



MICROSCOOP® / MINT Calibration Procedure:

Autoscoop™ Calibration 2.2.0.4 with SynCal Reagent

Overview

This document describes the recommended procedure to generate calibration files with Autoscoop™ Calibration 2.2.0.4 software and calibration samples prepared according to *Instructions for use of SynCal*. It expands on the workflow described in section 9.3 of the *User Manual* for Autoscoop™ software with best practices to achieve successful calibrations.

You must be familiar with the general usage and safety procedures of Microscoop Mint before following these instructions. Refer to the User Manual for details and contact Syncell if you are uncertain how to proceed.

Workflow

1. Prepare Calibration Sample

Prepare a sample as instructed in *Instructions for use of SynCal*. When finished, ensure that Parafilm® has not wrapped under the edges of the well slide, as this will prevent the sample from sitting flat on the microscope stage.

Holding lab tissue down on a flat surface, wipe the bottom of the well slide across the tissue several times in the same direction, using a new area of the tissue each time. This helps to remove any debris or spilled SynCal reagent from the bottom of the coverglass surface. Debris or liquid on the bottom surface may prevent successful calibration.

2. Place Sample on Microscope Stage

Open Autoscoop™ Calibration and ensure that the laser switch is set to Off (Fig. 1). Set the microscope to coarse focus by pressing one of the Z speed buttons on either the joystick or microscope focus knobs repeatedly until 3 upward arrows (^) are displayed to the right of Z on joystick screen (Fig. 2). If it is not already at a low Z position, focus the microscope down until the Z value displayed in the software is lower than 1000 μm (Fig. 3).

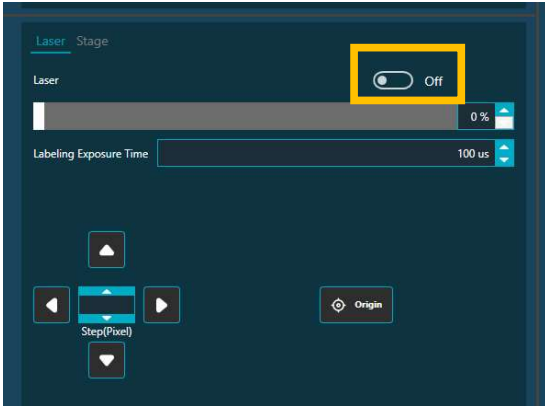


Fig. 1 - Laser Off



Fig. 2 – Focus Speed Buttons, Coarse Focus Enabled



Fig. 3 - Focus Lowered

Carefully place the sample on the stage as shown (Fig. 4), using the joystick to move the stage so the tip of the objective is centered in the well. When viewed from the sides, it should appear flat, with the surface of the lid parallel to the surface of the stage (Fig. 5).

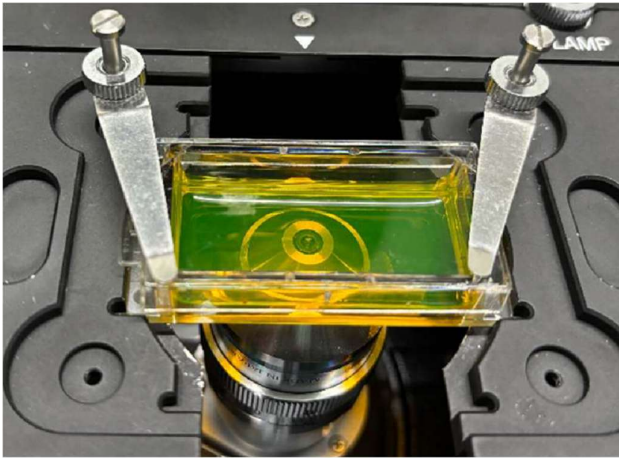


Fig. 4 - Sample Placement

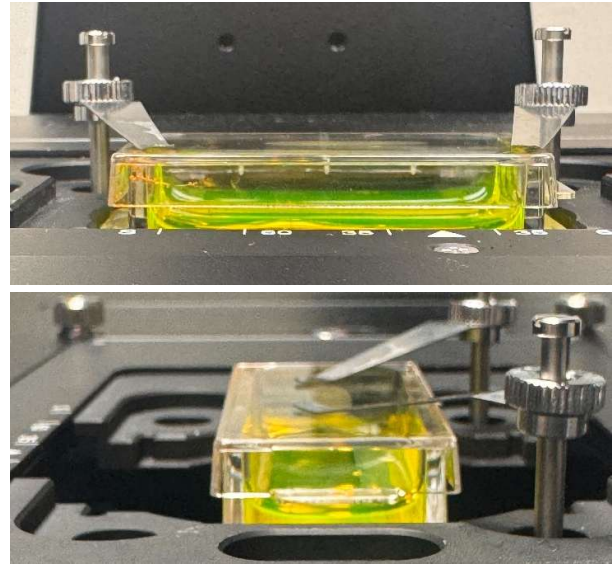


Fig. 5 - Sample Flat to Stage

Place the light shielding cover over the sample and return the transmitted light pillar to its fully vertical position (Fig. 6).

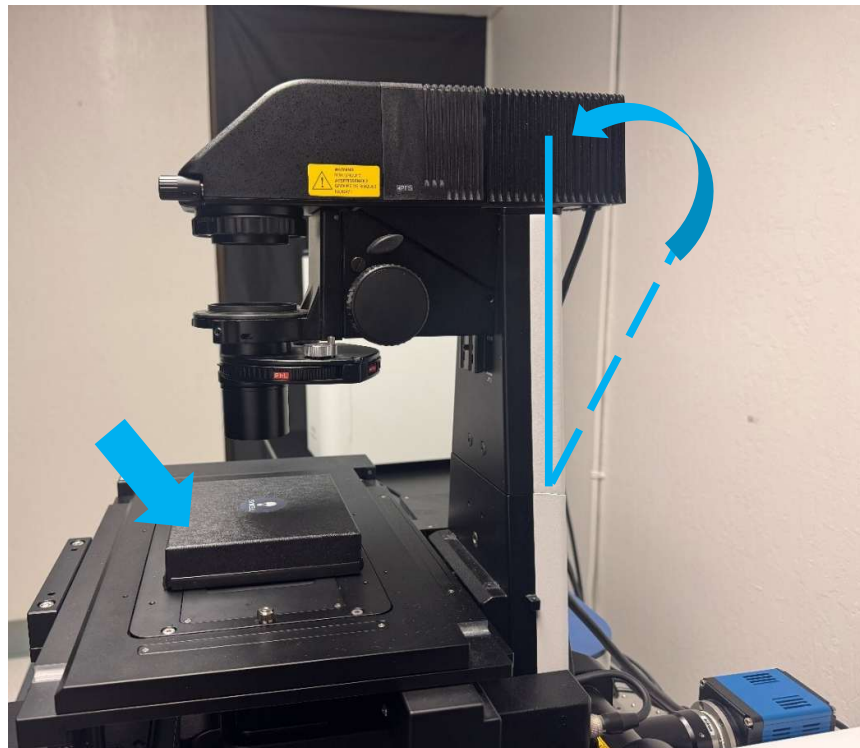


Fig. 6 - Sample Cover and Pillar in Place

3. Adjust Image and Laser Settings

Select the objective and resolution you wish to calibrate in the Image settings area at the upper left of the software. Set Exposure Time to 100 ms, Contrast to Manual, and use the full contrast range by ensuring 0 is shown to the left of the sliders and 65535 is shown to the right. Click the Live button to the right of the Image settings area (Fig. 7).

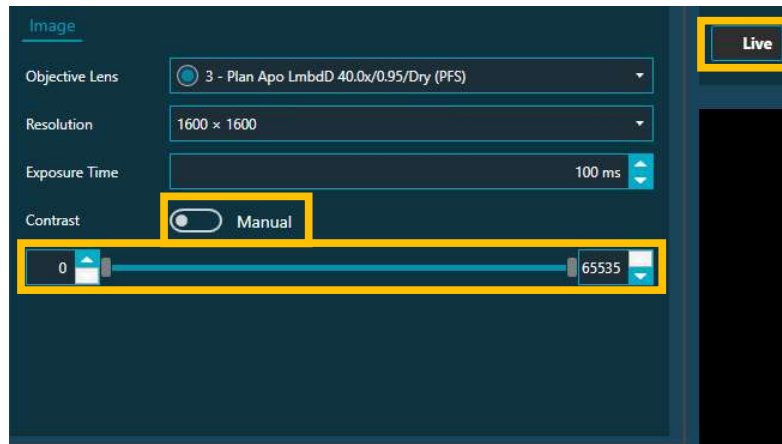


Fig. 7 – Image Settings and Live Button

In the Laser settings area at the lower left of the software (Fig. 8), switch the laser to On and adjust the power to 10%, leaving Labeling Exposure time set to the default 100 us. Click the Origin button to ensure that the laser will appear near the center of the live image shown at the center of the software. With the current settings and focus, the live image should remain black. If not, double-check your settings and that the light shielding cover is over the sample.

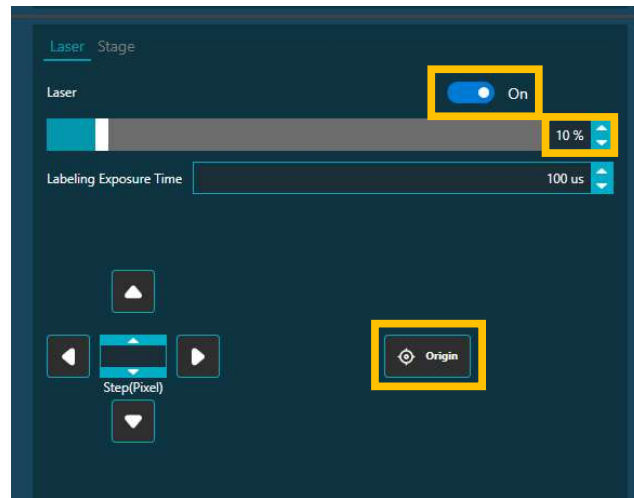


Fig. 8 – Laser Settings

4. Focus and Finalize Settings

Slowly focus up using the joystick or microscope focus knobs. You can be sure that you are moving up if the Z value shown in the software (Fig. 2) is increasing. Watch the live image carefully for a small gray or white spot to appear near the middle (Fig. 9). As soon as it appears, stop adjusting focus. Recording this Z value may help with future calibrations – on most systems it will be between 1700 – 2200 μm .

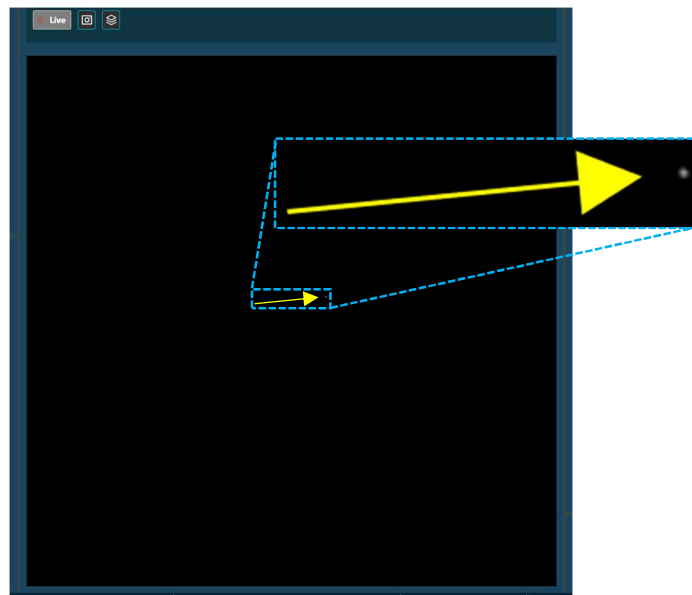


Fig. 9 – Laser Spot after Focusing

Hover the mouse pointer over the spot, then zoom in by pushing the scroll wheel forward until you can see individual square pixels. Adjust the laser power and / or camera exposure time (Image settings, Fig. 7) until 1-2 pixels in the laser spot appear pure white, and all others are gray (Fig. 10). If the intensity of the laser spot is unstable / flickers, check that Contrast is set to Manual (Fig. 7) and increase laser power until it appears stable. Then adjust camera exposure time to achieve 1-2 white pixels in the laser spot.

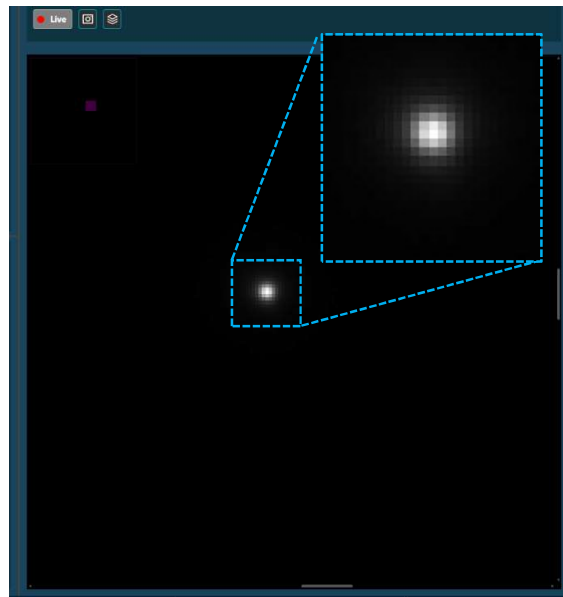


Fig. 10 – Laser Spot after Zooming and Final Adjustments

5. Check Sample Flatness

Zoom out completely by rolling the mouse wheel back while hovering over the live image. Enter a value of 1000 in the Step (Pixel) box, then click the arrows surrounding the box to steer the laser spot around the image (Fig. 11). Smaller or larger Step sizes may be used for finer or coarser movement of the spot. The laser spot may dim slightly as it approaches the edges, but it should not be difficult to see or disappear completely unless you step it past an edge. Check that the spot remains visible as you drive it near all four corners of the image. If it does not, see the troubleshooting section below.

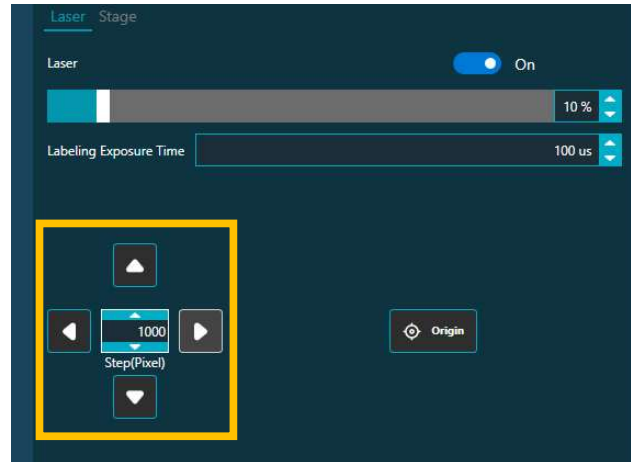


Fig. 11 – Laser Spot Movement Controls

6. Calibrate

1. Click the Calibration button at the lower right of the window (Fig. 12). The software will take about one minute to adjust fine focus of the laser and enter drift free mode (PFS). If an error occurs, see the Troubleshooting section below.

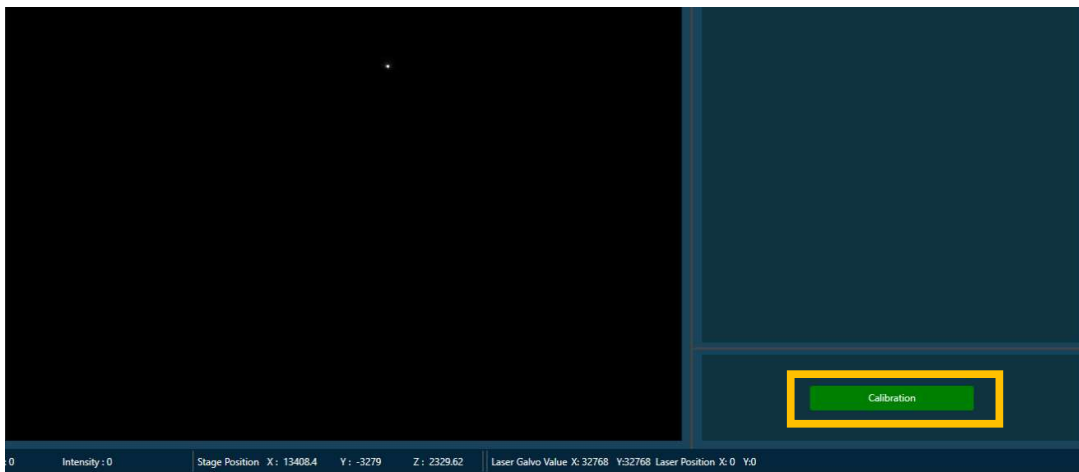


Fig. 12 – Calibration Button

2. A window will open asking “Recalibrate the search range?” Click OK. Recalibrating the search range will cause the laser spot to move from the origin to targets in the upper right and lower left of the image. This

matches the upcoming calibration to the camera's field of view. If this step fails to complete within 1 min, see the Troubleshooting section below.

3. On the right side, an indicator will show that you are at the Recursive Calibrate step, and the image window will be black with a whirling busy icon (Fig. 13). This step can take a few minutes to complete. Be careful not to touch the joystick or components on the optical table until the process is complete.

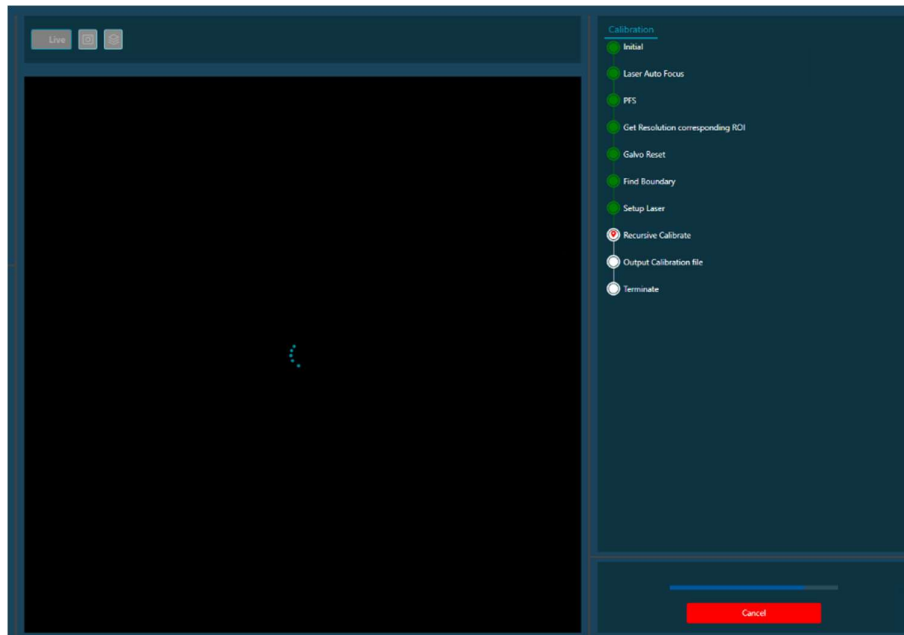


Fig. 13 – Calibration in Progress

4. When the calibration is complete, the system will generate the calibration file and open the folder containing the file. Bubbles for the Recursive Calibrate and subsequent steps in the workflow at the right of the screen will be filled green. If the calibration fails, the Recursive Calibrate bubble will fill red (Fig. 14), and you may see an error message. Use the Troubleshooting section below to find possible issues, then try again. If calibration continues to fail, please contact Syncell for support.

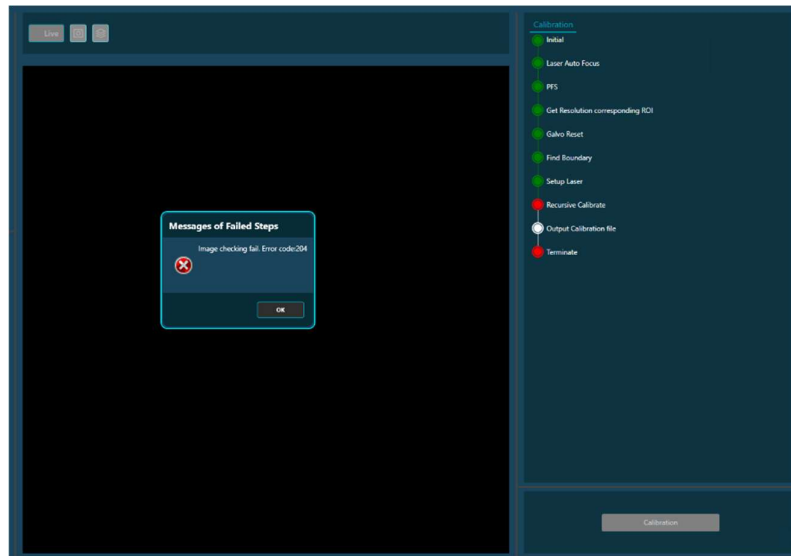


Fig. 14 – Calibration Error

7. Load Calibration Files in Autoscoop™

When you have finished generating calibration files, close Autoscoop™ Calibration then open Autoscoop™. Click the Settings menu at the top of the screen and choose System Configuration. In the System Configuration window (Fig. 15), select the objective for which you would like to apply a new calibration file. Click the folder icon to the right of Calibration File (High Resolution) to choose a 1600 x 1600 calibration file, or the icon to the right of Calibration File (Low Resolution) for an 800 x 800 calibration file. Browse to the path where your new calibration was saved (usually C:\Program Files\SynCell\Autoscoop Calibration\Calibration) and choose the file that matches the objective and resolution chosen. Files are named by date and time, and you can sort files by date modified to find your most recent calibration. After selecting the file, click Open, and remember to click Save in the System Configuration window before closing it.

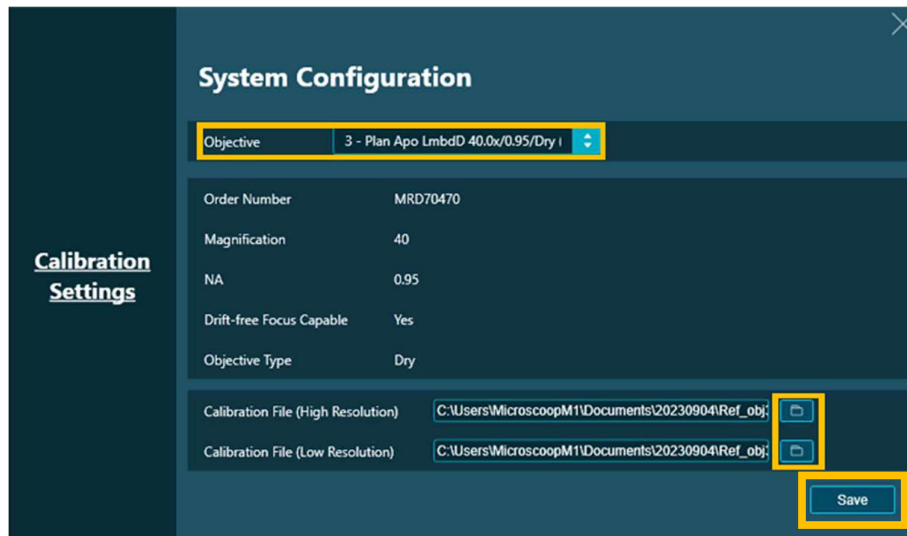


Fig. 15 – Loading Calibration Files

Troubleshooting

Laser spot disappears in some areas of the image when checking flatness

- **If the spot disappears as it approaches one side or corner, and does not reappear as it continues that direction**, the sample may not be flat. Turn off the laser and check again that the sample is seated flat on the stage. Ensure the sample is clean and no labels, Parafilm®, etc. are wrapped around the edges that contact the stage insert. Check that the stage insert is clean and undamaged. If you see small bends in the thin metal area where the sample is placed, contact your Syncell support team.

After correcting any issues you find, lower the focus by ~100 μm and return the sample to the stage, placing the light shielding cover over it. Sometimes, simply removing and returning the sample can cause it to sit flatter if it had not fully seated previously. Return the transmitted light pillar to its vertical position, then start at Workflow step 3 to try again.

- **If the spot disappears, then reappears while continuing in the same direction**, there may be debris on the chamber slide blocking the laser. Exit Autoscoop Calibration, and open Autoscoop. Turn on the FITC LED at 2% power, set the camera to a 10 ms exposure, click Live, and choose Auto contrast. Move the stage slightly with the joystick or software stage controls. If you notice objects moving as the stage moves, keep moving the stage until you find an area without this issue. Be careful not to travel so far that the objective hits the stage insert.

If searching for a short time does not yield a clean area, turn off the LED, remove the sample, then clean it, and try again. Note that you may need to refocus slightly after removing and replacing the sample and light shielding cover. Once you have found a clean area, return to Autoscoop Calibration, ensure the transmitted light pillar is in its vertical position, then follow the instructions above to set focus, laser power, and camera exposure, then attempt calibration again.

Errors during Laser Autofocus

- **“Image checking fail. Error code: 203”**: The laser may be switched off or at very low power, or a safety interlock closed the shutter and blocked the laser. Check that the laser is on, set to approximately 10% power, and the transmitted light pillar is in its fully vertical position. Then follow Workflow steps 3 and 4 to optimize focus and settings.

Calibration stalls at Find Boundary / Recalibrate Search Range

- **If the spot disappears as it approaches a corner target**, the spot is too dim, or the sample may be tilted. Cancel calibration by clicking the Cancel button at the lower right of the software if the Find Boundary step does not complete within one minute. Readjust laser power and camera exposure according to workflow step 4. Note that you may need to click the Live button and turn on the laser again. Check sample flatness according to step 5 in the workflow, correcting flatness issues if necessary. Once the sample seems flat, attempt Calibration again.

- **If the spot is very bright white and moves in and out of a target repeatedly**, the laser power is too high, or camera exposure is too long. If this occurs, cancel calibration by clicking the Cancel button at the lower right of the software if the Find Boundary step does not complete within one minute. Readjust laser power and camera exposure according to workflow step 4. Note that you may need to click the Live button and turn on the laser again. When finished, attempt Calibration again.