Introduction:

Scanning Probe Microscopy (SPM) is a general term referring to surface characterization techniques that utilize probes in close proximity to the sample surface. Surface topographical characterization is perhaps the most common use of SPM techniques. These devices are capable of characterizing the topography of a sample with vertical features ranging in size from $10^{-10}$ to $10^{-6}$ meters. The lateral dimensions of the scanned region may be on the order of $10^{-10}$ to $10^{-4}$ meters.

Various SPM techniques are being developed; among these are Lateral Force Microscopy, Magnetic Force Microscopy, and this manual is designed to serve as a supplement to training on the SPM and is not intended as a replacement for formal training sessions. To schedule training on the SPM, contact Gajendra Shekhawat (g.shekhawat@northwestern.edu, 1-3204/847-650-2918).

Who can assist you in BioscopeII AFM if you don’t find Manager:

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Six Rules of Safety

Here is a summary of precautions to follow during your learning phase. If you follow the six rules below, the MM-SPM can come to little harm.

1. **Read the manuals:** It is recommended that you read the instructions before doing any imaging work. Please consult with Manager if you have any issues.

2. **Follow good rules of engagement:** Engagement” refers to the process of bringing the tip and surface together. This is harder than it sounds, and the software routine for controlling the process is complex. Some probes (especially crystal silicon TappingMode™ probes) are prone to breakage if engaged too quickly or too hard. Ensure that engagement settings never exceed the limits of safety and never attempt to engage manually using the stepper motor.

3. **Never move the head while imaging:** The head contains the tip holder, laser and photodiode array. An X-Y translation stage is provided for moving the head and tip several millimeters across the sample for coarse adjustment. Even for relatively smooth samples, the head should NEVER be moved with the tip engaged. This almost always results in tip breakage. Always disengage first before using the X-Y stage to move the tip.

4. **Never leave your controller ON while the computer is turned OFF:** Operators are advised to turn OFF their controller when finished with imaging. If the controller is left ON for an extended period without an energized computer,
damage to the scanner may result. (This is especially true if the scan has been heavily offset in X and Y.)

5. **Do not unplug cables to/from energized hardware. Turn OFF first.** Unplugging energized hardware is not recommended and may result in damage to the MM-SPM. Always turn OFF hardware before making connections.

6. **Ask Permission.** Basically do not use the instrument in a way different than covered in this manual (i.e. basic contact or tapping mode AFM) without first asking a lab manager for permission

### Instructions for Operation:

#### Pretest Procedure

- All SPM Lab users **must log on to the Bioscope II first in order to turn the monitor ON.**
- Turn on the controller by flipping the switch on the back of the case. Make sure to do this before opening the software application.
- Turn on the power to the optical microscope monitor (the switch is located on the bottom left side of the microscope.
- Turn on the power on the electronics box by switching on the plugbox.
- Double-click on the NanoScope program (microscope-like icon) (Version 7.30).
- Double click on the yellow microscope icon located in the top lefthand corner of the software window. This will turn on the laser on the scanning head. When starting Realtime, the parameter’s values need to be selected with options between **PREVIOUS SESSION PARAMETERS** or **ORIGINAL DEFAULT PARAMETERS.** If a new session, selecting **ORIGINAL DEFAULT PARAMETERS** is recommended.
- Select all options from the checkbox in the window that appears next. Wake up the piezo motors by clicking on the appropriate button.

- Select an appropriate tip. For contact mode, use a silicon nitride probe tip. This probe appears gold in color and has two cantilever tips on either side. Remove the two tips on the side opposite of those to be used with a pair of tweezers. This is done because the tips on the opposite side are easily damaged and this ensures that future users will not be using potentially damaged tips.
- Mount the tip in the tip holder and slide the tip holder onto the 4 pins of the AFM head. The tip holder assembly must be flipped before being inserted, such that the tip is now pointing downwards. Make sure the tip holder is securely inserted on the 4 pins (you should feel a click when the tip holder is securely set in place).
- Place the AFM head on the EasyAlign so the 3 pins fit into the 3 holes of the Easy Align table. Power on the EasyAlign (the EasyAlign powers off after 15 minutes)
- Use the knobs on the right side of the EasyAlign to adjust the brightness and bring in focus the cantilever. Use the top screws on the EasyAlign to center on x and y the position of the cantilever in the display.
- Use the laser knobs to bring the laser spot onto the cantilever. Adjust the vertical and horizontal position for a maximum Sum signal. You will see on the digital display of the electronics box a SUM signal of around 6-8 V
• Adjust the dichroic mirror horizontal and vertical settings so that both values are each equal to zero on the E-Box Display.

• Move the z motors all the way up to the zero position by clicking on ‘Navigate’ from the left menu bar. This can be done either by enabling the joystick and pressing the Z button and the Joystick up, or by pressing the up button on the keyboard, or just keeping clicked the up arrow in the Navigate window.

• Tilt the microscope condenser.

• Place the sample to be imaged on the microscope stage. Use the magnetic white holders to secure the sample on the microscope table.

• Transfer the AFM head to the microscope stage and check the horizontal and vertical settings on the E-Box display to ensure that both values are still reading zero. Adjust these values back to zero if needed.

SOFTWARE
• Click on the microscope icon to go to Real Time.

• In “Add Views to Real Time 1” click Select All and then Ok, and close the Error Log message that pops up.

• From the left side of the screen select the scan type (Scan Dual shows up as default, but you have the options of “Scan Triple”, “Scan 8 Channels” or “Scan Single”), also, click to display the “Scan Parameter List”.

• In the “Other” section of the “Scan Parameter List” you can select the Microscope Mode, and chose between Contact, Tapping or STM. You can choose the Units between Metric and Volts.

CONTACT MODE
• In contact mode, horizontal difference (you will see on LCD display) should be made zero by turning the detector knob (on the back side of your left) until it is zero. You need to make sure that when you turn this knob, the SUM signal should not go down. If yes, then turn the knob in other direction.

• Use the top knob of the detector (top left) to get VERT DIFF of around -2 V. Here also, if SUM signal goes down, rotate the knob in other direction. The Horizontal DIFF. signal which you set to ZERO will change little bit, but you can adjust to ZERO again by adjusting that knob.

Select initial scan parameters
• Other Controls panel: set Microscope Mode to Contact if it is not there.

• Scan Controls panel: set the initial Scan Size to 1um, X and Y Offsets to 0, and Scan Angle to 0, Scan Frequency to 2 Hz.

• Feedback Controls panel: set Integral Gain to 1.0, Proportional Gain to 2.0, and Set point to 1.5.

• Channel 1 panel: set the Data Type to Height, Line Direction to Trace, Real Time Plane Fit to Line, Offline Plane Fit to Full.

• Engage the tip by just clicking the ARROW pointing downward.
- Wait until the tip engages the surface.

**Adjust scan parameters**
- Once contact with the sample surface occurs, check to see if Trace and Retrace are tracking each other well (i.e. look similar)- Trace and Retrace curves appear below the scanning image. If they are tracking, the lines should look the same, but they will not necessarily overlap each other, either horizontally or vertically.
- Once engaged, click on Set point and use the right arrow key to gradually increase the Set point value. Meanwhile, monitor the Z-Center position located on the right hand side of the monitor. If the line in the Z-Center moves close to the limit of the extended end, the tip has made a false engage. In such a case, withdraw the tip once, reduce the SUM signal to around 5-6 V by just offsetting the LASER and re-Engage.
- Adjust the Scan Rate, Gains, and/or Set point to improve the tracking.
- Set desired scan size, scan rate, scan angle, and offsets.
- Once the scan parameters are optimized, scan size and other features can be adjusted for capturing images for analysis.
- If you need to increase your scan size, before you do that, reduce your scan speed to less than 1 Hz. This will minimize the noise in your images.
- Increasing the set point voltage will increase your contact mode and decreasing it will lower it.
- You can always play with offsets to scan other close by locations.

**Image Capture**
- Select RealTime/Capture Filename to name the file (you should give it a path to save it in your own folder in the ‘users’ folder on the D drive).
- Select RealTime/Capture (this will save your image in the target directory). You might want to Capture Last (will capture the previous image after another scan has begun), Capture Now (will capture the image as it is) Capture Continuous (will capture consecutive images until the capture is stopped). For capturing you may use the icons (similar to a camera) on the toolbar.
- Once images are captured, they may then be analyzed and any modifications can be saved offline.

**Post test Procedure**
- Select Withdraw 4-5 times to sufficiently withdraw the tip from the sample surface (Click on the Red Arrow pointing upwards).
- Tilt the condenser and remove the scanning head. Place it securely in the holder on the table. Remove the cantilever holder.
- Return all SPM components to their appropriate locations.
- Exit the NanoScope software.
- Turn off power to the NanoscopeV controller, optical microscope, electronics box and log off.
Basics of Tapping Mode Operation:

Overview:
In tapping mode, the cantilever beam is oscillated at its resonant frequency. A prescribed amount of damping is used to represent sample contact. As the sample is moved below the cantilever tip, changes in surface topography cause the amplitude of the reflected laser spot on the photoelectric scanner to deviate from its target amplitude. The piezoelectric scanner then responds by vertically deflecting the sample such that the amplitude of the reflected laser spot is maintained at the set amplitude. This vertical deflection of the piezoelectric scanner is associated with a change in voltage. As such, the surface topography of the sample can be obtained by mapping out the corresponding deviations in this voltage.

Instructions for Operation:

- Follow the same procedure as described for Contact mode to initiate the program and to focus the laser onto the tip of the cantilever.
- **Other Controls panel:** set Microscope Mode to Tapping, on the Electronics Box display the Horiz panel will be replaced by a RMS signal
- Make the VERT signal zero by turning the Vertical knob (on the top left). When you rotate the knob, the SUM signal should not go down. You don’t need to adjust the Horizontal knob in Tapping Mode
- RMS output will only be displayed when we tune the cantilever.

Select initial scan parameters

- **Other Controls panel:** set Microscope Mode to Tapping (if it is not there)
- **Scan Controls panel:** Set the initial Scan Size to 1um, X and Y Offsets to 0, Scan Angle to 0, and Scan Frequency to 2 Hz (Note: Setpoint will be automatically set in the cantilever tuning procedure.)
- Feedback Controls panel: set Integral Gain to 0.5, Proportional Gain to 0.7
- Channel 1 panel: set the Data Type to Height, Line Direction to Trace, Real Time Plane Fit to Line, Offline Plane Fit to Full.

Tune the cantilever

- Click the Tuning Fork Icon, and then Auto Tune
- For Auto Tune Controls, make sure the Start Frequency is at 100kHz and the End Frequency is at 500kHz.
- Target Amplitude for smooth surfaces should be 2-3 V.
- Target Amplitude for rough surfaces should be 0.5-1 V.
- Click on Auto Tune (a "Tuning" sign should appear then disappear once Auto Tune is done).
- A single peak will appear with a resonance frequency between 200-450 kHz (If this does not occur, change the tip).
- If you are using MFM or longer cantilever, you set your range so it includes the nominal frequency.
Sometime the auto tune fails and all the peaks seem to be out off range. In such cases, put down the drive amplitude to Zero and tune it again.

When done auto-tuning (the Exit button becomes active), exit the cantilever tune menu and you will see a RMS value on the E-Box.

Engage the tip by clicking on the green arrow pointing downwards.

**Adjust scan parameters**

- Check to see if Trace and Retrace are tracking each other well (i.e. look similar). If they are tracking, the lines should look the same, but they will not necessarily overlap each other, either horizontally or vertically, if they are not tracking, adjust the scanning parameters until they do.
- Once engaged, click on Amplitude Setpoint and use the arrow keys (left ARROW) to gradually decrease its value. Meanwhile, monitor the Z-Center position located on the right hand side of the monitor. If the line in the Z-Center moves close to the limit of the extended end, the tip has made a false engage. Decrease Amplitude Setpoint until Trace and Retrace are tracking properly. (This parameter brings tip in close proximity to the sample).
- In conjunction with decreasing the amplitude setpoint, users can also increase or decrease the drive amplitude under the set point amplitude in OTHER controls and see the effect on the image quality. Increasing drive amplitude results in strong tapping while decreasing the drive amplitude it results in softer tapping.
- Adjust Gains to improve the tracking; for smaller features (.2-.3) and large features (1-2). Increasing gain settings will also increase the noise level.
- Scan Size, Scan Rate and Scan Speed are strongly dependent, Scan Rate giving the time it takes for the tip to complete one Trace and Retrace of the Scanning Size. When you have decided on the Scan Size, adjust the Scan Rate to improve the tracking.
- Set desired Data Scale for a good contrast.
- The scan parameters can be optimized at 256 Points/Line and 256 Lines, and once ready to record could be increased to 512 by 512 pixels or even higher for an increased resolution. Please notice that increasing the resolution, the scanning time also increases. Scan size and other features can be adjusted for capturing images for analysis.

**Image Capture**

- Select RealTime/Capture Filename to name the file (you should give it a path to save it in your own folder in the users folder on the D drive).
- Select RealTime/Capture (this will save your image in the target directory). You might want to Capture Last (will capture the previous image after another scan has begun), Capture Now (will capture the image as it is) Capture Continuous (will capture consecutive images until the capture is stopped)

Once images are captured, they may then be analyzed and any modifications can be saved offline.

**Offline Data Analysis/Modification:**
Please be referred to the Digital Instruments manual for a complete discussion of image analysis and modifying filters. A wide variety of analysis functions and image modifications are available in the **Offline** drop down menu. A few of the analysis functions are

- **Section**, which allows the analysis of the height, depth, and width measurements to be taken across a section of the sample. After selecting Section from the Offline drop off menu, draw a line on your scan, that includes the features of interest. In the right side a graph will appear with the profile of the selected line. Move the cursors accordingly and the information will be posted bellow in a color coded box matching the color of the cursors.

- **Roughness**, which allows for roughness measurements to be performed over the entire image or a selected region of interest.

**Typical image modifications listed in the Offline menu include**

- **Planefit Auto (x = 2, y = 2)** Planefit calculates a single polynomial fit for the entire image and then subtracts the polynomial fit from the image. Planefit is commonly used to remove tilt or bow from images.
- **Flatten** (order could be 0, 1, 2 or 3). Flatten of 0th order is used to remove image artifacts due to vertical scanner drift, image bow, skips and anything else that may have resulted in vertical offsets between scan lines.
- **Lowpass filtering** removes high-frequency noise (it is also called smoothening) by averaging adjacent pixels.

**CAUTION:** All Image Modifications options change the data. Don’t save the modified image over the original as the original data will be lost!