

MICROSCOOP[®] / MINT

Calibration Quick Reference Guide

You must be familiar with the general usage and safety procedures of Microscope Mint before following these instructions. Refer to the full Calibration Procedure document for details or if you encounter difficulty with any of the steps below.

1. Prepare Calibration Sample

Refer to *Instructions for use of SynCal*. Ensure that Parafilm[®] has not wrapped under the edges of the well slide and the bottom coverglass is clean.

2. Open Autoscoop[™] Calibration and Place Sample on Stage

- Ensure that the laser is switched off in the software
- Using coarse focus, lower the objective until $Z < 1000 \mu\text{m}$
- Tilt the transmitted light pillar back, place the sample on the stage, and ensure that it is sitting flat.
- Move the stage to center the sample over the objective.
- Place the light shielding cover over the sample and return the transmitted light pillar to its fully vertical position.

3. Adjust Image and Laser Settings

Select the objective and resolution you wish to calibrate, click Live, and switch on the laser. Click the Origin button, then adjust Image and Laser settings based on the table below. Use the starting points or optimized settings you determined from previous calibrations on your system.

	Starting Point	User Optimized			
Objective	20x / 40x	20x	20x	40x	40x
Resolution	Any	800x800	1600x1600	800x800	1600x1600
Image Exposure	100 ms				
Contrast	Manual, 0-65535				
Laser Power	10%				
Labeling Exposure	100 μs				

4. Focus and Finalize Settings

Slowly focus up until a small gray or white spot appears near the middle of the live image. As soon as it appears, stop adjusting focus. Record your Z value from the joystick display here for future reference:

Approximate Focus: Z = _____ μm

Zoom in, hovering over the laser spot and pushing the scroll wheel forward until you can see individual square pixels. Adjust the laser power and / or camera exposure time until 1-2 pixels in the laser spot appear pure white, and all others are gray. Record your settings in the table in step 3.

5. Check Sample Flatness

Zoom out completely, enter a value of 1000 in the Laser settings Step (Pixel) box, then click the arrows surrounding the box to steer the laser spot around the image. Check that the spot remains visible as you drive it near all four corners of the image. If it does not, refer to the full Calibration Procedure document for guidance.

6. Calibrate

1. Click the Calibration button and wait for the Laser Autofocus and PFS adjustment to complete (~ 1 min).
2. When prompted "Recalibrate the search range?" Click OK and wait ~30s for the laser spot to be moved to targets in opposite corners of the image.
3. During the Recursive Calibrate step, the image window will be black with a whirling busy icon. 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.
4. When calibration is complete, the calibration file will be generated and its folder will open.

7. Load Calibration File in Autoscoop™

Close Autoscoop™ Calibration then open Autoscoop™. Click the Settings menu and choose System Configuration. In the System Configuration window, 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) or Calibration File (Low Resolution) to open a 1600 x 1600 or 800 x 800 calibration file, respectively. Browse to C:\Program Files\Syncell\Autoscoop Calibration\Calibration, choose your new calibration file, then click Open. Click Save in the System Configuration window before closing it.