

The Applied Imaging Cytogenetic Workstation

Introduction to the Genus F.I.S.H. Imaging System.

The Genus Imaging System is a powerful and easy way to capture Fluorescence *in-situ* Hybridisation pictures for both analysis and presentation. It comprises the following components:

- An Olympus BX-51 upright microscope suitable for using with glass slides and both bright-field and fluorescence microscopy.
 - A cooled SenSys Charge Coupled Device (CCD) camera.
 - An Applied Imaging 10 filter excitation filter wheel.
 - A Pentium III based computer system to run the software and drive the hardware.
-

As with the other microscopes in the Molecular Cytogenetics and Microscopy Group, a booking sheet is located outside the microscope room (00/070) for users to book the times they wish to use the system.

Before using the system for the first time, we ask users to book a training session with one of the core staff in order to run through the system.

As with the other fluorescent microscopes, there are a few guidelines drawn up to help manage the system better. As normal practice, the mercury bulbs should be left to run for a minimum of 30 minutes before switching off, and allowed to cool for at least a further 30 minutes before switching on again. This helps to prevent bulb explosions. It is important for users to book the system in advance to avoid unnecessary switching off of the mercury bulb.

Once finished with the system, users should check to see if there are any bookings after their own. If there are, then the system should be left on. If not, users should turn everything off (log-off the computer but leave it running) and sign their name in the mercury bulb record book, together with the time and hours on the bulb (as read from the bulb power box).

These notes cover the basics of using Genus; from using the microscope, to capturing an image and analysing and enhancing it. It is not a comprehensive guide, but core staff are happy to assist as necessary and there is a comprehensive help file included in the software itself.

Contents:

1. Getting Started.
 2. Capturing Images.
 3. Basic Analysis.
 4. Quick Reference 1: Software.
 5. Quick Reference 2: Microscope.
-

Getting started.

Turn on the **mercury bulb** (Olympus box), the **filter wheel control box** (beige coloured box with orange power button, next to mercury bulb) and the **camera power** (small black box to the left of the monitor).

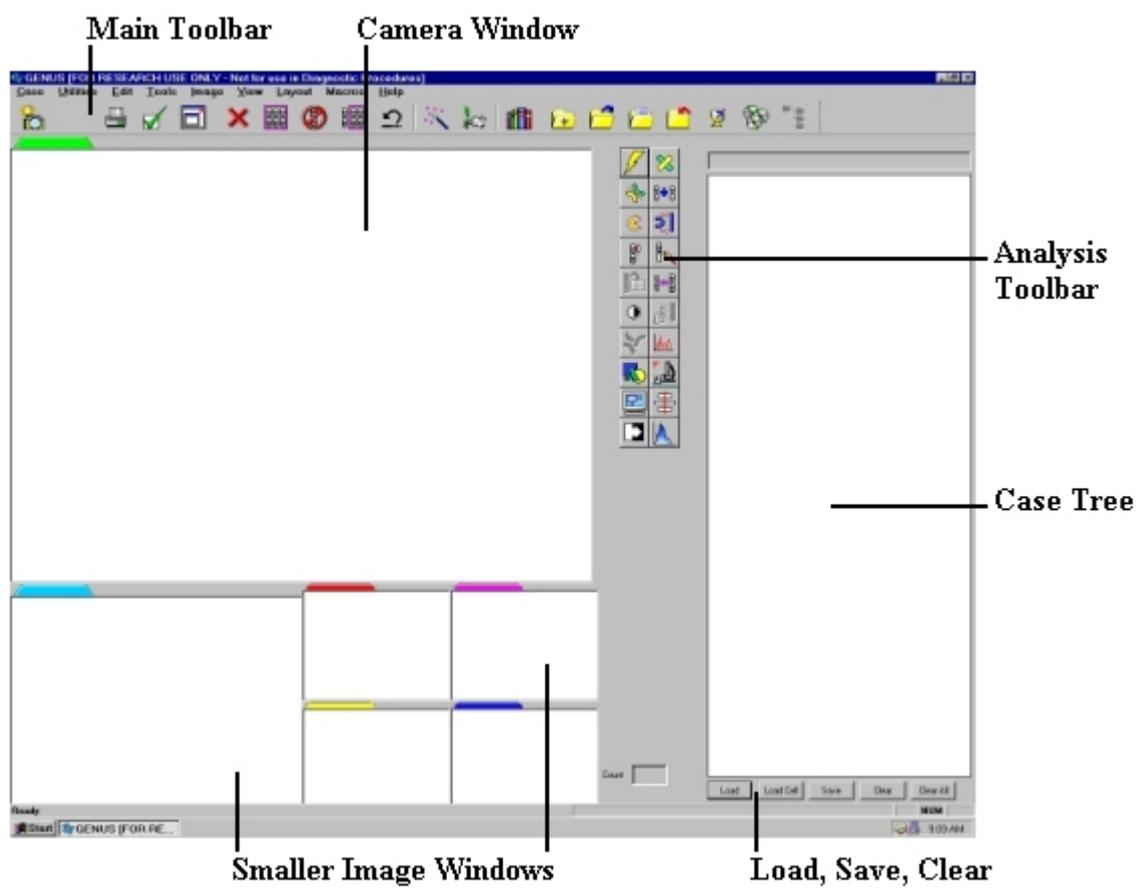
Log-on to the system with your normal username and password, ensuring that the domain is WHGC.

Once into the system, double click on the **Genus** icon located on the desktop and the program will load. (Although Genus loads quickly, the cursor may remain an hourglass; move over any icon to return to the pointer.)

To start work, an **Existing Case** needs to be opened (click ) , or a **New Case** created (click ). A case is split up into slides, which contain individual cells (each cell represents the images for a particular capture). It is suggested that the case name includes your own name (to simplify backing up on the network), while the slides can be named with the probe or specimen. If required, each cell can be renamed, and vernier references can be used as a cell name if so desired.

In Genus, there are essentially two screens; **Capture**  or **Analysis** . These two icons are at the left hand side of the main tool bar and the icon which appears "pressed" shows the screen you are currently in.

Upon loading the program, you will be presented with the following screen:



Capturing Images.

In the Capture screen, select **Capture Mode** , and select **Probe** and done. A **Fluorochrome Selection** window will open up with a list of fluorochromes. This list needs to be relevant to your particular work (i.e. probe and counterstain colours) and will be set up when you first use the system. New lists can also be built as and when required and saved in your user profile.

Once the correct fluorochrome list has been selected, the **Capture Setup** window should be opened by pressing the icon .

This window essentially gives you control over the camera whilst taking images. Brightness and Exposure (time the camera shutter is open) are controlled in this window. The "**binning**" should also be set to 2. For fluorescence work, click on the "**advanced**" box and more options will appear, including control over the filter wheel. By clicking on the "**Excitation**" box, the Filter Wheel Control window will open to give a list of currently installed excitation filters in the wheel as well as the option of controlling the wheel. This window can be left open all of the time.

Using the microscope.

Position your slide on the microscope stage. In order to view cells, the correct **excitation** wavelength light must reach the slide. Normally, DAPI is used as a counterstain for F.I.S.H. work and the excitation filter 1 (position 1 on the filter wheel) must be selected. This can be done by clicking on filter 1 in the filter control window, or by pressing "1" on the keyboard.

The correct **emission** filter in the microscope must also be selected and this is position 1 (triple pass filter) on the front of the microscope (filters are listed on the front of the microscope).

The neutral density filters can be opened to allow more light to reach the slide. These are located at the back left of the microscope, near the filter wheel).

The slider rod just below and right of the eye pieces should be pushed in all the way to allow light to reach the eye pieces.

The eye piece shutter should also be in the open position.

Initial scanning of the slide should be done under low power (10X objective). The slide can be scanned to find a cell of interest. To view at a higher power, oil emersion is required and a small drop of oil should be placed on the slide over the region of interest. The 100X objective is to the right and the 60X is to the left.

To view at higher magnification, pushing in one of the neutral density filters gives a more suitable light level.

Once a cell has been found, focusing should be done with the fine focus only. The signal can also be viewed at this time by selecting the appropriate excitation and emission filters. Once in focus and in the centre of the field of view, an image can be taken.

The slider rod should be pulled out all the way. This is to allow the light to reach the camera instead of the eye pieces.

Click "**New Cell**" in the Genus capture screen. If DAPI is selected as the counterstain, the filter wheel will move to position 1.

Click "**Live**". A live image from the camera will appear in the viewing screen. To increase or decrease brightness, the exposure slider bar can be moved. Ideally, the image will be as bright as possible without any over exposure; showed by red areas on the image.

If the image appears out of focus, the mouse pointer can be placed on the image and the left button held down and a box created by dragging the mouse. This will generate a "**region of interest**" which will be small and hence the fine focus can be used to focus the material in this region in real time. When happy with the focus, the region of interest box (in the Capture Setup window) should be un-checked.

When the image is bright and in focus, "**Capture**" can be clicked to capture the image. A "**threshold**" screen and slide bar will appear. Threshold is used to eliminate unwanted background. By sliding the threshold bar to the left or right, this controls the light intensity threshold at which the system differentiates between back ground (shown by the light blue) which is removed, and the signal which is retained. For chromosomes, threshold should cut out all the "empty" area up to the very edge of the chromosomes. Over threshold will remove the edges of the chromosomes, whilst under threshold will give a "haze" around the chromosomes. Once happy with threshold, click "**Accept**"

The coloured image will be displayed on screen and the filter wheel will automatically move to the next position. At this stage, the correct emission filter should be manually selected in the microscope and the neutral density filters can also be opened or closed to account for faint signals. When ready, click "**Live**" and adjust exposure and focus until happy with the image. Click "**Capture**" to save the image. Again, threshold is adjustable. After accepting the threshold, the probes will be visible on the coloured image.

Repeat the process for all fluorochromes, until every colour has been captured. The final image will be displayed and is called "**probe**" under the cell heading. RAW data images of each colour are also saved and can be re-thresholded if required. At this stage a "**New Cell**" can be selected to find and capture

more cells, or the **Analysis screen** can be entered by clicking .

Basic Analysis.

Once in the **Analysis screen**, image manipulation can be performed on any image captured with the system.

The **Analysis screen** is very similar to the **Capture screen** with the exception of an added toolbar in the centre and the fluorochrome list changes so that any of the captured components can be toggled on or off in the display.

For presentation or publishing, images can be enhanced using three features; **Contrast** , **Enhance**  and **Annotation** .

To work with any image, the components that require manipulation must be selected. This can be done manually by selecting with the mouse pointer and clicking on the box that appears around an object on the screen. Typically this is a single chromosome or group of chromosomes. Once selected, the box outline appears pink. By selecting to display/turn off particular colours on the image, only those that require enhancement can be selected.

If all parts of an image require work to be done, **Select All**  can be used to select all components of an image. **De-select All**  deselects again.

Contrast 

Contrasting can be done by sliding the two slider bars or dragging the graph to the desired position. Click "**Apply**" to view changes. If the change is undesirable, click "**Undo**" to reverse changes. Once happy with the image, click "**Accept**" to save changes.

Enhance 

Various enhancements can be performed including **Smoothing**, **Sharpening**, **Inverting** and a number of **advanced** filters can be applied to the image. As with contrast, click "**Apply**" to view changes, "**Undo**" to cancel changes and "**Accept**" when the image is as desired.

Annotation



By pressing this button, various **annotations** can be made to the image including **text**, **shapes** and **arrows**. Click on the appropriate icon and select click on the part of the image you wish to place that annotation.

Another option after accepting image changes is to click the main **Undo** icon  at the top of the screen.

Also, the original **Probe** image can always be restored from the **Case Tree** if required.

Saving and Exporting.

Once happy with your image, double click "**Save**" at the bottom right hand corner of the screen and a question marked pointer will appear. Click this on the image you want to save, i.e. the one you have been working on, which should be in the large image box. This will update the your case with the changes.

If you require a ***.TIF** file for viewing on another computer, right click on the image in question and a small menu will drop down which includes the **Export** option. Select **Export** and save your picture as a ***.TIF** file in your user space on the network for viewing outside the Genus environment.

Quick Reference 1: Software.

Commonly used icons on the **Main Toolbar**:



Analysis Screen: Enter Analysis mode.



Capture Screen: Enter Capture mode



Customise: General program settings



Capture Mode: Select bright-field, fluorescence etc.



Fullscreen: Gives a bigger image to analyse.



Capture Setup: Control exposure, brightness, filter wheel etc.



Threshold: Use to re-threshold RAW images.



Select All: Selects all components of an image.



De-select All: Deselects all currently selected components.



New Case: Creates a new case.



Open Case: Open an existing case.



Case Details: View case information.



Close Case: Closes case when work is finished.

Useful icons on the **Analysis Toolbar**:



Enhance: Smooth, sharpen image etc.



Contrast: Contrast, lighten or darken image.



Annotation: Add text, shapes or arrows.

Quick Reference 2: Microscope.

To see your cells down the microscope, the following steps need to be taken:

1. The correct Excitation filter must be in place. This is controlled within Genus.
2. The correct Emission filter must be in place. This is done on the front of the microscope.
3. The Neutral density filters can be adjusted to let more or less light to the slide.
4. The light must be diverted to the eye-pieces. This is done by sliding the rod all the way in, located underneath and to the right of the eye pieces (It must be all the way out when imaging).
5. The eye-piece shutter must be open.
6. Initially, scanning should be done with the 10X objective. The 60X and 100X are oil emersion objectives and should be used to image with.