



**MetaMorph<sup>®</sup>**

**Meta Imaging Series<sup>®</sup>**

**MetaMorph**

**3D Module User's Guide**

*Version 7.0 for  
Microsoft Windows XP<sup>®</sup>*

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## Acquire Z Series (Acquire Menu)

Acquires a series of frames at a specified Z-axis position (focal plane) using a Z-axis device. The acquired frames can be placed in a stack or stored on disk.

### Drop-in: 3D

If you are using a video camera, this command uses the acquisition settings from the Acquire Image command. If you are using a digital camera, Acquire Z Series uses the acquisition settings from the Acquire from Digital Camera command.

The units of measurement for the Z-axis position are based on the Z-axis device driver presently configured. Acquire Z Series uses the calibration, motor limits, and positions defined by the Focus command.

Acquire Z Series, Acquire Timelapse and Acquire Spectral Scan can be running at the same time. However, you may not perform a command within itself (such as running a timelapse within a timelapse). Acquire Z Series can also be performed from within Acquire Timelapse by selecting *Acquire a Z Series on Each Interval*.

### Acquiring a Z Series

To configure a Z-series acquisition, use the following procedure:

Step	Action
1	From the Acquire menu, choose Acquire Z Series. The Acquire Z Series dialog box opens.
2	Select the number of planes you want to acquire using <i>Number of Planes</i> .
3	Select the number of "Steps" between planes (the actual name of the option will be based on the name of the calibrated units used by your Z-axis device driver).
4	Select the starting location of the stage using the <i>Start At</i> drop-down list box.  The <i>Current Position</i> is the stage's present location. <i>Origin</i> , <i>Top</i> , <i>Bottom</i> , and <i>Home</i> are positions you will have set previously in the Focus dialog box.
5	Select the ending location of the stage from the <b><i>Move To</i></b> list.
6	From the <i>After</i> list, select the location to which you want the focus position to move after the acquisition is completed.
7	Select the desired type of storage from the <i>Image Storage</i> group. Select <i>None</i> to acquire a Z-series without acquiring or storing images.

**Note:** You should use *None* whenever you are using a journal to acquire images at each position.

- 8 If you selected *Stack*, select the destination stack using the *Destination* image selector.

**OR**

If you selected *Disk*, choose the *Save File Name* command button to select a file name. Type the file name in the *File Name* text box and select the desired file type using the *Files of Type* list. Choose *Save* when you have finished.

- 9 If you are using a shutter, select the MetaDevice associated with your illumination hardware from the *Shutter* list. (If you have already selected a MetaDevice with the *Install and Configure Devices* command (Devices menu), you can simply select "[Current Shutter].")

**OR**

Otherwise, select "[None]."

- 10 If you want to run a journal between acquisitions, choose the command button next to *Journal to Run*. Select the icon for the desired journal and choose *Open*.

**Note:** To deselect a journal, choose this command button again and choose *Cancel*. The command button will display *Press to Select* rather than the journal name.

- 11 Choose *OK*. The Z Series Acquisition dialog box will appear.

**Note:** If you are recording this command in a journal, choosing *OK* will display a dialog box that asks whether or not you want to record the journal without actually acquiring the images. Choose *Yes* if you want to merely save the journal without acquiring. Choose *No* if you want to both save the journal and acquire the images.

## Collecting Images Using Z Series Acquisition

To collect images using the Z Series Acquisition dialog box, use the following procedure:

<u>Step</u>	<u>Action</u>
1	Configure the acquisition using the procedure presented above.
2	The Z Series Acquisition dialog box will appear, and the acquisition will start immediately.
3	If you want to change the number of planes acquired during acquisition, choose <i>Planes</i> . Once you have selected the desired number of planes, choose <i>OK</i> to begin acquisition again.
4	To control the acquisition process, you can use the <i>Pause/Resume</i> and <i>Stop</i> command buttons.
5	MetaMorph will close the Z Series Acquisition dialog box when the acquisition is completed.

**Move To Options**

<u>Option</u>	<u>Description</u>
Planes * Spacing	<p>Specifies the ending location as the number of <i>Planes</i> * "<i>Steps</i>."</p> <p>EXAMPLE: Starting at a <i>Current Position</i> of 50, if <i>Planes</i> = 4 and "<i>Steps</i>" = 6, the following would occur:</p> <p>Plane 50...56...62...68...74</p>
Origin	<p>The ending location is the Origin position, as set with the <i>Focus</i> command. Uses the <i>Planes</i> option to determine how many planes to acquire.</p>
Top	<p>The ending location is the Top position as set with the <i>Focus</i> command. Uses the <i>Planes</i> option to determine how many planes to acquire.</p>
Bottom	<p>The ending location is the Bottom position as set with the <i>Focus</i> command. Uses the <i>Planes</i> option to determine how many planes to acquire.</p>
Home	<p>The ending location is the Home position as set with the <i>Focus</i> command. Uses the <i>Planes</i> option to determine how many planes to acquire.</p>

## Acquire Z Series - Dialog Box Options

### Number of Planes

Specifies the number of planes that will be acquired, using the spacing selected with the "Steps" option. The default is 1.

### "Steps"

Specifies the spacing between planes. This option is based on the Z-axis device driver presently configured. The units of measurements are calibrated using the *Focus* command.

### Start At

Specifies the starting location of the stage for the Z-series acquisition. *Current Position* is the stage's present location, *Origin*, *Top*, *Bottom*, and *Home* are positions set by the user with the *Focus* command.

### Move To

Specifies the ending location of the stage for the Z-series acquisition. *Planes \* Spacing* specifies the ending location as the number of *Planes \* "Steps."* The *Origin*, *Top*, *Bottom*, and *Home* options are positions set by the user with the *Focus* command.

### After

Specifies the location to which the stage will be moved after an acquisition is successfully completed. The *Origin*, *Top*, *Bottom*, and *Home* options are positions set by the user with the *Focus* command.

### Image Storage

Specifies the type of image storage for the Z-series acquisition. Select *None* if you want to perform an acquisition without acquiring or storing images. Use *None* when acquiring images with a journal. Select *Stack* to create a stack. Select *Disk* to store images in a file on disk.

### Save File Name

Specifies the file name for image storage when *Disk* is selected as the *Image Storage* type. If a file name is not selected, the words "*Press to Select*" will appear on the command button; otherwise the file name will appear on the command button.

### Destination

Specifies the destination stack if *Stack* is selected as the *Image Storage* type.

### Shutter

Selects the MetaDevice for the illumination hardware associated with your shutter. If you have already selected a MetaDevice with the Install and Configure Devices command (Devices menu), you can simply select "[*Current Shutter*]." If you are not using a shutter, select "[*None*]."

### Journal to Run

Specifies the journal to run between acquisitions. If a journal file name is not selected, the words "*Press to Select*" will appear on the command button; otherwise the journal name will appear on the command button.

**Note:** If you are recording this command in a journal, choosing *OK* will display a dialog box that asks whether or not you want to record the journal without actually acquiring the images. Choose *Yes* if you want to merely save the journal without acquiring. Choose *No* if you want to both save the journal and acquire the images.

### **OK**

Starts the Z-series acquisition and opens the Z Series Acquisition dialog box. This dialog box displays the progress of the Z-series acquisition. You can change the number of planes to be acquired using the *Planes* command. You can also *Pause*, *Resume*, and *Stop* the acquisition.

### **Close**

Closes the dialog box.

## Set Plane Z Distance (Stack Menu)

Specifies a between-plane Z-distance to a selected image stack.

### Drop-in: 3D

Use this command to assign an inter-plane distance value to a stack of images. This procedure is useful for calibrating the Z-axis of a series of through-focus images. The Z-distance value can be used by other commands that involve Z-distance, such as 3D Reconstruction, View Orthogonal Planes, Measure Volume, and Measure XYZ Distance. Calibrations can be applied to the entire stack or to just the current plane. (Whether the distance is considered to be that between the current plane and the plane below it, or the plane above it, depends on the command that uses the Z-distance value.)

Z-distance calibrations can be stored with an image stack by saving the stack with either the Save or the Save As commands (File menu). If you do not resave the image after applying the calibration, the Z-distance value will be used throughout the rest of your work session, but the default value of 1 will be reapplied when you next start MetaMorph.

### Setting a Plane Z-Distance

To create a 3D reconstruction from a stack, use the following procedure:

<u>Step</u>	<u>Action</u>
1	From the Stack menu, choose Set Plane Z Distance. The Set Plane Z Distance dialog box will appear.
2	With the <i>Image</i> selector, select the image stack for which you want to set the Z-distance. Then select either the <i>Current</i> plane to apply the calibration to just the plane currently being displayed, or select <i>All Planes</i> to apply the calibration to the entire stack.
3	Use the <i>Z Distance</i> spin box to specify the inter-plane Z-distance.
4	To apply the calibration, choose <i>OK</i> .
5	When you have finished, choose <i>Close</i> .

Remember to resave the image if you want to store the calibration permanently with the stack. To do so, choose Save from the File menu.

## Set Plane Z Distance - Dialog Box Options

### Image

Selects the image stack to which the Z-distance calibration is to be applied. Select *Current* to apply the calibration to just the plane currently being displayed, or select *All Planes* to apply the calibration to the entire stack.

### Z Distance

Specifies the inter-plane Z-distance.

### OK

Accepts the selected *Z Distance* and applies it to the stack.

### Close

Closes the dialog box.

## 3D Reconstruction (Stack Menu)

Creates a volumetric 3D reconstruction, consisting of rotated views, from a stack of through-focus images.

### Drop-in: 3D

Use this command for stacks that are composed only of planes belonging to a through-focus set of images. The angle, orientation, interpolation, Z-axis distance, and the reconstruction type for the model are configured in the 3D Reconstruction dialog box.

The 3D reconstruction process automatically applies interpolation between image planes. Interpolation automatically fills in the gaps between planes, so that the resulting 3D reconstruction will be smoother.

Fluorescence Z-series can be reconstructed with the *Maximum* or the *Self-Luminous Pixels* options:

*Maximum* creates views using the brightest pixel along the "line of sight," so that a bright feature hides a dim feature.

*Self-Luminous Pixels* uses all of the gray level information along each result pixel's "line of sight." This creates semi-transparent views with features that are slightly blurred compared to the *Maximum* option.

Each method has its advantages; it is recommended that when in doubt, you should try each method to determine which one gives the best result.

**After creation, the different viewing angles of the 3D model can be viewed using the *Select Plane* or *Movie* commands.**

**Note:** Accurate view-generation is a time-consuming process. Be prepared to allow MetaMorph to execute this function for an extended period of time while it creates the necessary views.

## Creating a 3D Reconstruction

To create a 3D reconstruction from a stack, use the following procedure:

Step	Action
1	From the Stack menu, choose 3D Reconstruction. The 3D Reconstruction dialog box will appear.
2	Select the desired stack using the <i>Image</i> selector.
3	Select <i>Shallow Tilt</i> , <i>Full Circle</i> , <i>Single</i> , or <i>Custom</i> from the <i>Pre-Defined Angles</i> group.  <i>Shallow Tilt</i> and <i>Full Circle</i> already have defined angles. Use <i>Single</i> if you want only one view created. Use <i>Custom</i> if you want to use settings that are different from the standard <i>Shallow Tilt</i> and <i>Full Circle</i> angles.
4	To adjust the angle settings for the <i>Custom</i> view, use the <i>Angle of First View</i> , <i>Angle of Last View</i> ,

and *Angle Between Views* options to select the desired settings.

To adjust the angle setting for the *Single view*, use the *Angle of First View* to select the desired angle of view.

**WARNING:**

These options can also be used to change the default settings for *Shallow Tilt* and *Full Circle* views. Because these changes are permanent, you should use *Custom* if you want to adjust the angle settings.

- 5 Select the desired reconstruction type from the *Reconstruction Type* group.

Use *Min* for brightfield planes (dark objects on a bright background). Use *Maximum* for darkfield or fluorescence planes (bright objects on a dark background). Use *Average of Min and Max* for stacks requiring that information from both lighter and darker regions be preserved. Use *Self-Luminous pixels* for stacks consisting of fluorescence planes.

- 6 Select *Horizontal* or *Vertical* from the *Orientation* group to select the orientation of the 3D reconstruction.

- 7 In the *Use Z-Distance* area, choose either *Calibration* or *User Specified*.

If your image stack is calibrated (you calibrated the distances between planes using the *Calibrate Distances* command), choose *Calibration* to apply the calibrated pixel ratio.

**OR**

If your image is not calibrated, or you want to apply a different XY to Z pixel ratio, choose *User Specified* to specify the ratio that you want to apply. The "Z" (ratio) box appears.

**Then,**

In the Z (ratio) box, type or select an appropriate Z step to XY pixel

size ratio value.

**Note:** If you enter a value that is too small or too large, the image will appear either flat or distorted. The default value is 1.00. However, increasing the ratio above 1.00 can enhance or exaggerate the three dimensional characteristics of the 3D image stack.

- 8 Type or select a different *Border Color* if you want to change the grayscale value of non-image areas of the 3D reconstruction.  
  
**Note:** If you select *Self-luminous Pixels* as the *Reconstruction Type*, the *Border Color* will always be 0 (Black).
- 9 Select *Repaint While Processing* if you want to view the reconstruction in process.
- 10 Click *Sharpen result*, to apply a sharpening algorithm to the resulting 3D reconstruction image.
- 11 Choose *OK* to create the 3D reconstruction.

## 3D Reconstruction - Dialog Box Options

### Image

Selects the image stack for the 3D reconstruction.

### Angle of First view

Sets the angle by which the first reconstructed view will be offset from zero degrees.

### Angle of Last view

Sets the angle by which the last reconstructed view will be offset from zero degrees.

### Angle Between Views

Sets the interval between adjacent views in the stack.

### Pre-Defined Angles

Selects the settings for one of the predefined sets of angles:

**Shallow Tilt** – Sets the First Angle of View, Last Angle of View, and Angle between views to **-30**, **30**, and **5**, respectively.

**Full Circle** – Sets the First Angle of View, Last Angle of View, and Angle between views to **0**, **345**, and **15**, respectively.

**Custom** – Enables you to specify settings for First Angle of View, Last Angle of View, and Angle between views.

**Single** – Enables you to specify a single angle of view.

**Note:** For 0 (or 180) degree *Maximum* or *Minimum* views, the respective Stack Arithmetic operation will give identical results and will be faster.

### Reconstruction Type

Selects the reconstruction type used for the 3D reconstruction based on the stack's planes.

Use **Min** for brightfield planes (dark objects on a bright background).

Use **Maximum** for darkfield or fluorescence planes (bright objects on a dark background).

Use **Average of Min and Max** for stacks requiring that information from both lighter and darker regions be preserved.

Use **Self-Luminous Pixels** for stacks consisting of fluorescence planes.

**Note:** *Maximum* and *Self-Luminous Pixels* gives similar results. *Maximum* gives a crisper view with bright features dominating overlapping dimmer features. *Self-Luminous Pixels* will result in a "softer" or blurred view, but has the advantage that overlapping features are additive.

### Orientation

Selects the horizontal or vertical orientation of the 3D reconstruction view.

### Use Z Distance

Chooses either the calibrated XY:Z ratio if the image stack was calibrated before acquisition, or a user-specified value if the image stack is not calibrated.

Choose *Calibration* if your image stack is calibrated. The calibrated values for X, Y and Z are shown below the *Use Z Distance* area.

Choose *User Specified* if your image is not calibrated or you want to specify a different Z ratio value. Initial recommended Z (ratio) value is 1.00.

**Note:** If you enter a ratio value that is too small or too large, the image will appear either flat or distorted. The default value is 1.00. However, increasing the ratio above 1.00 can enhance or exaggerate the three dimensional characteristics of the 3D image stack.

### **Border Color**

Changes the value assigned by MetaMorph for unfilled areas of the 3D reconstruction stack if the image data from the source stack does not fill all parts of every plane.

**Note:** The border will always be set to 0 if you select *Self-Luminous Pixels* as the *Reconstruction Type*.

### **Repaint While Processing**

Repaints the reconstruction image, plane by plane, while the reconstruction is in progress.

### **X:Y:Z Units**

Indicates the X, Y, and Z calibration units to enable you to use these values to more accurately reproduce a reconstruction.

### **Sharpen result**

Applies a sharpening algorithm to the resulting 3D reconstruction image.

### **OK**

Creates the 3D reconstruction.

### **Cancel**

Cancel the command.

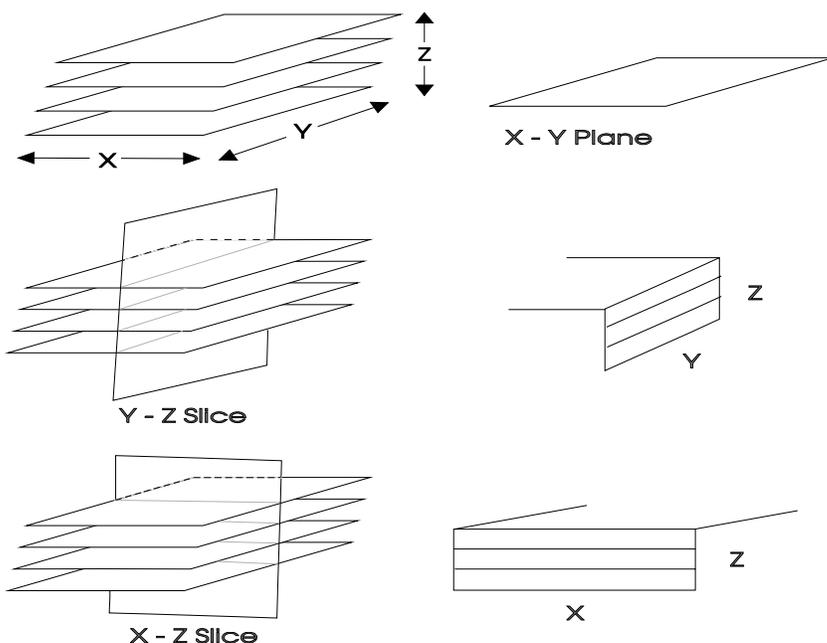
## View Orthogonal Planes (Stack Menu)

Creates a single plane XZ view and a single plane YZ view of a selected point in a stack or a stack of cross-section images.

### Drop-in: 3D

The View Orthogonal Planes command is an interactive image viewer which lets you examine cross-sectional slices through the image volume in the XZ, YZ, and XY planes. This helps you to visualize 3D structural information about a specimen. This command also allows you to determine whether appropriate optics are being used, by providing a rapid view of the axial (Z-axis) resolution of the system.

### Views Produced by the View Orthogonal Planes Command



This command provides a dialog box that allows you to use sliders to select the X, Y, and Z coordinates of the active pixel location as it is displayed in each image window. This location can be marked with a line or cross-hair marker, if you wish. In addition to the sliders, the command's interactive capabilities allow you to click anywhere in any of the three image windows to select the active pixel location. Each time you select the active pixel location, the views in the image windows will be updated to give the cross-sections at that position.

**Note:** This command can be used simultaneously with the Measure XYZ Distance Distance drop-in command (Measure menu) to draw "wireframe" tracking lines that follow objects or points through multiple planes of a stack. The tracking line can be observed as an overlay on both the original image stack and on the orthogonal plane stacks.

There are two ways to calibrate the Z-axis distance used by the View Orthogonal Planes command. If *Calibration* is selected in the *Use Z Distance* group, the Z-axis distance between planes will be determined when the stack is acquired with Acquire Z Series. The XY calibration will be determined by the settings in the Calibrate Distances dialog box. If a stack does not have the appropriate Z-axis distance between planes (for example, if a known spherical object does not appear to be circular when examining the three different views), you can select *User Specified* from the *Use Z Distance* group and select the desired Z-axis distance. The adjusted Z-axis distance can then be used in the 3D Reconstruction dialog box.

Although only one plane of the XZ and YZ views are displayed by the interactive image viewer, you can make a stack consisting of specified planes from either view. These stacks can be used for creating

movies, so that you can view the specimen as if you were looking at it from the front or side of the microscope slide. You can use the stack(s) to make side projections using Stack Arithmetic.

## Viewing Orthogonal Planes

To view orthogonal planes from a stack, use the following procedure:

<u>Step</u>	<u>Action</u>
1	From the Stack menu, choose View Orthogonal Planes. The View Orthogonal Planes dialog box and XZ View and YZ View image windows will appear. (The original image window is the XY view.)
2	Select the desired stack using the image selector.
3	From the <i>Use Z Distance</i> group, select <i>Calibration</i> or <i>User Specified</i> .  <i>Calibration</i> specifies that the Z-axis distance be determined from the conditions when the stack was created with Acquire Z Series.  <i>User Specified</i> allows you to specify the Z-axis distance.
4	If you selected <i>Calibration</i> , status text indicating the units of measurement and the calibration will be displayed below the <i>Use Z Distance</i> group.  <b>OR</b>  If you selected <i>User Selected</i> , select the desired value for the Z-axis distance using the <i>Z</i> text box.
5	Select the desired type of marker using the <i>Marker</i> list.  <b>AND</b>  If you selected <i>Line</i> or <i>Cross Hair</i> for the marker, select the desired color for the marker using the <i>Marker Color</i> list.
6	Click anywhere in any of the three image windows to select the active pixel location. The image windows will update to display the particular section (slice) in the image volume that you have selected.  <b>OR</b>

Select the desired location using the XY, YZ, or XZ options in the dialog box. If *Update While Tracking* is disabled, the image windows will not update until after the sliders have finished being moved.

- 7 If you want to create a stack consisting of XZ or YZ views, select *More >>* to expand the dialog box.

### **AND**

Select the YZ or XZ view from the *Views* group. You can use *Left Plane/Right Plane* (or *Upper Plane/Lower Plane*) to select a limited group of planes for the stack if you wish.

Choose *Create* to create the stack.

- 8 Choose *Close* when you have finished.

## View Orthogonal Planes - Dialog Box Options

### Source

Selects the stack for viewing orthogonal planes.

### XY

Selects the XY coordinate of the active pixel location.

### YZ

Selects the YZ coordinate of the active pixel location.

### XZ

Selects the XZ coordinate of the active pixel location.

### Use Z-Distance

Specifies the calibration method used for the Z-axis distance. If you select *Calibration*, the Z-axis distance between planes will be determined from the conditions when stack was acquired with Acquire Z Series. The XY calibration is determined by the settings in Calibrate Distances dialog box. If a stack does not have the appropriate Z-axis distance between planes (for example, if a known spherical object does not appear to be circular when examining the three different views), you can select *User Specified* from the *Use Z Distance* group box and select the desired Z-axis distance. In this situation, MetaMorph will assume that the X:Y calibration is 1:1. The adjusted Z-axis distance can then be used in the 3D Reconstruction dialog box.

### Z

If you select *User Specified* in the *Use Z Distance* group, this option will be used to select the desired value for Z-axis distance. **Note:** If you have not calibrated X, Y or Z, you may find that the calibration option is set to (1 X-pixel):(1 Y-pixel):(500 Z steps), or a similar large ratio. This will result in extremely slow processing and a distorted result. Try *User Specified* with Z in the range of 1.00 to 5.00 instead.

### Update While Tracking

If this option is selected, the image windows will update as you move the XY, YZ, or XZ slider to select the active pixel location in the dialog box. If this check box is cleared, the image windows will not update until after you have finished moving the sliders. This option does not affect the "point-and-click" interactive mode of selecting the active pixel location.

### Marker

Selects the marker type used to mark the active pixel location. You can select *Line*, *Cross Hair*, or *None*.

### Marker Color

Selects the color for the marker, if one is used.

### More >>

Expands the dialog box to display additional options.

### Less <<

Condenses the dialog box.

**Views**

Selects the view used to create a stack of either YZ or XZ images.

**Left Plane (or Upper Plane)**

Specifies the starting point of the stack that will be created from the YZ or XZ views.

**Right Plane (or Lower Plane)**

Specifies the ending point of the stack that will be created from the YZ or XZ views.

**Create**

Creates a stack consisting of either YZ or XZ images.

**Close**

Closes the dialog box.

## Topographic Surface (Stack Menu)

**Builds an 8-bit topographic surface image from a stack of brightfield or fluorescence images. A topographic surface map is one in which the brightness of the computed pixels corresponds to the Z-axis position of the surface of the object in the stack, relative to the first plane.**

### Drop-in: 3D

Use this command to build a topographic surface image from a stack of brightfield, fluorescence, or binary images. This command can be used with binary, 8-bit, or 16-bit stacks. The resulting image in each case will be an 8-bit image.

If you select *Fluorescent*, the topographic surface image will be derived from the stack by finding the brightest pixel at each location from all of the planes, and encoding it with a value that represents its plane number. If you select *Bright Field*, the topographic surface image will be derived from the stack by finding the darkest pixel at each location from all of the planes, and encoding it with a value that represents its plane number.

You can use *Fluorescent* when you are using image that are from a Z-series. The intensity of the fluorescence images represents height, with the brightest being the last plane of the stack and the darkest the first plane of the stack. The heights may be easier to distinguish if you select the Pseudocolor look-up table. *Fluorescent* also works well when working with a timelapse series of binary images. Objects that appear first in the first plane will be encoded as gray level 1; objects that first appear in later planes will be encoded with gray values that represent their plane number.

With small stacks, the values in the topographic surface can be difficult to see unless *Scale Resultant Image* is selected. This is because the values in the resulting topographic surface image are values which represent plane numbers. The total number of gray values in the topographic surface image will be equal to number of planes from the original stack. Thus, a topographic surface image from a 16-plane stack will consist of 16 gray values. *Scale Resultant Image* scales the maximum grayscale value in the topographic surface image to 255.

With stacks that consist of more than 255 planes, the topographic surface image will be scaled to a maximum of grayscale level 255.

**Note:** In addition to its typical use with stacks created by the Acquire Z Series command, Topographic Surface can be used with stacks created by Acquire Timelapse or Acquire Spectral Scan. The result image will be a map with gray values that represent time or wavelength.

**Note:** This command does not support 24-bit color images.

## Creating a Topographic Surface

To create a topographic surface map from a stack, use the following procedure:

<u>Step</u>	<u>Action</u>
1	From the Stack menu, choose Topographic Surface. The Topographic Surface Map dialog box will appear.
2	Select the desired stack using the <i>Image Stack</i> image selector.
3	Select <i>Bright Field</i> or <i>Fluorescent</i> from the <i>Image Source</i> group, depending on the optics used to acquire the image in the stack. Use <i>Fluorescent</i> with a binary stack.
4	Select <i>Scale Resultant Image</i> to scale the resultant image so that the maximum gray value is 255. If this option is disabled, the number of gray levels in the resulting image will equal the number of planes in the image.
5	Choose <i>OK</i> to create the topographic surface map. MetaMorph will display it in a new image window.

## Topographic Surface - Dialog Box Options

### Image Stack

Selects the stack for the topographic surface map.

### Image Source

Select either *Bright Field* or *Fluorescent* depending on the microscope mode used to acquire the images. Use *Fluorescent* if the images are fluorescence images or binary images.

### Scale Resultant Image

Scales the maximum grayscale value in the topographic surface image to 255. With small stacks, the values in the topographic surface can be difficult to see unless you select *Scale Resultant Image*. This is because the values in the resulting topographic surface image are values which represent plane numbers. The total number of grayscale values in the topographic surface image will be equal to the number of planes from the original stack, plus zero to represent no object present (thus a topographic surface image from a sixteen-plane image stack will consist of seventeen grayscale values).

### OK

Creates the topographic surface image.

### Cancel

Cancels the command.

## Remove Haze (Legacy)

**Note:** The Remove haze filter is now available through the Basic Filters command in the Process menu. The stand alone Remove Haze command is no longer available from the MetaMorph desktop and can only be accessed through the Journal Editor.

**Allows you to reduce the effects of out-of-focus haze from a plane in a stack of images.**

### Drop-in: 3D

Use this command when you want to reduce the effects of out-of-focus haze from a stack with three or more planes. Because this command is based on nearest-neighbor deblurring, it can only be applied to stacks with at least three planes. This command can be applied to 8-bit or 16-bit images.

The Remove Haze command depends on an estimation of the three-dimensional Point Spread Function (PSF) to compute the contributions from out-of-focus planes, and uses this to generate an image in which the effects of the out-of-focus haze are greatly reduced.

Stacks that lend themselves well to haze-removal will have planes that were acquired with the same analog adjustment settings, and will have a maximum dynamic range. This command requires that the planes in the stack be acquired very closely together (typically, one micron or less), so that the planes above and below contribute to the out-of-focus haze in the target plane. If the planes are too far apart, the Unsharp Mask filter should be used instead, as it will be difficult to compute accurately the contribution from distant planes.

MetaMorph allows you to change the size of the low pass filter kernel, which approximates the point spread function and is used to determine the contribution to blurring from the out-of-focus planes. For best results, experiment to see which values work best for a given specimen. As a general rule, the greater the scaling factor, the greater will be the amount of haze that is removed from the original, and hence the lower the amount of brightness from the original will be retained. If you select *Auto Result Scale*, MetaMorph will select a *Result Scale* that matches the selecting *Scaling Factor* so that the brightness of the resultant pixels will be roughly the same as in the original image.

**Note:** Results obtained from the Remove Haze command are not suitable for use in quantitative gray level analysis.

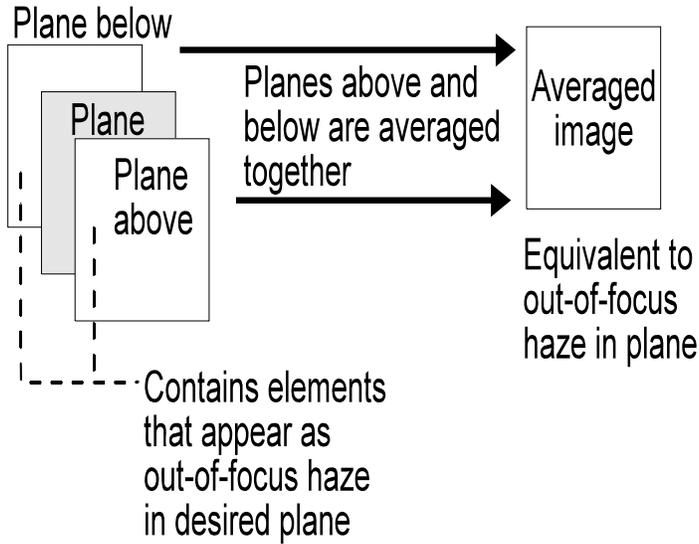
For best results, we strongly recommend scaling 8-bit image stacks to 16-bits by multiplying them by 100 prior to applying the remove haze operation. This will greatly improve the resultant image's quality.

### The Process of Removing Haze:

The process that MetaMorph uses to remove haze from a plane can be broken down into five steps.

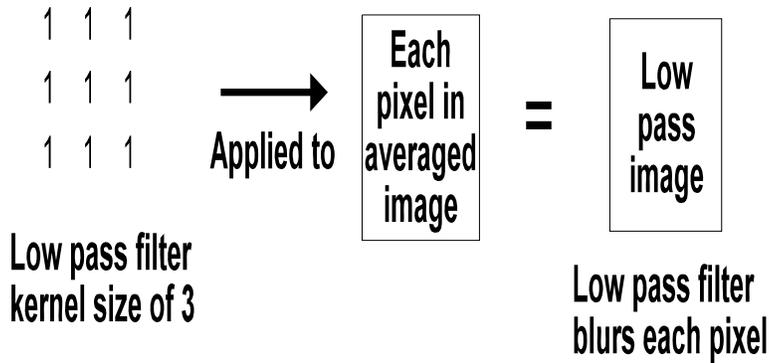
**Step 1:** The out-of-focus haze in the desired plane is computed by convolving the adjacent planes by the point spread function. To speed up computations, MetaMorph first averages the planes adjacent to the target plane to create an approximation of the haze in the desired plane.

1.



**Step 2:** After the plane above and below are averaged together, MetaMorph applies a low pass filter to the averaged image. The low pass filter blurs the image to simulate the effects of looking at those planes from the target plane. The low pass filter is a simple, and therefore a fast, substitute for a kernel derived from the PSF. A PSF kernel would have to be derived for each sample, and is computationally expensive.

2.



**Step 3:** Before MetaMorph subtracts the low pass image from the target plane, it first decreases the intensity range of the low pass image so that the result of the subtraction operation will be greater than zero (if the result was zero, the image would be uniformly black). Thus, rather than:

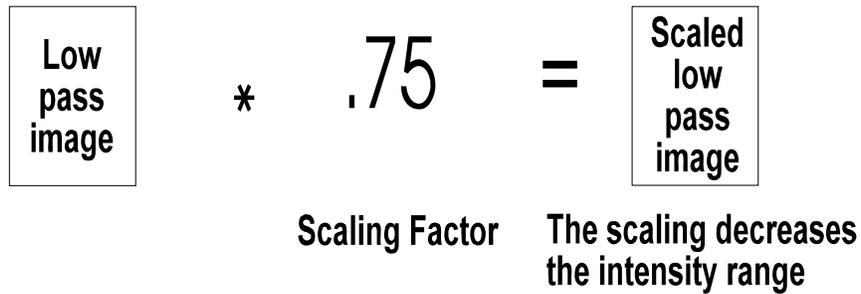
$$100\% - 100\% = 0 \text{ intensity range,}$$

the subtraction operations will look similar to the following if a *Scaling Factor* of 0.75 is applied:

$$100\% - 75\% = 25\% \text{ intensity range (approximately).}$$

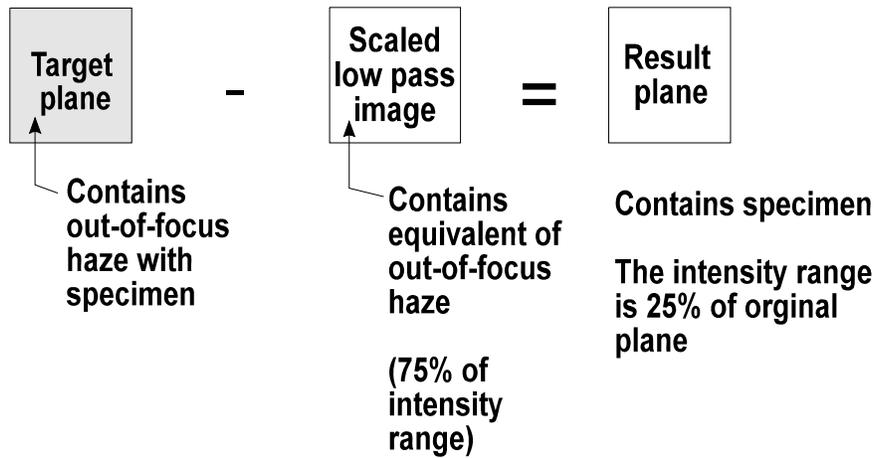
The *Scaling Factor* should be determined empirically, taking into consideration how much out-of-focus haze should be removed from the image and how much brightness range can be lost. The figure below uses the default *Scaling Factor* of 0.75 to illustrate this step.

3.



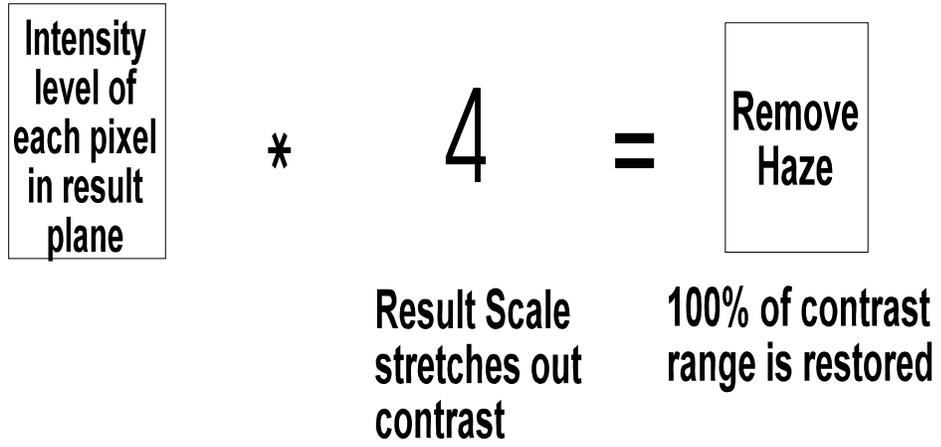
**Step 4:** Next, MetaMorph subtracts the scaled low pass image to remove the out-of-focus haze, creating a resultant image with the haze reduced.

4.



**Step 5:** After the out-of-focus haze has been subtracted, the contrast of the result plane can be "stretched" to restore about 100% of the contrast range. This will not restore the number of gray levels to that of the original image, but the range will be restored.

5.



## Removing Haze

To remove haze from an image, use the following procedure:

<u>Step</u>	<u>Action</u>
1	From the Process menu, choose Remove Haze. The Remove Haze dialog box will appear.
2	If the desired source stack is not displayed in the image selector next to <i>Source</i> , select it using the image selector.  <b>Note:</b> The source stack must contain at least three planes.
3	If the desired destination image is not displayed in the image selector next to <i>Dest</i> , select it using the image selector. You can overwrite or add to the existing image or you place the results in a new image window.
4	Select <i>Auto Result Scale</i> if you have MetaMorph select a result scale that matches the scaling factor you select.
5	Use <i>Low Pass Kernel Size</i> to select the pixel size of the low pass filter. Larger low pass kernel sizes leads to the detection of larger areas. The kernel size must be an odd number.  If the image is very wide and the selected kernel size is too large, MetaMorph will display a message dialog box requesting that you try a smaller kernel size.  If the horizontal size of the images are too large AND are much greater than their vertical size, you can use the Rotate command in the Graphics menu to rotate the images by 90 degrees, thereby creating a new stack that can then be processed by Remove Haze.
6	Select a <i>Scaling Factor</i> . If set to 1, only the edges are left after unsharp masking. Smaller numbers allow more of the image features to be present in the result image.

- 7 Use the *Result Scale* option to increase the contrast lost during the subtraction of the blurred copy from the original image.

**Note:** Skip this step if you selected *Auto Result Scale* in Step 5. Changing the *Result Scale* setting will disable *Auto Result Scale*.

- 8 Choose *Apply*.
- 9 Choose *Close* when you have finished.

## Remove Haze - Dialog Box Options

### Source

Selects the source stack from which you want to remove haze. This stack must consist of at least three planes.

### Dest

Selects the destination for the result image. You can place the results in a new image window. Or you can add the resulting image as a plane to an existing image or stack.

### Low Pass Kernel Size

Specifies the pixel size of the low pass filter applied the stack. Larger low pass kernel sizes lead to the detection of larger areas. The kernel size must be an odd number to maintain symmetry in the kernel.

### Scaling Factor

Specifies the scaling factor applied to the stack after the low pass filter is applied. If set to 1, only the edges will be left after unsharp masking. Numbers smaller than 1 allow more of the image features to be present in the result image.

### Result Scale

Restores the contrast lost during the subtraction of the blurred copy from the original image.

### Auto Result Scale

Allows MetaMorph to select a *Result Scale* value that matches the selected *Scaling Factor*.

### Apply

Applies the selected filter to the source image.

### Close

Closes the dialog box.

