# Standard Operating Procedure: Optical Microscopy

ZEISS Axio Imager

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## 1. Lab Safety Information

- ✓ All GMU NFF users are required to complete the Lab Safety Orientation (LSO) before performing any lab work.
- ✓ Proper Personal Protective Equipment (PPE) should always be worn before entering the clean room: safety glasses, hair net, shoe covers, gloves, and lab coat. Additional PPE is available for specialized chemical work as needed.
- ✓ No shorts, sandals, tank tops, or spaghetti-strap shirts are allowed in the clean room!
- ✓ Material Safety Data Sheets (MSDS) are available in a binder in the gowning room.
- ✓ Read the SDS for any chemicals you plan to use before proceeding with your work. Any materials used in the clean room for the first time should be brought in after the approval of NFF staff.
- ✓ A safety buddy is required in the clean room with you when doing chemical work. The safety buddy should be fully trained and qualified to work with the chemical you are using. They must remain in the clean room the entire time you are handling the chemical. Feel free to ask NFF staff if no one qualified is available!
- ✓ Prohibited clean room items: cardboard, pencils, cloth, hats/coats, and contact lenses.
- ✓ Accepted clean room items: plastic, pens, synthetic fabrics, clean room paper.

## 2. Optical Microscopy Safety Information

- ✓ Any irregular system behavior should be reported to NFF staff promptly. Never attempt to fix the system yourself! We are here to help.
- ✓ Fluids should not be spilled onto the microscope.
- ✓ The lamp should always be first-on and last-off.
- ✓ Make sure that you do not move the stage too far upwards while focusing. In such cases, the sample will press against the objective tip, and it may cause an expensive breakage of glass.
- ✓ When the microscope is not being used, make sure to cover it with the dust cover.
- ✓ Failure to use the system safely and properly may result in your access to the system being reviewed and/or revoked.
- ✓ Fill out the logbook before you begin.

## 3. Principles of Optical Microscopy

Optical microscopes are instruments that magnify specimens too small to see with the naked eye, ranging from simple single-lens tools to complex systems. The key component is the microscope objective, which forms the primary image. Modern microscopes use a dual-stage magnification system, combining an objective lens with an eyepiece. Magnification is achieved by multiplying their values, while resolution and contrast depend on various optical strategies and specimen preparation.

The ZEISS Axio Imager in our characterization laboratory is used for the inspection and analysis of structures. It features a motorized focus drive, nosepiece, and reflector turret, and is capable of performing motorized Z-stack, brightfield, darkfield, and circular DIC imaging. The microscope offers magnifications of 25X, 50X, 100X, 200X, 500X, and 1000X. The components of the ZEISS Axio Imager microscope are shown in Figure 1.

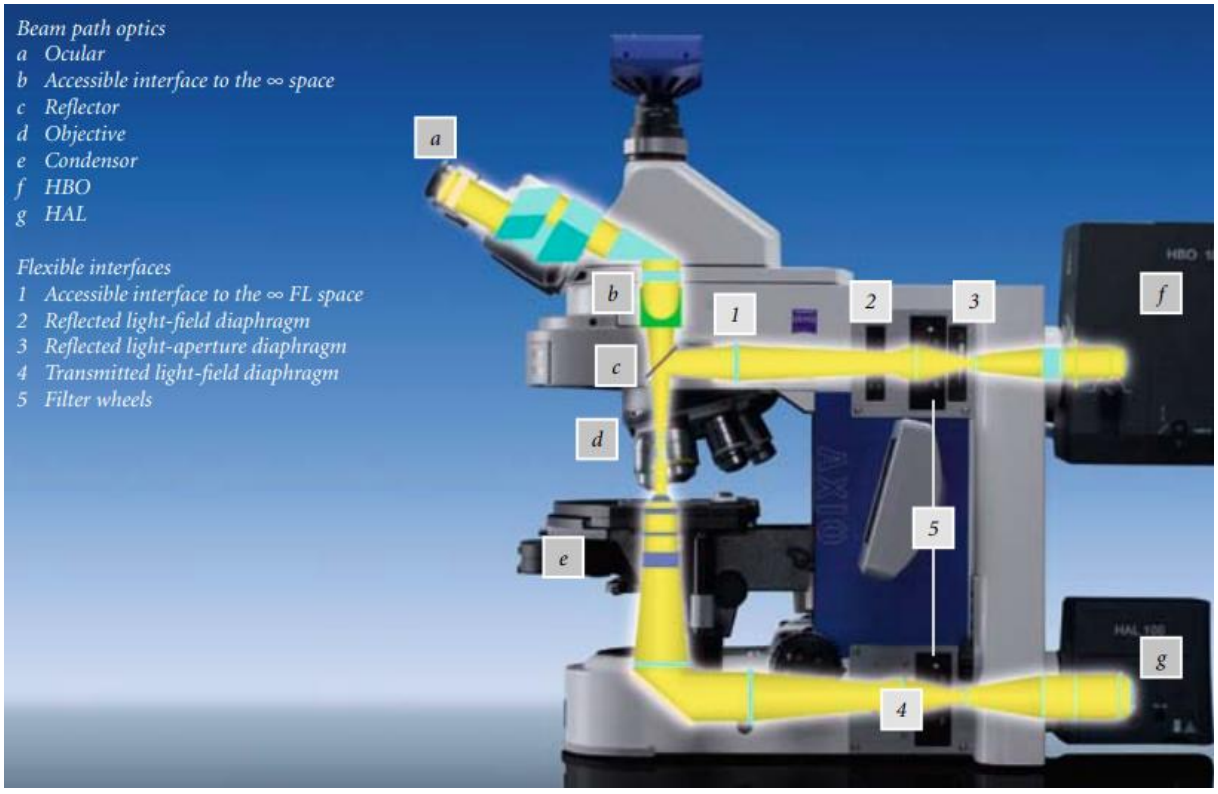



Figure 1. Optical Microscopy Setup and Components

# 4. Operation Manual

## 4.1. Turning on the Microscope and Starting with the Software

1. Remove the dust cover from the microscope, as shown in Figure 2.


Figure 2. Zeiss Axio Imager Optical Microscopy Setup with Dust Cover

2. Turn on the microscope using the button shown in Figure 3.

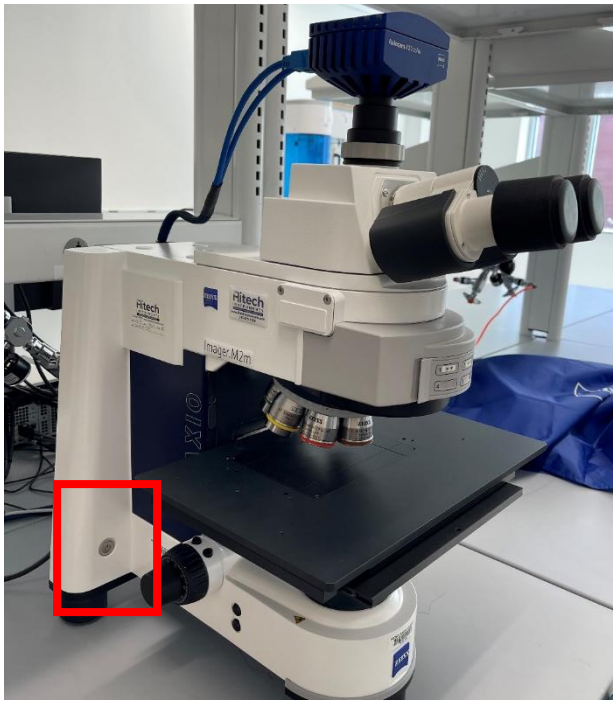



Figure 3. On/Off Button of the Microscope

3. Ensure the microscope is set to the lowest magnification (2.5X). If adjustment is needed, change the magnification from the monitor to the right of the microscope by selecting "Microscope" (Figure 4, left) and then the turret for 2.5X (Figure 4, right).

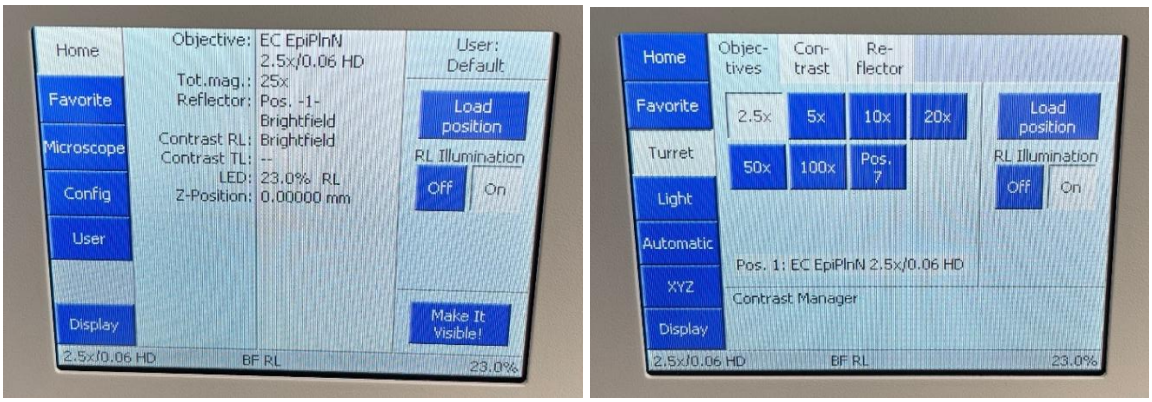



Figure 4. Magnification Adjustment on the Microscope Monitor

4. Open the Zen Core software. Select "Zen Core" as shown in Figure 5.

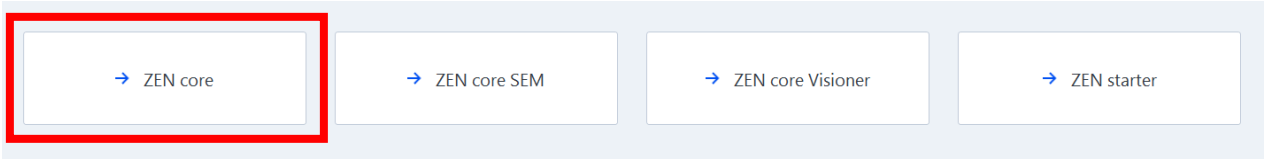



Figure 5. Zen Core Software Selection

5. Select "Free Mode" as in Figure 6.

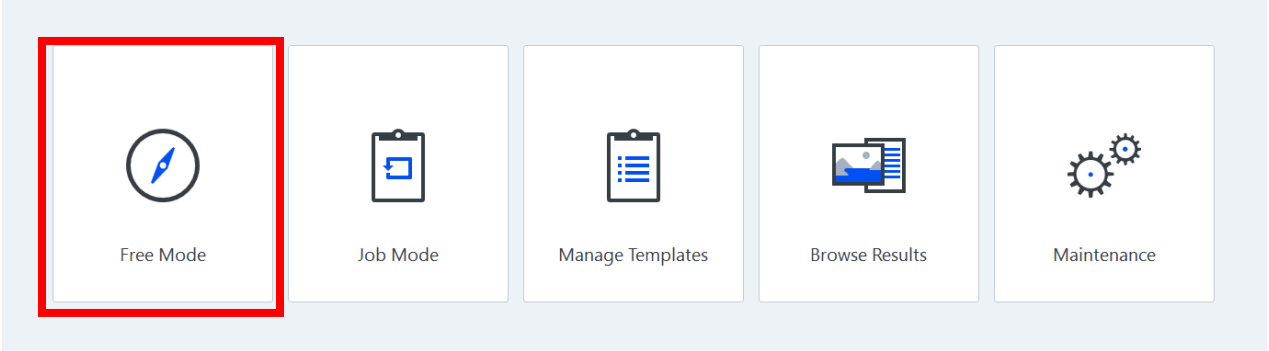



Figure 6. Software Mode Selection

6. Select "Calibrate Now" and wait until the calibration is complete and the software displays the imaging page, as shown in Figure 8.

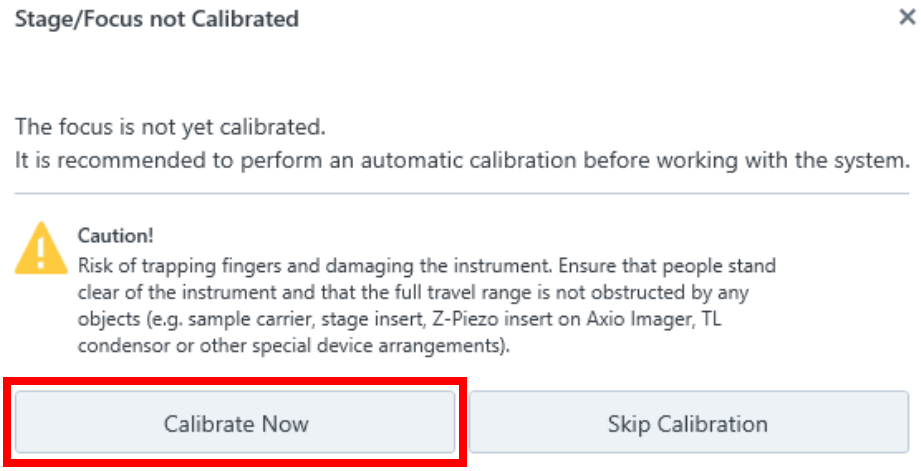



Figure 7. Calibration Selection

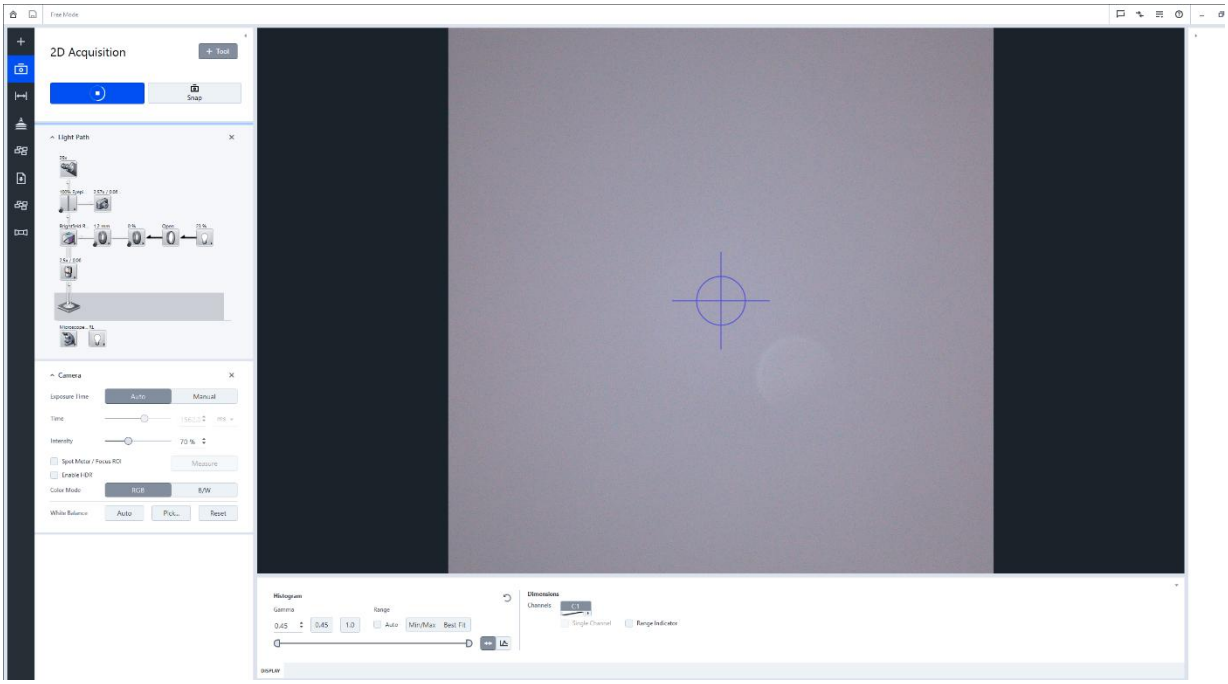



Figure 8. Software Imaging Page

## 4.2. Placing the Sample

7. Use the stage controllers, as shown in Figure 9 (#1), to move the stage closer to you by adjusting it in the X and Y directions.

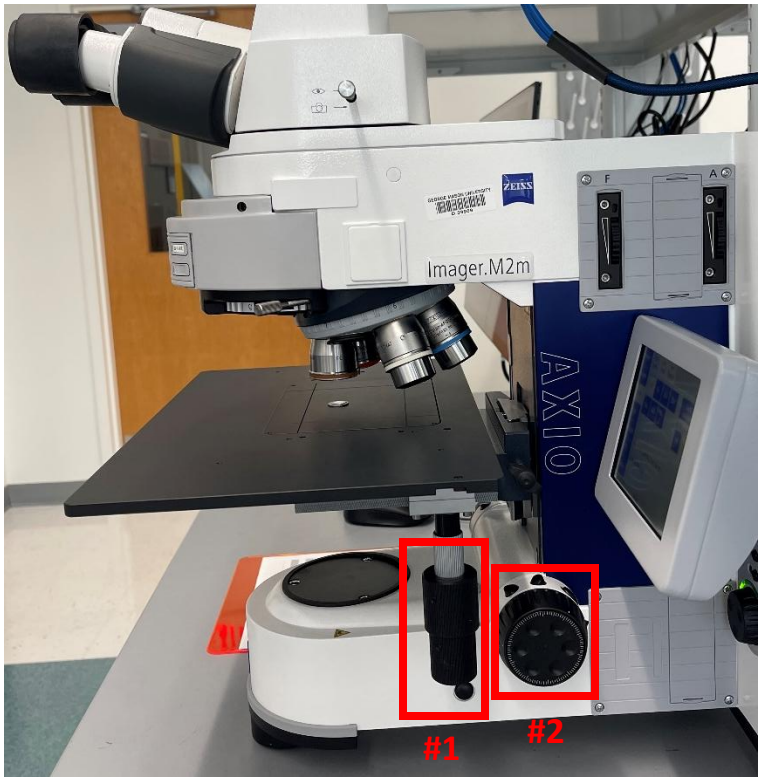



Figure 9. Microscope Showing Stage Controllers (#1) and Focus Adjustments (#2)

8. Carefully place your sample on the stage, as shown in Figure 10. Then, using the stage controllers, position the stage so that the sample is under the objective lens. Make sure there is enough space between the stage and the objective lenses.

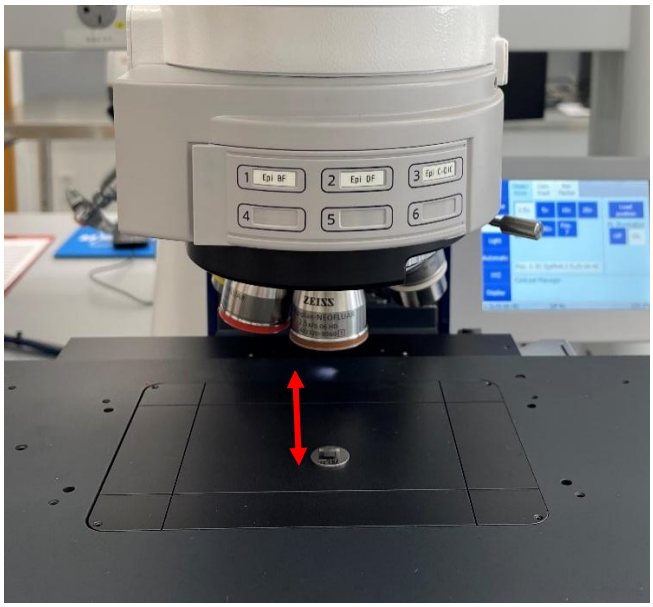
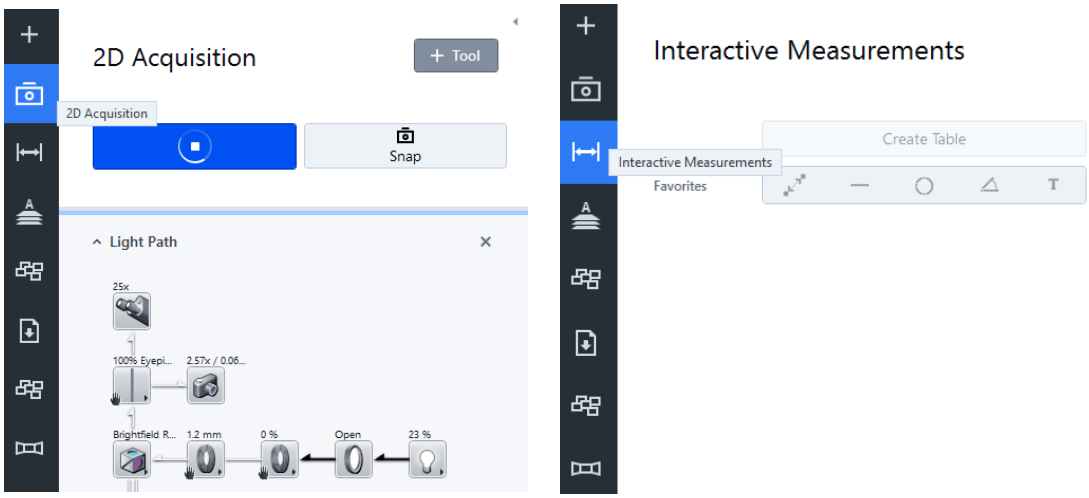


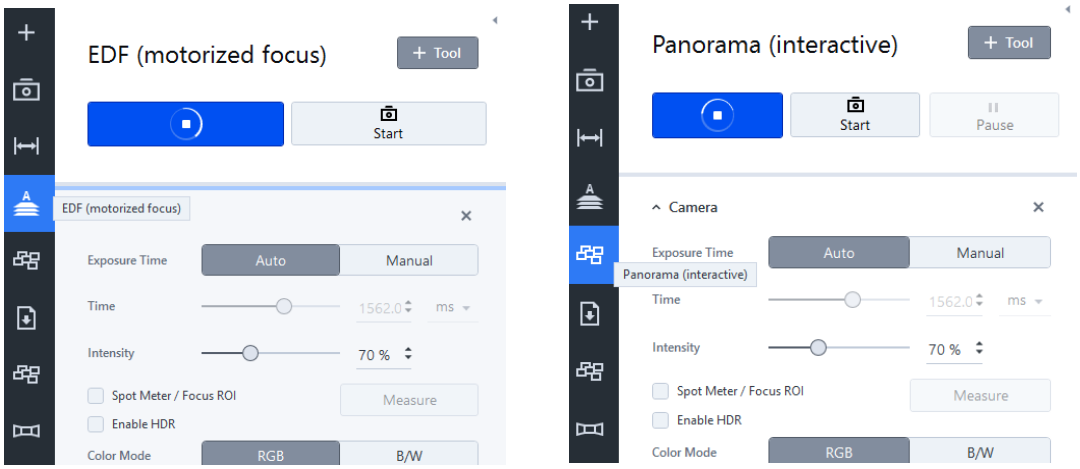

Figure 10. Sample and Sample Stage

### 4.3. Selecting the Imaging Mode and Adjusting the Parameters

9. The Zeiss Axio Imager Microscope offers multiple imaging modes, including 2D Acquisition, Interactive Measurement, EDF (Motorized Focus), and Panorama, as shown in Figure 11. Use the software to select the imaging mode that best suits your needs.




(a) 2D Acquisition (b) Interactive Measurements




(b) EDF (Motorized Focus) (d) Panorama (Interactive)

Figure 11. Software Imaging Modes: (a) 2D Acquisition, (b) Interactive Measurements, (c) EDF-Motorized Focus, and (d) Panorama-Interactive

10. Adjust the parameters for the selected imaging mode.

### 4.4. Image Acquisition

11. Image acquisition will be demonstrated using 2D Acquisition as an example. After selecting 2D Acquisition, focus the sample at 2.5X magnification. Use the coarse and fine focus adjustments, as shown in Figure 9 (#2), until the sample is focused as illustrated in Figure 12.

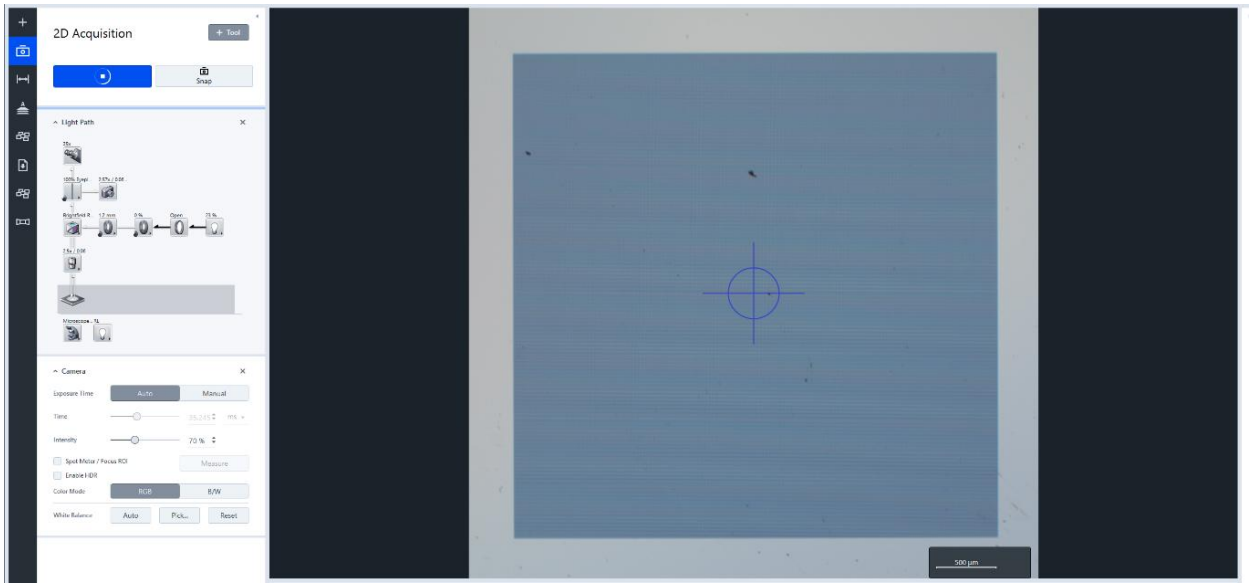



Figure 12. 2.5X Magnification Imaging Page Focused on the Sample

12. Magnification can be changed using the software, as shown in Figure 13 (#1), or as explained in Step 3. After each adjustment, make sure that the sample is properly focused and there is sufficient space between the stage and the objective lenses. As an example, Figure 14 shows images at two different magnifications: 2.5X on the left and 100X on the right.

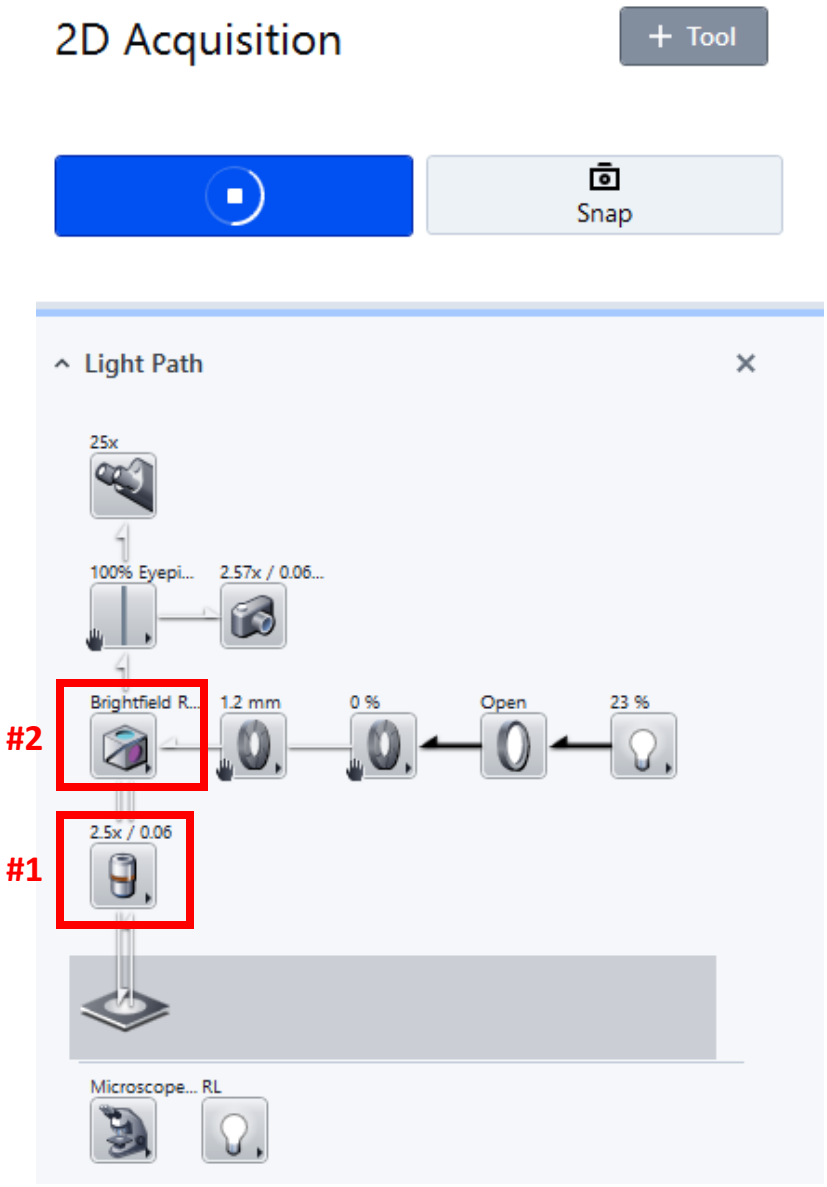



Figure 13. 2D Acquisition Parameter Selection: Magnification (#1) and Contrast Enhancement (#2)

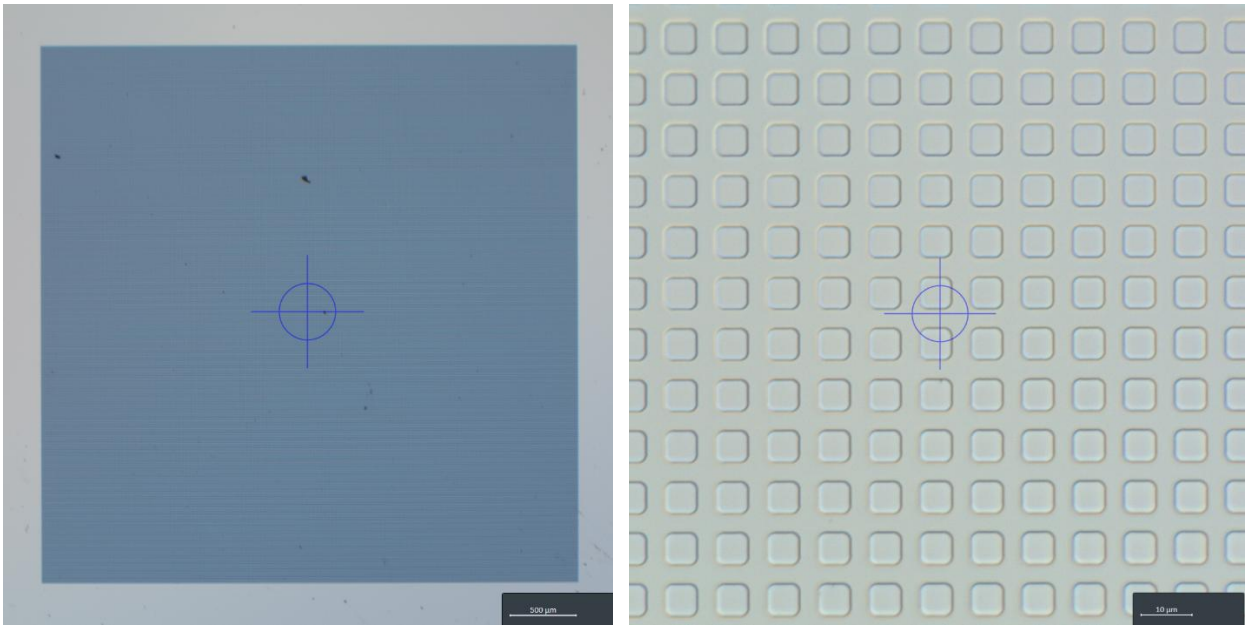


Figure 14. Sample Image at Two Different Magnifications: 2.5X (left) and 100X (right)

13. Additional adjustments, such as contrast and brightness, can be made based on your needs. Contrast can be adjusted using the aperture diaphragm shown in Figure 1. For further adjustments, you can enhance imaging by selecting bright-field or dark-field options from the software, as shown in Figure 13 (#2). The difference between bright-field and dark-field imaging is illustrated in Figure 15.

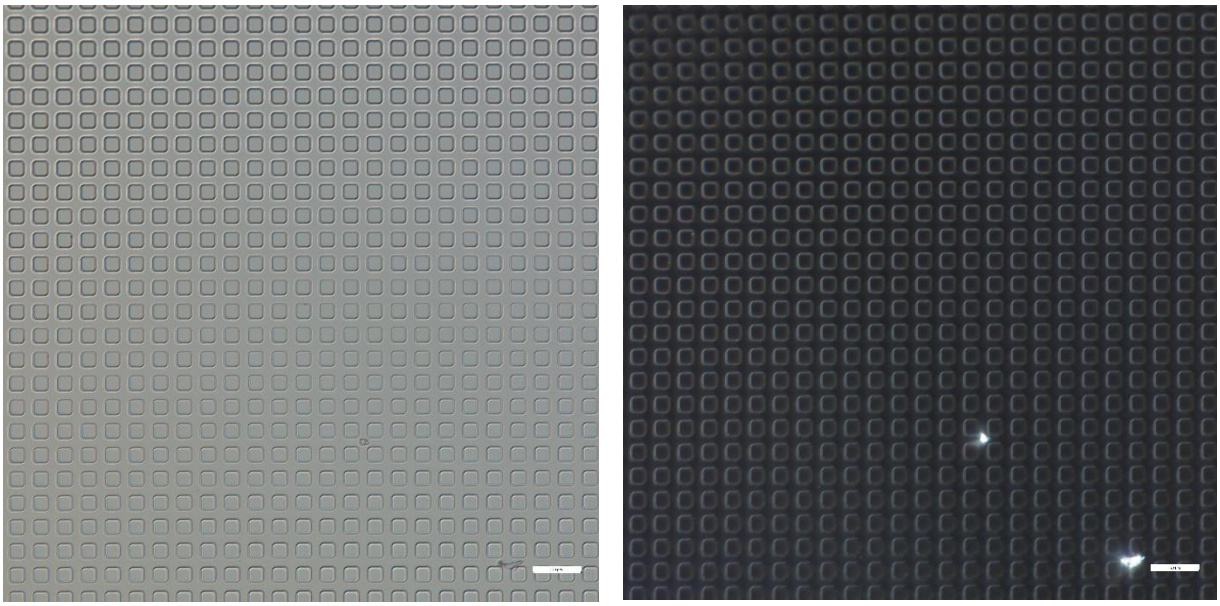


Figure 15. Sample Image with Bright Field (left) and Dark Field (right) Imaging Modes

14. Once all adjustments are complete, capture the image by selecting "Snap" in the software, as shown in Figure 16. After the image is captured, it will appear on the right side of the software interface, as shown in Figure 17, where you can save it by clicking on the image.

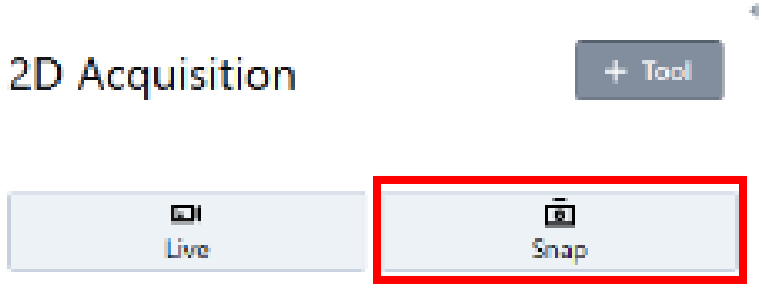



Figure 16. Capturing an Image by Selecting "Snap"

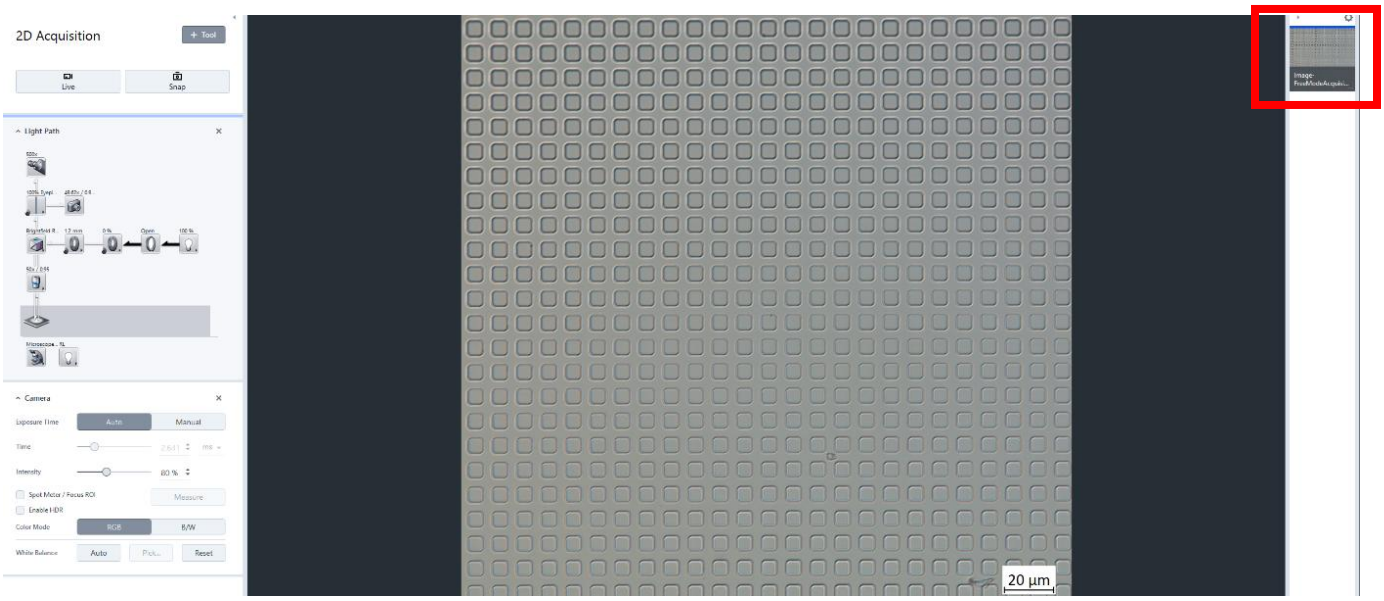



Figure 17. Captured Image