

Surveyor Autocalibration Guide

Objective Imaging Ltd.

1 Overview

The calibration in Surveyor defines a correlation between the pixel size of the camera to the same-sized area of the stage measured in microns, with the resulting value as the calibration factor in microns-per-pixel.

The formula for this calculation is as follows:

$$\text{Calibration factor} = (\text{camera pixel size/objective magnification}) / \text{c-mount magnification}$$

For example, the Lumenera Lt425 camera has a pixel size of 5.5 μm , and together with a 20X objective and 0.5X c-mount the calculation is as follows:

$$\text{Calibration factor} = (5.5/20) / 0.5 = 0.55 \text{ microns-per-pixel } (\mu\text{m:pixel})$$

Typically, a microscope setup will have multiple objectives, therefore the calibration factor must be defined uniquely for each objective, and the Surveyor software supports this.

Manual calibration of the system is possible within Surveyor, however in most cases the autocalibration process is preferred and is more straightforward for the end-user.

2 Prerequisites

Autocalibration requires the system to be fully setup and ready including the stage configuration, lens definitions and camera image settings. A suitable tissue sample is also necessary to execute the autocalibration procedure.

- Stage configuration requires that the lead screw pitch, microstepping and encoder settings are correctly defined in the flash memory of the Oasis hardware controller. The **system sales distributor** should assist with this during initial installation of the hardware and should be available to assist at other times where necessary.
- The stage should also be initialized prior to autocalibration.
- Hardware performance defects such as stage stalling and movement inaccuracies will interfere with the autocalibration process, and it is recommended to verify stage performance prior to calibration.
- The live camera image should be well-adjusted, with an appropriate exposure, white balance, gain etc., to show good contrast in the image, which is necessary for the autocalibration to succeed.
- The autocalibration procedure favors lots of detail and variation in the tissue sample, rather than repetition in the image. Geometric shapes or patterns, such as straight lines and artificial structures, can confuse the algorithm.
- Camera should be reasonably aligned perpendicular to the stage at the outset.

3 Autocalibration Procedure

The autocalibration process is typically an iterative one, in which several attempts are made before the final satisfactory result. Usually the camera requires minor rotation within the c-mount, and the process allows the user to observe the rotation factor either increasing or decreasing for each iteration.

1. Select the intended objective in the microscope.
2. Ensure the **same objective** is selected in Surveyor Joytools window:

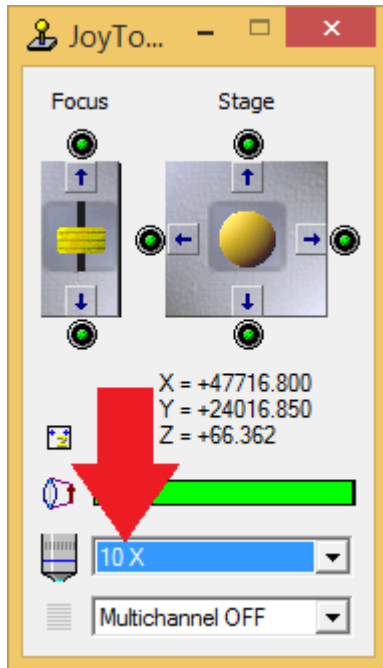


Figure 1 - Surveyor Joytools window, showing objective lens option.

3. Select the option *Calibrate Lens* in the *Control* menu of Surveyor:

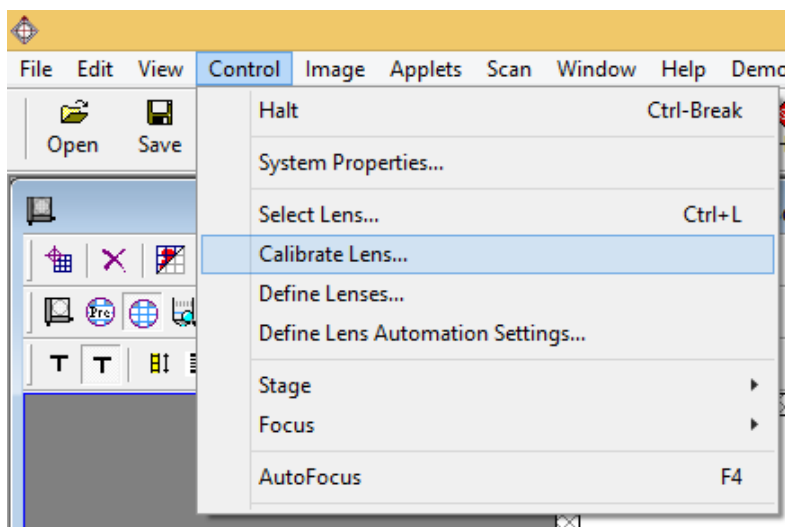


Figure 2 - Calibrate Lens menu option.

- Focus the live camera image on a suitable area of the tissue sample, along the lines described in the preceding section *Prerequisites* (contrast, detail and variation in the image). Ensure some detail is within the blue box:

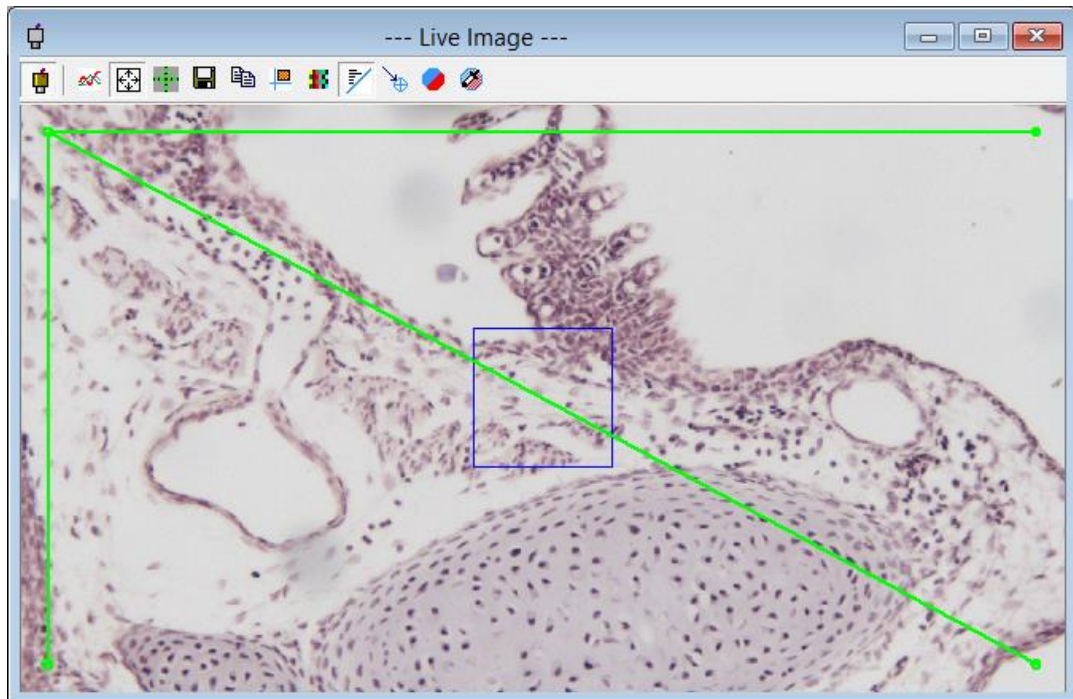


Figure 3 - Live image showing detail in the blue box.

- Click the *Auto Calibrate* button:

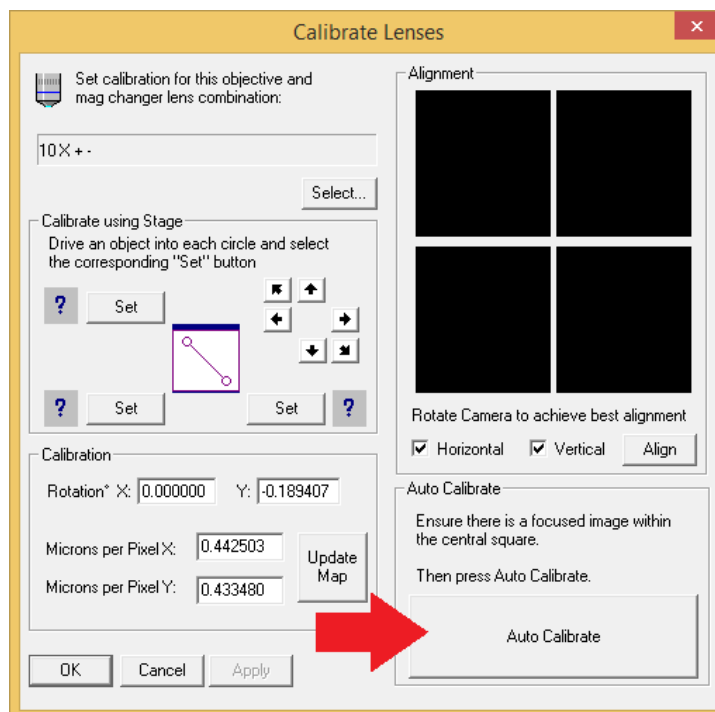


Figure 4 - Calibrate Lenses window, showing Auto Calibrate button.

- The process will execute and the stage will make some small movements.

- If the system requests to flip the live image either horizontally or vertically, click Yes:

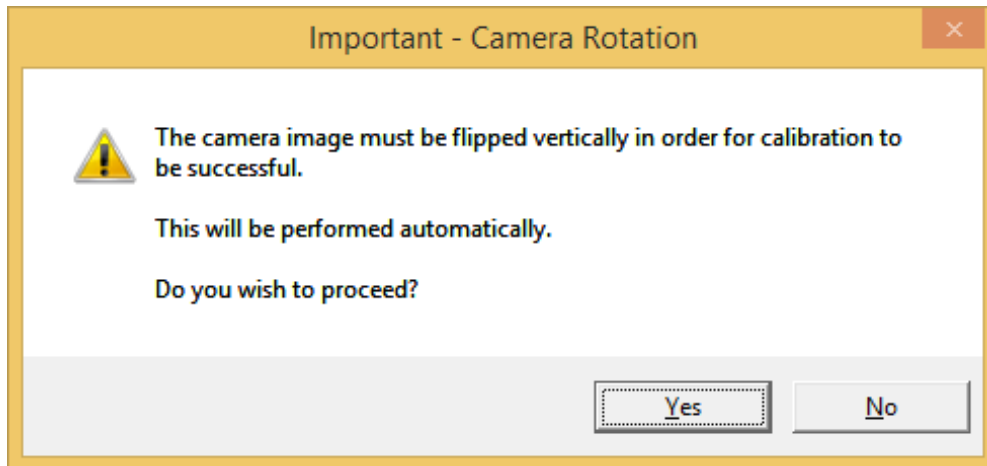


Figure 5 - Image flip request during autocalibration.

- If the system fails to calibrate due to excessive rotation, it is necessary to physically rotate the camera to be more perpendicular to the stage and restart the procedure:

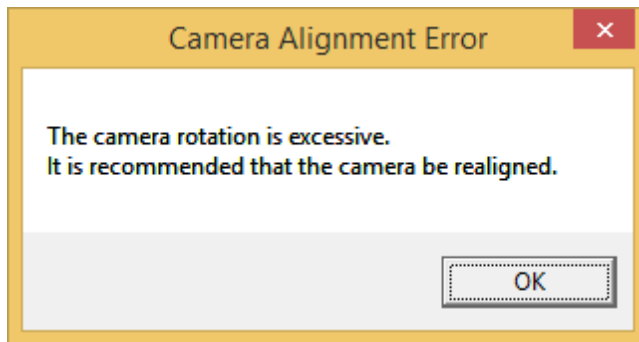


Figure 6 - Autocalibration failure, camera requires physical realignment.

- For each autocalibration attempt, observe the output of the rotation value. This can be positive or negative depending on the angle of rotation. The intention is to increment or decrement this value closer to 0.0 for each autocalibration attempt. To do this, it is necessary to physically rotate the camera by a very, very minor amount and repeat.

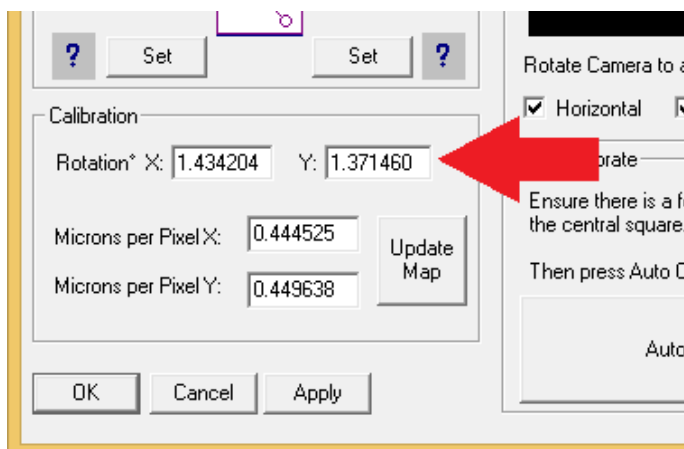


Figure 7 - Rotation measurement in degrees.

10. Initially it may be unknown in which direction to rotate the camera. Pay attention to the current rotation values, then rotate the camera and repeat the autocalibration. If the rotation has not moved closer to 0.0 then you need to rotate in the opposite direction.
11. The result is ideally between 0.0 and 0.5 degrees of rotation for optimal performance, and it is recommended to repeat the autocalibration until the values are somewhere closer to 0.0:

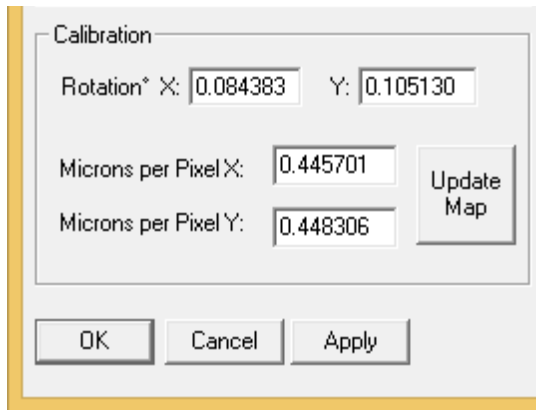


Figure 8 - Accepted rotation values, sufficiently close to target of 0.0.

12. Finally, click **Apply** and **OK** to commit these values.
13. Repeat procedure from the beginning for the next objective lens.