Standard Operating Procedure: Laser Microscopy

Olympus LEXT OLS4000

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Table of Contents

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. Laser 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.
- ✓ Do not insert or remove a sample while the laser is on.
- ✓ The system involves a Class II laser. Do not look directly at the laser light.
- ✓ Maintenance should only be performed by an authorized service representative.
- ✓ Liquid samples are not suitable for imaging.
- ✓ Toxic and corrosive samples are prohibited.
- ✓ The maximum sample thickness should be 10 cm and samples must not weigh more than 1 kg.
- ✓ Place the small samples on a glass slide to prevent cross-contamination.
- ✓ The motorized stage and height adjustment are pinch hazards. Always use caution while the stage is moving.
- ✓ Never move the stage or the objective lenses by hand. The system has software features that will move the stage/objective lenses.
- ✓ 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 Laser Microscopy

The laser microscope functions as a non-contact optical profilometer. Common applications include measuring step height and surface roughness. The laser confocal microscope captures high-resolution 3D images by taking multiple images of the sample at different heights. These images are then combined with both laser and color data to create a measurable 3D projection. It can assess sep height, surface/line roughness, and area/volume measurement.

The Olympus LEXT OLS4000 can capture high-resolution 3D images. Its objective lens magnification ranges from 5X to 100X; laser 3D image magnifications from 20X to 100X and digital magnification: 1X to 8X. The system can resolve features as small as 10 nm in the z-axis (sample height) and 120 nm in the x-y plane. The sample stage is designed for 4" wafers, but can accommodate up to 6" wafers. Figures 1 and 2 illustrate the Olympus LEXT OLS4000 Laser Microscope's setup and components.

Figure 1. Olympus LEXT OLS4000 Laser Microscope Setup

Figure 2. Olympus LEXT OLS4000 Laser Microscope Components

4. Operation Manual

4.1. Starting with the Software

1. Open the OLS4100 software. No password is required; simply click "OK."

Figure 3. Software Log On

2. A pop-up message will appear stating, "Please check the safety around the sample, objective lens, and stage." Click "OK."
3. Another pop-up will ask, "Do you want to move to the Z reference position?" Click "Cancel."

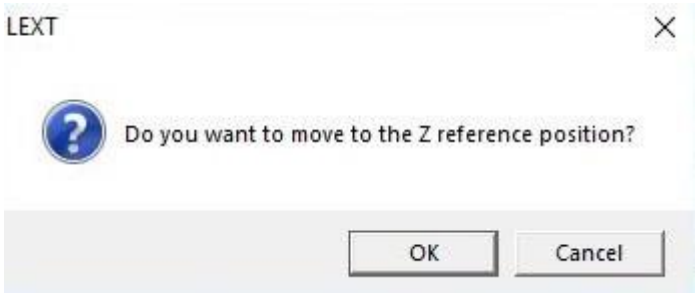

Figure 4. Cancel Z Reference Position

4. The software interface will display as shown in Figure 5.

   #1 represents live image controls, including focus adjustments, brightness control, accessories, and stage control.

   #2 includes imaging options such as microscope settings, advanced settings, color, laser, and dual-screen features.

   #3 contains magnification settings ranging from 5X to 100X.

   #4 represents acquisition settings for laser imaging.

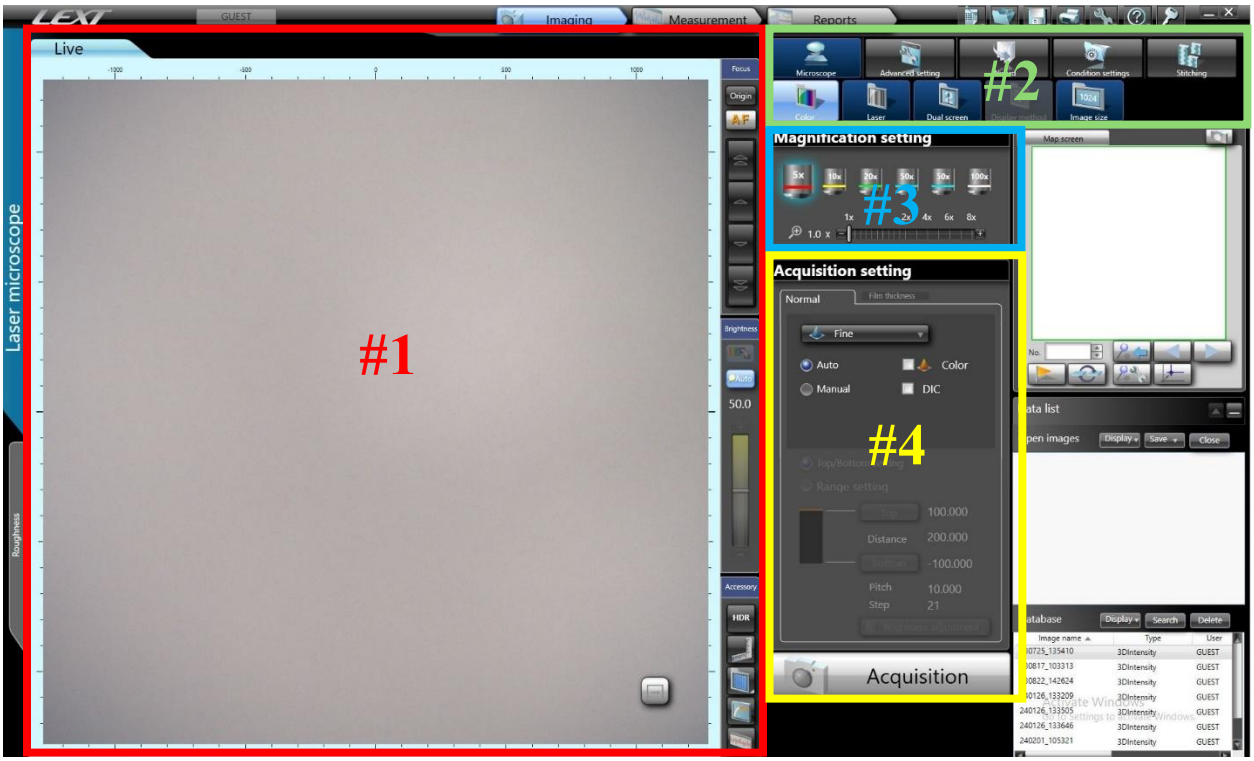

Figure 5. LEXT Software Interface

4.2. Placing and Focusing the Sample

5. Before loading the sample, ensure the microscope is set to the lowest magnification. If it is not, change the magnification to 5X by selecting it from the magnification settings, as shown in Figure 6.

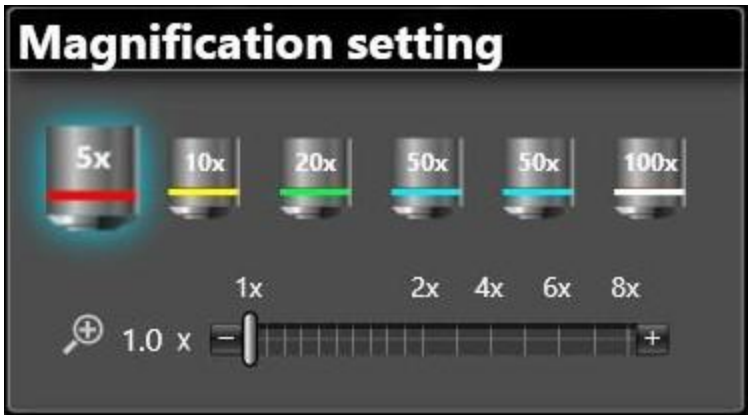

Figure 6. Magnification Setting

6. Place your sample onto the stage as shown in Figure 7. Avoid touching the optics or damaging the sample stage during placement.

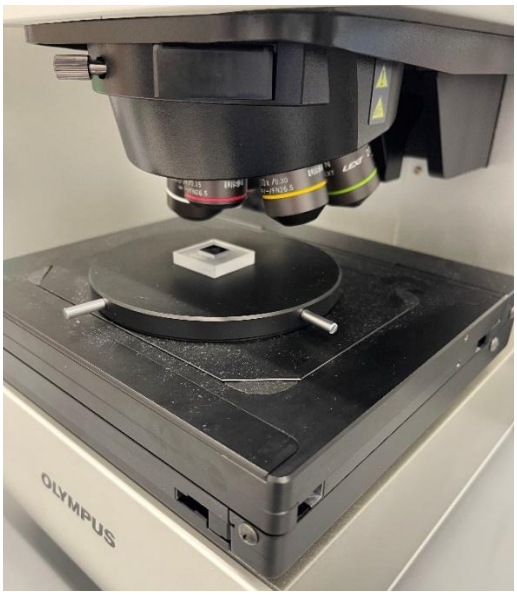

Figure 7. Sample Placement

7. After placing the sample, use the focusing knob to make a coarse focus adjustment. Once the sample is in focus, lock the knob by turning the locking mechanism counterclockwise, as shown in Figure 8.

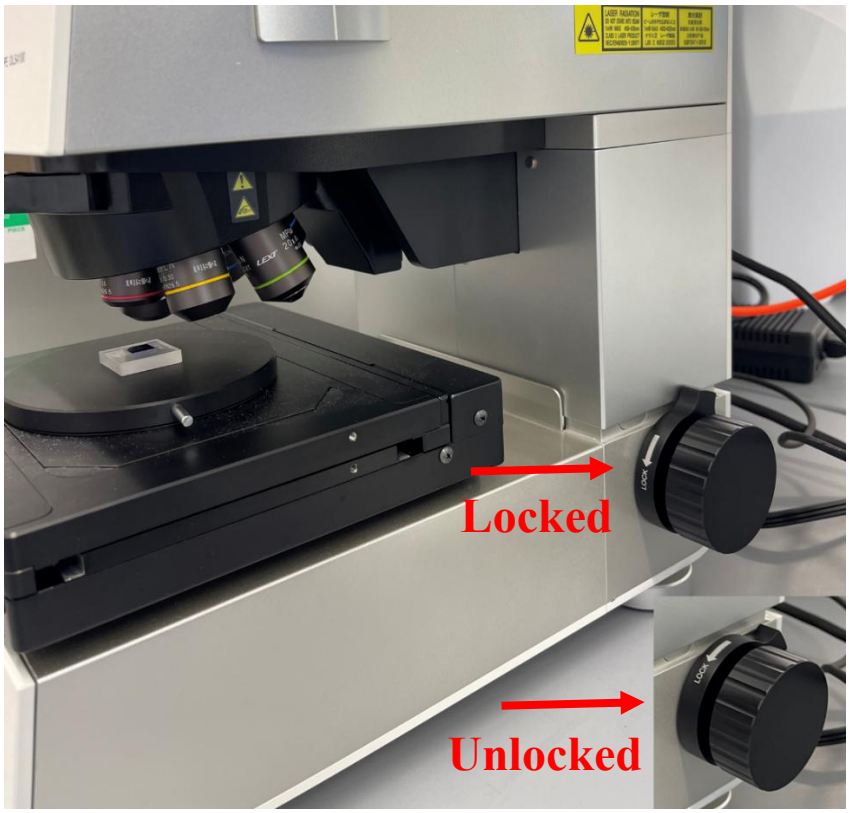

Figure 8. Locking Focus

8. Fine-tune the focus by using the arrows in the software, as shown in Figure 9.

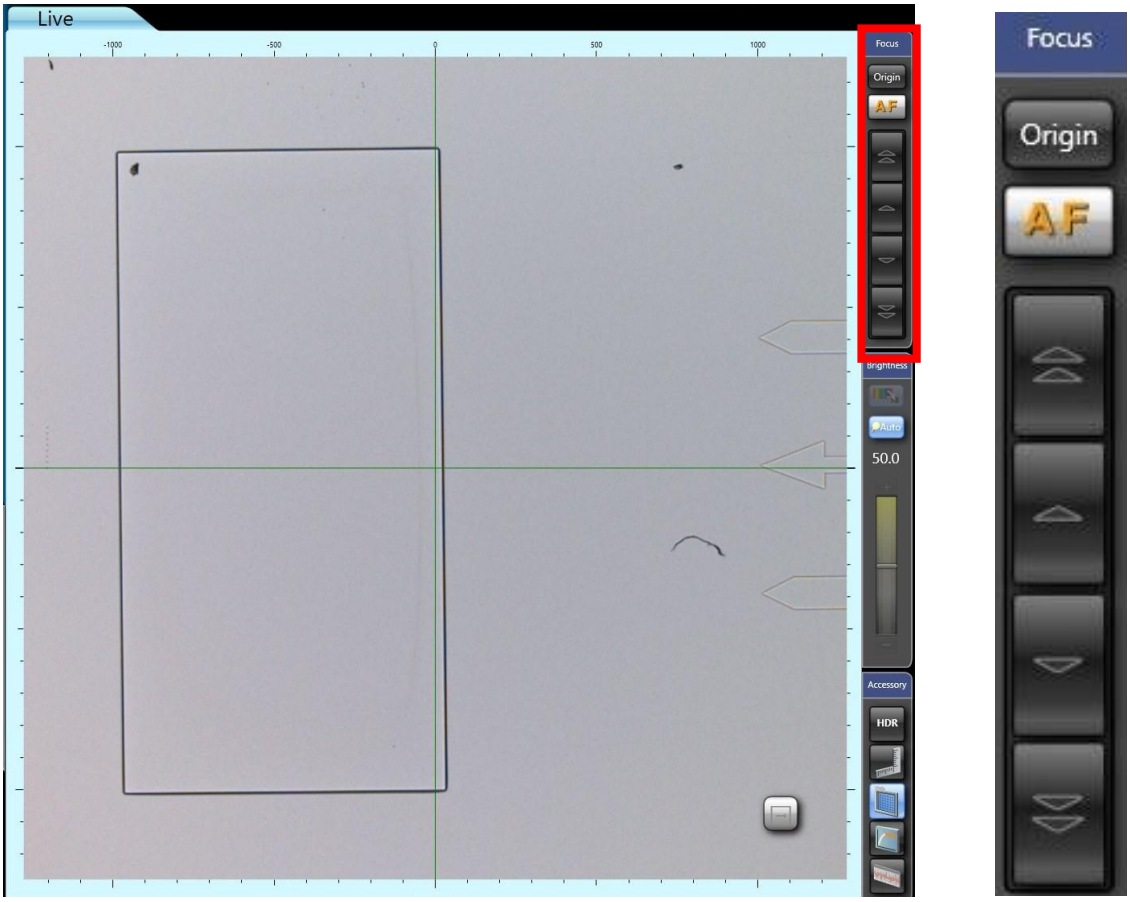

Figure 9. Fine-tuning Focus

9. In the bottom-right corner of the live image, there is a gray box. Click on it to reveal navigation arrows, which can be used to move the sample stage and navigate across the sample, as shown in Figure 10. You can also double-click on the live image to center the area you wish to focus on.

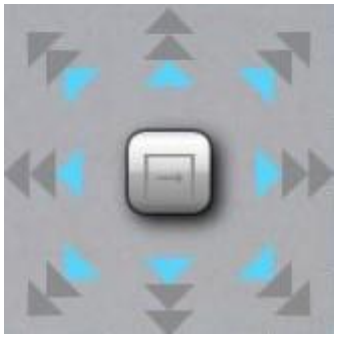

Figure 10. Image Navigation Icon

10. To zoom in, increase the magnification one step at a time, following the method described in Step 5. Ensure you refocus the sample after every magnification change, as explained in Step 8, to avoid damaging the objectives.

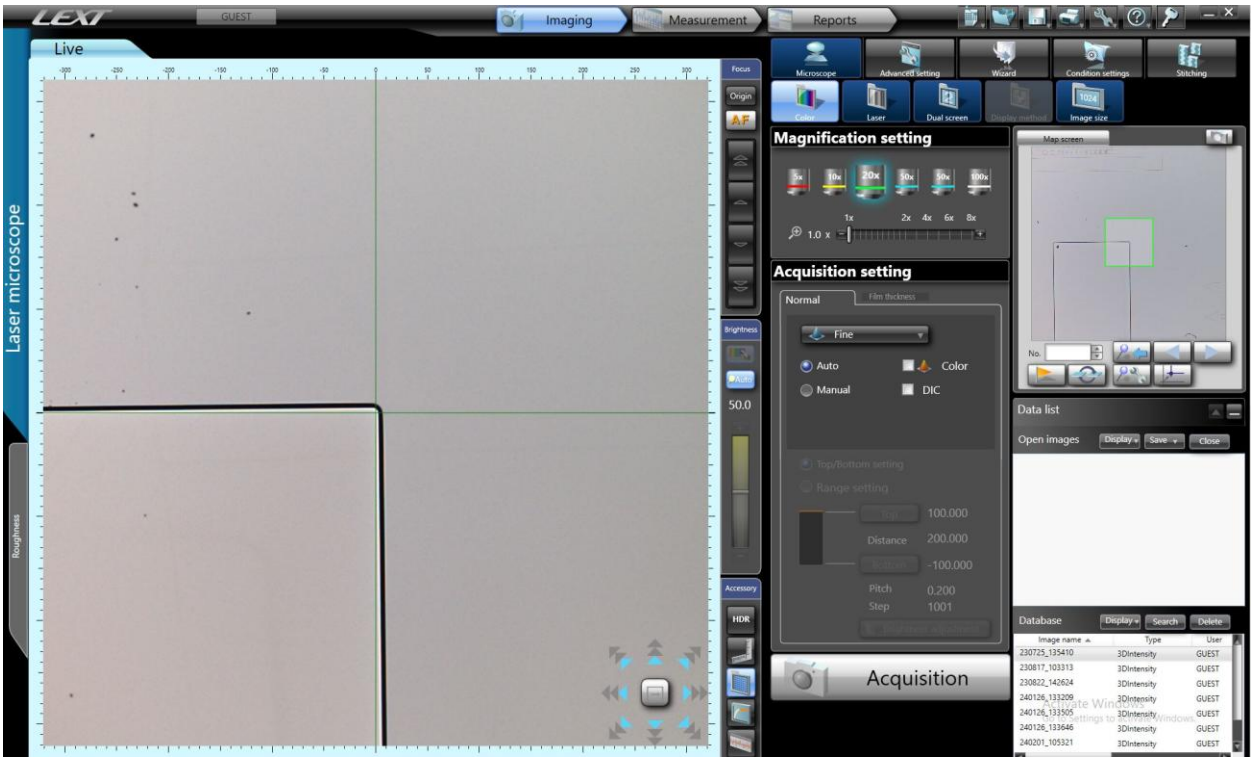

Figure 11. 20X Magnified Image

4.3. Laser Image Acquisition

11. After focusing on your sample, activate the laser imaging by clicking the laser icon, as indicated by the arrow in Figure 12. The hardware will display a second blue light to indicate that the laser is on, as shown in Figure 13.

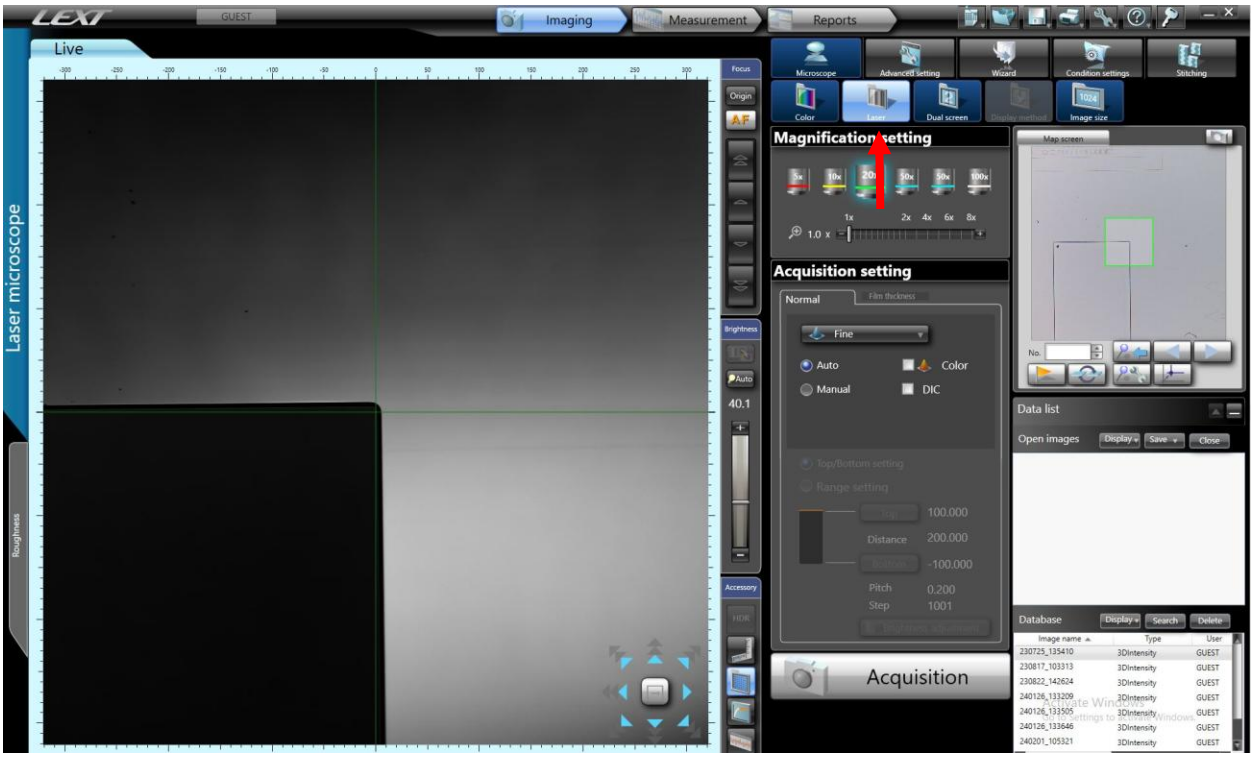

Figure 12. Laser Imaging Icon

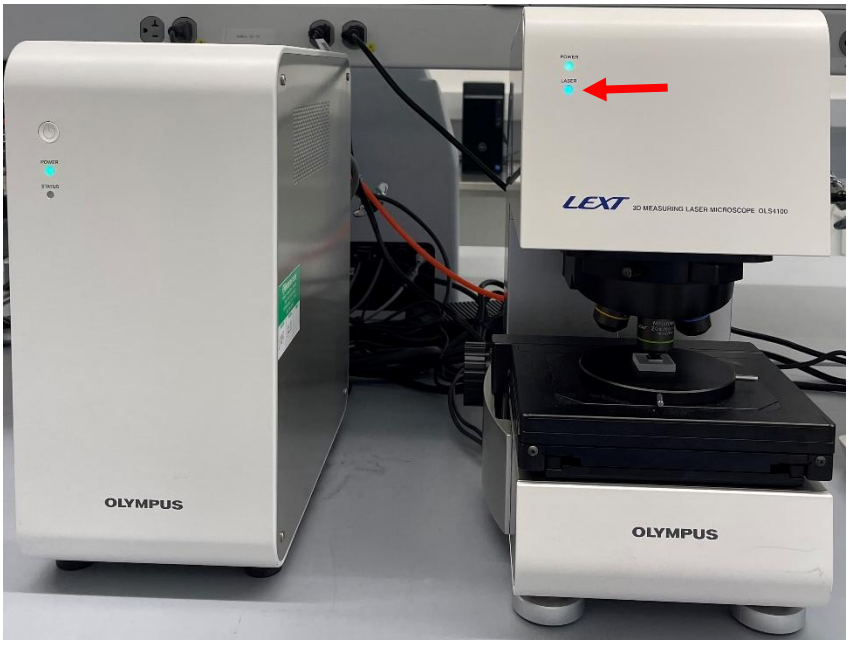

Figure 13. Hardware Showing Laser On

12. The laser live image will appear in grayscale, where bright areas are in focus and dark areas are out of focus. To check for oversaturation, enable saturation highlighting by clicking the

icon shown in Figure 14 (#1). Any saturated areas will appear red. Adjust the brightness to reduce oversaturation using the brightness controls shown in Figure 14 (#2).

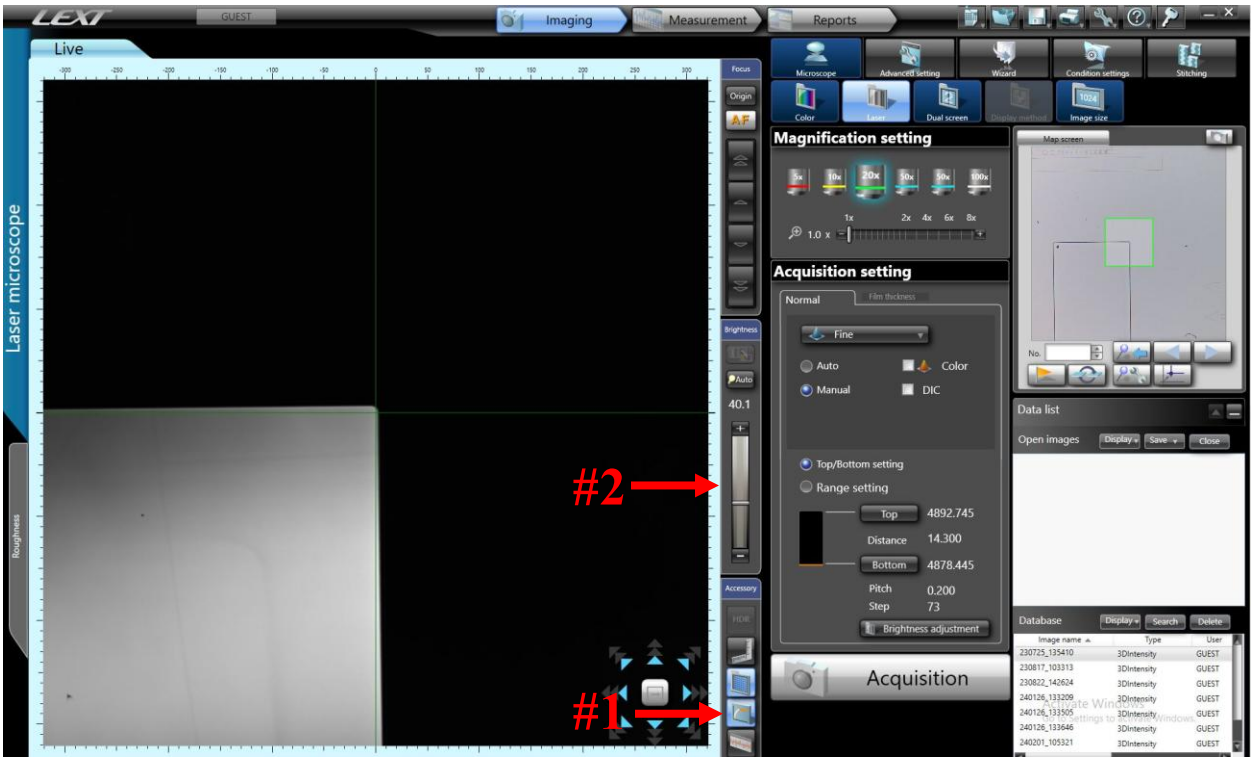

Figure 14. Saturation Highlighting Icon (#1) and Brightness Adjustment (#2)

13. In the acquisition settings, select "Manual." Increase the focus until the live image turns completely black. From the "Top/Bottom Setting," select "Top," then decrease the focus until the image is again completely black, and select "Bottom." Set the scan speed. "Fine" is used as an example in Figure 15.
14. Click "Acquisition."

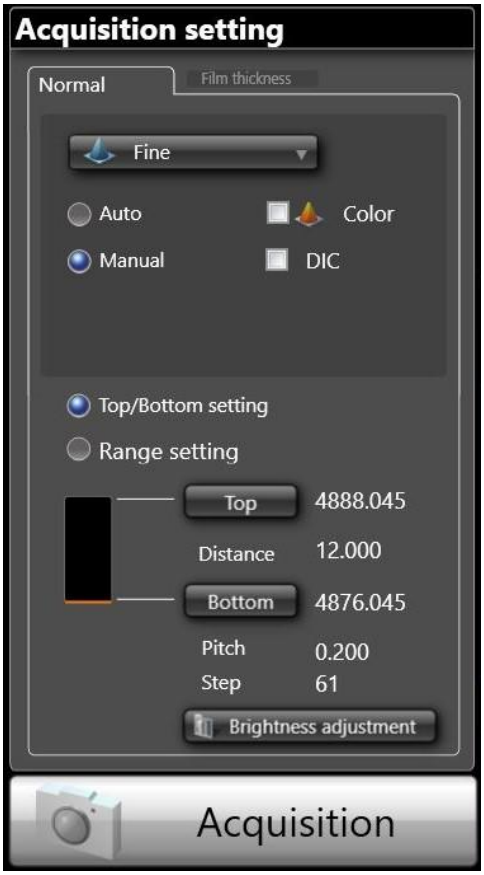

Figure 15. Acquisition Settings

4.4. Image Analysis

15. Once the acquisition is complete, you can switch between 2D and 3D views using the icon shown in Figure 16 (#1).
16. If you want to capture additional images, return to the live image by clicking "Live," as shown in Figure 16 (#2).

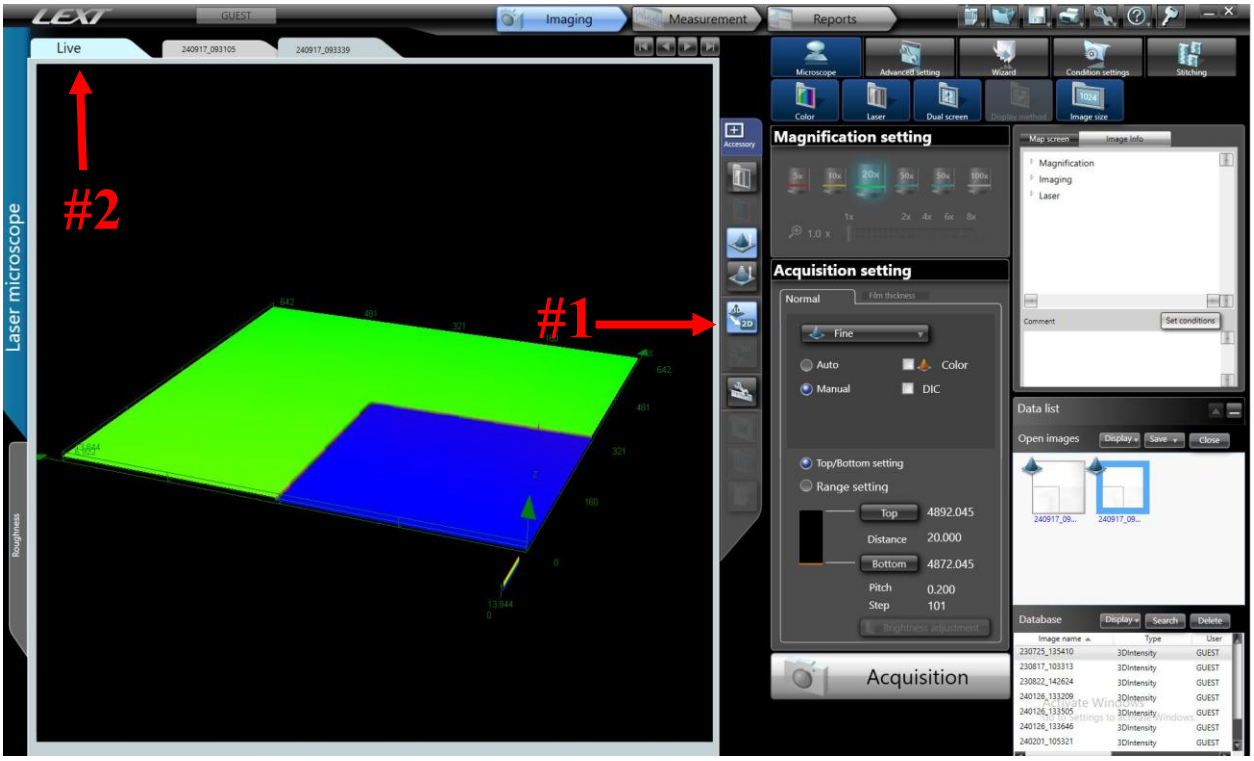

Figure 16. 2D-3D Imaging (#1) and Live Imaging (#2)

17. To save the file, click the disk icon and export the image, as shown in Figure 19.

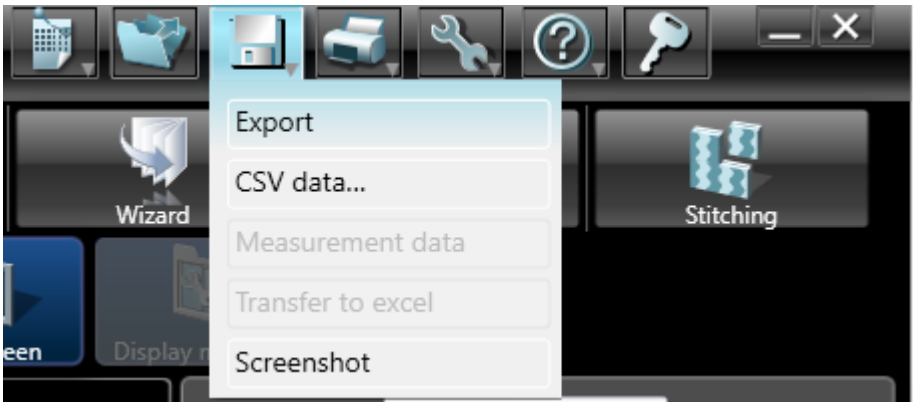

Figure 19. Saving the File

18. Further adjustments can be made in the advanced settings, as shown in Figure 20.

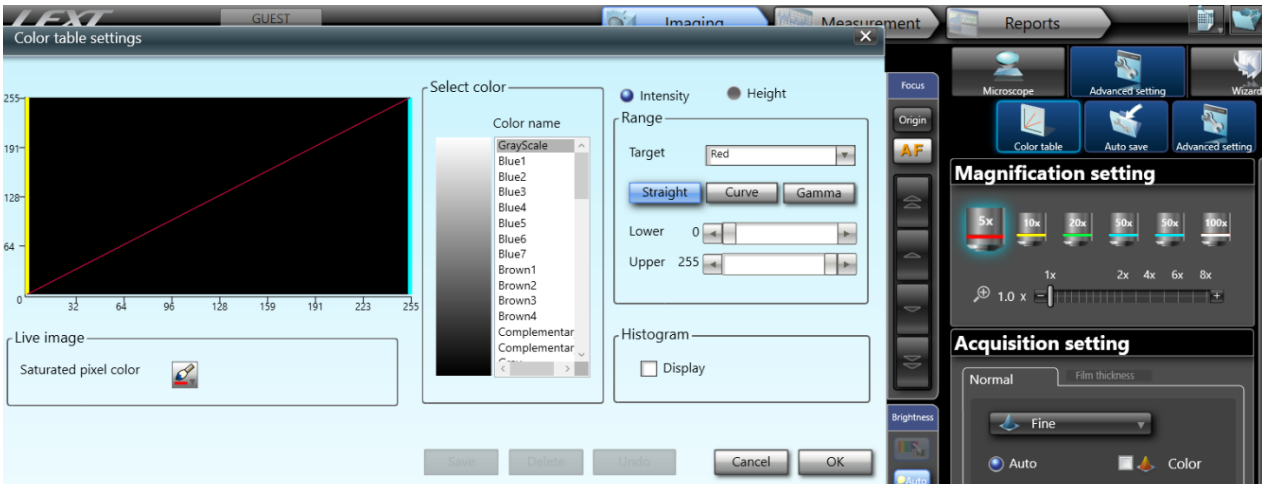

Figure 20. Advanced Settings

19. Measurements on the Intensity and Height graphs can be performed in the measurements tab, as shown in Figure 21.

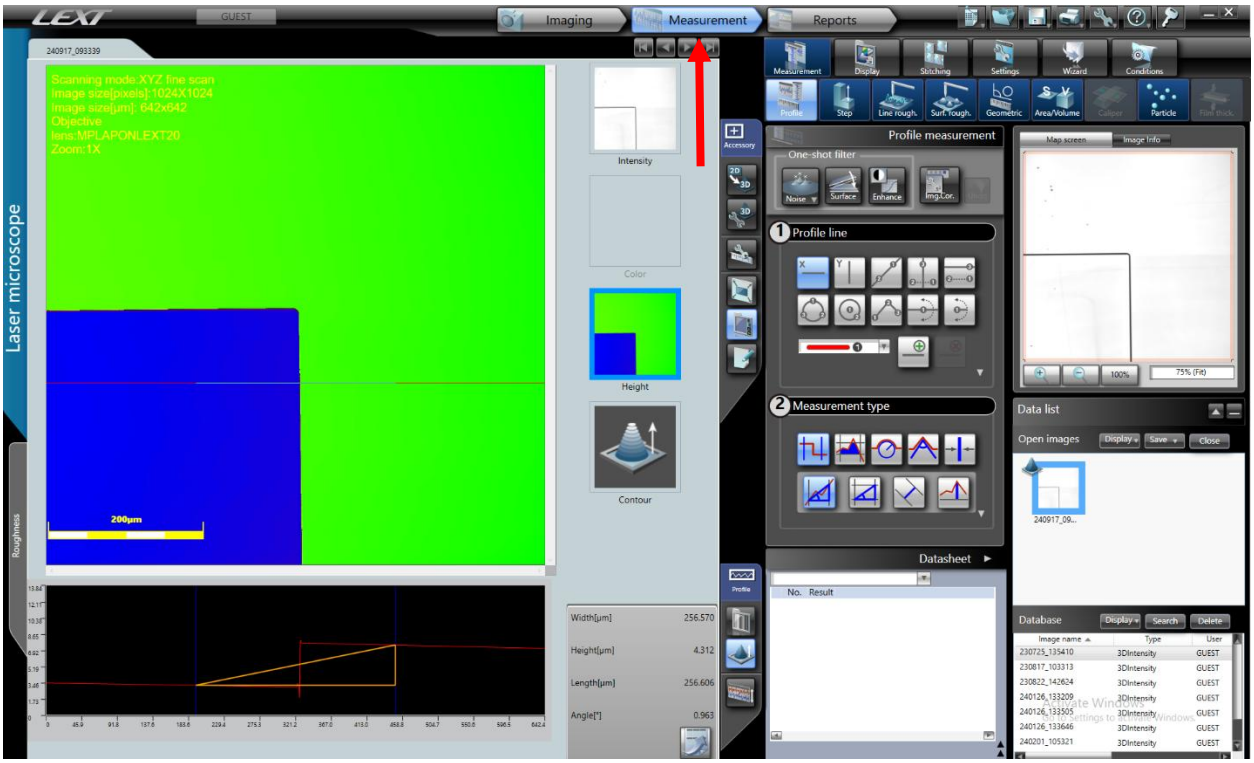

Figure 21. Measurements

4.5. Unloading the sample

20. After completing all scanning and imaging, turn off the laser by clicking the "Color" icon.

Figure 22. Color Imaging

21. Reset the magnification to 5X by selecting it from the magnification settings, as described in Step 5 and shown in Figure 6.
22. Remove your sample.
23. Close the software.