## Topic

The method for spatial calibration within Openlab changed with the release of Openlab 3.1. The Vernier caliper can no longer be supported therefore the straight line measurement tool now performs the calibration function in addition to measuring straight lines on images. Existing users will find it operates in exactly the same way as the Vernier caliper. This technical note serves as a correction to the Openlab and Openlab Darkroom User guide section on spatial calibration.

Discussion

1. Open a reference image that contains an object of a known size, for example an image of a stage graticule or micrometer. One image will be required for each magnification possible with your microscope.

2. Select the straight line tool from the tool bar at the left of the image document window.

3. Position the cursor over the start point of the known distance, click and drag a straight line to the end of the known distance. The straight line drawn will show the length as a number of pixels in a box near one end of the line. Note the actual physical distance shown by the micrometer.

4. Select **Calibration...** from the Image Menu to display the Spatial Calibration dialog. (You can also double-click on the Calibration tool in the Tools window or use the shortcut key combination.)
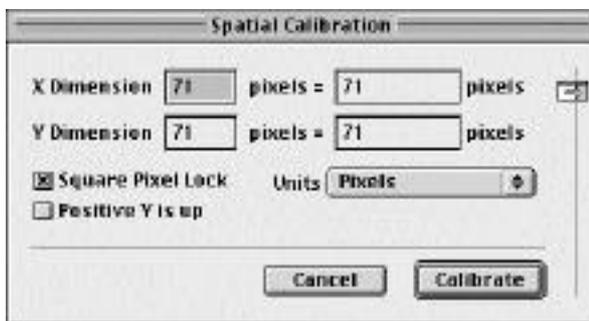
6. Check that the correct unit of measurement is displayed. If necessary, select the unit that is appropriate to your measurement from the **Units** pop-up menu.

7. The straight line tool provides the measured length in the **X Dimension** box. The **Y Dimension** automatically defaults to the same value. (The majority of camera chips have square pixels. However, you can override this to calibrate an image from a CCD camera that produces a rectangular pixel. Uncheck the **Square Pixel Lock** box first.)

8. Enter the real **X Dimension** value corresponding to the sample into the right-hand field. (If the **Square Pixel Lock** box is unchecked, do the same for the **real Y Dimension** value.)

9. Click on **Calibrate** to calibrate the image. This calibration will apply to all layers in the document, and will be retained when you subsequently open the document, as long as you save it as a LIFF file.

10. Once you have calibrated an image, all measurements on rulers and Calibration Bars will be displayed in calibrated units.

Positive Y is up

The Macintosh screen uses the top left-hand corner as the origin of its X and Y axes. This means that the Y co-ordinate increases as the mouse button moves down the screen, which is the opposite of most mathematical conventions. If required, you can invert the Macintosh Y-axis by clicking on the **Positive Y is up** box. The measurements will then follow the normal conventions.

Saving Settings

You can save settings if you want to use them on a batch of different images. Click the blue and white arrow at the right of the calibration dialog to access the Settings Saver area of the dialog. Here you can created new saved settings and name them so that you do not need calibration images again, but remember that calibration will be different for different objectives, Optovar settings, C-mounts and binning settings. Refer to the Openlab and Openlab Darkroom User Guide available to download from the Improvision web site for further details about saving settings.