Standard Operating Procedure: Atomic Force Microscopy (AFM)

Bruker Dimension Icon with ScanAsyst

December 2025

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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. AFM 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.
- ✓ The scanner should be handled carefully: hold it securely and avoid hitting the scanner end cap with other objects.
- ✓ The Z stage should be lifted to a clear position before moving the stage.
- ✓ The laser light should not be stared at directly.
- ✓ Excessive force should not be applied when mounting the probe holder on the scanner.
- ✓ Large offsets or maximum scan sizes should not be used for extended periods.
- ✓ Objects should not be placed on the granite base.
- ✓ The app mode screw should not be over-tightened on the scanner.
- ✓ Fluids should not be spilled onto the scanner.
- ✓ Probe holders and cantilevers should be returned to their respective boxes after the experiment.
- ✓ 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 AFM

AFM, developed in 1986 by Binning, Quate, and Gerber, emerged from the scanning tunneling microscope (STM) and has become more widely used due to its ability to study insulators, semiconductors, and conductors. An AFM uses a sharp tip on a cantilever to scan a sample surface. The forces between the tip and the sample cause the cantilever to deflect, which is measured by a photodiode detector. This deflection data is then used to generate a map of the surface topography.

As the tip approaches the sample, the interaction forces change, transitioning from attractive to repulsive as the atoms come closer together. Most AFMs detect cantilever position using optical techniques, typically involving a laser beam that reflects off the cantilever onto a quad photodiode (QPD). The QPD consists of four segments, each generating voltage signals based on the amount of light received. These signals are used to calculate vertical and lateral deflection, providing information on cantilever bending and torsion.

The AFM operates using Z feedback loop to control the distance between the tip and sample. In Contact Mode Imaging, the cantilever's DC deflection signal is maintained at a constant angle while scanning. Other modes, such as Tapping Mode AFM, involve oscillating the cantilever and monitoring the amplitude and phase of the deflection signal. Various AFM modes are used to measure different surface features, depending on the type of force being measured and the method used. The setup of the Bruker Dimension Icon AFM is shown in Figure 1.

Figure 1. Bruker Dimension Icon AFM Setup

4. Operation Manual

4.1. Probe Holder Preparation and Loading

1. To load the probe onto the probe holder, use the probe holder stand as shown in Figure 2. Attach the probe holder sockets to the pins on the probe holder stand at the appropriate loading station (fluid, STM, or standard AFM). Ensure the large spring clip of the probe holder is facing up.

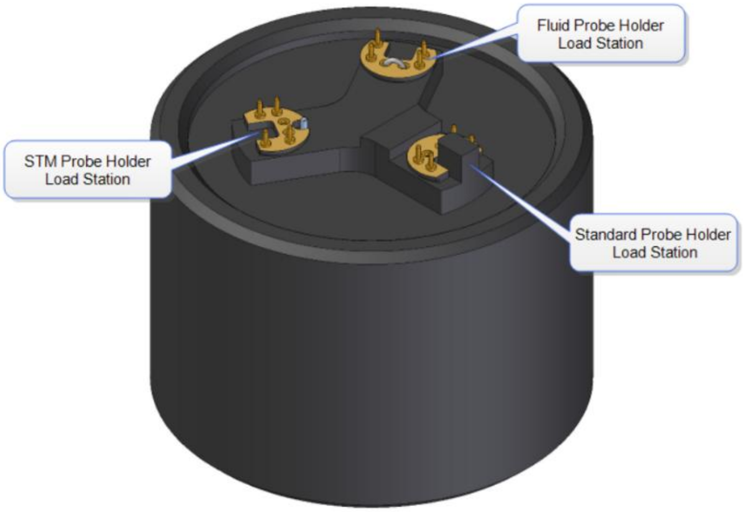


Figure 2. Probe Holder Stand.

2. To install a probe on the probe holder, use sharp tweezers to firmly grasp the probe (consisting of the cantilever and the tip) on the sides. Press down and slide back the spring clip of the standard probe holder, as shown in Figure 3.
3. Orient the cantilever intended for imaging away from the cantilever holder and place it in the probe holder groove.
4. Carefully maneuver the probe until it is flush against the back edge and lies flat in the probe holder groove.
5. Press the spring-loaded probe clip down, gently push it forward over the substrate, and release the spring clip to hold the probe in place in the probe holder groove.

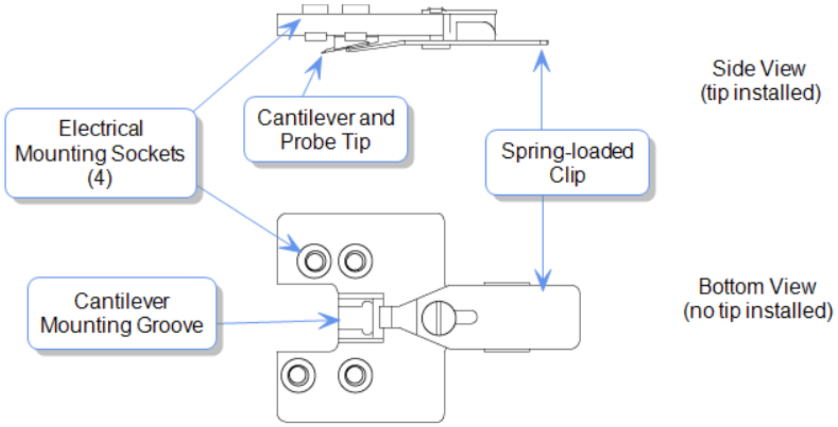


Figure 3. AFM Probe Holder

4.2. Installation of the Probe Holder on the SPM Scanner

6. Loosen the screw located on the right side of the Dimension Icon Scanner head, as shown in Figure 4.
7. To install the probe holder, gently remove the SPM scanner head, align the probe holder sockets with the pins on the Dimension Icon scanner, and install the loaded probe holder. Verify that the probe holder mounts flat against each pin on the end of the head.
8. Gently place the SPM scanner head back in position. Tighten the screw on the right side of the dovetail to secure the head.
9. Place your sample on the stage.

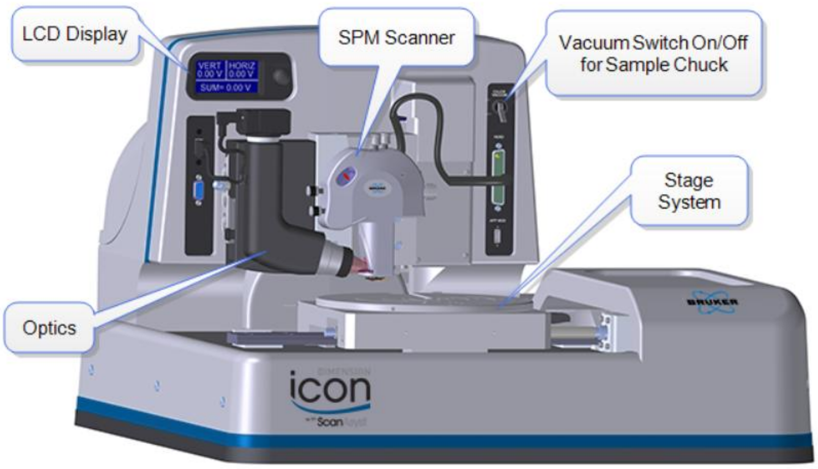
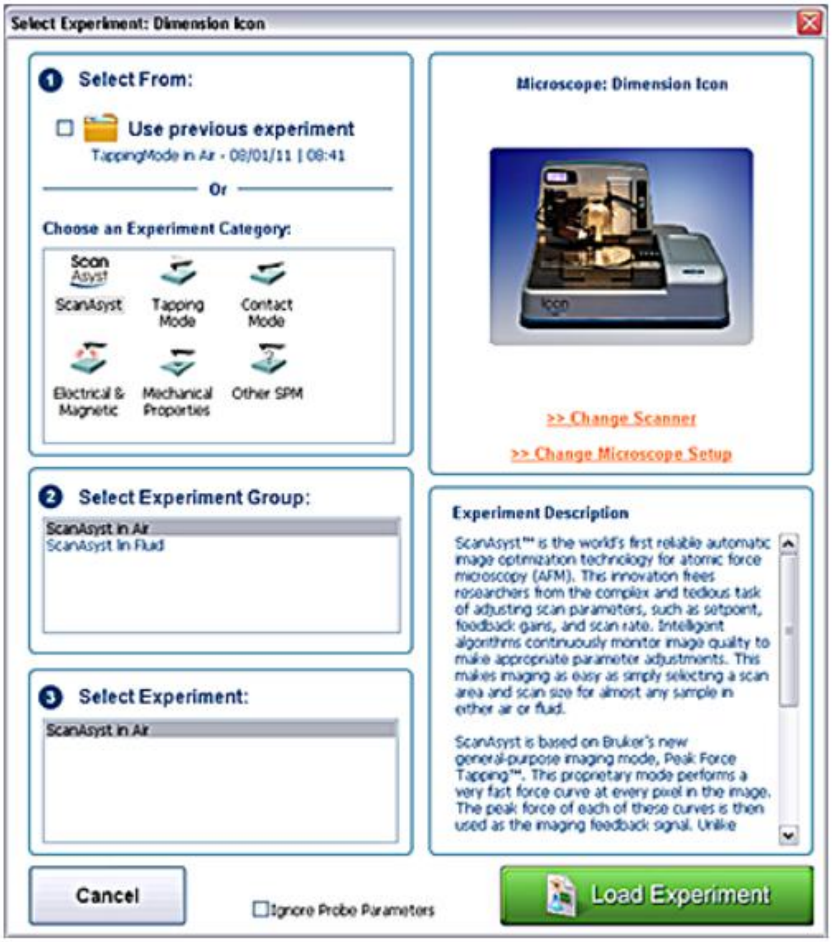


Figure 4. Bruker Dimension Icon AFM Setup

4.3. Introduction of the Software and Identifying the Probe

10. Open the Nanoscope 10.0 software.
11. In the Select Experiment window, choose an experiment category, experiment group, and experiment by clicking the icon for one of the Imaging Modes, as shown in Figure 5. (The default modes are ScanAsyst, ScanAsyst in Air, and ScanAsyst in Air for experiment category, experiment group, and experiment, respectively.)
12. Click "Load Experiment."



ScanAsyst™ is the world's first reliable automatic image optimization technology for atomic force microscopy (AFM). This innovation frees researchers from the complex and tedious task of adjusting scan parameters, such as setpoint, feedback gains, and scan rate. Intelligent algorithms continuously monitor image quality to make appropriate parameter adjustments. This makes imaging as easy as simply selecting a scan area and scan size for almost any sample in either air or fluid.

ScanAsyst is based on Bruker's new general-purpose imaging mode, Peak Force Tapping™. This proprietary mode performs a very fast force curve at every pixel in the image. The peak force of each of these curves is then used as the imaging feedback signal. Unlike

Figure 5. Select Experiment Dialog

13. After clicking "Load Experiment," the experiment page, as shown in Figure 6, will open. By default, the Workflow toolbar will appear on the left side, including the Setup, Navigate, Check Parameters, Engage, Scan, Ramp, and Withdraw sections, as in Figure 7. Scan and feedback parameters can also be found in this section.

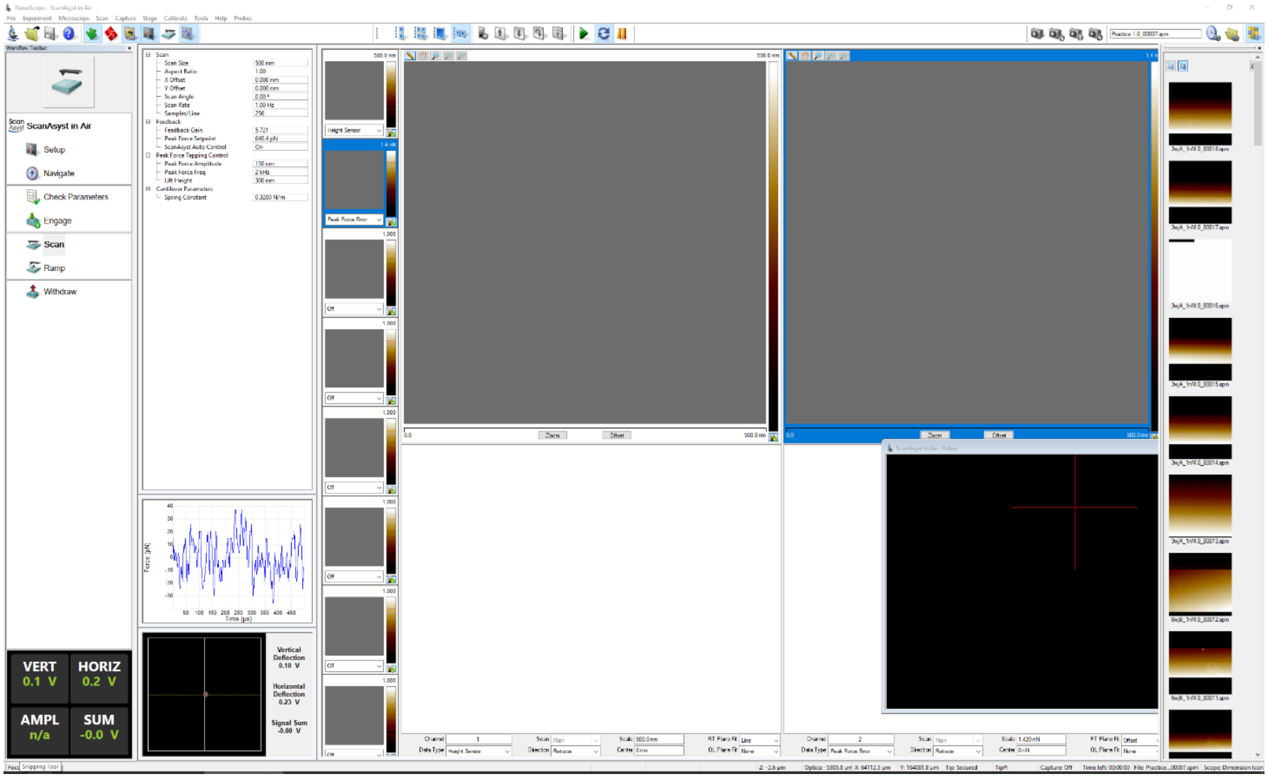
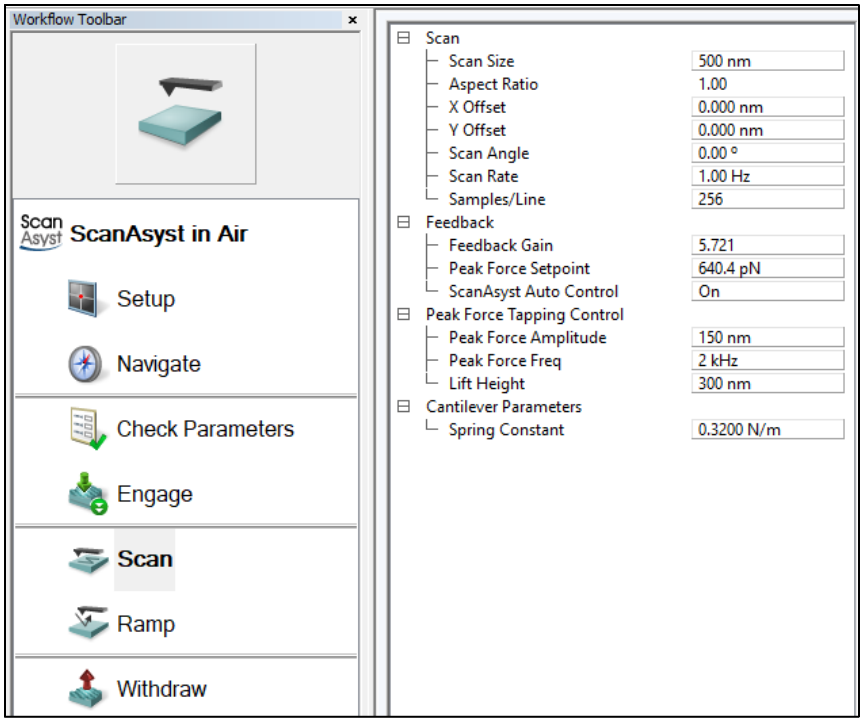


Figure 6. ScanAsyst in Air Experiment Page on NanoScope Software



Scan	
Scan Size	500 nm
Aspect Ratio	1.00
X Offset	0.000 nm
Y Offset	0.000 nm
Scan Angle	0.00 °
Scan Rate	1.00 Hz
Samples/Line	256
Feedback	
Feedback Gain	5.721
Peak Force Setpoint	640.4 pN
ScanAsyst Auto Control	On
Peak Force Tapping Control	
Peak Force Amplitude	150 nm
Peak Force Freq	2 kHz
Lift Height	300 nm
Cantilever Parameters	
Spring Constant	0.3200 N/m

Figure 7. Workflow Toolbar

14. In the Change Probe panel under Setup on the Workflow Toolbar, click the "Change Probe" button to open the Change Probe dialog box, as shown in Figure 8.
15. Select "New Probe of Type" and choose your probe type from the drop-down list. Save and close the dialog.

Change Probe

Recommended Probes: TESPA
FESPA
OTESPA

Probe I'll be using

○ New Probe of type: TESPA

● Other/Unknown probe

(Click Advanced to estimate Deflection Sensitivity) 0.00 nm/V

Tip Serial Number: Unknown

Recommended Probe Holder: DAFMCH

Figure 8. Change Probe Dialog

4.4. Laser Alignment

16. Select "Move to the Alignment Station."
17. Adjust the zoom and illumination.
18. Fine-tune the focus until the cantilever comes into view. Ensure the "Optics" reading on the lower right falls between -7000 µm and -8000 µm (around -7500 µm).
19. Figure 9 (left) shows the laser control knobs on the Dimension Icon scanner. Adjust the laser control knobs to move the laser onto the tip end of the cantilever. You can observe the movement of the laser in Figure 9 (right).
20. Continue adjusting until the maximum Laser SUM Signal, as shown in Figure 10, is achieved (around 5.00 V). The Setup view displays the laser signal values and a schematic

of the detector quadrants. The position of the laser is indicated by a red dot on the detector schematic.

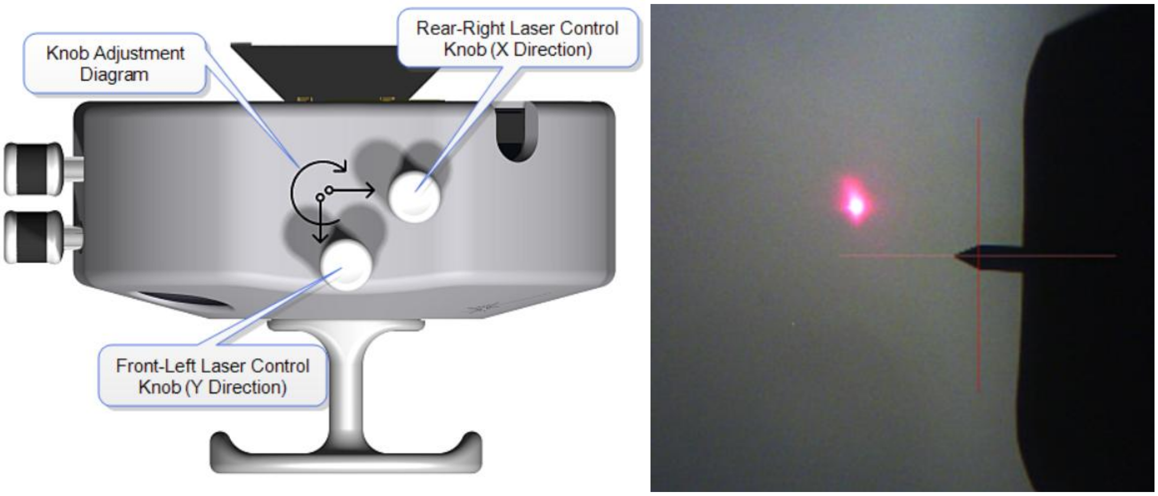


Figure 9. (Left) The Laser Control knobs on the Dimension Icon head and (right) The laser spot near the cantilever.

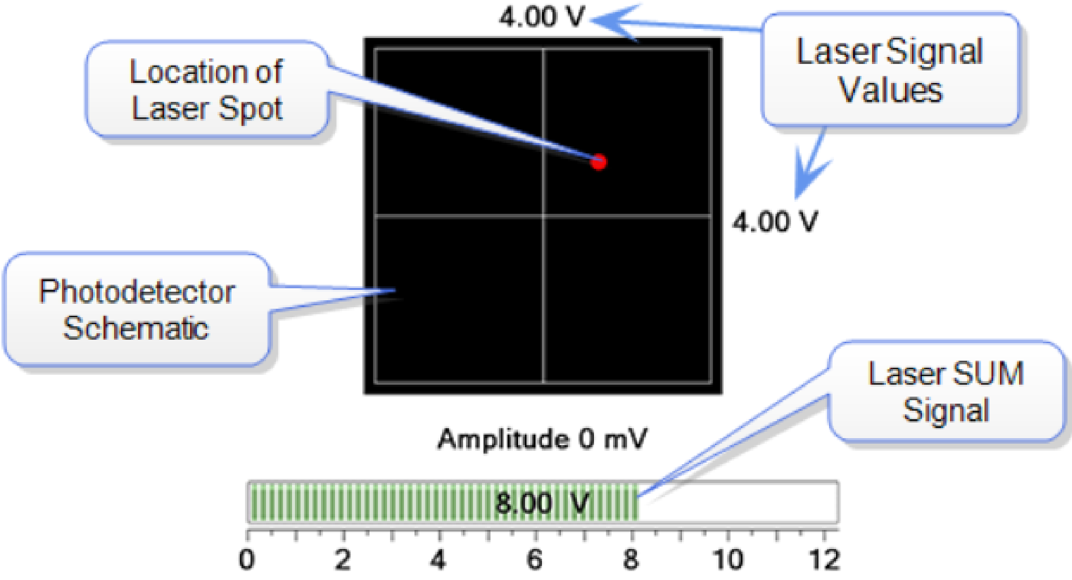


Figure 10. The Schematic of the video panel in the Setup view

4.5. Photodetector Adjustment

21. Center the laser detector signal in the photodetector schematic using the photodetector adjustment knobs located on the left side of the Dimension Icon scanner, as shown in Figure 11 (left).

22. Using the laser detector knobs, adjust the vertical and horizontal laser signal values to zero. Laser signal values can be observed both in the software Setup view, as shown in Figure 10, and on the Dimension Icon LCD panel, shown in Figure 11 (right).

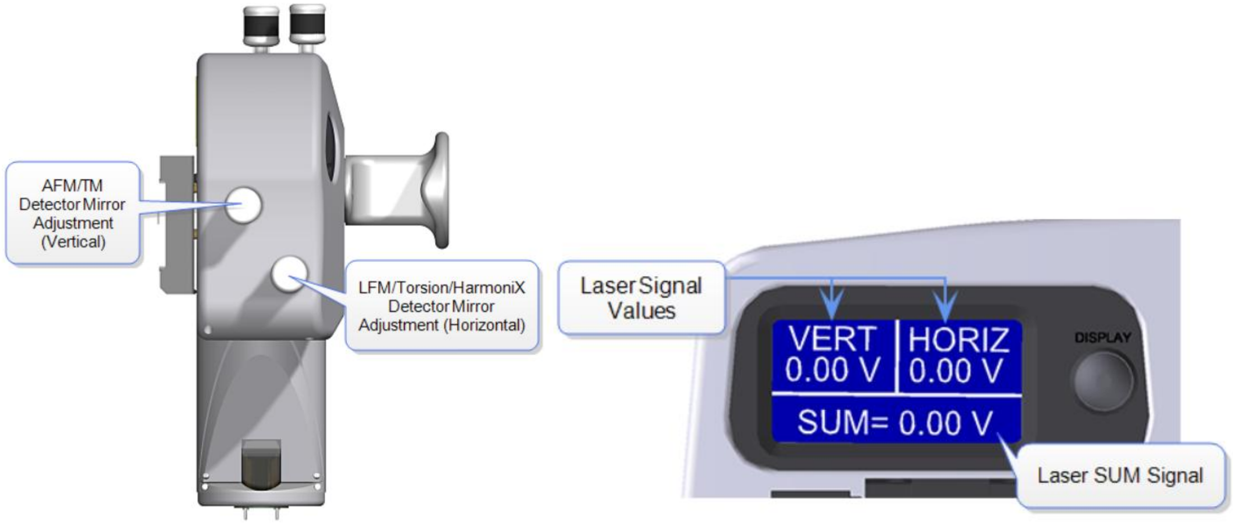


Figure 11. (Left) The Laser Detector knobs on the Dimension Icon head and (right) Dimension Icon LCD panel.

23. Click “Return from the Alignment Station”.
24. Fine-tune the focus until the cantilever comes into view. Ensure the "Optics" reading on the lower right falls between -4000 µm and -5000 µm (around -4200 µm).

4.6. Loading the Sample and Focusing on the Sample Surface

25. Click the "Navigate" icon in the Workflow Toolbar to open Navigate View.
26. From the Focus Sample, “Adjust focus by moving scan head up or down”, lift the scan head until you cannot lift it anymore.
27. From the "Navigate to Scan Centerpoint", use the XY Control buttons to navigate to the area of the sample you wish to scan, as shown in Figure 12.
28. Focus on the sample surface using “Adjust focus by moving scan head up or down”. Use fine adjustments by lowering the speed to prevent equipment damage. Ensure the "Optics" reading on the lower right falls between -5000 µm and -6000 µm (around -5200 µm).

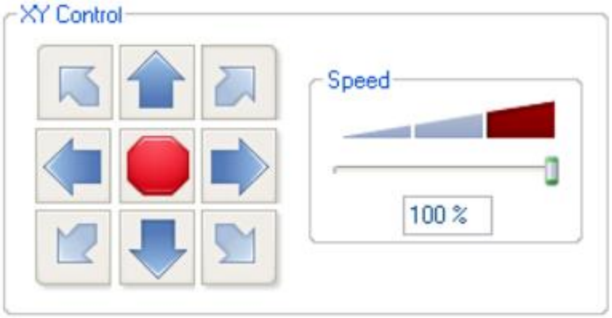


Figure 12. XY Control Buttons.

4.7. Checking the Scan Parameters

29. Either leave the parameters default and continue with your experiment, or adjust them according to the mode and application, using the Workflow Toolbar shown in Figure 13. When a parameter is changed from the workspace value, it will be highlighted in yellow. For details about initial parameter settings, refer to the specific Imaging Modes information on the Bruker Dimension Icon AFM website or consult NFF staff.

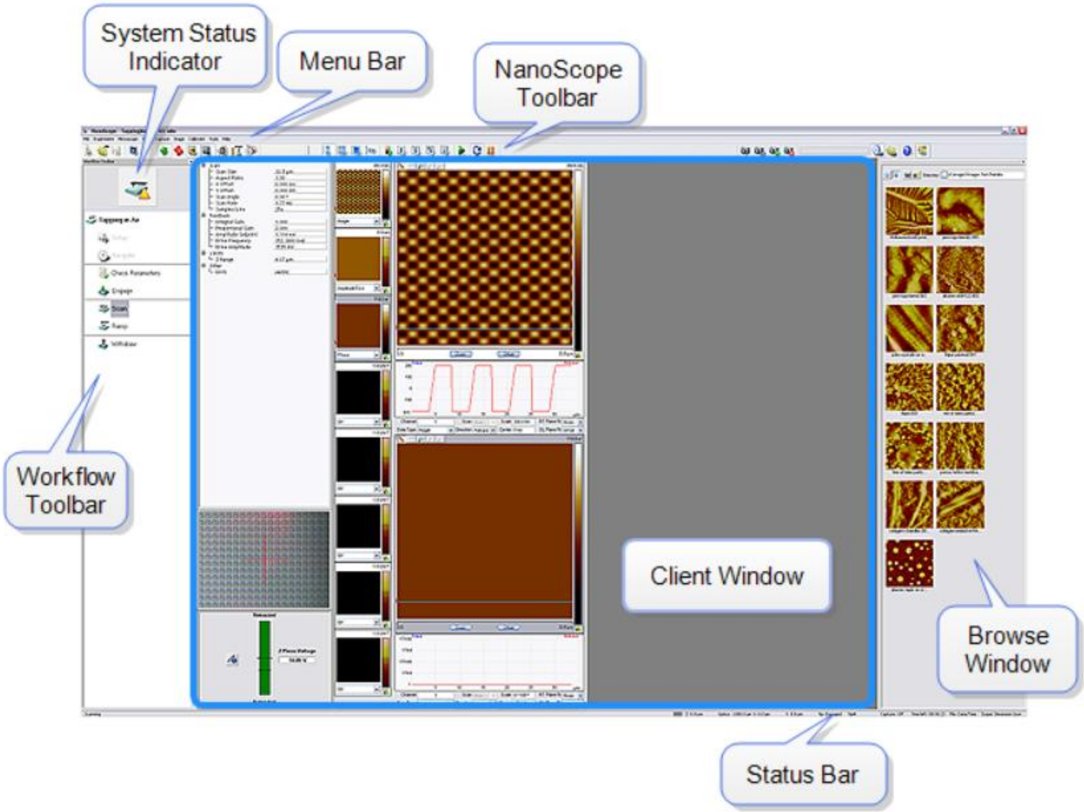


Figure 13. The Main Screen Elements of the Nanoscope 10.0 Software

4.8. Engaging, Scanning & Withdraw

30. After all adjustments, click the "Engage" icon in the Workflow Toolbar.
31. Once engaged, adjust the scan parameters as needed using the Scan view to acquire an optimized image. Images can be saved in the save directory > capture.
32. When imaging is complete, withdraw the tip by clicking the "Withdraw" icon in the Workflow Toolbar. This typically raises the tip 1 mm above the sample.
33. Before loosening and removing the SPM scanner, ensure that the stage is low enough. If necessary, lower the stage from the Navigate section.
34. Close the software. The laser will turn off automatically.
35. Remove your sample.

4.9. Analysis

Image analysis can be conducted using Nanoscope Analysis software. 2D and 3D imaging can be obtained. Be sure to select the proper color bar and redefine the minimum and maximum height from the data scale.