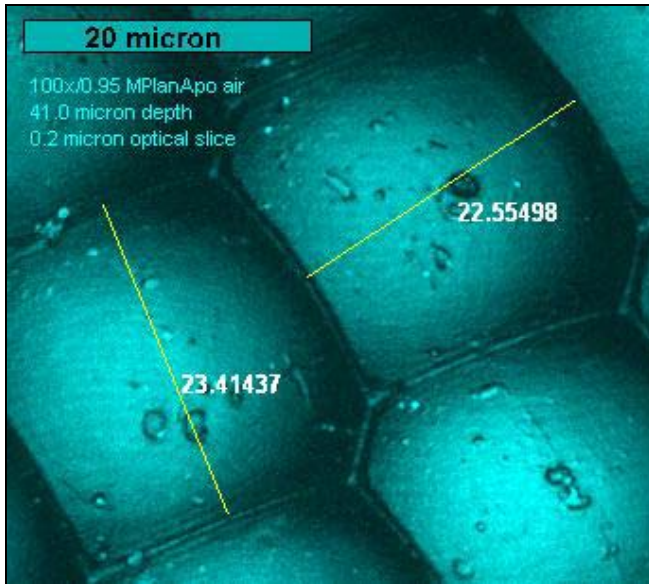


Image-Pro[®] Plus Product Note

Spatial Calibration in v. 5.0 and Higher



Digital image of a fly eye showing calibrated measurements. Image courtesy of Triptar Lens Company.

Introduction

A raw image does not have enough information to make any absolute measurements. Pixels by themselves say nothing about the sizes or intensities of the objects pictured, other than relative measurements such as, “object 1 is larger than object 2”. In order to measure objects in an image, it is necessary to know how large the pixels are. Once the pixel size has been established, any pixel based measurement can be converted to whichever units are required. This allows comparisons between images taken with different objective lenses, on different systems, on different days, all by referring the images to a standard measurement.

Applications and Examples

Calibrating images requires calibrating the optics used for acquisition, as well as tracking which optical system was used to capture the image. To do this Image-Pro Plus 5.0 creates two types of calibrations- reference calibrations and system calibrations.

Reference calibrations are created for a specific camera and objective lens combination and may be used repeatedly if the magnification properties do not change. This is very useful in microscopy, where

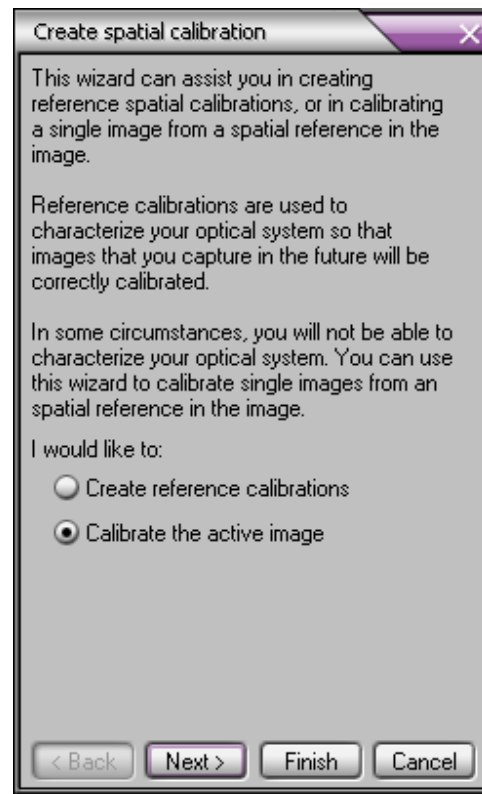
the sample is always at a fixed focal distance from the lens.

Individual image calibrations are created and used when the image has some form of reference marker in the image. This marker may then be measured to derive pixel/unit conversion factors.

Both Scope-Pro[®] and Stage-Pro[™] take the reference calibrations and apply them to images as they are captured, which makes re-entering such data unnecessary. This data is also used by Sharpstack[®] and 3D Constructor[®] for deconvolution and volume measurements.

Implementation

Measure|Calibration|Spatial Calibration Wizard...



The Spatial Calibration Wizard Dialog Box

Individual Image Calibration

- Click the 'Calibrate the active image' radio button. Supply a name and unit for the new calibration (Fig. 1)

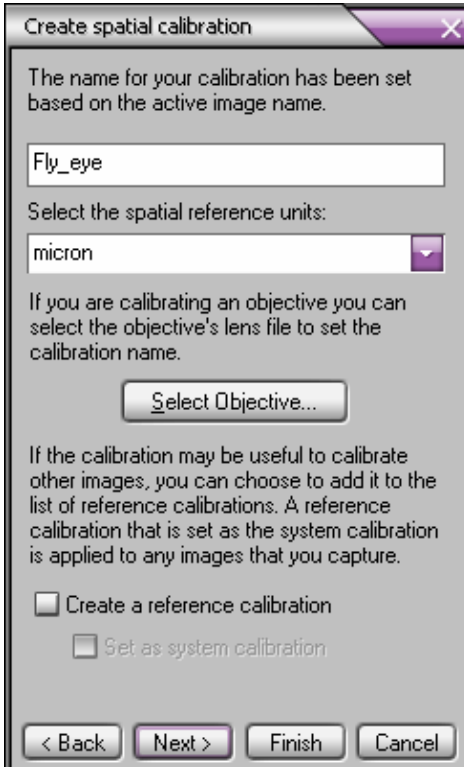


Figure 1. Naming Calibration Files and Assigning Units

- Decide whether this will be a reference calibration, and possibly a system calibration. Reference calibrations are a separate list of calibrations that can be used to generate derived calibrations. For example, a reference calibration may be generated for a camera binning of 1x1, and later used for a derived calibration using a binning of 3x3.
- If you are creating a reference calibration, click the 'Create a reference calibration' check box.
- If you would like to create a system calibration, click the 'Set as system calibration' check box.
- Draw reference lines across features of known sizes (Fig. 2). Multiple lines may be drawn and averaged to improve results. Note that the Local Zoom tool will appear. Turning on the crosshairs in the Local Zoom may be helpful in positioning your reference lines on the image.

- Click 'OK' to accept the calibration.

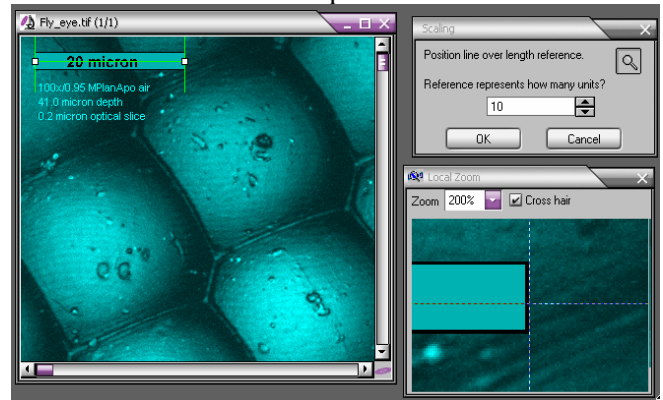


Figure 2. Drawing a reference line.

- Click 'Finish' to create the calibration. All spatial measurements created on the image will be calibrated in terms of the new units. If the image is saved as a TIFF file, the calibration file information will be retained with the image.

Reference Calibrations

- Click 'Create a reference calibration' The 'Create reference calibration' wizard appears:

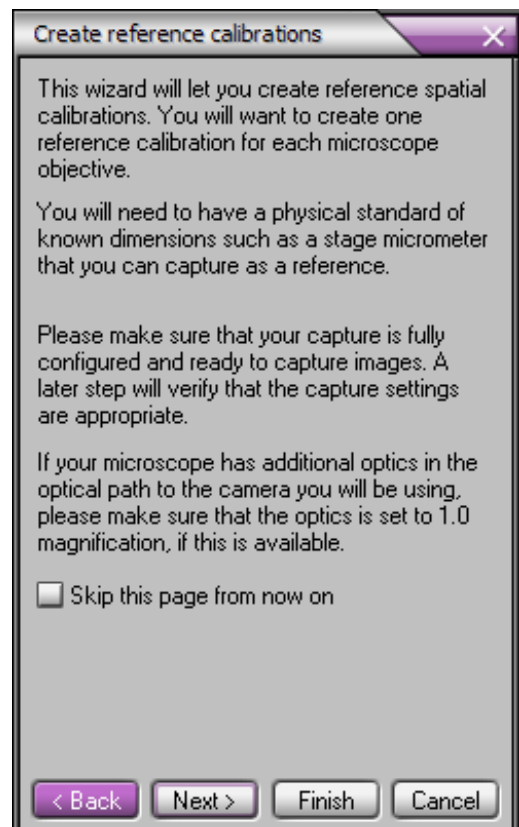


Figure 3. The Reference Calibration Wizard

- A message will appear indicating the system is ready for image capture. If you would like to test the capture settings, click ‘Test Capture’.
- Prepare your optical system to take a picture of a known reference, such as a stage micrometer. If your camera supports binning, it should be set to 1x1.
- Click ‘Next>’ to access the magnifier options (Figure 4).
- Input any additional optical magnification components such as magnification changers. If none are available, set the magnification to 1.0.

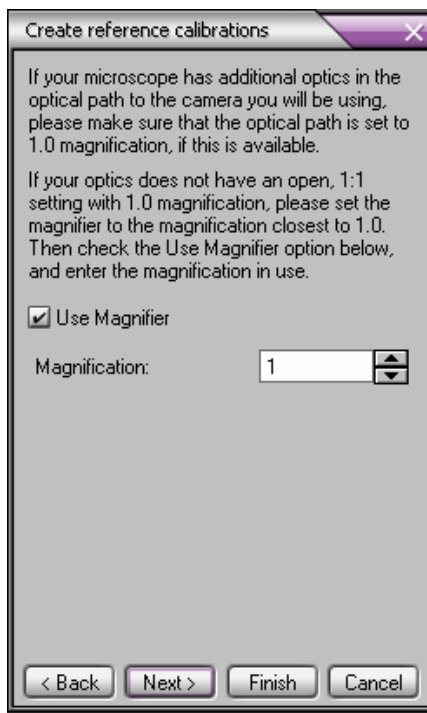


Figure 4. Indicating Additional Magnification

- Click ‘Next>’. The Add/Edit Objectives menu appears:

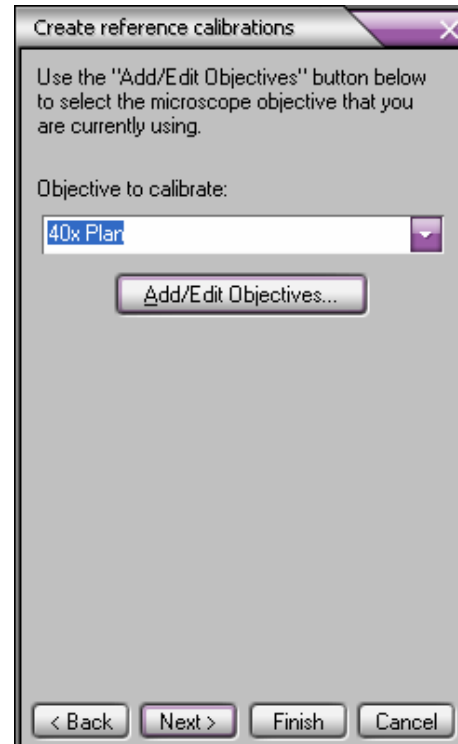


Figure 5. Selecting a lens for calibration

- The wizard will next ask you for a lens to calibrate (Fig. 5). If you are just setting up your system, click ‘Add/Edit Objectives...’ to add new objectives to the list (Fig. 6).



Figure 6. Adding Lens Information

System vs. Reference Calibrations

System calibrations are applied to images created in Image-Pro that do not otherwise have a calibration. If you will not be taking multiple images with the sample at the same distance, and hence the same pixel calibration, you don't need a system calibration – an individual calibration for that image is sufficient.

Reference Calibrations are used with microscopes by Image-Pro, Scope-Pro, and Stage-Pro, as the basis of measurements and some microscope stage movements. A reference calibration for a particular lens can be used to create derived calibrations for different camera binnings. It can also be used by Stage-Pro to tile multiple images together, moving just enough for the tiled images to abut each other. For example, when using the *Process\Tile Images...* command, you can set a guard frame in Stage-Pro to provide sufficient overlap for tiling images based on their calibrations.

Image-Pro Plus maintains a list of lens files, complete with numerical aperture, magnification, and immersion medium. This information is used by Sharpstack and 3D Constructor for deconvolution and volumetric measurements.

- To add a lens to the list, click ‘Add’ and fill in the lens information. Click ‘OK’ to add the lens information to the list (Fig 6).
- Click ‘Next>’. You can now capture reference calibration images (Fig. 7).

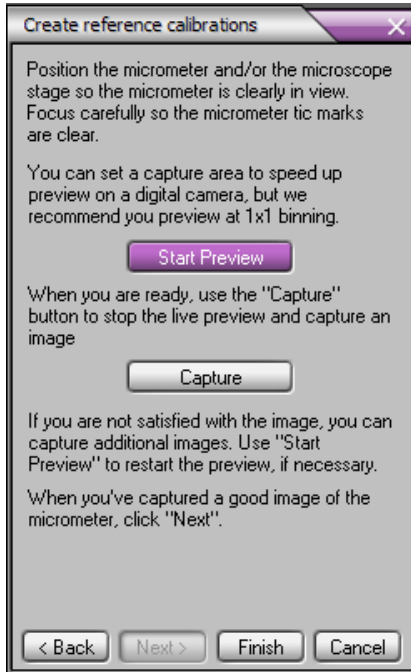


Figure 7. Previewing and Capturing a Reference Calibration

- Click 'Start Preview' to see how your image will look. Adjust settings as necessary to obtain a good image.
- Click 'Capture' to capture the image.
- Click 'Next>'. The 'Draw Reference Line' button appears in the accompanying menu (Fig. 8). Mark reference lines on the captured image as you would for individual image calibrations (Fig 2, above). You may create more than one line to improve the accuracy of the measurement.

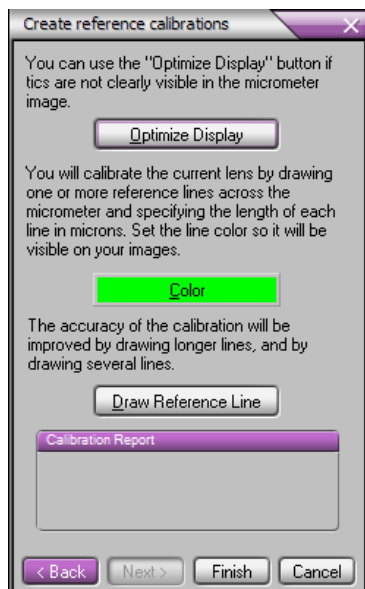


Figure 8. Creating Reference Lines

- Type in a value that represents the length of the reference units.
- Click 'Finish' to create the reference calibration.

In Stage-Pro and Scope-Pro you can associate lens calibrations with the positions on your lens turret using two equivalent commands. Stage-Pro uses the Calibrate Lens XY tool on the 'Lens/Mag' tab, while in Scope-Pro the same function can be completed using the Attach Calibration tool on the 'Scope' tab. When you capture an image with Stage-Pro or Scope-Pro set to a particular objective, that lens calibration is attached to the image. If the camera is binned, a derived calibration will be created for that image. Alternatively, you can at any time select a calibration for an image using **Measure|Calibration...|Select Spatial**.

Calibrated images permit all Image-Pro Plus tools to report measurements in calibrated units. With proper calibrations you can relate measurements taken at any time on any system, comparing results derived from all of your combined data.

See Also

Solutions Note 1578- Calibration Bar and Box
Solutions Note 449- Intensity Scale on Image

Related Products

- Scope-Pro Plug-In
- Advanced Fluorescence Acquisition (AFA[®])
Microscope and Peripheral Automation
Plug-In

- SharpStack Deconvolution Plug-In
- 3D Constructor Three Dimensional Display
and Rendering Plug-In

How to Order

For more information on Image-Pro Plus, and to locate a Media Cybernetics' reseller in your area, visit our website at www.mediacy.com.

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