**Note:** Selecting the **Auto setup** box in the Capture window will automatically set the camera settings (Bright, Black and Exposure) for both brightfield and fluorescent applications.

**Related topics**
- Define a Region of Interest (ROI)
- Binning options
- Capture with Gamma
- Capture with Shading

**Digital probe capture**

The maximum and minimum values of the sliders will depend on the camera you have installed. The **Black** slider is disabled for some cameras. Use Region of Interest to capture portions of the image. Use Binning to combine neighboring pixels. For a description of all other tools in **Capture and Fluorochrome Setup** see the topics under About probe capture.

A live **focus** button is located on the toolbar that enables you to focus on a live image without losing your **Bright** and **Exposure** settings.
To focus a live image

Live focus

1. Press Focus.
2. Focus the image. You can drag the focus area to a new location with the left mouse button.
3. Press the middle mouse button to return to your former settings.

Binning options

There are three binning states that can be used: 1X1, 2X2 and 4X4. Binning values can be changed while the image is live.

What is binning?

In this mode 2 or more horizontal and vertical pixels are "binned" together, that is, the accumulated charge is added. In case of a 2 x 2 (2 pixels horizontal and 2 pixels vertical) binning the number of output pixels is also reduced by 2 x 2, that is, the number of horizontal pixels is reduced by 2 and so are the number of vertical output pixels.
This results in a reduction of resolution but the frame rate, the sensitivity and the signal/noise ratio are increased. Binning is useful for capturing very faint signals but you will lose resolution.

**Using digital Region of Interest**

You can define rectangular regions of the image for capture. When the toggle is on the region of interest is used. Turn off **Region of Interest** to capture the entire visible image again. The defined area will remain the same until a new region is defined.

**To define a region:**

1. Click and hold the left mouse button at the top left corner of the area to define.
2. Drag the pointer out to define the region.
3. Let go of the mouse button.

**Rethresholding large digital images on a Cohu capture or review station**

There is a configuration file on the system that is used to set the size of the captured image and the type of camera and capture board used. If you have a Review station you will not have a capture board and your configuration file should be set up for a PseudoCapture Card.

If you have a system with a Cohu camera and want to rethreshold large digital images you can also temporarily change your system to these settings.

**Note for Cohu users** - Remember to switch the settings back after you have rethresholded the images as you will not be able to capture images until you do.

**To set up for PseudoCapture card:**

1. Login as Administrator. Make sure that CytoVision is NOT running.
2. Click on **Start** and select **Capture Config** from the **Applied Imaging** menu in **Programs**.
3. Select **PseudoCapture Card**.
4. Click on **Save to File**.
5. Click on **Done**.

**Capture Wizard**

**Capture Settings**

For each slide, the capture parameters and an initial focus point must be setup.

- To select a slide, left click on the slide in the wizard.
• Click on the **Advanced** button to open up additional set up selections related to autofocusuing:

• The slide border will turn red and the stage will move to the correct bay.

• Using the stage controls adjust the Z position so that the image is in focus.

• If this is metaphase autocapture, you may also have to adjust the X and Y positions to get the metaphase in the centre of the image.

• Click on **Set Offset** when done.

Now use the New Cell, Live and Capture buttons, to adjust camera settings, or with digital cameras use auto setup to quickly achieve the best contrast.

When a slide has been completed it will turn green.
Repeat this procedure for all slides.

**Advanced Parameters**

Also included in the capture setup page are some advanced parameters for autofocus. These are accessed by clicking the **Advanced** button.

These allow you to tune various focus parameters in order to get the best speed without compromising quality of autocapture. These focus parameters are used for both focus map building and for autocapture autofocus.

- **Skip focus mapping**
  
  Check the **Skip focus mapping** box to skip slides that have bad focus maps and begin autocapture on those slides that have passed focus map.

- **Plane spacing**
  
  This is the spacing between individual planes used to take focus measurements. It should be roughly equivalent to the depth of field for the objective. For 100x and 60x try a spacing of 4, for 40x try a spacing of 6 and for 20x try a spacing of 8.

- **Focus every nth**
  
  This parameter determines how many frames should elapse before a focus operation takes place. **NOTE** that this is only a rough way of specifying the focus frequency. In reality if autocapture moves a distance greater than "n" times the frame distance between two points then a focus operation will take place.

- **Focus Points**
  
  When the system builds a focus map it uses a number of focus positions to determine the focus map. This number determines the number of sample positions to use. The less points the faster the focus map building takes place, **however**, in order to calculate a focus map the system needs at least 5 good points otherwise the map building will fail.

- **Defaults**
  
  Clicking this button will revert the above settings back to some suggested default values.

Click **Next** to continue to Focus Map Building
Click **Back** to go to Objective Selection
Click **Cancel** to quit
Focus Map Building

Once all slides have been setup, on entry to the focus map page the wizard will automatically start to build the focus maps.

The wizard will move to the position of a specially selected cell or metaphase and begin focusing. This process is repeated for the number of focus points defined in the advanced parameters of the previous page.

When focus map building completes for a slide it will either show a green tick mark or a red cross depending on whether focusing was successful or not.

The system will then automatically move to the next slide.

Clicking the **Stop focus** button will stop the automated focus map building process for the current slide.

**If all focus maps have completed successfully for ALL the wizard will go straight into autocapture.**
**Note:** If skip focus mapping was checked, autocapture will proceed even if a focus map has failed.

- **Skip focus mapping**

If the focus map fails for any slide, if skip focus map is not checked, or is stopped for any slide:

Click **Next** to go to manual focus map building
Click **Cancel** to quit
Click **Back** to go back to capture settings

**Related Topics**

- The Capture Wizard
- Capture Settings
- Manual Focus Map Building

**Manual Focus Map Building**

Manual focus map building need only be done on those slides where the focus map failed. These will be indicated with a red cross. To select one of these slides left click on the slide and the stage will automatically move to the correct position.

Use the **Forward** and **Back** buttons to move between focus points in the focus map.

At each focus position using the z focus slider to adjust the focus so that the image is clear and sharp. Then click the **Set** button to fix the position.

When enough positions have been completed, the slide will turn green indicating the focus map is complete.
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Repeat this process for all failed slides.

Click Finish to go straight to autocapture
Click Cancel to quit
Click Back to go back to automated focus map building

Related Topics

- The Capture Wizard
- Capture Settings
- Focus Map Building

Objective Selection

The first page of the wizard allows you to choose the objective you wish to use for autocapture. This is accomplished by selecting an objective from the drop down list.

NOTE that for SPOT autocapture the objective must have been calibrated first using the SPOT calibration program.

Click Next to continue
Click Cancel to quit

Related Topics

- The Capture Wizard
- Focus Map Building

The Capture Wizard
The capture wizard allows you to build focus maps and setup capture settings prior to autocapture. There is no need to run the scan wizard first in order to do this.

The wizard can be accessed using the capture wizard button from the capture screen.

The wizard can be used for both metaphase autocapture or for SPOT autocapture.

**It is strongly recommended that the user gets familiar with capture before using the wizard.**

Click Next to continue

**Related Topics**

- The Scan Wizard
- Auto capture
Analysis

Analysis overview

The Analysis screen is the main view used to work with images. Switch to the Analysis view by clicking on the Analysis button on the main toolbar.

Analysis button

The Analysis toolbar is used to print, select and delete objects and switch between Fullscreen and the current layout. For Genus users there are buttons to load and create karyotype templates. Additional toolbars are displayed in the workarea that contain buttons specific to Analysis functions. The Analysis Operations toolbar is displayed next to the main image window and contains most of the tools used.

A small Annotation Toolbar is used to draw shapes or place text in an image. Users with Genus flexible karyotyping also have a toolbar specific to editing templates. Both of these additional toolbars are opened from buttons in Analysis Operations.

Cutting tools separate objects that are overlapped or touching.

Image appearance tools alter the appearance of objects. This can be through the application of filters or contrast stretches to improve visualization of bands or signals. Objects can be scaled and reflected.

Images of different types and from different cases can be combined to create montages for presentation. This type of image is referred to as a flex image and is saved as part of a cell.

Preferences for tool settings are selected in Customize.

The Analysis toolbar

The middle section of the main toolbar displays the following functions in Analysis.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Print</strong></td>
<td>Opens the print dialog used to send images to the printer.</td>
</tr>
<tr>
<td><strong>Customize</strong></td>
<td>Select options or preferences for the Analysis tools.</td>
</tr>
<tr>
<td><strong>Fullscreen</strong></td>
<td>A toggle button for switching between the large main image and normal views. When you are in Fullscreen mode, use the Navigator toggle to work with cases.</td>
</tr>
<tr>
<td><strong>Delete</strong></td>
<td>Used for deleting objects from the image. If modal operations are enabled, this enables delete mode, otherwise, it deletes the selected object(s) from the image. If you delete an object in a metaphase, it will remain visible but will not be included in the karyotype or in any image enhancements.</td>
</tr>
<tr>
<td><strong>Select All</strong></td>
<td>Selects all of the objects in an image. Any deleted objects in a metaphase will not be included. If a modal operation is active, the operation will be applied to the selected objects then they will be deselected.</td>
</tr>
</tbody>
</table>
The Analysis Operations toolbox

In the Analysis screen an additional set of buttons is displayed next to the main work window referred to as the Analysis Operations toolbox. The buttons shown change depending on the type of image that is loaded in the main window. All of the icons are drawn together here for demonstration purposes only. Unused buttons can be hidden from View > Toolbars > Customize > Analysis Ops Toolbar.

Click on the icon for any function to be taken to its topic in the help files.

<table>
<thead>
<tr>
<th>Icon</th>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>🚬</td>
<td>Automatic mode</td>
<td>A toggle switch with Manual mode, when this icon is shown Split and Overlap cutting is automatically.</td>
</tr>
<tr>
<td>👤</td>
<td>Manual mode</td>
<td>A toggle switch with Automatic mode, when this icon is shown Split and Overlap cutting is manually by drawing lines to cut or define objects.</td>
</tr>
<tr>
<td>🐝</td>
<td>Split</td>
<td>Separates touching objects.</td>
</tr>
<tr>
<td><strong>Overlap</strong></td>
<td>Separates objects that are overlapped in a simple X configuration.</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td><strong>Draw Axes</strong></td>
<td>Separates objects in complex overlaps.</td>
<td></td>
</tr>
<tr>
<td><strong>Chromosome</strong></td>
<td>Identify selected objects as chromosomes or part of a cell. They will be included in karyotyping and image enhancements.</td>
<td></td>
</tr>
<tr>
<td><strong>Join</strong></td>
<td>Join two or more objects together.</td>
<td></td>
</tr>
<tr>
<td><strong>Colorize</strong></td>
<td>Applies a random color to individual objects to help identify groups of connected chromosomes.</td>
<td></td>
</tr>
<tr>
<td><strong>Contrast/Enhance</strong></td>
<td>Contrast - Lighten, darken or stretch the contrast of an image. Enhance - Applies an S-shaped stretch or spatial filter to sharpen or smooth edges. Also to invert the banding pattern.</td>
<td></td>
</tr>
<tr>
<td><strong>Restore</strong></td>
<td>Removes all alterations made to selected objects and restores them back to their original captured form.</td>
<td></td>
</tr>
<tr>
<td>Feature</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Classifier</td>
<td>Select the template used to classify chromosomes automatically.</td>
<td></td>
</tr>
<tr>
<td>Auto Classify</td>
<td>Classify the chromosomes automatically to create a karyotype.</td>
<td></td>
</tr>
<tr>
<td>Manually Classify</td>
<td>Manually classify chromosomes to create a karyotype.</td>
<td></td>
</tr>
<tr>
<td>Karyotype Layout</td>
<td>Layout options for karyotypes in the default karyotype template.</td>
<td></td>
</tr>
<tr>
<td>Trim</td>
<td>Trim debris off of a chromosome in a karyotype.</td>
<td></td>
</tr>
<tr>
<td>Train</td>
<td>Used to add or edit data for creating custom trainable classifiers</td>
<td></td>
</tr>
<tr>
<td>Annotation Toolbar</td>
<td>Add text, shapes, arrows or ideograms to an image.</td>
<td></td>
</tr>
<tr>
<td>Background</td>
<td>In Flex images, functions as a toggle button that switches the background of a composite image between white and black. In probe images it is used to select any background color.</td>
<td></td>
</tr>
<tr>
<td>Scale to Fit</td>
<td>Scales an image down to a size the fits completely in the image window.</td>
<td></td>
</tr>
<tr>
<td>Edit Template</td>
<td>In Genus systems only, this replaces the Layout dialog when arranging flexible karyotype templates.</td>
<td></td>
</tr>
<tr>
<td>Alignment and Spacing</td>
<td>Contains alignment and spacing tools for arranging objects in a flex image.</td>
<td></td>
</tr>
<tr>
<td>Button</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>CGH Profile</strong></td>
<td>Opens the tools used to adjust CGH profile image display and settings.</td>
<td></td>
</tr>
<tr>
<td><strong>CGH Ratio Image</strong></td>
<td>Allows you to toggle on/off the amplification highlights and ratio slider for CGH images. Displays the threshold sliders for changing the way the red and green colors are displayed on the CGH ratio image.</td>
<td></td>
</tr>
<tr>
<td><strong>Fluochrome Selection</strong></td>
<td>Toggles the Fluochrome Selection Panel on or off. Displays the fluorochrome selection dropdown to allow you to switch on/off individual fluorochromes overlays, or simply display the inverted counterstain (usually DAPI) image. This works for <strong>Probe, M-FISH, or RXFISH.</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Manual Count</strong></td>
<td>Displays a counter which counts the number of objects manually identified in an image.</td>
<td></td>
</tr>
<tr>
<td><strong>Karyotype Result</strong></td>
<td>Used with scanning systems, it automatically calculates the karyotype result for each cell in the image and adds the result to the cell's notes field.</td>
<td></td>
</tr>
<tr>
<td><strong>Jump to Image Pro® Plus</strong></td>
<td>A shortcut to launching Image Pro Plus from Media Cybernetics®.</td>
<td></td>
</tr>
<tr>
<td><strong>Probe Measurements</strong></td>
<td>Opens the dialog that contains various probe measurement tools.</td>
<td></td>
</tr>
<tr>
<td><strong>MultiCell</strong></td>
<td>Displays all chromosomes within a slide for the selected class.</td>
<td></td>
</tr>
<tr>
<td><strong>Profile</strong></td>
<td>Creates a graph of the banding pattern in an object.</td>
<td></td>
</tr>
<tr>
<td><strong>3D Object Projection</strong></td>
<td>A 3-dimensional graph of the banding pattern in an object.</td>
<td></td>
</tr>
<tr>
<td>Action</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Straighten</strong></td>
<td>Straighten the selected chromosomes.</td>
<td></td>
</tr>
</tbody>
</table>
| **Scaling and Reflection** | Change the size of an object, or flip an object.  
| ![Image](image1.png) | ![Image](image2.png) |
| **Fluomap**        | Displays the map of fluorochrome ratios and distribution for M-FISH images.  
| ![Image](image3.png) | ![Image](image4.png) |
| **Registration**   | Allows you to reposition probe signals on counterstain to correct registration errors from capture.  
| ![Image](image5.png) | ![Image](image6.png) |
| **MFISH Cleanup**  | Opens the M-FISH Cleanup window - works only with combinatorial M-FISH and not COB FISH.  
<p>| <img src="image7.png" alt="Image" /> |</p>
<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tidy Karyotype</td>
<td>Allows for single button automatic tidying of karyotype - it can be used to space and align chromosomes after manual scaling.</td>
</tr>
<tr>
<td>Composite Viewer</td>
<td>Opens the M-FISH composite view window.</td>
</tr>
<tr>
<td>Pseudo Class</td>
<td>Allows you to toggle on/off the M-FISH classification pseudo-color display.</td>
</tr>
<tr>
<td>Tint Class</td>
<td>Allows you to toggle on/off the new M-FISH tint classification display. This shows the pseudo-class colors combined with the DAPI banding.</td>
</tr>
</tbody>
</table>

**Cutting tools**

**Analysis Tools**

There are four sets of tools used in the Analysis screen. The **Analysis toolbar** at the top of the screen contains tools that are available to all types of images. This includes printing, selection tools and preferences for Analysis.

The **Analysis toolbox** next to the main window contains tools used to work with individual image types. The contents of the toolbox will change depending on the image loaded in the main window.

The **Species template toolbox** will replace the Analysis toolbox whenever a template is loaded for editing. The template toolbox is used to arrange and edit classes in karyotype templates.

The fourth toolbar, floating **Annotation toolbar**, is used to add text, arrows and ideograms to images.
CytoVision 3.6

**Customize** is used to change settings for the cutting tools. There are three different cutting tools used for separating chromosomes in Analysis. **Split** is used to separate objects that are close or barely touching but not overlapped. Use **Overlap** for simple overlaps and **Draw Axes** for complex groupings.

**Manual vs. Auto mode**

Manual | Automatic
--- | ---
Working in manual mode tells the system that you want to draw the line yourself. This applies to any function that involves the drawing of a line.

The button functions as a toggle switch. Push it once to go to manual mode, the button will have a hand on it. Click on it again to return to automatic, the picture will return to a lightning bolt.

**Related topics**
- How to draw lines
- Using the cutting tools

**How to separate objects**

There are three different cutting tools used for separating chromosomes in Analysis. **Split** is used to separate objects that are close or barely touching but not overlapped. Use **Overlap** for simple overlaps and **Draw Axes** for complex groupings.

Split and Overlap have the option to use these tools manually or have the cut made automatically. Toggle **Manual** on to cut groups yourself. When using Split or Overlap in automatic mode more than one group can be selected and separated together.

**Draw Axes** is always done manually so the Manual/Automatic toggle has no effect.

Here are examples of groups and the tool used to separate them:

- Split
- Overlap
- Draw axes

**Tip** - Use **Full Screen** or **Zoom** to enlarge the work window and make cuts easier to visualize and perform.

**Related topics**
- How to use **Split**
Split touching objects

Split separates objects that are touching but not overlapped. The Split button is on the Analysis Operations toolbar.

Cuts are made automatically by the system or manually drawn. The quickest way to work is in automatic mode. After a split is made colored outlines drawn around each object show the results. An incorrect split can be reversed immediately by clicking on the Undo button on the main toolbar.

**Automatic method**
If not using modal operations: Select the group(s) then click Split.
If using modal operations: If Split mode is not already active, depress the Split button. Then click on groups to split them.

After a split, the boundary of each object will be displayed in a different color. If the split is performed incorrectly, click on Undo and use the manual method.

**Manual method**
1. Select Manual mode. If not using modal operations, select the group.
   a. Click on the Split button, unless using modal operations and Split mode is already active.
2. If using modal operations, select the group.
3. Position the mouse away from the chromosomes and click the left mouse button once.
4. Draw through the area that needs separating.
5. Click once with the right mouse button to stop drawing the line.
6. Repeat steps 4-6 if multiple cuts need to be made.
7. Click the right button a second time to exit drawing mode and perform the split.

![Separating objects using Split](image)

**Shortcuts for working faster:**
- Program a hotkey for the Split button
- Use the right-click context menu to choose Split

Separate simple overlaps

Overlap is used to separate chromosomes that are laying at approximately right angles to one another. Groups with more than two chromosomes can be split using overlap but the
command only separates one crossed section at a time. Overlap is on the Analysis Operations toolbar.

Cuts are made automatically by the system or manually drawn. The quickest way to work is in automatic mode. After a cut is made colored outlines drawn around each object show the results. An incorrect cut can be reversed immediately by clicking on the Undo button on the main toolbar.

**Automatic method**
If not using modal operations: Select the group(s) then click Overlap.
If using modal operations: If Overlap mode is not already active, depress the Overlap button. Then click on groups to split them.

A different colored boundary will be drawn around each object. If the split is performed incorrectly, click on Undo and use **Draw Axes**.

**Manual method**
The intersection of overlapped chromosomes are marked with mouse clicks. The clicks must be very close to the corners. For this reason the Draw Axes command is usually easier to use.

1. Select Manual mode. If not using modal operations, select the group.
   a. Click on the Overlap button, unless using modal operations and Overlap mode is already active.
2. If using modal operations, select the group.
3. Position the mouse pointer at one of the junctions in the overlap.
4. Click the left mouse button once. An X will appear. (See picture below)
5. Repeat steps 4-5 at each of the other 3 junctions.
6. At the end of the fourth click the cut will be made and different colored boundaries will be drawn around each chromosome.

![](image)

*Using Manual Overlap*

**Shortcuts for working faster:**
- Program a **hotkey** for the Overlap button
- Use the right-click **context** menu to choose Overlap

**Separating complex overlaps**

![](image)
Draw Axes can be used to separate any group of chromosomes. It works by drawing a line down the center of the chromosome while a boundary defines the edges. It is always set to a manual cutting mode so the Auto/Manual toggle has no effect. Lines are drawn using the Freehand or Rubberband method. The preference for this is set in Customize.

The width of the boundary is set in Customize in the setting Chromosome Width Factor. The value is a percentage of the length to width ratio. If too much material is included on the edge of a chromosome reduce the width factor. If the edges of chromosomes are being trimmed off increase the value. A Chromosome Width Factor of 115 works well for most metaphase chromosomes.

**To use Draw Axes:**

1. If not using modal operations, select the group.
   a. Click on Draw Axes, unless using modal operations and Draw Axes mode is already active.
2. If using modal operations, select the group.
3. Position the mouse pointer at the end of one of the chromosomes, it does not matter which end you choose.
4. Click the left mouse button once.
5. Draw a line down the middle of the chromosome.
6. Click the right mouse button once at the end of the chromosome.
7. Move to another chromosome and repeat steps 4-7 until all of the chromosomes in the group have been drawn.
8. Click the right mouse button a second time to exit drawing mode and perform the split. A different colored boundary will be drawn around each chromosome in the group.

When **Draw axes colour highlight** is selected in the Customize Analysis window, the axes will be drawn either red, green or blue.

**Notes:**

- DO NOT draw on chromosomes that are not part of the group. This will cause all cuts to be ignored and the group will not be separated. Turn Boundaries on in Customize to show group members if needed.
Shortcuts for working faster:
- Program a hotkey for the Draw Axes button
- Use the right-click context menu to choose Draw Axes

Joining objects

The main uses for Join are to connect satellites to their chromosomes and to fix inaccurate cuts. Multiple objects can be joined together. Deleted objects and small must be included in the cell using the Chromosome button ( ) before they can be joined.

Join is on the Analysis Operations toolbar.

If not using modal operations: To join objects select them then click Join.

If using modal operations: If Join mode is not already active, depress the Join button. Then select the objects to be joined. The join will happen when the second object is selected. Alternatively, multiple objects can be joined at once using the Select All or Select Group buttons.

Joining satellites

Undelete or include objects

Chromosome serves three functions:
- Undelete objects- Objects that have been deleted in a cell are not included in a karyotype or in image enhancements.
- Include small objects in a karyotype - Objects seen as too small to be chromosomes are excluded from karyotypes.
- Mark a curved chromosome as a single item that does not need splitting - Extremely bent chromosomes may be seen as a group that needs splitting. The classifier will not measure a chromosome marked as a group to determine its class.

The Chromosome button is located on the Analysis Operations toolbar .

If not using modal operations: To use Chromosome select the objects then click on the Chromosome button.

If using modal operations: If Chromosome mode is not already active, depress the Chromosome button. Then perform the operation on objects by clicking on them. Alternatively, the operation can be performed on multiple objects at once using the Select All or Select Group buttons.

Coloring objects in a cell

Colorize applies a color overlay on top of all objects that are included in a cell. Deleted objects are not colored. The Colorize button is located on the Analysis Operations toolbar.
The color overlay helps to identify groups of chromosomes that need to be separated. It also makes any debris that is being counted as a chromosome more obvious.

**Automatic object count field**

The Count field keeps a running total of the number of objects in a cell. Deleted objects or those automatically excluded due to a small size are not included in the count. This provides a way to check if groups of chromosomes need splitting or if there is debris that is included in a karyotype.

Count is located at the bottom of the screen when in Large Navigator or Fullscreen layout, or under the main image window in classic layout. Counts are only displayed for metaphases, karyotypes and fuse images.

**Manually count objects**

Manual Count keeps a running total of marks placed in an image manually. It is located on the Analysis Operations toolbar when metaphase or probe images are loaded in the main window.

A small dialog is opened when Manual Count is pressed. Each time a click is made in the image a small square marks the position and the count is increased by one. Remove a mark by clicking on it a second time. Remove all marks by clicking on Reset; the count will return to 0.

Click on the OK button to close the dialog and save the count. Both the count and marks can be recalled by loading the image and clicking on Manual Count.
Counting manually

Options

Customizing Analysis

Customize sets the preferences for working in Analysis. Settings are saved automatically.

Note: It is no longer necessary to open the Customize Analysis dialog to change the Probe, CGH, M-FISH or RxFISH display appearance. New icons have been added to the main vertical toolbar that include Fluorochrome Selection, CGH Ratio Image, Pseudo Class and Tint Class.

The Customize button is located on the main toolbar.

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zoom</td>
<td>Set the enlargement factor for zooming with the middle mouse button. The options are 2X or 4X larger than the original.</td>
</tr>
<tr>
<td>Drawing Style</td>
<td>Sets the preferences the functions that use line drawing. The differences between Freehand and Rubberband are described in the topic Drawing techniques and styles.</td>
</tr>
<tr>
<td>Chromosome Width Factor (%)</td>
<td>Sets the width of the outlines drawn when using Draw Axes. The value is a percentage with larger values being appropriate for short, fat chromosomes and smaller values for long, thin ones.</td>
</tr>
<tr>
<td>Highlights section</td>
<td>Used to select which highlight are drawn on the image. The selections vary depending on the type of image loaded. A list of each highlight and its function is shown below.</td>
</tr>
<tr>
<td>Metaphase Annotation</td>
<td>Labels chromosomes in a metaphase with their class number. See the topic Labeling metaphases for details.</td>
</tr>
<tr>
<td>Back-up n pixels</td>
<td>Selects the backup step size for freehand drawing styles. Press the middle mouse button to back up the number of pixels selected.</td>
</tr>
<tr>
<td>Freehand hold to draw style</td>
<td>When the Freehand hold to draw style box is checked, holding down the left button as you draw and releasing the mouse when finished completes the separation. This applies to any operation</td>
</tr>
</tbody>
</table>
where freehand drawing has been selected.

<table>
<thead>
<tr>
<th>Draw axes colour highlight</th>
<th>The option to highlight each chromosomes in a different color as they are drawn is available with the draw axes tool.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preview enhancements</td>
<td>Lets you immediately see the effect contrast and sharpen operations will have as you adjust their parameters. See the topic Preview enhancements.</td>
</tr>
<tr>
<td>Use modal operations</td>
<td>Enables modal operations. When using modal operations, instead of first selecting one or more objects then clicking a toolbar button to perform that operation, you first select the operation you want then click on objects to apply that operation to them. See the topic under Analysis - Options - Modal operations.</td>
</tr>
<tr>
<td>Scale to fit karyogram</td>
<td>Scales karyogram in the viewing windows to fit the window. A red box will appear around the image to indicate that the image has been scaled to fit.</td>
</tr>
</tbody>
</table>

### Customize Analysis

<table>
<thead>
<tr>
<th>Zoom</th>
<th>x2</th>
<th>x4</th>
</tr>
</thead>
</table>

**Drawing Style**

- Freehand
- Rubberband

**Select Many**

- Manual Split
- Draw Axes
- Straighten
- Trim

- Back-up n pixels
- Freehand hold to draw style
- Draw axes colour highlight
- Preview enhancements
- Use modal operations
- Scale to fit karyogram

**Chromosome Width Factor (%)**

---

<table>
<thead>
<tr>
<th>Highlight</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boundary</td>
<td>Place a check in the box to display blue outlines or boundaries around every object in the metaphase to visualize what groups need splitting, and if cuts were performed correctly.</td>
</tr>
</tbody>
</table>
**Centromeres**

Place a check in the box to display the location of the centromeres. A is placed at the centromere position. Centromeres can be moved within a chromosome by dragging them to a new position.

**Object type**

Place a check in the box to display the color coded outlines around objects.
Red is used for deleted objects and yellow for those too small to be considered chromosomes. A green band is drawn around groups that need splitting.
With object type toggled on (checked box), the F (fuse) flag will be displayed in the metaphase on chromosomes that came from a fused field. With object type toggled off, the F flag disappears.

**Flex Probe Frames**

Turn this option off to remove the rectangular frame around objects in flex images.

---

**Choosing magnification for Zoom**

You can choose to enlarge the zoom area to twice (X2) or four times (X4) the original size. Zoom in on an area by clicking the middle mouse button.
1. Click on **Customize**.
2. Choose X2 or X4.
3. Click on **Done**.

[Customizing Analysis]

**Selecting drawing styles for Analysis tools**

Different styles may be applied to each tool. Rubberband requires you to tack the line down with a mouse click when you change directions, Freehand follows all mouse movements without the need for clicks.

**To select a style:**

1. Click on Customize button to open the Customize Analysis window.
2. Choose Rubberband or Freehand style for each of the tools listed - Select many, Manual Split, Draw Axes, Straighten and/or Trim
3. Check the optional boxes for advanced drawing features
   1. Back-up in pixels - select pixel size
   2. Freehand hold to draw style - applies to any operation where Freehand is selected.
   3. Draw axes colour highlight - when selected, the draw axes tool button will display different colors for each chromosome outlined.
4. Click on Done.

**Related topics**

[General information about drawing lines]

**Setting the width of the Draw Axes outline**

**Chromosome Width Factor** determines the width of the outline drawn around chromosome when using **Draw Axes**. Numbers >100 are used for fatter chromosomes, <100 for narrower ones. If you are losing the edges of your chromosomes when using **Draw Axes** raise this
number. If too much extra material is included from the overlapping chromosomes, lower the number. As a guideline a width factor of 115 works well for the average cell of 550 band level. Change Chromosome Width Factor from Customize.

Display blue object boundaries

Blue outlines or boundaries can be displayed around objects to visualize what groups need splitting, and if cuts were performed correctly.

Turn Boundaries on from Customize Analysis - check the Boundary box.

Displaying color coded outlines

The system evaluates all objects in a metaphase for size, shape and banding pattern. Turning Object Type on in Customize shows the results of this evaluation by drawing color coded outlines around objects. This color coding assists with the karyotyping process.

- **Green borders** show groups of touching or overlapping chromosomes. Unidentifiable markers are also bordered in green.
- **Red borders** are drawn around deleted objects. The system never deletes objects automatically, this must always be done by a user.
- **Yellow borders** are used for objects that are seen as too small to be included as objects of interest.
- Red and yellow objects will not be included in the karyotype or in any image enhancements.
Related topics

Display blue boundaries around objects
Colorize the objects
Customizing Analysis

Fused field chromosome flag

The fused field flag, $F$, attached to a chromosome in a metaphase is now visible after the metaphase has been karyotyped and saved.

It can be toggled on and off in the Customize Analysis dialog by checking or unchecking the Object type box.

Click on the customize button to open the Customize Analysis dialog.

1. Toggle Object type on and off.
2. With Object type toggled on (checked), the fuse flag, $F$, will be displayed in the metaphase (not the karyotype) on chromosomes that came from a fused field.
3. With Object type toggled off (unchecked), the fuse flag, $F$, will disappear.

Display and move centromeres

Turning Centromeres on in Customize displays a small red plus sign (+) over the centromere. The reason for showing centromeres is to check the accuracy of their position. Accurate positioning is important for training classifiers and CGH analysis. It may also be necessary if karyotype layouts are based on the centromeres rather than the bottom of chromosomes.

One other reason to move a centromere is if class labels in a metaphase are drawn over an area of interest. Labels are drawn relative to the centromere position so moving the centromere will move the label.
Position is corrected by dragging the plus sign to the right location. The new positions are saved when the cell is saved.

Customizing Analysis

Display chromosome axes

The Axis highlight in Customize displays a line drawn the center of the chromosome. The accuracy of the axis is important for CGH, RxFISH and M-FISH analysis. is turned on, lines are drawn where along the axis of each chromosome is. This option is only available for these image types.

Axes are corrected by re-drawing them manually using the Draw Axes in the Analysis Operations toolbar.

To correct the axis:

1. Select the chromosome in the karyotype or metaphase.
2. Click on the Draw Axes button.
3. Draw a line down the chromosome, try to remain as close to the center as possible.

Redrawing an axis

Customizing Analysis

Flex Probe Frames

Flex Probe Frames is a setting in Customize.

- This option controls how objects are copied into a flex image.
- When turned off only the object is copied, when checked on, the bounding rectangle for the object is copied as well.
The background color of the rectangle is determined by the type of object copied.

**Note** - This option can be applied to previously copied objects. To see the change refresh the window swapping the image into another window zooming in and out on the image.

### Labeling metaphases

Chromosomes in a metaphase can be labeled with their class number manually or automatically based on the classifier. This is useful for marking chromosomes of interest in an unkaryotyped metaphase. An example would be a prometaphase cell taken to demonstrate the absence or presence of small bands in a microdeletion case.

The Metaphase Annotation options are found in the Customize dialog of Analysis. Show Class Labels displays the class labels. When on, the class label is ‘temporarily’ displayed when the mouse enters the canvas. A chromosome can be ‘permanently’ labeled by clicking on it with the right mouse button. To change the class number click on a labeled chromosome and choose the correct label from the list.

All chromosomes in metaphase can be labeled automatically by clicking on the Add Labels button. All overlapping or touching chromosomes should be split before doing this or the overlapping groups will be labeled with an A. Add Labels can be pressed again to force a relabeling if some overlapping groups were missed. Labels can be corrected by right clicking on the chromosome and choosing the correct class.

The Clear Labels button removes all labels from the image.

Labels are initially drawn based on the position of the centromere. If a label is drawn over an area of interest, its position can be changed by dragging the label where required.

### Preview enhancements

When the 'Preview enhancements' option in the Customize Analysis dialog is checked, the effect of a sharpen or contrast operation will be shown on the image as soon as the settings are changed by moving the sliders. Once the sliders have been moved, interaction with the rest of the program is disabled until the Apply, Undo or Done buttons are clicked.
If you have set up macros involving Enhance and Contrast, you will need to reprogram the macros after updating to CytoVision version 3.5 or later since the contrast / enhance dialog window has changed. See section of Preview Enhancement Macro Reprogramming.

**Related Topics**

- Customizing Analysis
- Using Enhance
- Using the Enhance dialog box
- Contrast stretches
- Using the Contrast dialog box

**Reprogramming a preview enhancement macro**

These are just two examples for reprogramming two macros with preview enhancement turned off (unchecked) in the Custom Analysis window.

The macros used by your lab may differ.

**Programming a simple one step sharpen macro with Preview Enhancements Off:**

**Macro description:** Macro designed to sharpen selected chromosomes @ level 3

1. Select F12
   a. Macro and hotkey window opens.
   b. Tick on macro bubble and tick in associated text box and type macro name to be programmed - *Sharpen @3*.
   c. Select Record (a little tape display appears) and the Macro window closes.
2. Select desired chromosomes.
3. Click on the contrast tool button to open the Contrast/Enhance window.
4. Move the *Enhance* slider to 0 and then to level 3.
5. Select *Apply*.
6. Select *Done*.
7. Hit F11
   a. Recording stops
8. Try replaying the macro.

**Programming a contrast and enhance macro with Preview Enhancements Off:**

**Macro description:** General macro to normalize all chromosomes, then sharpen at level 5, remove fuzz and improve final contrast.

1. In the Analysis mode, close all windows.
2. Open a karyotype in the main window.
3. Select the F12 key.
   a. The Macro and hot key window opens
   b. Tick on macro bubble and tick in associated text box and type macro name to be programmed - Norm/Sharpen 5 KT con
   c. Select Record (a little tape display appears) and the Macro window closes.
4. Move mouse to Select All in the analysis toolbar and click on button.
   a. All chromosomes are selected with pink boxes around them

5. Click on the Contrast tool button in the operations toolbox to open the Contrast / Enhance window.
6. Click on Reset button.
7. Click on the Contrast Apply button (next to Reset).
8. Move the Enhance slider from 0 to 5.
9. Select the Enhance Apply button.
10. Click on the Object bubble under Slider Range.
11. Move the Contrast brighten slide to +1, move the white cutoff slider to 5%, move the black cutoff slider to 1%.
12. Tick the Contrast advanced box to open the advanced contrast tools.
13. Click the blue slider bar up a couple of clicks.
14. Click on the Contrast Apply button.
15. Tick the Contrast Advance box (check mark disappears) to close the advanced contrast tools.
16. Select Reset.

17. Move mouse to Deselect all in the Analysis toolbar and click on button.
18. Select the F11 key.
   a. Recording stops
19. Try replaying the macro.

**Modal Operations**

Analysis can be configured so that many of the tools become modal. This is done by checking the 'Use Modal Operations' box in the Customize Analysis dialog.

When using modal operations, instead of first selecting one or more objects then clicking a toolbar button to perform that operation, you first select the operation you want then click on objects to apply that operation to them.

When a operation mode is active, the toolbar button for that operation is depressed. Only one mode can be active at a time, so selecting a different mode pops-up the button for the previously selected mode. If a toolbar button cannot be depressed, either 'Use Modal Operations' has not been selected or there is no mode available for that operation. To deactivate all modes, pop-up the button for the current mode by clicking on it. All modes are deactivated automatically in some situations, such as when a different image is loaded or when an incompatible action is performed.

Some modes allow you to apply an operation to multiple objects simultaneously by using the select all and select many buttons, whereas for other modes these buttons are disabled.

Customizing Analysis

**Karyotype cells**

**Karyotyping cells**

The system is provided with a standard human karyotype template and classifiers for G-banded and inverted DAPI chromosomes.

The usual workflow for karyotyping is:
1. Separate touching or overlapped chromosomes.
2. Select an appropriate classifier.
3. Click on the Auto Karyotype button.
4. Rearrange chromosomes in the karyotype if necessary.
5. Save and print the karyotype.

Users with Genus flexible karyotyping systems have the ability to create additional karyotype templates. The basic workflow is still the same but there are additional tools provided for working with these templates. See the topics in the section Species templates for information related specifically to flexible karyotyping.

Additional classifiers can be created to enhance the accuracy of classification in your laboratory. These are trained by adding cells to a training file. See the topics in the About Classifiers section for details on training and using classifiers.

Karyotypes remain editable even after they have been archived and restored on the system. Chromosomes can be joined and re-cut, missing chromosomes can be added. Chromosomes that do not fit in the field of view for the main metaphase are added from fuse images. Fuse images are designated as part of the same cell during capture. Chromosomes from fuse images in the same cell can be copied into their metaphase.

The topics in this section cover classifying and arrangement of cells in a karyotype. The tools used to separate chromosomes and instructions for their use are covered in the Analysis/Tools topics.

You can change an existing karyotype to the extended format in the

**Adding fuse or stray chromosomes to a metaphase**

Only Fuse images from the same cell can be pasted into a metaphase.

**To copy chromosomes from a fuse:**
1. Load the metaphase and any fuse images you need.
2. Select the objects you want from the fuse image.
3. While holding the Ctrl button drag them into the metaphase.
4. Drag the fuse chromosomes to a place in the metaphase where they won't cover any areas of interest. Fuse chromosomes cannot be moved after they have been split in the metaphase or after the karyotype has been generated.

**Note** - All of the cutting tools are functional in fuse images. Use them to split off unwanted objects before copying needed stray chromosomes.

Capture fuse images

**To automatically classify a metaphase**

Once your chromosomes have been separated click on Auto Classify. The cell will be classified using the last template and classifier selected. The exception to this is CGH, M-FISH and RxFISH cases; their template is attached before capturing the cell. The karyotype will automatically be placed in the main work window, and the metaphase will be moved to a smaller window. You will also find Auto Classify in the right-click shortcut menu for metaphases.
You can return to the metaphase and classify the cell again if you want to overwrite the previous karyotype. This allows you to use a different classifier to identify the chromosomes. After the karyotype is created you can return to the metaphase to join or re-cut chromosomes.

When the karyotype is first generated a bottom row will be created to handle chromosomes that could not be placed in their appropriate area. Once all of the chromosomes have been moved up, the rows can be spread out to cover the entire page. Use **Auto** in the Karyotype Layout dialog for cells in the normal human template. Use the species template toolbox for cells in a species template.

**Correct and rearrange a karyotype**

When a karyotype is first created (or when a chromosome is first moved into a different class) the curve of chromosomes are automatically used to determine their best position in their class. Left curves are placed on the left and right curves to the right. If two chromosomes curve the same direction the most bent chromosome is placed on the outside.

You will probably need to make some corrections and adjustments once your karyotype has been created. Chromosomes can be rotated, inverted, moved or exchanged using the normal mouse button commands.

The tools used to align arrange the chromosomes are different depending on the template the chromosomes are in. If you used a species template to create the karyotype then the species template toolbox is used to rearrange the karyotype.

If you used the built-in normal human template then the Karyotype Layout dialog box is used. After a karyotype is correctly arranged you may want to **Trim** any unwanted debris from the chromosomes and **enhance the image** to maximize the banding.

**Related topics**

Learn to move, rotate, invert and exchange chromosomes

**Trim debris from karyotyped chromosomes**

1. If not using modal operations, select the chromosome you want to trim.
2. Click on the **Trim** button, unless using modal operations and Trim mode is already active.
3. If using modal operations, select the chromosome you want to trim.
4. **Draw** around the debris. The ends of the line will automatically join when you click the right button. Make sure the ends are clear of the chromosome.
5. Draw around all of the debris you want to remove from the selected object then click the right mouse button a second time.
6. If not using modal operations, you will need to de-select the object.
Trimming debris off of a chromosome

Tip - Image enhancements can affect the appearance of cytoplasm and cellular material around the chromosomes. If you enhance the image first you may save some time and avoid unnecessary trimming. See the topic image enhancement for details.

Related topics

Restoring a chromosome to its original appearance

Manually classify a metaphase

Manually Classify
You can create a karyotype by manually assigning chromosomes to their correct place. The cutting tools can be used while you are manually karyotyping.

To manually classify an image:

1. With a metaphase in the main window click on Manually Classify. A blank karyotype template will be loaded into an empty window.
2. Place the cursor over a chromosome and click on the right mouse button to open a list of numbers.
3. Move the cursor down the list until the correct number for your chromosome is highlighted and select it. The chromosome will be placed in the karyotype and a magenta outline is drawn around it in the metaphase.
4. When you are finished, select Done from the numbered list. Any chromosomes that have not been assigned to a number will be placed at the bottom of the karyotype.

To manually classify in a species template:

1. With a metaphase in the main window click on Classifier to select the right species template.
2. Click on Manual Classify. A blank karyotype template will be loaded into an empty window.
3. Place the cursor over a chromosome and click on the right mouse button to open a list of numbers.
4. Move the cursor down the list until the correct number for your chromosome is highlighted and select it. The chromosome will be placed in the karyotype and a magenta outline is drawn around it in the metaphase.
5. When you are finished, select Done from the numbered list. Any chromosomes that have not been assigned to a number will be placed at the bottom of the karyotype.
**Note** - If you swap the metaphase with the karyotype, or click on **Done**, you will not be able to return to the metaphase and classify chromosomes. You will still be able to perform any cutting functions in the metaphase. If this happens you can start again by clicking on **Manually Classify** or you can manually move the chromosomes to their correct class groups.

**Saving a karyotype**

Click on **Save** from the Navigator or **Edit** menu, then click on the karyotype.

You can also find **Save** in the right click menu for images. Position the cursor so that it is in the cell, but not on an object. Then click with the right mouse button and choose **Save**.

**Using the Karyotype Layout dialog box**
From the **Karyotype Layout** window you can spread the chromosomes out over the entire page, choose how to align the chromosomes and how you want to label the sex chromosomes.

**Default**
- Returns the chromosomes to the default layout.

**Unstack**
- Takes all of the chromosomes from the overflow row at the bottom and puts them into their classes.

**Auto**
- Lines up the chromosomes in a karyotype based on what options have been chosen and spaces them evenly within each class.

**Labels**
- Place a check in the boxes you want to use to label the sex chromosome groups.

**Q-arms**
- Aligns chromosomes along their bottom edges.

**Centromeres**
- Aligns chromosomes by their centromeres.

**Always Auto Layout**
- Dynamic scaling is used to automatically accommodate different types of metaphases (chromosome number and or length) to scale the chromosome sizes in the main window.

**Short**
- Sets the Height of the main view window to a default height. It can be applied to the currently selected karyotype or for new acquired karyotypes.

**Extended**
- Sets the height of the main view window to a longer window (maximum 800) to accommodate viewing longer chromosomes. It can be applied to the currently selected karyotype or for new acquired karyotypes. See below for details.

**Aligning chromosomes in a karyotype**

You can choose to align chromosomes by either their centromeres or their Q-arms. Click on Karyotype Layout. Select Centromeres or Q-arms. Click on Auto. Click on Done.

💡 **Tip** - Unless you correct the position of the centromeres manually, you will get a better aligned karyotype using the Q-arms option.
Extended karyotype display mode

<table>
<thead>
<tr>
<th>Height</th>
<th>This karyotype</th>
<th>New karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short</td>
<td>Extended</td>
</tr>
<tr>
<td></td>
<td>Extended</td>
<td>Short</td>
</tr>
</tbody>
</table>

If you have long chromosomes but do not want to use the Autoscale feature (which may be compressed in the conventional or Short layout), you can extend the main work window by selecting the Extended format for the Karyotype template. Select Short to return to the default size window.

- **The Extended option can be selected to apply to only the currently selected karyotype or for new karyotypes.**
- If you are in the Classic Layout, species templates with less than a height of 800 will not have scroll bars. The main window will appear elongated to accommodate viewing longer chromosomes.
- If you are in the Large Navigator or Fullscreen layout, the extended template will appear with scroll bars to allow access to the entire karyotype display.
- It will be necessary to design new Print templates or formats to accommodate these extended Karyotypes.

Intelligent karyotyping

- **Always Auto Layout**

In CytoVision, dynamic scaling will size the chromosomes automatically to fit the Karyotype template selected when the Always Auto Layout box is checked in the Karyotype Layout window.

This feature allows different types of metaphases (chromosome number and or length) to automatically be sized to fit in the main window or template. As chromosomes are moved the layout is extended both horizontally and vertically to accommodate any size chromosome.

**Automatically scale large chromosomes**

The Autoscale feature is automatically done as you edit the karyotype display when the Always Auto Layout box is checked - there is no need to use Autoscale.

To re-label marker chromosomes

You can only label chromosomes that are unknown and labeled with an A. If a chromosome has been classified and is labeled as something else, just drag it into one of the other classes. Then drag it back to the bottom of the karyotype.

**To re-label a marker:**

1. Click on the A label.
2. Enter a new label in the text field.
3. Press the Enter key.

Karyotype results
Karyotype Results

This feature is mainly used by scanning system users to automatically add the results of a karyotype to the slide report. To turn Karyotype Result on click on the button in the Analysis toolbox. To turn the feature off immediately just click the button a second time. To turn it off after the current cell unloads, place a check in Don't show this dialog again before closing the window.

If required, this feature will be setup as part of your installation such that when a karyotype is loaded in the main window the count, sex and any aneuploid abnormalities will be automatically calculated from the positions of the chromosomes. As you move chromosomes between classes in the template the result will update. The lower field can be used for adding any text such as a rearrangement. This text will be used subsequent cells and will remain in the field until you delete it.

If your system was not set up to update the automatic result whenever the karyotype changes, a button named Auto will appear in the dialog. Clicking this button will create an automatic result, however this will not be automatically updated.

About classifiers

Classifiers are tied to specific species templates and kept within their folders. The Genus will come with a human directory and four installed classifiers attached to a normal human template. All classifiers within a species will be available for all templates within that species.

You can create other human templates and customize the layout and labels of the classes. Separate classifiers will have to be generated to use with custom human templates. You can create your own classifiers to be used with the installed normal human template using Train.

Auto Classify will use the last template and classifier selected as long as you are karyotyping cells of the same species and stain type.

Related topics

- Create a new classifier
- Select a classifier
- Edit the data in a classifier
- Delete a classifier

Using the Train dialog box
Classification templates are data files that contain the algorithms used by the system to identify which class a chromosome belongs in when using the **Auto Classify** function. Using your own data to create classifiers can increase the accuracy of automatic classification. Use **Train** to create new custom classifiers, or edit the data of existing classifiers.

### Train Classifier

<table>
<thead>
<tr>
<th>Current training data</th>
<th>Displays the name of the classifier you are working with.</th>
</tr>
</thead>
<tbody>
<tr>
<td># Cells</td>
<td>Displays the number of cells used to generate the classifier data.</td>
</tr>
<tr>
<td>Class Count</td>
<td>Displays the number of chromosomes in each class.</td>
</tr>
<tr>
<td>Add</td>
<td>Press to add the data from the karyotype in the main window to the current classifier.</td>
</tr>
<tr>
<td>Edit</td>
<td>Opens a dialog which allows you to add, replace or remove cells from a classifier.</td>
</tr>
<tr>
<td>Generate</td>
<td>Generates and saves the classifier after any data has been changed. You must have at least 6 cells to generate a classifier for the first time.</td>
</tr>
<tr>
<td>Restore</td>
<td>Any changes made to a classifier will be removed. <strong>Restore</strong> must be used before <strong>Generate</strong> is pressed if you want to cancel any changes made.</td>
</tr>
<tr>
<td>Delete</td>
<td>Deletes the classifier displayed in the <strong>Current Training Data</strong> field.</td>
</tr>
<tr>
<td>Refresh</td>
<td>Immediately updates the list of classifiers across a network.</td>
</tr>
</tbody>
</table>

### To create a new classifier

1. Load a karyotype into the main window. It must be attached to the species you want to create a classifier for.
2. Check the position of the **centromeres** and correct them if necessary.
3. If any chromosomes are not of good quality morphology (e.g. they are very curled or overlapped) delete them before adding the cell. You do not need to save a cell before adding it.
4. Click on **Train**.
5. Enter the name you want to use for the classifier in the text box.
6. Click on **Add**.
7. Continue to add cells or click on **Done** to close the window. You can add more cells at any time.
8. You can either save the cell or discard the changes made. The classification data has already been added.
9. When at least 3 cells have been added to a classifier, click on **Generate** to create the classifier. Your new classifier is now ready for use in karyotyping. For the best results, use at least 12 cells in a classifier.

**Tips for creating a successful classifier:**
- Use cells with that are of similar length, width, and banding level.
- Delete any extremely bent or overlapped chromosomes before adding to the classifier.
- Make sure that the centromeres are drawn accurately.
- Make sure heterogamous cells are included.

**To select a classifier**

1. With a metaphase in the main window click on **Classifier**.
2. Choose one of the installed options, or to choose custom classifiers choose their name in the list on the right.

Any classifier other than the installed ones will have to be created using **Train**.

**Classifiers**

```
Classifiers
G-band short
G-band long
R-band
Inverted DAPI
```

**Classifier selection dialog**

**Editing cell data of an existing classifier**

Edit or delete classifiers from the Train dialog box.

**To replace cell data:**

1. Load the corrected karyotype in the main window.
2. Click on **Train**.
3. If the machine is on a network choose All machines in the drop down list.
4. Select the classifier name from the list.
5. Click on Add. If you are replacing an existing cell, choose Replace when asked.
6. Click on Generate to save the data.

To remove a cell:
1. Load a karyotype in the main window to enable the train button.
2. Click on Train.
3. If the machine is on a network choose All machines in the drop down list.
4. Select the classifier name from the list.
5. Click on Edit.
6. Select the cell to remove from the list.
7. Click on Remove.
8. Click on Done.
9. Click on Generate to save the changes.

Related topics
- To delete a classifier
- Using Train

To delete a classifier

1. Load a karyotype in the main window to enable the train button. It must be attached to the species the classifier is used for.
2. Click on Train.
3. If the machine is on a network choose All machines in the drop down list.
4. Select the classifier name from the list.
5. Click on Delete.
6. Click on Done

Related topics
- To delete cells from a classifier
- Using Train

Image appearance

Image appearance

You can change the appearance whole images of just of selected objects within an image. Enhance filters and contrast stretches will alter the appearance by changing the range of gray levels present in the images or objects.

Geometry tools are used to change the size, shape and reflection of objects. The original captured image can be restored at any time, even after the cell has been saved or archived.

Related topics
- Change the range of gray levels
- Change the geometry
- Restore the original image
**Restoring objects to their original appearance**

**Restore** will remove all contrast, size and reflection changes made to an object. It will also rejoin any material that was split from a chromosome by the **Trim** command. You can restore an object at any time, even after it has been saved or archived.

**To restore an image:**
If not using modal operations: Select the object(s) you want to restore, then click on **Restore**.

If using modal operations: If Restore mode is not already active, depress the **Restore** button. Then restore objects by clicking on them. Alternatively, multiple objects can be restored at once using the **Select All** or **Select Group** buttons.

**Tip** - Performing numerous enhancements and restores on a karyotype can result in the chromosomes looking different than they do in the metaphase. You can avoid this problem by applying a scale of 100% to the chromosomes after using **Restore**.

**Image enhancement**

**Image enhancement overview**

By using a combination of enhancements and contrast stretches in **Capture** and **Analysis**, you can maximize the ability to distinguish individual bands. These enhancements do not change the actual data in the image, they just change how the image is displayed in order to make detail more visible and distinct to the human eye.

The best final results are obtained when cells are captured with good quality contrasts and thresholds. If a cell is captured poorly, it will be more difficult to obtain quality results in **Analysis**.

The enhancement tools in **Analysis** can be used to improve a cell, or to help compensate for a poorly captured, or hard to capture cell. All image enhancements can be applied to single objects or to entire cells. The objects that are selected are the ones that are changed.

![Image of chromosome enhancements](image)

This is the same chromosome before any image enhancements and after.

The different contrast and enhance features can be used as many times as desired before the window is closed.

**Important:** Macros involving enhance and contrast will need to be reprogrammed!

**Preview Enhancements**
Operation is slightly different depending on whether you have checked Preview Enhancements in the Customize Analysis dialog.

WITH PREVIEW ENHANCEMENTS OFF:
1. Open the contrast / enhance window.
2. Move the Contrast slider bars, select Apply under the contrast window to see the effect of the changes.
3. Move the Enhance slider bar to the desired effect and select Apply to the right of the Sharpen slider to see the effect of the changes.
4. Click on Done to close the window.

WITH PREVIEW ENHANCEMENTS ON:
- Moving the slider bars in the contrast section of the window immediately shows the changes.
- Open the contrast / enhance window.
- Move the Contrast slider bars to see the immediate effect.
- To accept the changes, select Apply under the contrast window. This must be done before moving to the Enhance sliders.
- Move the Enhance sharpen slider bar to see the immediate effect.
- To accept the changes, select Apply to the right of the Sharpen slider.
- Click on Done to close the window.

Related topics
Learn about using Enhance filters
Learn about using Contrast
Learn more about what a contrast stretch does
Minimize the appearance of cytoplasm
Bring out some bands in black areas

Enhancement filters

Applying image filters

Enhance contains image filters used to sharpen or smooth edges. It also provides a way to invert an image.

The Sharpen command increases the contrast between pixels, so that detail is more visible.

Advanced options - check the advanced box in the Enhance section in the Contrast / Enhance window.
- The Smooth command reduces the contrast between pixels, so that detail is less visible.
- Invert produces a negative image.
- **Spatial filters** change the appearance of an image. Some commonly used filters are built-in. When one of these filters is selected in the list, the template or mask is displayed to the right. You can create your own filters by selecting one of the undefined templates. Fill in the values of the cells, enter a name for the filter above the template and click on **Save**.

**Related topics**

- Use the Enhance dialog box
- About using Contrast
- Use spatial filters
- Sharpen an image
- Smooth an image
- Invert an image

**About using the Enhance tools in the Contrast/Enhance window**

Click on the contrast/enhance icon in the operation toolbar to open the **Contrast/Enhance** window.
The controls are grouped as follows:

**Standard**

<table>
<thead>
<tr>
<th>Sharpen</th>
<th>Strength</th>
<th>Apply</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makes visualizing bands easier by increasing the difference between gray levels. See topic Sharpen an image. The <strong>Strength</strong> slider determines the level of sharpening - the higher the number the greater the effect on the image. The <strong>Apply</strong> button applies a sharpen operation of the selected strength.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Normalization**
### Object
Objects will be individually contrast stretched after each **Sharpen** or **Advanced** operation.

### Full
Objects will not be individually contrast stretched.

### Undo
The last action performed will be undone. Clicking the Apply button twice will only undo the last action.

---

**Advanced Enhance features** - Check the enhance advanced box to open additional controls

---

**Spatial filter list**
When you click on a filter in the list, the template for that filter will open to the right. The HiGauss filters sharpen images. Laplac filter enhance edges. Smooth filters soften or blur edges. Create your own filters by selecting one of the undefined templates and filling in the cells. See topic Spatial filters.

**Apply**
Applies the advanced spatial filter to the selected objects.

**Invert**
Inverts the banding pattern. See topic Invert banding pattern.

**Smooth**
Lessens the difference between the gray levels of bands smoothing out the appearance of objects. See topic Smooth an image.

**Spatial filter template**
Used to define a small image structure or mask used for filtering. The template is created from the Enhance filter dialog which opens when a filter is selected in the spatial filter list.

**Save**
Used to save your own spatial filters in the Enhance filter dialog.

**Delete**
Used to delete a spacial filter in the Enhance filter dialog.

---

**Sharpen an image**
CytoVision 3.6

Sharpen makes bands of selected objects easier to see by stretching the colors farther apart. The darkest grey in the chromosome is turned black and the lightest value is turned to white. All the grays in the lower or upper half of the spectrum are either darkened or lightened respectively.

To sharpen an image:
1. Select the objects you want to sharpen.
2. Open Enhance.
3. Select the sharpen Strength by moving the slider.
4. Click on the Apply button next to the slider.

![Original and Sharpened images]

Original  Sharpened at level 5

If 'Preview enhancements' is turned on, the Apply button will not be enabled until the slider has been adjusted. Once the slider has been adjusted, other operations in the Enhance dialog will be disabled until Apply, Undo or Done have been clicked.

💡 Tip - A level of 3-5 works with most G-banded cells. Probe and fluorescent images use 4-7.

Related topics
-about using Enhance

Smooth an image

Smooth evens out the appearance of selected objects by pushing the colors of the objects closer together. The smooth feature is available when the Enhance advanced box is checked in the Contrast/Enhance dialog box.

To smooth an image:
1. Select the chromosomes you want to smooth.
2. Open Enhance.
3. Click on Smooth.
Tip - Smooth is useful for making over trypsinized chromosomes or cells look less jagged.

Related topics

About using Enhance

To invert the banding pattern

Inverts the image producing a negative of the cell. This function can be used to see pale areas such as telomeres better by making them more visible against the background. The invert feature is available when the Enhance advanced box is checked in the Contrast/Enhance dialog box.

To invert an image:
1. Select the objects you want to invert.
2. Open Enhance.
3. Click on Invert.

About Spatial filters

Spatial Filters are filters that convolve one image with another. The filter images are typically small with respect to the target image and are defined by spatial masks or templates.

<table>
<thead>
<tr>
<th>-1</th>
<th>-1</th>
<th>-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>9</td>
<td>-1</td>
</tr>
<tr>
<td>-1</td>
<td>-1</td>
<td>-1</td>
</tr>
</tbody>
</table>

3x3 sharpen mask

The center cell of the mask is placed over the target pixel in the image. Then each cell of the mask is multiplied with its underlying pixel. The sum of these values becomes the new value of the target pixel. This is repeated for every pixel in the image.

These are the three types of spatial filters that are installed with the system. You can create your own filters in the Enhance dialog using the undefined masks.
To create a filter:
1. Choose the size of the kernel you want to build from the filter list. The Enhance filter dialog will open.
2. Enter the values in each cell.
3. Enter a name in the filter field above the template.
4. Click on Save.

To apply a spatial filter:
1. Select the objects to enhance.
2. Click on the Contrast / Enhance button.
3. Select the filter from the list.
4. Click on Apply.

To delete a filter:
1. Select the filter in the list.
2. Click on Delete.

Related topics
- HiGauss filters
- Laplacian filters
- Smooth filters

Contrast stretches

Contrast stretches

The contrast tools in Contrast/Enhance allow you to change the range of grey levels within the screen image. You can lighten or darken an image overall, or stretch the values present over a larger range. Contrast stretches can be applied to whole images or to selected objects only. Stretching the contrast of an image will make the individual bands easier to see.

There are two ways to calculate and stretch the gray levels in an image. Either by treating each object individually (object method), or treating the whole image as a single object (global method).
There are three installed contrast profiles or you can create and save your own.

Related topics
- Use the contrast dialog box
- To apply a contrast stretch
About using the Contrast tools in the Contrast/Enhance window

Click on the contrast/enhance icon in the operation toolbar to open the Contrast/Enhance window.

<table>
<thead>
<tr>
<th>Standard contrast controls</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brighten/Darken</strong></td>
<td>This slider is used to push the contrast curve up or down. When you push it up the number increases and the image will get lighter in color. When pushed down the number decreases and the image will get darker. The range is from -10 to +10 but normally only values from -3 to +3 are used.</td>
</tr>
<tr>
<td><strong>Black Cutoff</strong></td>
<td>Takes the selected section of dark grays and turns them to black. The contrast is then stretched across the remaining grays. If the black or top slider is set at 5 and the Object option is used, then the darkest 5% of grey levels will be changed to black. If Full is used and the slider is set to 5 then gray levels from 0-5 would be changed to black.</td>
</tr>
<tr>
<td><strong>White Cutoff</strong></td>
<td>Takes the selected section of light grays and turns them to white. The contrast is then stretched across the remaining grays. If the white or bottom slider is set at 5, and the Object option is used, then the lightest 5% of grey levels will be changed to white rather than just the top one. If Full is chosen and the slider is set at 250, then gray levels from 250-255 would be changed to white.</td>
</tr>
<tr>
<td><strong>Object</strong></td>
<td>Forces the cutoff sliders to act on a percentage value for the range of grays present in the image.</td>
</tr>
<tr>
<td><strong>Full</strong></td>
<td>Forces the cutoff sliders to act on absolute gray values. In Full mode the cutoff range is from 0-255.</td>
</tr>
<tr>
<td><strong>Global</strong></td>
<td>Place a check in the box if you want the contrast to be calculated based on the whole image rather than within individual objects. Use this feature if you want to maintain the relative intensities of objects.</td>
</tr>
<tr>
<td><strong>Apply</strong></td>
<td>Set the sliders then press Apply to change the contrast of selected objects.</td>
</tr>
<tr>
<td><strong>Advanced</strong></td>
<td>A toggle switch that hides or displays the more advanced contrasting tools.</td>
</tr>
<tr>
<td><strong>Reset</strong></td>
<td>Resets all sliders to 0.</td>
</tr>
</tbody>
</table>

**Advanced Contrast features - Check the contrast advanced box to open additional controls:**
**Blue sliders**
Limit the upper and lower limits of grey when a stretch is performed. The left slider prevents areas from turning to white, the right keeps areas from becoming black. Move the left blue slider down if you want to prevent pale areas from blending into a white background. For fluorescence, move the right blue slider up to prevent dark areas from blending into a black background.

**Pink sliders**
Limit the range that curves are applied to during a contrast stretch. Areas outside of the pink slider are stretched linearly. For example, you could lighten most of a cell by moving the **Brighten** slider up and the white **Cutoff** in, but then move the left pink slider down to prevent the pale ends from being lost.

**Save**
Used to save custom profiles, click on **Save** then type the name you want to save it under in an empty text field. Place a dot in the box next to the name and click on **OK**.

**Load**
Press to load a saved profile. Select the profile you want to apply from the list and click **OK**. The profile will load and you are ready to click on **Apply**.

**Low**
Applies the built-in, low contrast profile to the selected objects. There is no need to hit **Apply**.

**Medium**
Applies the built-in, medium contrast profile to the selected objects. There is no need to hit **Apply**.

**High**
Applies the built-in, high contrast profile to the selected objects. There is no need to hit **Apply**.

**Show graph**
Place a check in the box if you want to see the profile applied by the preset contrast stretched **Low**, **Medium** and **High**.

**Using the Brighten/Darken slider**

This slider is used to push the contrast curve up or down. When you push it up the number increases and the image will get lighter. When pushed down the number decreases and the image will get darker. The range is from -10 to +10 but normally only values from -3 to +3 are used.
Using the Cutoff % slider

Uses the value selected as the darkest or lightest band and calculates the contrast stretch based on that value. Any grays outside the slider will be changed to white or black. Two different ranges of gray levels can be used. Choose the **Object** option to base the contrast stretch on the levels of gray that are present in the current image. Choose the **Full** option to specify actual values of the gray scale from 0-255.

**White Cutoff**

Takes the selected section of light grays and turns them to white. The contrast is then stretched across the remaining grays. If the white or bottom slider is set at 5, and the **Object** option is used, then the lightest 5% of gray levels will be changed to white rather than just the top one. If **Full** is chosen and the slider is set at 250, then gray levels from 250-255 would be changed to white.

**Black Cutoff**

Takes the selected section of dark grays and turns them to black. The contrast is then stretched across the remaining grays. If the black or top slider is set at 5 and the **Object** option is used, then the darkest 5% of gray levels will be changed to black. If **Full** is used and the slider is set to 5 then gray levels from 0-5 would be changed to black.

**Tip** - Use the white slider at values 5-10 (**Object** scale) to lessen the appearance of cytoplasm in G-banded cells, and the black slider at 5-10 (**Object** scale) to make the edges of fluorescent images sharper.

Using the advanced contrast sliders

**Blue sliders**

Determine the darkest and lightest colors in an image. Move the left blue slider down to determine the lightest color of gray in the image. Move the right blue slider up to set the darkest color of gray as something other than black. These sliders are useful if the edges of chromosomes are hard to see against the background.

**Pink sliders**

Limit the output range of contrast stretches. Any contrast curve will only be applied between the pink sliders. Areas outside of the slider will be stretched linearly.

**Tips:**

- If you find that brighten at +1 makes most of your cell look good, but your pale ends are hard to see, move the left pink slider down a bit. This will prevent your pale ends from being lightened.
- If you have black areas with no details, move the right blue slider up a small amount.

Methods of contrast, Global vs. object

There are two ways to calculate and perform a contrast stretch. One is to calculate the range of grays within each object and maximize it. Because each object has its darkest band
changed to black and the lightest to white, the relative intensity or darkness between objects will change.

The other method is to take the range of grays within the whole cell and base the contrast stretch on that. This is what the Global contrast option in Contrast does. When using Global contrast, the relative intensities of objects within the cell are kept the same.

Regular or non-Global contrast will maximize banding and is best for G-banded chromosomes. Use Global when the relative intensities of objects are important, such as in probe images, C-bands or if you have small marker chromosomes.

Related topics

- What is a contrast stretch

To apply a contrast stretch

Objects that have been deleted will not be included in the contrast calculations.

1. Select the objects you want to change and click on Contrast.
2. Move the sliders to the position you want and click on Apply.
3. If you do not like the results, click Undo to remove the last change made.

To see the effect a contrast stretch will have as soon as the sliders are moved, turn on Preview enhancements in the Customize dialog.

About custom or built-in contrast settings

There are two ways to avoid having to set the options in Contrast for every cell. One way is to use the three built-in profiles. The other is to save your settings as custom profiles. These profiles can then be loaded and applied to images.

To use a built-in contrast profiles:

Three profiles have been built into the program that will produce images with low, medium or high contrast.

1. Select the objects for contrast and click on Contrast.
2. Place a check in Advanced to display the buttons.
3. Click on Low, Medium or High depending on the contrast you want. You do not need to press Apply.

If you want to see the graph each profile is using place a check in the Show graph box.

To save a custom contrast profile:

You can create and save up to 5 custom contrast profiles. If the buttons Load and Save are not visible, place a check in the Advanced box.

1. Open Contrast and set the slider positions.
2. Click on Save.
3. Select the option box for one of the rows.
4. Type the name in the text field.
5. Click on OK.
Custom profile dialog

To load a saved profile:
1. Open the contrast window.
2. Click on Load.
3. Select the profile you want by clicking in the option box.
4. Click on OK.

Contrast Stretch explained

Stretching the contrast means that you are taking an image with a set number of grey levels or colors, and spreading them out over all possible values of grey, from black to white. There are 256 grey levels possible in the image, black is 0 and white is 255. If no contrast curve is applied then a grey level coming in at 50 will be sent out at 50. No lightening or darkening will occur.

If you capture a cell with a contrast of 70%, you only have 70% of these grey values in your cell. For this example we will say they go from about 30-210. That would mean the darkest grey you have has a value of 30, and the closest value to white you have is 210. If you perform a contrast stretch with a straight line, your grey level of 30 is changed to 0, and 210 is changed to 255 with the other values being spread out evenly across the range.
You can use contrast to bring out bands in chromosomes that would otherwise be too dark or too light. If the curve is bent up then in the cell, the darkest band will be changed to black, the lightest to white and every grey in between will be lightened.

Likewise if a cell is too light, the curve can be bent down. All of the mid-range grays will be darkened.

Size, shape and reflection

There are three ways to change the geometry of chromosomes or other objects. You can straighten them, scale their size or change their reflection.

Related topics

Learn about the Scaling and Reflection dialog box
Straighten chromosomes

Straightening chromosomes

Automatic method
If not using modal operations: Select the chromosome(s) you want to straighten and click on **Straighten**.
If using modal operations: If Straighten mode is not already active, depress the Straighten button. Then click on chromosomes to straighten them. Multiple chromosomes can be straightened at once using the Select All or Select Group buttons.

![Straightening an object](image)

**Manual method**

1. Turn on **Manual**. If not using modal operations, select the chromosome you want to straighten.
2. Click on **Straighten**, unless using modal operations and Straighten mode is already active.
3. If using modal operations, select the chromosome you want to straighten.
4. **Draw** a line down the center of the chromosome.
5. When you finish your line the chromosome will be straightened.

**Related topics**

ℹ️ Restore an object back to its original appearance

**Change the size and reflection of objects**

![Scaling and Reflection](image)

You can vary the size, or scale of an object from between 25-200% of its original size. The numbered boxes will change the size by the amount on the box. Use the slider to scale by other amounts. If the slider is already set at the size you want, just click on the arrow to apply the scale factor. Scale factors are applied to the original size of the object, not the current size.

The reflection tools are **Mirror** and **Flip**. **Mirror** flips the chromosome so the mirror image is displayed. **Flip** will also invert the chromosome as well.
Scaling and Reflection

<table>
<thead>
<tr>
<th>Mirror</th>
<th>Flips the object horizontally to its mirror image.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flip</td>
<td>Displays an inverted mirror image of the original object.</td>
</tr>
<tr>
<td>Preset boxes</td>
<td>Select the objects you want to re-size and click on one of the boxes. The number of the box is the scale factor that will be applied. The size will change as soon as the box is clicked.</td>
</tr>
<tr>
<td>Sliding scale</td>
<td>Select the objects you want to change the size of. Slide the pointer to the scale factor you want to apply. The size will change as the slider is moved.</td>
</tr>
</tbody>
</table>

**To change the size of an object:**

1. Select the object.
2. Push the numbered button that is labeled with the desired scale percentage. Or use the slider for infinite adjustment between 25-200%.

**To reflect an object:**

1. Select the objects.
2. Click on Mirror or Flip.

![Mirror]

Original

Mirror image

![Flip]
Composites, profiles and annotation

Flexible images allow you to combine chromosomes or objects from more than one case and from different image types. The alignment and spacing tools will make it easier for you to position the objects in the flexible image.

The MultiCell viewer provides a way to quickly compare all of the chromosomes from a single class. These panels of chromosomes can then be copied into flex or other images.

The profile tools provide you with graphic representations of the gray levels present in an object.

Annotation is used to add text and ideograms to images. You can also draw arrows or geometric shapes to highlight specific areas.

Related topics

- Create a flexible image
- Learn about the alignment and spacing dialog
- Create a 3D object projection
- Create a standard profile
- Use MultiCell to compare chromosomes
- Annotate an image with text or graphics

Working with Flex images

Flexible images allow you to combine chromosomes from different cases, cells and image types. Copy the objects you want to include into an empty window - see topic under Working with images - Edit commands.

When you save the image it will appear as a Flex icon in the Navigator - see topic under CytoVision Basics - About the Navigator. Flex images are saved with the source images in the same cell.

To combine objects from more than one case, start with the cell you want to save the Flex into. Select the objects you want and copy them to an empty window. Then add the objects from the other case or cell and save the flex image. It will automatically save into the first cell.
Note: If your system uses a digital camera, depending upon the objective used to acquire an image, some captures will be scaled to fit the display window. Objects from a scaled to fit window and a non-scaled window will be scaled to fit in the flex window and a view will have a red border.

Things you can change or add to flex images:

- Toggle the background color between white and black
- Remove the background boxes from objects
- Add ideograms, text, arrows or shapes
- Align or space objects equally
- Export the image in TIFF format

Background color selection

If a flex image is loaded, the color tool toggles the background color of between black and white.

If a probe image is loaded, change the background color using the standard Windows color palette.

Alignment and spacing tools
**Alignment and Spacing**

These tools make it easy to arrange objects in a Flex or composite image. The top row of buttons contains tools for lining the objects up by their edges or centers. The bottom row contains tools for spacing objects evenly across a row or column.

**To align objects:**

1. Position one object to use as an anchor. The anchor is the object that you want to use as the basis for placement of the other objects.
2. Select the objects you want to align.
3. Click on an alignment option.
4. Click on the anchor object.

**To space objects evenly:**

1. Position one object to use as an anchor. The anchor is the object that you want to use as the basis for placement of the other objects.
2. Select the objects you want to space.
3. Use the slider to select a distance to use between the objects.
4. Click on horizontal or vertical spacing.
5. Click on the anchor object.

**MultiCell viewer**

MultiCell provides an easy way to visualize all examples of a chromosome class contained in a slide. Load a karyotype in the main window and press the MultiCell tool button. Select the class of interest in the combo selector or use the vertical arrows to scroll through the chromosome numbers. Only chromosomes from karyotyped cells are included. Each chromosome will be labeled with the name of the cell it belongs to.

The Case, slide and cell information is displayed above each chromosome pair.

Chromosomes from probe, CGH, RxFISH and M-FISH cells are drawn using the display options selected in Customize. You can change these options while the chromosomes are loaded in the MultiCell viewer. Copy the panel of chromosomes into a window if you want to print the display.
| Chromosomes | When Ideogram is enabled, choose this option to load chromosomes instead of ideograms. |
| Show Ideograms | Place a check in this box to display the Ideogram associated to the chromosome selected. |
| Autoscale | Three options to select from - Autoscale off, Autoscale by cell. Select **Autoscale** to scale all the chromosomes at the same height as the ideogram. Select **Autoscale off** to display each chromosome at the size used in the karyotype. Select **Autoscale by cell** to, for each karyotype, scale the leftmost chromosome in the class group to the height of the ideogram and the remaining chromosomes in the class group are scaled by the same factor. |
| All Slides | Place a check in the box to include chromosomes from all cells acquired within the selected case (all slides), not just cells associated to a single slide. |
| Ideogram combo box | Use the combo box to select the ideogram set - combo box displays saved ideograms |
| Pseudo Ideograms | Used to display the pseudo color ideograms for CGH or RxFISH cases. This option is disabled when other cell types are loaded. |
| Clear lines | Click on the Clear lines button to clear all horizontal band lines in the MultiCell display. See Band level lines below for more details. |
| Vertical Arrows | Use the vertical arrows to scroll through and display the chromosomes in the karyotype. Alternately, use the combo box selector arrow to select the chromosome number you want to display. |
**Horizontal Arrows**

Use the horizontal arrows to scroll across the chromosome display in the MultiCell window.

**Update**

Click on the update button to refresh the MultiCell display to reflect any changes made to the metaphase/karyotype.

---

**Chromosome display in the MultiCell window**

- To display a specific chromosome in the MultiCell display window, click on the vertical arrows or select the chromosome number from the drop-down box.
- All case, slide and cell text is at the bottom of the MultiCell window. The associated cell number or name is displayed at the bottom of the left chromosome with it’s paired chromosome directly to the right. The chromosome pairs from different cells are slightly separated. Use the horizontal arrow keys to scroll through the chromosomes in the MultiCell window.
- The selected chromosomes MultiCell display reflects the karyotype display. For example, chromosomes are at the same spot and orientation as seen in the karyotype. The two homologous chromosomes from one cell need to be close together, with a little extra space between the ones from a next cell.

**Display ideogram**

- Click on the Show Ideogram box to display the ideogram associated to the selected chromosome. Select the ideogram from the drop down combo box.

**Open selected chromosome karyotype**

- Double click on a chromosome in the MultiCell window to open the associated karyotype and metaphase images in the main windows.

**Autoscale**

- Place a check in this box to display all of the chromosomes at the same height.
- Select **Autoscale off** to display the chromosomes, without scaling, at the same size used in the karyotype.
Autoscale off

Select **Autoscale** to scale all chromosomes to the same height as the ideogram.

Autoscale

Select **Autoscale by cell** to, for each karyotype, scale the leftmost chromosome in the class group to the height of the ideogram and the remaining chromosomes in the class group are scaled by the same factor (to maintain relative size within the class group) - this helps identify abnormalities (which may be longer or shorter).
**Analysis**

![MultiCell interface](image)

**Autoscale by cell**

**Band level lines**

- Horizontal reference lines can be added to the MultiCell window by clicking, with the *middle mouse button*, on any band in the window.
- Middle mouse click on the line again to delete the line or, alternately, click on the Clear Lines button to clear all added lines in the window.

![MultiCell interface with band lines](image)

**Additional features**

- Double-click on a chromosome in the MultiCell window to open the related karyotype & metaphase for the associated cell in the main window.
- The karyotype and metaphase are fully editable with MultiCell open. The operations from the operations toolbar (such as reflect, enhance, restore, etc.) are active and working for the karyotype in the main working window.
- The MultiCell window will stay open until the operator closes the window, even when a different metaphase (that has not been karyotyped) is loaded.
- Print functions are active for the karyotype window even when the MultiCell window is open.
**Tip:** With the MultiCell window open, you can open the Gallery display to look for all metaphase associated with the case. The Gallery will display all cases in the Navigator.

**Suggested workflow for reviewing cases using MultiCell:**
1. Reviewer opens a case that needs to be reviewed.
2. Load karyotype in the main working window.
3. Click on the MultiCell button to open the MultiCell window.
4. MultiCell window will display the chromosomes of the selected class from all karyotypes in the case.
5. Start reviewing chromosomes by using vertical arrows to scroll through the chromosome classes.
6. **To see a specific karyotype associated to a chromosome, double-click on the chromosome of choice.**
7. The karyotype and related metaphase open in the main windows.
8. Reviewer performs operations to improve/modify karyotype. This includes (but is not limited to):
   1. Realignment of the chromosomes
   2. Chromosome separation
   3. Annotating the karyotype
9. Save changes to karyotype
10. Print the karyotype
11. Close the case

**Banding profiles**

Draws a graph of the banding, or grey levels in an object. Press **Profile** to open the dialog. Select the object of interest and press **Set**. Copy banding profiles by holding the **Ctrl** key down while dragging the profile into another window.

**Set** Generates the profile of the selected object. The profile is drawn from left to right where left is the top of the object. The profile is based on a line drawn...
<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flip</td>
<td>Flips the profile horizontally so it is drawn from the other end.</td>
</tr>
<tr>
<td>Arrows</td>
<td>Use the arrows to scroll across profiles. The center arrow will return the profile to its original position.</td>
</tr>
<tr>
<td>Blue alignment bar</td>
<td>Draws a line through all three profile windows. Place a check in the box to display the line. Move the bar on the slider to change its position.</td>
</tr>
<tr>
<td>Zoom</td>
<td>Zoom in to enlarge the profile to make it easier to see detailed areas. Zoom out to return to normal view.</td>
</tr>
<tr>
<td>Manual line</td>
<td>Place a check in the box if you want to draw the axis manually. The cutting tool will appear when Set is hit. Draw the axis as you would normally. The profile is based only on the area under the drawing line.</td>
</tr>
<tr>
<td>Mean over width</td>
<td>Place a check in the box to calculate the mean intensity across the width of the chromosome before drawing the profile. If Manual line is turned on, make sure you draw your line down the center of the chromosome.</td>
</tr>
</tbody>
</table>

**3D Object Projection**

3D Object Projection draws a 3-dimensional image of the banding or grey levels in an object. Copy 3D projections by holding the Ctrl key down while dragging the profile into another window. Use the options to determine how your profile will look.

**To draw a 3D projection:**

1. Select an object and press **3D Object Projection**.
2. Choose your display options and press **Set**.
### 3D Object Projection

- **Contours**: Adds black stripes to help identify curves.
- **Elevation**: Raises or lowers the Y-scaling of the profile so you can choose the level of vertical detail seen.
- **Gradation**: Adds gradated stripes to help identify height.
- **Orientation**: Moves the profile around so you can see all sides of it.
- **Show base**: Colors the base black in order to make the bottom edge clearer.
- **Set**: Generates the profile of the selected object.
- **XY scaling**: Lets you display the profile at actual, half or double its size.

### Adding text, shapes or arrows

### Overview of Annotation toolbar

![Annotation Toolbar]
You can add text, arrows, ideograms, shapes and symbols to any image from the **Annotate** window. When you click on the Annotate tool button, a separate toolbar opens. This floating toolbar can be moved to any part of the screen by dragging it with the mouse. Close it when you are finished by clicking on the X in the top right corner.

The line thickness of the arrows and shapes can be edited in the **Color select window**. The shape also can be set to a solid or outline fill type. See Topic - *To select line thickness or fill type* for details.

While you are adding elements to an image the selection tools will be disabled. Click on the pick tool to enable object selection in an image again.

**Ideogram** opens a separate program used to edit and copy ideograms. This program will remain open until you press its Done button or until you exit CytoVision.

---

**Related topics**

- To add text to an image
- Copying ideograms to image
- Creating abnormal ideograms
- To draw a line
- To draw a polygon
- To draw a rectangle
- To draw a circle
- To draw arrows
- Male and female symbols
- To select a color for annotations
- To select line thickness or fill type

**Using the pick tool**

Use the **Pick** tool to close any dialogs and stop using a specific annotation tool. You must click on the pick tool before any objects can be selected in the image.

**To add text to an image**
1. Click on the **Text** tool button to open the text style dialog.

2. Choose the font you want to use from the drop down list. To use bold or italic font styles, push the **Bold** button and/or the **Italic** button(s). Deselect both buttons to use regular font style.

3. Choose the text size you want to use from the drop down list.

4. There are two options for placing text on the image:
   1. To save text in the drop down selection, click in the text box and type the desired text. Text becomes part of the drop down selection. Click in the image with the left mouse button where you want to place the text and the text is copied onto the screen in the selected size and font. The text box drop down selection will display all text entered in this manner. To use the same text again, or select a different saved text, use the drop down selection.

   **Note:** The image window does not scroll. For larger font sizes, click on the far left hand corner of the window to start your text entry box.

   2. To enter a one time only text, select the text size and font and left click in the image window where you want to enter the text. The cursor will appear inside brackets at the selected point. Type the text and press **enter** on the keyboard to place the text in the image window. This text is not displayed in the text drop down selection.

CytoVision 3.6 and later versions allows visualization of the entire text that is typed, even in huge text mode, in normal and full-screen displays.

**Moving text annotations**

The text can be moved in the window by a click and drag action. Left mouse click on the text (a blue box will appear around the text) and drag it to the desired location in the image.

**To delete text in the image window**

Left mouse click on the desired text (multiple texts can be selected), a colored box will appear around all text selected. Click on the delete button in the analysis toolbar. All selected text boxes will be deleted from the image view.
Deleting the text list
To delete the text list, click on the drop down arrow, select the desired text then click on the Delete button.

Copying ideograms to images

Band labels can be attached to the ideogram before you copy it. Click on the band you want labeled with the right mouse button. Repeat for any additional bands you want labeled. Remove a label by clicking on it a second time.

Ideograms can be inverted or scaled like other objects. You can also use the cutting tools to create abnormal ideograms.

To learn more about creating ideogram sets see the topic About the Ideogram Editor.

To copy an ideogram:
1. Click on Ideogram.
2. Click on Load and select the ideogram set you want to use.
3. Use the drop-down list in Ideogram Class to select the class number.
4. Select the size using the Percent Zoom combo list or by entering any value between 1-1000.
5. Click in the image with the mouse to place an ideogram in an image.
Creating abnormal ideograms

Creating an ideogram that matches an abnormal chromosome can be a useful tool for illustrating the abnormality. Use the Split command in Manual mode to cut through the ideogram at the breakpoints of the rearrangement. These pieces can then be removed, inverted or joined into other ideograms. When the pieces are rearranged correctly, select them and use Join to paste them together. This will also smooth the edges of the joined areas.

Note - Ideograms are normally vector objects which allows you to resize and rotate them without loss of quality. Cutting them will convert them to bitmaps that will become blurry if rotated or resized. Perform any scaling on the ideograms before cutting them to avoid this problem. You can also use the Ideogram Editor to create marker or abnormal chromosomes by adding, deleting or changing bands.

Tip - You will not be able to save abnormal ideograms created in the editor, so for commonly seen abnormalities create an image library case. Then you can paste the abnormal ideograms into a flex image within that case. That way, when you need one, just use copy and paste the ideogram from the saved flex into your new image.

Creating an inv(16) ideogram

To draw an annotation line

1. Click on Polyline.
2. Click with the left mouse button to start drawing the line.
3. To change direction tack the line down with another click of the left button.
4. Click the right mouse button to finish the line.

Lines can be moved by dragging them with the mouse.

To draw a polygon
1. Click on **Polygon**.
2. Click in the image at the point where you want to place your polygon.
3. Draw your sides by tacking the line down with the left mouse button.
4. Click the right mouse button to stop drawing. Any open faces of the polygon will be connected.

![Polygon](image)

**To draw a rectangle**

1. Click on **Rectangle**.
2. Click in the image once with the left mouse button to start the rectangle.
3. Move the cursor until you have the rectangle sized the way you want, then click with the right mouse button to stop.

![Rectangle](image)

**To draw a circle**

1. Click on **Circle**.
2. Click in the image with the left mouse button at the point where you want the center of the circle to be.
3. Move the cursor away from the staring point until the circle is the size you want.
4. Click the right mouse button to stop.
To draw arrows

1. Click on Arrow.
2. Click in the image at the point you want the arrow tail to start.
3. While holding the mouse button down, drag the pointer until your arrow ends where you want it.

Once an arrow is in the image it can be moved, rotated or scaled just like any other object.

Male and female symbols

1. Click on the symbol you want to add.
2. Click in the image to add.
Symbols can be scaled like any other object.
To select a color for annotations

1. Click on the **Color Select** tool button.
2. Choose one of eight colors to draw objects and text in. The current color is shown at the bottom of the selection panel.

3. Click on the **Pick tool** to close the color palette.

To select line thickness or fill type

The line thickness of the arrows and shapes can be edited in the Color select window. Click on the Color Select button in the Annotation Toolbar to open the Drawing Colour window.
To set Line Thickness

1. Click on the **Line Thickness** drop down arrow and select the desired line thickness (range from 1 to 10).
2. Select the arrow or shape tool in the Annotation tool bar.
3. Click with the left mouse button to start drawing the line or shape.
4. Click the right mouse button to finish the line or shape.
5. Click on the Pick tool in the annotation toolbar to close the Drawing Colour window.

**Note:** Line thickness does not work for the circle or male/female symbols.

To set Fill Type

1. Select the **Fill Type** from the drop down selection - Solid fill or Outline.
2. Select the shape tool (polyline, closed polyline, rectangle or ellipse tools).
3. Click with the left mouse button to start drawing the shape.
4. Click the right mouse button to finish the shape.
5. Click on the Pick tool in the annotation toolbar to close the Drawing Colour window.

**Note:** Fill type does not work for the circle or male/female symbols.
Examples of line thickness, outline and solid fill with Black color selected.

About the Ideogram Editor

The Ideogram Editor is used to load, create and edit sets of ideograms. The editor can be run from within Genus from the Annotation toolbar or as a stand alone program through Start -> Programs->Applied Imaging->Ideogram Editor.

The ideograms are drawn in the window to the left. Use the Percent Zoom combo box to select the size they are drawn in or type in a value directly. The controls are separated into three groups. Ideogram Sets is used to load, select and create whole sets of ideograms. Once a set is loaded, use Ideogram Class to create or edit classes within the set. Edit Bands is used to build the ideograms for each set.

Note - CytoVision users cannot save any changes made to ideograms. This feature is only enabled on Genus systems.
Load an ideogram set
Select, create or edit an ideogram class
Select, create or edit bands
Paste an ideogram into an image window

Load, create and edit ideogram sets

Your system will come with some normal human ideograms of different banding resolution. To load a set click on Load and select the set you want to use.

Once loaded, select classes from the Ideogram Class section. The Save and New features are only available on the Applied Imaging Genus systems.

Properties allows you to change the width of the ideograms.

Load, create and edit ideogram sets
Ideogram sets are organized by species. Each species can have several different sets so you can base them on different banding resolution and stain. To load a set click on Load and navigate to the species folder and set you want to use. To create a new set click on New. The on-screen instructions will walk you through the creation of the set. Once created add classes to the set from the Ideogram Class section.

If you have made any changes to a set click on Save to save the changes. You will be given the choice between overwriting the current set or using the file to create a new set. If you choose Save as new set a wizard will open allowing you to select the species folder and filename.

**Properties** allows you to change the width of the ideograms and add any notes about resolution and type of banding.

Ideogram sets are attached to species templates using **Select Ideograms** in the species template toolbox.

**Note** - Ideogram sets and bands can be deleted and can be time consuming to recreate. After creating an ideogram set archive the data to keep as a backup.

**Select, create and edit ideogram classes**
Select the class you want to work with using the Current combo list.

Although you cannot save ideogram sets in CytoVision systems, you can create abnormal or marker chromosomes to copy into an image window. To add a class to an ideogram set click on **New** in the **Ideogram Class** section. The Ideogram dialog will appear where you assign sex or autosome status and the class number or sex designation. After adding a class, use the controls in the **Edit Bands** section to build the ideogram.

💡 **Tip** - Create a case with a flex image used to store common abnormal ideograms. That way you can copy them from the flex image rather than having to recreate them every time.

**Select, create and edit ideogram classes**

To add a class to an ideogram set click on **New** in the **Ideogram Class** section. The Ideogram dialog will appear where you assign sex or autosome status and the class label or sex designation. Ideograms are drawn in their corresponding class boxes using the class label as identification; an ideogram labeled A1 will be drawn in a class box labeled A1.

After adding a class, use the controls in the **Edit Bands** section to build the ideogram.
Select, create and edit ideogram bands

As a CytoVision user you will not be able to save any changes made to ideograms but you can still use the editor’s tools to create marker or abnormal chromosomes. Alternatively you can cut the ideograms after you paste them into the image.

When you create a new class it automatically creates the first band for you. To add bands to the ideogram click on New to open the New Band dialog.

Select between band or centromere and whether you want to add the band above or below the selected band.
Click on Properties to set the band type, size, color and label. The length is an arbitrary number only so you can use any units you want. A common method is to print a set of ideograms drawn in another image format and measure the bands in millimeters. At 100% zoom you can fit 785 total units in the window.

The Type options are used to designate the way a band is drawn. Heterochromatic bands, stalks and satellites will be drawn with hash patterns and the color cannot be selected. You cannot change a band’s designation to centromere once it has been created. Instead you must insert a centromere using the New Band dialog.

To set the color of a band use the Black and White buttons or use Select to pick a color from the Windows color palette.

The Name field is used to enter the band label. The Show option when turned on, will display the label of the selected band in the window. Use this option if you always want a band labeled. To label bands only when needed, click on them with the right mouse button.

The Select arrows are used to scroll through the bands as you work. Any changes made will be saved automatically when you move to another band. Undo will remove all changes made to a band from the time it was selected. You must use Undo before moving to another band if you do not want to save the changes.

💡 Tip - You will not be able to save abnormal ideograms created in the editor, so for commonly seen abnormalities create an image library case. Then you can paste the abnormal ideograms into a flex image within that case. That way, when you need one, just use copy and paste the ideogram from the saved flex into your new image.

**Select, create and edit ideogram bands**
When you create a new class it automatically creates the first band for you. To add bands to the ideogram click on **New** to open the **New Band** dialog.

Select between **Band** or **Centromere** and whether you want to add the band above or below the selected band.

Select a band by clicking on it with the left mouse button. Then click on **Properties** to set the band type, size, color and label. The length is an arbitrary number so you can use any units you want. A common method is to print a set of ideograms drawn in another image format and measure the bands in millimeters. At 100% zoom you can fit 785 total units in the window.

The **Type** options are used to designate the way a band is drawn. Heterochromatic bands, stalks and satellites will be drawn with hash patterns and the color cannot be selected. You cannot change a band’s designation to centromere once it has been created. Instead you must insert a centromere using the **New Band** dialog.

To set the color of a band use the **Black** and **White** buttons or use **Select** to pick a color from the Windows color palette.

The **Name** field is used to enter the band label. Display the label of the selected band in the window by putting a check in **Show** or by clicking on a band with the right mouse button.

The **Select** arrows are used to scroll through the bands as you work. Any changes made will be saved.
automatically when you move to another band.

**Undo** will remove all changes made to a band from the time it was selected. You must use **Undo** before moving to another band if you do not want to save the changes.

**Copying ideograms to images**

Band labels can be attached to the ideogram before you copy it. Click on the band you want labeled with the right mouse button. Repeat for any additional bands you want labeled. Remove a label by clicking on it a second time.

Ideograms can be inverted or scaled like other objects. You can also use the cutting tools to create abnormal ideograms.

To learn more about creating ideogram sets see the topic About the Ideogram Editor.

**To copy an ideogram:**

1. Click on **Ideogram**.
2. Click on **Load** and select the ideogram set you want to use.
3. Use the drop-down list in **Ideogram Class** to select the class number.
4. Select the size using the **Percent Zoom** combo list or by entering any value between 1-1000.
5. Click in the image with the mouse to place an ideogram in an image.
Exporting images

You can use the Export tool to save an image in Bitmap (.bmp), Graphics Interchange Format (.gif), Jpeg (.jpg), Portable Network Graphics (.png), or Tagged Image File Format (.tif) format or you can copy it to the Windows NT® clipboard and paste it into another program. If you subscribe to CytoNet, you can convert an image to .TIFF format while sending it to another CytoNet user.

Export dialog

Related topics
- Export an image to File
- Export images using CytoNet

To export to file
1. Load the image into the main window of Analysis.
2. Select Export from the right mouse click menu.
   
   **Note:** For raw or un-thresholded images you will need to position the cursor at the far right edge of the image.
3. Select to File.
4. Move the slider bar to select the export size of the image. The percentage and the pixel size (aspect ratio) will adjust accordingly. Alternately, select the Image size in pixels arrows.
5. Click on OK.
6. The browser opens to select the location, name and type (format) of the file to save the image to.
7. Click Save. File is saved to selected location with the selected name and image file type.

To export using CytoNet

1. Load the image into the main window of **Analysis**.
2. Select **Export** from the right mouse click menu.
3. Choose **to CytoNet**.
4. Choose a location (Public Archive, Service: USA/Canada or Service: Rest of World).
5. Enter a description of the image.
6. Click on **OK**.

**Image Pro® Plus shortcut**

Image Pro Plus shortcut

Image Pro Plus is a powerful image analysis package from Media Cybernetics®. You must purchase and install this program separately. When installed, pressing this button will launch the Image Pro Plus program.
Note: This shortcut only works with Image Pro Plus version 4.0. Please check with your local sales representative or Applied Imaging before purchasing any other version.
Species templates

Species templates overview

The Genus system allows you to define your own templates and classifiers for creating karyotypes. These templates are saved and stored in an Explorer type directory structure. Multiple templates can be saved for any species of animal or plant. Templates are not limited to a cell type and can be used to karyotype brightfield, probe, RxFISH, M-FISH, and CGH metaphases.

The display option for an extended karyotype display mode allows for a species template with a height of 800 microns or less. See topic under Analysis - Karyotype cells - Using the Karyotype dialog (Extended Karyotype) for more details.

Related topics

?- Create a new template
?- Edit template layouts
?- Using species templates during capture
?- Use a template to classify a cell

Ideograms and Species Templates

The Genus system will come installed with normal human templates and ideograms. For any other species you can either create your own sets or share sets with other labs that have a Genus using the archive and restore tools. You will also find a list of templates, classifiers and ideogram sets on your software disks that can easily be restored onto your system. As Applied Imaging gathers more of this data from our customers this list should grow.

Ideogram sets are created using a separate program called the Ideogram Editor. When an ideogram set is attached to a species template these ideograms will be used in CGH profile images, CGH expanded profiles and MultiCell displays. They also make setting class properties easier by automatically setting chromosome lengths and centromeric indices.

Ideogram sets should be attached to templates before the templates are used to capture and karyotype cells. If you want to change the template associated with cells after they are captured or karyotyped you will have to reprocess the cells (for color banded cells) or re-classify cells (for brightfield or probe karyotypes).

Related topics

- Learn about the Ideogram Editor
- Learn about Species templates

Creating and editing templates

Creating and editing species templates

The standard installed layout in Genus is for normal human cells. To karyotype a different species, or to create a layout to accommodate abnormal human lines, a species template will need to be created. New species templates are created using the Species wizard.
Once they are created, they are stored in a file accessed through the **Species Browser**. These are the default versions of the templates that are attached to metaphases before karyotyping. These default templates can be loaded, edited and deleted from the **Species Browser**. A CGH version of each template will automatically be created.

Once a cell is karyotyped using a species template, you can change the layout of that cell without affecting the default version. Just load the karyotype and you can open the editing tools to make any changes.

**Related topics**

- Learn about the Species Browser
- Create a new template
- Edit the default layout
- Re-label classes

**Create a new species template**

The wizard walks you through the creating a new species template. Templates are organized within species group directories. Choose an existing group or create a new one and follow the on-screen instructions.

The maximum number of classes allowed is 200 and the maximum size or length of the template is 1200 pixels. When you finish the template will open allowing you to specify the properties and placement of each class.

**Note:** If you create a species template with a height of less than 800 pixels, use the Extended Height (from the Karyotype Layout window) option and are in the Classic layout, the entire window is displayed without scroll bars.
**New Species Template Wizard**

This wizard is to help create a species template file. Step 1 is to select an existing species group or add a new species group.

- Select an existing species group from the list below
- Type in a name for a new species group

---

**Species Wizard**

**Changing class properties**

Properties you can change include whether it is an autosome or sex chromosome and the centromere position and index. Change the properties by clicking on each class box with the right mouse button and choosing Properties from the menu.

Choose between **Autosome** or **Sex** chromosome. You can only identify one class for each sex chromosome. The **Centromere Type** is important for CGH, RxFISH and M-FISH cells.

💡 **Tip** - If you have an ideogram set for this species then you do not need to set the length and Centromeric Index. These settings will be changed automatically when the ideogram set is attached.

⚠️ **Note** - Ideograms are placed in class boxes according to their label and sex designation not the class number, so if your species has classes such as A1, you will want to label the classes first.
Chromosome Class Properties dialog

To label classes

1. Right click on the class in the custom template and select **Rename**.
2. Enter a new label and press the **Enter** key.

To change a label after a template has been saved, first load the template using the **Species Browser**.

Attach an ideogram set to a template

Select Ideograms

You will only be able to use ideograms that have been saved within the same species folder as the karyotype template. The ideograms will be placed in the class boxes according to their label and sex designation. Labels and designations can be changed after a template is attached so if you forget to mark a class correctly you can make any changes without having to reattach the ideogram set.

To remove a set from a template click on **Remove existing set from template** in the **Ideogram Set** dialog.

To attach a set:
1. Click on Select Ideograms to open the Ideogram Set dialog.
2. Select the set you want to attach; only sets from the same species folder will be listed.
3. Click on Use Selected.
4. Click on OK.

Tip - For CGH cases that have already been captured, if you want to change the ideogram set you must attach the new set to the template using the Species Browser, then reprocess the cells to apply the change.

Resizing and moving classes

Resize boxes by dragging their edges with the mouse. If an ideogram set is loaded all classes will scale uniformly. Move a box by dragging the box to a new location when you see the 4 pointed cursor. There are alignment, spacing and sizing tools in the species toolbox that will help arrange the classes.

Tip - If you attach an ideogram set to the template the class heights will be adjusted automatically. When you change the height of one class all the remaining class will be changed as well.

About the Species Template Browser

Species Template Browser

The browser provides a way to view and select the species templates you have created. Each species heading will contain all templates that have been created for that species. Each template is created as a standard and CGH pair automatically. You cannot delete either half of the template pair. The CGH templates will only be displayed if Show CGH templates is turned on.

In Capture the Species Browser button will be in the Fluorochrome Selection panel for modes of capture that require you to select a template before capturing the cells. In Analysis the Species Browser button will be in the Analysis toolbar at the top of the screen. Select a name from the list and that template will be used to create the karyotype.

Edit saved templates in Analysis by double-clicking on its name in the list. The template will be loaded into the main window where you can change the properties and arrangement of the group boxes.
Delete a template by selecting its name and clicking on the Delete button.
To edit the layout of a default template

1. Open the Species Browser.
2. Double click on the name of the layout you want to edit.
3. Make any changes and click on Save.

Any changes made will automatically be written to the CGH pair. The only changes that can be made from the CGH template are class width and position.

Learn more about the layout tools
Change the layout of a karyotyped cell

About CGH templates

The CGH template is used for the CGH profile image. A CGH template is automatically generated whenever a species template is created. Most properties are linked between the two templates and cannot be changed from the CGH template. Only the width and position of each class can be different in the CGH template. If you want to edit any other properties, you must do so from the standard template.

CGH templates are listed separately in the species browser and are marked with a CGH profile icon. The ideograms used in the profiles of custom CGH templates are created using the Ideogram Editor. When you attach an ideogram set to a template the generic gray ideogram placeholders will be replaced with your ideograms.

Example of CGH template classes
Related topics

- About the Ideogram Editor
- To attach an ideogram set to a template

Copying species templates

You can use an existing template to create a new template for a species.
1. Load a karyotype that is attached to the template you want to copy in the main window of Analysis.
2. Click on Edit Template.
3. Click on Save as New Default.
4. Enter a different name in the text field.
5. Click on OK.

Sharing data

Sharing data such as species templates and ideogram sets with other laboratories can be done easily through archiving. The Archive Species Data section allows you to save and restore this data with the necessary directory structure intact. The data can be archived to a common media such as floppy or ZIP disks and mailed. Or you can zip the archived data into a single file using a program such as WinZIP® which can then be sent via the Internet using email.

To zip species data for sending:
- The uppermost directory is called arctemplates. You will find it on the disk selected when archiving.
- Zip this directory using the settings for including subfolders and paths. Refer to the program's instructions for how to do this.
- Extract the arctemplates directory to a temporary folder or blank removable disk and you can restore the data using the Archive and Restore utility.
- Using this method will safely copy the data while checking that no files are overwritten.

Notes about sharing individual files

The recommended method for sharing data is through the archive facilities, however if you are a knowledgeable Windows user and plan on sending individual files the following notes are important:
- The file structure and permissions are very important for species data
- Ideogram sets must be in directories with the same species name as the one they were originally created in
- Species templates must be copied as karyotype and CGH template pairs. i.e. both the .karytemplate and .cgtemplate files must be copied
- There will be no safety checks for duplicate file names

Disclaimer

Manually copying files and creating directories on your system can lead to significant problems and/or loss of data. Applied Imaging does not recommend or condone anyone manually creating directories or copying files on their system.

Using the species template toolbox

Using the species template toolbox
Whenever you are in template editing mode the species template toolbox will replace the normal Analysis tools. Use these tools to arrange the class boxes, assign certain properties to groups and to save the templates.

### Alignment, spacing and size

Use these tools to align and space the class boxes across the rows, or use the specific alignment options. Position one class to use as the anchor, or point of reference. Select the classes to align and click on one of the options. Click on the anchor when prompted with the cursor and the classes will be moved.

<table>
<thead>
<tr>
<th>Tool</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Auto Arrange</strong></td>
<td>Will line classes up along the bottom edges and space the boxes in each row evenly.</td>
</tr>
<tr>
<td><strong>Align Left</strong></td>
<td>Align classes vertically along their left edges.</td>
</tr>
<tr>
<td><strong>Align Bottom</strong></td>
<td>Align classes horizontally along their bottom edges.</td>
</tr>
<tr>
<td><strong>Space Vertically</strong></td>
<td>Space classes vertically.</td>
</tr>
<tr>
<td><strong>Space Horizontally</strong></td>
<td>Space classes horizontally.</td>
</tr>
<tr>
<td><strong>Make Same Height</strong></td>
<td>Makes all selected classes the same height as the anchor class.</td>
</tr>
<tr>
<td><strong>Make Same Width</strong></td>
<td>Makes all selected classes the same width as the anchor class.</td>
</tr>
</tbody>
</table>

### Karyotype layout

Some tools are only used if the template is attached to a karyotype.

<table>
<thead>
<tr>
<th>Tool</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unstack Chromosomes</strong></td>
<td>Forces extras chromosomes in the bottom row to be placed in the class they are labeled with.</td>
</tr>
<tr>
<td><strong>Align Qters</strong></td>
<td>Aligns chromosomes by their Qters.</td>
</tr>
<tr>
<td><strong>Align Centromeres</strong></td>
<td>Aligns chromosomes by centromere position.</td>
</tr>
<tr>
<td><strong>Autoexpand</strong></td>
<td>Automatically scales chromosomes down in size if they are too big to fit in the template.</td>
</tr>
<tr>
<td><strong>Auto Adjust</strong></td>
<td>Will space chromosomes out evenly within classes.</td>
</tr>
</tbody>
</table>

### General properties and saving

<table>
<thead>
<tr>
<th>Tool</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Template Properties</strong></td>
<td>Provides an easy way to review the properties of a template and all of its classes.</td>
</tr>
<tr>
<td><strong>Template Height</strong></td>
<td>Change the height, or length of the template page. Drag the bar in the dialog that opens to select the new height.</td>
</tr>
<tr>
<td><strong>Ideogram Set</strong></td>
<td>Used to attach a custom set of ideograms to a template.</td>
</tr>
<tr>
<td><strong>Save</strong></td>
<td>Saves the changes for the current karyotype.</td>
</tr>
</tbody>
</table>
Species templates

View Template Properties

Template Properties

The Template Properties dialog provides an easy way to review the properties for each class in a template. It is organized in a file tree structure. If you want to change any of the properties you must do so from the class box itself. You must have a template loaded in the main window to access this dialog.

Change template height
Change the default height or length of a template by loading it using the Species Browser. Change a single instance of a template attached to a karyotype by using Edit Template when the karyotype is loaded. The height is set in pixels.

**To change template height:**

1. Click on **Template height**.
2. Move the horizontal slider in the pop-up dialog to set a new page length for the template.
3. Click on **Apply**.
4. Click on **OK**.

**Note:** If you create a species template with a height of less than 800 pixels, use the Extended Height (from the Karyotype Layout window) option and are in the Classic layout, the entire window is displayed without scroll bars.

![Template Screen Height](image)

Using the species template toolbox

**Karyotype using species templates**

**Karyotype using species templates**

For CGH, RxFISH and M-FISH cells the template must be attached before the cells are captured. For brightfield, fluorescent and standard FISH cells the template is attached as part of the karyotyping process.

A classifier must be generated for each species before cells can be automatically classified. Until a classifier is generated the chromosomes will have to be identified manually or automatically using size as the criteria. The exception to this is M-FISH, which uses the map of fluorochrome labeling for classification. Once you have three karyotypes for a species you can generate a classifier. Classifiers will be available to all templates within a single species folder.

The Extended karyotype display mode is useful with species templates where longer chromosomes may be seen. See topic under Analysis - Karyotype cells - Using the Karyotype Layout dialog (Extended Karyotype).
Related topics

- Attach a template to CGH, RxFISH or M-FISH cells
- Attach a template to brightfield, fluorescent or standard FISH
- Manually classify a cell
- Generate a classifier for a new species

Select a template and classifier

Classifier

Classifier opens the Template and Classifier dialog. Each species folder contains templates created for that species. List the classifiers by selecting a template. Only those classifiers created using cells for a specific template are listed.

If you have trained more than one classifier for a species their name will appear in the right column. Just select the specific classifier before you click OK.

To select a template and classifier:

1. Load a metaphase into the main work window and click on Classifier.
2. Select the species and classifier you want to attach to the metaphase.
3. Click on OK.
4. Click on Auto Classify and the cell will be automatically classified into the format of the chosen species.

Note - If it is a new species, or you have not trained a classifier you will be asked if you want to manually classify the chromosomes or have them placed in classes based on size. When you have three karyotypes then you can create a classifier for the species group.
**Related topics**

Attach a template to CGH, RxFISH or M-FISH templates

**To automatically classify a metaphase**

**Auto Classify**

Once your chromosomes have been separated click on **Auto Classify**. The cell will be classified using the last template and classifier selected. The exception to this is CGH, M-FISH and RxFISH cases; their template is attached before capturing the cell. The karyotype will automatically be placed in the main work window, and the metaphase will be moved to a smaller window. You will also find **Auto Classify** in the right-click shortcut menu for metaphases.

You can return to the metaphase and classify the cell again if you want to overwrite the previous karyotype. This allows to you use a different classifier to identify the chromosomes. After the karyotype is created you can return to the metaphase to join or re-cut chromosomes.

When the karyotype is first generated a bottom row will be created to handle chromosomes that could not be placed in their appropriate area. Once all of the chromosomes have been moved up, the rows can be spread out to cover the entire page. Use **Auto** in the Karyotype Layout dialog for cells in the normal human template. Use the species template toolbox for cells in a species template.

**Manually classify a metaphase**

**Manually Classify**

You can create a karyotype by manually assigning chromosomes to their correct place. The cutting tools can be used while you are manually karyotyping.
To manually classify an image:

1. With a metaphase in the main window click on **Manually Classify**. A blank karyotype template will be loaded into an empty window.
2. Place the cursor over a chromosome and click on the right mouse button to open a list of numbers.
3. Move the cursor down the list until the correct number for your chromosome is highlighted and select it. The chromosome will be placed in the karyotype and a magenta outline is drawn around it in the metaphase.
4. When you are finished, select **Done** from the numbered list. Any chromosomes that have not been assigned to a number will be placed at the bottom of the karyotype.

To manually classify in a species template:

1. With a metaphase in the main window click on **Classifier** to select the right species template.
2. Click on **Manual Classify**. A blank karyotype template will be loaded into an empty window.
3. Place the cursor over a chromosome and click on the right mouse button to open a list of numbers.
4. Move the cursor down the list until the correct number for your chromosome is highlighted and select it. The chromosome will be placed in the karyotype and a magenta outline is drawn around it in the metaphase.
5. When you are finished, select **Done** from the numbered list. Any chromosomes that have not been assigned to a number will be placed at the bottom of the karyotype.

⚠️ Note - If you swap the metaphase with the karyotype, or click on **Done**, you will not be able to return to the metaphase and classify chromosomes. You will still be able to perform any cutting functions in the metaphase. If this happens you can start again by clicking on **Manually Classify** or you can manually move the chromosomes to their correct class groups.
Edit the layout of species template karyotypes

Load a karyotype that is attached to a template in the main window of Analysis and the Edit Template tool will become active. Press it to open the template toolbox and begin editing. You can rearrange the chromosomes, re-label the classes or alter the properties of a class. You will not be able to change the height of class boxes while the karyotype is loaded.

Unstack Chromosomes to force the chromosomes in the bottom row to be placed in a class box.
**Template height** opens a small dialog with a small bar used to change the length or height of the template. Just drag the bar up or down. The height of the template is shown in pixels to the right.

Use **Align Qters** to line the chromosomes up by their terminal ends. Aligning by terminal ends usually results in a better layout as centromere positions can be incorrect.

**Align Centromeres** will line them up by their centromeres. Centromeres can be moved if the chromosomes are poorly aligned using this option.

**Auto Adjust** spaces chromosomes out evenly within each class.

**Save** saves the changes for the current cell only.

**Save as New Default** gives you the option to apply any of the changes to the default version of the template or to save the template under a new name.

**Autoscale** reduces the size of large chromosomes to fit in the template.

**Attach an ideogram set to a template**

**Select Ideograms**

You will only be able to use ideograms that have been saved within the same species folder as the karyotype template. The ideograms will be placed in the class boxes according to their label and sex designation. Labels and designations can be changed after a template is attached so if you forget to mark a class correctly you can make any changes without having to reattach the ideogram set.

To remove a set from a template click on **Remove existing set from template** in the **Ideogram Set** dialog.

**To attach a set:**
1. Click on Select Ideograms to open the Ideogram Set dialog.
2. Select the set you want to attach; only sets from the same species folder will be listed.
3. Click on Use Selected.
4. Click on OK.

Tip - For CGH cases that have already been captured, if you want to change the ideogram set you must attach the new set to the template using the Species Browser, then reprocess the cells to apply the change.

Karyotyping with a template

Karyotype using species templates

For CGH, RxFISH and M-FISH cells the template must be attached before the cells are captured. For brightfield, fluorescent and standard FISH cells the template is attached as part of the karyotyping process.

A classifier must be generated for each species before cells can be automatically classified. Until a classifier is generated the chromosomes will have to be identified manually or automatically using size as the criteria. The exception to this is M-FISH, which uses the map of fluorochrome labeling for classification. Once you have three karyotypes for a species you can generate a classifier. Classifiers will be available to all templates within a single species folder.

The Extended karyotype display mode is useful with species templates where longer chromosomes may be seen. See topic under Analysis - Karyotype cells - Using the Karyotype Layout dialog (Extended Karyotype).

Related topics

Attach a template to CGH, RxFISH or M-FISH cells
Attach a template to brightfield, fluorescent or standard FISH
Manually classify a cell
Generate a classifier for a new species

Select a template and classifier

Classifier

Classifier opens the Template and Classifier dialog. Each species folder contains templates created for that species. List the classifiers by selecting a template. Only those classifiers created using cells for a specific template are listed.

If you have trained more than one classifier for a species their name will appear in the right column. Just select the specific classifier before you click OK.

To select a template and classifier:

1. Load a metaphase into the main work window and click on Classifier.
2. Select the species and classifier you want to attach to the metaphase.
3. Click on OK.
4. Click on Auto Classify and the cell will be automatically classified into the format of the chosen species.
**Note** - If it is a new species, or you have not trained a classifier you will be asked if you want to manually classify the chromosomes or have them placed in classes based on size. When you have three karyotypes then you can create a classifier for the species group.

**Related topics**

- Attach a template to CGH, RxFISH or M-FISH templates

**Edit the layout of species template karyotypes**
Load a karyotype that is attached to a template in the main window of Analysis and the **Edit Template** tool will become active. Press it to open the template toolbox and begin editing. You can rearrange the chromosomes, re-label the classes or alter the properties of a class. You will not be able to change the height of class boxes while the karyotype is loaded.

**Unstack Chromosomes** to force the chromosomes in the bottom row to be placed in a class box.

**Template height** opens a small dialog with a small bar used to change the length or height of the template. Just drag the bar up or down. The height of the template is shown in pixels to the right.

Use **Align Qters** to line the chromosomes up by their terminal ends. Aligning by terminal ends usually results in a better layout as centromere positions can be incorrect.

**Align Centromeres** will line them up by their centromeres. Centromeres can be moved if the chromosomes are poorly aligned using this option.

**Auto Adjust** spaces chromosomes out evenly within each class.

**Save** saves the changes for the current cell only.

**Save as New Default** gives you the option to apply any of the changes to the default version of the template or to save the template under a new name.

**Autoscale** reduces the size of large chromosomes to fit in the template.

**Add ideogram sets to karyotyped cells**

If you karyotyped a cell using a template that did not have an ideogram set attached to it you will have to re-classify the cell to get the ideograms to draw in MultiCell.
To add an ideogram set:
1. Open the template using the Species Browser.
2. Attach an ideogram set using Select Ideogram.
3. Save the template.
4. Load the metaphase for the cell in the main window.
5. Click on Classifier and choose the template you just saved.
6. Click on Auto Classify or Manual Classify.
7. Choose to overwrite the karyotype.

Using templates in capture

Using templates in Capture

When capturing cell types that involve background processing of images, the system needs to know what template will be used to karyotype cells before they are captured. This applies to CGH, RxFISH and M-FISH cells. The number of classes and their properties is needed for the background processing of the cells. The Species Browser is accessible through the Fluorochrome Selection panel and the current template name will be in the title bar. Once a template is selected, it will be attached to each cell as it is captured.

The last species template used will be applied to any capture mode unless it is changed. The Save List function will save both the fluorochrome list and the template name.

Related topics

- Specifics of CGH capture
- Specifics of M-FISH capture
- What to do if you captured cells with the wrong template

What to do if you capture cells with the wrong template

1. Open the case in Analysis.
2. Click on the affected slide in the Navigator.
3. Switch to the Capture screen.
4. Click on Reprocess in the Image Capture dialog.
5. Select the new template you want to use from the Species Browser.

If you notice the template is wrong during capture you can press Reprocess while the cells are being processed. You do not need to wait until the batch is complete.

Notes
- Everything in the cells except the raw images will be deleted.
- Use this method to replace blue ideograms with a species ideogram set. Just attach the ideogram set to the template first, then reprocess the cells.

To capture CGH cells with species templates

Select a species template before capturing the cells when you configure the Fluorochrome Selection panel. Use the Species Browser to select the species. If you want to include ideograms for the species attach an ideogram set to the template before capturing the cells. If you do not the generic blue ideogram place holders will appear in your CGH Profile image. To
replace the generic ideograms with your set after capture, attach the ideogram set to the template then reprocess the cells.

Save the fluorochrome list and the template name using Save List. Then whenever the list is loaded the template will already be selected.

If you select the wrong template and want to change see the topic What to do if you capture cells with the wrong template for help.

**Capture in M-FISH**

The first step in M-FISH capture is adding the fluorochromes to the Fluorochrome Selection panel. Then if you are using a template other than Normal human you must select it from the Species Browser. Once you have done this you are ready to configure the Fluomap.

The Fluomap is a table that contains the hybridization details for each chromosome class. It is also used to select the pseudocolors used to label the classes. You can save time by saving commonly used sets of fluorochromes, species templates and Fluomaps using Save List.

As the raw images are captured they are processed and tested for quality. If there is not enough data in the raw images to create a metaphase they will fail testing. The test results for each cell will be shown in the Image Capture dialog box.

**Related topics**

- See the topic in Probe capture > Fluorochromes > Configure the Fluorochrome Selection panel
- Select a species template for MFISH
- Configure the Fluomap
- Work with the Image Capture dialog box
- Read tips for capturing M-FISH cells

**Capture in RxFISH**

If you want to use a custom template rather than the default, you must select the template you want to use for your karyotypes before you begin capturing cells. Open the Species Template Browser with the button located in the Fluorochrome Selection panel.

If you make a mistake and select the wrong template, see the topic What to do if you capture cells with the wrong template for help.
Importing images and files

Importing images and files

There are three activities that involve importing files from external sources. The file type and end use determine how they are imported.

Third party files such as documents or non-CytoVision image types can be stored as part of a case. This provides a way to keep all material relevant to a patient or project in one location. These files are viewed in their native software and remain editable. Files stored in a case are archived with the case.

Image files in various popular formats can be captured into a slide as if they were a live image. These files may have been created in third party graphics applications from scans of photographs or other file types.

Image from SatCAP, Genevision and Probevision are converted to CytoVision format from the import dialog. The case structure of the imported images is kept. When images are imported, the system will check to see if the case already exists. If it does the images will be placed in the existing directory. If not a new case directory will be created.

Capturing images from file

Capturing images from file provides a way to use chromosomes, cells or even graphics from external sources with the capture and analysis tools of CytoVision. Sources can be scanned photographs or pictures from a graphics application like Paint Shop Pro™. Metaphases can be karyotyped. Objects in imported files can be combined in flex images.

The source images can be Bitmap (.bmp), Graphics Interchange Format (.gif), Jpeg (.jpg), Portable Network Graphics (.png), or Tagged Image File Format (.tif). Single images can be captured in brightfield or fluorescent mode. Multiple layer composites can be captured in probe. The image can be thresholded, segmented, karyotyped or enhanced just like a captured image. The file is imported by treating it like a live image in the Capture screen.

Capture From File is a setting in Customize. When on, pressing the Capture button opens a common Open file dialog used to select the file. Once a file is selected, it is placed in the image buffer like a captured live image. All options selected in Customize are then applied. Standard thresholding tools can be used to eliminate background and create a metaphase that can be karyotyped. Graphics can be captured with a threshold level of zero to retain the entire image, or the threshold mask can separate them. For example, an ideogram for a species can be saved as a tif, captured and thresholded so each class can be copied or moved as a separate object.*

To capture from file:
1. Open a case and slide.
2. Select the appropriate stain type in Capture Mode.
3. Click on Customize and turn Capture From File on. Check any other options in Customize that you want to use.
4. Click on New Cell.
5. Click on Capture.
6. Select the file for import.
7. Click on Open.
8. Threshold the image if you want separate objects for karyotyping or analysis.

*Tip* - If you are planning on thresholding the image, remember that threshold works on grey level values. Anything you want to keep should be a different color than the background. In the ideogram example above, the white bands of the ideogram should actually be a very light gray (RGB values of 250, 250, 250 are fine).

**Importing third party files**

Third party documents and images can be stored in a case. These files are displayed in the Navigator with an identifying icon and file extension. Files remain editable and are opened and viewed in their native software. To view a file type the appropriate application must be installed on the system; for example Acrobat Reader must be installed to view a PDF file.

To add a file open the case in the navigator, then drag the file onto the navigator. Files can be stored in any level; case, slide or cell. The original file remains intact, only a copy of the file is added to the case folder. A file can also be dragged from its current navigator location to another location. The following file types are supported:

<table>
<thead>
<tr>
<th>Navigator Icon</th>
<th>File types</th>
</tr>
</thead>
</table>
| ![Icon] | - Bitmaps (.bmp)
- Graphics Interchange Format (.gif)
- Jpeg (.jpg)
- Portable Network Graphics (.png)
- Paint Shop Pro (.psp)
- Tagged Image File Format (.tif) |
| ![Icon] | - Microsoft Word (.doc)
- Rich text format (.rtf) |
| ![Icon] | Adobe Acrobat (.pdf) |
| ![Icon] | Plain text (.txt) |
| ![Icon] | Microsoft Excel (.xls) |
| ![Icon] | Zipped archives (.zip) |

*Note* - Files cannot be dragged directly from an email. They must first be saved to disk, then copied to the case.

**Import from SatCAP or GeneVision**

**Import from SatCAP or Genevision**

The Import dialog is used to convert images from SatCAP stations, Genevision or Probevision cases. SatCAP images are transferred to the CytoVision from the satellite station. Genevision or Probevision archives are connected to a SatCAP station for transfer using a special application called GV2CV. Details about transferring images can be found in the SatCAP user manual or from GV2CV support.
Using the Import dialog

The Import dialog is used to convert images transferred from a satellite station to CytoVision format. Click on Import Images in the main toolbar to open the dialog.

The combo list at the top of the dialog selected the type of images for import. Images that have been transferred are listed along with their case name, slide and cell number, and their status. If the image status is OK, import the images by clicking on the Import button in the dialog.

Clear deletes all images in the list. It is used to remove any images that are not needed; for example they are duplicates or must be renamed. Cancel closes the dialog without making changes. Use it if slides or cells need to be renamed, or if cases need to be restored before importing.

If a case already exists the images will be added as they are imported. If the case does not already exist, it will be automatically created using the case name from the export machine.

In order to import a case, certain conditions must be met:

- If the case already exists in the Library, it must currently be on a hard drive. If it has been archived off it must be restored before additional images are imported.
- The cell number should be unique. If there are duplicate cell names, either rename the one that already exists, or the rename the imported image at the satellite station.
- Two metaphases of the same type cannot be saved into the same cell.

Tip - On networks, to select the system a case is imported to, create the case on that system first. Then import the images.

Related topics

List of status messages and their meanings
Importing cases from a SatCAP

1. Transfer the images from the SatCAP.
2. Click on the Import Images button in the main toolbar.
3. Select Satellite from the drop down list.
4. Click on Import.

Importing Genevision/Probevision cases

1. Transfer the images from the Genevision/Probevision reader.
2. Click on the Import Images button in the main toolbar.
3. Select Genevision/Probevision from the drop down list.
4. Click on Import.

The karyotypes will be imported as flex images. Kdata will be imported into the variable fields section of Case details.

Import status messages

<table>
<thead>
<tr>
<th>Message</th>
<th>Meaning</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>exists</td>
<td>A cell of this name already exists in the slide</td>
<td>• If it is a different cell, you can rename the existing cell.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Rename the cell at the export machine and transfer it again.</td>
</tr>
<tr>
<td>archived</td>
<td>The case has been archived or removed from the hard drive.</td>
<td>• If archived you must restore the case before you can import the image.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If the case was deleted from the hard drive but not from the Library,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>use the Case and Library Manager to delete the library entry.</td>
</tr>
<tr>
<td>Unknown can’t read</td>
<td>The file cannot be read.</td>
<td>Either the file is corrupted, or the transfer partially failed. Try clearing the images and transferring them again.</td>
</tr>
</tbody>
</table>
Printing

Printing Images Overview

The Print dialog is used to arrange page layouts and print images. There are three different formats which appear as tabbed pages. Karyogram is used to print the karyotype and metaphase of a single cell. Image Montage allows you to print 1 to 6 images of any type, and combinations of images from different cases on the same page. Case Details is a text only option for printing the case details.

The page layouts are WYSIWYG (what you see is what you get : a display generated by word-processing or desktop-publishing software that exactly reflects the appearance of the printed document) examples of what the printed page will look like. Image windows can be moved and resized. You can select which fields from Case Details you want printed with the image. The fonts size and color can be changed in the Karyogram and Image Montage layouts.

Prints are sent to the system default printer using the properties set for that printer. To change these settings permanently you must change the properties for the printer. To change them temporarily use Page Setup in the Print dialog.

The logo can be easily changed by importing a .bmp file. See Selecting a logo topic under Printing > Page layout > Customizing print layouts for details.

Related topics

- Change printers and settings
- Customize page layouts
- Adding a logo
- Annotation phrase lists
- Share a printer over a peer-peer network
- About network printers on a server
- Cancel a print
- Image Montage layout
- Karyogram layout
- Case Detail layout

Printing images

The Print panel is used to load and print images. Open it by clicking the Print button on the Analysis toolbar.

Load images into Print by holding the Ctrl button, then dragging the image from a work window into the print window. For items in dialog boxes such as CGH profiles or Fluomaps, drag them from the dialog into a work window first.
When you are ready, click on the **Print** button in the dialog to send the image to the default printer. If you want to select a printer other than default use **Page setup** and choose the name from the drop down list. Installed network printers will appear in the list as if they were local. **Page setup** is also used to change page orientation, copy number and print quality settings temporarily.

**To cancel prints**

1. Whenever a print is sent a printer icon appears in the Windows taskbar. Double click on this printer icon.
2. Select the print job you want to cancel from the list.
3. Choose **Cancel** from the **Document** menu.

If you want to clear all jobs choose **Purge Print Documents** from the **Printer** menu.

**Note** You can only cancel jobs going to a local printer. If the printer is on a networked system you must cancel the prints from there.

**Page layout**

**Customizing Print Layouts**

The print dialog contains a WYSIWYG page editor used to set the layout of pages. Print or hide fields from Case Details using the check boxes on the right. The relative size and position of images, text fields and fonts will be shown on the page.

**Border** prints a rectangular border around each image. Check the **Grid** box to display a grid pattern to help with lining detail boxes up. When on, objects will snap to the grid.

- ☑ Border  ☐ Grid

Customized print layouts can be saved and loaded. Changes you can make to the layout include the size and placement of images, what fields are printed and the fonts used for fields. Text entered in the **Title** field can be saved with a layout but text in the **Comments** field cannot.

Create and save as many of these custom layouts as you want for each print format. The print window will open with the last layout used for printing.

If you have selected the **Extended Height** option in the **Karyotype Layout dialog**, you will need to reformat the print layout to allow for the extended karyotype window.

**Selecting a logo**

A new Logo can be easily added to all print layouts. Only BMP files may be used and only one logo can be selected (i.e. not different logos for each print layout.)

1. Select the Logo box in the detail list.
2. A blank box appears in the print window. Move the mouse inside the box and press the middle mouse button.
3. Browse to a logo saved as a bitmap (bmp) image. Select open or just double click on the bmp file. This file will be remembered by the system until a new logo is selected.
4. The bitmap image appears the logo box in the print window.
5. To resize the image, move the mouse cursor over edge of the logo box - the cursor becomes a beveled arrow - click and drag the box to resize the image.
6. To move the logo image, click within the logo box, the cursor changes to a cross arrow, drag the window the different location.

To move a window:
Move image windows by dragging them with the mouse like any other object. Position the cursor over an image window then click and hold the left mouse button to drag the window to a different location.

To resize a window:
Place the cursor over the border of the image window. When the cursor changes to double arrows, click and hold the left mouse button. Drag the borders to the size desired then let go. The aspect ratio must remain the same so you cannot change the height and width separately.

To change fonts:
1. Placing a check in a data field displays a box with the field name in the image window. Click on this box with the middle mouse button and the standard Font dialog box will appear.
2. Select a font size, color and style. The font selected will be applied to the field name in the text box allowing you to judge size and appearance.

Tip - The font size is relative to the Print panel. If needed, sizes other than those listed can usually be entered by typing over the font Size field. For example you can print with Arial 6 by typing a 6 over the smallest size listed which is usually 8. You will find this to be true for many Windows based software.

To create and insert an Annotation Phrase list
Insert items from the saved annotation phrase list to an image. Type text in the Phrase list box and click on Insert. The text will be added to the detail box list. Check the associated box to add the text to the layout. If the template is saved with the user phrase checked, it will reappear when the template is reloaded. If it is checked off when the template is saved, it will disappear from the field list when the template is reloaded.

Note: The text will be available in the drop down combo box but is not saved to the report template detail list unless the layout is saved.

To save a layout:
1. Arrange the page layout.
2. Enter a name for the layout in the combo box at the bottom of the format page.
3. Click on Save.

To load a layout:
Load a layout by selecting it in the drop down combo box.

Selecting case details fields
Click on the box of the detail you want to include in the layout.

**Annotation Phrase list**

A new case detail can be added by creating a new detail in the Phrase list box and clicking on the Insert button. The new detail will be added to the list and when checked, will be added to the layout. It must be saved to the layout to permanently add phrase to the template. It will be stored in the Phase list combo box unless deleted.

**Image Montage layout**

Print images from more than one case or of different types in Image Montage. If all of the images are from a single case, then you will be able to print any of the case details fields. If images from more than one case are printed together, then only non-specific fields like the title will be available.

The six buttons at the bottom of the page are used to select the number of image windows. Choose between 1, 2, 3, 4, 5 and 6 images. Once you select the number of image windows you can resize them and move them to suit your needs. See the topic under *Printer - Page layout - Customizing print layouts* for more information.
Karyogram layout

Prints a karyotype and its associated metaphase. See topic under Printing - Page layout - Customizing print layouts for more details.
Case Details layout

Prints text only containing the details of the active case. You can draw horizontal lines to mark off sections of the page. Click the right mouse button wherever you want a line the page. You
do not need to draw the line it will automatically go from one side of the page to the other. Right click on the line again to remove it.

Check the boxes of the details you want to include in the Case Details report. Click and drag boxes to the location you want them in the report. Click on the Grid box to help align the boxes.

See the topic under Printer - Page layout - Customizing print layouts for more information.
Printers and settings
It is customary to have one printer set as the default printer. This is the printer that all of your programs will use unless you specifically select a different one. When you click on the Print button the image will be sent to the default printer. Your printing preferences such as paper size, resolution and copy number are saved in the Document Defaults of each printer.

If you want to temporarily change the printer used, page orientation and margins, number of copies or other printer settings click on Page setup to open the Windows Print dialog box. The system will use these temporary settings until the program is exited.

**Related topics**
- [Set the default printer](#)
- [Set the Document Defaults](#)
- [Select a printer other than the default](#)
- [Temporarily change printer settings](#)
- [Print multiple copies](#)

**To set the default printer**

1. Click the Windows NT Start button, point to **Settings**, and then click **Printers**.
2. Right click on the printer you want.
3. Choose **Set As Default** from the menu.

**To share a printer**

1. Click on **Start** then point to **Printers** in the **Settings** menu.
2. Click the right mouse button on the printer you want to share and select **Sharing**.
3. Click on the **Sharing** tab and choose the option **Shared**. If you want to use a name other than the default, replace the text in the **Share Name** field.
To set the Document Default properties

1. Click the Windows **Start** button, point to **Settings**, and then click **Printers**.
2. Right click on the printer you want.
3. Choose **Document Defaults** from the menu and enter the new settings.

**Notes**
- Changing the printer properties in the Printers folder will change the default settings for all documents you print on this printer. To change these settings for one document, click **Page Setup** in the Print window.
- The document defaults must be set for each login or user name separately. The exception is that some systems only allow users with Administrator privileges to change Document defaults. In this case login as Administrator to make the changes.

To select a printer other than default

1. Click on **Page setup**. The standard print dialog will open.
2. Choose the printer from the **Name** combo list.
3. Click on **OK** to start printing.
Related topics

To change the default printer

Changing printer settings

Click on Page setup to open the standard Windows NT Print panel. Here you can change page orientation, number of copies and other printer settings depending on the printer driver. Many printers also allow you to change resolution and color settings as well. Click on Properties to access any additional settings available on your printer.

Note Any changes made to the printer from Page setup will only be temporary and will be lost when the program is exited. If you want to save the settings as the new defaults, then you must change them from Document Defaults.

To print multiple copies

1. Click on Page setup to open the printing dialog box.
2. Enter the number of copies you want and click OK to start printing.

Note The actual location of the copy number will change depending on the printer and the driver used. Some printer drivers require you to use the Properties button to access copy number. If you always want more than one copy, most printers will let you specify a number in Document Defaults.
Probes

For Research Use Only. Not for Use in Diagnostic Procedures.

Overview of probe images

Probes are captured as black and white images. The colors seen in the final image are for display only and do not affect the data. The color is applied as part of the thresholding process.

It is common to assign colors to fluorochromes that match those seen in the microscope, but any color that makes visualizing the signals easily can be used. One image is captured for each fluorochrome and then they are layered to produce the final image. This allows you to work with fluorochromes individually for image enhancements and displays.

When probe images are karyotyped a metaphase image is created as part of the process. This allows you to keep the original probe image which can still be enhanced, contrasted or have the fluorochrome colors changed.

Related topics

- Capture probe images
- Work with probe images in Analysis
- Karyotype a probe image
- How to copy probe images

Probe capture

About probe capture

There are three parts to the process of capturing probe images. The first part is the selection of fluorochromes used in your image. This is done with the Fluorochrome Selection panel.

The second is the actual adjustment of the live image. The Capture Setup dialog becomes the Capture and Fluorochrome Setup dialog in probe capture. This is because separate settings are saved with each fluorochrome. These settings include the camera setup and which filters are used to capture the fluorochrome. If you have a filterwheel or motorized microscope the correct filters will automatically be chosen during capture.

Finally the captured image is thresholded and an image is created. Any area not covered in the green thresholding mask will have the designated color applied to it. Fluorochromes are captured as separate images which are layered to create the final probe image.

Related topics

- Setup for probe capture
- Learn about tools and options
- Learn about motorized filterwheels
- Capture images
CytoVision 3.6

**Fluorochromes**

**Fluorochromes overview**

Start by making sure that your microscope is properly set up then select the fluorochromes used in the cell and add them to the Fluorochrome Selection panel.

The settings used to capture fluorochromes are saved in two places. The default settings are saved in the main Build List file. Default settings are only loaded when the fluorochrome is initially added to the Fluorochrome Selection panel using **Build List**.

The second group of settings is saved in the Fluorochrome Selection panel. These can be thought of as the current settings. Any changes made while capturing images will be saved here.

Once the Fluorochrome Selection panel is configured, the list of fluorochromes used can be saved. Each probe capture mode has its own directory where lists are stored. When a list is saved, the fluorochrome's current settings are the ones saved.

**Notes**

- As you work in different modes of probe capture there is an important point to remember. When you create a fluorochrome you are actually creating a file. That file contains the configuration settings that you want to use to capture the fluorochrome with. The fluorochrome name is actually a file name. This is important because like any file, if you have the name wrong it won’t find the information you want. This is most relevant in samples with CGH, M-FISH or spot counting, where the system must go back to that file for information.
- Naming is case sensitive so the system sees DAPI and Dapi as two different files. If you capture a CGH image with a fluorochrome named FITC, then delete that fluorochrome from the Build List. If the CGH image is reprocessed it will not know what color to use to render the image and will display it as white. This is the type of problem you could see.

**Related topics**

- Set up the microscope
- Configure the Fluorochrome Selection panel
- Learn about saving a loading fluorochrome lists

**Setting up the microscope for probe capture**

- It is important to make sure that your fluorescent bulb is in good condition and centered properly. Uneven illumination from an un-centered bulb can make it very difficult to threshold an image.
- It is also important that your objectives and oil are optimized for fluorescent work. Some objectives reduce the amount of fluorescent light significantly, forcing long exposure times to be used. If you are unsure, check with your microscope supplier.
- Neutral density filters will improve the resolution of DAPI metaphases. They will also help reduce fading of the image particularly with very bright lamps.

**Tip** - Use Pseudocolor to check for uneven illumination.

**Using the Color Panel**
There are two ways to assign colors to probe images. The Color Panel, and the Windows color palette. The same methods are used for assigning colors to fluorochromes in both **Capture** and **Analysis**. Move the RGB sliders to create a color. Click on OK to assign the color displayed to the fluorochrome. Use Gamma to make a color brighter or darker in an image. Use **Counterstain Attenuation** to make signals more visible against the counterstain.

<table>
<thead>
<tr>
<th><strong>RGB sliders</strong></th>
<th>Use these sliders to build your color. The higher the number the more of a color is added.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Color</strong></td>
<td>Opens the Windows color palette</td>
</tr>
<tr>
<td><strong>Gamma</strong></td>
<td>Changes the brightness of the color. Values &gt;1 will darken that color, &lt;1 will lighten it.</td>
</tr>
<tr>
<td><strong>Counterstain Attenuation</strong></td>
<td>Subtracts the counterstain color from any overlying probe signals. This makes the signals easier to see and print.</td>
</tr>
<tr>
<td><strong>Redraw</strong></td>
<td>Place a check in the box if you want to see the changes in the probe image immediately. If <strong>Redraw</strong> is not on you must hit <strong>Apply</strong> to effect the changes.</td>
</tr>
</tbody>
</table>

**Related topics**

- Using the Windows color palette

**Using the Windows color palette**

Select one of the Basic colors by clicking on it and pressing OK. To build a custom color first select a box then use the pointer and slider to build a color or enter values directly. When you have the color you want click on **Add to Custom Colors**.
<table>
<thead>
<tr>
<th><strong>Basic colors</strong></th>
<th>The basic 48 colors in a palette. Select the color you want and click on <strong>OK</strong>.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Custom colors</strong></td>
<td>Color boxes used to store custom colors. Create a custom color by selecting one of these boxes, then use the color matrix or RGB values to create your custom color. Press Add to custom color to assign the new color to the custom box. Use a custom color by selecting the color you want and click on <strong>OK</strong>.</td>
</tr>
<tr>
<td><strong>Define custom colors</strong></td>
<td>Opens the color matrix and controls for defining custom colors. It will be disabled if these controls are already displayed.</td>
</tr>
<tr>
<td><strong>Infinite pointer</strong></td>
<td>Grab the crosshairs with the mouse and move them to any area of the color matrix to create a custom color by sight. The color in the Color/Solid box will change to reflect the area under the crosshairs. Use the slider to the right to adjust its brightness.</td>
</tr>
<tr>
<td><strong>Luminosity slider</strong></td>
<td>Allows you to change the Luminosity of a color without changing the Hue or Saturation.</td>
</tr>
<tr>
<td><strong>Hue</strong></td>
<td>Hue is the value in the color wheel. 0 is red, 40 is yellow, 80 is green, 120 is cyan, 160 is blue and 200 is magenta.</td>
</tr>
<tr>
<td><strong>Saturation</strong></td>
<td>The amount of a color present. The higher the number the brighter the color will be. 240 is the maximum level.</td>
</tr>
<tr>
<td><strong>Luminosity</strong></td>
<td>Sets the brightness of the color. The higher the number the brighter the color will be. 240 is the maximum level.</td>
</tr>
<tr>
<td><strong>Add to Custom Colors</strong></td>
<td>Adds the color selected to the Custom colors palette. If you do not have a specific box selected it will overwrite an existing color.</td>
</tr>
</tbody>
</table>

**Related topics**

- Using the Color Panel
Working with the Fluorochrome Selection panel

The Fluorochrome Selection panel is used to select fluorochromes for probe capture. This dialog appears whenever probe capture modes are selected. Select one fluorochrome as the counterstain by placing a check in the left column; the others will be treated as signals. A ✓ is displayed by the name of each fluorochrome as it is captured.

The fluorochrome with a check next to its name, is the current or active fluorochrome. Any actions will be performed on this fluorochrome. Delete a fluorochrome from the panel by making it the active fluorochrome then clicking on Delete.

Commonly used configurations can be saved and loaded to prevent having to setup fluorochrome lists every time.

![Fluorochrome Selection](image)

**Probe Fluorochrome Selection window**

<table>
<thead>
<tr>
<th>BRfluors</th>
<th>Place a check in the box to switch to BRfluor mode of capture.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counterstain</td>
<td>Place a check in the box of the fluorochrome that is the counterstain. One fluorochrome must be designated as a counterstain before capture can proceed.</td>
</tr>
<tr>
<td>Auto sequence</td>
<td>Eliminates the need to click on Live between fluorochromes. If a filterwheel is attached to the microscope, or the system uses an automated dichroic turret, Auto Sequence will automatically change filters between fluorochromes. Auto Sequence is selected in the Fluorochrome Selection dialog window.</td>
</tr>
<tr>
<td>Build List</td>
<td>Opens the Fluorochrome List dialog which is used to add fluorochromes to the list or to create new ones.</td>
</tr>
<tr>
<td>Load List</td>
<td>Used to load any saved sets of fluorochromes.</td>
</tr>
<tr>
<td>Delete</td>
<td>Removes the selected fluorochrome from the panel.</td>
</tr>
<tr>
<td>Save List</td>
<td>Used to save the current group of fluorochromes as a set.</td>
</tr>
</tbody>
</table>

Related topics

- To create a new fluorochrome
- Auto sequence
- Capture Customize

235
To change default fluorochrome settings

Each fluorochrome can have its own unique settings. Change the color, filter position, slider settings or Background Subtraction level for any fluorochrome.

1. Place a check next to the name of the fluorochrome in the Fluorochrome Selection Panel.
2. Click on Capture and Fluorochrome Setup.
3. Change the settings to the new values.
4. Save the changes by clicking on Save as Default.

Related topics
- Fluorochrome setup options

To create a new fluorochrome

1. In the Fluorochrome Selection panel box click on Build list. The Fluorochrome list select dialog will open.
2. Enter the name of the new fluorochrome in the text field and click on New.
3. Highlight the name in the fluorochrome list and click on Add.
4. Select the new fluorochrome in the Fluorochrome Selection panel and open Capture and Fluorochrome Setup.
5. Click on Color and select the color you want to use then click OK.
6. If you have an Applied Imaging filterwheel click on Excitation, select the filter you want to use and click on OK. For motorized microscope use Dichroic to call up the Filter Control dialog for the microscope cubes.
7. Change any other settings in Capture and Fluorochrome Setup and click on Save as Default.

Once a fluorochrome has been created, you can add and delete it from the Fluorochrome Selection panel without having to reconfigure the settings.

Related topics
- Using the color palettes

To add fluorochromes to the selection panel

1. Click on Build List.
2. Select a name from the list.
3. Click on Add.
4. Click on Done.

If the order fluorochromes are captured in is important, add them to the list in the order you want them. The first name added will be the first captured.
Related topics

- Selecting fluorochromes for probe capture
- To create a new fluorochrome
- To delete a fluorochrome

Working with fluorochrome lists

Commonly used lists of fluorochromes can be loaded and saved. The capture settings saved are the current or temporary settings that are in the Fluorochrome Selection panel. This enables you to have custom lists with settings that may be different than the default settings for the fluorochromes. The only time default settings are loaded is when a fluorochrome is initially added to the Fluorochrome Selection panel.

The following settings are saved with a fluorochrome list:
- Bright, Black and Exposure levels
- Color settings
- Background Subtraction level
- Fluomap
- Z-Stack settings

To save a list:
1. Add your fluorochromes using Build List.
2. Mark the one used as the counterstain.
3. Click on Save List.
4. Enter a name for the list and click on OK.

To load a list:
1. Click on Load List in the Fluorochrome Selection panel.
2. Choose the name of the fluorochrome configuration you want to load.
3. Click on OK.

To delete a list:
1. Click on Load List in the Fluorochrome Selection panel.
2. Select the name of the list you want to delete.
3. Click on Delete.
Related topics

- Add a fluorochrome to the list
- Delete a fluorochrome from the main list

To delete fluorochromes from the build list

1. Click on Build List in the Fluorochrome Selection panel.
2. Select a name from the list.
3. Click on Delete.

Related topics

- Delete a fluorochrome from the selection panel

Tools

Probe capture tools

The Capture and Fluorochrome Setup dialog changes to include tools and settings specific for probe capture. You can change the settings manually or use the automatic capture tools. If you have a focus motor attached to the microscope, you can have the system automatically adjust for the difference in focal planes between fluorochromes. Or use Z-Stack to capture multiple focal planes and combine them to create one image. This is a useful tool for capturing 3 dimensional objects like nuclei.

Related topics

- Learn about the Capture and Fluorochrome Setup dialog
- Use automatic capture
- Automatically adjust for focal planes
- Use Z-Stack to capture layers

Using Capture and Fluorochrome Setup

The Capture Setup dialog becomes Capture and Fluorochrome Setup in probe capture modes. The capture tools for probes allow each fluorochrome to have unique capture settings. These settings are saved in the Build List file and loaded whenever the fluorochrome is added to the Fluorochrome Selection panel. Once loaded, a second file is saved as part of the Fluorochrome Selection panel. Any changes made to the settings will be saved to this second file. If you want to make the settings part of the build list use Save As Default.

Default settings are only loaded when the fluorochrome is initially added to the Fluorochrome Selection panel using Build List.

Note - The controls of this dialog are slightly different if you have a digital camera.
### Color
Used to select the color you want to use for the fluorochrome. See Topic under Probes - Fluorochromes - *Select fluorochrome colors - the Color Panel*.

### Link Sliders
When you place a check in this box, the Bright and Exposure sliders are linked. Linking the sliders prevents Exposure from moving to the right of Bright. This forces the system to increase the Bright level before it increases the exposure time. Use this tool when you want to keep your exposure time as short as possible.

### Bright and Black sliders
Used to control the gain and offset camera settings. These are the main tools used to determine the contrast range of the live image. Use the field to the right of Bright to set a maximum level if you want to limit the amount of gain in an image. See Topic under Capture - Tools and options - About capture setup - *Using the Bright and Black slider bars*.

### Exposure
Used to set the exposure or integration time. Use the field to the right to set a maximum level for Exposure. Times can be set from 0-40,000. The camera’s capabilities should be considered when setting the maximum exposure. Not all cameras are capable of long exposure times without a significant increase in noise. See Topic under Capture - Tools and options -- About capture setup - *Exposure*.

### Save As Default
Used to make the current settings the new default settings for the fluorochrome. The default settings are saved in the build list.

### Advanced
Opens the bottom half of the panel to expose all of the capture tools.

### Excitation
Opens the Filter Control dialog. Use this panel to select the correct filter from the Applied Imaging filterwheel that is needed to capture the fluorochrome. The filter position and name are listed to the right.
## CytoVision 3.6

<table>
<thead>
<tr>
<th>Feature</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transmission</strong></td>
<td>Opens the transmitted light Filter Panel. Transmission filterwheels are used to capture colored brightfield images. See topic under - Probes - Probe capture - Using motorized filterwheels - Transmission filterwheel.</td>
</tr>
<tr>
<td><strong>Dichroic</strong></td>
<td>This button is only active if you have a motorized microscope interfaced with the program. Opens the Filter Control dialog used to select the correct filter from the microscope's filterwheel that is needed to capture the fluorochrome. The filter position and name are listed to the right. See topic under - Probes - Probe capture - Using motorized filterwheels - Motorized microscope filters.</td>
</tr>
<tr>
<td><strong>Gamma</strong></td>
<td>You can apply a gamma stretch to a fluorochrome during capture. If you find that you must consistently brighten signals in Analysis you can save time by applying a Gamma correction to the fluorochrome color settings. See topic under Capture - Tools and Options - About capture set up - Use Gamma to capture light or dark images.</td>
</tr>
<tr>
<td><strong>Registration</strong></td>
<td>If a fluorochrome is consistently out of registration, you can apply offset values during capture. Use Registration in Analysis to determine the best values to use.</td>
</tr>
<tr>
<td><strong>Z-Stack</strong></td>
<td>This button is only active if a focus motor is installed on your microscope. Opens the Z-Stack settings dialog where the number of planes and the distance between them is set. See topic under Probes - Probe capture - Tools - Capture and Fluorochrome Setup.</td>
</tr>
</tbody>
</table>

### Related topics

- [About probe capture](#)
- [Step by step probe capture - using filterwheels, using manual filters](#)
- [To create a new fluorochrome](#)

### Automatic capture in probe modes

Automatic capture features function the same way in probes as they do in brightfield or fluorescent capture.

**Auto Sequence** will eliminate the need to click on Live between fluorochromes. If there is a filterwheel it will automatically advance to the next position and continue until all of the components have been captured. If you do not have a filterwheel, a message box will appear telling you when it is time to move the filter blocks.

**Auto Camera Setup** adjusts the Bright, Black and Exposure sliders until the target contrast is reached. You can change the target contrast for probe capture.

**Auto Threshold** will threshold the image automatically after Capture is pressed. Turn Predict Threshold on if you want the system to estimate the best threshold level, leave it turned off to use the level last used.

💡 **Tip** - Speed up the automatic capture process by lowering the Contrast target value in signal fluorochromes. Signals do not usually have much detail and can be captured with lower
contrast levels such as 40-50%. This can speed up the capture process as lower contrast levels are usually quicker to achieve.

**Capturing layers using Z-Stack**

Z-Stack is used to capture probe signals in different focal planes. You can specify how many planes you want to capture and the distance between them. You must have a focus motor attached to your microscope to use Z-Stack.

To setup a fluorochrome for Z-Stack capture, click on **Z-Stack** in the Capture and Fluorochrome Setup dialog. The Z-Stack dialog will open. Choose the **Number of planes** and the Spacing between them. The spacing is given in micrometers with increments of 0.1µm.

If you want to specify an area for capture place a check in Use Region of Interest. When the first fluorochrome is captured you will be prompted to define a rectangular region of interest. Click once with the left mouse button at the top left corner of the region. Then move the cursor to define the region. Click once with the right mouse button to set the rectangle.

**Probe Size** is used in processing to remove out of focus signals and background. Use a number that is about the same size as the width of the signals in pixels. As a guideline try 10-14 for medium signals like centromeric or YAC probes and 7-10 for small signals like cosmid probes.

Click on **Apply** after you have selected all of the settings and the fluorochrome is setup to use Z-Stack. Click on **Save as Default** in Fluorochrome setup to make these the default settings.

To remove Z-Stack set the **Number of Planes** to **Z-Stack is off** and click on **Apply**.

![Z-Stack dialog](image)

- **Tip** - If you capture the same fluorochrome and don't always use Z-Stack, create a new fluorochrome for using with Z-Stack, e.g. FITC and Z-FITC. That way you don't have to configure the settings every time. Or save the list once it is configured for Z-Stack.

**Using motorized filterwheels**

**Using motorized filterwheels**

There are three types of motorized filterwheels that can be installed on your system. The excitation filterwheel is installed between the lamp housing and the microscope body and is
used for fluorescent work. It contains excitation filters that are used in conjunction with a special multi-band emission block. Transmission filterwheels are mounted over the lamp in the base of the microscope used for brightfield work.

If you have microscope with a motorized filter turret or slider then a plug-in utility application will allow you to control the microscope from within the program. There are specific microscopes that this applies to so check with your local sales representative or Applied Imaging for details.

All types use the Filter Panel to designate filters for capture and to control the filterwheel. There is a separate filter panel for each filterwheel on the system. The filters used are assigned to each fluorochrome from the Filter Panel. The **Excitation**, **Dichroic** and **Transmission** buttons in **Capture and Fluorochrome Setup** open their respective Filter Panels.

### Using the Filter Control dialog

The Filter Panel is opened by clicking on Excitation, Dichroic and/or Transmission in the **Capture and Fluorochrome Setup** dialog. Names are assigned to help identify the position filters. Change a name by first selecting it, then clicking on it once. Enter the new name and hit Enter. You can move to a filter by clicking on it in the list or by using the keyboard shortcuts.

**Warning** It is important to use Cancel to close the panel unless you want to assign a filter position to a fluorochrome.

![Filter Control dialog](image-url)

<table>
<thead>
<tr>
<th>Filter name</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAPI</td>
<td>1</td>
</tr>
<tr>
<td>NIU</td>
<td>2</td>
</tr>
<tr>
<td>492 Green</td>
<td>3</td>
</tr>
<tr>
<td>BLANK</td>
<td>4</td>
</tr>
<tr>
<td>522 Med</td>
<td>5</td>
</tr>
<tr>
<td>NIU</td>
<td>6</td>
</tr>
<tr>
<td>Red-Green</td>
<td>7</td>
</tr>
<tr>
<td>Triple</td>
<td>8</td>
</tr>
<tr>
<td>405 Blue</td>
<td>9</td>
</tr>
<tr>
<td>Clear</td>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>On</strong></th>
<th>Place a check in the box to tell the software to activate the filterwheel.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reset</strong></td>
<td>When pressed, the filterwheel will find home position, then return to the active filter.</td>
</tr>
<tr>
<td><strong>OK</strong></td>
<td>Is used to define which filter will be used to capture a fluorochrome. Select a filter and click on OK. The filter name and position will be entered into the filterwheel position of Fluorochrome Setup.</td>
</tr>
</tbody>
</table>
Tip - To capture with a separate, single excitation cube, move the filterwheel to the CLEAR position and remove the check from the On box. This will prevent the filterwheel from spinning during capture.

Turning a filterwheel on or off

The filterwheel can be turned on or off from the within software. When turned off in this way, the system will not try to communicate with the filterwheel when entering probe capture modes.

Open Capture and Fluorochrome Setup and open the filter panel. A check in the On box means that the filterwheel is on or active.

Tip - To capture with a separate, single excitation cube, move the filterwheel to the CLEAR position and remove the check from the On box. This will prevent the filterwheel from spinning during capture.

Excitation filterwheel

The Filter Control dialog is used to drive the filterwheel manually and to assign specific filters to fluorochromes. Once a filter is assigned to a fluorochrome, the system will automatically turn to that filter whenever Live is pressed during capture. Open the Filter Panel by clicking on Excitation in the Capture and Fluorochrome Setup dialog.

Move the filterwheel manually by selecting filters in the list, or use the keyboard shortcuts listed below. When using the Filter Control dialog to control the filterwheel, make sure you use Cancel to close the panel.

Filters can be labeled with any name. The only requirement is that one is named BLANK and one is named CLEAR. This is because the filterwheel will go to the BLANK position during the thresholding process to prevent fading, and will go to the CLEAR position when the program is exited to allow the other cubes in the microscope to be used.

Keyboard shortcuts

Filters 1-9 press the numbered key.
Filter 10 press 0 (zero)
Filter 11 press –
Filter 12 press =/+  

To assign a filter to a fluorochrome:

1. Select the fluorochrome’s name in the Fluorochrome Selection Panel.
2. Click on Excitation in the advanced section of Capture and Fluorochrome Setup.
3. Select the filter you want to use with that fluorochrome and click on OK in the Filter Panel.
4. Click on Save as Default to make the change permanent.

Tip - If the filterwheel control window is left open during capture, the filterwheel will not go to the BLANK position during the thresholding stage. It will proceed immediately to the next fluorochrome. To make use of the automatic slide saving feature close the window before going live.

Motorized microscope filter blocks
If you have a microscope with a motorized filter turret or slider then the filters blocks are moved using the Microscope Control application, the Filter Control dialog or the keyboard short cuts listed below.

Open the Microscope Control application by clicking on Microscope. Move to a cube by selecting it in the diagram or scroll through the cubes using the arrow keys.

Filter positions are assigned to fluorochromes using the Filter Panel. Once a block is assigned, the system will automatically move to a cube whenever Live is pressed during capture.

Filters are usually named as part of the microscope configuration but you can change names from the Filter Panel as well. Change a name by first selecting it, then clicking on it once. Enter the new name and hit Enter.

**Keyboard shortcuts**

Press Alt followed by the number of a filter. e.g. Alt-1 to move to filter 1 The shutter will automatically open after the filter moves.

💡 Tip - If you also have an Applied Imaging filterwheel, assign the Clear position from the Applied Imaging filter panel (accessed through the Excitation button) in conjunction with a cube from the microscope panel.

**To assign a filter block to a fluorochrome:**

1. Select the fluorochrome’s name in the Fluorochrome Selection panel.
2. Click on Dichroic from the advanced section of Capture and Fluorochrome Setup.
3. Select the filter you want to use in the Filter panel.
4. Click on OK in the Filter Panel.
5. Click on Save as Default to make these the permanent settings.

**Transmission filterwheel**

The transmission wheel is used to put colored filters in the path of the brightfield lamp. When used with BRFluor capture you can automate colored brightfield capture. Once a filter is assigned to a fluorochrome, the system will automatically turn to that filter whenever Live is pressed during capture. Open the Filter Panel by clicking on Transmission in the Capture and Fluorochrome Setup dialog.

Move the filterwheel manually by selecting filters in the list, or use the keyboard shortcuts listed below. When using the Filter Control dialog to control the filterwheel, make sure you use Cancel to close the panel.

Filters can be labeled with any name. The only requirement is that one is named BLANK and one is named CLEAR. This is because the filterwheel will go to the BLANK position during the thresholding process to prevent fading, and will go to the CLEAR position when the program is exited to allow the other cubes in the microscope to be used.

💡 Tip - If the filterwheel control window is left open during capture, the filterwheel will not go to the BLANK position during the thresholding stage. It will proceed immediately to the next fluorochrome. To make use of the automatic slide saving feature close the window before going live.

**To assign a filter to a fluorochrome:**
1. Select the fluorochrome’s name in the Fluorochrome Selection Panel.
2. Click on Transmission in the advanced section of Capture and Fluorochrome Setup.
3. Select the filter you want to use with that fluorochrome and click on OK in the Filter Panel.
4. Click on Save as Default to make the change permanent.

Capturing cells

Probe capture process

Once your fluorochromes are configured you are ready to start capturing cells. Decide the method you will use to change filters during capture. This will depend on the hardware installed, and the options you have selected in Customize.

The goal is to get a live image with good contrast and a dark, even background. The target contrast will depend on whether you need any banding information from a fluorochrome. The thresholding dialog contains additional tools for probe capture that can be used to eliminate background noise and to isolate areas or signals of interest.

Fluorochromes can be recaptured individually if most of a cell is good, but one or more fluorochromes were poorly captured.

Note: If the Change Filter message pops up during probe capture, the message can be disabled when it appears the first time you capture a probe image. This is login specific.

- Check the Do not show this message again box.
- The message will never appear again for your login.

If you want to reset the message, left click on Help while you are in CytoVision. Select Reset optional messages.

Related topics

- Change filters during capture
- Adjust the live image contrast
- Threshold a probe image
- Recapture a fluorochrome
- Troubleshoot probe capture

Changing filters during capture
CytoVision 3.6

If you have a motorized filterwheel, the filters will be changed automatically as **Live** is pressed. The filters used are assigned to each fluorochrome from the **Dichroic** button **Capture and Fluorochrome Setup**. The filter used to capture a fluorochrome is saved in the fluorochrome's default settings file. When used with the automatic capture options found in Customize, the system can change to each filter as needed without any user interaction.

If you have an excitation filterwheel from Applied Imaging it will contain a blank filter position. The filterwheel will return to this blank position between fluorochromes during capture, while the image is thresholded and saved. This helps minimize the bleaching of the slide. If you do not have a filterwheel or motorized microscope move the next filter block into place before hitting **Live**.

**Related topics**
- About the excitation filterwheel
- About the dichroic filterwheel
- About the transmission filterwheel
- Using the Filter Panel

**Adjusting the live image**

The **Bright**, **Black** and **Exposure** sliders are used to adjust the contrast of the live image. Higher exposure times result in slower capture and the bleaching of fluorochromes. Higher **Bright** values can increase the noise in an image though it is usually not a problem.

It is recommended that you start with a fairly high **Bright** value, around 60-80 with **Black** set at around 170. Then increase the exposure time until the image is bright and clear. Use the **Black** slider to blacken the background by moving it to the left. You may need to adjust each of the sliders a bit more to achieve the desired results.

It is important not to saturate the image with too high an exposure as this will make thresholding difficult and will reduce the amount of data that can be extracted from images. Over exposed images are a flat, light gray with low contrast.

Generally high contrast values are not as important for probe images with the exception of counterstain or signals where you need to see banding. For cosmid or alpha signals ranges of 30-50% contrast can give good results.

**Related topics**
- Thresholding probe images
- What the Bright and Black sliders do
- What Exposure does

**Capturing brightfield fluor images**

**BRfluor** allows you to capture cells using the layering of probe capture with brightfield cells. This method is only used if you capture brightfield images using colored filters. To use it select probe mode and configure the **Fluorochrome Selection panel** as you would for normal probe capture. If you have a transmission filterwheel then select the appropriate filters as described in the topic **Transmission filterwheel**. Turn the **BRfluor** option on then proceed with capture. The **threshold bar** will work in the opposite direction to its normal function.

**To recapture a fluorochrome**
1. Select the fluorochrome you want to recapture in the Fluorochrome Selection panel.
2. Click on Live.
3. Choose Recapture from the warning box.
4. Proceed with capture as normal.

**Troubleshooting probe capture**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Troubleshooting Tip</th>
</tr>
</thead>
<tbody>
<tr>
<td>The filterwheel is not turning when live is pressed</td>
<td>• Make sure that the power box is turned on.&lt;br&gt;• Check that the software for the filterwheel is turned on in the Filter Panel.</td>
</tr>
<tr>
<td>The filterwheel is going to the wrong position</td>
<td>• Use Reset in the Filter Panel to reinitialize the filterwheel motor.&lt;br&gt;• Check the fluorochrome's settings in Capture and Fluorochrome Setup and make sure that the correct filter and position are assigned to it.</td>
</tr>
<tr>
<td>The signals are not in the right place on the counterstain</td>
<td>Load the image in Analysis and use Registration to correct the position. If the placement is consistently wrong, use the Registration offsets in Capture and Fluorochrome Setup to apply the correction as part of the capture process.</td>
</tr>
<tr>
<td>There is a lot of background noise in the image</td>
<td>Try using Counterstain mask or Region of Interest as part of the thresholding procedure.</td>
</tr>
<tr>
<td>The microscope is not turning to the right filter block</td>
<td>Check the fluorochrome’s settings in Capture and Fluorochrome Setup and make sure that the correct filter and position are assigned to it in the Dichroic row.</td>
</tr>
<tr>
<td>The probe modes are disabled</td>
<td>When starting CytoVision there are two buttons with options for using the software; in Research Use Only or In Vitro. The Research Use Only option must be selected to enable probe capture. Try closing the software and restarting making sure the correct button is pressed.</td>
</tr>
</tbody>
</table>

**Thresholding probe images**

**Thresholding probe images**
Thresholding probe images is similar to thresholding for brightfield. The difference is that when thresholding probes, any area not covered by the blue mask will have the color chosen for that fluorochrome applied to it. Loose threshold values can result in large irregular signals. Background noise and debris can also make thresholding difficult therefore there are additional tools for thresholding in probe capture.

While you are capturing, individual threshold levels and Background Subtraction settings will be remembered for each fluorochrome. This means you can apply a subtraction level of 20 to your counterstain and 7 to your signal, thus eliminating the need to keep setting the level for each image. Background Subtraction levels can also be saved as part of a fluorochrome's default settings or as part of a fluorochrome list.

Counterstain Mask eliminates all parts of the image that do not contain counterstain. This is a good tool to use if you have a lot of starry background noise.

Region of Interest lets you define one or more areas for thresholding. This is a good tool to use for isolating small signals from artifacts or debris.

Probe Isolation looks for signals that match the size chosen and isolates them from the rest of the image.

Related topics

- Use Counterstain Mask
- Use Region of Interest
- Use Probe Isolation

Use Counterstain Mask

Use the Counterstain Mask arrows to expand or contract the boundaries of the counterstain. This allows you to compensate for slight offsets of signals.

Use Undilated to have the mask fit tightly around the counterstain. Use Dilate 2, 4 or 6 to have the mask leave a border around the counterstain. The Dilate value is roughly equal to the number of pixels the border will be in width.

Threshold with Region of Interest

ROI (Region of Interest) is used to isolate parts of an image for thresholding. Any area outside of the defined regions will be treated and background.

1. Click on ROI; the cursor will change to the standard cutting tool.
2. Draw around one or more areas of the image.
3. When finished the thresholding mask will appear allowing you to adjust the sliders.
4. Click on Accept to complete the capture.

Click on Restore Image to remove the regions and return to the original image.

Note - Select the drawing style for ROI from Customize; hide or display ROI from the threshold Customize dialog.
To use Probe Isolation

1. Select a spot size in the list and press Isolate. After a moment the thresholding mask will appear with only spots of the chosen size or less showing through the mask.
2. Move the slider until the background has been removed then press Threshold. The spots will appear as white dots in the blue mask.
3. Press Dilate if you want to enlarge the spots.
4. Click on Accept when finished.

To threshold raw probe images

Threshold
Raw probe images can be used to overwrite existing fluorochrome images of cells using the Threshold tool.

1. Load the raw image of the fluorochrome you want to overwrite.
2. Click on Threshold.
3. Choose Rethreshold to replace a fluorochrome image, if a raw DAPI image is loaded, choose Metaphase to create an inverted DAPI metaphase.
4. Adjust the threshold as you would for regular probe capture.

Related topics
- Work with image display and enhancements
- Karyotype a probe image
- Create flexible images and profiles
- Measure probes

Karyotype a probe image

To karyotype a probe image first separate the chromosomes using the standard cutting tools as described for brightfield. Then click on either Auto Classify or Manually Classify. The Counterstain Dilation slider will open allowing you to adjust the width of the counterstain to include any probe signals that are slightly off of the counterstain. When you click on OK to accept the dilation the metaphase and karyotype will be created and appear in the navigator.
When using **Auto Classify** the system will automatically choose the Inverted DAPI classifier as the default. You can create custom classifiers using your own probe images.

If you want to use a classifier other than the default, select it from the Classifier dialog before classifying the cell.

**Tip** - To distinguish between the probe image and the probe metaphase, use the label in the Fluorochrome Selection panel. When the probe metaphase is loaded you will see this label:

![Fluorochrome](image)

**Notes**

- It is better to separate the chromosomes in the probe image before classifying.
- Perform any image enhancements on the fluorochromes before classifying. Once the karyotype is created, you will only be able to alter the inverted DAPI layer.
- Click on the fluorochrome button ![Fluorochrome](image) in the analysis toolbar to turn the displays on or off. This works for **Probe**, **M-FISH**, or **RxFISH**.

**Related topics**

- **About classifiers**

**Image display and enhancement**

**Image display and enhancement**

The Fluorochrome Selection panel in Analysis is used to turn the display of individual fluorochromes on and off, select fluorochromes to work with and to change the color of signals after capture.

Registration can be used to correct the placement of signals on counterstain caused by filter shifts. The enhancement and contrast tools can be used to sharpen images or change the brightness of fluorochromes.

**Related topics**

- **Use the Fluorochrome Selection panel**
- **Change the color of a fluorochrome**
- **Change the background color**
- **Correct registration errors**
- **Enhance or contrast probe images**

**Working with the Fluorochrome Selection panel in Analysis**

![Fluorochrome Selection toggle](image)
When a probe image is loaded in the main window of Analysis, the Fluorochrome Selection panel appears. This panel allows you to work with specific fluorochromes individually. Place a check next to the names of the fluorochromes you want to work with. You can then use the selection tools to pick all or some of these fluorochromes. For example, placing a check next to FITC only, then clicking on Select All would select all FITC signals but nothing else. This is particularly useful when contrasting or enhancing an image. The Fluorochrome Selection panel can be hidden or displayed using the toggle button on the Analysis toolbox.

<table>
<thead>
<tr>
<th>Fluorochrome toggles</th>
<th>Place a check next to the name of any fluorochromes you want to work with.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Display toggles</td>
<td>Placing a check in the toggle turns the display for that fluorochrome on.</td>
</tr>
<tr>
<td>Select All toggles</td>
<td>Placing a check in the toggle selects all objects in the cell that are labeled with that fluorochrome.</td>
</tr>
<tr>
<td>Change Color</td>
<td>Change the color of a fluorochrome after it has been captured by selecting it then click on Change Color to open the color selection dialog.</td>
</tr>
<tr>
<td>All Components</td>
<td>Makes all components of a cell active.</td>
</tr>
<tr>
<td>Deselect All</td>
<td>Makes all of the components inactive.</td>
</tr>
<tr>
<td>Display All</td>
<td>Displays all components of the cell</td>
</tr>
<tr>
<td>Hide All</td>
<td>Turns the display for all of the layers off.</td>
</tr>
<tr>
<td>Invert</td>
<td>Use to create an inverted DAPI image on a white background.</td>
</tr>
<tr>
<td>Counterstain</td>
<td>A ◆ is used to mark the component that was captured as the counterstain.</td>
</tr>
<tr>
<td>Image type label</td>
<td>This label will tell which type of probe image is loaded in the main window. This is useful for other types of colored chromosomes such as RxFISH or M-FISH, where it can be hard to tell whether the metaphase or probe image is loaded.</td>
</tr>
</tbody>
</table>

To change the color of captured fluorochromes

1. Load the image in the main window of Analysis.
2. Deselect all but the component you want to change in the Fluorochrome Selection panel.
3. Click on Change Color in the Fluorochrome Selection Panel.
4. Move the sliders or click on Color and use the Windows color palette to choose a new color.
5. Click on OK. Save the image to keep the changes.

Related topics
- Using the color palettes
- Changing the color used for capture
- Change the background color

Change the background color

1. Load the probe image.
2. Click on Background Color.
3. Select a new color using the standard Windows color palette.

Image enhancements in probes

All of the Enhance and Contrast tools are available for probe images. Work with all of the fluorochromes in an image or with individual components. Place a check next to the names of the fluorochromes you want to work with in the Fluorochrome Selection Panel.

Tips for enhancing probes
- If you find you must consistently brighten your signals, try lowering gamma in the fluorochrome's color settings. This will brighten the signals as part of the capture process.
- Keep the relative brightness of signals by using the Global method of contrast and not Sharpening the signals.
- Usually the counterstain signals should be enhanced or contrasted separately from the signals.
• If you are going to karyotype a metaphase, perform any enhancement on the fluorochromes before classification. You will not be able to change their appearance once the karyotype is created.

Related topics

- Using the Enhance filters
- Using Contrast

Registration errors

Registration errors are when the signals are offset from the counterstain. They occur because each filter cube has its own dichroic mirror which can alter the light path slightly. Also the cubes may not be placed in exactly the same position with a manual slider. Registration moves the offset of an entire layer, not just the signals selected.

Display a small area of the screen in the Registration dialog box by selecting one object. This allows you to enlarge the area while moving the signal.

<table>
<thead>
<tr>
<th><strong>X and Y offsets</strong></th>
<th>The relative position changes will be shown.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scale</strong></td>
<td>Choose to display the image at actual, double or four times normal size.</td>
</tr>
<tr>
<td><strong>Redraw</strong></td>
<td>Place a check in the box to redraw the whole image as you move the signal.</td>
</tr>
</tbody>
</table>

💡 Tip - If the offset positions are consistently wrong with a fluorochrome, enter the amount used in the Registration correction panel into the X and Y Registration fields of Capture and Fluorochrome Setup.

Related topics

- To correct registration

To correct registration
1. Place a check to the left of the fluorochrome you want to move in the Fluorochrome Selection Panel.

2. Click on Register. 

3. Place a check in Redraw if you want to view the changes live in the main window.

4. Click on the directional arrows to move the counterstain to the right position.

5. Click on OK and the image will be redrawn.

Using the Registration dialog box

Composites and profiles

Flexible images and profiles

Probe objects can be copied into flexible images two different ways depending on what you want to do with them. You can copy the fluorochromes as separate objects that can be moved and worked with individually, or you can copy them as a single joined unit.

Band profiles can contain the histograms for each fluorochrome separately, or combined in the same graph.

Related topics

- Copy probe objects
- Create a profile

Copying probe objects

Probe objects are copied the standard way but selecting those objects differs slightly. 
Fluorochromes can be copied independently of each other or together to form a single unit. Place a check by the names of the fluorochromes you want to copy in the Fluorochrome Selection panel.

To copy them as a single unit use Select Group to draw around the objects. To copy them as separate objects use Select All or click on individual objects. Here are some examples of flexible images and how you would create them.

To copy only the DAPI chromosomes in a metaphase:

1. Place a check next to DAPI and remove the checks from the other fluorochromes. 
2. Click on Select All and drag the chromosomes to another window.

To copy a single chromosome with all of its fluorochromes:

1. Click on All Components in the Fluorochrome Selection panel. 
2. Click on Select Group and draw a line around the chromosome.
3. Drag the selected chromosome into another window.

When you rotate or move an object copied using Select Group, it is treated as a single image and the fluorochromes move together.

Tip - If you are copying probe objects and do not want the black bounding box turn off Flex Probe Frames in Customize.

Creating profiles of probe images
Profile works the same way for probe images as it does for brightfield. A different banding profile is created for each fluorochrome displayed. Display the fluorochromes you want to profile in the Fluorochrome Selection panel and then hit Set. The profiles will be drawn with the display colors of the fluorochromes.

Profile of an RxFISH labeled chromosome

Related topics

- Using Profile

Measurements

Measurements

The system comes installed with some measurement tools for probe images, or if you have Image Pro Plus installed you can export the images to their package.

Related topics

- Use the measurement tools
- Export the images to Image Pro Plus
- Export the images in .TIFF format

Probe measurement tools

Probe Measurement

Takes various measurements from a probe image and displays the results in a table and on a graph. The values are not absolute units but relative values. The horizontal scale will change depending on the returned values of the measurement. A peak will be drawn for each value. The vertical scale shows the number of objects that have the same value in a measurement. The scale will change depending on the object counts returned.

To use a tool select the items you want to measure and click on the tool. If you are using Distance or Fipter, you can only choose one item at a time. Place a check in Keep if you want to use the Statistics dialog to calculate mean, minimum or maximum values of your measurements.
### Probe Measurements

The image shows a window titled "Probe Measurements" with a histogram graph displaying intensity values against distance. Below the graph, there is a table with columns for Distance, Diter, Intensity, Area, and Count. The table contains data for different distances and their corresponding intensity values.

#### Statistics

Statistics opens the Statistics window which shows (n), minimums, maximums, and mean values. You must have Keep turned on before you make any measurements to use Statistics.
### Statistics

<table>
<thead>
<tr>
<th></th>
<th>n</th>
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<tbody>
<tr>
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- **Export**
  Exports the data to a file in comma delineated format. You can then import the data into a spreadsheet or database application.

- **Keep**
  Place a check in this box to save any measurements made between cells, and add them to the statistics dialog. This allows you to calculate statistical data for a group of cells or objects.

- **Graph selection**
  Choose which measurement to display in the graph by selecting the name from the combo list.

- **Bins**
  Designates the number of bins a section is divided into. Choose from 32, which will give very broad peaks, to 512 which will display very narrow peaks on the graph.

- **Distance**
  Draw a line over the distance you want to measure. The length of the line will be shown in pixels.

- **Flpter**
  Measures the distance between the signal and the p-terminus as a ratio of the total length. Start your line at the p-ter by clicking the left mouse button, click on the signal with the left button, then on the q-ter with the right mouse button.

- **Intensity**
  Measures the intensity of the selected object, as a percentage of the average saturation in the image. Contrast adjustments will affect this measurement so use the restored image to perform the measurements first. It is also recommended that you capture cells with **Probe Background Cut** turned on in **Customize**.

- **Area**
  Measures the area of the selected objects and displays the value in square pixels.

- **Count**
  Counts all of the selected objects.
CGH

For Research Use Only. Not for Use in Diagnostic Procedures.

CGH overview

Comparative Genomic Hybridization (CGH) is a method of assessing aneuploidy changes in total genomic DNA. Raw images of the fluorochromes are captured, then the background noise is subtracted from the image and the ratio of test to reference DNA is calculated. After a cell has been karyotyped these ratios are calculated for individual chromosomes and the resulting profiles are used to determine the presence of abnormalities.

High resolution CGH was developed to improve detection of genomic gains and losses by increasing specificity and sensitivity. It also allows you to include regions such as telomeres in the analysis which are normally excluded using traditional methods.

A special note

Applied Imaging wish to thank Tommy Gerdes, Jan Maahr and Claes Lundsteen from Rigshospitalet in Copenhagen, Denmark for their assistance and the information they provided to us in the writing of the CGH on-line help.

They are the developers of high resolution CGH and the dynamic standard reference interval method of analysis. A document written by them describing this method in detail has been included in the topic High Resolution CGH Analysis.

Capturing CGH images

Getting started

Two things are critical for good quality CGH images. Quality preparations and proper microscope setup.

Begin CGH capture by deciding which species template you are going to use to karyotype. If you are capturing normal human cells and want to use the installed template you do not need to choose a template now. If you are capturing a species other than human and have not created any templates you must do so before capturing your images.

Configure the Fluorochrome Selection panel and you are ready to begin capturing cells.

Remember when capturing that the number of cells used to calculate the results will affect the accuracy of interpretation. You should capture at least 15 chromosomes for each class to use confidence levels of 99%. The number of cells needed to get this number will depend on your sample type and quality.

If you are unfamiliar with capturing probe images you will find general information in Capturing Probes. The process is similar with some exceptions:

- The Navigator is disabled to prevent capturing into the wrong slide. If you want to select a new, or different slide you must do this from Analysis or from another capture mode.
- It is not necessary to select New Cell, this happens automatically after the last fluorochrome of the current cell is captured.
- CGH thresholds the images automatically. There is no manual thresholding option for CGH capture. You can manually load and re-threshold the raw counterstain image.
- The cells are measured against quality control parameters to determine whether any data can be extracted from the images.
- If a species template other than normal human is going to be used you must select it as part of configuring the Fluorochrome Selection panel.

**Related topics**

- Read the overview of species templates
- Read tips and hints for successful CGH
- Configure the Fluorochrome Selection panel
- Customize CGH capture

**Fluorochrome configuration for CGH capture**

When CGH mode is selected the Fluorochrome Selection panel appears with options specifically for CGH.

Add the fluorochromes needed in the order in which you want to capture them. Mark which fluorochromes will be used for the counterstain, test and reference DNA. Standard CGH capture is performed with DAPI set as the counterstain, FITC set as test and TRITC used for reference DNA. Capturing a fourth fluorochrome such as Cy5 is an option for helping to differentiate between chromosome pairs when karyotyping.

Use the Species Template Browser button to select the template you want. A CGH template will have been created automatically when you created the template. The template will be attached to each cell as it is captured. When you save a list of fluorochromes the template attached will also be saved.

**Notes**

- If you want to include a set of ideograms with the species template you should attach it before capturing the cells.
- If you do not see the Species Browser button open a case first, then select CGH mode.

![Fluorochrome Selection Panel](image)

*Fluorochrome Selection Panel set up for three fluorochrome CGH capture*
Tip - Optimum fluorochrome settings for CGH may differ from other types of probe capture. Creating duplicate fluorochromes with settings specifically for CGH will prevent you from having to alter settings each time you change capture modes. For example, have both DAPI and CGH-DAPI in the list of available fluorochromes when you click on Build List.

Related topics

To create new fluorochromes

Customize CGH capture

Most Customize options are disabled in CGH mode as they should not be changed. The Automatic Capture options and Auto Register Images are the only enabled options. Turning Auto Sequence on eliminates the need to click on Live to proceed to the next fluorochrome. Auto Camera Setup will eliminate the need for manually adjusting the contrast of the live image. There is no manual thresholding in CGH capture but you can manually re-threshold the counterstain image after capture. If Mode settings is turned on your CGH options will be remembered and loaded the next time you select CGH mode.

Important Note: Gamma for capturing test and reference has to be set to 1.0 for CGH. The gamma is adjusted using the Capture and Fluorochrome Setup window. See topic Probes - Probe capture - Tools - Capture and Fluorochrome Setup
To manually threshold the DAPI image

1. Click on the cell name in the Image Capture dialog.
2. Click on **Threshold**.
3. Enter a value in **Background Subtraction** that is equal to half of the chromosomes width in pixels (usually 15-20).
4. Click on **Subtract**.
5. Adjust the sliders with or without **Contrast Stretch**.
6. Click on **Accept**.

To bring up the Image Capture dialog if you are not currently capturing the cells click on the slide in Analysis. Switch to the Capture screen.

**Note** - It is not mandatory that you use background subtraction when thresholding, however it is recommended. If you do not use it, the fluorescence ratios and CGH profiles will be affected.

**Tips and hints for successful CGH**

- Even illumination is necessary for accurate, quality CGH images. Ensuring that your lamp is centered, and that the bulb is in good condition will make CGH capture easier, and your results more accurate. The **Pseudocolor** display option is a good tool to use to determine if there are "hotspots" in your image or if the illumination is uneven.
- Good quality preparations are important for successful CGH. Metaphases should be of a reasonable length and have as few overlaps as possible. Chromosomes that are too short, curled or overlapped will not produce accurate results.
• When a case is completed it is no longer necessary to keep the raw images. If you wish to save disk space you may use Delete to remove them.
• Profiles are calculated for every karyotyped cell in a slide. Therefore you can only capture CGH images into a slide devoted solely to CGH. If the data is not from the same slide then it is important to choose New slide before entering CGH mode in the capture screen.
• Gamma for capturing test and reference has to be set to 1.0 for CGH.

Quality control and review

Quality control and review

Once all of the fluorochromes in a cell have been captured the processing of the cells begins. This process runs in the background so you can continue to capture cells. The background is subtracted and the median intensity of one fluorochrome is normalized to the other. Then the values of the quality tests are compared to threshold values of known good cells. If one or more of these tests fails the cell is marked as rejected by the system.

The Image Capture panel is used to view CGH cells and open the Cell Measurements panel. Cell Measurements is used to accept or reject cells and to see the results of the tests. Part of the background subtraction process is the elimination of large objects that are judged to be interphase cells. If a clump or chromosomes is thrown out as part of this process re-threshold the image using manual Background Subtraction.

Related topics

☐ View the CGH ratio image
☐ Use the CGH Image Capture panel
☐ Use the Cell Measurements panel

CGH Ratio image

During capture when you select a cell in the CGH Image Capture window the inverted DAPI image will be displayed along with the CGH ratios. Green and red areas indicate the relative intensity of the test to reference DNA. Green areas indicate amplification and red areas indicate deletion. By default, the colors are displayed when the relative intensity is above 1.25 (green) or below 0.75 (red). You can change the thresholds for displaying colors by moving the ratio slider bar under the main window. This will increase or decrease the stringency of the relative fluorescence display.

Display the ratio image in Analysis by toggling the CGH ratio button to display the threshold sliders under the image.
**Note:** Changing the ratio slider in the capture screen does not affect the profiles, only the visual portrayal of them.

**CGH Image Capture dialog**

Click on a cell to load the inverted DAPI chromosomes with the CGH Ratio image in the window and to open the CGH Cell Measurements dialog box. Change the pass or fail status of a cell from CGH Cell Measurements.

Even though CGH allows you to change the pass or fail status of a cell if you think it is good enough to analyze, if the system is unable to create a valid metaphase from the raw images, the cell will be deleted even if it is marked for keeping.

If you have chosen the wrong species template you can click on **Reprocess** any time during capture. This will stop the background processing and bring up the Species Browser. Just select the correct template and the cells will be re-evaluated.

Click on **Batch Complete** to create the metaphase, CGH profile and probe images.
CGH Cell Measurements

When a cell is selected in the CGH Image Capture dialog the CGH Cell Measurement panel will open with the list of quality control tests and how the captured cells' parameters compare with the preset thresholds. To display the definition of a test from within the program click on the ? to the left of its name.

The preset thresholds of the tests can be changed by typing over the existing value. To restore the original values click on Defaults then Continue.

If a cell has failed but in your opinion it is good enough to analyze, click on the Keep button. A ✓ will appear by the cell in the CGH Image Capture dialog. Likewise if a cell has passed but you do not want to keep it, click on Discard and a ✗ will be displayed.
Note: Marking a cell as passed does not guarantee that the system can create a metaphase from it. If the quality is too poor the cell will be deleted.

Related topics
- Descriptions of quality control tests
- General use of Cell Measurements

CGH in Analysis

The first step to getting results is to karyotype your cells. Then use the CGH profiles to determine whether an abnormality is present.

Use the display options in Customize to turn the fluorochrome or ratio displays on and off.

Related topics
- Generate karyotypes
High Resolution CGH analysis

CGH Research Group: Tommy Gerdes, Jan Maahr and Claes Lundsteen
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Department of Clinical Genetics,
Juliane Marie Center,
The National University Hospital,
Copenhagen, Denmark
http://www.cgh-group.rh.dk

High Resolution CGH (HRCGH) analysis is developed for improving detection of genomic gains and losses by increasing specificity and sensitivity and at the same time include analysis of chromosome regions (e.g. telomeres) which normally are excluded from CGH analysis, and it includes:

- Correction for unreliable hybridizations:
  - Improved normalization during CGH ratio profile computation. The normalization is improved by 1) skipping fluorochrome intensities from known problematic parts of the chromosomes (i.e. telomeres, centromeres and boundary pixels), and 2) for human chromosomes by skipping chromosomes with fluorochrome intensities known to deviate from the normal levels of all other normal chromosomes.
  - Improved automatic exclusion of unreliable fluorescent intensities. This mostly happens for low intensity pixels at boundaries, telomeres, centromeres, and heterochromatic regions, but unreliable extreme high intensity pixels are excluded as well. Displayed ratio profiles will clearly show totally excluded parts of the profile by grey bands.
  - Automatic correction for unsuppressed repetitive sequences.

- Base confidence intervals on proper student t-statistics instead of 2*SEM and 3*SEM for 95% and 99% confidence intervals (SEM is Standard Error of the Mean). Furthermore two new optional levels of confidence intervals are added 99.5% and 99.9%.

- Use of Dynamic Standard Reference Intervals as reference to the slide average of CGH profiles.
  The CGH ratio levels of normal DNA has shown to vary systematically on each side of ratio 1 along the CGH profiles. The systematic variation, which from slide to slide represents different degrees of the same general pattern, depends on the laboratory doing the analysis and on the protocol they use. The systematic variation is modelled on basis of statistics of representative slides, and used as reference for whether or not ratio variations for a CGH analysis represent possible gains or losses in the test DNA. We use the name “Dynamic Standard Reference Intervals” for this model. It models the average of the systematic deviations along the CGH profiles and the corresponding confidence intervals. Any degree of systematic variation along the CGH profiles for a test slide can then be modelled by scaling the systematic deviations (ratio distances to ratio 1.0) of the model by a constant factor, which fits the model to the ratios of the test slide at locations representing normal test DNA. I.e. the scaling factor is found dynamically for each analyzed slide.

- The core of High Resolution CGH analysis is to compare confidence intervals of the slide average of CGH profiles to the corresponding Dynamic Standard Reference Intervals. The comparison for a case is done at the same level, i.e. 99.5% confidence intervals are compared to 99.5% Dynamic Standard Reference Intervals etc. Each profile point is compared individually as it has its own confidence interval and its own Dynamic Standard Reference Interval.
**Dynamic Standard Reference Intervals** have the following properties:

- They have to be generated on basis of statistics of CGH ratios of normal DNA analyzed by the same protocol and wet methods as the slides they are going to be compared to, and therefore tools for training the statistics is incorporated into the CytoVision program. Preferably each laboratory should generate Dynamic Standard Reference Intervals for each CGH protocol they use on basis of analysis of at least 10 representative slides of 10 metaphases each. The "normal" fluctuations of the CGH ratios from ratio 1.0 should not be to tiny or too large compared to the laboratory standard for these "representative" slides, and it is expected that the "normal" fluctuations represent different degrees of the same general pattern. For X an Y chromosome analysis individual sets has to be generated for analysis of female test DNA versus female reference DNA, and for male DNA versus Male DNA, etc.

- A default set (female DNA versus female DNA) suitable for laboratories using the same direct labelling protocol and laboratory methods as RH is provided. But before being used the laboratory should test at least 20 normal cases, and no false positive results should be found, preferably each laboratory should generate their own sets as mentioned above.

- They look like CGH profile confidence intervals for an "average" case based on analysis of 10 metaphases. I.e. they reflect that the basic pattern of CGH ratios in a systematic way often deviates from ratio 1.0 (clearly seen on human chromosomes 1p, 19 and 22), and they are especially wide at profile areas where CGH measurements are known to be unreliable (heterochromatic regions, the centromeres and the telomeres).

  The explanation why they look like confidence intervals for an "average" case of 10 analyzed cells is that the statistics are adjusted to assume that a maximum of 20 chromosomes (10 metaphases) have contributed to the computation. Otherwise the confidence intervals can get extremely narrow if the statistics is based on e.g. 100 cells.

- They can be scaled automatically or manually for a case to fit the degree of systematic deviation from ratio 1.0. The automatic scaling requires that there are only a limit number of gains and losses in the test DNA, otherwise there is not much to base the scaling on.

- **Set up user adjustable thresholds for detection of possible gains or losses in the test DNA on basis of the slide average of the length normalised CGH profiles.** The user can choose to use either fixed (adjustable) thresholds placed symmetrically around ratio 1.0, or Dynamic Standard Reference Intervals to define thresholds individually for each profile location (point). When the chosen thresholds do not overlap either (optional) the slide average of the CGH profiles or the corresponding confidence intervals for the average, the location is marked and designated as being either a loss (red) or a gain (green). It is up to the user to select an appropiate level of thresholds and of confidence intervals for interpreting these marks, depending on the use of the CGH analysis.

  The following considerations should be made when the user sets up the confidence levels for the Dynamic Standard Reference Intervals:

  - By choosing narrow confidence intervals (95%) non-aberrant fluctuations (false positives) may be marked as aberrant.
  - By choosing the widest confidence interval (99.9%) small abnormalities may be missed (false negatives). Deletions down to 3 Mbp (in 82% of the cells) have been detected without detection of false positives by using a 99.5% confidence level.

- **Provide pseudo ideograms based on the average of all chromosome profiles of the current slide.** E.g. the default pseudo ideograms based on the inverted DAPI counter stain band profiles can be used to define the location of possible gains and losses in the test DNA.

  These "CGH Expanded Profile" views give an illustration of the method:
**All:** Pseudo ideograms based on the inverted DAPI counter stain band profiles for the case, which has a deletion at the telomeres of 2q.

<table>
<thead>
<tr>
<th>Left: The two CGH ratio profiles of chromosome 2 of a metaphase. Unreliable CGH ratios are marked with grey.</th>
<th>Middle: 99.5% confidence intervals (yellow) versus fixed ratio thresholds 0.8 - 1.20 (black).</th>
<th>Right: 99.5% confidence intervals (yellow) versus the corresponding Dynamic Standard Reference Intervals. The two sets of intervals clearly deviate at the deletion. Notice how well the two sets of intervals otherwise fits.</th>
</tr>
</thead>
</table>

The Dynamic Standard Reference Intervals have been scaled automatically to fit all chromosome classes, but these characteristic patterns could easily and with great certainty have been fitted manually as well.

**Documents cited:**

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**Student t-statistics**

Rather than using simple approximation of t-statistics (based on the Standard Error of the Mean (SEM)) to generate confidence intervals for the slide average of CGH ratio profiles, CytoVision uses student t-statistics. The simple approximation gives confidence intervals that are too narrow when only a few cells are analyzed. This could lead to a false conclusion.
regarding a CGH gain or loss. To overcome this statistical tables of t-statistics are built into
the program. At the same time wider confidence intervals representing 99.5% confidence and
99.9% confidence are added in addition to the common 95% and 99% as they are needed for
the Dynamic Standard Reference Intervals. For high sample numbers it will give approximately
the same result as using the standard error of the mean (SEM), however for smaller numbers
you will be less likely to get false positives or negatives.

The exact number of non-missing ratio values behind each mean ratio profile value along the
profiles is used during the computation of each mean value and standard deviation (used for
standard error of the mean). Tables of two-tailed t-statistics (the Student distribution) for 1 to
19 degrees of freedom are built into the program for 95%, 99%, 99.5%, and 99.9% probability limits. All mean ratio values based on more than 20 ratio values are treated as if
there is only 20 ratio values with respect to the t-stat-table look up.

\[ t = \frac{\bar{X} - \mu}{s / \sqrt{N}} \]

This formula is used to calculate a value for \( t \) based on a "true" or comparison mean, and the
measurement mean, standard deviation, and number of data. The actual "test" is performed
using the following steps.

1. Determine a \( t_{\text{calc}} \) using the above formula with the "true" mean and the measurement
   statistics using the formula given above.
2. Compare the calculated \( t \) \( (t_{\text{calc}}) \) to one from the table of Student's-t values (see below)
   for a particular confidence level, and the degrees-of-freedom of the measurement.
3. Test the null hypothesis by comparing the two \( t \) values. If the calculated value is
   greater than the table value \( (t_{\text{calc}} > t_{\text{table}}) \), then the null hypothesis is false to within the
   confidence level of the table value. In this case, the means are different. That is, the
   variation from the reported value is greater than you would expect from random error
   alone, and gain or loss is likely to be real. Else, \( (t_{\text{calc}} < t_{\text{table}}) \), the null hypothesis is not
   shown to be false, and the two means are not different at the chosen confidence level.

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<th>Degrees of Freedom</th>
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Generating karyotypes

Generating CGH karyotypes

Karyotypes will be placed in the template chosen during capture. If you want to change the template used you must reprocess the cells which will destroy all karyotypes you have in the slide.

If you attached a custom species template you will need to generate a CGH classifier before you can automatically classify the cells. If you used the Normal human template you can use the default Inverted DAPI classifier or train a classifier using your own cells.

Related topics

- Karyotype a CGH cell
- Change the species template
- Select a classifier
- Train a new classifier

Karyotyping a CGH cell

Overlapped or twisted chromosomes will not produce accurate profiles and should be deleted. Large objects are deleted from the image automatically so the ratio is not affected by non-chromosomal material. If groups of touching or overlapped chromosomes are deleted as part of this process they may be included in the karyotype by selecting them and clicking on Chromosome. If they have been removed from the cell during processing re-threshold the cell and manually subtract the background.

When the karyotype is generated, ratios of test to reference DNA will be calculated for each chromosome pair. The resulting profiles will be drawn in the CGH Profile Image. There are two measurements that are very important for accurate profiles. Centromere and axes positions. The profiles of p and q arms are calculated separately so the positions of centromeres are very important. Check their positions by turning them on in Customize and adjust their placement if needed.

The position of chromosome axes is critical for calculating profiles. Place a check in the Axis box of Customize to display the axes. To correct or re-draw an axis, select the chromosome and click on Draw axes. Draw a line down the center of the chromosome using standard drawing methods. The profile will be updated whenever an axis or centromere is re-positioned.

Tip - It is easier to karyotype a metaphase with only the inverted DAPI image displayed. Turn off the fluorochrome and ratio display options from Customize before karyotyping. The exception to this is the fourth fluorochrome which is used to help identify pairs.

Creating custom CGH classifiers

You can build custom classifiers that use your cells as the template for automatically classifying chromosomes. Axis and centromere positions are critical for profile calculations. Move centromeres to the correct position by dragging them, then re-draw any incorrect axes.
using the **Draw Axes** command. If you build a custom classifier, make sure the position of your centromeres and axes have been checked and corrected if necessary.

**To redraw an axis:**

1. Place a check in the **Axis** box of **Customize**. When a CGH karyotype is loaded in the main window this command will draw a line where the axis of the chromosome should be.
2. Check through the chromosomes for axes that are not drawn right.
3. Select the chromosome that needs correcting.
4. Click on **Draw Axes**.
5. Click the left mouse button to start then draw a line down the center of the chromosome.
6. Right click to end drawing. The new axis will be displayed.

![Correcting the axis](image)

**Related topics**

- Training Standard Reference Intervals

**Profile and display options**

**Profiles and display options**

The profiles calculated from the ratio of test to reference DNA are displayed in the CGH Profile image. The **CGH Profile Display** dialog box controls the way the profiles are drawn and which profiles are displayed. It also allows you to choose the method you want to use for identifying aberrations; using fixed limits (CGH) or dynamic standard reference intervals (High Resolution CGH).

You can enlarge the profile of a single class by clicking on it with the right mouse button. This enlarged view, or **CGH Expanded Profile**, shows the same graph and profiles as the Profile Image but in larger format. Within the **Expanded Profile** the **Thresholds** view provides another way to interact with the profiles to determine the thresholds of amplification and deletion.

**Related topics**

- Work with the CGH profile image
- Use the CGH and HRCGH Profile Display dialog
- Work CGH Expanded Profiles view
- Work with the Expanded Thresholds view
Manual registration of CGH fluorochromes

CGH and HRCGH Profile display dialog options

Load a CGH profile image in the main window and click on the CGH Profile tool button. The CGH Profile Display dialog will open with options for what profiles are displayed and for the range of lines or axes drawn on the graphs.

When the dialog is closed, the current set of display settings is saved and the next cell that is loaded will be displayed using the same settings. Also the settings for Slide option and Axes will be applied to all subsequent cells analyzed.

The HRCGH-2 features are enabled via toggle buttons in the CGH Profiles Display window. HRCGH-2 processing modifications will be applied to all new cases captured when HRCHG-2 is toggled on. When the dialog is closed, the current set of display settings is saved and the next cell that is loaded will be displayed using the same settings. Also the settings for Correct for unreliable hybridizations and Confidence limits will be applied to all subsequent cells analyzed.

New HRCGH features in CytoVision 3.6 are circled in red:
The new **HRCGH-2 features** are enabled via toggle buttons in the Slide Option section of the dialog.

HRCGH-2 can be turned on/off for the actual case by the **HRCGH-2 This Case** toggle. When **HRCGH-2 This Case** is toggled on, the case will be automatically converted to use the HRCGH-2 processing modification but be sure to have **be sure to have an archived copy ready if you have done many corrections to chromosome axes and afterwards want to restore the original analysis**, because all chromosome axes of all cells are recomputed during the conversion.

HRCGH-2 can be set as default for all new cases captured and processed on the system by the **HGCAGH-2 New Cases** toggle.

<table>
<thead>
<tr>
<th>Chromosomes</th>
<th>Displays the individual chromosome profiles for the current cell.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell average</td>
<td>Displays the average of each chromosome pair in the current cell.</td>
</tr>
<tr>
<td>Slide average</td>
<td>Displays the average of each chromosome pair for every cell in the slide. The n=7 number under each pair is the actual number of chromosomes used to generate this profile.</td>
</tr>
<tr>
<td>All chroms</td>
<td>The All chroms toggle button is provided to display profiles for all chromosomes (yellow) in the current slide.</td>
</tr>
<tr>
<td>Confidence</td>
<td>Displays the confidence limits used to determine the presence of aberrations. Choose a value between 95-99.99%. Please see the topic <strong>About Confidence Limits</strong> if you are unsure about which value to use.</td>
</tr>
<tr>
<td>Thresholds</td>
<td>These adjustable thresholds are used for marking possible gains and losses in the test DNA using the confidence limits for comparison. Locations along the profiles will be marked when the confidence limits and the threshold ranges do not overlap. Red bars to the left indicate deletions; green bars to the right indicate amplification. The comparison is done with the slide average when no confidence limits are displayed. Either <strong>Fixed Limits</strong> or <strong>Std. Reference Intervals</strong> can be used as threshold limits. See <strong>Interpreting CGH results</strong> for details about using thresholds.</td>
</tr>
<tr>
<td>Fixed Limits</td>
<td>When selected, two straight lines will be placed symmetrically around ratio 1.0. Use the spin controls to set the limits.</td>
</tr>
<tr>
<td>Std. Reference Intervals</td>
<td>Uses dynamic <strong>Standard Reference Intervals</strong> to mark aberrations. Select the appropriate set of intervals in the combo box. You should generate a separate custom reference interval for each protocol used. For high resolution CGH you should use <strong>Std. Reference Intervals</strong>.</td>
</tr>
<tr>
<td>Fit</td>
<td>Fits the standard reference interval automatically to the sample. This</td>
</tr>
<tr>
<td><strong>Automatically</strong></td>
<td>should only be used for samples with a limited number of gains or losses in the test DNA. Removing the check from this option will display the slider that is used to <strong>manually fit the intervals</strong> for samples that cannot be used with <strong>Fit Automatically.</strong></td>
</tr>
<tr>
<td>-------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>HRCGH-2 This Case</strong></td>
<td>The <strong>HRCGH-2 This Case</strong> toggle identifies if a case is currently HRCGH-2 or using the previous CGH mode. When used in conjunction with Change Options it allows the user to convert between the two. This provides a quick mechanism to confirm the effects of the new changes on old cases. (The conversion does not affect chromosome classification or centromere location - only chromosome axis and ratio profile- have an archive copy ready if you have done many corrections to chromosome axes and afterwards want to restore the original analysis.)</td>
</tr>
<tr>
<td><strong>HRCGH-2 New Cases</strong></td>
<td>The <strong>HRCGH-2 New Cases</strong> toggle instructs CytoVision to apply HRCGH-2 processing modifications to all new cases captured and processed on the system as default. If this toggle is switched off all cases will continue to be created using the previous CGH mode.</td>
</tr>
<tr>
<td><strong>Correct for Unreliable Hybridizations</strong></td>
<td>Selecting this option automatically excludes areas where the results are unreliable.</td>
</tr>
<tr>
<td><strong>Apply to all cells</strong></td>
<td>Applies the option <strong>Correct for Unreliable Hybridizations</strong> to all cells in the slide. <strong>Note</strong> - When analyzing cases processed by an earlier version of CytoVision, this processing will also generate the pseudo ideograms displayed in <strong>CGH Expanded Profile</strong> and the <strong>MultiCell</strong> viewer.</td>
</tr>
<tr>
<td><strong>Fixed Axes</strong></td>
<td>Displays the same fixed ratio ranges for all of the pairs. Use the minimum (left arrows) and maximum (right arrows ) controls to specify the range displayed. This can be useful for determining the approximate amount of amplification or deletion as you can see the value of the threshold where the peaks occur.</td>
</tr>
<tr>
<td><strong>Minimum spin controls (left)</strong></td>
<td>Controls the minimum threshold used to display deletions. Use the arrows to select increments of 0.25, or enter a value from 0-1.00 in the text field.</td>
</tr>
<tr>
<td><strong>Maximum spin controls (right)</strong></td>
<td>Controls the maximum threshold used to display amplifications. Use the arrows to select increments of 0.25, or enter a value from 1-8.00 in the text field.</td>
</tr>
<tr>
<td><strong>Full range</strong></td>
<td>Allows variation of threshold ranges between chromosome pairs. Each</td>
</tr>
</tbody>
</table>
Related topics

- About Expanded CGH profiles

Manual registration of CGH fluorochromes

Manual registration of CGH fluorochrome images is enabled whenever a CGH metaphase is placed in the main display window.

Clicking the Registration tool button opens the control shown below and displays the metaphase with the Reference color and the DAPI object boundaries. This control allows you to modify alignment of the fluorochrome images in relation to each other and the DAPI objects to correct for any automatic registration errors or to specifically allow exact registration on one metaphase area or chromosome. When you click OK, the chromosome ratio profiles are recalculated and updated in the profile diagram.

Keyboard controls are available for manual registration.

- Alt+R selects "Register Reference"
- Alt+T selects "Register Test"
- Arrow keys move the fluorochrome images
- 0 (or Ins) resets the movement
CGH Profile image

A CGH profile image is created for each cell at the end of capture when the metaphase and probe images are created. It is represented by the icon in the Navigator. The actual profiles that show the ratio of test to reference DNA, will not be drawn until the first karyotype is produced. Each karyotyped cell is automatically added into the profile calculations for the slide.

Graphs are drawn for each chromosome pair that consist of lines or axes. The lines are drawn in increments of 0.25 to represent ratios of test to reference DNA. The default limits of these ratios are 0.50 and 1.50. Red lines are used to show levels of deletion, green lines are for amplified regions. Profiles of the ratios are drawn on these graphs. Different profiles are available for depicting the cell, chromosome and slide average along with confidence levels and thresholds.

If the profiles extend beyond the lines of the graph, the limits of the graphs can be changed. There are a variety of options for displaying profiles and determining threshold limits available in the CGH Profile Display dialog.

The profiles can be displayed in a larger format by clicking on an individual graph with the right mouse button. These Expanded profiles have additional features to define the levels and position of aneuploidy and allow you to view the profiles in increments of 0.05.

CGH profile with Slide average and 99% confidence level displayed

Related topics
- About the CGH Ratio Image
- Display options for CGH chromosomes
- Generating CGH karyotypes
- Interpreting CGH results
Correcting for unreliable hybridizations

This option allows you to exclude regions of the genome that do not provide accurate, reliable profiles. Profile locations excluded from further analysis are displayed as gray bands for Chromosomes, Cell average and Slide average. For well hybridized slides these bands should only appear at centromeres, heterochromatic regions and telomeres, and confidence limits and trained standard reference intervals will be wide at these locations. Default exclusion (classes 19, 22, X and Y) is based on the following:

- Normalization is improved by skipping known problematic areas (i.e. telomeres, centromeres and boundary pixels; and human chromosomes with fluorochrome intensities that are known to deviate from normal levels) during ratio profile calculation.
- Automatically excludes unreliable fluorescent intensities. These are usually at locations where both test and reference DNA fluorescent intensities are low, but extremely high intensities are also excluded.
- Automatic correction for unsuppressed repetitive sequences.

Select the All option to use every class in your interval; select the Selected option to choose which classes you want to exclude, then select the classes you want to use.

Examples of CGH profiles

HRCGH-2 Features

Image processing and measurement changes (HRCGH-2 features)

- Improved background subtraction during capture processing
  This results in slightly less overall background subtraction applied for the test and reference images, but with more accurate subtraction on the metaphase chromosomes when compared to earlier CGH versions. (This part of HRCGH-2 is not applied to cells where the raw images have been deleted.)
- Improved chromosome axis determination
HRCGH2 has an improved object trimming which generates slightly longer axes on the chromosomes and improves CGH detection towards the telomeres.

- **Improved image sampling for more accurate ratio profiles**
  More regular and frequent ratio measurements increase the accuracy of the profiles for all chromosomes in the metaphase regardless of shape, size or orientation.

HRCGH-2 uses a new set of default Standard Reference Intervals, which are based on the slightly longer chromosome axes generated by the new software.

will only see one set of "Default" SRIs depending on which mode you are in. When you switch on HRCGH-2 CytoVision automatically uses these new files instead of the original shorter versions. If you turn off HRCGH-2 CytoVision automatically reverts to using the original versions.

**Important**
If you have built your own Standard Reference Interval (SRI) files and wish to make use of HRCGH-2 you will need to re-generate your SRI files. See CGH topic - Using standard reference intervals. This can be done by the following simple procedure:

1. Load the case(s) used for the original SRI files
2. Switch on HRCGH-2 This Case in the CGH Profile Display dialog, and Apply Options
3. Reload each cell and correct any axes if necessary.
4. Add each cell to a new SRI file - use a new name to distinguish from your old file.

The CGH profiles due to the slightly longer chromosome axes generated by HRCGH-2 are illustrated below. Note that the current set "Default" SRIs expects that most of the CGH profiles looks like the illustration, it is therefore not advisable manually to make the chromosome axes so short that the short grey band at the profile ends disappears.

![CGH Expanded Profiles](image-url)

HRCGH-2 is off  
HRCGH-2 is on
**CGH Expanded Profiles** allow for more detailed viewing of the profiles. Click on a chromosome pair in the **CGH profile image** with the right mouse button and an enlarged view of the profile will be displayed. The axes are labeled and the increased size makes it easier to view the relationships between the axes and the profiles. Then number of cells used to calculate the profiles is shown underneath (n=?). The standard ISCN ideogram and a pseudo ideogram will be displayed next to the graph.

Copy an expanded profile by holding the **Ctrl** button down while dragging it into a window. Print an expanded profile by copying it into an image window first.

Additionally expanded profiles have the **Thresholds** option which displays the peaks next to an ideogram in bar graph format. This allows for rough estimates of the relative position of abnormalities. It also allows you to see if any individual cells differ significantly from the slide average. When you open the **Thresholds** view the CGH Profile display options will open as well so you can adjust the settings.

The **Confidence** option can be used to quickly identify which chromosomes profiles lie outside the slide confidence intervals. For example, to identify "stray chromosomes" which can be deleted from the karyotype.

<table>
<thead>
<tr>
<th>Profile</th>
<th>Displays the same profile seen in the CGH Profile Image but in a larger format with labeled axes.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thresholds</strong></td>
<td>This is an alternative method for viewing the ratios of amplifications and deletions on a finer scale than with the profiles. Use the threshold limit selectors to visualize the actual values in increments of 0.05. See topic - Threshold view of CGH expanded profiles for details.</td>
</tr>
<tr>
<td><strong>Confidence</strong></td>
<td>For identification of chromosome profiles that lie outside the slide confidence intervals. For example, to identify &quot;stray chromosomes&quot; which can be deleted from the karyotype. See topic - Confidence view of CGH expanded profiles</td>
</tr>
<tr>
<td><strong>Show chromosomes</strong></td>
<td>Place a check in the box to display the chromosomes from the group selected in the profile image. The chromosomes will be shown with the display options that are selected in <strong>Customize</strong>.</td>
</tr>
</tbody>
</table>
Related topics

- Interpreting CGH results
- About CGH profile display options

Thresholds view of CGH expanded profiles

<table>
<thead>
<tr>
<th>Profile</th>
<th>Switches back to the profile view.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual chromosome bars</td>
<td>The black lines represent the areas where the actual profile is outside the thresholds. Each set of lines is labelled with the cell's name.</td>
</tr>
<tr>
<td>Red bars</td>
<td>Red bars represent the areas where the slide average profile crosses the deletion threshold.</td>
</tr>
<tr>
<td>Green bars</td>
<td>Green bars represent the areas where the slide average profile crosses the amplification threshold.</td>
</tr>
<tr>
<td>Ideogram</td>
<td>An ideogram of the chromosome class you are working with is displayed so the relative position of the peaks can be compared to bands.</td>
</tr>
</tbody>
</table>

Confidence view of CGH expanded profiles
### CGH Expanded Profile

![CGH Expanded Profile Diagram](image)

<table>
<thead>
<tr>
<th>Profiles outside: 99.5% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Profile icon]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Profile</th>
<th>Switches back to the profile view.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thresholds</td>
<td>Switches to the thresholds view.</td>
</tr>
<tr>
<td>Individual chromosome bars</td>
<td>The black lines represent the areas where the actual profile is outside the thresholds. Each set of lines is labeled with the cell's name.</td>
</tr>
<tr>
<td>Red bars</td>
<td>Red bars represent the areas where the slide average profile crosses the deletion threshold.</td>
</tr>
<tr>
<td>Green bars</td>
<td>Green bars represent the areas where the slide average profile crosses the amplification threshold.</td>
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<tr>
<td>Ideogram</td>
<td>An ideogram of the chromosome class you are working with is displayed so the relative position of the peaks can be compared to bands.</td>
</tr>
</tbody>
</table>

### Related topics

- [About CGH Expanded profiles](#)
- [Interpreting CGH results](#)

### Customize CGH Display options

Additional display options are available for CGH metaphases. The DNA fluorochrome images can be overlaid on the inverted DAPI chromosomes. Turn display options on or off from Customize.

<table>
<thead>
<tr>
<th>CGH ratio</th>
<th>Green and red are displayed in areas of amplifications and deletions. These are merely visual tools to show where areas of abnormalities are on the actual chromosomes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CGH test</td>
<td>Overlays the probe image of the test DNA fluorochrome on the metaphase.</td>
</tr>
</tbody>
</table>
**CGH reference**

Overlays the probe image of the reference DNA fluorochrome on the metaphase.

**CGH fluo4**

Overlays the probe image of the fourth fluorochrome on the metaphase. This display option will only be enabled if the cell was captured with a fourth fluorochrome to help in identifying chromosome pairs. This option only appears if fluo4 has been captured.

### About pseudo ideograms

The pseudo ideogram is based on the average of all chromosome profiles for the inverted counterstain, test or reference image depending on the currently selected display options in Customize. Pseudo ideograms are used in the CGH Expanded Threshold dialog and MultiCell viewer to help locate the position of possible abnormalities.

![Pseudo ideograms](image)

*Inverted counterstain only*  
*CGH test and reference fluors turned on*

**Note** - If the pseudo ideograms are not displayed, apply the **Correct for Unreliable Hybridizations** option in the CGH Profile Display dialog.

### Interpreting CGH results

Confidence limits or slide average profiles can together with a suitable set of selected thresholds automatically mark possible gains and losses along the ideograms. Results are determined by comparing the ratio of test to reference DNA for a sample against a set of thresholds considered to be representative of a normal sample. There are two types of thresholds you can use. Fixed limits use adjustable ratio threshold lines placed symmetrically around ratio 1.0. For example ratios of less than 0.8 would be marked as a loss and 1.2 or above would be marked as gain if you chose Fixed Limits of 0.80-1.20.

The recommended method uses dynamic standard reference intervals which use the systematic variation seen in normal samples. That is, a normal sample does not usually give a straight line profile of ratio 1.0. The pattern of this systematic variation will vary between laboratories and protocols used so the ability to generate your own reference intervals has been provided. When the chosen thresholds do not overlap either the slide average of the CGH profiles or the corresponding confidence limits, the location is marked and designated as being either a loss (red) or a gain (green) in DNA.

The following are examples of abnormalities detected with high resolution CGH using standard reference intervals that are not seen when using fixed limits.
You should select an appropriate level of thresholds and confidence values for interpreting these marks depending on the use of the CGH analysis. Remember that narrow limits and intervals may yield false positives, and wide limits and intervals may yield false negatives.

CGH profile with 99.5% confidence level (yellow) and corresponding Standard Reference Intervals (black). Note the red bar where these do not overlap indicating a loss of DNA in that region.

Related topics

- About Confidence limits
- Using fixed limit thresholds
- About standard reference intervals
- Using MultiCell to compare chromosomes on a slide

About confidence limits

Confidence limits provide the most accurate information and you should base your analysis on these limits. They are a recognized statistical indication of how accurately the slide profile reflects the real abnormalities in the test DNA. Confidence limits are calculated using student t-statistics.

For any given point on the slide average ratio profile, the width of the confidence interval (distance between the confidence limits) indicates the variability of the ratio measurement about that point. A narrow interval indicates small variation and a high degree of confidence in the ratio value. A wide interval indicates that there is too much variability between the chromosomes analyzed to get accurate, reliable results.
The confidence values (95%-99.9%) allow us to refine this measurement further. They indicate what proportion of the measurements are represented by the confidence interval. At higher values you can be assured that the intervals are a true representation of the variability around that point. However, when test and standard reference intervals are used to identify aberrant regions (where the intervals do not overlap), choice of confidence value can affect the detection: at 95% small non-aberrant fluctuations may be marked as aberrant (false positives), but at 99.9% small abnormalities might not be marked (false negatives). Confidence values are selected in the CGH Profile display dialog.

Related topics

- Using fixed limits
- Standard reference intervals
- Interpreting CGH results

Using Fixed Limits

When you choose the Fixed Limits option for displaying the threshold adjustable fixed ratio threshold lines are placed symmetrically around ratio 1.0. Use the slider provided to select the proper ratio thresholds based on the ratio of tumor to normal DNA for your sample.

If there is a trisomy in the test DNA, a ratio of 3(test):2(control) or 1.5:1 would be expected in the area of the amplification. For a monosomy the ratio would be 0.5:1.

These ratios would work for samples with purely tumor DNA. It is assumed that there will be 50% contamination of the sample with normal cells. The default thresholds of 1.25 for amplification and 0.75 for deletion are chosen to compensate for this contamination. If your samples are not 50:50 tumor to normal DNA, the ratio thresholds should be recalculated.

**Fixed limits set at 0.80-1.20**

Related topics

- Use dynamic Standard reference intervals

Standard Reference Intervals

About standard reference intervals
It has been shown that the CGH ratio levels of normal DNA vary systematically on each side of ratio 1.0 along the CGH profiles. This systematic variation can be used as a reference for whether or not CGH ratio variations represent gains or losses in test DNA. This model is termed dynamic standard reference intervals. The pattern of variation is found to be similar between slides that have been processed using the same protocol by the same laboratory. The degree of variance (ratio distance to 1.0) can change but the pattern remains similar.

The standard reference intervals look like an average of the confidence intervals of the cells which were used for the training. For some CGH protocols the systematic variation appears as different degrees of the same general pattern and have therefore to be fitted dynamically to the actual case. This can be done either automatically or by a user adjustable scale factor -3 to +3, of which value 0.0 means no scaling. The width of the Standard Reference Intervals will correspond to the chosen confidence limit.

**New HRCGH-2 Standard Reference Intervals**

**HRCGH-2** uses a new set of default Standard Reference Intervals, which are based on the slightly longer chromosome axes generated by the new software. You will only see one set of "Default" SRIs depending on which mode you are in. When you switch on HRCGH-2, CytoVision automatically uses these new files instead of the original shorter versions. If you turn off HRCGH-2, CytoVision automatically reverts to using the original versions.

**Important**

If you have built your own Standard Reference Interval (SRI) files and wish to make use of HRCGH-2 you will need to re-generate your SRI files. This can be done by the following simple procedure:

1. Load the case(s) used for the original SRI files
2. Switch on HRCGH-2 This Case in the CGH Profile Display dialog. and Apply Options
3. Reload each cell and correct any axes if necessary.
4. Add each cell to a new SRI file - use a new name to distinguish from your old file.

**Related topics**

- Interpreting CGH results
- CGH Profile Display options
- Using standard reference intervals
- Training standard reference intervals
Using standard reference intervals

When you use standard reference intervals, any degree of systematic variation along the CGH profiles is modelled by scaling the systematic deviations by a constant factor which fits the model to the ratios of the test slide at locations representing normal DNA. This scaling factor is dynamically set for each slide analyzed. The scaling factor can be fit automatically or adjusted manually.

**Fit Automatically** will automatically calculate the scaling factor for you but should only be used when there is a limited number of gains and losses in the test DNA. For samples with large numbers of aberrations you should fit the reference intervals manually.

When you remove the check from **Fit Automatically** a slider will appear. Use the slider to adjust the thresholds manually. Use chromosome classes that you know to be normal to determine the best setting. Adjust the slider so that the known normal classes are not marked for any abnormalities.

A default set of standard reference intervals has been provided with the system. This set is based on female DNA using the direct labelling protocol from Rigshospitalet where the method was developed. Before using this set of intervals you should test at least 20 normal cases and find no false positives or false negatives. It is recommended that you train your own standard reference intervals for the most reliable results.

**Important**

If you have built your own Standard Reference Interval (SRI) files and wish to make use of HRCGH-2 you will need to re-generate your SRI files. This can be done by the following simple procedure:

1. Load the case(s) used for the original SRI files
2. Switch on HRCGH-2 This Case in the CGH Profile Display dialog, and Apply Options
3. Reload each cell and correct any axes if necessary.
4. Add each cell to a new SRI file - use a new name to distinguish from your old file.

**Related topics**

- About standard reference intervals
- CGH Profile display options
- About high resolution CGH analysis
- Training standard reference intervals

**Training standard reference intervals**

As the pattern of systematic variation for normal CGH profiles will vary between protocols and laboratories it is important to train your own standard reference intervals. When training standard reference intervals the recommendations are:

- Each set of intervals should be trained using at least 10 representative slides with 10 metaphases analyzed per slide.
- Train a set of intervals for each protocol you use.
CytoVision 3.6

- The normal fluctuations of the CGH ratios should not be too small or too large compared to the laboratory standard. Don't include slides that differ significantly from your norm.
- Normal fluctuations should represent different degrees of the same general pattern.
- For X and Y chromosome analysis, individual sets should be generated for female test DNA vs. female reference DNA and for male test DNA vs. male reference DNA.

To train a standard reference interval, individual cells are added using the same Train dialog as is used for classification. Once an interval has been generated its name will appear in the combo box in CGH Profile Display.

💡 Tip - Backup your CGH standard reference intervals using the archive and restore tools.

🔍 About standard reference intervals
RxFISH

For Research Use Only. Not for Use in Diagnostic Procedures.

RxFISH overview

RxFISH uses standard probe capture techniques to generate color banded chromosomes for
karyotyping. This is done by hybridizing labeled primate DNA to human DNA. A raw image is
captured for each fluorochrome then the images are processed and combined to produce the
color banded metaphase. This metaphase can be easily karyotyped using standard methods
and tools.

The kit comes with four fluorochromes. Each fluorochrome is captured as a black and white
image. The colors seen in the final image are assigned by you during initial capture setup, and
are applied as part of the thresholding process.

In normal FISH capture it is common to assign colors to fluorochromes that match those seen
in the microscope, but in RxFISH the colors assigned are different in order to maximize the
number of bands you can see.

Capturing RxFISH images

Capturing RxFISH images

Capturing RxFISH is a three step process. Fluorochromes are configured to optimize the
visibility of the bands. Then raw images are captured, processed and tested for quality. Those
cells that have passed the quality control tests will have metaphase and probe images built
from the raw images.

No special methods are used to adjust the contrast of the live image but the amount of
banding data that can be seen is important. If you are unfamiliar with capturing probe images
you will find general information in Capturing Probes.

Related topics

- Set up for RxFISH capture
- Learn about the quality control tests

Capturing the cells

Capturing the cells

Configuring the fluorochromes correctly is necessary for RxFISH. If the wrong fluorochromes
are used you will not be able to karyotype the cells.

If you want to use a custom template for your karyotypes you must select it before capturing
the cells. Use the Species Browser button in the Fluorochrome Selection panel to select a
template.

Customize contains some options that can improve the quality and speed of capture. There are
some other tips that will make capture easier and help keep exposure times short.
Configuring RxFISH fluorochromes

The fluorochromes used for capturing RxFISH images must have specific names or a karyotype cannot be generated. If they do not exist in the selection list already, create fluorochromes with the names RX-DAPI, RX-FITC, RX-Cy3 and RX-Cy5. When creating the fluorochromes, use the following color settings. Different color settings can be used, but these have been selected as the best for viewing all of the bands.

### Recommended settings for RxFISH fluores

<table>
<thead>
<tr>
<th>Fluorochrome</th>
<th>Red</th>
<th>Green</th>
<th>Blue</th>
<th>Gamma</th>
</tr>
</thead>
<tbody>
<tr>
<td>RX-Cy5</td>
<td>255</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>RX-Cy3</td>
<td>0</td>
<td>255</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>RX-FITC</td>
<td>0</td>
<td>0</td>
<td>255</td>
<td>1</td>
</tr>
<tr>
<td>RX-DAPI</td>
<td>60</td>
<td>60</td>
<td>60</td>
<td>1</td>
</tr>
</tbody>
</table>

The order that fluorochromes are captured in can be very important in RxFISH. Capturing DAPI first can severely bleach the Cy5 making it difficult to capture. For this reason it is best to capture the fluorochromes in the order shown. To set the order of fluorochromes, add them to the Fluorochrome Selection panel in the order you want to capture them in.

#### Fluorochrome Selection Panel setup for RxFISH

![Fluorochrome Selection Panel](Image)

### Customizing RxFISH capture

The automatic capture tools that control contrast adjustment and thresholding are enabled as well as two additional features used in RxFISH. Some options in Customize are disabled when capturing in RxFISH mode as they are not needed.

If you are using a quad dichroic block and an Applied Imaging filterwheel to capture all of the RxFISH fluorochromes, you should not see any registration errors. Switch the Auto Register Images toggle off to speed up image processing during capture. Leave it on if different blocks are being changed manually.

If you have a Z-axis motor and controller fitted to your microscope switch the Auto Focus Offset toggle on to automatically remember the focus adjustment for each fluorochrome.
Switch Use Last Capture on to prevent double exposures. This can be particularly important when working with long exposure times.

Turn Mode settings on to have your RxFISH settings automatically remembered and loaded.

**To manually threshold the DAPI image**

1. Click on the cell name in the Image Capture dialog.
2. Click on Threshold.
3. Enter a value in Background subtraction that is equal to half of the chromosomes width in pixels (usually 15-20).
4. Click on Subtract.
5. Adjust the sliders with or without Contrast Stretch.
6. Click on Accept.

To bring up the Image Capture dialog if you are not currently capturing the cells click on the slide in Analysis. Switch to the Capture screen.

**Note** – It is not mandatory that you use background subtraction when thresholding, however it is recommended.

**Tips for RxFISH capture**

- Use the FITC filter for scanning slides. Other filters can be used, but DAPI can severely bleach the Cy5 making capture difficult.
- Make sure the Fluorochrome Selection panel is configured for the RxFISH fluorochromes.
- If you are using a quad-dichroic block for capture, turn off Auto Register Images in Customize.
- Focal planes are usually slightly different between the fluorochromes used for RxFISH. If a focus motor is installed on your microscope then use Auto Focus Offset automatically make focus adjustments for you during capture.

**Quality control and review**

**Quality control and review**

Once all of the fluorochromes in a cell have been captured the processing of the cells begins. This process runs in the background so you can continue to capture cells. The background is subtracted and the raw images are checked for quality and content of data. If one or more of these tests fails the cell is marked as rejected by the system.

The Image Capture dialog box is used to view the cells and open the Cell Measurements panel. Cell Measurements is used to accept or reject cells and to see the results of the tests. Part of the background subtraction process is the elimination of large objects that are judged to be debris. If a clump of chromosomes is thrown out as part of this process rethreshold the image using manual Background Subtraction.

**Related topics**

- Use the RxFISH Capture panel
- Work with Cell Measurements

**RxFISH Image Capture**
CytoVision 3.6

Click on a cell to load the unprocessed metaphase image in the window and open RxFISH Cell Measurements. RxFISH allows you to change the pass or fail status of a cell if you think it is good enough to analyze. Change the status from RxFISH Cell Measurements. If the system is unable to create a valid metaphase from the raw images, the cell will be deleted even if it is marked for keeping.

Click on Batch Complete to create the metaphase and probe images.

💡 Tip - If you want to restart processing click on Reprocess any time during capture. This can be used to reevaluate cells with Auto Register Images on if you have cells fails because of registration errors.

**RxFISH Cell Measurements**

When a cell is selected in the RxFISH Image Capture panel the RxFISH Cell Measurements dialog will open with the list of quality control tests and how the captured cells' parameters compare with the preset thresholds. To display the definition of a test from within the program click on the ? to the left of its name. Some test definitions also list the results for each fluorochrome individually.

The pre-set thresholds supplied with the software have been derived empirically from good quality cells. You may find that these thresholds are too stringent or possibly too loose. Change the preset thresholds by entering a new value over the existing number. To restore the original values click on Defaults then Continue.

If a cell has failed but in your opinion it is good enough to analyze, click on Keep. A ✅ will appear by the cell in the RxFISH Image Capture panel. Likewise if a cell has passed but you do not want to keep it, click on Discard and a ✗ will be displayed.
**Related topics**

- General use of Cell Measurements

**RxFISH in Analysis**

**RxFISH in Analysis**

Once your cells are captured you are ready to start karyotyping. Sometimes identifying the boundaries of chromosomes can be easier if you turn the color display off and work with just the inverted DAPI image.

Use the **Fluorochrome Selection** panel to select and view fluorochromes, and to determine whether the metaphase or probe image is in the main window. They look identical in RxFISH.

If you want to train your own classifiers make sure that you correct the axes of the chromosomes first. This will affect the accuracy of the classifier.

**Related topics**

- Turn the color display off
Generate karyotypes
Correct the axes of chromosomes
Train your own classifier

To display inverted DAPI only

Click on the Fluorochrome selection button display just the inverted DAPI image without any of the probe colors.

Using the Fluorochrome Selection panel

To display the fluorochromes individually or in different combinations, use the Display toggles of the Fluorochrome Selection panel. Place checks in the Display column by the names of the fluorochromes you want to see or work with. This is helpful if you need to see the actual banding of each fluorochrome image.

The label in the Fluorochrome Selection panel changes depending on the type of image that is loaded. When the metaphase is in the main window it will read RxFISH, when the probe image is loaded it will say Probe. Use this label to easily deduce which image is loaded.

Related topics

General information about the Fluorochrome Selection panel

Generating a karyotype

The methods and tools used to create RxFISH karyotypes are the same as they are for normal karyotyping. The classifier automatically defaults to the built-in RxFISH classifier whenever a RxFISH metaphase is loaded.
Tip - If an overlapped area is hard to see through the fluorochrome colors, turn off the Show fluors display option in Customize. Then only the inverted DAPI image will be displayed.

Related topics
- Using the cutting tools
- Training a classifier
- Manually classifying cells

Correcting the axes

You can build custom classifiers that use your cells as the template. Since the axis is used in identifying chromosomes, you can correct the axes of RxFISH chromosomes. If you build a custom classifier, make sure the position of your centromeres and axes have been checked and corrected if necessary.

1. Place a check in the Axis box of Customize.
2. Check through the chromosomes for axes that are not drawn right.
3. Select the chromosome that needs correcting.
4. Click on Draw Axes.
5. Click the left mouse button to start then draw a line down the center of the chromosome.
6. Right click to end drawing. The new axis will be displayed.

Correcting an axis
MFISH

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Introduction to M-FISH

You can use either the combinatorial or a combinatorial ratio (COBRA) labeling method to do M-FISH.

- The combinatorial method looks for the presence or absence of a fluorochrome.
- COBRA M-FISH also looks at the amount, or ratio of the fluorochromes in conjunction with a binary label.
- The fluorochromes used and the way they are combined is setup by the user before capture. This information is kept in the Fluomap.

There are two different color display images in M-FISH. One is the combination of the colors used to capture the probes. The other is the class image. The class image assigns a unique pseudocolor to each chromosome. The color used is chosen and assigned in the Fluomap along with the combination of fluorochromes.

COBRA M-FISH

COBRA M-FISH (combinatorial ratio) uses proportional labeling to achieve more labels out of a given number of fluorochromes versus combinatorial M-FISH where fluorochromes are either 100% labeled or not labeled at all. With COBRA M-FISH, just 2 labels FITC and TRITC for example, 5 chromosomes could be labeled with the following ratios:

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>FITC</th>
<th>TRITC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>67</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

- Individual histogram peaks can be created for each of the 5 chromosomes. In order to get 24 distinct peaks, COBRA M-FISH uses 12 ratios of 3 fluorochromes plus a binary label. This gives 12 peaks with the label on and 12 with it off.
- From these peaks, classes can be set for each the chromosomes, and M-FISH style classification colors can be painted over the chromosomes.

Combinatorial M-FISH

The combinatorial approach to M-FISH uses 5 fluorochrome labels to achieve 24 unique combinations. For example, the probe which attaches to chromosome 1 could be labeled with FITC alone, chromosome 2 with Cy7 alone, chromosome 5 with Cy3 and Cy7 and so on building up binary combinations as shown.
So anything which was imaged with five filters and found to contain fluorescence from TRITC, Cy3, and Cy5 must be chromosome 19 material. The pixels in the original counterstain image could be classified with a certain marker color to reflect this. Alignment of the captured fluorescent images is obviously important here, data from a pixel in one fluorochrome image must be compared with the equivalent pixels in the other fluorochrome images.

### Set-up and capture cells

#### Capture in M-FISH

The first step in M-FISH capture is adding the fluorochromes to the **Fluorochrome Selection** panel. Then if you are using a template other than Normal human you must select it from the **Species Browser**. Once you have done this you are ready to configure the **Fluomap**.

The **Fluomap** is a table that contains the hybridization details for each chromosome class. It is also used to select the pseudocolours used to label the classes. You can save time by saving commonly used sets of fluorochromes, species templates and **Fluomaps** using **Save List**.

As the raw images are captured they are processed and tested for quality. If there is not enough data in the raw images to create a metaphase they will fail testing. The test results for each cell will be shown in the **Image Capture** dialog box.

### Related topics

- ![See the topic in Probe capture > Fluorochromes > Configure the Fluorochrome Selection panel](image)
- ![Select a species template](image) for MFISH
- ![Configure the Fluomap](image)
- ![Work with the Image Capture dialog box](image)
- ![Read tips for capturing M-FISH cells](image)

### Selecting a species template for M-FISH

In M-FISH the system must know how many classes of chromosomes there are and what sex the sample is before it can process the captured cells. You must select a species before configuring the Fluomap and capturing the cells. The current species will be displayed at the top of the panel.

Use the **Species Template Browser** button to select the right template. The Fluomap will open after your selection is made. The sex chromosomes will be labeled correctly if you marked them as sex chromosomes in their **Class Properties**.
If you mistakenly choose the wrong template or want to change the template during capture see the topic What to do if you capture cells with the wrong template for help.

**Notes**

- In M-FISH centromere location is important for processing and for configuring the Fluomap. Make sure that the appropriate option for Metacentric, Submetacentric or Acrocentric has been chosen in the **Class Properties**.
- If you do not see the Species Browser button open a case first, then select M-FISH mode.

---

**About the Fluomap**

**About the Fluomap**

The system needs to know which fluorochromes are used to identify each chromosome class. The Fluomap is table that allows you to setup your own combinations. When M-FISH mode is selected in Capture, the Fluomap button will appear in the Fluorochrome Selection panel.

Before setting up the Fluomap, the Fluorochrome Selection panel needs to be configured and if you are capturing a species other than normal human the template needs to be attached using the Species Browser button. The Fluomap will open automatically.

Click on Fluomap to open the table directly. Your fluorochromes will appear in the left column. The numbered columns represent each of the chromosomes. You can split a column by clicking on the number at the top. When split, the p and q arms are treated separately. Make sure you change the class color for one of the arms.

Choose which method of M-FISH you are using and whether the sample is male or female. If combinatorial is chosen just click in the fluorochrome boxes that each chromosome is labeled with. If you are using the COBRA method, you must tell the label the fluorochrome used as the binary label by clicking on its name with the right mouse button. Then use the slider to enter the percentage of fluorochromes that each chromosome is labeled with. Move the slider to the value you want, then click in a box to enter the value. Clear all will remove all entries from the table.

The colors used for the classification image can be changed. Select the colored box and move the red, green and blue sliders until you have the color you want. If you want to return to the original colors click on Default colors.

Save a Fluomap by clicking on Save List in the Fluorochrome Selection panel. The list of fluorochromes, species template and the Fluomap will all be saved. This is a Fluomap configured for combinatorial M-FISH.
Fluorochrome names

The names in this column come from the Fluorochrome Selection Panel. You must build your list of fluorochromes there before you can create the Fluomap.

Map cells

A column of cells will be displayed for each chromosome group present in the species. If you label the p and q arms separately click on the chromosome number in the top row to split the column (combinatorial method only). Mix a new class color for one of the arms. Recombine them by clicking on the number again.

Binary label

You must mark one fluorochrome as the binary label when using COBRA method. Mark the binary label by clicking on the name of the fluorochrome with the right mouse button.

Class colors

Each chromosome class has an associated unique pseudocolor used in the classification image. Use the default colors or create your own with the RGB sliders.

RGB sliders

To change the class color of a group click on the chromosome number and use the red, green and blue sliders to mix a new color.

Default colors

Restores the default class colors.

Ratio slider

When configuring a COBRA Fluomap use this slider to enter percent labeled values for fluorochromes. Just click in a cell to apply the selected value.

Combinatorial/COBRA method

Choose between combinatorial or COBRA M-FISH methods before configuring the map cells. Switching modes will clear any data in the cells.

Sex designation

Used to specify whether the sample is male or female.

To configure a combinatorial Fluomap

1. Add your fluorochromes to the Fluorochrome Selection panel.
2. Click on Species Browser to select a template other than Normal Human.
3. Select Combinatorial in the Fluomap.
4. Select the sex of the sample.
5. For each chromosome class, click in the rows of the fluorochromes used to label that class.

If you will be using this combination of fluorochromes again, use Save List to save the Fluomap and species template with the list of fluorochromes.

To configure a COBRA (combinational) Fluomap

300
1. Add your fluorochromes to the Fluorochrome Selection panel.
2. Click on Species Browser if you want to select a template other than Normal Human.
3. Select COBRA in the Fluomap.
4. Select the sex of the sample.
5. Click on the name of the binary label with the right mouse button. The entire row should turn black.
6. Set the slider to 100.
7. For each row click in the columns of the classes that are labeled 100% with that fluorochrome.
8. Set the slider to another percentage and mark those columns. Continue until the cells are all marked.

If you will be using this combination of fluorochromes again, use Save List to save the Fluomap and species template with the list of fluorochromes.

**To change chromosome class colors**

1. Open the Fluomap.
2. Click on the class color box.
3. Move the red, green and blue sliders until you have the desired color.
4. Click on Done and use Save List if you want to save the change to the stored file.

**Show chromosomes by M-FISH class**

Show a selection of chromosomes in the M-FISH class display using the M-FISH Fluomap dialog.

1. Load an M-FISH karyotype into the main display window.
2. Click on the pseudo class button to toggle on the pseudo class color display.
3. Click on the Fluomap button to open the Fluomap window.

![Fluomap Window](image)

*Chromosome 1 selected in Fluomap*

4. Click in the color tab of the class you want to display.
5. A gray arrow is now positioned under the selected colored class box in the M-FISH fluormap.
6. Click on the gray arrow. A black arrow is show on the Fluomap window above the class and only that class is now displayed in the karyotype image.
Chromosome 4 selected in Fluomap

**Note:** Chromosomes that have regions of the selected chromosome pseudo colors will also appear in the karyotype window. See topic M-FISH Cleanup on how to minimize this cross over pseudo color.

7. To display more than one class, click in the color tab of another class. A gray arrow will appear under the selected class.
   a. Click on the gray arrow under the class. The selected class is now added to the karyotype display and a black arrow appears above each selected class.

8. To remove a selected class display, click on the associated black arrow. The class will disappear from the karyotype view.

9. To display all pseudo classes in the karyotype window, remove all black arrows from the M-FISH fluomap.

10. Click on Done to close the Fluomap window and return to the original pseudocolor karyotype display.

**Save a Fluomap**

1. Select the fluorochromes and configure the Fluomap.
2. Close the Fluomap and click on Save List in the Fluorochrome Selection panel.
3. Enter a name for the list.
4. Click on Save.

Fluorochrome lists and Fluomaps are saved as a single file. You cannot change the fluorochromes selected for capture without reconfiguring the Fluomap as well.

**Tips for capturing M-FISH images**

- Scanning slides with the DAPI filter can bleach some fluorochromes like Cy5. Use a filter like FITC instead.
- If your chromosomes are getting cut by the automatic background subtraction and thresholding, you can manually threshold the DAPI counterstain image as you capture an M-FISH cell by switching off Auto Threshold in the Customize Capture dialog.
- After configuring a Fluomap, save it as part of a fluorochrome list. Then when the list is loaded your Fluomap will already be configured.
- If you are using a single cube for capture or do not have problems with registration errors leave Auto Register Images turned off. Image processing is quicker if the system is not trying to correct registration.

**M-FISH Image Capture dialog**

Click on a cell name to load an unprocessed class image into the main window. You can load the raw images and manually subtract the background if the images have had too much or too little background removed. You cannot change the status of M-FISH images.

When Batch Complete is pressed the metaphase and probe images will be built from the raw images.

**To manually threshold M-FISH counterstain**

M-FISH cells are automatically thresholded during capture, however you can load and rethreshold the counterstain image by doing the following:

1. Click on the cell name in the Image Capture dialog.
2. Click on Threshold.
3. Enter a value in Background Subtraction that is equal to half of the chromosomes width in pixels (usually 15-20).
4. Click on Subtract.
5. Adjust the sliders with or without Contrast Stretch.
6. Click on Accept.

To bring up the Image Capture dialog if you are not currently capturing the cells click on the slide in Analysis. Switch to the Capture screen.

The DAPI counterstain image can also be manually thresholded as you capture an M-FISH cell. This helps to ensure that terminal ends of chromosomes are not removed. To access this feature, switch off Auto Threshold in the Customize Capture dialog.

**Note** - It is not mandatory that you use background subtraction when thresholding, however it is recommended.

**Analysis**
CytoVision 3.6

M-FISH in Analysis

The Fluomap has some additional features in Analysis that can aid in identifying abnormalities. You can change the class (pseudo) colors on a live display, limit the classes displayed and quickly label sections of chromosomes in karyotypes. These features are enabled when a karyotype is generated.

There are four ways that you can view an M-FISH metaphase. With the class (pseudo) colors, the tint colors, the probe colors or the inverted DAPI image.

Related topics
- Generate M-FISH karyotypes
- Work with the Fluomap
- Change the display options
- Pseudo Class
- Tint Class
- Expected class color bars
- M-FISH composite viewer
- M-FISH Cleanup

Using the Fluomap in Analysis

In Analysis you can use the Fluomap to change the class colors and attach labels to the chromosomes. This can be helpful if you have similarly colored chromosomes involved in an abnormality. You can return to the default colors by clicking on Default colors.

Use the Fluomap to display a single class color. This makes it easier to see if there is a translocation in the cell. You can also attach the class labels to chromosomes with a single mouse click.

If you want to print the Fluomap or add it to an image, just hold the Ctrl key down and drag the Fluomap to an image window. You can copy it to the clipboard as a bitmap for use in other applications.

To change class colors:
1. Click on Fluomap.
2. Click on the chromosome class you want to change.
3. Move the red, green and blue sliders until you have the color you want. The display will change as you move the sliders.
4. Click on Done and use Save List if you want to save the change to the stored file.

Return to the default colors by clicking on Default colors.

To attach class labels:
1. Open the Fluomap.
2. Move the mouse pointer over the chromosome you want to label.
3. The number will change as you move over the different colors.
4. Click with the left mouse button to attach the label to the image.
5. Click the right mouse button to flip the label horizontally.

Labeling M-FISH chromosomes

To display a single class:
You can only use this feature if M-FISH class is turned on in Customize.

1. Click on Fluomap.
2. Click on the class number you are interested in. The arrows will move to that group.
3. Click on one of the arrows.

To print a Fluomap:
1. Open the Fluomap.
2. Place the mouse pointer over the map.
3. Hold the Ctrl key down and drag the Fluomap to an image window by holding the left mouse button down.
4. Open Print.
5. Load the Fluomap into the print window by repeating steps 2-3 with the image window.

Now you can save the Fluomap as part of an image or clear the window.

Generating an M-FISH karyotype

The methods and tools used to create M-FISH karyotypes are the same as they are for normal karyotyping. The classifier automatically defaults to the built-in M-FISH classifier whenever a M-FISH metaphase is loaded. The trainable classifier does not function for M-FISH cells.

Tip - If an overlapped area is hard to see through the colors, turn them off by removing the check in M-FISH class and Show fluors in Customize. This will display only the inverted DAPI image.

Related topics
- Using the cutting tools
- Manually classifying cells

Display options

Use the toggle buttons in the operations toolbar to change the image display to the probe colors, tint colors, class colors (pseudocolor) or inverted DAPI.

Pseudo Class toggle button- (formerly called M-FISH class) is used to toggle between the pseudo class display and the inverted DAPI image.
CytoVision 3.6

The Tint Class toggle button - is used to toggle between the pseudo class colors combined with the DAPI image and the inverted DAPI image.

Related topics

M-FISH in analysis

Pseudo Class

Click on the pseudo class button in the operations toolbar to display the M-FISH class colors (pseudocolors).

Pseudo class window

Click the pseudo class button again to display the inverted DAPI image.
Pseudo class inverted DAPI window

Related topics

M-FISH in Analysis

Tint Class

The tint class view shows the pseudo-class colors combined with the DAPI banding. This can be used with both M-FISH metaphase and karyotype displays.

Click on the tint class button to display a tinted image.

1. The Tint Gamma slider below the karyotype image allows you to adjust the gamma setting to bright or darken the image.
2. Moving the slider to the far right will result in pure pseudocolor display.
3. Clicking the tint button again will display the inverted DAPI image.
Tint class window

Tint class inverted DAPI window
Related topics

M-FISH in Analysis

Using the Fluorochrome Selection panel

To display the fluorochromes individually or in different combinations, use the Display toggles in the Fluorochrome Selection panel. Place checks in the Display column by the names of the fluorochromes you want to work with. This is helpful if you need to see the actual banding of each fluorochrome image.

Fluorochrome Selection panel setup for viewing DEAC only

Tip - The label in the Fluorochrome Selection panel changes depending on the type of image that is loaded. When the metaphase is in the main window it will read M-FISH, when the probe image is loaded it will say Probe. Use this label to easily determine which image is loaded.

Related topics

General information about the Fluorochrome Selection panel

Expected Class Color Bars

The expected class color bars (based on the M-FISH fluomap) are shown on either side of the class number. These help you quickly distinguish abnormal from normal regions.
Related topics

M-FISH Composite Viewer

This feature allows you to display a composite view of a selected chromosome in the karyotype. The dialog displays the chromosome in a variety of formats:

- A composite fluorochrome image
- A pseudo colored M-FISH class image
- An inverted DAPI image
- Each individual fluorochrome image
- A profile plot of each of the fluorochromes along the length of the chromosome.

The composite view window:

1. Load a karyotype into the main display window
2. Select a chromosome
3. Click on the Composite view button.
4. The Composite Viewer dialog opens:
   - The selected karyotyped chromosome is displayed as a color composite image as well as all the individual fluorochrome images of the chromosome. The profile displays the various intensities of each fluorochrome involved.
   - The expected color of the M-FISH class is shown to the left of the classification name.
   - The expected fluorochromes of the chromosome is shown to the right of the classification name.
• The profile lines are drawn in the respective fluorochrome colors
  Example: In the dialog shown, the bottom half of the chromosome appears in
  the correct classification color (orange). The colored boxes to the right of the
  classification name and the individual fluorochrome displays show that the
  chromosome is labeled with MFISH-Green, and MFISH-Aqua but there is an
  abnormality to the top of the chromosome labeled with MFISH-Red.

5. To zoom the window:
   • Press the middle mouse button.
   • Use the scroll bars to move around the display.

6. One or more horizontal lines may be drawn across the window to follow a certain band
   or area:
   • Right click where you want the line drawn.
   • The line will be displayed across the whole window.
   • To remove the lines, select ‘Clear all lines’.

7. One or more of the fluorochrome profiles can be toggled on/off in the profile plot
   • Left click on the fluorochrome to be removed.
   • Left click again on the fluorochrome to return it to the profile.

8. The composite view can be quickly changed to a different chromosome by selecting
   another in the karyotype window.

9. The composite view can be copied to a flex screen.
   • Place the cursor in the composite view window. Hold down the Ctrl key and left
     mouse button, drag the cursor into a blank image window. (If there are no
     available blank windows, clear one of the windows before copying the
     composite view.)

   • With the flex in the main display window, select the background button, ☑,
     to display a black background.

10. Click on the Done button to close the Composite View window.

**Related topics**

M-FISH in Analysis
M-FISH Cleanup

The M-FISH class pseudocolor display is often blotchy or patchy due to background and cross-hybridization. The M-FISH Cleanup tool allows for the cleanup, in the display, of any of the chromosomes on the karyotype. This is done by reclassification of pixels that only make up a small percentage of the chromosome.

Note: This feature is designed to work with combinatorial M-FISH and does not apply to COBRA M-FISH.

Below is an example of a patchy karyotype

Patchy Karyotype

To improve the M-FISH karyotype display:

1. Select a chromosome in the karyotype window for clean up.

2. Click on the M-FISH cleanup button in the operation toolbar -

3. The dialog lists the class composition of the selected chromosome, with the highest percentage at the top and a breakdown of other classes displayed underneath. The inclusion of other classes may possibly be due to cross-hybridization, background, an abnormality, or an edge effect.
4. Move the slider to choose the percentage you wish to remove from the display. Example - setting the slider to 15% will remove all chromosomes that have less than 15% in the list.

5. Select Adjust to cleanup the selected chromosome.
   Select Reset to return the chromosome to its original display.
   Select Apply to All to apply the % cleanup to all chromosomes in the karyotype.
   Select Reset All to return all chromosomes in the karyotype to their original display.

6. Clicking the right mouse button on a chromosome in the karyotype window will clean up that chromosome using the percentage set in the slider in the MFISH Cleanup window.

7. To clean up several chromosomes using the same percentages, click the chromosome in the karyotype window with the right mouse button.

8. To reset a certain chromosome, click on the chromosome in the karyotype window with the middle mouse button.

9. Select Done to close the MFISH Cleanup window.

For Example:
An adjustment using a 15% cutoff has been applied to only the two #4 chromosomes (15% was chosen because the window above shows the highest % that is not chromosome number 4 to be 9.17%). The % display for chromosome #4 changes to 100%:
Only chromosome #4 now appears smooth:

Selecting **Apply to All** will apply the selected % (15% in the example) to all chromosomes in the karyotype image and results in a new display for all chromosomes. The chromosomes that are patchy will have a mixed chromosome class composition of greater than 15%:

Select **Reset All** to return all chromosomes to the original karyotype image.
Related topics

M-FISH in Analysis
Scanning systems

Scanning systems overview

The scanning package has features for finding cells or metaphases on brightfield or fluorescent slides. The software packages adds the Scan and Review screens in addition to Capture and Analysis. The Scan screen contains controls for scanning slides and calibrating the system hardware. Review has controls for reviewing the cells found, sorting them and selecting cells for capture. Buttons for Scan and Review will be placed next to Capture and Analysis on the toolbar at the top of the screen.

There are two ways to start scanning - using the Scan Wizard or use the Scanning for Cells method.

Tip: The Scan Wizard is the recommended scanning method.

Once the cells have been found you can view, sort and mark them in the Review screen, then capture them automatically in Capture. There are two methods of analyzing scanned cells on-screen and recording the data. In Capture use the Count dialog to quickly record the count, sex and results of analysis for cells; use Aberration Scoring to record abnormalities in breakage studies.

Related topics

- Get started
- Scan for cells
- Review scanned cells
- Capture cells
- Use the Count dialog
- Use Aberration Scoring

Hardware basics

Hardware basics

There are a few basic things to learn before you start scanning for cells. The calibration of the stage is important for accurate relocation of cells. You can use the hand controls on the stage to reposition before you do any scanning but make sure that you never adjust the stage manually during a scan. Whenever you use the hand controls always Reset the stage before using the system again.

The correct camera position is important for cell relocation. This is critical if you are doing any spot counting.

To control the stage use the on-screen controls or the keyboard shortcuts.

Related topics
CytoVision 3.6

- Control the stage
- Move to and select bays
- Position the camera correctly
- Reset the stage

**Controlling the stage**

There are two ways to control the stage; using the on-screen controls and/or the joystick. Not all systems will have a joystick.

**On-screen controls**

Control the stage and focus motor with the slider controls on-screen or with the keyboard. Move the stage from left to right using the slider underneath the main window. Or use the left and right arrow keys on the keyboard.

Move the stage along the length of the slide using the slider to the right of the main window or with the up and down keys on the keyboard. Adjust the focus using the slider set out away from the main window or with the less-than and greater-than arrow keys.

The keyboard controls move the stage and focus motor by set amounts or step sizes. There are 4 levels of movement for the keyboard controls, 1μm - 10μm - 100μm and 1000μm. You can cycle through these levels using the right Ctrl and Shift keys. Press the Shift key on the right side of the keyboard once to increase the step size for the stage. Each press will increase the amount a bit more. Decrease the amount of movement by pressing the Ctrl key on the right side of the keyboard. Like the Shift key, this is additive so each press will decrease the step size a bit more.

Focus has three step sizes; 0.6μm, 3μm and 10μm. To decrease the step size press the Alt key on the left side of the keyboard. To increase it press the Shift key on the left side of the keyboard.

**Stage controls**

- ![Left](left_icon.png)
- ![Right](right_icon.png)
- ![Up](up_icon.png)
- ![Down](down_icon.png)

**Stage increment of movement (right side of keyboard)**

- ![Ctrl](ctrl_icon.png)
- ![Shift](shift_icon.png)

Decrease stage Increase stage

**Focus controls**

- ![Focus out](focus_out_icon.png)
- ![Focus in](focus_in_icon.png)
Focus increment of movement (left side of keyboard)

<table>
<thead>
<tr>
<th>Alt</th>
<th>Shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease focus</td>
<td>Increase focus</td>
</tr>
</tbody>
</table>

**Note** - If you move the stage manually click on **Reset** before setting any scan areas. You can focus manually without having to hit reset, but once the focus map has been set DO NOT adjust the focus manually.

**Joystick**

The joystick posts control stage movement and focus. The four buttons move the excitation and transmission filterwheels.

**Warning** - Never use the joystick and the on-screen controls simultaneously. This can cause erratic behavior in the system.

Moving to and assigning bays to slides

Depending on your stage there are four or eight pictures shaped like slides at the bottom of the screen. Each one represents a bay on the stage. Move to a bay by clicking on one of the slides with the right mouse button.

The small crosshairs mark the position of the bay datum. You can move to the bay datum by clicking on the crosshair.

Assign a slide to a bay by selecting the slide in the **Navigator** and clicking on **Set** in the bay control picture. Slides with saved scans are attached to a bay in the same way. Detach a slide by clicking on **Clear**, then on the bay control picture. The shaded area will be green when a slide is set for scanning and red if it has already been scanned. When a slide is attached to a bay the case name and slide number will be displayed above the bay picture.

Bay control pictures
The bays are numbered starting from the left. As you look at the microscope the bay at the farthest left side is bay 1.

**Note** - A slide must be attached to a bay before the scan area and focus can be set.

**Camera position**

If the camera is not turned correctly, the system will not be able to accurately relocate cells after a scan. The front face of the camera should be parallel to the stage. If you want to turn the camera for some reason, then the system *XY-scaling* should be calibrated.

*Front of a COHU camera*

**Reset the stage**
**Reset** will force the stage to find its home position. Always reset the stage after using the hand controls to move the stage. Every time the system is switched on you should reset the stage position and focus.

💡 **Tip** - If you have an external focus motor you can manually focus the microscope before clicking on Reset. This will 0 the focus on the correct plane. **Note** - This should be the only time you adjust the microscope manually without resetting the Home position.

**Calibrating the system**

**Calibrate the system**

There are 4 different system calibrations depending upon use of the system.

- The **Wizard calibration** is used outside of the CytoVision program for accurate relocation in the Review program. This calibration is described in a different section of the help.
- The **Bay Datum** calibration sets a fixed position for each bay in the stage.
- The **System calibration** is used to calibrate the scanning portion of the application.
- The **Microscope conversion** is optional and is used for relocation within the CytoVision program.

Launch the CytoVision software, open the scan screen 📽️ and click on the calibration button 🛠️

**Calibration tools**

These are the tools that calibrate the hardware used by the scanning package. Calibration should only need to be done at initial installation or if the stage and been removed and re-installed. Click on the Calibration button in the tool bar to open the Finder Calibration dialog.

The dialog box contains two options; **Set Bay Datum** and **System Calibration**.

- **Set Bay Datum** sets the exact position of each slide bay.
- **System Calibration** contains the setting for stage position and image size relative to on-screen pixels. If you have moved your camera or stage and are having difficulty in relocating cells, then re-calibrate the system.
CytoVision 3.6

What to you want to do?

- Set the bay positions
- Calibrate the stage

Setting bay datum positions

Calibrate the system using the Applied Imaging Calibration A slide. This calibration procedure is done in each bay of the stage. Calibration should only need to be performed when the system is first setup, or if the stage has been moved.

Launch the CytoVision program - Click on the CytoVision icon and Log in as administrator

Note: The 'A' image will appear upside if the camera orientation is correct.
1. If the CytoVision program is not open, launch the CytoVision software, then open the scan screen.

2. Click on **Reset (Home) Stage**.
3. Place the Applied Imaging Calibration A slide in bay 1; the first bay on the left side of the stage. (diagram of a stage)
4. Adjust the focus and light to get an image. Only adjust the focus using the joystick or onscreen controls.
5. Move the stage to position bay datum position on the calibration slide (England Finder position C59) using the on-screen stage controls. Do not move the stage manually once **Reset Stage** has been pressed.

6. Click on the **Calibration button** to open the Finder Calibration dialog.
7. Click on **Set Bay Datum**.
9. Enter a 1 in the **Bay Number** field.
10. Press **Save**.
11. Place the slide in the second bay just to the right of the first.
12. Repeat steps 2-9. Then enter a 2 in the **Bay Number** field.
13. Repeat steps 1-12 for the other bays entering their corresponding number in the **Bay Number** field.

Or, alternately, the user can calibrate the bay datum positions using an England Finder slide.

1. Click on **Reset (Home) Stage**.
2. Place an England finder in bay 1; the first bay on the left side of the stage. (diagram of a stage)
3. Adjust the focus and light to get an image. Only adjust the focus using the joystick or onscreen controls.
4. Move the stage to position C59* on the England Finder using the on-screen stage controls. Do not move the stage manually once **Reset Stage** has been pressed.
5. Click on **Calibrate**.
6. Click on **Set Bay Datum**.
7. Enter C59 for the England Finder Reference (or W21 if a different slide orientation is used).
8. Enter a 1 in the **Bay Number** field.
9. Press **Save**.
10. Place the slide in the second bay just to the right of the first.
11. Repeat steps 1-6. Then enter a 2 in the **Bay Number** field.
12. Repeat steps 1-10 for the other bays entering their corresponding number in the **Bay Number** field.

**System calibration**

This calibration is done after setting the **bay datum positions**. This step only needs to be done for one bay.

Stage calibration is necessary for proper travel of the stage and accurate relocation of cells.

1. If CytoVision is not open, launch the CytoVision software, open the scan screen.
2. Place the Applied Imaging calibration slide in a bay.
3. **Reset** (home) the stage.
4. Click on the Calibration button.
5. Click on **System Calibration** to open the dialog window (see below).
6. Move the stage to bring the 32 micron image calibration pattern into view. Focus and adjust the contrast so the pattern is clearly visible.

7. Click on **Live** in the System Calibration window.
8. Click on **Capture** in the System Calibration window.
9. A line should be drawn between two rectangles in the same column. If not, reposition the image calibration pattern and repeat steps 4-7. The length (in units of pixels) will appear in the **Feature Separation (pixels)** field.
10. Enter *64* in the **Feature Separation (microns)** text field.
11. Move stage to a cross hair most commonly used for calibration (see image above).
12. Click on **X-Y Scaling** in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.
13. Click on **X-Y Backlash** in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.
14. Click on **Focus Backlash** in the System Calibration window. The focus will be adjusted until the setting is found and entered in the field.
15. Enter a name for the calibration data in the **Filename** text box.
16. Click on **Save**.
17. Click on **Apply**. This will apply the currently selected calibration settings to the system.
18. Click on **Done** to close the window.
System Calibration dialog

Scan Wizard

The Scan Wizard

The scan wizard provides a simple way to setup scanning on a number of slides. It allows the setup to be saved and then reused as a template at a later date. This system of saving scan settings is called a SuperScan.

Brightfield and fluorescent scans may be created using the wizard and scanning may be set for metaphase or interphase finding (SPOT) applications.

The wizard may be started by clicking the wizard button within the finder screen.

What do you want to do?
Creating a scan with the scan wizard

After you have clicked the wizard button in the finder screen you will be presented with the following dialog:

Open an existing superscan by clicking on the open an existing scan radio button and selecting a scan from the list.

To create a new superscan:
Click the Create a new scan radio button and type in a name to save the scan under.

If you wish you can click in the Notes field and type some text to act as a reminder about the content of the scan. This can be useful when opening a superscan for later use but it is not compulsory. Notes can be added or amended at a later date.

Now click either Fluorescent or Brightfield depending on the kind of scan you wish to do:

<table>
<thead>
<tr>
<th>Mode</th>
<th>Available finder applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brightfield</td>
<td>Metaphase finder blood \ Metaphase finder bone marrow</td>
</tr>
<tr>
<td>Fluorescent</td>
<td>Metaphase finder blood \ Metaphase finder bone marrow \ Interphase finder</td>
</tr>
</tbody>
</table>
(prescan)
Interphase finder
(framelist)

Now click the slide orientation button † to indicate the way you prefer to orient your slides on the stage.

You can now click Next to continue to the next part or Cancel if you wish to stop and do nothing more, in which case the superscan will not be saved.

Related Topics

❓ The Scan Wizard
❓ Scan Wizard Case Assignment

Finder Application Assignment

For each slide with a case and a scan area it is possible to assign a finder application. To the right of the page is a list of available applications and the content of this list will be dependent upon the Mode assignment.

⚠️ Note - You cannot mix metaphase and interphase applications in the same scan.

⚠️ Note - You cannot mix metaphase blood and metaphase bone marrow applications in the same scan.

⚠️ Note - You can mix interphase finder prescan and interphase finder framelist applications in the same scan.

Left click on a slide to assign it the currently highlighted finder application.

Click the Clear button to remove a finder application from a slide.
If the finder application is an Interphase application, then the assay selector dialog will appear. Clicking on an assay in the assay selector grid will assign the assay to the slide. The name of the assigned assay will appear on the slide.

If the finder application is a Metaphase application, then the classifier selector will appear. Selecting a classifier from the drop down list will assign it to the slide. The assign classifier will appear on the slide. If no classifier is selected then the default classifier will be used.

Note - If all slides are setup as interphase framelist slides, clicking Next will move to the Interphase Framelist page in the scan wizard.

Slides will turn green once they have been assigned an application.

Click Back to go back scan area setup page.
Click Next to continue.
Clicking Cancel will stop and do nothing more.

Related Topics
- The Scan Wizard
- Scan Area Setup
- Focus and Camera Setup

Focus and Camera Setup

Once the slides have been assigned a finder application, the next step is to setup camera parameters for each slide and also set an initial focus position for each slide.
For each slide, left click to highlight the slide. The stage will move to the correct bay position, and the camera setup dialog will appear.

Adjust the focus position to get the slide image in focus using the Z slider focus control in the finder screen. Adjust the camera parameters for the slide using the camera setup dialog.

If desired you also set the focus frequency so that an autofocus operation is carried out every few frames during scanning. Leaving the value as zero will prevent any autofocus from taking place. It is best to use autofocus when the slide is very uneven or a large scan area is being used.

If you wish to stop scanning after a specific number of cells or metaphases have been captured you can enter a value in the **Stop After** field. This will tell scanning to stop and move onto the next slide when this number of cells or metaphases have been scanned. Leave it at zero to scan the whole scan area.

- **Apply to all** - sets the focus frequency and stop after settings to all slides in the setup.
- **Make Default** - sets the settings as the default.
- **Ignore bad focus slides** - check the box to continue scanning slides that pass and skip the slides that fail the focus map. Failed slides are flagged so the user can scan the slides at a later date.

Repeat the procedure for all slides.

- **Note** - The slide will turn green when it is setup correctly.
- **Note** - The camera parameters and stop counts are automatically and individually recorded for each slide. These are automatically saved as part of the superscan.

Slides will turn green once they have been assigned an application.

Click **Back** to go back finder application setup page.
Click **Next** to continue and start automatically focus map building. The slides will automatically be created in the navigator and attached to their assigned cases. Clicking **Cancel** will stop and do nothing more.

**Related Topics**
- The Scan Wizard
- Finder Application Assignment
- Focus Map Building
- Opening an existing superscan

**Focus Map Building**

Once the required slides have had their camera, focus and scanning parameters set the wizard will automatically start building the focus maps for the slides.

When a focus map has completed successfully, the stage will automatically move to the next slide. If the focus maps for all slides are built successfully, the wizard will automatically move to the **scan page** and start scanning.

- **Ignore bad focus slides** If the Ignore bad focus slides check box is checked, the scans will proceed on those slides that have built successful focus maps.

Clicking the **Stop Focus** will stop focusing for the current slide. You will be asked whether you wish to continue with the next slide.

If one or more slides have had their focus maps stopped or the focus map failed in any way, clicking **Next** will go to the **manual focus map page**.
Clicking **Back** will go back to the **Focus and Camera Setup Page**. This will allow you to alter any focus and camera settings and retry automated focus map building on those slides that have failed or have been stopped.

**Related Topics**
- The Scan Wizard
- The Scan Page
- Focus and Camera Setup Page

**Interphase Framelist**

If all slides are interphase framelist slides then the following page will appear:

![Prescan Setup Wizard](image)

All slides are framelist slides. No further setup required. Click Finish to save or cancel to quit without saving.

This indicates there are no further setup steps required.

Clicking **Finish** will save the superscan setup to disk and close the wizard. The slides will automatically be created in the navigator and attached to their assigned cases. Clicking **Back** will go back to the **Finder Application Assignment page**. Clicking **Cancel** will stop and do nothing more.

**Related Topics**
- The Scan Wizard
- Finder Application Assignment

**Building A Manual Focus Map**
To build a manual focus map for a slide, left click to highlight the slide.

Use the **Forward** and **Back** buttons to move between focus points in the focus map.

At each focus point use the z focus slider to adjust the focus so that the image is clear and sharp. Then click the **Set** button to fix the position.

When enough positions have been completed, the slide will turn green indicating the focus map is complete.

Click **Next** to start scanning. Clicking **Cancel** will stop and do nothing more.

**Related Topics**

- The Scan Wizard
- The Scan Page

**Opening an Existing Superscan**

To open and load an existing superscan, click the **Open an existing scan** radio button and then highlight any superscan in the adjacent list of superscans.

As soon as a superscan is highlighted any superscan notes attached to the scan will appear in the **Notes** window. These notes can be edited if required and will automatically be updated.
To find out more information about the superscan click the **Info** button. This will give additional information about the content of the superscan.

Click **Next** to continue. This will move you to the case assignment page. Clicking **Cancel** will stop and do nothing more.

**Related Topics**

- The Scan Wizard
- Scan Wizard Case Assignment
Scan Area Setup

Once a case is assigned to a slide, you can assign a scan area to it.

On the right of the page is a list of predefined scan areas. Left clicking on a slide and then clicking on one of these scan areas will assign the predefined area to the slide. Left clicking on another slide will assign the same currently highlighted scan area.

Each slide will turn green once a scan area has been assigned to it and the scan area will appear on the slide.

**Note** - You cannot assign a scan area to a slide that has no case.
Clicking **Edit** will allow you to edit or create a new predefined scan area for the slide (see Set the scan area).

Clicking **Clear** will clear the scan area for the current slide.

Click **Back** to go back to the case assignment page.
Click **Next** to continue.
Clicking **Cancel** will stop and do nothing more.

**Related Topics**

- The Scan Wizard
- Scan Wizard Case Assignment
- Assigning Finder Applications
Scan Wizard Case Assignment

Each slide must have an associated case. This page allows you to assign cases to slides.

To do this, left click to highlight a slide. The border of the slide will turn red and in the middle of the slide will appear the currently highlighted case in the navigator. In the diagram above, slide 2 is assigned case Wiz2.

To change the case for the current highlighted slide simply click on a different case in the navigator.

To clear the case for the current slide, click the Clear button.

Right clicking on a slide will allow you to name it.

Slides are green when a case has been assigned to them and grey otherwise.

If this is a new superscan:

Clicking Next will move on to the next stage of the superscan setup.
Clicking Back will move to the wizard start page.
Clicking Cancel will stop and do nothing more.

If an existing superscan has been loaded:

Clicking Next will move on Focus and Camera Setup page. If all the slides in the superscan are framelist slides then clicking Next will move to the Interphase Framelist page.
Clicking Back will move to the wizard start page.
Clicking Cancel will stop and do nothing more.

Related Topics

- The Scan Wizard
- Creating a superscan with the scan wizard
Scanning

For all slides with a focus map, scanning will automatically begin.

**During scanning:**

Clicking the stop button ■ in the scan controls will stop scanning the current slide. Hitting the start button ▶ will continue with the next slide.

Clicking the pause button ■ will temporarily pause scanning on the current slide. Hitting the start button ▶ will continue with the current slide.

**After scanning:**

Click **Back** to go back to the focus and camera setup page. This will allow you to tune any camera settings for any of the slides and then allow you to rescan.

Click **Next** will apply if you are performing a metaphase scanning application. This will take you to the finder application screen where you can re-assign classifiers to slides. Click **Next** from this screen will rescan the slides.

Clicking **Finish** will save the superscan to disk.

**Note** - Interphase framelist slides will not be scanned.

**Related Topics**

- [Focus and Camera Setup](#)
- [Classifier selection](#)
- [The scan wizard](#)
CytoVision 3.6

Scan for cells

Scan screen

The Scan screen contains the tools used to look for metaphases. The Scan toolbar is used to set scanning preferences and camera settings. Before starting you must set the slide area, sample type and focus points. After that you can start the scanning process.

The system scans for cells by measuring the contents of the current field of view against parameters saved in a classifier. You can use the default classifier or create your own.

Scan work area

The main image window is used to display the live image when setting up for a scan or to view the progress of a scan. The toolbar is used to set up the parameters used to scan.

The bay control pictures serve a couple of functions; to connect slides on the stage with slides in the navigator and to provide a way to quickly move between slide bays.

Scan controls start, stop and pause the scan. The progress bar shows how much of a scan area has been completed. The slide graphic shows information for the active slide. This includes case information, scan area and markers for items found.

The buttons in the center of the screen control the live image display, filterwheel and hardware reset. Live video turns the live image on so you can adjust camera settings and scan area if needed before starting a scan. If have a motorized filterwheel it will remain in the blank

338
position until a scan is started. Use the filterwheel button to move to a filter for displaying a live image to set up for a fluorescent scan.

**Using the Scan toolbar**

These are the tools used to setup scanning preferences, settings for individual scans and the system calibrations tools.

<table>
<thead>
<tr>
<th>Icon</th>
<th>Tool</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>✅</td>
<td>Customize</td>
<td>Used to set mode, number of cells and sample type.</td>
</tr>
<tr>
<td>📊</td>
<td>Classifier Selection</td>
<td>Used to select the scanning classifier.</td>
</tr>
<tr>
<td>📷</td>
<td>Camera Setup</td>
<td>The tools used to adjust live image contrast in scanning.</td>
</tr>
<tr>
<td>🕵️</td>
<td>Scan Area</td>
<td>Used to select an area of the slide to scan.</td>
</tr>
<tr>
<td>🕵️</td>
<td>Focus Map</td>
<td>Creates a focus map used to maintain focus throughout the scan area.</td>
</tr>
<tr>
<td>🕵️</td>
<td>Calibration</td>
<td>The tools used to calibrate the stage after installation.</td>
</tr>
</tbody>
</table>

**Setting up to scan manually**

**Setting up to scan**

Before you can start scanning a slide the system needs to know:
- which slide the cells belong to
- the bay that slide is in
- the type of sample you are scanning
- the classifier you want to use
- the proper camera settings
- the area of the slide you want to scan
- the focal plane for several points in the scan are

The sample type and scan area can be saved as default settings. If you use default settings you only need to attach a slide to a bay, adjust the camera settings and set the focus points before starting a scan.

To view a live image during set up press the **Live Video** button in the center panel.

For fluorescent slides the filterwheel button will move to the correct filter or allow you to select another filter.

**Related topics**

- Attach a slide to a bay
- Customize the scan settings and sample type
Select a classifier

Classifiers are templates that tell the system exactly what you want to look for on the slide. You may only want to find well spread, long metaphases, or you may want to find anything on the slide that is a metaphase. Click on the Name drop down list and select the classifier that matches what you are looking for before beginning a scan. A default classifier is installed on the system. You can build your own classifiers with cells from different types of samples.

For Default or classifiers created using Train, there are three buttons that change how stringently the templates will be used. Select Fine to accept only small variances from the template, Medium for average differences, and Coarse to accept large variances. If you have created a classifier manually these settings will not affect the classifier.

Camera setup

The contrast of the live image needs to be at least 50% for the system to accurately scan for metaphases. The same sliders used for adjusting image contrast in capture are used in the Scan screen. Click on Camera Setup to display the Bright, Black and Exposure sliders. These sliders work the same way in Scan as they do for regular capture. Adjust the sliders manually to achieve a good contrast. If you want the contrast adjusted automatically, click on Auto Setup. You can change the target contrast in the Scan screen by entering a new value in the Desired Contrast field. When the image looks good click on Done.

In Fluorescent mode, open the Filter panel by clicking on Change Filter. This allows you to choose which fluorochrome you want to scan for.

If you change any settings and want them to be the default, click on Save as default before clicking on Done.
Related topics

- Using Bright and Black
- Using Exposure
- Using Auto Setup

**Note** - if using a digital camera then the camera setup dialog will look like this:
Additional options include:

**Binning** - set the camera binning level. Care must be taken when changing the binning level for scanning. Make sure that the system is calibrated for the chosen level.

**Stretch** - checking this box stretches the camera image to fit the screen. This option allows you to see the whole field of view when setting camera levels and scan areas.

**Select a filter for scanning**

Filters are selected from the **Scan** screen.
1. Click on **Camera Setup**.
2. Click on **Change filter**.
3. Click on the name of the filter you want to use for scanning.
4. Click on **Apply Filter**.
5. Click on **Save as default** if you want this to be the default filter. If you only want to use the filter for this scan then just click on **Done**.

Under normal operation the filterwheel will stay in the blank position unless the slide is being scanned or the Capture Setup and focus are being adjusted. If you want a live image, place a check in **Filter** to move the filterwheel to the correct position.
Related topics

Using motorized filterwheels

Set the scan area

Use Scan Area Setup to define the region of the slide you want to scan. The slide map to the right of the main window will show the relative area you have selected. If you do not define a scan area, the default area will be used. Create a default area by setting an area and clicking on Apply As Default. To apply the change to all bays press Apply to all bays. You can save multiple default scan areas in a list using Scan Areas.

The white, arrow buttons will move the stage to the top, right, bottom and left edges of the slide. Set the position of an edge by moving to a new location and pressing the associated red set button.

Scan Areas

Used to save and load default scan areas.

Reset scan area

Reverts the settings back to the loaded default.

Move

Moves the stage to the edge of the scan area.

Set

Sets the current position of the stage as the edge of the scan area.

Note A slide must be attached to a bay before the scan area can be set. For instructions on how to attach a slide to a bay click here.

Map the focus points

Focus Map

Use Focus Map Setup when not using the Scan Wizard.
Slides are uneven surfaces and different areas of a slide will have different focal planes. In order to stay in the correct focal plane throughout the entire scan area, the system will take nine readings around the scan area and use them to determine the correct focus to use for scanning. Click on Auto Focus to have the system automatically find the focus at each point.

You can set the focus manually by using the on-screen focus slider. When the image is focused press Set. If Move is turned on the slide will advance to the next point. Use the arrows to scroll through the points to check focus in either mode.

**Note** A slide must be attached to a bay before the focus can be set.

**How to scan more than one case at once**

You can load slides from different cases and scan them at the same time. Open all of the cases you are going to scan slides for. Attach each bay to a slide using Set Case. Make sure that the correct case and slide are selected in the Navigator before you click on Set case. Set the scan area and focus maps for each slide. Turn Multi-bay on in Customize if you do not normally use it and you are ready to begin.

**Customize scanning options**

**Customize scanning options**
Use Customize Scanning when not using the Scan Wizard.

The settings in Customize are used to choose sample type and the number of cells you want to find with each scan. This is also where you tell ChromoScan whether you are scanning more than one slide at a time. The settings will be saved automatically when you exit the program.

If a whole slide is to be scanned, or if large numbers of objects are expected, then it may be useful to lower the Pause value. This tells the ChromoScan to pause the scan when it finds the number of cells entered and lets you review the thumbnail list in Review. If no value is entered here then a default of 1000 images is used. During a multi-bay scan the system will move to the next slide when it reaches the Pause after value - it is not possible to continue scanning the slide after this.

### Customize Scanning

<table>
<thead>
<tr>
<th>Mode</th>
<th>Select between brightfield or fluorescent mode of scanning. <a href="#">See topic - Choosing scan modes</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-Bay Scan</td>
<td>Turn on if you are scanning more than one slide simultaneously. <a href="#">See topic - Single or multi-bay scans</a></td>
</tr>
<tr>
<td>Remove/merge overlapping cells</td>
<td>Turn on to automatically apply Remove Overlaps. <a href="#">During a scan, the system will overlap areas of the slide slightly to avoid missing any cells. Click on Remove Overlaps to reduce the number of thumbnails that contain the same cell.</a></td>
</tr>
<tr>
<td>Pause After</td>
<td>Enter the number of cells you want the system to find before it stops scanning.</td>
</tr>
<tr>
<td>Focus Frequency</td>
<td>If you are scanning an uneven surface you can force the system to focus during the scan. <a href="#">See topic - Set the focus frequency</a></td>
</tr>
<tr>
<td>Save as Default</td>
<td>Press to save the current settings as the new default.</td>
</tr>
</tbody>
</table>

**Choose brightfield or fluorescent scan mode**
Select between brightfield and fluorescent mode from the Customize dialog. **Fluorescent** mode will work for all types of fluorescent scans.

### Scanning multiple or single bays

You can scan one or more slides together. Place the slides in any bay you want, there is no order that must be used. If you are scanning more than one slide, you will need to place a check in the Multi-Bay of Customize before beginning your scan. If you are only using one slide then leave this option turned off.

### Select an application

Select an application to determine how the default classifier will be used. Click on Customize to select an application type. **Metaphase finder (bone marrow)** uses a more advanced classification algorithm allowing for detection of metaphases in the poorest preparations, however it does significantly slow down scanning. For most preparation types (especially using a 20x lens) use **Metaphase finder (blood)**.

In spot counting capture mode two more sample types are available; **Interphase finder (Prescan)** and **Interphase finder (Framelist)**. See the section on Automatic capture of spot counting for details about these applications. These options will only appear when Fluorescent mode is selected.

### Set the Focus Frequency

If you do not want to rely on the focus map, or if you are scanning an uneven surface such as plastic slides, you can tell the system to perform an auto-focus every few frames. Just enter the frame interval in the text field. Any focus adjustments will be applied to the focus map settings. This setting can usually be left at 0.

### Slide orientation

Set the orientation of the slide graphics to match your preference for slide orientation in the stage. Both the graphic to the right of the live window, and those of the bays will change.

#### Note - Slide orientation in scanning must be the same as that used for calibrating the system, and should not change between slides or scans.

### Starting the scan

After setting up you can start scanning for cells. As the slide is scanned the progress bar will update and the number of cells found will be shown. The locations of the cells are marked with
dots on the slide graphic to the right of the window. You can switch to the Review screen and start reviewing the cells while the scan is in progress.

The length of time needed to complete a scan will depend on the sample type and the size of the area being scanned. View the live image during a scan by pressing the Live Video button. The scanning process is faster if the live video display is off.

The scanning controls start, stop and pause the scan.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="start.png" alt="Start" /></td>
<td>Starts the scanning process.</td>
</tr>
<tr>
<td><img src="pause.png" alt="Pause" /></td>
<td>Temporarily stops the scanning process. Press Start to resume scanning.</td>
</tr>
<tr>
<td><img src="stop.png" alt="Stop" /></td>
<td>Stops the scan completely.</td>
</tr>
</tbody>
</table>

### Related topics

- ![Review the cells](review.png)
- Reviewing during scanning

### Review cells during a scan

During scanning, the Review screen displays the thumbnail images that have been captured. These will vary in number and quality depending on the classifier used and preparation type. You can start reviewing these thumbnails while scanning by selecting cells and marking them for keeping or deleting.

If the number of thumbnail images becomes too large the scanning process may slow down. If this happens you can remove unwanted images in Review by marking as Delete and clicking on the Save button in Review to save the list. See the topic Marking cell status for details.

### Scanning classifiers

### Scanning classifiers

Classifiers are the files that contain the settings used by the scanning algorithms to find cells. You can create a classifier using cells from a scan or by entering the parameters manually. Once a classifier is generated you can edit any of the parameters and re-save it.

### Related topics

- ![Train classifier using cells from a scan](train.png)
- ![Edit or create a classifier manually](edit.png)

### Edit scanning classifiers

![Edit Classifier](edit.png)
The system uses a numerical template when scanning a slide to determine whether or not a field contains a metaphase. This template is known as the classifier. Custom classifiers can be created for finding different types of metaphases. Classifiers are created and edited from the **Review** screen. Choose the classifier used for a scan in the **Scan** screen.

Edit an existing classifier’s data by choosing its name in the drop down list and changing its parameters. Create a new classifier by entering a name and the values you want to use for finding metaphases. If you do not know what values to use to create a classifier, use **Train** to create a new classifier based on cells found in a scan.

### Parameter Description

**Object size**
Limits the size of each individual object within a cell. If nuclei or other large objects are being found in the scan reduce the Maximum value - if small background spots are being found try increasing the minimum value.

**Influence**
This distance in pixels determines whether or not objects are seen as being from the same cell. Very well spread metaphases may need a larger maximum value.

**Object Number**
Total number of individual objects identified. For x20 scanning a good metaphase should show between 20 and 40 objects, but normal variation is between 10 and 40 depending on spread.

**Area**
Total amount of object material the cell contains.

**Spread**
Area enclosed by the edges or borders of the cell.

---

**Tip** - If you want to create a classifier try the following values:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object size</td>
<td>5</td>
<td>400</td>
</tr>
<tr>
<td>Influence</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Object Number</td>
<td>8</td>
<td>50</td>
</tr>
<tr>
<td>Area</td>
<td>5</td>
<td>300</td>
</tr>
<tr>
<td>Spread</td>
<td>800</td>
<td>22000</td>
</tr>
</tbody>
</table>

---

**Train a classifier with images**

---

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The easiest way to create a classifier is to use cells from a scan. Use a slide with cells that are typical for the sample type you want to create a classifier for. Perform a scan and select at least 10-15 cells that look like cells you want to find with the classifier. Mark them as **Keep**. Click on **Train** and the **Train Classifier** dialog appears. The total number of cells scanned is displayed along with the number that have been marked as **Good**.

Enter a name for your classifier in the **Classifier Name** field. Click on **Make**. The data from the selected cells will be averaged and used to create a new classifier. When a classifier is created using this method, all of the data used to create the classifier is kept. This allows you to use the Fine, Medium and Coarse adjustments to alter the way the data is applied to scanning.

![Train Classifier dialog](image)

**Reviewing the cells**

**Reviewing the cells**

The **Review** screen is used to sort, label and select cells that were found during a scan.

**Related topics**

- View the images
- Sort the cells
- Work with the cell data
- Use the Review toolbar

**Review toolbar**

<table>
<thead>
<tr>
<th>Button</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Select All" /></td>
<td>Selects all thumbnails.</td>
</tr>
<tr>
<td><img src="image" alt="Deselect All" /></td>
<td>Deselects all thumbnails.</td>
</tr>
<tr>
<td><img src="image" alt="Zoom" /></td>
<td>Enlarges the thumbnail images.</td>
</tr>
<tr>
<td><strong>Edit Classifier</strong></td>
<td>Edit existing classifiers or create classifiers manually.</td>
</tr>
<tr>
<td><strong>Train Scanning Classifier</strong></td>
<td>Create scanning classifiers using cells from a scan.</td>
</tr>
<tr>
<td><strong>Sort</strong></td>
<td>Sort cells by different criteria.</td>
</tr>
<tr>
<td><strong>Remove Overlaps</strong></td>
<td>Removes overlapping or duplicate thumbnails.</td>
</tr>
<tr>
<td><strong>Microscope Coordinate Conversion</strong></td>
<td>Convert coordinates to another microscope or to England finder coordinates.</td>
</tr>
<tr>
<td><strong>Aberration Scoring</strong></td>
<td>Use a tally system to score aberrations in the cells.</td>
</tr>
</tbody>
</table>

**Viewing the images**

**Viewing the images**

The small images known as thumbnails, can be enlarged for easier viewing. Click on them to relocate cells quickly. Use the slide map view to see the relative locations of the cells on the slide graphic.

Navigate through the lists using the scrollbars or the **PgUp** and **PgDn** button on the numeric keypad.

You can load scans that have been saved and cleared using the buttons under the Navigator.

**Related topics**

- Enlarge the thumbnails
- Relocate cells
- View the Slide map
- Load a saved scan

**Enlarge the thumbnail images**

Enlarge all of the thumbnail images by clicking on **Zoom** in the **Review** toolbar.

Magnify single thumbnails by clicking and holding the middle mouse button on them. The area under the cursor will be enlarged in a small window. You can move the mouse while holding the button to change the view.
Magnified thumbnail

To relocate cells

Move the stage to a cell’s coordinates by clicking on the thumbnail image with the right mouse button.
If you are having trouble relocating cells check the following:
- The camera is correctly positioned.
- The slide is placed on the stage correctly.
- If you have removed and replaced the slide it will probably be slightly offset from its original position. Apply an offset to correct for the difference.

To load cells from a saved scan

1. Select the slide in the Navigator.
2. Click on Load.
The relocation of cells and the ability to create classifiers will be disabled. If you want to enable these features, place the slide from the scan in a bay and attach it to the bay.

View the slide map in Review

Slide view

Slide view will replace the cell data fields with a slide diagram similar to that in the Scan screen. Each cell will be shown as a color coded dot on the slide. As you change the status of a cell the color will change to reflect its new status. This provides a quick way of determining which part of the slide contains the cells you are interested in.
Sorting and marking cells

It is likely that you will want to delete some of the cells found during a scan. You also need to mark cells for capture if you want to use the automatic capture feature or for counting and slide reports. Mark the cell status using the three buttons in the Review screen.

To make reviewing cells easier you can sort them based on different criteria. There is also a feature used to delete any overlapping or duplicate images.

When you are finished reviewing the cells save the results of the scan.

Related topics

- Remove duplicate cells
- Sort cells
- Mark the status of cells
- Save a scan

Remove duplicate cells

During a scan, the system will overlap areas of the slide slightly to avoid missing any cells. Click on Remove Overlaps to reduce the number of thumbnails that contain the same cell.

Sort cells
The sorting function sorts the cells and displays both their data and the thumbnails in order. Click on **Sort** in the **Review** screen to access the sorting functions. Choose the sorting option you want then click on **Apply** to sort the cells. Place a check in **Reverse order** to display the cells from lowest to highest value.

**Sort dialog**

These are the different parameters you can use to sort the cells:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Objects</strong></td>
<td>Use to sort cells based on the number or objects.</td>
</tr>
<tr>
<td><strong>Area</strong></td>
<td>Use to sort cells based on the total area covered by chromosomal material.</td>
</tr>
<tr>
<td><strong>Spread</strong></td>
<td>Use to sort cells based on the total area covered between the edges of the cells.</td>
</tr>
<tr>
<td><strong>Objects/Area</strong></td>
<td>Use to sort cell based on the number of objects seen per unit area.</td>
</tr>
<tr>
<td><strong>Objects/Spread</strong></td>
<td>Use to sort cells based on the number of objects seen per unit spread.</td>
</tr>
<tr>
<td><strong>Area/Spread</strong></td>
<td>Use to sort cells based on the amount of chromosomal material per unit spread.</td>
</tr>
<tr>
<td><strong>ID</strong></td>
<td>Sort cells by their ID numbers.</td>
</tr>
</tbody>
</table>

**Mark cell status**

Change the status of a cell by selecting its thumbnail and clicking on **Keep**, **Delete** or **Unclass**. Click **Keep** if you want to mark a cell for **Auto Capture**, to be included in the **Slide Report**, for **Counting** or to be used for training classifiers. A green border will be drawn around the thumbnail.

Click on **Delete** to remove a cell from the scan. Cells are deleted when **Save** is pressed. A red border will be displayed around the thumbnails of deleted cells.
CytoVision 3.6

If you do not want to mark a cell for capture, but do not want it deleted from the list, leave it unmarked. If you decide to change the status of a cell marked as Keep or Delete to unclassified, select it and click on Unclass. The colored border will be removed.

The number of cells marked as Keep, Delete and Unclass will be continuously counted and displayed in the text fields to the right of the status buttons.

To choose which thumbnails to display based on their status, place a check in the display column next to the type you want to see.

![Cells status controls]

**Save results of a scan**

Save the locations, data and thumbnail images from a scan by clicking on Save. Any cells marked as Delete will be removed. You can re-load saved scans for capture and review but for the most accurate re-location of cells it is recommended that you capture your cells before removing the slide from the stage.

**Cell data**

**Cell data**

The Notes view in Review will show the coordinates of the cells along with some of their properties. Add comments to the cells and save them with the data. Much of this data will be added to the slide report along with whether a cells has been captured or analyzed from the Count dialog.

You can convert these coordinates to England finder coordinates or to coordinates for another microscope. Use Aberration Scoring to relocate cells and tally abnormalities.

**Related topics**

- Use the notes view - see topic Data fields
- Convert coordinates
- Use Aberration Scoring

**Data fields**

![Notes view]
Data fields provide information about each cell as well as an area for you to enter any notes or comments you want to save with the cell. This is the default view for the Review screen. Any colored border that is drawn around the thumbnail images is also drawn around the data for that cell. Green borders mean that the cell has been marked for keeping, red borders mean it is marked for deleting. The magenta borders are currently selected cells.

You can save comments in the Notes column to the right of a cell. Just click in the row and enter your text, then click on Save.

<table>
<thead>
<tr>
<th>Rank</th>
<th>Cell</th>
<th>X</th>
<th>Y</th>
<th>Obj</th>
<th>Area</th>
<th>Spread</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>8634</td>
<td>-29730</td>
<td>36</td>
<td>18</td>
<td>11340</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>9094</td>
<td>-30468</td>
<td>37</td>
<td>16</td>
<td>8466</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>9095</td>
<td>-31267</td>
<td>35</td>
<td>21</td>
<td>10509</td>
<td>Karyotype</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>9496</td>
<td>-39032</td>
<td>39</td>
<td>17</td>
<td>8820</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>9448</td>
<td>-37144</td>
<td>35</td>
<td>21</td>
<td>8840</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>9488</td>
<td>-35812</td>
<td>41</td>
<td>20</td>
<td>13407</td>
<td></td>
</tr>
</tbody>
</table>

About Slide Reports

When a cell is marked as Keep in the Review screen it is automatically added to the slide report. The slide report keeps track of cells that have been counted, captured and karyotyped. This information will also be added to the Notes section of the Review screen. The data is added from the Count and Karyotype Result dialogs.

Open a slide report by selecting the slide in the Navigator with the left mouse button, then double click the middle mouse button anywhere in the Navigator window. In Auto Capture, when the Navigator is disabled, click on Report in the Auto Capture dialog box.

You can edit the Analysis and Result fields by clicking on them with the right mouse button. Enter the new data and click outside of the edit box to save the change.

You can select which fields you want in the slide report by turning them on or off in the Options menu of the Slide Report dialog. Only the selected fields will be printed. Print the report by selecting Print in the Print menu of Slide Report.

To remove a cell from the report just de-select it from the Keep list in Review. However, if a cell has been captured or karyotyped you cannot remove it from the report directly. Instead you must delete it from the Navigator.

Export the data in the report as a text file by selecting Export to text file in the Print menu. You will be prompted with the standard Windows Save As dialog that allows you to name the file and select its location.

Karyotype results
This feature is mainly used by scanning system users to automatically add the results of a karyotype to the slide report. To turn Karyotype Result on click on the button in the Analysis toolbox. To turn the feature off immediately just click the button a second time. To turn it off after the current cell unloads, place a check in Don’t show this dialog again before closing the window.

If required, this feature will be setup as part of your installation such that when a karyotype is loaded in the main window the count, sex and any aneuploid abnormalities will be automatically calculated from the positions of the chromosomes. As you move chromosomes between classes in the template the result will update. The lower field can be used for adding any text such as a rearrangement. This text will be used subsequent cells and will remain in the field until you delete it.

If your system was not set up to update the automatic result whenever the karyotype changes, a button named Auto will appear in the dialog. Clicking this button will create an automatic result, however this will not be automatically updated.

Counting and analyzing cells

Counting and Analyzing cells

Count and Analyze

The Count dialog in Capture allows you to scroll through a list of cells recording the count, sex and any comments or results. This information will be added to the Slide Report automatically.

Start by marking cells as Keep in the Review screen. Then click on Count in the Capture toolbar. Using this dialog you can count and sex a cell, add stray chromosomes from fuse fields, use Inspect Cell to fully analyze a cell and capture the image.
### Scanning systems

#### Count Interface

| **Next Cell** | Saves the data for the current cell and moves to the next cell in the list. |
| **Remove Current Cell** | Removes the current cell from the list and moves to the next cell. The cell will be marked as Unclass in Review. |
| **Reset Cell** | Clears all data for the current cell. |
| **Reset List** | Clears all data for the current list. |
| **Previous Cell** | Saves the data and moves back to the previous cell. |
| **Count** | Click to begin counting a cell. **Marker Size** sets the size of the square markers. |
| **Inspect Cell** | Click to begin analyzing a cell visually. |
| **Fuse Field** | Used to add stray chromosomes to the main cell image used for counting. |
| **Live Image** | Used to search for stray chromosomes while counting or analyzing cells. Return to counting by clicking on Count or save the data and move to the next cell by clicking on Next if no stray chromosomes are found. |
| **Capture Current Image** | Click to capture the count image. |

<p>| <strong>Current cell</strong> | Displays the ID number of the current cell. |
| <strong>Count</strong> | Automatically increments for each marker placed on the image. This number is added to the Slide Report. |
| <strong>Sex</strong> | Use the buttons to record the sex of the cell. This information is automatically added to the Slide Report. |
| <strong>Notes</strong> | Enter any comments or results that you want to save with the cell. This text will be saved in the Results field of the Slide Report. If <strong>Retain</strong> is on, the same entry will be made for each additional cell until it is changed or deleted. |</p>
<table>
<thead>
<tr>
<th><strong>Progress</strong></th>
<th>Shows how many cells have been done and how many are left in the list.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Summary</strong></td>
<td>Calculates the number of cells that have been counted, analyzed with <strong>Inspect Cell</strong> and captured.</td>
</tr>
</tbody>
</table>

**Counting**

1. **Count**

Click on **Count** to begin counting a cell. The live image will be frozen. Place markers on the screen by clicking with the left mouse button. You can change the size of the markers using the **Marker Size** field. Each time a marker is added the count will automatically increase by 1. Remove a marker and decrease the count by clicking on it a second time.

When you are finished counting click on **Next**, or on the image with the right mouse button to save the data and move to the next cell.

If you think there might be stray chromosomes out of the field of view click on **Live** and use the stage controls to look. If there are stray chromosomes they can be pasted into the count image using **Fuse Fields**. If not you can click on **Count** again to continue counting or **Next** to move on to the next cell.

**Capturing fuse fields**

**Fuse Fields** is used to add stray chromosomes into the main count image. This is done by drawing a small rectangle around only the area with the chromosomes which is then pasted into the main image.

To capture a fuse field:

1. From the main image click on **Live**.
2. Search for stray chromosomes and click on **Fuse Fields** when found.
3. To draw a rectangle around the chromosomes move the cursor to one corner of the area of interest. While holding the mouse button down drag the rectangle out to define the area.
4. Release the button to save the area for pasting.
5. Repeat steps 1-4 for each area to be pasted. When finished click on the image with the right mouse button to return to the count image.
6. When you return to the count image the first paste fragment will be attached to the cursor. Position the cursor and paste the fragment by clicking with the left mouse button. The next fragment will appear at the cursor. Once the fragment is pasted you cannot move it again.
7. The system will automatically return to count mode when all of the fragments have been pasted. You can end pasting manually by clicking on the image with the right mouse button.

**Analyzing a cell**
Inspect Cell

Inspect Cell assists in the on-screen analysis of cells by providing a way to show which chromosomes have been checked. This is done by first counting the cell, then removing the markers as each class is checked. Any cell analyzed in this way will be listed as Inspected in the Slide Report.

To use Inspect Cell:
1. You must first complete the count of the cells.
2. Click on Inspect Cell.
3. Click on the marker of a chromosome class to remove the marker and show it has been checked.
4. When finished click on Inspect Cell a second time to register that the cell has been analyzed.
5. Enter any data in the Sex or Notes fields or adjust the count if it is wrong.
6. Click on Next to move to the next cell.

Note - Clicking Inspect Cell again at any time marks the cell as done. Once you have started to remove the count markers do not press Inspect Cell again until you have removed them all, as it will save the current count number - not the initial one.

Counting and analyzing cells

Capture a cell from Count

Capture Current Image

You must count the cell first then click on Capture Current Image to capture the cell as a metaphase image. Cells captured from the Count dialog will automatically be labeled with their England finder coordinates and cell ID. They will also be listed as Captured in the Slide Report. Any cell that is not captured from the Count dialog will not be included in the Summary calculation for cells captured in the Slide Report.

Any options selected in Customize will be applied during capture.

Note - Once you start to count the cell it is not possible to re-adjust the image contrast for capture - this has to be done BEFORE pressing Count, or you have to reset the cell and start again.

Counting and analyzing cells

About Aberration scoring

About aberration scoring

Aberration Scoring

Used to quickly count the aberrations in cells found during a scan. Load a metaphase list and click on Aberration Scoring. The scoring data fields will replace the cell data fields. Create custom templates with the titles of each field and save them from the scoring dialog. The data
generated can be exported as a text file that can be read by database or spreadsheet applications.

Pressing an F key on the keyboard will increment the tally for that field by 1. **Clear** will reset the data for the current cell. Use the arrows or keyboard controls to scroll through the cell list. Turn **Stage Move** on to have ChromoScan relocate the cells as they are scored. **Stage** will bring up the stage controls. Turn **Filter** on to move to the selected filter for viewing cells. Use **Change Filter** to select a different filter.

The **Print** menus enable you to print the results of the scoring. **Export** will export the data as a text file which can then be used in other applications such as spreadsheet.

**Related topics**

- Create or load a title template
- Score the cells
- Export the data
- Print the data

**Create scoring templates**

1. Click on **Set**.
2. Select the F key you want to enter a title for.
3. Enter the title in the **Title** field.
4. Click on **Set**.
5. Continue until all titles have been entered.
6. Click on **Done**.
7. Click on **Save titles** in **Aberration Scoring**.
8. Enter a name in the Titles box and click on **OK**.
Load a title by pressing **Load titles** and selecting a name from the list.

**About Aberration Scoring**

To score cells

1. Load a metaphase list and attach it the bay holding the slide.
2. Click on **Aberration Scoring**.
3. Click on a cell or use the arrow buttons to scroll through the list.
4. Press the F key that represents the abnormality you want to score.
5. Proceed to the next cell using the arrows or keyboard shortcuts.
6. Click on **Save** in the File section when finished.

**Clear** will reset all fields for a cell to null.

**Keyboard Shortcuts**

- **Shift-F(n)** will decrease the count by one for the field associated with that F key.
- * on the numeric keypad will set all values to 0 and advance to the next cell.
- + on the numeric keypad moves to the next cell
- - on the keypad moves to the previous cell

**About Aberration Scoring**

To export data

**Printing Aberration Scoring results**

There are two menus at the top of the Aberration Scoring dialog. **Print Options** lets you choose which fields you want to include in the print. **Print** lets you choose which cells you want to include and sends the results to the default printer.

<table>
<thead>
<tr>
<th>Print Options</th>
<th>Print</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔ CellID</td>
<td></td>
</tr>
<tr>
<td>✔ Stage Co-ords</td>
<td></td>
</tr>
<tr>
<td>✔ England Finder</td>
<td></td>
</tr>
<tr>
<td>✔ Comment</td>
<td></td>
</tr>
</tbody>
</table>

**Print Options menu**

- **CellID** includes the cellID.
- **Stage Co-ords** includes the position of the cell in the scanning system's stage.
- **England Finder** includes the position of the cell in England Finder coordinates.
- **Comment** includes any text written in the Comment field.
The system will test to see if the data will fit on the page in portrait layout. If it is too wide the page layout will switch to landscape automatically. The orientation will switch back to portrait at the end.

If the data is still too wide to fit on a single page in landscape orientation a warning dialog will appear. Reduce the width of the field titles or the number of fields selected and try printing again. Alternatively you can export the data as a text file and print it from another application.

Export scoring data

Data is exported a comma delineated ASCII text. You can import this type of data easily in most spreadsheet or database applications.

1. Click on Export.
2. Select a name and location for the file.
3. Click on OK.

About Aberration Scoring

Microscope conversion

Convert cell coordinates

Microscope Coordinate Conversion

The coordinates of each cell can be converted to England finder coordinates, or the Vernier units of specific microscopes. You can choose between printing the file or saving the results as a text file. Choose Good to convert only those cells marked as Keep or choose All to convert all cells.

Note: Before you can convert cell coordinates, the microscope must be added to the microscope list in the Microscope Coordinate Conversion window.

To convert cell coordinates:

1. Load a metaphase list and click on Microscope conversion.
2. Select the name of the microscope you want to convert the coordinates for.
3. Enter the length of your slide and of the England finder.
4. Choose whether you want to print or save the file.
5. Choose Good to convert only the cells marked as Keep.
6. Click on Convert.
Related topics
See topics under Metaphase finding scanning systems > Hardware basics > Calibrating the system > Microscope Coordinate Conversion

Add a new microscope

Note: Slide Calibration for Microscope Coordinate conversion
1. Click on Add in the Microscope Coordinate Conversion dialog box. A new dialog will open.
2. Enter a name for the microscope.
3. Select the units you want to convert to. Verniers are the units most commonly used.
4. Use an England finder to find the X and Y coordinates for A15 and Z50. Enter them in the fields.
5. Do the same for the scanning system microscope if they were not entered as part of the system setup.
Related topics
See topics under Metaphase finding scanning systems > Hardware basics > Calibrating the system > Microscope Coordinate Conversion

Capturing the cells

Capturing the cells

Read the topic Overview of Capture for general information about capturing cells. These topics will only deal with the capture features of the scanning system package.

There are 3 different ways to link in the scanned metaphase list and each allows different data to be used or saved.

- Manual capture using the Stage Control window.
- Automatic Capture using the Auto-capture window.
- Visual Count and analysis data recording using the Count window.

Auto Capture provides a way to mark cells for capture in the Review screen, then have the system relocate and capture the cells automatically. On-screen stage controls are provided in capture. It is important to use these or the keyboard to control the stage rather than the hand controls. The Auto Capture dialog contains the controls used to start capturing cells. Often objects that are centered under low power need to be moved slightly to be centered under high power. Save this stage offset value using Set Offset in the stage control panel.
You can also capture cells from the Count and Analyze dialog that provides additional tools for counting cells and adding data to the slide report.

**Related topics**
- Use Auto Capture
- Use the Count and Analyze dialog
- Use the stage control dialog
- Set stage offset

**Stage Control in Capture**

Access the stage controls by clicking on **Stage Controls**. A dialog with a picture of a stage will appear. Use the small sliders next to the picture to move the stage and focus. Scroll through cells marked as Keep in the **Review** screen using the **Previous** and **Next** arrows.

Use **Set Position** to change the coordinates of a single cell. Use **Set Offset** to adjust for centering between low and high power objectives.

Use **Step size** to adjust focus control. The step size can be adjusted between 0.1 and 1 micron (µm). Place a check in **Focus Enabled** to adjust focus offset as well as position. If **Focus Enabled** is off, the focus will not change when you move to another cell.

**Setting stage offset**
CytoVision 3.6

There are two reasons why you may need to apply a position offset to the coordinates of a cell. One is if a slide is removed and replaced in the bay. The second is that a cell that is in the center of the field of view under low power is often not centered when seen with a high power objective. These differences can be saved as an offset value to eliminate the need to manually center images in the capture screen.

**To save a position offset:**

1. In the **Review** screen click on a thumbnail with the right mouse button. Choose a cell that will be easy to center.
2. Go to the **Capture** screen and click on **Live**.
3. If you are correcting the offset because the slide has been moved or replaced use the 20X scanning objective. To correct for a difference between the scanning and high power objective, change to the objective you will use for capture.
4. Center the cell using the screen or keyboard controls but DO NOT change the focus. DO NOT use the stage or focus knobs on the microscope to adjust the position.
5. Click on **Stage**.
6. Click on **Set Offset**.

**Note** If you are using probes, the focus offset applied here is the difference between low and high power. To save different focal planes between fluorochromes use **Auto Focus Offset**.

**To change a cell’s coordinates**

If a cell is on the edge of the field of view and you can change its coordinates.

1. Load the cell in the capture window.
2. Click on **Live**.
3. Click on **Stage** to open the stage controls.
4. Center the cell.
5. Click on **Set Position**.

**Using Auto Capture**

**About Auto Capture**

There are two different modes for Auto Capture. One for metaphase auto capture and one for SPOT auto capture. If using the capture wizard auto capture will be started automatically on completion of the wizard.

**Metaphase Auto Capture**

**Metaphase Auto Capture** will automatically locate and capture cells that have been marked as **Keep** in the **Review** screen. Press **Auto Capture** to open the dialog box. The same buttons used in the Find screen are used to start, pause and stop **Auto Capture**.

**Pause Every Frame** allows you to stop the capture process between every cell. During the pause you can center the cell or make a focus adjustment. **Auto focus** will automatically focus each frame before capture. When it is off, you must focus manually using the slider. The range of focus for each objective is stored in a file. This range limits the distance the Z-axis will move during auto focus thus shortening the amount of time it takes to capture a frame. Use **Objective** to select which objective is being used for capture and to edit these files.

Each cell captured using **Auto Capture** will be named automatically by the system. The name will be derived from the England Finder position of the cell and its cell id from the list. For example, the cell 23 from a list which may be found at England Finder coordinates H17/2 will be given the name H17/2 _ cell 23.
It is very important to determine the relationship between the stage coordinate system and England Finder coordinate system before using this feature. To do this, use the Microscope Conversion feature to create a microscope named ChromoScan, entering the coordinates for the positions A15 and Z50 in both parts of the dialog box.

Auto Capture

Remove Current Cell
Delete the current image from the list of cells to capture, marks it as Unclass in Review and advances to the next cell.

Capture Fuse Field
Tells the system that you want to capture fuse fields for the next image.

Live Image
Gives you a live image without starting auto capture.

Slide Report
Opens the slide report.

SPOT Auto Capture

SPOT auto capture will automatically locate and scan a slide that has been setup in the finder screen as either an interphase prescan slide OR an interphase framelist slide. If the slide was setup as an interphase framelist slide SPOT auto capture automatically divides the scan area into a list of frames for automatic capture. If the slide was setup as an interphase prescan slide, the cells captured by the prescan will be optimised into a list of frames to minimise the amount of frames that need to be captured.

See Using SPOT Autocapture for more details.

Related topics
- To use Metaphase Auto capture
- Using SPOT Auto capture
- To add an Objective offset
- The capture wizard
To use Auto Capture

1. Mark the cells you want to capture as Keep in the Review screen and save the metlist. If you are capturing cells from a previous scan make sure that you attach the list to the right bay and check the position of the cells.
2. Right click on one of the cells to relocate to it. Try to choose one that is centered in the thumbnail image.
3. Switch to the Capture screen.
4. Select the capture mode you are going to use.
5. Switch to the objective you will use for capture and bring up a live image.
6. Open the Stage Controls and center the cell.
7. Click on Set Offset in the Stage Controls. You can use the scrolling arrows to check the position of the cells marked for capture and correct the offset again if necessary.
8. Click on Auto Capture.
9. Click on Start and the stage will move to the first cell. If you have selected Auto Camera Setup, Auto Threshold and Auto Sequence then the system will continue capturing cells until all cells marked as Keep are captured.
10. If you have not selected the automatic capture features then adjust the contrast and threshold as you would for normal capture. The stage will advance to the next cell as soon as thresholding is completed. Click on Pause if you want to temporarily stop the capture process. Start will begin it again. Click on Stop to cancel the capture process completely.

Capture fuse fields in Auto Capture

Capture Fuse Field

You must turn Pause Every Cell on to capture fuse images during Auto Capture. Capture Fuse Field tells the system that the next cell you capture has stray chromosomes.

To capture a fuse field:

1. When a cell with stray chromosomes comes up for capture click on Capture Fuse Field.
2. Click on Start.
3. Capture the metaphase image.
4. If there is more than one fuse needed click on Capture Fuse Field again. If not click on Start.
5. Center and focus the stray chromosomes.
6. Click on Start to resume auto capture of the next cell.

Tip - You can always add fuse fields after all the cells have been captured. This is probably easier than during Auto Capture. Just relocate to the cell, select the metaphase in the navigator and click on Live in the Capture screen. When asked choose Join (Fuse) as you would for any manually captured cell.

Objective depth of field

Suzanne will confirm windows

The system uses these settings to determine the correct range of Z-axis positions to use for focusing. A default list of your objectives will be built during the installation. You can add to
this list using the following procedure. Use the focus slider to measure the depth of field for an objective, or enter a value directly. Values are usually between 2-4.

SPOT users can access this dialog from the Assay Interface dialog. Click on the Objective button to select or add an objective.

To add an Objective:
1. Open Auto Capture.
2. Click on Objective.
3. Click on Add.

Add Objective

4. Enter a name for the objective and its depth of field.
5. Click on Apply.

To measure depth of field and offset:
1. Switch to the objective you are adding.
2. Focus on a cell.
3. Use the focus slider to determine the upper limit or plane where objects would be in focus and note the position.
4. Move the focus to the lowest likely plane and note the position.
5. Enter the difference between these values in the Depth of Field text field.
Spot counting

For Research Use Only. Not for Use in Diagnostic Procedures.

Spot counting overview

SPOT includes some additional software used to analyze and store data. This software quantifies signals in cells found in captured frames. Spot counting data is kept in a SQL database either locally or on a server. The additional software includes Entry which is used to manage the SQL database. Review which processes the frames and cells captured and does the analysis. For scanning system users there is also some additional calibration and configuration that needs to be done with Microscope Configuration and Microscope Calibration. Shortcuts to open these applications can be found in Start > Programs > Applied Imaging.

Each additional software package has its own help files for information that is related to their general use. These files only contain information specifically related to spot counting. For automated spot counting you should also familiarize yourself with the use of the scanning system. See the topics in the Scanning systems section and also the Scan Wizard.

There are two methods of cell capture; automatic (for SPOT AX users only) or manual. Automatic uses the motorized hardware used for scanning along with automatic capture settings. Manual uses the standard method of probe capture. Both methods send the cells to the Review application for analysis and classification.

Note - In these help files, the names of the additional software will be italicized to differentiate between the Review application and the Review screen.

The CEP XY Assay has been FDA approved. See topic under Spot > CytoVision CEP XY for the intended use and instructions for setting up this assay.

There are three ways to access the SPOT Review application:

- Follow the pathway: Start > All Programs > Applied Imaging > Review
- In Spot capture, open the fluorochrome selection window - click on the Review icon to launch the Review application
CytoVision 3.6

Fluorochrome Selection

- From the active desktop if the Review application has been placed on the active desktop by your product support specialist.

Related topics

Fluorochrome setup

Getting started

Getting started

Before you can start capturing cells for spot counting some setup needs to be done. Assays and scripts need to be configured for the DNA test analyzed and the fluorochromes used in the scripts should be on the system. You should be familiar with general probe capture procedures. You can find out about probe capture in the topics under Probes.

SPOT AX systems with motorized stages must configure and calibrate the system. This is done for you on installation but the calibration needs to be repeated periodically for accurate relocation and finding.

SPOT SA systems do not need to be calibrated. Just create a script to begin capturing cells.

Configuring the system

The hardware capabilities of the system are setup through Configure ( ). This should be done:

- In addition to the Microscope Control application setup for your microscope.
- In addition to the Stage and wheel configuration.
- For systems with no motorized microscope objectives, add the objective nose piece and set the controller type to Manual and the COM port to None. Enter the correct number of objectives.

Detailed information about how to add and configure components is in the help files for Configure. Unless the hardware changes from when it was installed you will not have to configure the system.
Note - This information only applies to users with a SPOT AX automated system.

Calibration for SPOT counting application

Scanning systems must be calibrated from both the Scan screen calibration and the Calibration application. The system needs to be calibrated if hardware has been moved or changed; for example if the camera is cleaned or rotated, or if relocating begins to lose its accuracy.

The SPOT counting application uses several different calibration procedures.
- The Wizard calibration is used outside of the CytoVision program for accurate relocation in the Review program.
- The Bay Datum calibration sets a fixed position for each bay in the stage.
- The System calibration is used to calibrate the scanning portion of the application.
- The Microscope conversion is optional and is used for relocation within the CytoVision program.

Notes
- Before calibrating the system the Configure application must be setup for the capabilities of the microscope and stage. See Configuring the system.
- Adjust the C-Mount for parfocality before calibration as it is likely the camera will be rotated during adjustment.
- Make sure there is no dirt on the camera or C-Mount before calibration.
- The camera must be positioned squarely to the stage. This is critical for spot counting. Use the crosshairs on the calibration slide to align the camera. There is a step in the calibration wizard devoted to this alignment.
- Once calibrated, do not rotate the camera. If you do the system will have to be calibrated again before performing any scans.
- Recalibrate the system periodically (try once a month to start) or when cells are not relocated accurately.
- A map of the Applied Imaging calibration slide A can be found here. This information only applies to users with a SPOT AX automated system.

Step 1 - Wizard system calibration:

1. Close down all Applied Imaging software if it is running.
2. Open the Calibration program ( ). Microscope connection made - configured components are displayed orange in image window.
   Note: Use the online help (main menu - Help > Contents) if additional help is needed.
3. Launch calibration wizard - click on Wizard icon in main toolbar.
4. Prompt that you will need the Applied Image calibration slide during the operation of this wizard.
5. The Calibration wizard welcome window opens - press 'skip' to begin calibration and follow the directions in the wizard, selecting Next to move to each section in the wizard.

Notes:
  a. Calibration needs to be done for only one bay on the stage.
  b. Details of this process can be found in the help files for the program (main menu - Help > Contents).
  c. You must use the Applied Imaging calibration slide A supplied with your system.
d. Skip the sections for XY backlash, Z backlash and Idealised coordinates. It does no harm to calibrate these settings but they are not used by SPOT and you can save time by skipping them.

6. Skip the wizard sections (these are done in the: XY backlash, Setting Ideal coordinates, and XY limit switches.

7. Click on Finish - save calibration data. The Wizard window will close.

Notes

- Before you begin make sure all of the objectives are clean and free of oil. The calibration slide must also be clean and free from dust and oil.
- Oil is not necessary for calibration, even for high power oil objectives. If the lens is clean, the image can be focused well enough and the calibration slide will remain cleaner and easier to work with.
- Once calibration is started none of the motorized components should be moved or adjusted manually. Use the on-screen controls or the joystick controller.

Step 2 - Setting bay datum positions - this is done in each bay of the stage

Calibrate the system using the Applied Imaging Calibration A slide. This calibration procedure is done in each bay of the stage. Calibration should only need to be performed when the system is first setup, or if the stage has been moved.

1. Launch the CytoVision software, open the scan screen \* and click on the calibration button \* to open the Finder Calibration dialog.

2. Click on Reset (Home) Stage

3. Place the Applied Imaging Calibration A slide in bay 1; the first bay on the left side of the stage. (diagram of a stage)

4. Adjust the focus and light to get an image. Only adjust the focus using the joystick or onscreen controls.

5. Move the stage to position bay datum position on the calibration slide (England Finder position C59) using the on-screen stage controls. Do not move the stage manually once Reset Stage has been pressed.

Bay datum point

\*Note: The 'A' image will appear upside if the camera orientation is correct.

6. Click on the Calibration button \* to open the Finder Calibration dialog.
7. Click on **Set Bay Datum** to open the dialog.

9. Enter a 1 in the **Bay Number** field.
10. Press **Save**.
11. Place the slide in the second bay just to the right of the first.
12. Repeat steps 4-9 but enter a 2 in the **Bay Number** field.
13. Repeat steps 4-11 for the other bays entering their corresponding number in the **Bay Number** field.
14. Click on Done to close the Set bay Datum window.

**Step 3 - System calibration**

This calibration is done after setting the bay datum positions. This step only needs to be done for one bay.

Stage calibration is necessary for proper travel of the stage and accurate relocation of cells.

1. Place the Applied Imaging calibration slide in a bay.
2. **Reset** (home) the stage.

3. Click on the **Calibration button**.

4. Click on **System Calibration** to open the dialog window.

5. Move the stage to bring the 32 micron image calibration pattern into view. Focus and adjust the contrast so the pattern is clearly visible.
6. Click on Live in the System Calibration window.
7. Click on Capture in the System Calibration window.
8. A line should be drawn between two rectangles in the same column. If not, reposition the image calibration pattern and repeat steps 4-7. The length (in units of pixels) will appear in the Feature Separation (pixels) field.
9. Enter 64 in the Feature Separation (microns) text field.
10. Move stage to a cross hair most commonly used for calibration (see image above).
11. Click on X-Y Scaling in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.
12. Click on X-Y Backlash in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.
13. Click on Focus Backlash in the System Calibration window. The focus will be adjusted until the setting is found and entered in the field.
14. Enter a name for the calibration data in the Filename text box.
15. Click on Save.
16. Click on Apply. This will apply the currently selected calibration settings to the system.
17. Click on Done to close the window.

Step 4 (optional in SPOT) - Slide Calibration for Microscope Coordinate conversion

1. After Bay Datum and System Calibration have been completed
2. Place the England finder (provided with the system) in Bay 1 of the stage and adjust the focus and camera/light settings if necessary.
3. Select the Reset home button, resetting the stage (c59)
4. Move to position A15, record the X and Y coordinates displayed at the bottom of the screen. These will be used later.
5. Move to position Z50, record the X and Y coordinates displayed at the bottom of the screen. These will be used later.
6. Switch to the Review screen and open the Microscope Coordinate conversion window. Enter the slide lengths in the spaces provided.
7. Click on Add to create a new "Microscope" entry. Type a name in the New Microscope field, this will be the Default entry for printing out England Finder coordinates from a scan.
8. Select Units as England Finder and input the A15 and Z50 coordinates that were noted previously (put them in both New and ChromoScan microscope fields).
9. Select Done to save this data, the new name will appear in the list.

See related topics Metaphase finding scanning systems > Hardware basics > Calibrating the system > Microscope Coordinate conversion.

The system has now been set up with the necessary Calibration and configuration data to enable you to successfully use it for scanning.

Configuring a slide

Configuring a slide for spot counting involves two things; marking a slide as containing a spot counting sample, and selecting which assays should be used to find and count signals.

Scanning systems mark a slide for spot counting from Customize in the Scan screen. You must be in fluorescent mode then select one of the Interphase sample types. For manual capture, Spot Counting mode is selected for a slide in the Capture screen from Capture Mode. When this mode is selected the system knows the contents of the slide are for spot counting analysis.

An assay is selected using the assay selector. For more information see the topic The Assay Selector.

When a slide is marked for spot counting you must choose an assay that defines how the slide should be scanned and/or analyzed. The assay selector dialog is used to select assays. The dialog is opened when Spot Counting mode is selected in capture or as part of scan setup when one of the Interphase sample types is selected in the Customize dialog of Scan.

See the topic Editing and creating assays for information about this.

Alternatively, use the scan wizard to load an scan configuration or create a new one specifically for the slide or slides.

About assays

Assays are used to store all the required information to perform the scanning and spot counting task. This includes the selection of fluors to capture, the kind of assay, the classes for each pattern of spots and the number of cells for each class to capture.

Assays define the characteristics of what you are looking for. When performing a DNA FISH test on a sample you want to know how many spots of each fluorochrome are in a cell. In fusion or breakaway studies you also want to know if signals are joined or not. The spot counting scripts in conjunction with the assay files contain the image processing algorithms and settings information used to determine how many signals are present, if signals are fused and to classify the cells based on the results.

The assay selector dialog is used to select assays, create new assays and copy or delete existing assays. The content of the assays are edited via the assay creator dialog.

Creating and editing assays
The Spot Counting **assay creator dialog** is used to build and edit assays. Open it by selecting the required assay in the **assay selector dialog**, then clicking on the edit assay button in the **assay selector dialog**. Alternatively, if the assay is a new one, then click on the correct grid location in the assay selector grid and you will be asked if you want to create a new assay. Select yes and the assay creator dialog will appear.

The dialog is constructed like a grid; each column contains the settings for the signals, each row contains the settings for the classes.

The **Description** field provides a place to briefly describe the purpose of the script. **Number of probe Fluors**. sets the number of fluorochromes in addition to the counterstain. The maximum number is 5. A column will be placed in the configuration table for each fluorochrome. **Fusion enabled** tells the system to look for signals that are joined. When turned on a **Fusion** column is added to the grid.

**Add Class** adds a new row to the grid. Add as many classes as you want to score, the maximum number in 33. Select the number of probe fluoros and whether the script is fusion enable before you begin adding classes. Delete a class by clicking on the button in the **Delete** column.

The names for the counterstain and fluorochromes are selected from the same list of fluors found in Build list of probe capture. A combo box is used to select the name. The counterstain is set in the **Counterstain** combo box. A **Fluor Name** combo box is used to select the fluorochrome for each signal. If Fusion Enabled is turned on a check box is drawn next to each fluorochrome name. Place a check next to the fluorochromes that are involved in the fusion.

The way the grid works is for each class added enter the number of signals expected for each fluorochrome (and fusion if it is enabled). The name for each class is entered under **Class Name**. Class colors are selected by clicking on the colored box next to the Class Name. For example, when using dual-fusion BCR/ABL a normal or Ph- cell would have 2 Orange signals, 2 Green signals and no fusion signals. Enter a 2 under each fluorochrome name and a 0 in the Fusion field. In a cell with a fusion or Positive you would see 1 Orange, 1 Green and 2 Fusion signals. Enter a 1 under each fluorochrome name and a 2 in the Fusion field.

Every script has one normal class. The label can be anything you want; in this example the normal class has been called Negative. The Normal designation is only used by the script algorithms to help in processing. Mark a class as normal by clicking in the **Normal** radio button.
The wildcard character (*) specifies any number other than those listed. If Review finds cells that do not fall under designated classes an Ignore class is added. This class can be built into the script as an option.

**Fine tuning the assay**

The size and separation settings for fluorochromes make the script more accurate at finding and counting spots. Each fluorochrome has **Spot area in pixels** sliders to set the minimum and maximum size of spots based on the area in pixels. Minimum size is between 0-40, maximum is 0-700.

This dot is 4X4 pixels or 16 on the slider scale.

The **Separation distance** sliders set the distance at which signals are seen to be separated. The **Fluorochrome** slider is used to set the distance between spots of the same fluorochrome. The **Fusion** slider is only present when Fusion Enabled is on. It sets the distance for determining whether two signals are fused or just close together. Choose between distances as a percentage of the % nuclear diameter or % spot diameter. Distances are measured between the centres of each spot.
Tip - Use reprocessing to fine tune your assays. Modify the assay settings and reprocess as a new scan. That way you can see the affect the assay changes have on classification.

Saving assays
When you click the Done button you will be presented with three options if you have made changes to your assay:
1. Save changes and exit - this option will save changes to your assay and exit from the assay creator dialog.
2. Exit without saving - this will ignore any changes you made to your assay and exit from the assay creator dialog.
3. Continue editing - this will allow you to continue making changes to your assay.

Notes
- When a setting is changed for a default fluorochrome file it will also change when the script is run. This means if you change a render color or Z-Stack setting the scripts that use the file will be updated automatically.
- Before the Min slider can be moved up the Max slider must be moved up.
- Use the fusion settings to work with breakaway probes where cells with the fusions are classes as normal.

The Assay Creator

The diagram shows the component parts of the assay creator dialog. The assay creator allows new assays to be created with up to five probes which may either be enumeration, break apart or fusion assays.
**Number of probes and assay type:**

When a new assay is created the dialog will appear blank. In the top left corner of the dialog there is an edit box where the desired number of fluors can be entered:

```
<table>
<thead>
<tr>
<th>Description</th>
<th>Number of Probes</th>
<th>Fusion enabled</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEPXY_AML</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>
```

The **Number of Probes** can be anything from 1 to 5. If 2 or more probes are entered, the **Fusion Enabled** box becomes active. Checking this box indicates that you are creating a fusion assay and that two probes will be involved in the fusion analysis. **NOTE** the rest of the dialog will be relatively blank at this stage.

**Fluorochrome Selection:**

Clicking the **Add Class** button for the first time will bring up a series of drop down list boxes depending on the number of probes entered previously:
Spot counting

For each probe the following information can be setup:

1. The probe name, this is a drop down list of CytoVisions' default fluorochromes.
2. The overlay color for each fluor, this color is used to highlight signals of this probe type in Review. The current color is indicated by the box to the right of the probe name. Clicking on this box allows the user to change the current color selection.
3. If the **Fusion Enabled** check box has been checked, then a check box appears to the very right of each probe name. Any two boxes may be checked indicating that these are the two probes involved in the fusion (in the example above, the spectrum green and spectrum orange probes are involved in a fusion).
4. The number of planes for the Z stack capture.
5. The spacing for each plane.
6. The min and max spot areas for the probe. Signals outside of the min and max limits will be ignored by the analysis.

**Counterstain Selection:**
Clicking the **Add Class** button also gives you access to the counterstain drop down. This enables you to choose the counterstain and also choose a color for the overlay outline that will highlight the counterstain in Review.

**Class Configuration Table:**
This is the most important part of the assay creator dialog:

The numerical values entered into the table in the column underneath each fluor represent the pattern of spots that constitute a class of nuclei. Up to 32 individual classes may be added. Clicking on the colour square next to the class name allows to assign a colour for each class so
that nuclei belonging to the class are easily identifiable in Review. Clicking the Normal radio button defines which of the classes represents the class you would normally expect or the default pattern of signals in normal nuclei.

The example above has two classes setup. The "Normal" class has 0 fusions, 2 spectrum green, 2 spectrum orange and 2 spectrum aqua signals and will be marked with a green tab in Review and this is the default for a normal nucleus. The "Positive" class has 2 fusions, 1 spectrum green, 1 spectrum orange and 2 spectrum aqua signals and will be marked with a red tab in Review.

**NOTE** It is also possible to use wildcards to specify signal patterns for a class. For example:

<table>
<thead>
<tr>
<th>Probes</th>
<th>Spectrum Green</th>
<th>Spectrum Orange</th>
<th>Spectrum Aqua</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Planes</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Spacing (nm)</td>
<td>1.330</td>
<td>1.440</td>
<td>0.550</td>
</tr>
<tr>
<td>Min Spot Area in Pixels</td>
<td>10</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Max Spot Area in Pixels</td>
<td>340</td>
<td>340</td>
<td>270</td>
</tr>
</tbody>
</table>

There are two classes set up in the assay creator. The "Normal" class has 2 of signals of each type of fluor.

For the first "Positive" class there is an asterisk (*) in the Spectrum Green count. This means that any number of Spectrum Green signals are allowed for this class. The Spectrum Orange box has a greater than sign (>) meaning that any number greater than 2 Spectrum Orange signals are allowed for this class. The same applies for Spectrum Aqua.

The second "Positive" class there is a less than sign (<) in all the boxes. This simply means that if there are less than 2 Spectrum Green signals AND less than 2 Spectrum Orange signals AND less than 2 Spectrum Aqua signals then the nucleus is classed as "Positive".

**NOTE** there are 2 definitions of a "Positive" nucleus in this setup. This simply means that if either signal pattern is detected in a nucleus then the cell is classed as "Positive".

**Classifier Setup:**
This allows you to assign a shape classifier to the assay. In order to assign a shape classifier, one or more classifiers must have been created using Review.
To assign a classifier, simply double click on the DAPIShape text in the classifier box. Once assigned to an assay, the classifier will be applied when the system is scanning and will filter out nuclei depending on whether they meet the shape criteria defined by the classifier.

To clear a shape classifier simply click on the Clear button. See Creating SPOT classifiers for more information.

Separation Distances:
There are two separation distance settings that apply to assays. This determines how signals are merged together to form a spot within the nuclei. The distances are specified as a percentage of the overall nuclei diameter or as a percentage of the average spot diameter.

The fluorochrome separation distance applies to signals of the same fluorochrome. It says that if the centers of two or more signals are within X% of the nucleus or average spot diameter (where X is set by the slider) then count these signals as part of the same spot.

The fusion separation distance, used when Fusion Enabled is checked, applies to signals of the two fluorochromes involved in the fusion translocation. It says that if the centers of two or more signals of different probes involved in the fusion are within X% of each other then these signals are part of a fusion or greater than X% of each other then these signals are just close together.

Tip - Use reprocessing to fine tune your assays. Modify the assay settings and reprocess as a new scan. That way you can see the affect the assay changes have on classification.

Spot area in pixels
The size and separation settings for fluorochromes make the script more accurate at finding and counting spots. Each fluorochrome has Spot area in pixels sliders to set the minimum and maximum size of spots based on the area in pixels. Minimum size is between 0-40, maximum is 0-700.

This dot is 4X4 pixels or 16 on the slider scale.
**Scan Stop Counts:**
These counts tell scanning when to stop. Scanning will **Stop after N abnormal cells** have been captured i.e. cells not included in the class marked as normal OR scanning will **Stop after N total cells** have been captured i.e. cells that are marked as normal or abnormal where N is any positive value. Entering a value of zero means that scanning will continue until the whole scan area is complete.

**Class Stop Counts:**
Entering a value for the class stop counts will tell scanning to stop when that number of cells for that class have been captured. For example, entering a value 50 for the "Normal" class would mean that scanning stops after 50 nuclei classified as "Normal" have been captured. Entering a value of 20 for your "Positive" class means that scanning will stop after 20 nuclei classified as "Positive" have been captured.

*It is important to note that when entering class stop counts for multiple classes, scanning will stop on whichever count is reached first.*

Entering a value of zero for any class count simply means that class counts do not apply for this class.

**Advanced Parameters:**
Clicking the **Advanced Parameters** button will bring up the dialog shown below:
Normally, the default settings will cover most scanning and processing situations and these parameters will suffice. The parameters and their meanings are listed below:

**High background detection:**
Checking this box will enable high background detection. As the capture integration is extended to enable the capture of faint probe spots, so the overall background intensity within the cell in the probe channel increases. This is designed to indicate to the user that the overall background intensity is high, and that the spot count for this cell may be suspect.
**Remove weak signals:**
This option allows suppression of noise signals found to be weaker in intensity than the most intense signal detected in a cell.

**Cross talk removal:**
With some signals (especially bright ones) there may be some cross talk between the channels. This means that a signal may get incorrectly detected in other probe images. By checking this box, weaker signals in other probe images in the same spatial location will be removed.

**Context based merge:**
Checking this box simply means that if only two spots are detected in this cell, then the merging distance (at which two spots are considered to be the same signal) is decreased 25% of its original value. Thus the chance of merging spots is decreased.

**Counterstain threshold:**
This is specified as a percentage of the overall intensity of the counterstain for the nucleus. If captured nuclei are really dull, then this value can be lowered to obtain a more accurate representation of the nuclei. Similarly, this value can be reduced to obtained better nuclei segmentation when nuclei are really bright.

**Boundary dilations:**
Once a nucleus has been segmented it is possible to increase the size of the nucleus by dilating it. Increasing nucleus size will help include probe signals on the boundary of the nucleus that may otherwise be excluded by counterstain thresholding.

**Debris min size:**
Setting to value greater than zero will enable debris detection. Debris detection will detect and remove nuclei that intersect with large debris objects that may exist in one or more of the probe images.

**Minimum spot intensity:**
This is an absolute value and any signal with an intensity less than this will be removed from consideration before spot counting begins.

**Minimum fusion intensity:**
This is a percentage value of the maximum signal intensity for signals within a nucleus. Any signals less than this intensity level will be removed prior to fusion analysis.

**Minimum spot intensity after merge:**
This is a percentage value of the maximum signal intensity for signals within a nucleus. Any signals with an intensity less than this value that do not constitute part of a fusion or part of a larger spot will be removed.

**Aggressive split:**
In most cases this option is best left on as it separates nuclei in close proximity to another. However, if you find your nuclei are getting broken up then this option can be turned off. Broken up nuclei normally occur when the appearance of nuclei is mottled.

**Contrast stretch probe images:**
This will enhance the probe signal images viewed in *Review*. It does not affect analysis in anyway and can be used in situations where the probe images contain high levels of background.

*Also See:*
The Assay Selector and Configuring a slide, Using SPOT Auto capture
**Saving assays**

When you click the **Done** button you will be presented with three options if you have made changes to your assay:

1. **Save changes and exit** - this option will save changes to your assay and exit from the assay creator dialog.
2. **Exit without saving** - this will ignore any changes you made to your assay and exit from the assay creator dialog.
3. **Continue editing** - this will allow you to continue making changes to your assay.

**Notes**

- When a setting is changed for a default fluorochrome file it will also change when the script is run. This means if you change a render color or Z-Stack setting the scripts that use the file will be updated automatically.
- Before the Min slider can be moved up the Max slider must be moved up.
- Use the fusion settings to work with breakaway probes where cells with the fusions are classes as normal.

**The Assay Selector**

The assay selector is used to select assays specifically for a scan. It presents the assays as a series of rows against disorders as a series of columns in the grid.

**To select an assay:**

Simply click on the corresponding icon in the grid position for the assay. The icon for the selected assay will turn orange. Click **Done** when you have made the right choice (In the picture below, the CEP XY BMT assay will be become the currently selected assay when the Done button is hit).

![Assay Selector Grid](image)

New rows and columns may be added or deleted from the grid at any time.

**To add or delete a row in the grid:**

Left click the desired row in the grid, then right click and a menu will appear. Click **Add Row** to add a row to the end of the grid or **Delete Row** to delete the current row.

**To add or delete a column in the grid:**
Left click the desired column in the grid, then right click and a menu will appear. Click **Add Column** to add a column the end of the grid or **Delete Column** to delete the current column.

**Creating a new assay:**
A new assay is created by left clicking on a blank location in the grid and you will be asked wether you wish to create a new assay. Click yes to create one and the assay creator dialog will appear.

**Editing an existing assay:**
Highlight an existing assay in the grid. Click the **Edit Assay** button. The assay creator dialog will appear with the settings for the selected assay.

**Deleting an existing assay:**
Highlight an existing assay in the grid. Click the **Delete Assay** button. The assay will be deleted from the grid.

**Copying an existing assay:**
Highlight an existing assay in the grid. Right click and a menu will appear. Select **Copy** to copy the assay. Then move the mouse to an new grid location and right click again. Select **Paste** to paste the assay. This will copy the entire contents of the assay to the new location. **NOTE** if you elect to **Paste** into a grid location where an assay already exists you will be asked wether you want to overwrite the existing assay or not. If you overwrite the existing assay, the contents of that assay will be lost.

*Also See:*
Creating and Editing assays and Configuring a slide

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**For Research Use Only. Not for Use in Diagnostic Procedures.**

**UroVysion**

To set up a UroVysion assay perform the following steps:

1. Set up a case as usual
2. From the finder screen select the scan wizard
3. Create a new superscan with the name of your choice:
4. Click next and then define a suitable scan area covering as many cells as possible (see the scan wizard scan area setup for more information on how to do this).
5. Click next to go to the assay screen. Highlight the slide and select Interphase Prescan in the finder application window.
6. If there is not already a UroVysion assay this will have to created from scratch.
   - Left click in the left most column and type the assay a name e.g. UroVysion.
   - In top most grey row left click in the right most column and assign a disease name e.g. BC for bladder cancer.
   - Now click in the grid on the UroVysion row and at the BC column and you will be asked to create a new assay.

7. Create the assay as follows:
   - Enter 4 for the number of probes.
   - Then select each probe from the drop list box in each column - the probes should be Spectrum Red, Spectrum Green, Spectrum Aqua and Spectrum Gold.
   - Enter the z - stack settings and min and max spot areas for each of the probes.
   - Enter your preferred probe separation distance.
   - Now create 5 classes as shown in the table below. Make sure the correct stop counts are entered for each class.

<table>
<thead>
<tr>
<th>Class</th>
<th>Red</th>
<th>Green</th>
<th>Aqua</th>
<th>Gold</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Gain</td>
<td>&gt;2</td>
<td>&gt;2</td>
<td>*</td>
<td>*</td>
<td>8</td>
</tr>
<tr>
<td>Gain</td>
<td>*</td>
<td>&lt;2</td>
<td>&gt;2</td>
<td>*</td>
<td>8</td>
</tr>
<tr>
<td>Gain</td>
<td>&gt;2</td>
<td>*</td>
<td>&gt;2</td>
<td>*</td>
<td>8</td>
</tr>
<tr>
<td>Loss</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

   - Enter zero for the stop after N abnormal cell count.
   - Enter 200 for the stop after N total cell count.

8. Click done when finished and then make sure each slide is setup to use the newly created assay.

9. In the focus and camera set up screen, highlight each slide in turn and set its camera settings. Enter 500 for the prescan stop count.
10. Click next and let the wizard build the focus maps for each slide.
11. 20x prescanning should then begin.
12. After 20x prescanning has finished, click finish in the wizard.
13. For each slide, select the slide in the finder screen by clicking on it. Then go to the Review screen in CytoVision where you will see all the cells that have been found by the prescanning process.
14. Click on the sort by button and select area. Click the Apply button and then the Done button.
15. Now deselect cells that are multiple cells joined together or that look like debris. Keep 200 or more cells for autocapture.
16. Once this has been completed for all slides, go to the capture screen.
17. Use the capture wizard to setup the fluorochromes and capture settings for each slide. Make sure the 60x objective is selected when the capture wizard is started.
18. Once the focus maps have been successfully completed, the wizard will go straight into autocapture and start capturing the cells selected in the CytoVision Review screen. Scanning will stop when one of the preset stop counts has been reached or all cells have been scanned.
19. Once scanning has completed switch to the SPOT Review application and review abnormal cells and reclassify false positives if necessary.
20. Select 4 cells for printing, select UroVysion report format and Print.

**See Also**
The scan wizard
The capture wizard
SPOT Review

**Database**

**Database overview**

Spot counting data is uploaded to an SQL database. The database contains the measurements, location and classification of a cell along with its image. The contents of the database are organized into a hierarchical file tree. Each level in the tree is a node in the database. The hierarchy is as follows: Study > Case > Slide > Scan > Cell/BLOB. A navigator similar to that of CytoVision or Genus is used to access the nodes. All cell measurements and their associated images are loaded together as a scan file in the Review application.
Entry ( ) is used to archive and delete the contents of the database. It also provides access to the case details fields that transfer with the cells captured for spot counting.

Case management

When cells are captured in spot counting mode a case is created in the database. When a slide is completed it will be placed in CVStudy.

The standard case details will be sent to the database when the case is created. Additional case details can be added in the Entry application. See the Entry help files for information about this.

Entry is used to delete any files that are unwanted or finished. It is also used to archive spot counting data. Nodes can be marked as completed in the navigator. Completed nodes have a red tick. This completed flag can be used to filter data for archiving.

Archiving data
Spot counting cases store application data in two places; the SQL database has all of the spot counting data, the standard cases directory contains other types of images. This means that a case with a mixture of spot counting slides and other types of images will need to be backed up in two places. In addition to archiving, users with an SQL database should also perform regular backups of the database. See the topic Database backup notes.

You must use the archiving tools in the Entry program to save the spot counting SQL database. See the topic Archive and Restore overview for information about backing up all other case data.

The Archiving dialog is opened by clicking the Archive and Restore button on the toolbar of Entry.

The Archive to box is used to select which drive to back-up to. The amount of free space on a disk is read when the drive is selected. The Case Filter tools are used to find a case.

To mark a case for archiving put a check in the box by its name; multiple selections can be chosen. The amount of space needed to archive the case will be calculated; this can take quite a bit of time if the case is large. The disk space required will be calculated as each name is chosen. Click on the OK button to begin archiving.

Restoring data
CytoVision 3.6

Restore spot counting data by clicking on Archive and Restore in the Entry application. Select the Restore tab and the drive that contains the archive disk. Select the case you want and click on the OK button to copy the data back into the database.

Database backup notes

Who these notes apply to
Users with data held in a Microsoft SQL Server™ database. These include SPOT™ AX and SPOT SA systems.

Where to find information
The online help files for Microsoft SQL Server contain information about backup and maintenance strategies as well as how to recover a database. These topics can be found in the section Administering SQL Server > Backing Up and Restoring Databases. The online help files can be opened from Start > (All)Programs > Microsoft SQL Server > Books Online.

FAQs

What is the difference between archiving and backing up?
Archiving from your software package (CytoVision or Genus) is used to save application data and to clear completed items from the case directories and database.

Backing up the database saves all information in the SQL database both completed and work in progress. This avoids loss of data due to an event such as hard disk failure or database corruption.

Do I have to do both?
No, backing up is not a requirement. If you do not back up, however, your data is at risk and in the event of hard disk failure or database corruption all of your data may be lost. It is therefore strongly recommended.

How often should I backup?
There are recommendations for backup strategies in the Books Online that will help you decide how and when to backup. Large sites with a database hosted on a server may already have daily backups scheduled. Check with your IT department or system administrator. Small sites that do not have these resources will need to examine their quantity of data and resources for managing the database. Daily backups are always the safest strategy and there are ways to automate this task.

What kind of media should I use?
That will depend on the amount of data stored and your backup strategy. Make sure you choose a media format that can handle the size of your database.

Does Applied Imaging provide the hardware and software for backups?
No. Applied Imaging does not provide software or hardware for SQL Server backups. You will need to determine your individual needs for this.

Capturing

Capturing overview

In spot counting frames are captured, not individual cells. Then, using the parameters defined in the script files, the system looks for interphase cells in the frames. When a cell is found the
signals are located and counted according to the analysis script. Each cell found is given a row in the Review data grid.

There are two methods that can be used to capture cells; automatic or manual. The automatic method makes use of the scanner’s motorized stage and automatic capture, and can perform a low-power prescan to determine the best frames for capture. The manual method uses the normal probe capture methods but is done from the Spot Counting capture mode.

Cells must be marked for spot counting analysis before they are captured. When capturing manually this is done by selecting Spot Counting in Capture Mode. For scanning systems this is done by choosing one of the Interphase sample types in Customize for the Scan screen. Slides scanned in this way will be automatically set for Spot Counting when switching to the Capture screen.

The cells are captured using the probe capture tools. The fluorochrome names are setup the analysis pass script. If they are not the fluorochromes you want to use you must select a different script or create a new one. See the topic Creating and editing assays. You can change the Z-Stack or settings.

Signals in interphase cells are usually captured with Z-Stack layers. The Z-Stack settings will depend on your sample type, probe size and objective. Usually 4-8 layers at 0.8-0.5μ apart will yield the best results. Capture will be quicker and the slide will suffer less bleaching if a lower number of layers is captured. If you do reduce the number of layers you may need to increase the distance between them. Z-Stack Probe size is not used, this is set in the spot counting script. See the topic Capturing layers using Z-Stack for general information about this subject.

Customize options in Capture are set when spot counting is selected.

Notes

- The raw images captured for spot counting are deleted from the CytoVision/Genus directory after they are sent to the Review application at the end of capture. Do not be alarmed by this. The slide will remain in the navigator for two reasons; to let you know the slide has been processed already and so the system can number new slides correctly as they are created.
- When a script configures the Fluorochrome Selection panel it pulls information about the fluorochromes from the build list. If a new default color Z-Stack setting is saved to the default file it will automatically be applied the next time the panel is configured.
- If a fluorochrome file is used in a script it should not be deleted from the build list.

Related Topics

Using SPOT Auto Capture

Customize options for SPOT

Spot counting mode automatically sets the necessary settings in Customize. These are Save Raw Image, Zero Threshold and Auto Threshold. The only other options relevant to spot capture are Auto Sequence, Auto Focus Offset and Auto Camera Setup. Turning Auto Camera Setup on is not advised as it increases the amount of time needed to capture. The most efficient way to capture cells is to start with Auto Sequence off. Then capture a couple of frames to determine the best camera settings and focus for each signal. Then turn Auto
Sequence on and continue capturing. If the camera settings need adjusting just repeat this process.

If the Z-Stack settings cover a large enough range then Auto Focus Offset is not necessary. If the range of stacks has been limited to save time then Auto Focus Offset may be necessary to compensate for signal to counterstain offset.

There is an option specific to spot counting called Upload images to database. When turned on the raw images are uploaded to the SQL database. This allows reprocessing. If it is turned off the raw images are not sent and the images can not be reprocessed in the Review application. See the topic Reprocessing scans for more information about this function.

Fluorochrome setup

The Fluorochrome Selection panel is configured by the assay and should never be edited manually. The name and the order of the fluorochromes must match the assay or the cells cannot be processed.

When the fluorochromes are loaded the default settings are used. To save setup time create fluorochromes with the correct Z-Stack settings and use them to build the script. If the Z-Stack settings are not saved in the fluorochrome's default file you will have to change the Z-Stack every time a new slide is captured. See the topics Fluorochromes overview and Capturing layers using Z-Stack for general information.

If you want to change the render color of a fluorochrome in a script the new color can be saved to the default file. Then the next time a script is used the new color settings will be applied.

![Fluorochrome Selection](image)

Spot Fluorochrome Selection window

Using SPOT Auto Capture

SPOT auto capture is used to perform the high magnification capture and analysis of interphase nuclei. It is strongly recommended that users are familiar with assay creation and various assay creator options.

Prerequisites
In order to use SPOT auto capture, one or more slides must already have been prescanned or set up as framelist slides using either the customize scan options in the finder screen OR by use of the scan wizard.

**Setting up and running SPOT auto capture**

The recommended and easiest route is to use SPOT auto capture is via the capture wizard. The wizard will perform all the setup steps required and automatically launch SPOT auto capture on completion.

**The Control Panel**

The SPOT auto capture control panel is shown below:

![Spot AX Auto capture panel](image)

**The Controls**

- This button will start auto capture after it has been stopped or paused.

- will temporarily pause scanning.

- the stop button stops scanning, allowing access to New Cell, Live and Capture buttons for capture adjustments.

- fast forward will allow moving from the current bay to the next bay. All capture on the current bay ends.

**Options**

Checking the Frame pause box will pause scanning at the start of next capture frame.

Checking the Autofocus box will turn on or off autofocus on every nth frame.

**Buttons**

Clicking the Focus options button will allow you to fine tune the autofocus parameters. This may be done on the fly without having to stop autocapture.
**Plane spacing**

This is the spacing between individual planes used to take focus measurements. It should be roughly equivalent to the depth of field for the objective. For 100x and 60x try a spacing of 4, for 40x try a spacing of 6 and for 20x try a spacing of 8.

**Number of planes**

This is the number of measurements used to measure and then calculate the optimum focus position. Generally, the lower the magnification of the objective, the less focus planes are required as the depth of field between them will be greater.

**Focus every nth**

This parameter determines how many frames should elapse before a focus operation takes place. NOTE that this is only a rough way of specifying the focus frequency. In reality if autocapture moves a distance greater than "n" times the frame distance between two points then a focus operation will take place.

Clicking the **Mon** (short for class monitor) will bring up the class monitor dialog. This dialog shows the classes as defined in the assay used for auto capture and gives an early indication of the outcome of the slide. An example is shown below:

Classes names are shown on the left hand side. The total number of informative nuclei captured so far is shown at the top of the dialog. The progress bars show the percentage of the total captured informatics for each class. The **% of total** column shows these values as a numerical percentage. The **% of class** shows the percentage of the stop count for the class if this has been defined. Otherwise it shows **N/A**. For more information on stop counts see the Assay Creator Dialog.

Clicking the **Quit** button will end SPOT auto capture for the current bay. The **Quit** button is only enabled once the Stop **button** has been pressed.
**Stopping SPOT auto capture**

SPOT auto capture will stop when one of the following conditions is met:

- One of the class stop counts has been reached i.e. % of class in the class monitor dialog reaches 100%.
- The Stop After N Total Cells has been reached.
- The Stop After N Abnormal Cells has been reached.
- It is the end of the scan area or all cells have been captured.

**NOTE** If multiple bays have been setup, if SPOT auto capture stops automatically due to one of the above reasons, then auto capture will continue with the next slide in the next bay.

**Related topics**
- About Auto capture
- The capture wizard
- The scan wizard
- The assay creator

**Automatic capture options**

For users with scanning systems you have a choice between methods of scanning and capture; Prescan and Framelist. Both methods make use of the motorized stage, filterwheel and focus motors on the system to automate both scanning and capture. These options are listed in the Customize dialog of Scan as Interphase Finder (Framelist) and Interphase Finder (Prescan). Prescan is done using a 20X objective. The analysis pass captures frames from the Auto Capture dialog in the Capture screen using a high power objective; 40X or above can be used though 100X objectives usually yield the best results for analog cameras. High resolution digital cameras get better results with 40-60X objectives.

**Prescan**

This method is best for slides with low density cell populations or rare event detection. A low magnification scan is performed to find interphase nuclei followed by high magnification acquisition and spot counting. The assay file for the prescan method uses both passes in the assay.

**Framelist**

Framelist method simply divides the scan area into frames. Every frame in the scan area is captured. This method works well with slides that contain a high density of cells and a smaller scan areas. Only the Analysis Pass of the assay file is used in the framelist method.

**Focusing options**

Before switching to the Capture screen another focus map must be created with the high power objective used for capture. This focus map is used as a starting point for auto focusing. This speeds up the capture process and helps prevent the system from focusing on the wrong plane.

The Auto Capture dialog has an option to focus before every frame is captured called **Auto focus**. This provides well focused images but can be time consuming. An alternative is to rely solely on the focus map by turning the **Auto focus** option off. This eliminates focusing from
the analysis pass and speeds up the process. This option can be turned on and off during Auto Capture so if you find that focus is not accurate enough using the focus map alone turn Auto Focus back on.

**Objective depth of field**

The objective selected as part of creating an assay helps to limit the range auto focus moves through. The narrower the depth of field the quicker auto focus will be. If the range is set too low then the correct plane may not be found. Try a setting of 3 for a 60 or 100X.

**Scan screen**

Prescan

![Diagram of scan screen process]

**Capture screen**

Path for capturing with a focus map

- Auto capture-Auto focus off

Path for focusing every frame

- Auto capture-Auto focus on

**Prescan capture**

In prescan capture two passes are made over the scan area. The purpose of doing a prescan is to avoid capturing frames that do not contain interphase cells so the first pass is done with a 20X objective and an interphase finding script. The script and objective used are defined in the Prescan pass tab of the Assay dialog.

In Capture the Fluorochrome Selection panel is configured by the script. Do not change the fluorochromes in the panel. You can change the Z-stack settings for the fluorochromes.

See topic - prescan flowchart for a printable flowchart.
To capture using Prescan:

1. Create a new slide and assign it to a bay.
2. Click on Customize in Find and select Interphase finder (Prescan). You must be in Fluorescent mode to see this option.
3. Click on Assay select the assay you want to use. Make sure the high power objective is selected for the Analysis pass.
4. Click on Save.
5. Switch to the 20X objective.
6. Setup the scan area, camera settings and focus map. Unless the cell density on the slide is extremely thin the scan area should be small.
7. Click on Start.
8. The normal Review screen can be used to view the frames that were captured. Mark the frames you want captured and analyzed as Keep.
9. Switch to the high power objective.
10. Click on Focus Map and map the focus points under high power.
11. Switch to the Capture screen. The Fluorochrome Selection panel will be configured by the script.
12. Turn Auto Sequence and Auto Camera Setup off and perform one or two captures to get the right camera settings. The Auto Setup toggle below the image can still be used to automatically determine the best settings. When the camera settings are right turn Auto Sequence back on. This will be faster than using Auto Camera Setup for every frame.
13. Click on Auto capture.
14. Use the arrows to check that the frames are relocated correctly. Use the Set Offset function if necessary to center the cells in each frame.
15. Start Auto Capture.
16. When all the frames have been captured click on Done to finish processing and upload the cells to the SQL database. The cells are now ready to be analyzed in the separate Review application. A shortcut for launching Review is located on the Fluorochrome Selection panel.

**Note** - The interphase finding script was written for used with a 20X objective. If a different power objective is used the finding algorithm may not find interphase cells.

**See Also**
The scan wizard
Prescan capture flow

Find

Create a slide and set it to the bay. → In customize choose Interphase Prescan → Click on Assay and select an assay. Click on Save.

Click on OK to close Customize

Set the camera settings and scan area

Set the 20X focus map

Start the scan

Review

Switch to 100X and right click on one of the firstframes found → Mark the frames for capture in the Review screen. Save.

Find

Return to Find and focus on the image

Do a focus map for the 100X

Capture

Adjust camera and Z-Stack settings so the images produce bright, discreet signals

Go to capture and click on New Cell-Live

Open Auto Capture. Turn Auto Focus off. Click on Start.

Framelist capture

Framelist captures the entire scan area of a slide. When the framelist is created the area is divided into frames based on the power of the objective saved in the assay. The higher the power the more frames there will be. There is only one pass of the scan area and that is capture. The Prescan pass information in the assay is ignored so the same assay can be used regardless of which method of capture you are using. A focus map is only created for the high power capture objective as no prescan is done.
In Capture the Fluorochrome Selection panel is configured by the script. Do not change the fluorochromes in the panel. You can change the Z-stack settings for the fluorochromes.

See topic Framelist flowchart for a printable flowchart click.

To capture using Framelist:

1. Create a new slide.
2. Assign it to a bay in the Scan screen.
3. Click on Customize and select Interphase Finder (Framelist). You must be in Fluorescent mode to see this option.
4. Click on Assay and choose the assay you want to use. Make sure the high power objective is selected for the Analysis pass.
5. Click on Save. The start scan controls will be removed.
6. Switch to the high power objective used for capture if it is not already in place.
7. Set the scan area and camera settings.
8. Click on Focus map and map the focus points.
9. Switch to the Capture screen. The Fluorochrome Selection panel will be configured by the script.
10. Turn Auto Sequence and Auto Camera Setup off and perform one or two manual captures (New cell > Live > Capture) to get the right camera settings and focus offsets; when the settings are right click on Save as Default in Capture and Fluorochrome setup. This is a good chance to make sure the Z-Stack settings are also correct. The Auto Setup toggle below the image can still be used to automatically determine the best settings. When the camera settings are right turn Auto Sequence back on. This will be faster than using Auto Camera Setup for every frame.
11. Click on Auto Capture.
12. Click on Start.
13. When all of the frames have been captured click on Done to complete processing and uploading to the database.
14. Use the separate Review application to analyze the cells found. A shortcut to Review can be found on the Fluorochrome Selection panel.

See Also

The scan wizard
Framelist capture flow

Find

Create a slide and set it to the bay. → In customize choose Interphase framelist → Click on Assay and select an assay. Click on Save. → Click on OK to close Customize → Set the scan area → Do a focus map for the 100X

Capture

Adjust camera and Z-Stack settings so the images produce bright, discrete signals → Go to capture and click on New Cell-Live → Open Auto Capture. Turn Auto Focus off. Click on Start.

Manual capture

Manual capture for spot counting is done in the Capture screen. When you create a slide and select Spot Counting in Capture Mode the Assay Interface dialog will open. This is how you select which assay and script will be used to process and analyze the cells. When an assay is selected both the Fluorochrome Selection panel and the Customize options will be configured for you. Do not change the fluorochromes from the Fluorochrome Selection panel.

Analysis is done using the raw images. For this reason thresholding and contrast options are not necessary. You can turn them on if you want but the more functions performed during capture, the slower the process will be. To finish capturing switch to the Analysis screen. This will complete the cell processing.

To capture cells manually:
1. Open a case and create a new slide.
2. Click on Capture Mode and select the Spot Counting option.
3. Select the assay you want and click on Save.
4. The Fluorochrome Selection panel should show the correct fluorochromes. Z-Stack settings can be changed at this point.
5. Begin capturing cells as you would for normal probe capture. They will be uploaded to the database as they are captured.
6. When you are finished capturing switch to the Analysis screen. This will complete processing of the cells so they are ready to analyze in the Review application.

Tips
- To capture signals with Z-Stack create a fluorochrome with the Z-Stack settings you want and save it as a default fluorochrome. Then, using the Spot Counting Configuration utility, create a script using the fluorochromes with the Z-Stack settings.
- Turn Auto Sequence and Auto Camera Setup off and perform one or two captures to get the right camera settings. The Auto Setup toggle below the image can still be used to automatically determine the best settings. When the camera settings are right turn Auto Sequence back on. This will be faster than using Auto Camera Setup for every frame.

Analysis

Spot Analysis overview

The classification and counting are done by the separate Review application. Using the parameters in your finding script, the signals will be located, counted and the cells classified based on the findings. The results are shown in a spreadsheet type format in Review.

Since all of your analysis is done in Review you should familiarize yourself with its use. See the help files in Review for more information.

Related topics

Spot counting overview

Opening, loading and saving files

Open
Results are loaded and analyzed in the Review application. There is a Review shortcut button on the Fluorochrome Selection panel in spot counting mode. When you are finished capturing switch to Review to analyze and view the results.

Open the whole study or an individual case by clicking on the Open button in the main toolbar. A dialog with all studies in the database will open. Spot counting cases are located in the CVstudy file. Open an individual case by expanding the contents of the study and selecting the case name. Frames that are still being processed are kept in the case CV_WIP. When processing is finished they are moved to the normal CVstudy case.
The Review application uses a similar navigator to load files. To load a scan file expand the contents of a case in the navigator and double-click on the scan file. The data grid for the scan will open in a document window. Multiple scans can be opened allowing you to compare results side-by-side.

Changes made to the measurements or classification are saved automatically as you work. If you edit an image you will be prompted as to whether you want to save the changes or not.

**The data grid**

The data grid has two different views; a montage of images only or a table view with images and measurements in a spreadsheet format. When a scan file is loaded the Classes dialog opens with a list of each class in the script file. The small triangular markers in the corner of each image are color coded to show which class it is marked as. The table view also has a Class column to show the class name.

In both table and montage views, measurements are shown to the right hand side for the current selected image in the grid. The current selected image will be shown magnified in the middle of the Review screen.
Review application

See also
Viewing the images

Classification of images

The images will be classified automatically based on the settings in your assay. Classifications can be changed manually using the Classes dialog. This dialog opens whenever a scan is loaded in the Review application.

<table>
<thead>
<tr>
<th>Classes</th>
<th>Count</th>
<th>Sel.</th>
<th>Hot Keys</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>12</td>
<td>✔</td>
<td>Num 0</td>
</tr>
<tr>
<td>Positive</td>
<td>22</td>
<td>✔</td>
<td>Num 1</td>
</tr>
<tr>
<td>BCR Del</td>
<td>5</td>
<td>✔</td>
<td>Num 2</td>
</tr>
<tr>
<td>ABL Del</td>
<td>18</td>
<td>✔</td>
<td>Num 3</td>
</tr>
<tr>
<td>Ignore</td>
<td>240</td>
<td>✔</td>
<td>Num 4</td>
</tr>
</tbody>
</table>

Total: 297

The class names entered in your script file are listed in the dialog. The images and class names will be color coded with the color selected in the script. The small triangle in the corner of the image shows the color. These settings can be changed for an individual scan file from the Classes dialog.
Change the classification of a cell by selecting it in the grid, then clicking on a Class button or pressing a hotkey. Groups of objects can be marked together. If you use the hotkeys to mark the class pressing the key automatically moves to the next cell in the grid. To view the hotkey list or program a different key click on the expansion arrow on the right edge of the Classes dialog.

Tip - If you want to mark all objects in the grid with the same class, from Table view click on the upper left box to select the whole table.

Note - The default list of hotkeys uses the number pad keys 0-9. In order to use the number pad Num Lock must be turned on.

Reprocessing scans

The entire scan can be classified using a different script by reprocessing it. Reprocess is located on the Review application toolbar.

The Reprocess Assay dialog shows the assay and script originally used to process the scan. Select a different script by clicking the browse button ( ) Edit the current script or create a new one through the Spot Counting Config dialog. When a scan is reprocessed you have the options to overwrite the current scan or save the reprocessed scan as a new file. (Reprocess new regions is not applicable for spot counting assays.)
A Note - You must capture images with **Upload images to database** turned on in Capture to customize to reprocess a scan.

**To reprocess and overwrite a scan:**
1. Capture images with **Upload images to database** turned on.
2. Select the scan node in the navigator or load the scan.
3. Click the **Reprocess** button on the **Review** toolbar.
4. Use the **Assay Creator** to modify or create a new assay to reprocess with.
5. Select the option **Overwrite this scan**.

**To reprocess the scan as a new file:**
1. Capture images with **Upload images to database** turned on.
2. Select the scan node in the navigator or load the scan.
3. Click the **Reprocess** button on the **Review** toolbar.
4. Use the **Assay Creator** to modify or create a new assay to reprocess with.
5. Select the option **Reprocess into new scan**.
6. Click the OK button.
7. The new scan is saved as the next consecutive number in the navigator.

**Creating SPOT classifiers**

Classifiers are used to help define the objects found by the **Review** application. They work in conjunction with the parameters set in the script and are best used to define the size and shape of the cells. Only the cells that are visible in the grid are added to the training data so you can use the selection and filtering tools in the **Classes** dialog to select cells for training.

Classifiers are selected in the Assay Interface dialog.

A full description of all the classifier tools can be found in the **Review** help files.

**To create a classifier:**
1. Load a scan with cells that are good examples of the type of cells you are interested in.
2. Mark all of these cells with the same classification.
3. Using the **Classes** dialog show only the cells of interest.
4. Choose **Classifier > Train**.
5. Select the Spot folder.
6. Click on the **New** button at the right.
CytoVision 3.6

7. Enter a name for the classifier.
8. Select those measurements that are appropriate.
9. Click on OK.

To select a classifier:
1. Open the Assay Interface dialog and select the assay to modify.
2. Click on the Analysis pass tab.
3. Double-click on the name (DAPIShape) in the Type column.
4. Select the new classifier.
5. Click on Open. The new classifier should appear in the Path column.

Tips
- Use Reprocess to see the effects a classifier has on processing.
- To find round cells of the same size use the AREA and CIRC measurements.
- To find irregularly shaped objects add the AXISRAT, AREA and CIRC measurements.
- Use the scatter plots to quickly select cells based on measurements. Then mark them all with the same class. For example if you plot AREA to CIRC and selected a group of small to medium round cells you get a plot like this

![Scatter Plot](scatter_plot.png)

and the cells selected are about the same size and very round in shape. Notice how the irregular cell is not selected.
**Viewing the images**

Images are opened in the larger Image Viewer window by clicking on them in the grid. The Image Viewer window has its own toolbar used to add text or graphics, and to enhance the images. See the help topics in the Review application for information on how to edit an image. Annotations show the location of spots, the outline of the cell and any fusions if you are working with fusion or breakaway probes.
CytoVision 3.6

When an image is open the layer selection panel is shown in the lower left corner of the screen. A separate layer is created for each signal and its annotation. Fusion signals have their own layer and distinguished by circles, single probe signals are square.

| Image Components |  
|------------------|------------------|
| **DAPI**         |  
| Spectrum Green   |  
| Spectrum Orange  |  
| DAPI Mask        |  
| Spectrum Green Signals |  
| Spectrum Orange Signals |  
| Fusion signals   |  

**Layer selection panel**

Select a layer by clicking on its name. Hide a layer by clicking in the middle column to remove the black square. Click on the colored squares to the right to change the render color for a layer. Render color for fluorochromes is taken from the analysis script. Annotation overlays are drawn in the inverted color of their fluorochrome.

Scroll through the images in the grid using the left and right arrow keys. The up and down keys will scroll through the layers or components in the layer selection panel. The spacebar will toggle a component on or off. The End key can be used to switch between color and monochrome for the current selected component.

**CytoVision CEP XY**

**CEP® XY_ENG (Engraftment) Intended Use**

**INTENDED USE**
The Applied Imaging CytoVision™ system is an automated scanning microscope and image analysis system. It is intended for in vitro diagnostic use as an aid in chromosomal analysis. CytoVision assists in the location of interphase and metaphase nuclei on standard microscope slides using both brightfield and fluorescent microscopy.

This particular CytoVision software application, SPOT counting, is an accessory to the CEP® X Spectrum Orange™/CEP® Y Spectrum Green™ DNA Probe kit (Vysis, Inc. Downer’s Grove, IL) and is limited to the analysis of CEP XY probes via high magnification capture and analysis of interphase nuclei. CEP XY is indicated for use to assess the effectiveness of bone marrow transplantation in opposite-sex transplants.

**CYTOVISION™ CEP XY_ENG SUMMARY EXPLANATION and PRINCIPLE OF OPERATION**

The CytoVision Interphase FISH system is an integrated system from Applied Imaging combining the scanning, re-location, capture and analysis functions of CytoVision (CV) with a FISH signal analysis database and Review program.

There are 3 main steps in the user interaction on the system. Steps 1 and 2 are carried out using the CytoVision interface, with step 3 using the Review program.

1. **Assay selection and slide set-up.**
   An Assay is a CytoVision Interphase FISH classifier specific to the probe kit being
used, allowing set-up of fluorochromes, expected signal combinations and other factors involved in scoring captured images. In the Scan screen a **Superscan Wizard** takes you through choosing scan set-up modes, selecting (or creating) Assays, assigning them to cases and slides and customizing aspects of scan and capture. The review features allow you to discard any objects not of interest, and then keep the rest for the high magnification (usually x60) Auto-capture.

2. **Auto-capture.** A capture Wizard takes you through the necessary objective lens focus and capture set-up procedures required by the Auto-capture feature to acquire the cells for processing. The capture procedure is NOT capturing the individual cells as seen in the Review screen. Instead the scan area is broken down into a series of field-of-view sized “frames” which the system re-locates to. Each Frame is captured sequentially, possibly containing several individual interphase cells (depending on cell size and preparation density).

The aim of the auto-capture is to save raw images from the individual fluorochromes signals, which are then processed using the Assay parameters.

3. **Review application.** As soon as auto-capture is initiated the stand-alone **Review** program can be used to view results of the assay processing (although in reality it is usual to wait until a large amount of the capturing is done). Of the cells being captured by Auto-capture only those passing the systems quality threshold procedure will be displayed in Review, classified as to the Assay settings.

Once in Review a range of advanced data display, management and reporting options are available to view, edit and interpret the results of the experiment.

At the end of the procedure you will have 2 sets of case information:

a. A standard CytoVision case and slide structure that contains case details but NO cells – these are deleted after processing by auto-capture to save disk space. However the raw image data being captured can be uploaded to the system SQL database as an option.

b. A Review "Case Study", which contains each single processed list of cells sent through. If the raw images have been saved in the SQL database then this allows reprocessing of the cell lists with modified Assay parameters.

In addition to these main sections or programs there are also associated support applications necessary for the system to calibrate, function and run routinely. See topics Materials and Equipment,

**CEP Materials and Equipment**

**MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED**

- Reagent kits for FISH DNA XY probes kits, e.g., CEP® X SpectrumOrange™/Y SpectrumGreen™ DNA Probe Kit for Fluorescence In Situ Hybridization, Product # 30-161050 and 32-161050 (Vysis, Inc., Downers Grove, IL). Please refer to the specific package inserts for instructions and recommendations related to:
  - staining principles
  - description of reagents
  - reagent kit storage and handling
  - specimen preparation
  - reagent precautions
  - reagent preparation, slide preparation and staining procedure
  - staining quality control
  - interpretation of staining/signal enumeration
  - reagent kit limitations
  - warnings and precautions
  - reagent kit troubleshooting
CytoVision Requirements

System components

There are 3 motorised system requirements for the microscope to be used for the CytoVision Interphase FISH system.

1. Motorized stage. Fitted in place of a standard microscope mechanical stage to allow motor control of the stage X and Y movements. The stage will have a 4 or 8 bay insert from the manufacturer Prior.
2. Motorized microscope focus. Scan and auto-capture on the system require direct focus motorisation. This will use the internal mechanism of microscopes such as the Bx61 or Axioplan 2 ie MOT or by means of an externally connected Applied Imaging Corp focus motor onto manual microscopes such as the Bx51.
3. Motorised fluorescent filter changer. This controls the type fluorescent light reaching the slide and is essential to allow automated capture. Microscopes such as the Bx61 and Axioplan 2 allow direct control of their 6 and 8 position turrets. If turret motorisation is not available then an Applied Imaging Corp excitation filterwheel can be used instead.

The microscope and the 3 sub-systems are interfaced with a CytoVision system (Camera, PC and Monitor) via the Applied Imaging Corp Motor Control Unit (which can also connect with a Joystick device) and, if applicable, a microscope controller.

Software requirements

The CytoVision Interphase FISH system requires the following software components to be installed for correct functionality.

1. Applied Imaging Corp CytoVision v3.1 software or greater.
2. Crystal reports, for the report function.
4. Security key (Dongle) programming for the CytoVision Interphase FISH capability.

Configuration of CytoVision Interphase FISH

Using the Capabilities config program confirm that the System Type is set to Spot. Z-stack is essential for the CytoVision Interphase FISH system function to work correctly and the IMS setting needs to be available for the motorised stage. If a motorized microscope focus motor is to be used this is configured from the separate Microscope setup program and requires Microscope to be active in capabilities. All associated settings are as CytoVision.

If any of these settings are incorrect please contact the Applied Imaging Corp Service and Support department for a re-activation code.

Connection and installation

The microscope and attachments will usually be manufactured at Applied Imaging Corp but will need to be re-assembled at installation. The most important aspects, which may need re-adjustment following assembly on-site are;
• Z-axis limit switch. When an Applied Imaging Corp external focus motor is fitted onto a manual microscope an electronic limit (“kill”) switch is supplied. This is not necessary for a motorised microscope internal focus. The switch consists of 2 components, the sensor, which attaches onto the main body of the microscope, and an activation arm, which attaches onto the microscope sub-stage. The position of the sub-stage arm stops the stage moving up when it reaches the activation point of the sensor. This can be set using the focus controls from the CV Scan screen and adjusting the mount to where the highest magnification objective lens is just touching the slide surface. When doing this, do not have a slide in the bay until you are sure that the limit is not set too high, and be careful that the objective lenses do not get damaged. It may be useful to start with a low magnification lens (x20), set the limit lower than desired and then gradually work up through the lenses. The upper limit should eventually be no higher than where the slide just starts to physically contact a high objective lens (x63 or x100) and for routine working should only be a little above the standard focal plane of a x20 objective (enough to allow for normal variation in slide and coverslip thickness).

• Motorized stage X and Y limits. Stage limits are set by Applied Imaging Corp at manufacture of the system. The Prior stages only have internal adjustment of the limits, although older Marzhauser 4 bay stages have external limit bars on the front and side of the stage, controlling the extreme X (left/right) & Y (front/back) positions.

  • The X limits should be set so that all of the slides on the stage can be covered by a scan and that England Finder positions A through Z are accessible.
  • The Y limits should be set so that the front is just into the frosted area of a standard microscope slide (approx. England Finder position *62), while the back limit is set to prevent the condenser or objective lenses hitting the stage and being damaged. This is probably the most important limit to set up since contact may shift the alignment of the stage and necessitate re-calibration. It may require several attempts to re-set the limit if it required.
  • Do not adjust the X – Y limits after system calibration as this will affect all defaults and scan data, requiring a full system re-calibration.

\[\text{Note.}\] There are also additional “failsafe” switches inside a Marzhauser stage. To access these you would need to unscrew the front or side plates from the stage to adjust the sensor arm positions inside. Do not do this unless instructed by an Applied Imaging Corp representative.

Slide holder level. The microscope slides are placed into a special insert that fits onto the motorized stage. Make sure this is correctly fitted to the motorized stage. There are one or two spring-grips (depending on stage model) at the bottom left of the stage, which the insert pushes into. Once correctly fitted the insert should not move or “rock” if gently tapped at the 4 corners.

Prior stages allow permanent securing of the insert using 6 PVC screws. These should be carefully adjusted, in combination with the 4 grub-screws at the corners of the insert, to produce an even flat focus plane on the stage. This is generally done during system manufacture.

**CEP XY_ENG Quality Control**

Differences in cell processing and staining in the user’s laboratory may produce significant variability in results, necessitating regular performance of in-house controls.

\[\text{Note:}\] These quality control directions are in few package inserts. That’s why we suggest you have them in your help section.

**ProbeChek slides** supplied by Vysis are designed for use as controls for interphase FISH, and laboratory quality control, specifically, for the CEP X/Y IVD Kit. ProbeChek slides may also be used as FISH assay controls for appropriate hybridization conditions and as controls for proper
reagent preparation. All Vysis ProbeChek Control Slides are designed for use with Vysis FISH reagents and protocols.

**Control low-level - male: 95% XX, 5% XY**
ProbeChek Low Level Male Control Slides are tested and optimized for use in Fluorescence *in situ* Hybridization experiments. Vysis slides are manufactured using standard cytogenetic slide preparation methods that are optimized for FISH. These slides are manufactured from a mixture of cultured normal male and female lymphoblast cell lines. If the results using the control slide are not as expected, results with the patient specimens should be considered invalid.

**Control low-level - female: 95% XY, 5% XX**
ProbeChek Low Level Female Control Slides are tested and optimized for use in Fluorescence *in situ* Hybridization experiments. Vysis slides are manufactured using standard cytogenetic slide preparation methods that are optimized for FISH. These slides are manufactured from a mixture of cultured normal male and female lymphoblast cell lines. If the results using the control slide are not as expected, results with the patient specimens should be considered invalid.

**CytoVision Interphase FISH start procedure**

**Need to review - a cut & paste mess from the FDA submission!**

Before scanning sample slides, the system should be calibrated. See topics under Scanning systems - Hardware basics - Calibrating the systems for details. Also review topic under Spot counting - Getting started - Calibration for spot counting.

Confirm that the Motor Control Box is switched on (if a motorized microscope is being used also switch this on) then login to the system using the standard cyto login.

Start the CytoVision program using the desktop icon or from the Start>Programs>Applied Imaging CytoVision menu.

The application starts into the Analysis screen and there will be 3 screen icons available to choose from the main toolbar, Capture, Scan and Review.

[Capture, Scan and Review icons]

**Capture Basics**

Check the color and filterwheel set up for each Fluorochrome from the Capture and Fluorochrome Set Up. Select the ‘Spot Counting’ option. Create an Assay – see topic under Spot counting - CytoVision CEP XY - Creating a CEP XY Spot Assay for details on creating an assay.

Select the Scan screen. On entering the screen the filterwheel (if present) is initialized and a Live image is presented in the display window.
Every time the CytoVision program is started you should re-link software, stage position and controllers by setting Home.
This moves the stage to its mechanical X and Y limit points, sets the current focus position to 0 (external focus motors only) and then moves to the bay datum point. In software version CV 3.0 or later, this is automatically carried out on opening the Scan screen Wizard, which detects if the stage has been homed. Only if you experience a stalling of the stage or switch of the power to the control box during CytoVision operation should you manually re-home using the main screen icon.

Before the Home function is used check the following;

1. If you have an external focus motor manually adjust the focus of the microscope until you find the approximate focal plane of the slide for the scanning lens (x20) before selecting Home. This should be the only time you adjust the focus manually without resetting the Home position.
2. If you are using an Axioplan 2 you should also move the focus to the correct focal plane for your slides before selecting Home. This also resets the position to zero, although after the Home it is still possible to further adjust the microscope focus “manually” as there is a 2-way link with the system.

For microscopes like the Bx61 the focus position is “hard-coded” into the bay-datum calibration setting. Any adjustment of the microscope focus will be electronically linked to the CytoVision through the interface.

Moving to the SCAN screen opens all the controls necessary to setup, adjust and initiate interphase scanning through the microscope. Microscope, stage, focus, filterwheel and keyboard controls are as for CytoVision, including Homing the stage position.

Begin by creating a case - see topic under Working with cases and the library - Opening and creating cases.

The Scan Wizard - Setting up a Scan

All aspects of a CytoVision Interphase FISH setup are controlled though the “Superscan” Wizard which is opened from the icon on the main toolbar. See topics under Scanning systems - Scan Wizard - Creating a scan with the scan wizard, and related topics, for more details.

The first page of the Wizard allows you to create a new scan name or select an existing scan set up which has pre-defined values for scan area, scanning mode, assay type etc.

- Select **Fluorescent** for CytoVision Interphase FISH, Brightfield is for metaphase finding only (if available). Using an existing scan still allows modification of the saved parameters by using the Back option from whichever page you require.
- Select **Next** to move to the next page, slide and case settings. Remember that through the Wizard new slides are created at the end of the procedures, and existing Navigator slides are NOT used.
- Left click on the bay icon representing where your slide is placed on the stage. This will be assigned to the active case in the Navigator and will go green. Right click to change the slide name from the default numerical value.
- To set multiple slides from different cases make sure that the correct cases are open in the navigator and click on them to adjust the displayed case name in the Wizard. Confirm that the case and slide names shown in the Wizard are appropriate for the slides in place on the stage, then select **Next** to move to the scan area page.
- Select a slide and assign a scan area from the pre-defined area window to the right. This contains all scan areas previously saved through the CytoVision “Scan Area” function. To open this function directly, to modify or create a new scan area, select **Edit**. (This can also be opened outside of the Wizard through the main Scan screen toolbar to set default areas separately).
The shaded area in the slide icons shows, approximately, the scan area currently selected.

Move the stage and set the position to be one of the 4 sides of the scan area rectangle (repeat for the other 3). Clicking on the Default Areas button can save a new scan area into the list. By doing this you can have multiple “default” scan areas which can be quickly applied to different preparation or slide types and accessed from within the Superscan Wizard.

**Note:** Even a very small scan area can produce hundreds, if not thousands, of objects and create an auto-capture list that may take several hours. Do not set a large scan area unless you have a sample with an interphase density of less than 1 or 2 cells per field of view (at the scanning magnification).

Once a valid scan area is set the slide icon turns green again. When all slides are green select Next to proceed to the Application selection window.

Select a slide and choose the appropriate Interphase Finder application, Prescan or Framelist. CytoVision capability, if this is available, will also allow Metaphase Finder selections.

**Interphase Finder (Framelist)** allows selection of the appropriate Assay, however scanning is not done and the Auto-capture moves through each single frame on the selected scan area and attempts capture regardless of it’s content.

**Note:** If you have a sample with high cell density with multiple objects in a field of view then this may be appropriate, otherwise there is a risk of empty frames (fields of view) being present and increasing the overall capture time. In this instance Prescan is more appropriate.

**Interphase Finder (Prescan)** allows selection of an Assay with a view to carry out a low magnification (pre-) scan of the selected area. The resultant scan list, which can be viewed in the Review screen, modifies the auto-capture framelist to exclude capture where there would be no interphase cells present, and so speeds up the process.

The Assay selector opens when an Interphase Finder application is chosen. An assay is a classifier, containing all the important information on how to scan, capture and process a case. See topic Creating a CEP XY Spot assay for more details.

- The table can be used to store different assays (rows) and, if required, disease types (columns). The rows and columns intersect and here the assay itself can be selected for use (turning red), edited or a new one created.

Once appropriate Assays have been assigned for each slide in the scan (again indicated by going green) select Next. If Framelist was chosen then no further interaction is required and Finish saves the Superscan (which can be selected for future use). Proceed to Capture screen, see related topics under Spot counting - Capturing.

If Prescan is selected then the next page allows the camera control adjustment and focus map creation necessary to successfully complete the scan.

- The Focus Frequency box allows for re-focusing during the scan (leave at 0 to use the default focus map – sufficient for most samples) and the Stop After box sets a maximum interphase find number for the Prescan.

- The contrast of the live image may need improving for the system to accurately scan for interphase cells. Focus the live image and adjust the Bright, Black and Exposure settings to obtain good visual contrast. As a guideline try to aim for bright white nuclei with some starting to show a little (red) saturation. The background should be dark, but not showing excessive (blue) saturation.

- The change filter button allows access to the filters window to change the microscope (Dichroic) or Filterwheel (Excitation) filter to be used. Under normal operation the fluorescent light will be off unless the slide is being scanned or the camera settings and focus are being adjusted.

- Once the single focus point and camera values have been adjusted for the (DAPI) image, for all of the bays to be used, then select Next to move into move into the Auto-focus procedure.

420
Slides can be uneven and different areas of a slide may have different focal planes. In order to stay in focus throughout the entire scan area the system takes several points, in an elliptical pattern, within the scan area. These are then used to create a 3D Focus Map for the system to determine the correct focus to use during the scanning. The default settings for the Prescan Focus Map are 9 points with 10 planes of focus per point. These are set in the “scan.conf” file in Program Files/Applied Imaging /CytoVision/Defaults folder and can be modified if only small Prescan areas are used (6 or 7 points with 7 or 8 focus planes would be appropriate).

The system automatically moves to, and sets, the focus points. Once done, the slide will go green and the focus map will continue for any further slides in the Superscan. If all focus maps are set correctly then the Prescan will start. However if for any reason the focus map fails, or the “Stop Focus” button is selected for manual intervention, then the 9 points will need to be set manually.

Selecting the Set button saves the current focus point. It may be useful to check camera settings during a manual focus map, as you are able to see the cell intensities as you pass through the focus points.

Once all focus points are completed the slides will go green and “Next” will move into the Prescan.

During Prescan a “Cell count” displays the number of objects found and the scan progress shows what percentage of the scan area has been covered. Note: In a high density preparation this may not reach the end, depending on the “Stop After” number set.

CytoVision Interphase FISH Auto-Capture Wizard

The Auto-capture Wizard links directly into a Superscan that has been chosen from the Scan screen.

Clicking on the Wizard icon opens the first page, the capture objective lens selection. This links into the specific calibration files already created and saved through the separate Microscope Calibration application (above). If you do not see the objective lens you want in this list this means that it has not been calibrated correctly.

Note: If you are working with a manual microscope you will need to move to the correct objective yourself.

Next moves onto the capture set-up, where focus and offsets can be selected and camera controls adjusted if necessary. In addition the Advanced button allows control of the required focus map and auto-focus settings useful for advanced user control.

The Stage controls and Camera setup windows will open automatically. For each slide get the live image in focus (use the sliders or joystick to move a little from the initial point if necessary) and then press the Set Offset button in the Stage Controls window. This then changes the help text below the slides to the pre-capture details.

Use the normal CytoVision New Cell, Live and Capture commands to take a probe image of the cell, adjusting the camera settings to get optimum image contrast for each of the fluorochromes. Z-stack settings for the probe signals could also be modified at this time to ensure that during the capture the whole focal plane of the signals is covered.

Once it is decided that the capture values are appropriate then left click on any further slides in the display (if set) to repeat the Set Offset procedure before New Cell, Live and Capture again. Do not select Next in the Wizard until all slides set have been through both procedures.

The use of fixed and constant camera values during auto-capture is essential, as any attempt to continuously auto-adjust the settings for each cell would add too much time onto the procedure. Also a typical FISH slide preparation is most likely to be uniform in
fluorescent intensity and therefore it should be unnecessary to modify the camera values once capture has started.

- The Wizard then takes over to run the high power focus map necessary before the auto-capture can start. This uses the number of points set in the Advanced section above. 6 should be set for a very small area, 15 for a very large area, 9 for intermediate.

- If auto-focus fails then manual re-focusing will be necessary, however generally auto-focus should be successful and once complete the system should go straight into Auto-capture. From now on manual interaction should not be necessary, although adjustment of the camera, Z-stack and auto-focus parameters are still possible should the user decide fine-tuning is necessary.

**Auto-capture control window**

- Once auto-capture is started the window tells you the number of captured frames and those remaining to be captured. Remember that these values refer to frames and do not necessarily mean the number of cells processed, as this depends on several factors (preparation density, capture lens magnification, raw image quality and assay script parameters).

- The Auto focus option options are adjustable through the Focus Options button, opening a control window. These contain the same controls (and settings) as used for the initial focus map - the Number of Planes the system checks during an auto-focus on the DAPI image and the Spacing between each plane. It is the Focus every nth value that is important in auto-capture, as this determines how regularly auto-focus activates based on the number of intervening planes between one capture and the next. If you want the system to auto-focus on every frame to be captured change this to 1.

- As cells are captured by the system the raw images are processed by the assay and then sent to the separate Review program for analysis.

Review can be accessed any time during auto-capture by clicking on the icon in the capture list window –

A Score Monitor window can be opened using the Mon button in the Auto-capture window. This displays the number of Informative cells processed so far in the Review program.

Informative means the number of cells that fit into one of the pre-defined classes set into the Assay, so it does not include processed but Uninformative cells otherwise seen in the Review program thumbnail list.

Once auto-capture is finished and the cells are processed then all images in the CytoVision slide will be deleted. All that will remain is the slide name (used for reference in the Review program) and the Spot icon created which indicates the slide has been used for Spot-counting.

The raw images captured will be permanently stored in the SQL database and can be used for reprocessing later in the Review program as long as the “Upload images to database” option is selected in Capture customize.

There are no further image analysis options from within CytoVision.

**Review**

The Review screen can be used to check and edit the thumbnail display of the Interphase cells found by a CytoVision Interphase FISH Prescan.
Spot counting

All cells are automatically marked as "Good" – so entry into the screen is not necessary before moving into Auto-capture - however deletion of cells from the list can further modify the auto-capture. If cells are marked for deletion selecting "Save" from below the Navigator will remove these from the list.

Please note that deletion of single cells from the thumbnail list is unlikely to make a large difference to capture, as the “frame” they belong to may still be represented by nearby cells. Only deletion of large numbers of thumbnails, or several close to each other, is likely to have a noticeable effect on auto-capture.

There are no additional or modified features or commands in the Review screen that are required for CytoVision Interphase FISH function. See related topics under Spot counting - Analysis for using the Analysis window.

Creating a CEP XY_ENG (Engraftment) Spot assay

The core of the CytoVision Interphase FISH counting system is the Assay, which includes all the relevant information needed by the system to determine the parameters used for counting signals and scoring the cells.

The Assay contains;

- A Capture List – with the fluorochrome names and Z-stack values.
- The control panel for defining a fluorescent signal – with signal size, spacing, separation and intensity settings
- The count table location, determining how the scored cells are displayed and grouped.
- The DAPI classifier to determine acceptable cell size and shape for scoring.

In addition the Assay contains stop values which interact with the Auto-capture to finish when a fixed number of cells is reached. See topic under Spot Counting - Getting started - Creating and editing assays and the Assay Creator for detail about the Spot Counting Conf window.

In CytoVision the CytoVision Interphase FISH Assays are set through the Assay Selection table. This is entered through:

- The Customize window in the scan screen, selecting a Fluorescent Interphase Finding mode and clicking on Assay.
- The Scan screen Wizard, once an interphase finder mode is selected.

In the Review program the reprocess command links into a copy of the Assay used for the original capture of the cells, which can be modified independently to the default Assay used in CytoVision.

To do this click the spot icon.

Before scanning sample slides, the system should be calibrated. See topics under Scanning systems - Hardware basics - Calibrating the systems for details. Also review topic under Spot counting - Getting started - Calibration for spot counting.

Confirm that the Motor Control Box is switched on (if a motorized microscope is being used also switch this on) then login to the system using the standard cyto login.

Start the CytoVision program using the desktop icon or from the Start>Programs>Applied Imaging CytoVision menu.
Procedure to create, or edit, a CEP-XY_ENG assay in CytoVision.

NOTES:
- The Description will match the assay and disease name in the table - the CEP XY_ENG is a default assay only.
- You will have to adjust the assay settings for your system and slides.

1. Enter the Assay Selector through the CytoVision capture screen, selecting a new slide and then “Spot counting” capture mode.
2. Select and empty space where an Assay and Disease name intersect in the table (or create a new row or column to do so) – the option to create a new Assay is presented, select Yes. To edit an existing assay, click on the associated box in the table.
3. The Spot Counting Config window opens to present the empty starting layout if a new assay is selected or the existing assay layout to allow editing. The step by step described below will be for a new assay.
4. Enter 2 into the Number of Probes section. You can have a maximum of 5 fluorochromes in the list (not including the DAPI) and if the number of fluoros is greater than 2 then a fusion check box appears, allowing creation of classes where 2 fluorochrome signals overlap.

5. Once the fluoros have been entered the Add Class button becomes active. Selecting this creates the initial class for the script and allows selection of the fluorochromes and counterstain. An Assay must contain at least one class before it can be saved.
6. From the pull down list next to the Counterstain select the appropriate DAPI name.

These are taken directly from the CytoVision “Build List” so if you want to use fluorochrome names not present in the list you need to create them before-hand. The color box next to the name will be used for the DAPI mask outline seen in the Review screen (or for the scoring boxes overlayed onto the Probe signals below)

7. Repeat for the Probe names. For a Fusion assay check boxes will be present and need to be activated for the fluorochromes which are part of the fusion test.

For CytoVision Interphase FISH Assays it is advisable to create specific fluorochrome names not used in other capture CytoVision modes (Probe or CGH) to reduce the risk of other users inappropriately modifying colours and capture values.

8. Below each probe name are the starting settings for the Z-Stack to be used during capture. Use an appropriate Number of Planes and Spacing for the objective lens and sample type – if unsure 5 Planes at 1 microns is a typical 40x starting value.

9. Spot Area. This is possibly the single most important factor in the Assay which affects the accuracy, allowing a range which removes small and large background but defines the spots present on the cell. The sizes are in pixels and are approximately the size as
10. **Class values.** For each class created you can define Review Program Display characteristics and set the scoring results necessary for a cell to be classified. From left to right; Class Name; Normal check box (only one); 0 signals expected; Fluorochrome 2 signals expected; Fluorochrome 2 signals expected; Auto-capture Stop Count for the class.

<table>
<thead>
<tr>
<th>Class Name</th>
<th>Normal</th>
<th>Count</th>
<th>Delete</th>
</tr>
</thead>
<tbody>
<tr>
<td>101G_XY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20_Y%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10_Y0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11. With these 3 classes present then all other scores will go into an Uninformative class automatically created in the Review program.

12. Separation distances. This sets how close together 2 spots have to be before they are counted as one in the classifier. Distance can be set as a percentage of nuclear diameter (good if signals vary in size) or % spot diameter (good if signal sizes are consistent) and is based on measurements from the centre of one signal to the other. In the table shown below, the Fluorochrome separation is set at 200% of Spot Diameter. Therefore the minimum distance between the 2 spots must be at least the same as the size of the spots themselves, otherwise they will be counted as one merged signal. If the separation distance is set to 100% then the 2 spots can be touching before they are merged.

<table>
<thead>
<tr>
<th>Separation Distances</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Nuclear Diameter</td>
</tr>
<tr>
<td>% Spot Diameter</td>
</tr>
</tbody>
</table>

Fluorochrome

13. Stop scan criteria. This sets a limit to the number of informative cells to be captured in auto-capture. This is based on abnormal cells (all but the normal class) or total cells. Remember that this does not include Uninformative cells, only cells matching the relevant scoring classes.

Stop Scan Criteria

Stop after 0 abnormal cells

Stop after 400 total cells
14. Classifier selection. If a DAPI classifier has been created it can be assigned for default use for this assay.

15. For a CEP XY_ENG assay, follow the above image settings

16. The advanced Assay section contains features that are more likely to require modification during specific Review Program re-processing procedures to accommodate variations in sample type (DAPI staining, signal intensity etc). The settings shows below are the suggested default settings only.

The advanced button opens a window with 2 sections. First a radio box list which has options either on or off, the second is a slider bar section allowing more interactive user control of some of the counting parameters. These features are described in detail through the system Help files. For routine Spot use the default setting. The first four are not usually necessary for an Interphase assay.

![Assay Configuration](image)

17. Slider controls. The sliders relate to adjusting the DAPI boundary (and how signals are counted in relation to it) and several advanced features to allow fine-tuning of background removal and signal intensity levels for scoring.
### Counterstain threshold

<table>
<thead>
<tr>
<th>Value</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>40</td>
</tr>
</tbody>
</table>

### Boundary dilations

<table>
<thead>
<tr>
<th>Value</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

### Debris min size

<table>
<thead>
<tr>
<th>Value</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Minimum spot intensity

<table>
<thead>
<tr>
<th>Value</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>255</td>
</tr>
<tr>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>

### Minimum fusion intensity

<table>
<thead>
<tr>
<th>Value</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

### Minimum spot intensity after merge

<table>
<thead>
<tr>
<th>Value</th>
<th>Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>0</td>
<td>50</td>
</tr>
</tbody>
</table>

- **Counterstain Threshold.** This sets the systems stringency for determining the boundary of the DAPI nucleus – as shown by the “DAPI Mask” in the Review program. 50 is the default but if captured nuclei are really dark or low contrast then the boundary may not be accurate. Lower the value to expand the DAPI mask, increase it to tighten it, which may be necessary with clusters of cells or to obtain better segmentation when nuclei are really bright. Suggested setting for the CEP XY_ENG assay = 40.

- **Boundary dilations.** This expands the DAPI mask by a set number of pixels above the Threshold level, effectively increasing the size of the nucleus. This can help if you have probe signals on the boundary of the nucleus that may otherwise be excluded by counterstain thresholding. Suggested start setting for the CEP XY_ENG assay = 1.

- **Debris min size.** This feature deletes from the display (and score) any DAPI cell that contains “debris” above the size value set by the slider. This is different to the probe size ranges which affect the scoring of signals but keep the cell on display for manual re-scoring if desired. In routine use it is envisaged that this feature would only be used at the high extremes of debris size, 700 – 1000 where such large objects of background could obscure any legitimate signal and negate even manual scoring. Suggested start setting for the CEP XY_ENG assay = 0.

- **Minimum spot intensity.** This is an absolute value taken after background subtraction where any signal with a grey level intensity less than this number will not undergo any count processing. If you have clearly defined signals (compared to background) then you can increase this value to reduce the risk of background spots being counted. Suggested start setting for the CEP XY_ENG assay = 20.

- **Minimum fusion intensity.** This is a percentage value of the maximum signal intensity for signals within a nucleus. If you have 2 signals of the same colour in the cell the weaker signal must be at least this value (%) compared to the brightest before it will be counted. This measurement is before fusion analysis (before the end of all the processing being carried out to determine how many individual signals are present). Not applicable for the CEP XY_ENG assay.
• **Minimum spot intensity after merge.** This is a similar measurement for signals with a % intensity less than the brightest one. This measurement is after fusion processing for signals which are not merged as part of a larger spot. Not applicable for the CEP XY_ENG assay.

• **Aggressive split.** This is the default option for the system to identify single DAPI stained cells compared to background or when cells are part of clusters or surrounded by debris. In most cases this option is best left on as it improves separation of nuclei in close to each other. However, if you find your nuclei are getting broken up then this option can be turned off. Broken up nuclei normally occur when the appearance of nuclei is mottled.

• **Contrast stretch probe images.** This will enhance the probe signal images displayed in Review. It does not affect analysis in anyway and can be used in situations where the probe images contain high levels of background.

18. Once all of the Assay fields and parameters have been decided on then select **Done** to close the window and either Save or Discard your changes or continue with the editing.

19. The Assay will now be visible in the Selector table and can be used for new Scan and Captures and for re-processing in the Review program.

20. For image re-processing through Review a new button will be available – **Load.** This allows you to quickly restore a pre-built Assay rather than modification of the original Assay used for the scoring (to be able to compare Assay accuracy, etc.).

The Load Assay Window will show you all Assays saved which contain the same number of fluorochromes to be scored as the current set of data. Therefore you can quickly load a pre-saved assay containing new class data or modifications to the scoring parameters rather than modifying the original one used.

**BEWARE** – this allows you to select Assays which have different fluorochrome names – if you do so it is important that you change the fluorochromes to the correct ones captured for this data otherwise data corruption could occur.
**Microscope Control Application**

**Microscope Control Application**

This application allows you to control motorized microscopes and take full advantage of automatic features such as filter, objective and focus adjustment.

**Related topics**
- Configure a new microscope
- Control the microscope

**Getting started**

**Getting started**

If you have purchased this package then you will already have the capabilities installed on your system. If not please contact your local sales representative or Applied Imaging for details.

Before using the microscope you must configure its capabilities and set the default microscope.

**Related topics**
- Configure the capabilities
- Set the default microscope

**Configuring a new microscope**

Before configuring a new microscope you will need to know its capabilities, the number of filters and objectives it has, and the maximum number of focus steps.

1. Click on **Start** then point to Applied Imaging in the Programs menu and select Microscope.
2. Select the microscope you are using from the list and the COM port it is plugged into.
3. Click on **Setup**.
4. Check off the capabilities of your microscope.
5. Enter the number of filter positions and objective sockets.
6. (Optional) Click on **Filter Names** to enter names or labels for the filters.
7. (Optional) Click on **Nosepiece Names** to enter names or labels for the objectives.
8. Enter the maximum range of focus steps possible.
9. Click on **Default** if this is the default microscope.
10. Click on **Save**.

If you want the setup program to determine these for you, place a check in **Use Auto Detect**. When on, **Auto Detect** will check the capabilities each time the microscope is initialized.

**Note** - Some microscopes do not detect their own capabilities well. If there is a problem with detection, turn **Use Auto Detect** off and configure the capabilities manually.
Setting the default microscope

1. Click on Start, and then select Microscope from the Applied Imaging group in the Programs menu.
2. Select the microscope from the list.
3. Click on Setup.
4. Click on Default.
5. Click on Save.

XY Stage control

This feature allows control of the motorized stage through the Applied Imaging controller along with control of a motorized microscope through a separate COM port and cable. This configuration is only found when a scanning system is used with a motorized microscope.

To use the feature check the XY-stage box in the XY-stage group on the configuration page and select the communication port used for the stage. The port chosen must be different from the one selected for the microscope itself. Standard configuration would be the stage controller on COM1 and the microscope on COM2.

See topic - common configurations for a list of common configurations.

Common configurations

In order to properly control the stage, motorized microscope and filterwheel there are three files that must be set:
- .mic file using the Microscope Control Application
- Capabilities using **Capabilities config**
- Filter wheels file using **Stage and Wheel Config**.

All of these programs can be found in the Applied Imaging menu (Start-Programs-Applied Imaging). The following is a list of systems of differing capabilities and the settings used to configure them.

<table>
<thead>
<tr>
<th><strong>scanning system with:</strong></th>
<th><strong>Program</strong></th>
<th><strong>Settings</strong></th>
</tr>
</thead>
</table>
|                          | Microscope Control Application | 1. Check the XY stage box  
2. Set ComPort to COM2 for stage  
3. ComPort to COM1 for microscope |
|                          | Capabilities | Select the Z-Stack, Finder, IMS stage and Microscope options |
|                          | Stage and Wheel | Set the filter wheel COM port to the same port selected for the XY stage in Microscope Control Application (usually COM2). |

<table>
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<th><strong>Settings</strong></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Microscope Control Application</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Capabilities</td>
<td>Select the Z-Stack, Finder, IMS stage. Deselect the microscope option.</td>
</tr>
<tr>
<td></td>
<td>Stage and Wheel</td>
<td>Set the filter wheel COM port to the same port selected for the XY stage (usually COM2).</td>
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</tbody>
</table>

<table>
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<tr>
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<th><strong>Settings</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microscope Control Application</td>
<td>Uncheck the XY stage box. Set ComPort to COM1 for microscope</td>
</tr>
<tr>
<td></td>
<td>Capabilities</td>
<td>Select the Z-Stack, IMS stage and Microscope options. Deselect the Finder option</td>
</tr>
<tr>
<td></td>
<td>Stage and Wheel</td>
<td>Set the filter wheel COM port to a different setting from the microscope; usually COM2.</td>
</tr>
</tbody>
</table>

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<th><strong>Settings</strong></th>
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<td>Microscope Control Application</td>
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<tr>
<td></td>
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</table>
CytoVision 3.6

<table>
<thead>
<tr>
<th>Capabilities</th>
<th>Select the Z-Stack and IMS stage options. Deselect the microscope and Finder options.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage and Wheel</td>
<td>Set the filter wheel to COM2</td>
</tr>
</tbody>
</table>

**Controlling the microscope**

**Controlling the microscope**

There are two ways to control the microscope. One is through the control application itself which can be launched from within the CytoVision program and separately as a stand-alone application. The other involves the filter blocks and focus only. The automatic filter block and focus adjustment functions of CytoVision can be set to use the default microscope.

**Related topics**

- Launch the program from CytoVision
- Launch the program as a stand-alone application
- Use automatic filter adjustment
- Use automatic focus adjustment

**Using as a stand-alone application**

If the main program is not running or you want to control a different microscope, use the Microscope program. Click on Start then point to Applied Imaging in the Programs menu and select Microscope. Select a microscope from the list and click on Connect.

The capabilities of the microscope are displayed on the first page. Click on the tabs to change to the other controls. The tabs shown will change depending on the capabilities of the microscope.

Click on Reset to send a reset command to the microscope. Click on Manual ON to switch the microscope into manual mode so that certain functions such as the Z focus drive are controllable by hand. The need for this command will vary depending on your microscope.

**Note** - Manual ON may disable control of certain microscope functions by the driver as some microscopes may not allow for both manual and automated control over the communications interface.
**Microscope Control Application**

**Integration with the main software**

The ability to drive motorized microscopes has been integrated into capture. Depending on the capabilities of the microscope, you can assign filter blocks to specific fluoros so the program automatically positions the blocks during capture, use the focus motor with **Auto Focus Offset** for probe capture or just for focusing on-screen. These functions can be performed without starting the Microscope application.

There are keyboard shortcuts for driving the filter turret or slider. Press the **Alt** key along with the number of the filter you want. After the filter is in position, the shutter will open. E.g. Alt-2 would move the turret to position 2 and open the shutter. Alt-0 will open and close the shutter.

If you would like to drive the other features of your microscope, click on the Microscope control tool in the Capture toolbar.

**To change filter blocks**

First choose the filterwheel you want to control from the Wheel options. Select a filter by clicking on the blocks in the diagram. Scroll through the filters by clicking on the arrows. The names of the filters can be entered from the **Setup** page, or from the **Filter panel**.

Click on **Flip** to open or close the shutter.

**Keyboard shortcuts**

Press **Alt** followed by the number of a filter. e.g. **Alt-1** to move to filter 1 The shutter will automatically open after the filter moves.
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Press Alt-0 to close the shutter. Pressing any filter key will open it again.

*Filter block controls*

**To change objectives**

Select an objective by clicking on the diagram or moving the slider. Scroll through the objectives with the arrows. The names of the objectives can be entered in the Setup page.
**Objective nosepiece controls**

**To change the focus**

Focus movement is controlled in units called steps. Each microscope determines the size of each step. The range of movement or **Drive Absolute** scale, is entered as part of setting up the microscope. If you want to limit movement when manually controlling the stage at the microscope, click on **Change** and enter new limits.

Use the **Drive Absolute** slider to move the focus position to a specified point. The stage will move when **Move** is pressed.

To move the focus relative to current position use the **Relative Control** slider. The stage will move when **Move** is pressed.
Focus controls

To change the light level

Move the slider to adjust the brightness of the lamp.

Lamp control

To change the light path
Choose the option to direct the light path. All microscopes may not be capable of every option.

**Light path**

**To adjust the field diaphragm**

Use the slider or the spin controls to open the aperture diaphragm. Click on **Flip** to move the condenser in or out of the light path.
To adjust the aperture diaphragm

Use the slider or the spin controls to open the aperture diaphragm.

Aperture diaphragm controls
CytoNet

Introduction to CytoNet

CytoNet uses the Internet to send and receive cases between users. When a case is sent through CytoNet, it can be karyotyped or processed like any other case. It also provides a way to review cases remotely from a standard PC or laptop. All cases are sent through the Applied Imaging server at http://www.cytonet.com. You will need a separate account with an Internet provider or a site network with Internet access in order to use CytoNet.

The CytoNet dialog is made up of three tabbed pages. Transfer is used to send cases, Receive is for retrieving them from the server. Settings is where your user name and log on are tested. Switch pages by clicking on the named tabs.

Related topics

- Configure the connection settings
- About sending or transferring cases
- About receiving cases
- To test connection settings

Configuring CytoNet

Configuring CytoNet

CytoNet is configured in a separate utility called CytoNet Setup. This utility is accessed by clicking on Start then pointing to Applied Imaging in the Programs menu, and selecting CytoNet Setup.

The local server is the machine that will make the Internet connection either through a modem connected to it or through a site network connection. Any networked systems will be labeled as remote.

A User ID and password will be given to you when you subscribe to CytoNet. These along with the IP address of the server are entered in Connection Settings.

Choose the sites that you will send cases to, and receive cases from in Other Users. This ensures that you have control over who can and cannot send cases to you. See the topic CytoNet user permissions for more information.

Steps to configure CytoNet:

1. Click on Start then pointing to Applied Imaging in the Programs menu, and selecting CytoNet Setup.
2. Identify whether a machine is a local server or if the server is remote. The local server is the machine that will make the Internet connection either through a modem connected to it or through a site network connection.
3. Click on Connection Settings to enter your user ID and password.
4. Choose the sites that you will send cases to, and receive cases from, in Other Users. This ensures that you have control over who can and cannot send cases to you.
Use a remote Local Server

Select this option if another machine on the network is used to connect to the Internet. Enter the name of the machine in the Name field.

Make this machine a Local Server

Select this option if this machine is the one used to connect to the Internet.

Connection Settings

Contains information needed to connect with the CytoNet server.

Other Users

Opens the dialog that allows you to choose who you can send cases to and receive cases from.

To enter connection settings

1. Enter the user ID given to you by Applied Imaging.
2. Enter your password.
3. Enter the Server IP address. This will be given to you when you receive your login ID and password.
4. Click on Save and Verify. A message will tell you if you successfully connected to the server.

If connection fails:

- Check that the IP address is typed correctly. Look for double dots (..) or spaces.
- Make sure that your user name and password are typed correctly, and that Caps Lock is not turned on.
- Check that the modem is turned on.
- Check that the modem is plugged into an analog phone line. Many modern phone systems are digital and cannot be used for modem connections.
- Try to connect again. Sometimes the connection will be successful on a second attempt.
- If the modem shares a line with a FAX machine, make sure that the FAX is not using the line.

**About setting user permissions**

You cannot send cases to a site unless they have setup permission for you to do so on the Applied Imaging server. Likewise, no-one can send cases to you unless you have allowed them to. This is done to prevent any unknown or unwanted files from being received on your system.

Setup this user list from the **Connection settings** of **CytoNet Setup**. Click on **Start**, then point to **Applied Imaging** in the **Programs** menu and select **CytoNet setup**. Click on **Other Users** to open the **Links with other users** dialog. The column on the left contains a list of all users who have given you permission to send cases to them. The column on the right contains a list of all users that have permission to send cases to you. The middle column lists all CytoNet subscribers.

**To give someone permission to send cases to you:**

1. Select their name in the middle column and click on **Add**.
2. Click on **OK** to save the settings and send the changes to the Applied Imaging server. To prevent them from sending cases to you, click on there name in the column on the right and click on **Remove**.

![Links with other users](image)

**About Configuring CytoNet connections**

**To test your CytoNet connection**

1. Click on **CytoNet**.
2. Click on the **Settings** tab.
3. Click on **Test login** and the Applied Imaging server will be contacted. If the test fails check that your modem or Internet connection is turned on and plugged in.
If connection fails:

- Check that the IP address is typed correctly in Connection Settings of the configuration utility. Look for double dots (..) or spaces.
- Check that the modem is turned on.
- If you are on a remote system, check that the auto dial feature in Dial up Networking is enabled on the local server.
- Check that the modem is plugged into an analog phone line. Many modern phone systems are digital and cannot be used for modem connections.
- If the modem shares a line with a FAX machine, make sure that the FAX is not using the line.

Related topics

About configuring the CytoNet connection

Sending and receiving cases

Sending cases

You can only send cases to other CytoNet users who have given you permission to do so. This is done from Other Users in the CytoNet Setup program. There are two formats that cases can be sent in; one is as working CytoVision images, the other as TIFF images. Send cases confidentially by placing a check in Confidential. This prevents the patient’s name from being sent with the case.

To send cases, select the cases you want to send and who you want to send them to. Choose a format and click on Send Selected Cases. A small dialog will appear on the screen that shows a status bar for the Send process. When the system is finished sending cases, the status dialog will close. If you want to stop the send process, click on Cancel in the status dialog.

If a case is going to be returned, make sure that no changes are made to the case until it has been received back again. When a case is received and imported back into CytoVision it will overwrite the existing copy.
CytoNet Transfer

<table>
<thead>
<tr>
<th>Machine</th>
<th>Select the name of the machine who's cases you want to display. All machines will display every case on the network in the case list.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filter</td>
<td>Use the filter to limit the cases in list or to find specific cases.</td>
</tr>
<tr>
<td>Select all</td>
<td>Selects all cases visible in the list. If a filter has been used, only those cases whose names appear in the list will be sent.</td>
</tr>
<tr>
<td>Send To</td>
<td>Choose the recipients name from the drop down list. If you do not see the site you want to send cases to, check that they have given you permission to send to them.</td>
</tr>
<tr>
<td>Format</td>
<td>Choose between sending the images in CytoVision format or as TIFF images.</td>
</tr>
<tr>
<td>Confidential</td>
<td>Place a check in this box to prevent the Patient name from being sent with a case.</td>
</tr>
<tr>
<td>Send Selected Cases</td>
<td>Click on this button to activate your modem or network connection to start sending cases. You can stop the process by clicking on Cancel in the status dialog.</td>
</tr>
</tbody>
</table>

**Note** If a case was sent with Confidential on, it is important to make sure that it is also on when you receive them. If not the patient’s name will be erased from the system.

**Related topics**

- Step by step transfer

**Formats for sending cases with CytoNet**
There are two formats for sending cases. In both formats the Cases Details file is sent with the images. **CytoVision** will send the cases in a working CytoVision/Genus format. Use this option if the recipients need to be able to modify the cases. They will need to have a CytoVision or Genus to receive these cases.

To send images across the web to a remote PC, use the **Web** option. This converts the images to a TIFF format for review only. This is the format used to send cases to someone who is not physically connected to your system through a network, or to someone who wants to use a web browser to view the images. Only the Results field of Case Details can be modified when this format is used.

**Receiving cases**

When cases have been sent or returned to you, use the **Receive page** to import them back into the cases directory. Click on **Get Case List** to see a list of all cases that have been sent to you. Select the cases you want and click on **Receive Selected Cases**. If you want to stop the process for any reason click on **Cancel** in the status dialog.

<table>
<thead>
<tr>
<th>Case list</th>
<th>A list of all cases sent to you is shown with their place of origin and their file size. Make sure that you have enough disk space left on your hard drive to store cases before receiving them.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Get Case List</td>
<td>Contacts the Applied Imaging server and displays a list of all cases that have been sent to you. Click on this button anytime you want to refresh the list of cases available to receive.</td>
</tr>
<tr>
<td>Filter</td>
<td>Use the filter to limit the list of cases or to find a specific case.</td>
</tr>
<tr>
<td>Select all</td>
<td>Selects all cases visible in the list. If a filter has been used, only those</td>
</tr>
</tbody>
</table>
cases whose names appear in the list will be downloaded.

| **Confidential** | Prevents the patient name from transferring with the case. If you send cases with **Confidential** checked make sure that you receive them the same way. Otherwise the name will be erased. |
| **Receive Selected Cases** | Downloads any selected cases from the server and imports them into your machine. A status bar will show the progress of the receive process. |

**Note** - If a case was sent with **Confidential** on, it is important to make sure that it is also on when you receive them. If not the patient’s name will be erased from the system. You can leave **Confidential** turned on in the Receive page. That way there is never a chance of losing any patient data.

**Related topics**

- Permit other users to send cases to you
- Step by step Receiving
Lab Management interface

Lab management overview

The Lab Management software package will enable you to have more flexible Case Details fields and the power of using a database for managing patient data. Rather than using the standard Case Details files, you can use a third party database to manage your patient data. With this system there are more fields available, and the titles of the fields are created by you. For the purpose of this manual the computer that houses the Lab Management software will be referred to as the LMS.

Data is sent between the Applied Imaging system and the LMS as ASCII text files. There are 3 types of files:
- Interface file (.txt suffix) – The file on the LMS that contains the original data and determines both field names and type of field displayed on CytoVision.
- Results file (.res suffix) – file generated by CytoVision when an LMS case is closed. Contains any data altered by CytoVision user.
- Completion file (.cmp suffix) – generated when a case is marked as complete at the CytoVision.

Related topics
- Setup the interface and files
- Work with LMS cases

Getting started

Getting started

There are three steps needed to start using the LMS package:
1. Create a database that will read and write the text files used by the LMS package. It is advisable to seek professional help by someone familiar with database construction.
2. Physically connect the systems. A network specialist can help you with this connection.
3. Configure the LMS package connection settings.

Note - The connection is slightly different if you have an Applied Imaging client-server network so please choose the correct instructions accordingly.

Related topics
- Read about building the text interface files
- Configure the LMS connection settings for a non-client-server network
- Configure the LMS connection settings for a client-server network

Network connection for non-client-server networks

Before configuring the program to connect to an LMS, you must network the PC that hosts your Lab Management database with the Applied Imaging systems. The way the systems are networked will vary depending on the hardware and software involved, so consult with a
network specialist for help with the physical connection and the network configuration of the operating system. This procedure must be done for every system on the network.

1. On the Lab Management System (LMS), share the directory that contains the case text files.
2. Click on the Windows™ Start button then point to Applied Imaging in the Programs menu and select Network Config.
3. The Network configuration dialog will open.
4. Select the name of the LMS in the left column.
5. Click on Add.
6. Click on LMS.
7. Enter the System name in the LMS field and the shared directory name in the export directory field.
8. Click on Add.
9. Click on the X in the upper right-hand corner to close the LMS setup dialog.
10. Click on OK in Network config to save the settings.

**LMS Setup dialog**

**Related topics**

- Building the ASCII interface files

**Network connection for servers**

Before configuring the program to connect to an LMS, you must network the PC that hosts your Lab Management database with the Applied Imaging systems. The way the systems are networked will vary depending on the hardware and software involved, so consult with a network specialist for help with the physical connection and the network configuration of the operating system. If you have an Applied Imaging client-server network then only the server is set to communicate with the LMS. Then the server is used by the other systems to access the LMS files.

**To connect from a fileserver network:**

1. On the server create a directory for the interface files and share it.
2. On the LMS map a network drive to the shared directory you just created on the Applied Imaging server.

**then at each workstation.**

1. Click on the Windows™ Start button then point to Applied Imaging in the Programs menu and select Network Config.
2. The Network configuration dialog will open.
3. Click on LMS.
4. Enter the server name in the LMS field and the shared directory name in the export directory field.
5. Click on Add.
6. Click on the X in the upper right-hand corner to close the LMS setup dialog.
7. Click on OK in Network config to save the settings.

**Related topics**

- Building the ASCII interface files

**Configuring interface files**

**Building the ASCII Interface files**

The files sent to CytoVision by the Lab Management System (LMS) must be ASCII based text files. The text must be continuous (no paragraph markers), enclosed in quotes and separated by commas. There are two sets of information that are used to build the interface files for each case; Key records and Test records. Key records contain the basic information about a case such as the patient’s name and date of birth. There are sixteen key record fields found in the Key Fields and Additional Key fields pages. You must have one, and only one set of Key records in each case.

The second set of information is the Test records. Test records contain information related to the tests performed, and the results from these tests. There is no limit on the number of Test records you can have in a case. Test records are optional and do not need to be included.

The final interface file must be built in the following order:
1. Key record header
2. Key record fields
3. Additional key fields
4. Test1 record header
5. Test1 record fields
6. Additional test fields
7. Test2 record header
8. Test2 record fields
9. Additional test fields

The interface files for the tabbed pages are built in this order:
1. Key Fields

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*Lab Management interface*

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2. Additional Key Fields
3. Test Fields
4. Test Fields - Tally Box
5. Test Fields - ID Time
6. Test Fields - Banding
7. Additional Test Fields (1)
8. Additional Test Fields (2)

Each field on a page is make up from three parts:
"type","title","data"

The first part tells the CytoVision if the field is a header, or if it is a text field, whether or not the data in that field can be edited. Use "H" to specify a header, "F" for a fixed or non-editable field and "E" for fields that you want to edit from the CytoVision.

The second part is for setting the title for each field. A maximum of 64 characters can be used. The third is for any data you want to be entered in the field itself. Data fields can contain up to 16,384 characters. For example this text file:

"H","LabIntID","1","F","LabNo","2test","F","Date Of Birth","1960/12/25","F","Patient Name","Bond, Jane A","F","Specimen Type","Blood - Peripheral","F","Alert","Alert message","F","Indication","Cogenital anomalies/Clinical features Large Ears, Family History of chromosome or genetic disorder. Uncle with CF","F","Client Service Comment","Login comments"

would produce a Case Details file that looked like this:

<table>
<thead>
<tr>
<th>LabNo</th>
<th>2test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Of Birth</td>
<td>1960/12/25</td>
</tr>
<tr>
<td>Patient Name</td>
<td>Bond, Jane A</td>
</tr>
<tr>
<td>Specimen Type</td>
<td>Blood - Peripheral</td>
</tr>
<tr>
<td>Alert</td>
<td>Alert message</td>
</tr>
<tr>
<td>Indication</td>
<td>Cogenital anomalies/Clinical features Large Ears, Family History of chromosome or genetic disorder. Uncle with CF</td>
</tr>
<tr>
<td>Client Service Comment</td>
<td>Login comments</td>
</tr>
</tbody>
</table>

Instructions on how to setup the fields for each page are listed separately to make it easier to follow. The actual file will be one, long, continuous text file. The pages are listed in the order that they must be sent to the CytoVision.

Related topics
Key Fields

The Key field header along with nine defined fields create the Key Fields page. Four other fields are stored on the CytoVision and are not included in the LMS interface files. These are Case, Keywords, Archived On and Complete. These are the only fields that are required for a basic interface file.
You can only have one Key Field section in each case. The header must be: "H","LabIntID","1"
The remaining nine fields are from the LMS.
Fields 1-3 - Non-scrolling fields designed for small amount of data such as laboratory case number, patient name or date of birth.
Fields 4-7 - Large fields with scroll bars.
Fields 8-9 - Small fields designed for numerical data like priority numbers.
Fields 1,2,3 and 7, along with the CytoVision fields at the top of the page, are the only fields that you can include with your images when printing.
Building the ASCII interface files

### Additional Key Fields

These fields must immediately follow the Key Fields. You do not need to include these files. If you do not want to use them, insert the Test header after field 9 of Key fields. Fields 10-15 - Large fields with scroll bars; they create the Additional Key Fields page.
Test Fields

There is no limit as to the number of Test Field sections you can include in a case. Immediately following your key fields (field 9, or if you have additional key fields, number 15) you must insert the Test Field header: "H", "TestIntID", "1" would be the header for the first section. Any additional test sections would change to "2", "3", etc. The number of the header is the number that appears in the Test field at the top of the page. The name that follows is picked up from field 16 data.
You do not need to include any test fields in your interface file. If you do not want to use them, do not insert the test header, and end the file with your key fields. If you do include a test header and any of test fields, then you must include fields 16-59 (end of the Banding page). If you want to use some, but not all of these fields, format the unused ones as blanks; e.g. "F", "", "".
Fields 16-17 - Non-scrolling, single line fields designed for small amounts of data.
Fields 18-21 - Large, scrolling fields designed for data related to any tests run on a sample.

**Test Fields - Tally Box**

Fields 22-46 configure the Tally Box and are formatted the same way as other fields. They immediately follow field 21 of Test Fields. The test number and description are picked up from the test header inserted before field 16. If you are including Test records, then these fields must be included in the interface file. If you do not want to use them leave them blank; E.g. ""'","'","

Field 22 - The title part of this field is ignored and can be left blank but must be included. E.g. ""E"","","

Fields 23-26 - The titles for these fields are used to create the text that labels the rows of the tally box. E.g. ""E",""Counted",""
Fields 27-46 - The titles for these fields are ignored. Only the state and data are displayed. E.g. "F", "", "46" would label a column 46, and would not allow the CytoVision user to change that label.

![Image of LMS interface with Test fields - ID/Time page]

Building the ASCII interface files

**Test Fields - ID/Time**

Fields 47-56 create the Test Fields ID/Time page. The fields alternate between the upper and lower section; or between a regular field and an auto-dating field. If you are including a Test record, then these fields must be included in the interface file. If you do not want to use them leave them blank; e.g. "F", "", ""

Field 47 - Non-scrolling field designed for a name or initials.
Field 48 - Non-scrolling field designed for a date. Enter the current date and time automatically by pressing the button to the right.
Field 49 - Non-scrolling field designed for a name or initials.
Field 50 - Non-scrolling field designed for a date. Enter the current date and time automatically by pressing the button to the right.
Field 51 - Non-scrolling field designed for a name or initials.
Field 52 - Non-scrolling field designed for a date. Enter the current date and time automatically by pressing the button to the right.
Field 53 - Non-scrolling field designed for a name or initials.
Field 54 - Non-scrolling field designed for a date. Enter the current date and time automatically by pressing the button to the right.
Field 55 - Non-scrolling field designed for a name or initials.
Field 56 - Non-scrolling field designed for a date. Enter the current date and time automatically by pressing the button to the right.

Test Fields - Banding
Fields 57-59 create the Test Fields - Banding page. They are large scrolling fields designed for information related to banding. If you are including a Test record, then these fields must be included in the interface file. If you do not want to use them leave them blank; e.g. "F" 

If you do not want any Additional Test Fields records you can stop the interface file here, or if you want to include another test section, insert another test header after field 59.

Building the ASCII interface files

**Additional Test Fields (1)**

Fields 60-65 create the Additional Test Fields (1) page. They are large fields with scroll bars. These fields do not need to be included in the interface file unless you want to use them. If you stop the interface file after these records and want to include another test section, insert your next test header after field 65.
Building the ASCII interface files

Additional Test Fields (2)

Fields 66-71 create the Additional Test Fields (2) page. They are large fields with scroll bars. These fields do not need to be included in the interface file unless you want to use them. If you want to include another test section, insert your next test header after field 71.
Building the ASCII interface files

**Working with LMS cases**

Cases are created by contacting the LMS and importing a case details text file. If you want to create a case that is local to the machine and not from the LMS you are given that option.

You can delete LMS cases from the hard drive and the Library but you cannot rename them.

The Case Details dialog box for LMS cases is multi-tabbed to make navigation easier. Because of the number and types of fields available in the Case Details file not all fields can be printed.

**Related topics**

- [Browse the Case Details pages](#)
Creating cases

To create an LMS case:
LMS cases are created by contacting the LMS and importing the data into a new case. Click on New case. After a few seconds the list of cases on the LMS will appear.

Select the name of the case you wish to create and click on New Case. The filter may be used to find a specific case.

To create a local case:

1. Click on New Case.
2. Enter a case name in the Name field at the top of the dialog and click on Create.
3. The system will look for the filename on the LMS and then ask if you want to create a non-LMS case. Click on OK.
Local cases are not associated with the LMS and will use a local Case Details file.

**Creating local cases**

**New Case**
Local cases are not associated with the LMS and will use the standard Case Details file.
1. Click on New Case.
2. Enter a case name in the Name field at the top of the dialog and click on Create.
3. The system will look for the filename on the LMS and then ask if you want to create a non-LMS case. Click on OK.

**Renaming and deleting LMS cases**

You cannot rename an LMS case. As the file name is determined by the LMS this feature has been disabled for LMS cases. You can still rename ordinary cases. You can delete LMS cases. Use the Case and Library Manager if you want to delete the library entry with the case. Otherwise use the Navigator to delete the case from the hard drive only.

**Opening and closing LMS cases**

The Lab Management System (LMS) should always be on whenever you open a case. If the LMS is turned off and you need to open a case, make sure that you do not alter the Case Details. Any changes made will not be saved.

When a case is closed, a .res text file will be returned to the LMS with any new case details that have been entered.

**Printing LMS images**

The fields available for printing are different for LMS cases. When you load an image from an LMS case into the print window the fields that you can print are:

- Title
- Comment
- Case Name
- Slide/Cell
- Field 1
- Field 2
- Field 3
- Field 7

These fields are from the Key Fields page. You may design and create layouts for each type of case as you would normally. Depending on which type of case is loaded in the print window, the data fields displayed will change.

**Related topics**

- Loading and printing images
LMS Case Details

LMS Case Details

The Case Details dialog for Laboratory Management System (LMS) cases differs from that of normal cases. Click on Case Details and a multi-page dialog will appear. The data fields are grouped and placed on eight tabbed pages to make navigating through the data easier.

The titles or labels for the data fields are set by the text file sent over from the LMS. Fields that are background colored are not editable from CytoVision. Change the data in these fields from your Lab Management software. Data in white text fields can be changed from the CytoVision. Any new data will be saved and returned to the LMS when the case is closed. The editable state of a field is set at the Lab Management system.

Page views

- Key Fields
- Additional Key Fields
- Test Fields
- Test Fields - Tally Box
- Test Fields - ID/Time
- Test Fields - Banding
- Additional Test Fields (1/2)

Key fields

The main page of details, Key fields contains the Case number, Keywords, Archive history and the Complete toggle in addition to ten user defined fields. These 4 fields from CytoVision cannot be changed. The remaining fields can have any title you want and are defined by the LMS.

Place a check in the box to mark the case as completed. When an LMS case is marked as complete a .cmp file is returned to the LMS.
Additional Key fields

Provides six additional user defined Key fields. Unused fields should have a blank title and be non editable. For example "F", "", "".
Test Fields

The top row has spin controls used to select the test.
About LMS case details

**Tally box**

A grid or table format is provided for recording counts and numbers easily. The top row can be used to create column headings.
About LMS case details

ID/Time and Date

You can either enter the date and time a case is reviewed, or you can use the Date buttons to the right of the fields to automatically enter the current time and date.
### Banding

<table>
<thead>
<tr>
<th>Test</th>
<th>1</th>
<th>Cell Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capture Karyotype ID</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Counts ID</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-Review By ID</td>
<td></td>
<td></td>
</tr>
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<td>Counts Date/Time</td>
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<tr>
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**Current date and time buttons**

- Current date and time buttons
  - Date

**About LMS case details**
About LMS case details

Additional Test Fields 1 and 2
About LMS case details
Step by step procedures

Step by step procedures

These topics can also be found under their related headings. Each one will give instructions on how to perform a function using the most common options. Other options can be used for most settings, these are given a guidelines just to get you started.

Capture

Step by step brightfield capture

1. Open or create a case and select a slide in the Navigator. If you need to create a slide click the right mouse button of the case and choose New slide from the menu.
2. Click on New cell.
3. Choose Brightfield in Capture Mode.
4. Click on Live.
5. Center and focus the image.
6. Click on Capture setup.
7. Adjust the light level on the microscope with Bright set about 10 and Black set about 160. Pale ends should be clearly visible. If the background is a little gray that is OK, it will be removed in thresholding.
8. Move Black left until the dark bands are almost black. You do not want any blue to appear in areas of interest.
9. Move the Bright slider to the right until the pale ends and background have lightened to an acceptable level. There should not be any red visible in areas of interest.
10. If necessary adjust the Black and Bright sliders more until a good image contrast is achieved.
11. If a good contrast cannot be achieved try using Gamma correction.
12. Click on Capture. The thresholding panel will appear.
13. Move the slider until the blue mask is wrapped tightly around the chromosomes. If it is too loose the chromosomes will look fuzzy and lack detail. Do not cover any pale areas with the mask or they will be lost. See Thresholding for details of this process.
14. Click on Accept.
15. If you have stray chromosomes click on Live and choose Join(Fuse). Then proceed with capture, trying to keep all of the settings the same if possible.

Step by step fluorescent capture

The Bright and Black sliders work they same way in fluorescent mode as they do in brightfield. Exposure replaces adjusting the light on the microscope.

1. Open or create a case and select a slide in the Navigator. If you need to create a slide click the right mouse button of the case and choose New slide from the menu.
2. Click on New cell.
3. Choose Fluorescent from Capture Mode.
4. Click on Live.
5. Center and focus the image.
6. With the **Bright** slider about 10 and the **Black** set about 160, move the **Exposure** up until the image is clearly visible. The background should have a smooth dark appearance. If vertical lines are visible try a longer exposure. If the image has a flat, light gray appearance you may have too much light, move the exposure level down.

7. Move the **Bright** slider to the right until the chromosomes are bright. You do not want any red to show in areas of interest.

8. Move the **Black** to the left until the background is black and you have a good contrast level. No blue should be seen in areas of interest.

9. If necessary adjust these sliders more until a good contrast is achieved.

10. Click on **Capture**. The thresholding panel will appear.

11. Move the slider until the blue mask is wrapped tightly around the chromosomes. If it is too loose the chromosomes will look fuzzy and lack detail. Do not cover any of the chromosomes with the mask or they will be lost.

12. Click on **Accept**.

13. If you have stray chromosomes click on **Live** and choose **Join (Fuse)**. Then proceed with capture.

**Related topics**

- Using the Bright and Black sliders
- Using the Exposure slider
- About thresholding

**Probe capture using filterwheels**

1. Click on **Capture mode** and choose Probe.

2. Set up the fluorochromes you will be using for capture in the **Fluorochrome Selection Panel**.

3. Select which options you want to use from **Customize**.

4. Close the Filterwheel panel if you have had it open.

5. Click on **New cell**.

6. Click on **Live**.

7. Focus and center the image. If **Auto Camera Setup** is on, click **Capture** once the image is focused. For manual capture, with the **Bright** slider about 10 and **Black** set about 160, move **Exposure** up until the image is clearly visible. The background should have a smooth dark appearance. If vertical lines are visible try a longer exposure. If the image has a flat, light gray appearance you may have too much light, move the exposure level down.

8. Move the **Bright** slider to the right until the signal is bright. You do not want any red to show in areas of interest.

9. Move **Black** to the left until the background is black and you have a good contrast level. No blue should be seen in areas of interest.

10. If necessary adjust these sliders more until a good contrast is achieved.

11. Click on **Save as Default** if you want to save these settings as the new default. If not click on **Capture** and continue with the thresholding process.

12. If **Auto Threshold** is on, the image will be processed and capture will continue. If not, the thresholding screen will appear. Adjust the threshold to eliminate any unwanted background. If a good threshold cannot be obtained try using the **special tools** to isolate specific regions.

13. If **Auto Sequence** is on, the system will continue to the next fluorochrome after thresholding. If not, click on **Live** to capture the next fluorochrome.

14. Repeat steps 6-13 until all of the fluorochromes have been captured. If you have a filterwheel and **Auto Camera Setup**, **Auto Sequence** and **Auto Threshold** are
turned on, then the system will continue to capture the rest of the fluorochromes for you.

**Probe capture with manual filter changes**

1. Click on **Capture mode** and choose Probe.
2. Set up the fluorochromes you will be using for capture in the **Fluorochrome Selection Panel**.
3. Select which options you want to use from **Customize**.
4. Click on **New cell**.
5. Move the filter for the first fluorochrome into position.
6. Click on **Live**.
7. If **Auto Camera Setup** is on then click capture once the image is focused. For manual capture with the **Bright** slider about 10 and the **Black** set about 160, move the **Exposure** up until the image is clearly visible. The background should have a smooth dark appearance. If vertical lines are visible try a longer exposure. If the image has a flat, light grey appearance you may have too much light, move the exposure level down.
8. Move the **Bright** slider to the right until the signal is bright. You do not want any red to show in areas of interest.
9. Move **Black** to the left until the background is black and you have a good contrast level. No blue should be seen in areas of interest.
10. If necessary adjust these sliders more until a good contrast is achieved.
11. Click on **Save as Default** if you want to save these settings as the new default. If not click on **Capture** and continue with the thresholding process.
12. If **Auto Threshold** is on, the image will be processed and capture will continue. If not, the thresholding screen will appear. Adjust the threshold to eliminate any unwanted background. If a good threshold cannot be obtained try using the **special tools** to isolate specific regions.
13. If **Auto Sequence** is on, the system will continue to the next fluorochrome after thresholding. If not, click on **Live** to capture the next fluorochrome.
14. Repeat steps 5-13 until all of the fluorochromes have been captured.

**Step by step CGH capture**

These are instructions for capturing CGH images using the most common fluorochromes for test and reference DNA.

1. Create a **New slide** in the case.
2. Select CGH from **Capture mode**.
3. Delete any unwanted fluorochromes and add DAPI, FITC and TRITC to the **Fluorochrome Selection Panel**. (Note - A CGH fluorochrome selection panel will say CGH).
4. Mark DAPI as counterstain, FITC as Test and TRITC as reference.
5. Click on Species Browser and select the species template you want to use.
6. Turn on any automatic capture tools you want to use from **Capture Customize**.
    - If you have a filterwheel, turn **Auto register images** off.
7. Click on **Live**, center and focus the image. Adjust the contrast and click on **Capture** when ready. If **Auto camera setup** is on contrast will be adjusted for you when **Capture** is clicked.
8. Click on **Live** to proceed to the next fluorochrome. If **Auto sequence** is turned on this will be done for you. When all of the raw images have been captured for the first cell the Image Capture panel will appear and start processing the cell.

9. Continue capturing cells until you have the number of cells you want with a ✓ by them.

10. If you want to view the cells before exiting capture, click on their name in the Image Capture panel. The unprocessed metaphase will be displayed and the CGH Cell Measurements dialog will open with the test results. You can decide to change the pass or fail status of the cells from CGH Cell Measurements.

11. Click on **Batch complete**. If you have any failed cells choose **Discard** to have them deleted from the system. The cells that have passed will have metaphase, probe and ratio images created from the raw image data. You are ready to generate your karyotypes.

**Step by step M-FISH capture**

These instructions will be written for capture with a filterwheel. If you do not have a filterwheel turn **Auto Register Images** on in **Capture Customize**. You will have to position your filters before pressing **Live**.

1. Choose M-FISH from **Capture Mode**.

2. Add your fluorochromes to the **Fluorochrome Selection** panel.

3. Click on **Species Template Browser** and select the correct template.

4. Click on **Fluomap** and select the method of M-FISH you are using and whether it is a male or female sample.

5. Map the flours to the chromosomes they will label. If you are using the COBRA method, make sure that the binary label has been marked.

6. Use a filter like FITC filter to find a good quality metaphase. Scanning with DAPI can bleach fluorochromes.

7. Click on **Live**. The filterwheel will turn to the first filter.

8. Focus the image.

9. Adjust the contrast and click on **Capture**. If **Auto Camera Setup** is turned on you do not need to adjust the contrast, just click on **Capture**.

10. The image will be thresholded automatically.

11. If **Auto Sequence** is on, capture will proceed to the next fluorochrome. If not click on **Live**.

12. Repeat steps 6-10 until all fluorochromes have been captured. The M-FISH Image Capture dialog will appear after the last fluorochrome is captured. Cells that pass the quality control tests will be marked with a ✓, those that have failed will be marked with a ✗.

13. Continue capturing cells until you have as many as you want.

14. If you want to view the cells before exiting the capture screen click on their name in the M-FISH Image Capture dialog. The class image of the cell will be displayed in the window.

15. Click on **Batch complete**.

16. Choose **Continue** if you want to discard the cells that failed the quality control tests. Choose **Keep all cells** if you want to keep all of the cells. Metaphase and probe images will be created for each of the cells. You are ready to begin karyotyping.

**Step by step RxFISH capture**

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1. Choose RxFISH from **Capture Mode**.
2. **Configure** the Fluorochrome Selection Panel for RxFISH.
3. Use the FITC filter to find a good quality metaphase.
4. Click on **Live**. The filterwheel will turn to the first filter.
5. Focus the image.
6. Adjust the contrast and click on **Capture**. If **Auto camera setup** is turned on you do not need to adjust the contrast, just click on **Capture**.
7. If **Auto sequence** is on the system will continue to capture the rest of the fluors. If not then repeat steps 4-6 until all 4 fluorochromes have been captured.
8. The **RxFISH Image Capture** dialog will appear after the fourth image is captured. Cells that pass the quality control tests will be marked with a ✔, those that have failed will be marked with a ✗.
9. Continue capturing cells until you have as many as you want. View the cells by selecting their name in the **RxFISH Image Capture** dialog. If you want to change a cell’s pass or fail status use the **Keep** and **Delete** buttons in the RxFISH Cell Measurements dialog.
10. Click on **Batch complete**.
11. Choose **Continue** if you want to discard the cells that failed the quality control tests. Choose **Keep all cells** if you want to keep all of the cells.

Metaphase and probe images will be created for each of the cells. You are ready to begin karyotyping.

**Archive and Restore**

**Step by step archiving**

These are step by step instructions for archiving to a single disk and deleting your cases as part of the process. If you want to create a back-up disk, skip step 7 for the first disk and include this step when archiving on your back-up disk.

1. Insert a **formatted, labeled** disk into the drive.
2. Click on **Archive and Restore**.
3. Click on the **Settings** tab.
4. If the drive you want is not displayed, select it from the **Archive Device** list.
5. Click on the **Archive Cases** tab.
6. Select the cases you want. Make sure that the space needed is less than the space available on the disk.
7. Place a check in the **Delete after archiving** box.
8. Click **Archive** and archiving will start.
9. A status dialog box will appear showing the progress of archiving. When the cases have been copied to the archive disk, the status dialog will close and a message will tell you that archiving is finished.

**Note** If you do not want your cases deleted after archiving, do not turn on **Delete after archiving**. You will have to delete them manually.

**Step by step restoring**

1. Insert the disk into the drive.
2. Click on **Archive and Restore**.
3. Click on the **Settings** tab.
4. Select the drive with the disk from the **Archive Device** list.
5. Click on the **Restore Cases** tab.
6. The disk will be read and a list of the cases on it will be displayed.
7. Select the cases you want.
8. Click on **Restore**.
9. A status dialog box will appear that shows the progress of the restore process. When all of the cases have been copied back to the hard drive a message will appear telling you that restore is finished.

**CytoNet transfers**

**Step by step sending**

1. Click on **CytoNet**.
2. Click on the **Send Cases** tab.
3. Choose the machine that has the cases from the **Machine list**.
4. Select the case names individually or by using **Select All**. Use the Filter if you want to limit the names displayed before selecting them.
5. Select who you want to send cases to in the **To** list.
6. Select the **Format** you want to send the case in. Use **To CytoVision** if they need to modify the images, or **To Web** if the images are going to be sent across the Internet for reviewing only.
7. If you don't want to send the Patient name place a check in **Confidential**.
8. Click on **Send Selected Cases**. A status dialog will appear showing the progress of send.
9. When finished, a message will appear showing the results of the send process.

**Step by step receiving**

1. Click on **CytoNet**.
2. Click on the **Receive Cases** tab.
3. Click on **Get Case List**.
4. Select the names of the cases you want. Make sure you have enough disk space on your hard drive to download the selected cases.
5. If the cases were sent with **Confidential** on make sure a check is placed in the box.
6. Click on **Receive Selected Cases**.

**Scanning systems**

**Calibration of the Metaphase finding systems (Chromoscan)**

There are 3 parts to calibrating a system, setting the bay datum positions, system calibration, and microscope coordinate conversion.

**Step 1 - Setting bay datum positions**

Calibrate the system using the Applied Imaging Calibration A slide. This calibration procedure is done in each bay of the stage. Calibration should only need to be performed when the system is first setup, or if the stage has been moved.
**Note: The 'A' image will appear upside if the camera orientation is correct.**

1. If the CytoVision program is not open, launch the CytoVision software, then open the scan screen.
2. Click on **Reset (Home) Stage**.
3. Place the Applied Imaging Calibration A slide in bay 1; the first bay on the left side of the stage. (diagram of a stage)
4. Adjust the focus and light to get an image. Only adjust the focus using the joystick or onscreen controls.
5. Move the stage to position bay datum position on the calibration slide (England Finder position C59) using the on-screen stage controls. Do not move the stage manually once **Reset Stage** has been pressed.
6. Click on the **Calibration button** to open the Finder Calibration dialog.
7. Click on **Set Bay Datum**.
9. Enter a 1 in the **Bay Number** field.
10. Press **Save**.
11. Place the slide in the second bay just to the right of the first.
12. Repeat steps 2-9. Then enter a 2 in the **Bay Number** field.
13. Repeat steps 1-12 for the other bays entering their corresponding number in the **Bay Number** field.

**Step 2 - System calibration**

This calibration is done after setting the bay datum positions. This step only needs to be done for one bay.

Stage calibration is necessary for proper travel of the stage and accurate relocation of cells.

1. Place the Applied Imaging calibration slide in a bay.
2. **Reset** (home) the stage.

3. Click on **Calibrate** and select **System Calibration** to open the dialog window (see below).

4. Move the stage to bring the 32 micron image calibration pattern into view. Focus and adjust the contrast so the pattern is clearly visible.

5. Click on **Live** in the System Calibration window.

6. Click on **Capture** in the System Calibration window.

7. A line should be drawn between two rectangles in the same column. If not, reposition the image calibration pattern and repeat steps 4-7. The length (in units of pixels) will appear in the **Feature Separation (pixels)** field.

8. Enter 64 in the **Feature Separation (microns)** text field.

9. Move stage to a cross hair most commonly used for calibration (see image above).

10. Click on **X-Y Scaling** in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.

11. Click on **X-Y Backlash** in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.

12. Click on **Focus Backlash** in the System Calibration window. The focus will be adjusted until the setting is found and entered in the field.

13. Enter a name for the calibration data in the **Filename** text box.

14. Click on **Save**.

15. Click on **Apply**. This will apply the currently selected calibration settings to the system.

16. Click on **Done** to close the window.
Step 3 - Slide Calibration for Microscope Coordinate conversion

1. After Bay Datum and System Calibration have been completed:
   1. Place the England finder (provided with the system) in Bay 1 of the stage and adjust the focus and camera/light settings if necessary.
   2. Select the Reset home button, resetting the stage position (C59)
   3. Move to position A15, record the X and Y coordinates displayed at the bottom of the screen. These will be used later.
   4. Move to position Z50, record the X and Y coordinates displayed at the bottom of the screen. These will be used later
   5. Click on the Review tool button to switch to the Review screen.
   6. Click on the Microscope Coordinate conversion button to open the Microscope Coordinate conversion window.
7. Enter the slide lengths in the spaces provided.
8. Click on **Add** to open the Microscope Coordinate Conversion Data dialog to create a new "Microscope" entry.
   Type a name in the **New Microscope** field, this will be the Default entry for printing out England Finder co-ordinates from a scan.
9. Select England Finder from the drop down **Units** selector and input the A15 and Z50 X and Y coordinates that were noted previously (put them in both **New** and **Chromoscan** microscope fields).

10. Select **Done** to save this data, the new name will appear in the list.

For converting See topic under Metaphase finding scanning systems > Reviewing the cells > Microscope conversion > Convert cell coordinates & Add a new microscope.

The system has now been set up with the necessary Calibration and configuration data to enable you to successfully use it for scanning.

**Step by step scanning for cells**

1. Select sample type and whether you will scan more than one slide in **Customize**.

2. Select the name of the classifier you want to use.

3. Open the cases you want to scan.

4. In the **Scan** screen click on **Reset**.

5. Click on **Camera Setup** and adjust the contrast of the live image until it is at least 50%.
6. Select a slide in the Navigator then click on the correct bay image for that slide. Click on **Set**.

7. If you want to change from the default scan area, click on **Scan Area** to select a new area for scanning. This step is optional.

8. Click on **Focus Map**. Click on **Auto focus** or set the focus points manually.

9. Repeat steps 6-8 for each slide you are going to scan.

10. Click on **Start**.

11. When the scan is finished, review the cells and save the results from the **Review** screen.

**Spot counting**

**Step by step SPOT counting calibration**

All of this can be done in brightfield mode using the Applied Imaging Slide A calibration slide.

There are **4 separate calibration routines** required for calibrating a SPOT system:

A. **SPOT**, auto capture and objective lens calibration - Requires Applied Imaging calibration slide A.

B. **ChromoScan Bay Datum position** - Requires Applied Imaging calibration slide A.

C. **ChromoScan system calibration**. Applied Imaging calibration slide A.

D. **Coordinate conversion setup** - Requires England Finder. (not necessary for Spot only systems) - see topic under Metaphase finding scanning system > Hardware basics > Calibrating the system > **Microscope Coordinate conversion** for instructions on this calibration procedure.

**Notes**

- For the most accurate calibration all of these procedures should be done without removing the calibration slide.
- We recommend calibrating the system once a month or if problems with relocation occur.
Step by step procedures

- A map of the calibration slide:

**Calibration slide A**

**Step 1 - Wizard system calibration - SPOT, auto capture and objective lens calibration**

1. Close down all Applied Imaging software if it is running.
2. Open the *Calibration* program ( ). A microscope connection made - configured components are displayed orange in image window
3. Launch calibration wizard - click on Wizard icon in toolbar.
4. Prompt that you will need the Applied Image calibration slide during the operation of this wizard.
5. Calibration wizard welcome - press 'skip' to begin calibration and follow the directions in the wizard, selecting Next to move to each section in the wizard.
Calibration needs to be done for only one bay on the stage.

**Notes:**
1. Details of this process can be found in the help files for the calibration program (In the main menu, click on Help > Contents).
2. You must use the Applied Imaging calibration slide A supplied with your system.
3. **Skip the sections for XY backlash, Z backlash and Idealised coordinates.** It does no harm to calibrate these settings but they are not used by SPOT and you can save time by skipping them.

6. Click on Finish and save calibration data. Close the Calibration program.

**Notes**
- Before you begin make sure all of the objectives are clean and free of oil. The calibration slide must also be clean and free from dust and oil.
- Oil is not necessary for calibration, even for high power oil objectives. If the lens is clean, the image can be focused well enough and the calibration slide will remain cleaner and easier to work with.
- Once calibration is started none of the motorized components should be moved or adjusted manually. Use the on-screen controls or the joystick controller.

**Step 2 - ChromoScan Bay Datum Positions**

Launch the ChromoScan program and switch to the Scan screen.

**Setting bay datum position**

Calibrate the system using the Applied Imaging Calibration A slide. This calibration procedure is done in each bay of the stage. Calibration should only need to be performed when the system is first setup, or if the stage has been moved.

![Bay Datum dialog](image)

**Note:** The 'A' image will appear upside if the camera orientation is correct.
Step by step procedures

1. If the CytoVision program is not open, launch the CytoVision software, then open the
   scan screen.

2. Click on **Reset (Home) Stage**.
3. Place the Applied Imaging Calibration A slide in bay 1; the first bay on the left side of
   the stage. (Diagram of a stage)
4. Adjust the focus and light to get an image. Only adjust the focus using the joystick or
   onscreen controls.
5. Move the stage to position bay datum position on the calibration slide (England Finder
   position C59) using the on-screen stage controls. Do not move the stage manually
   once **Reset Stage** has been pressed.
6. Click on the **Calibration button** to open the Finder Calibration dialog.
7. Click on **Set Bay Datum**.
9. Enter a 1 in the **Bay Number** field.
10. Press **Save**.
11. Place the slide in the second bay just to the right of the first.
12. Repeat steps 2-9. Then enter a 2 in the **Bay Number** field.
13. Repeat steps 1-12 for the other bays entering their corresponding number in the **Bay
    Number** field.

**Step 3 - ChromoScan Scanning Calibration**

**Calibration tools**

These are the tools that calibrate the hardware used by the scanning package. Calibration
should only need to be done at initial installation or if the stage and been removed and re-
installed. The dialog box contains two options; **Set Bay Datum** and **System Calibration**. **Set
Bay Datum** sets the exact position of each slide bay.

**System calibration** contains the setting for stage position and image size relative to on-
screen pixels. If you have moved your camera or stage and are having difficulty in relocating
cells, then re-calibrate the system.

1. With the Applied Imaging Calibration slide still in bay 1 adjust X, Y and Z so that the
   calibration image is clearly focused and centred in the live image window.

2. **Reset** (home) the stage.

3. Click on the Calibration button.
4. Click on **System Calibration** to open the dialog window (see below).
5. Move the stage to bring the 32 micron image calibration pattern into view. Focus and
   adjust the contrast so the pattern is clearly visible.
6. Click on Live in the System Calibration window.
7. Click on Capture in the System Calibration window.
8. A line should be drawn between two rectangles in the same column. If not, reposition the image calibration pattern and repeat steps 4-7. The length (in units of pixels) will appear in the Feature Separation (pixels) field.
9. Enter 64 in the Feature Separation (microns) text field.
10. Move stage to a cross hair most commonly used for calibration (see image above).
11. Click on X-Y Scaling in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.
12. Click on X-Y Backlash in the System Calibration window. The lines will be moved around the screen until the scaling settings are found. They will be entered in the fields labeled X and Y.
13. Click on Focus Backlash in the System Calibration window. The focus will be adjusted until the setting is found and entered in the field.
14. Enter a name for the calibration data in the Filename text box.
15. Click on Save.
16. Click on Apply. This will apply the currently selected calibration settings to the system.
17. Click on Done to close the window.

- The SPOT AX is now calibrated and ready for scanning.

#### Building a fusion assay

In this exercise we will build a script for analyzing dual color, dual fusion BCR/ABL translocation probe. Before starting make sure that the fluorochromes DAPI, Spectrum Green and Spectrum Orange exist and ideally, that the signal fluorochromes have the correct render color. Color settings can be changed from Capture if they are not set. See the topic Fluorochromes overview for information about creating and editing fluorochrome settings.
When you create your own assays you will change the values to suit your sample types and fluorochromes used but the general workflow will remain the same.

1. Open the Spot Counting Configuration dialog.
2. Enter a 2 in the **Number of probe fluor**s, field.
3. Place a check in the **Fusion Enabled** check box.
4. Click on **Add Class**.
5. In the **Counterstain** combo box select DAPI.
6. Click on the box on the right hand side of the Counterstain name and choose a color.
   This color will be used to highlight the boundary of the nuclei in Review (in fusion assays it is also used to highlight the fusion signals).
7. In the first column select Spectrum Green in the **Fluor names** combo-box.
8. Place a check in the box next to the name. This tells the script the fluorochrome is part of a fusion.
9. Check the Z stack settings are correct.
10. Click on the box on the right hand side of the Fluor name and choose a color. This color will be used to highlight the Spectrum Green signals in Review.
11. In the second column select Spectrum Orange in **Fluor names** combo-box.
12. Place a check in the box next to the name.
13. Check the Z stack settings are correct.
14. Click on the box on the right hand side of the Fluor name and choose a color. This color will be used to highlight the Spectrum Orange signals in Review.
15. Enter a 2 in each of the fields under the Spectrum Green and Orange names.
16. Enter a 0 in the Fusion column.
17. Enter Negative (or your own designation) in the Class Name column and click in the Normal radio button.
18. Click on the black box at the end of the row. Select a color for your Normal class.
19. Click on **Add Class**.
20. Enter a 1 in each of the fields under the Spectrum Green and Orange names.
21. Enter a 1 in the Fusion column.
22. Enter Positive (or your own designation) in the Class Name column.
23. Click on the black box at the end of the row. Select a different color for your Positive classes.
24. Click on **Add Class**.
25. Enter an * in the signal and fusion columns. This is a wildcard character that tells the system any number other than those listed in the other classes.
26. Enter Undefined in the Class Name column. Select a color for the undefined cells or leave them at the default black color.
27. Now set the spot sizes. Set the max size in both signal columns to 100. Leave the minimum sliders set to 1.
28. Now set the signal separation distances. Choose the **% nuclear diameter** option.
29. Set the Fluorochrome separation to 10.
30. Set the Fusion separation to 10.
31. Enter sgI1BCRABL in the Script Names combo-box at the bottom of the screen.
32. Click on **Save**.

This is how the assay looks at the end.
CytoVision™, Genus™ and SPOT AX Systems are manufactured and distributed by:

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Intended Use:
CytoVision Karyotyper and CEP XY_ENG – For In Vitro Diagnostic Use

CytoVision Karyotyper is a rapid metaphase finder and computer aided chromosome analysis system, which assists the cytogeneticist in viewing the chromosome displays and looking for cellular anomalies. The Karyotyper enables the qualified cytogeneticist to rapidly and accurately analyze the chromosome banding pattern. All diagnostic decisions are made by the qualified clinician.

CytoVision CEP XY_ENG is limited to the analysis of CEP XY probes via high magnification capture and analysis of interphase nuclei. CEP XY is indicated for use to assess the effectiveness of bone marrow transplantation in opposite-sex transplants.

Genus is For Research Use Only. Not for Use in Diagnostic Procedures

The applications CytoVision Fluorescence In-Situ Hybridization (FISH), Comparative Genomic Hybridization (CGH), SPOT AX, RxFISH Color Chromosome Analysis, and M-FISH are For Research Use Only. Not for Use in Diagnostic Procedures.

RxFISH (Patent Pending GB 2330907 A)
CytoVision 3.6

Hazards:
Applied Imaging has not validated use of the CytoVision system outside of the standard CytoVision operation as described in this release note and the online help. It is important for the user to recognize that product validation does not include unauthorized modifications of the system’s hardware or software. Applied Imaging bears no responsibility for the performance of the CytoVision system when it is utilized in a manner other than its intended use, or when unauthorized modifications have been made by the user. Finally, operators should follow standard laboratory safety procedures for handling laboratory materials and electronic equipment.

The release of CytoVision 3.5 was the first in a series of CV releases designed to increase efficiency and functionality of the software, as well as implement features requested by customers. The CV 3.5 release included improvements to Capture and Analysis to minimize mouse clicks and increase speed and efficiency. M-FISH has great new capture and display features that really advance this application. The CV 3.6 release provides new features to software modules within the CytoVision suite and resolves certain software bugs encountered while using existing versions of the software. The intended use statement above applies.

**NOTE:** Be advised that loading this software will overwrite some macros or function keys.

CV 3.6 will be suitable for existing users with CytoVision running on Windows NT 4.0 or Windows XP, as field upgrade as part of the normal warranty/contract facility.

version 3.6
Glossary

A

Assay: A collection of scan passes that include information, including filters, scripts, objectives, etc., used to define how a slide is scanned.

Axis ratio: One thousand times the ratio of the minimum to maximum axis lengths for a region.

B

Background: The regions in an image that are not part of a region of interest.

Build list: The list of fluorochromes on a system. The list can be viewed by clicking on Build List in the Fluorochrome Selection panel in Capture.

C

Calibration: The set up of the system hardware, by defining it's parameters.

Class: A text label describing a fragment.

Context menu: A menu that is opened by right-clicking on the item, it contains a list of commonly used commands

F

Flex: Image type that allows objects to be selected from different cases, cells and stain types. They are used to create montages for publication or illustration.

Fluorochrome: Any fluorescent dye used to stain tissues and cells for examination by fluorescent microscopy, such as a fluorescently labeled or tagged genetic marker.

Frame: A field of view.

Frame List: A series of frames, captured in succession, in a defined area.

Freehand: Drawing method where the line follows the mouse. No clicks are needed to change direction. See also Rubberband.

Fuse image: Additional images connected to a metaphase during capture. They are used to include stray chromosomes in a cell.

G

Gallery: thumbnail views of case images opened in the Navigator

Gamma: Gamma affects the relative value of output vs. input. A gamma of 1.0 does not change the image. Values greater than 1.0 will lower the output relative to the input and darken the image. Values less than 1.0 will lighten the image.

H

Hue: Related to image dominant color. Hue is presented on a scale of 0 to 255.
I

Intelligent Karyotyping: Dynamic scaling to automatically accommodate different types of metaphases (chromosome number and or length).

Intensity: Related to image brightness. Intensity is presented on a scale of 0-255.

L

Layout: A redefined plot template containing plot properties, example - background color and title font.

M

Mask: A set of pixels representing a region of interest on an image.

Modal: Analysis can be configured so that many of the tools become modal. This is done by checking the 'Use Modal Operations' box in the Customize Analysis dialog. When using modal operations, instead of first selecting one or more objects then clicking a toolbar button to perform that operation, you first select the operation you want then click on objects to apply that operation to them. When a operation mode is active, the toolbar button for that operation is depressed. Only one mode can be active at a time, so selecting a different mode pops-up the button for the previously selected mode. If a toolbar button cannot be depressed, either 'Use Modal Operations' has not been selected or there is no mode available for that operation. To deactivate all modes, pop up the button for the current mode by clicking on it. All modes are deactivated automatically in some situations, such as when a different image is loaded or when an incompatible action is performed.

R

Raw image: The un-enhanced and un-thresholded image.

Region: An area of a slide to be scanned and/or processed.

Registration error: Registration errors are when the signals are offset from the counterstain. They occur because each filter cube has its own dichroic mirror which can alter the light path slightly. Also the cubes may not be placed in exactly the same position with a manual slider. Registration moves the offset of an entire layer, not just the signals selected.

Reprocess: To re-analyze the data, applying different parameters, without having to recapture the image.

Review: A database which shows all captured cells or objects.

Rubberband: Method of drawing lines that used straight lines. A mouse click tacks the line down to change direction. See also Freehand.

S

Saturation: Related to the image relative purity of a color. Saturation is presented on a scale of 0 to 255.

Scan: A collection of data captured by the system from a slide, comprising both images and measurements.

Script: A set of instructions which performs an image analysis.
**SpectraChrome:** A collection of settings used to capture a monochrome image from the camera mounted on the microscope hardware. Settings include: camera settings (example - exposure time), filterwheel positions (example - red, green or blue filter) and microscope objective settings.

**Streamlined capture:** Enables one-button capture in brightfield and fluorescent mode.

**Superscan:** A predefined set up assisting wizard functions - a SuperScan is a new system of saving scan settings.

**T**

**Table:** A database item holding information arranged in rows and columns.

**W**

**Wizard:** Automated series of setups or instructions that facilities setup up a scan or calibrating the system.

**WYSIWYG:** Stands for "what you see is what you get." A way of referring to document layouts that graphically represent the way the document will print.

**Z**

**Z-Stack:** A collection of images taken at different focal planes at the same position on the slide.
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