

Spatially calibrating images in MetaMorph

OVERVIEW

This document describes how to use MetaMorph's Calibrate Distance dialog box to build a list of spatial calibrations that can be applied to images, as well as link a spatial calibration to a specific hardware condition. There are several reasons why you need to configure spatial calibration in MetaMorph:

- By default, all of the values obtained from MetaMorph's measurement functions are reported in pixels. The term pixel (or picture element) does not refer to any particular physical dimension. For example, what is 20 pixels in length on one system might be 17 pixels in length on another system. Once an image is calibrated, measurement functions will report the data in calibrated units.
- The number of pixels can be related to the chip of the camera that you are using. For example, if you are using a camera with a 1 cm x 1 cm chip and each pixel is 6 microns x 6 microns, then the total number of pixels on the chip is roughly 1666 x 1666. For this camera, an object that is 36 microns in length would cover 6 pixels at a magnification of 1x.
- Within MetaMorph, pixels can also mean two different units. If you are looking at an image in MetaMorph at 100%, then the relationship of a pixel in the image window to a pixel on the camera is 1:1. If you increase or decrease the magnification of the image, then MetaMorph has to interpolate more or less MetaMorph pixels per camera pixel.

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PRODUCTS
MetaMorph® 6.0 or later

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To obtain meaningful measurement values, you must normalize the measurements into 'real' units of dimension, such as microns, mm, cm, or inches. This involves creating a calibration for each objective that contains the relationship between a certain objective and the number of microns per pixel. This calibration is then used to report numbers in 'real' units. The instructions below contain the following procedures:

- Building a calibration set
- Linking a spatial calibration to a hardware condition.
- Applying a calibration setting to an image

NOTES:

- For more information about the procedures and dialog boxes described in this document, refer to the MetaMorph online help system. Press [F1] to access help for the active dialog box.
- You will need to acquire a reference image with known measurements to complete the procedures in this document. Examples of valid reference images include an image of a stage micrometer or a grid. If your camera does not have square pixels, you will need to use a grid or a crosshair micrometer. The sample image used in this document is of a stage micrometer. Reference images should be acquired using 1 x 1 binning.
- Linking a spatial calibration to a hardware condition requires MetaMorph version 6.0 or later.
- This document assumes that the camera used in the procedure uses square pixels.

- A calibration setting should be created for each unique magnification configuration (objective plus any zoom lenses, manual or motorized, placed in the optical path).

INSTRUCTIONS

There are two tabs in the Calibrate Distance dialog box: the *Setup* tab and the *Apply* tab. The *Setup* tab is used to create calibrations or calibration sets and to edit an existing calibration setting. A calibration set is a collection of calibrations that are used together and saved to a single calibration file [*.cal]. You should create a calibration set for a microscope that contains calibration settings for each magnification configuration and save it to a calibration file. The *Apply* tab is used to apply the selected calibration to a single image or to all open images.

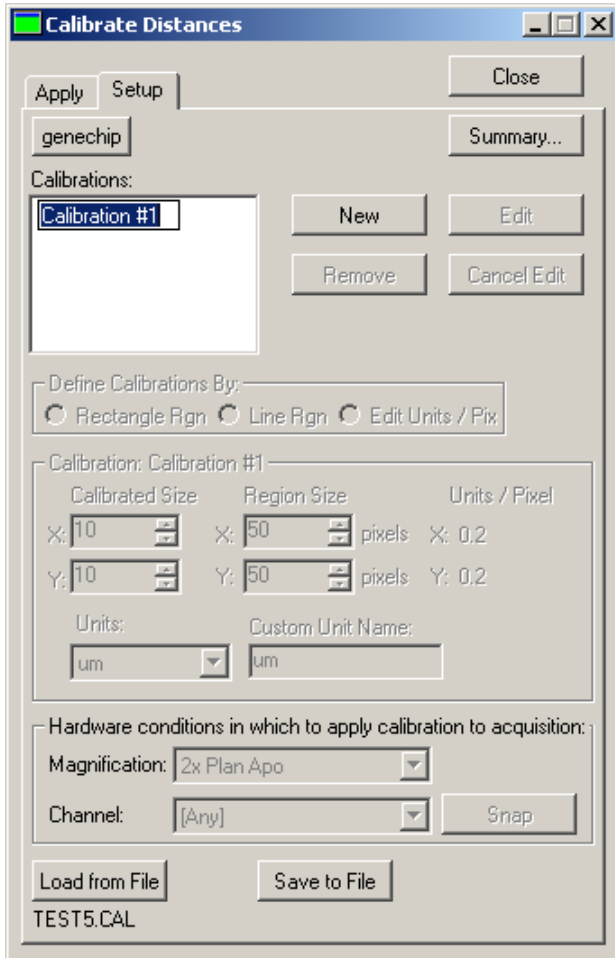
Complete the following procedure to create a calibration set:

1. Open your reference image.

NOTE: You will need to acquire a reference image with known measurements to complete the procedures in this document. Examples of valid reference images include an image of a stage micrometer or a calibration grid. The sample image used in this document is of a stage micrometer.

2. From the Measure menu, select Calibrate Distances. The Calibrate Distance dialog box opens.
3. Click the *Setup* tab to select it and then click *New*. A calibration region appears on the image and a new calibration name appears in the *Calibrations* list, as shown in Figure 1:

**FIGURE 1
CALIBRATE DISTANCES DIALOG BOX**

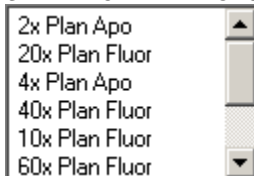


4. Type a name for the new setting and click in the white area outside the text box to set the name.

NOTE: If you are creating a unique calibration setting for each magnification configuration, MDC recommends naming each calibration after the magnification setting for the corresponding objective.

Figure 2 illustrates a *Calibrations* field with listings for each objective:

**FIGURE 2
SAMPLE CALIBRATIONS FIELD**



5. Select *Rectangle Rgn* in the *Define Calibrations By* field. A small rectangular region of interest opens on the active image.

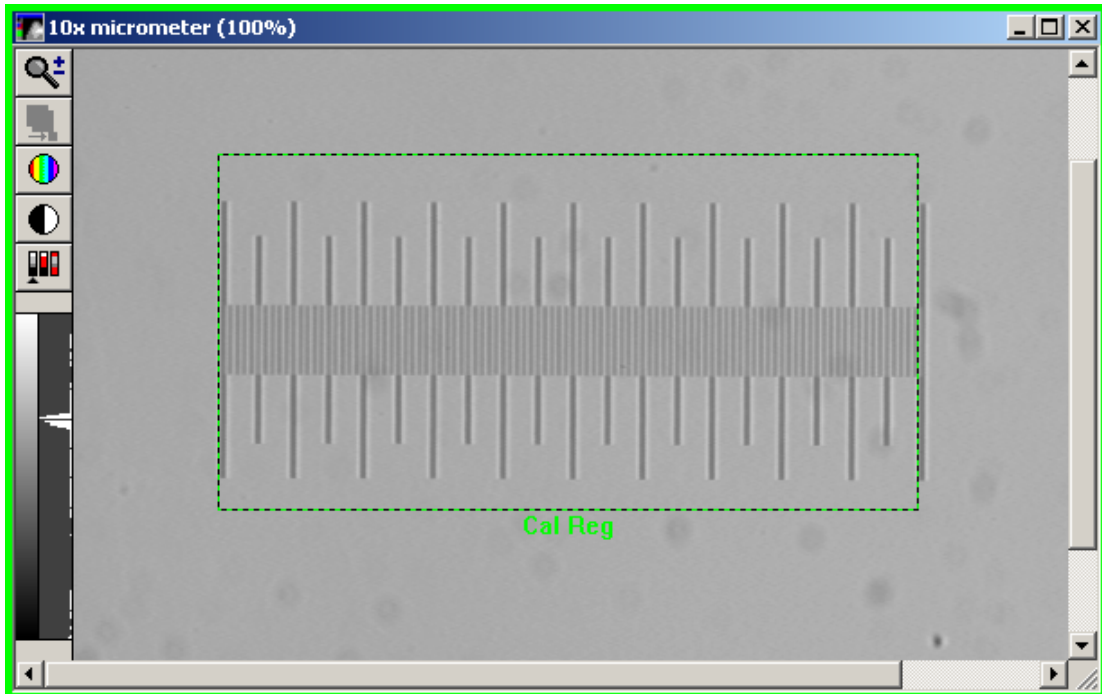
NOTE: Calibrations can be defined by a rectangular region, a line region, or entering a known calibration using the *Edit Units/Pix* option. This procedure uses the *Rectangle Rgn* option.

For cameras with square pixels, a rectangle or line region may be used for calibration. A rectangle region provides two lines (vertical and horizontal) by which to align the region along the reference image. The rectangle region will also help you to ensure that the micrometer is straight.

If your camera does not have square pixels, a rectangle region must be used to set up your calibration.

6. Move and resize the region to cover the maximum known distance along the micrometer in the x axis, as shown in Figure 3:

FIGURE 3
ALIGNING THE REGION



7. Use the Zoom tool to increase the magnification of the image to check for proper alignment of the region.

NOTE: In order for your calibration to be accurate, you must start and end on the same edge of the micrometer bars. If you start on the left side of the leftmost bar, you must place the right most point of the region on the left side of the right most bar, as shown in Figure 3.

NOTE: As you adjust the size of the active region, the values in *Region Size X* and *Y* fields will change, displaying the region's width and height. You can use these settings boxes to fine-tune the region's size.

8. Note the value in the *Region Size X* field. This is the width of the micrometer in pixels.
9. Enter the value from the *Region Size X* field in the *Region Size Y* field if you are using a camera with square pixels. The pixel to micron ratio is the same for the X- and Y-axis for cameras with square pixels. If your camera does not have square pixels, do not alter the *Region Size Y* field.

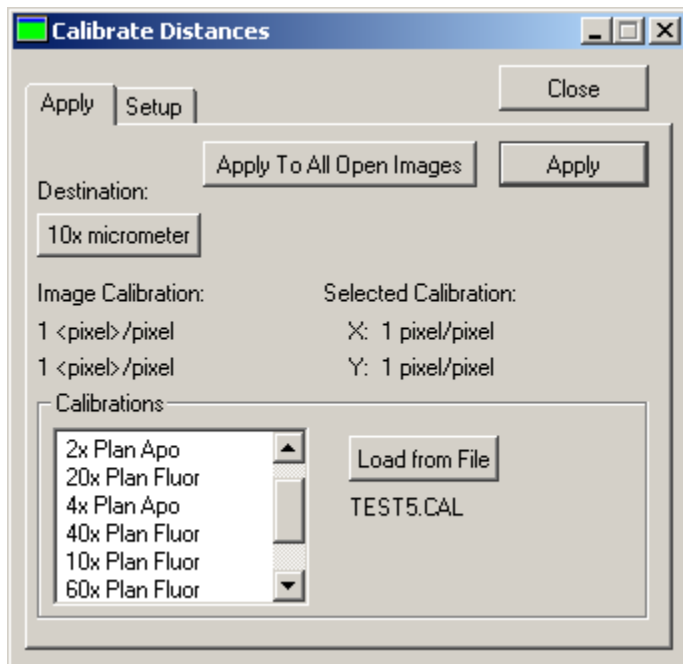
NOTE: Sometimes it is not possible to enter the same region size into the *Region Size Y* box. This is because the region would have to be drawn off of the image. In order to overcome this you need to either reduce the size of the region in the X-Axis or move the entire region up in the image. If you are using a camera with square pixels, you can also choose to use the line region option instead of the rectangle option.

10. In the *Calibrated Size X* field, enter the actual distance of the known X-axis size of the object used for calibration (in this case the length of the entire micrometer).
11. Enter the same value that you entered in the *Calibrated Size X* field into the *Calibrated Size Y* field. If your camera does not have square pixels, enter the actual distance of the known Y-axis size of the object used for calibration in the *Calibrated Size Y* field.
12. From the *Units* drop-down box, select *um* to calibrate your image(s) in microns. This is the most common unit selection. There are other options available depending on your magnification setting and calibration reference.
13. To use the current calibration settings on images acquired with a unique magnification and camera combination, select a magnification setting (if applicable) and acquisition channel from the *Magnification* and *Channel* drop-down boxes. The magnification setting must be predefined before it is available in the dropdown box. The calibration will automatically be applied when using the selected magnification and channel combination during acquisition.

NOTE: For more information about magnification settings, refer to the online help for the Configure Magnification dialog box.

14. Click *Done* to finish editing the calibration.
15. Repeat steps 3-14 for each objective on your microscope.
16. Click the *Apply* tab to activate it. The calibrations you created are listed in the *Calibrations* list, as shown in Figure 4:

FIGURE 4
APPLY TAB



17. Select the appropriate calibration setting from the *Calibrations* list.
18. Open the image(s) you want to calibrate and select it from the *Destination* drop-down list.
19. Click *Apply* to apply the calibration setting to the image listed in the *Destination* field.

NOTE: If you want the calibration setting to be applied to the image whenever it is opened, you must save the image after you have applied the calibration.

20. To apply the calibration setting to all images currently open, click *Apply To All Open Images*.
21. To save the complete list of your current calibration settings to a file, go back to the *Setup* tab and click *Save to File*. The Save Spatial Calibrations dialog box opens.
22. Enter a name for the calibrations file in the *File name* field and click *Save*. These setting can be loaded at anytime using the *Load from File* command.
23. Click *Close* to exit the Calibrate Distances dialog box.

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