Guidelines for Fluid Operation with a MultiMode AFM

290.1 Overview

This support note addresses scanning probe microscope (SPM) imaging of samples in fluid using a MultiMode atomic force microscope (AFM). Refer to the following for your specific area of interest:

- **Introduction:** Page 2
- **General Fluid Operation:** Page 3
- **TappingMode in Fluids:** Page 15
- **Troubleshooting Tips:** Page 20

290.2 Conventions

In the interest of clarity, certain nomenclature is preferred. An SPM *probe* is comprised of a *tip* affixed to a *cantilever* mounted on a *substrate*, which is inserted in a *probe holder*.

Three font styles distinguish among contexts. For example:
Window or Menu Item / BUTTON OR PARAMETER NAME is set to VALUE.

Document Revision History: Support Note 290

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Rev. C	9-Oct-01	All	0434	Alan Rice
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112 Robin Hill Rd.
Santa Barbara, CA 93117
(805)967-1400

290.3 Introduction

Imaging of samples in fluid is a growing application of AFM technology. This may be prompted by a desire to minimize surface forces on delicate samples, the need to observe biological specimens in their natural, fluid environments, and/or the necessity to make real time observations of samples undergoing electrochemical reactions (ECAFM). In order to conduct ECAFM observations with electrical potentials, it is necessary to connect an external potentiostat unit. Contact Digital Instruments/Veeco Metrology Group for more information.

Imaging samples under fluid eliminates attractive forces due to surface tension. This enables the sample surface to be imaged with a minimum of cantilever tip force—a decided advantage when imaging biological specimens and delicate materials.

Essentially, the procedure for observing samples under fluid is the same as that for Contact Mode or TappingMode™ AFM in air; however, special hardware is utilized to contain the fluid. In addition, minor adjustments must be made to correct for refractive effects as the laser beam transits air-fluid boundaries.

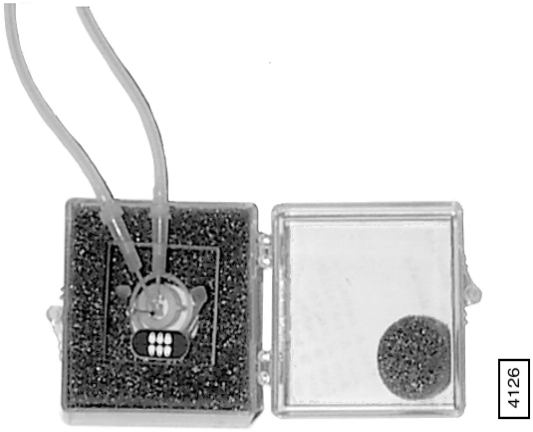
This support note describes Contact Mode and TappingMode AFM operation of the MultiMode SPM in fluid, including loading the probe into the probe holder, mounting the probe holder into the head and aligning the laser on the cantilever portion of the probe and then engaging the probe tip with the sample.

This support note assumes familiarity with Contact Mode AFM operation of the MultiMode in air. If you are not familiar with air operation of the MultiMode, refer to the procedures outlined in your *Multimode SPM Instruction Manual* before attempting to operate the AFM with a fluid cell.

290.4 General Fluid Operation

The *fluid cell* consists of a small glass assembly with a wire clip for holding an AFM probe. The glass surfaces provide a flat, beveled interface so that the AFM laser beam may pass into the fluid without being distorted by an unstable fluid surface.

Figure 290.4a: Tapping Mode Fluid Cell in Case



290.4.1 Clean Fluid Cell and O-ring

To reduce contamination problems and to obtain high-quality images, clean the fluid cell, and O-ring if applicable, as follows:

1. While soaking the fluid cell and O-ring in warm, soapy water, place a few drops of liquid dish soap on them.

2. Gently rub the fluid cell and O-ring with a cotton swab or finger. Avoid scratching the glass surface with abrasive material.

3. Using distilled water, rinse the fluid cell and O-ring of all soap.

4. Using 0.2mm-filtered, compressed air or dry nitrogen, blow dry the fluid cell until all moisture evaporates.

290.4.2 Select the Probe

AFM probes featuring low stiffness cantilevers produce the best results for biological applications. We recommend sharpened tips to start: 100µm ("short"), V-shaped cantilevers with oxide-sharpened silicon nitride tips; for example, models NP-S (standard) or NP-STT (oriented twin tip). Stiffer etched silicon probes (model FESP, single beam, 225µm long) used at very small oscillation amplitudes also obtain good results. Experiment to find which probes work best for your sample.

Note: For additional information on selecting a probe, please refer to the Digital Instruments/Veeco Metrology Group website, www.di.com.

290.4.3 Remove Organic Contamination from the Tip

Contaminants on the tip may limit AFM resolution. You may use ultraviolet (UV) light to remove contaminants, as follows:

1. Place the fluid cell with installed tip face-up on a clean surface.
2. Position a UV lamp very close (3-5 mm) to the fluid cell and irradiate the probe for 15-30 minutes at full intensity.

290.4.4 Load the Fluid Cell with a Probe

The probe is held in a small pocket on the bottom side of the fluid cell by a gold-plated, stainless steel wire clip. A tiny coil spring mounted on the top of the fluid cell holds the wire clip against the probe.

Load a probe into the fluid cell by performing the following procedure:

1. Turn the fluid cell upside down, and gently raise the wire clip by pressing from beneath.

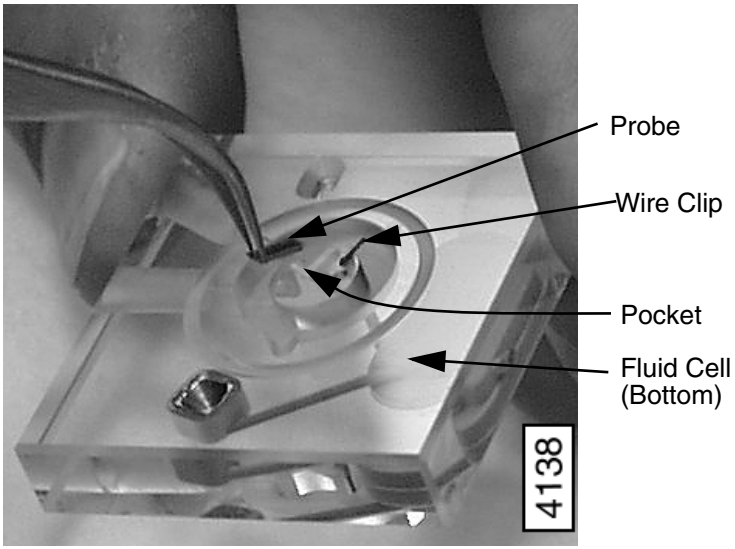
   Note: Do not press harder than enough to completely compress the spring!

2. With the wire clip raised, use tweezers to slide a probe into the pocket. Lower the clip to hold the probe (see Figure 4b).

3. Verify that the probe is squarely set against one side of the pocket and flush against the back. Verify the probe is held firmly by the wire.

ATTENTION: Avoid scratching the fluid cell glass surface with the tweezers or the probe, especially in the area under the probe.

Figure 290.4b: Load Probe into Fluid Cell



290.4.5 Sample Mounting

Secure a sample support (e.g., mica) to a magnetic stainless steel sample puck. Supports may be secured to the puck with epoxy. Select epoxy as follows:

- For non-critical applications, use Devcon 2-Ton Epoxy or 5-Minute Epoxy.
- For applications where contamination control is more critical, use a more inert, solvent-free epoxy such as Master Bond EP21LV or EP21AR or a hot melt adhesive.

  Note: Follow the manufacturer's directions for mixing and curing to obtain the best resistance to leaching and chemical attack.

- For lower-resolution applications requiring quick imaging of samples without waiting for adhesive to cure, hold the sample in place using double-sided tape.

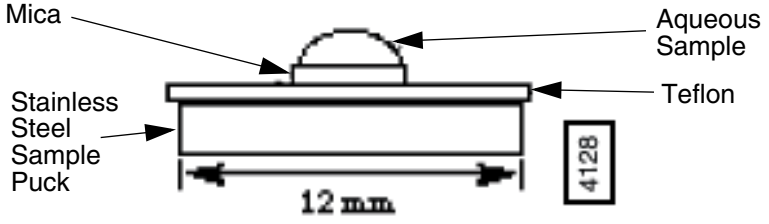
ATTENTION: Do not use cyanoacrylate glue (e.g., Superglue) for mounting samples in fluid.

Limit risk of leakage as follows:

- Use a sample support larger than the puck.
- Use the recommended adhesive Loctite 770 to affix a Teflon cover over the steel sample puck. The Teflon should extend slightly over the edge of the sample puck. Use epoxy to attach the sample support to the Teflon.

The hydrophobic Teflon helps confine the solution without installing an O-ring (see Figure 4c).

Figure 290.4c: Stainless Steel Sample Puck with Teflon Cover



You may load the sample on the support now, or you may inject it when the fluid cell is installed inside the AFM head.

In any case, limit the volume of liquid in the fluid cell to 30-50µl, if possible, to limit thermal drift.

Method 1 (with an O-ring)

1. Install the protective O-ring into the fluid cell.

   a. Insert the O-ring into the recessed groove in the underside of the fluid cell. The O-ring slides up into the recessed groove.

      Note: The O-ring protects the AFM scanner tube from spilled liquids.

   b. Position the O-ring so that it forms a seal around its periphery and does not overlap any edges.

CAUTION:	Do not add excessive amounts of fluid or the cell will overflow and damage the piezo tube scanner.

ATTENTION:	N'ajoutez pas trop de liquide. Sinon, la cellule débordera et le liquide endommagera la céramique piézo-électrique.

2. Pre-wet the fluid cell.

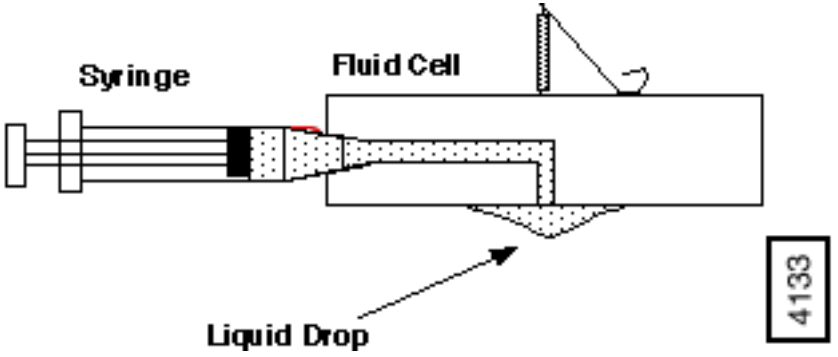
Occasionally air bubbles form in the fluid cell and block laser light. Reduce the chance of forming bubbles as follows:

a. Before installing the fluid cell into the head, insert a syringe filled with liquid solution into a fluid port, or connect the syringe to the inlet tubing on the fluid cell.

b. Push enough fluid through the fluid chamber to flood the fluid cell port with buffer solution, allowing liquid to drip out the bottom of the cell.

c. Leave the buffer-filled syringe inserted. A small amount of buffer solution should be held to the bottom of the cell by surface tension (see Figure 4d).

Figure 290.4d: Flush the Fluid Cell Before Installation to Reduce Bubble Formation



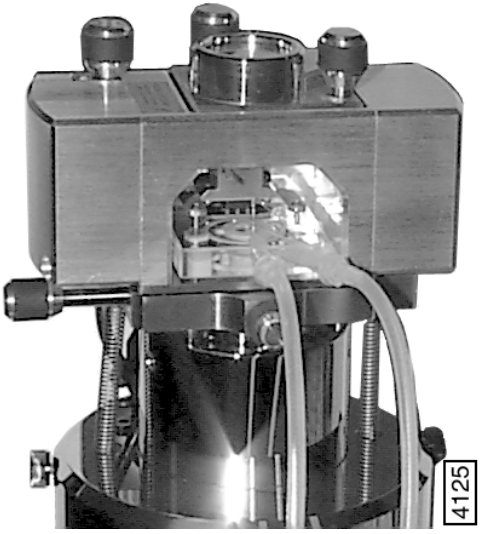
3. Install the sample or sample support in the fluid cell.

4. Carefully install the fluid cell in the AFM head, lowering the fluid cell on the O-ring until seated securely.

5. Tighten the clamp to hold the fluid cell in place, making certain that the O-ring positions properly between the sample and fluid cell.

Note: If the O-ring shifts, remove the fluid cell and try repositioning the O-ring. If shifting continues, see Chapter 15 of the *Multimode SPM Instruction Manual* for help. Try clamping the outlet tube, though it is not necessary for operation and may increase noise in the image.

Figure 290.4e: Fluid Cell Inserted into Microscope Head



6. Verify the head is leveled side-to-side and that the head is tilted slightly forward, so the tip is level when it contacts the surface.

   Note: If you are using a vertical-engage scanner, leveling problems are eliminated.

7. Fill the fluid cell with liquid.

   a. Attach a drain line to the other fluid cell port.

   b. Slowly flush the fluid cell with buffer solution from the syringe.

      - Check for leaks and wick away any spilled liquid with filter paper until AFM components are dry.

CAUTION: Do not add excessive amounts of fluid or the cell will overflow and damage the piezo tube scanner.

8. Remove bubbles and clamp-off fluid cell lines.

Bubbles inside the fluid cell near or on the probe can interfere with the laser beam. Remove them by performing the following procedure:

a. Observe the fluid cell and probe through the viewing port using an optical microscope.

b. Rapidly push liquid through the cell with a syringe. If sufficient force is applied, the bubbles will be carried out of the fluid cell.

> **Note:** To prevent spillage and the introduction of air, maintain a good seal between the syringe and fluid cell port.

c. Clamp off the drain line with a pair of hemostats or similar clamp.

9. Adjust the laser aiming (if necessary).

a. If the presence of fluid causes the laser beam to refract, slightly adjust the laser aiming screw, to move the laser spot onto the end of the cantilever.

b. If air bubbles become trapped near the probe, interfering with the laser beam path, use the syringe to force liquid quickly through the cell to break the bubbles loose.

10. **Troubleshooting;** symptom: drift in AFM image. Cause: O-ring slides across sample surface.

Set up the fluid cell so there is minimal lateral movement of the optical head with respect to the sample once the O-ring is installed. Keep the head level while positioning the tip close to the surface, to minimize tip lateral motion during engagement. Other countermeasures:

a. Unlike three-point supports, the vertical engage scanner allows the tip to approach samples without lateral offset, eliminating stress on the O-ring during engagement. For more detail, call Digital Instruments/Veeco Metrology Group.

b. Lightly coating the area of the O-ring which contacts the sample surface with white petrolatum or vacuum grease allows the O-ring to slide across the surface, minimizing lateral stress. This also forms a fluid-tight seal between the O-ring and sample. However, some

solvents (i.e., nonpolar organic solvents) may dissolve some of the lubricant into the fluid.

c. Substitute an alternative for the O-ring:

- Replace the O-ring with a slice of thin-walled glass, plastic or stainless steel tubing.
- Choose the diameter and thickness of the ring of tubing so that it does not contact the inner or outer walls of the circular groove in the fluid cell. This allows the optical head to move laterally during engagement and helps in positioning the tip over the sample surface.
- Choose the length of the ring of tubing so that it is sufficient to reach the sample surface and to reach the bottom of the fluid cell before engagement.
- Glue the ring of tubing to the steel sample puck or to the sample to prevent leaks.

d. When positioning the fluid cell over the sample surface, adjust the positioning knob at the base of the optical head to move it slightly forward. This will counter some of the lateral stress on the O-ring resulting from the optical head moving back during engagement.

Method 2: Without an O-ring

In many cases, it is possible to image a sample under a drop of fluid without use of a closed fluid cell. This technique is recommended for aqueous buffers, when evaporation is not an issue; (for example, in short experiments and those involving no use of volatile solvents).

WARNING: Without the O-ring, this method poses a potential spill hazard to microscope electronics and must always be undertaken with extreme caution.

Two variations of this method are possible: starting with a dry sample and starting with a sample in solution.

1. Complete the following if starting with a dry sample:

a. Attach the sample support (e.g., mica) to a puck (see Section 290.4.5).

b. Deposit your sample on the support.

> **Note:** It is easier to first align the AFM head and mounted fluid cell (probe installed) with the dry sample puck before installing the liquid-coated sample. Allow 0.5mm clearance between the tip and dry substrate surface.

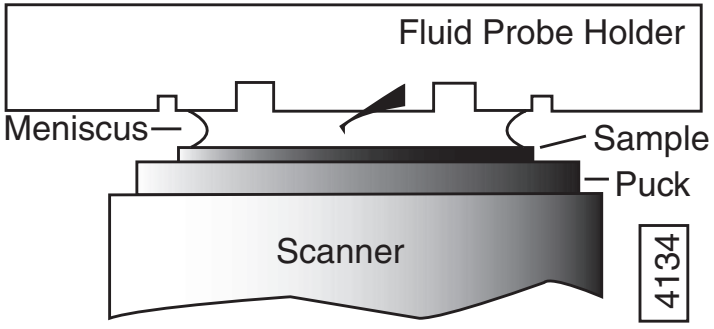
c. Align the optics and laser (see also Section 290.4.6).

- For coarse adjustments, tilt the deflection mirror.
- For fine adjustments, adjust the photodetector knobs on the left-top and left-rear of the head (See Section 290.4.7).
- Adjust the photodiode position for a deflection signal of `0V`.

d. Using a syringe, inject the fluid (buffer) underneath the fluid cell.

> **Note:** The liquid is held by surface tension between the sample surface and the fluid cell (see Figure 4f). Because the aqueous sample changes the light deflection, the laser photodetector must be re-adjusted.

Figure 290.4f: Imaging a Sample Covered by a Drop of Fluid



2. Complete the following if starting with a sample in solution:

a. Incubate 30-40µl of your sample on the support mounted on the puck (the liquid should form a small dome over the support). During this incubation the sample should adhere to the support (e.g., mica).

b. Install the sample support in the AFM head.

c. Carefully install the fluid cell inside the head.

- Inspect from the front to make sure the fluid is well confined to the mica area only.
- Aim the laser on the cantilever and adjust the photodiode detector position (see Section 290.4.7).

> **Note:** Always verify that the microscope is dry and that all MultiMode surfaces are free of spilled fluid. Wick away moisture and droplets with filter paper.
>
> **Note:** Over time, evaporation of the fluid may necessitate replenishing the fluid cell using a standard micropipette or syringe.

290.4.6 Align the Laser

Use either of the techniques for aligning the laser on the probe in the Digital Instruments/Veeco Metrology Group *Multimode SPM Instruction Manual*. (Refer to Chapter 5, Section 5.2, *Laser Alignment*.) The following considerations apply:

- Refraction causes the laser beam path to bend slightly entering and exiting the fluid surrounding an immersed probe as compared to the path in air. However, the basic process is essentially the same.
- In the fluid cell, the probe rests flat on an angled, glass surface. This surface produces a false laser reflection, even when the laser is not aimed at the probe. This reflection from the glass surface does not affect operation of the MultiMode, but it can be a source of confusion when aligning the laser. Ignore this faint reflection and focus on the much brighter reflection from the cantilever.
- The **SUM** signal on the display monitor typically displays less than `1V` when the laser is not aligned on the probe. The **SUM** signal should rise well above `1V` when the laser is reflecting off the probe.

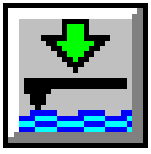
290.4.7 Adjust the Detector Offsets and Setpoint (Contact Mode)

Adjust the detector mirror adjustment screws:

- In Contact Mode (i.e., **Other Controls/MICROSCOPE MODE** set to **CONTACT** and **Feedback Controls/SPM FEEDBACK** set to **DEFLECTION**), to achieve a vertical deflection signal of roughly `-1.0V`. Set the **Feedback Controls/DEFLECTION SETPOINT** to `0V` to begin.
- The difference between the vertical deflection signal before engaging and the setpoint determines the amount of force that the probe applies to the sample.
- Typically, samples are softer in liquid than in air. Before engaging, verify that there is not so large a difference between the setpoint and the vertical deflection signal as to damage the sample.
- Verify reasonable values for scan parameters (e.g., **SCAN RATE**, **SCAN SIZE**, **INTEGRAL GAIN** and **PROPORTIONAL GAIN**).
- Once engaged, reduce the setpoint to minimize the tracking force.

290.4.8 Engage the Surface

1. Using the coarse adjustment screws and **MOTOR DOWN** switch, lower the tip until it is just above the level of the sample surface.
   - The **MOTOR DOWN** switch is located on the MultiMode base.
   - Watch the engagement closely through the 25× magnifier or using the vertical optical microscope.
2. In the **Real Time/Motor** menu, click **ENGAGE**, or click the **Engage** icon (shown).



   - The motor begins to move the AFM head and probe down to the sample.
   - When the tip reaches the surface, the system should automatically stop, beep, and begin to image the sample.

- In Contact Mode, if the system engages immediately or before the tip reaches the surface, try increasing the **SETPOINT** approximately **2.0V**, then repeat step 2.

290.4.9 Adjust Scan Parameters (Contact Mode)

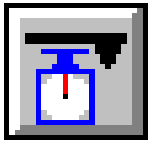
1. Once engaged, adjust the scan parameters to obtain the best image.

   Note: This procedure is similar to operation in air, except that samples are often softer in fluid. Adjusting the applied force can be critical.

2. To avoid sample damage, reduce the **DEFLECTION SETPOINT** as low as possible:

   a. Stop when the tip pulls off the surface and the **Z CENTER POSITION** on the display monitor jumps to **LIMIT** (**-220V**).

ATTENTION: Adjust the setpoint using the numeric keypad on the keyboard. Do not use the arrow keys.



3. Increase the setpoint until the tip begins to touch the surface again and an image appears.

   - As an alternative, use the **Force Calibration** command to select the setpoint and estimate the contact force (refer to Chapter 11 of the *Multimode SPM Instruction Manual*).
   - Because the tip typically adheres to the sample surface much less in fluid, it is possible to image at much smaller contact forces in liquid than in air.
   - The optimal integral and proportional gains and scan rate may be different from air operation, because the dynamics of the cantilever change in fluid.

4. Set the two gains as high as possible (starting with the integral gain) without causing oscillation distortion to appear in your image.

5. Choose a scan rate that is sufficiently slow to image without degrading your data.

290.4.10 Clean and Dry Parts When Done

1. When sample imaging is complete, drain the fluid cell and carefully remove it from the head. Avoid spilling fluid.

2. Rinse and dry the fluid cell, and O-ring if applicable, to prevent the buildup of salts or other contaminants on these parts.

ATTENTION: When cleaning the fluid cell, use care to avoid scratching the glass surfaces in the center of the fluid cell.

290.5 TappingMode in Fluids

Operation of TappingMode in fluid provides the same advantages of TappingMode in air, with the additional ability to image samples under native liquid conditions. In fluid TappingMode, the probe is oscillated so that it only intermittently contacts the sample surface. This can reduce or eliminate lateral forces that can damage soft or fragile samples in Contact Mode. The following sections provide general instructions for TappingMode imaging in fluid.

Note: Before attempting TappingMode in fluids, it is recommended that the user becomes familiar with standard TappingMode operation in air and contact AFM in fluid (refer to Chapter 7 in the *Multimode SPM Instruction Manual*).

290.5.1 Procedure for TappingMode Imaging in Fluid

1. Load the sample in the AFM and fluid in the fluid cell (see Section 290.4.5).

2. Align the laser on the free end of the cantilever portion of the probe.

3. Center the photodiode to give a deflection signal near **0V**.

4. Choose the TappingMode operation in software.

   - Set **Other Controls/MICROSCOPE MODE** to **TAPPING**.

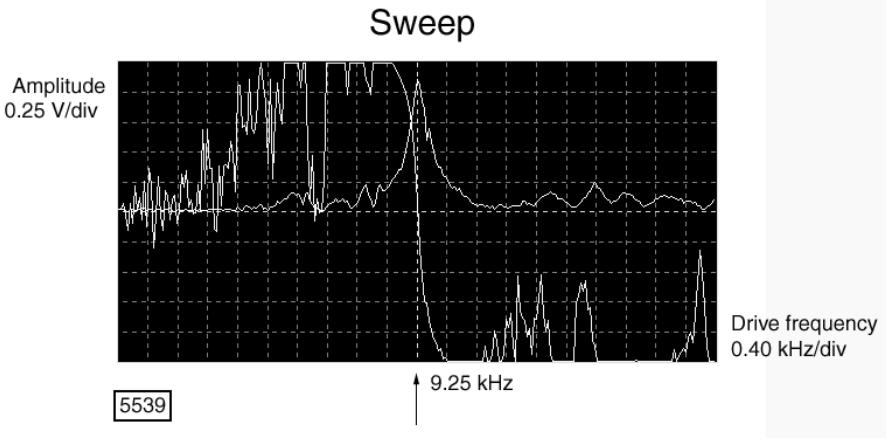
5. Set the initial scan parameters, using the following settings:

   a. Enter the **View/Sweep/Cantilever Tune** menu to select a drive frequency.

   - This is similar to the Cantilever Tune process used for standard TappingMode in air. Unlike operation in air, the cantilever resonance will be largely damped by liquid and the AutoTune function cannot be used.
   - When viewing a wide bandwidth cantilever tune sweep, observe that there is no single, well-defined resonance peak, but, rather a number of broader maxima. Manually select a peak.

> **Note:** For the short, narrow Si_3N_4 probe recommended for soft samples (e.g., models DNPS or OTR4), the resonant frequency in fluid is a broad peak centered around 10kHz (see Figure 5a). Best results are achieved by tuning the cantilever to a peak between 7-12kHz. Higher and lower frequencies have also been used depending on the type of probe employed. Start with a **SWEEP WIDTH** of **20kHz** and a **DRIVE FREQUENCY** of **9kHz** in the **Sweep Controls** menu.

Figure 290.5a: A 100µm, Narrow-legged, Si_3N_4 Cantilever Fluid Tune Curve



- Manually adjust the **ZOOM IN** and **OFFSET** functions above the Cantilever Tune display.

ATTENTION: If the expected peak does not appear in the spectrum, choose another peak, engage on the surface and disengage immediately. With the tip closer to the surface, the peak at 8 to 9 kHz appears.

b. Adjust the **DRIVE AMPLITUDE** until a desired probe RMS amplitude is obtained.

- An RMS amplitude of **0.5V** is appropriate for soft samples and typically results from a **DRIVE AMPLITUDE** of **250-500MV**. A **DRIVE AMPLITUDE** >**1.0V** generally works poorly with soft samples.
- For rougher samples, target **1-2V** RMS amplitude. Experiment to find what level of probe oscillation gives best results for specific applications.

ATTENTION: The RMS amplitude must be adjusted when the AFM tip is near the sample surface (<50µm).

c. Set the **SCAN RATE** to **1Hz**.

d. Set the **INTEGRAL GAIN** to **0.5**.

e. Set the **PROPORTIONAL GAIN** to **0.75**.

f. Set the **Z RANGE** to **50 NM**.

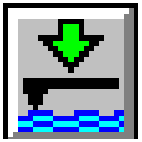
g. Select the Channel 1 **Data type** as **HEIGHT**.

h. Select the Channel 2 **Data type** as **AMPLITUDE**.

i. Optional: Select the Channel 3 **Data type** as **PHASE**.

- Adjust and optimize these settings for each imaging condition and sample.
- Verify that the tip is very close to the sample surface when tuning the probe under fluid.

6. Center the laser spot on the photodiode detector.
    - Adjust the photodiode until deflection is roughly zero.
    - The deflection signal can drift when the probe is first in fluid, so it is best to adjust just prior to engaging.



7. Click the **Engage** icon (shown) to bring the tip into tapping range.
    - The NanoScope software automatically selects a setpoint, then stops the engagement when the surface is detected.
8. Adjust the setpoint when engaged.
    - The best topographic images are usually obtained at setpoints 10-20 percent less than the RMS amplitude before engaging.
    - The setpoint may be optimized using the **FORCE CAL** (**View/Force Mode/Calibrate**) command and by optimizing the image quality. Both techniques are described in the next section.

290.5.2 Plotting Amplitude vs. Distance (Force Cal Curve)

The **View/Force Mode/Calibrate** command plots the cantilever oscillation amplitude versus the sample position. The curve shows a mostly flat region where the probe has not yet reached the surface and a sloped region where the amplitude is being reduced by the tapping interaction.

1. Set up **Force Calibrate** as described for TappingMode in air (experienced users may prefer the **Force Step** command instead).

CAUTION: To protect the tip and sample, do not reduce the cantilever oscillation amplitude to zero.

2. Adjust the setpoint until the green setpoint line on the graph is just barely below the flat region of the Force Cal curve.
    - This setpoint applies the lowest force to the sample.

Note: The slope of the Force Cal curve during probe interaction with the sample surface is defined as the *sensitivity* of the fluid TappingMode measurement. In general, higher sensitivity results in better image quality. If the sensitivity is poor, check the mounting of the sample and fluid cell.

290.5.3 Optimizing Image Quality

Adjust the setpoint by monitoring image quality, as follows:

1. Select a **SCAN SIZE** of **500 NM**.
2. Increase the setpoint in small increments until the probe pulls off the surface and the **Z CENTER POSITION** voltage goes to retracted (i.e., **-220V**).
3. Reduce the setpoint in small increments until an image appears.

ATTENTION: Increase or reduce the setpoint by typing numerical voltage values with the keyboard. Do not use the keyboard arrow keys.

4. Continue reducing the setpoint until the image is optimized.
   - The best images are obtained at setpoints just below where an image appears.
   - The NanoScope Controller attempts to keep the cantilever oscillation amplitude constant during the scan.
5. Optimize the **INTEGRAL GAIN** and **PROPORTIONAL GAIN** so the **HEIGHT** image shows the sharpest contrast and there are minimal variations in the **AMPLITUDE** image (the error signal).
6. Optimize the **SCAN RATE** to obtain the sharpest image.

290.6 Troubleshooting Tips

290.6.1 Cantilever Tune Plot Looks Poor: Loose Probetip

The **Cantilever Tune** plot can be used as a diagnostic tool. Become familiar with its characteristics when good images are obtained. If the plot looks substantially different from previous successful experiments, there may be a problem with the fluid cell. For example, the probe may be loose in its holder.

Check the clip which holds the probe in place, and verify the probe is not loose. Contact Digital Instruments/Veeco Metrology Group for assistance if needed.

290.6.2 Laser Sum Signal Absent or Weak: Air Bubbles

Verify that all bubbles are removed from the probe. Bubbles may attach themselves to the probe, causing the laser beam to be diffracted. While bubbles can sometimes be removed by forcing fluid through the fluid cell, it is often necessary to dry the fluid cell with absorbent paper (e.g., Kimwipe).

Degassing your imaging fluid prior to use in the AFM reduces bubble problems.

290.6.3 Poor Image Quality

Contaminated Tip

Some types of samples (e.g., certain proteins) may adhere to the cantilever or tip. This reduces resolution, resulting in blurred images.

If tip contamination is a problem, it is necessary to protect the tip against contamination. There are two ways this may be accomplished:

1. If the sample is adhered to a surface through absorption (e.g., diffusion of protein onto mica):

    a. Diffuse the sample substance into the substrate, then rinse with buffer.

    b. Lower the tip into a fluid containing little or no stray substances which may adhere to the tip.

2. If the sample is short-lived and must be imaged quickly, it may be possible to mask the tip against contamination by bringing the tip into gentle contact with an uncontaminated substrate surface:

    a. Put the MultiMode into Contact Mode: set **Other Controls/ MICROSCOPE MODE** to **CONTACT** and engage the substrate surface using a **ZERO SCAN SIZE**.

    b. While the tip is kept in gentle contact with the substrate surface, add the sample substance to be imaged and allow it to diffuse/settle onto the substrate.

    c. After a diffusion/settling period has lapsed, quickly lift the tip from the substrate surface.

    d. Switch **Other Controls/MICROSCOPE MODE** to **TAPPING** and image the sample before it becomes contaminated.

Dull Tip

Change to a new probe. AFM probe tips can become dull during use and some unused tips may be defective. Check the probe type being used. Oxide sharpened silicon nitride probes are usually much sharper than standard silicon nitride probes.

Multiple Tip

Change or clean the AFM tip. Probes can have multiple protrusions at the apex of the tip, which result in image artifacts. Features on the surface appear two or more times in an image, usually separated by several nanometers.

290.6.4 Lost Particulate Samples: Attracted to Cantilever

Some particulate samples such as proteins may prove difficult to find directly beneath a cantilever if the cantilever has remained stationary during a diffusion or settling period. This may be due to the fact that some types of particulates are more attracted to the cantilever than to the substrate intended to support them. The result is a “shadow” on the substrate directly beneath the cantilever where fewer sample individuals can be located; they are stuck to the cantilever instead. If you suspect this problem, shift the imaging site to a new location away from where the probe had remained motionless.