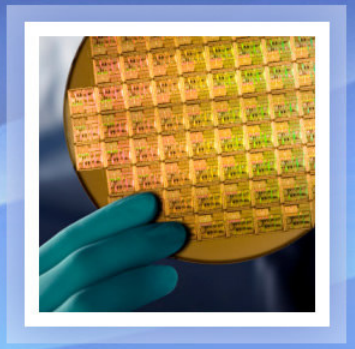






Icon Service Training: Imaging in Fluid

January 22, 2009
Santa Barbara, CA

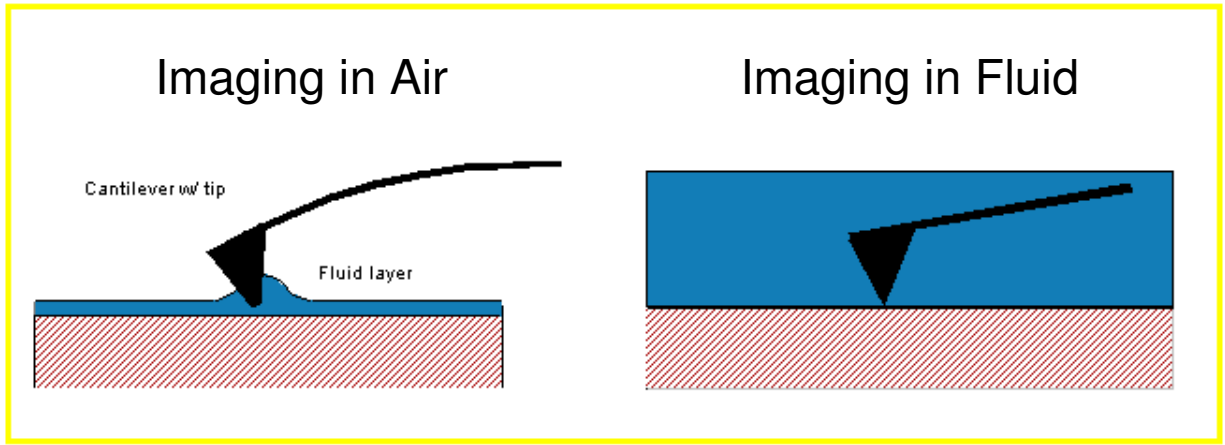
Air Vs. Fluid Imaging

IN AIR:

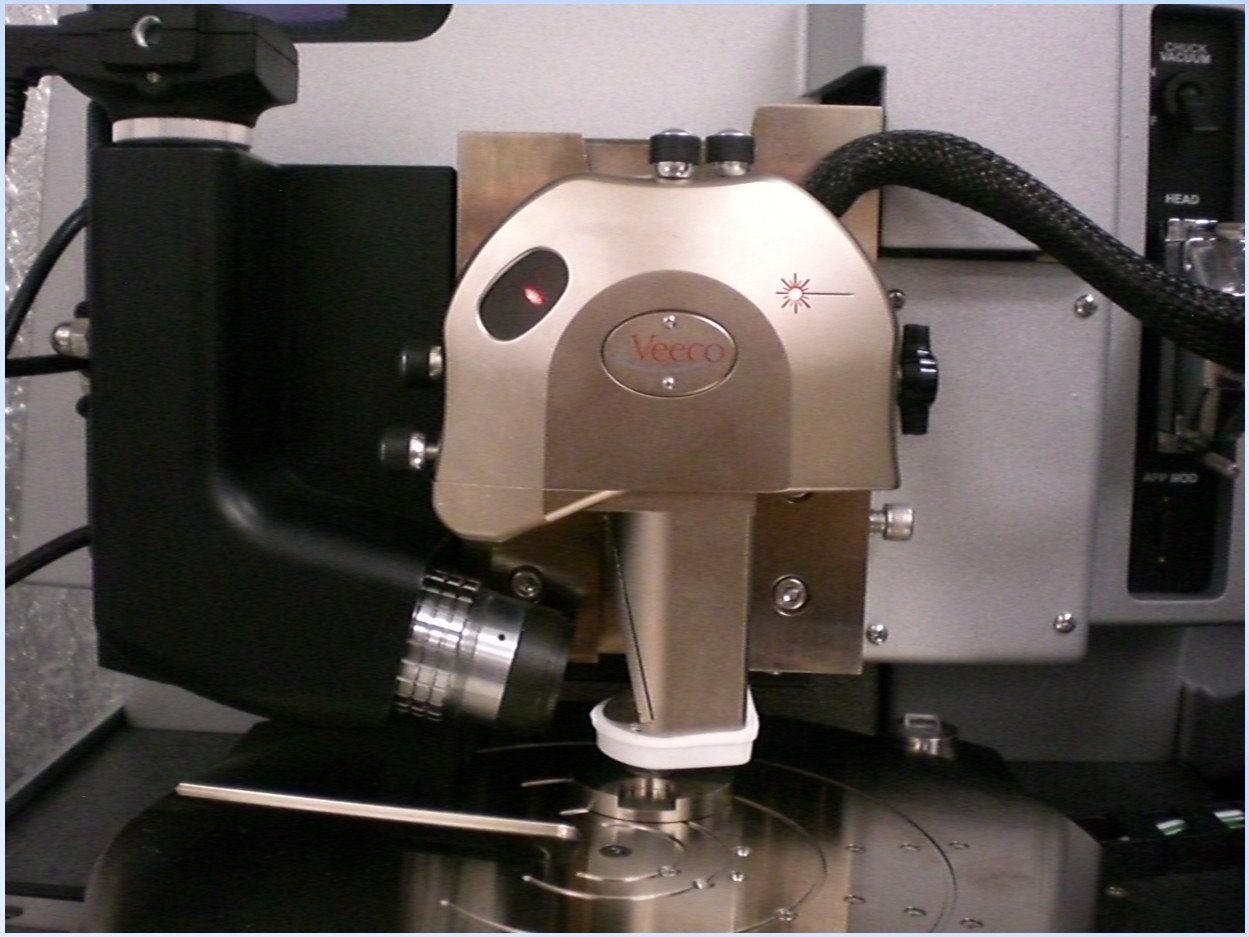
- Increased imaging (vertical) force due to:
  - Surface tension of adsorbed fluid layer
  - Electrostatic forces
- Shear (lateral) forces can distort/damage soft samples

IN FLUID:

- Reduces surface tension (meniscus) forces
- Partial reduces shear forces (contact mode)
- Allow measurements in native environment (many polymer and majority of biological samples)



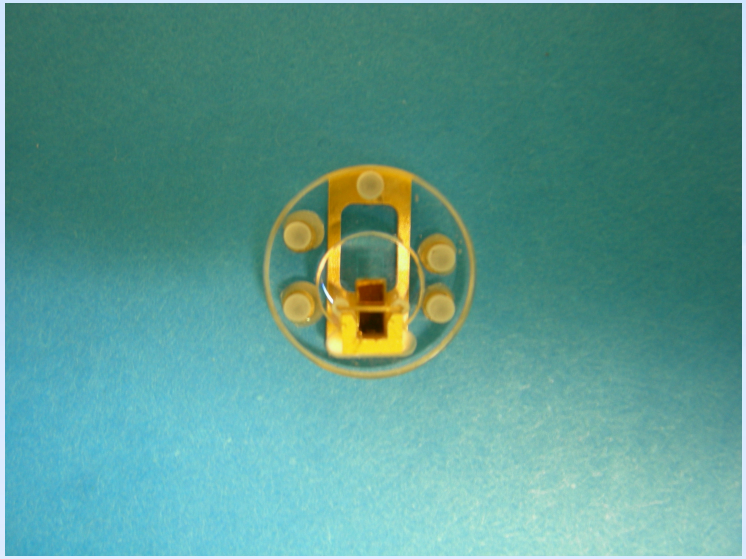
Icon Scanner with Direct Drive Fluid Cell



The splashguard is placed on the end of the scanner to protect the piezo tube from potential damage by fluid.

Icon Fluid Cantilever Holders

Z Modulation Fluid Cell With Side Port (DTFML-PC)

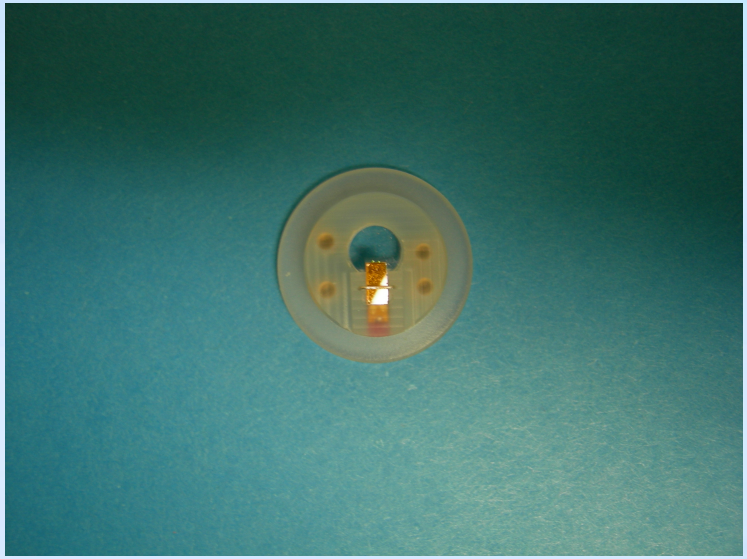


Uses Z Modulation to oscillate the Z tube scanner

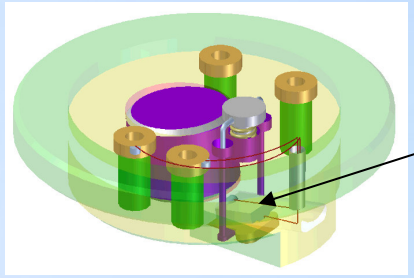
The side port allows access to samples for:

- routing electrode wires for electrochemistry experiments
- delivering fluids or for patch-clamping experiments

Direct Drive Fluid Cell (DTFML-DD)



Uses piezo in cantilever holder to oscilate the probe

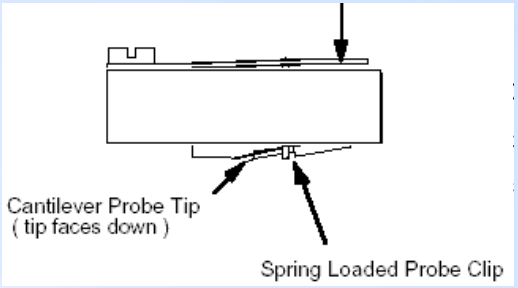


Icon Setup for Fluid Imaging

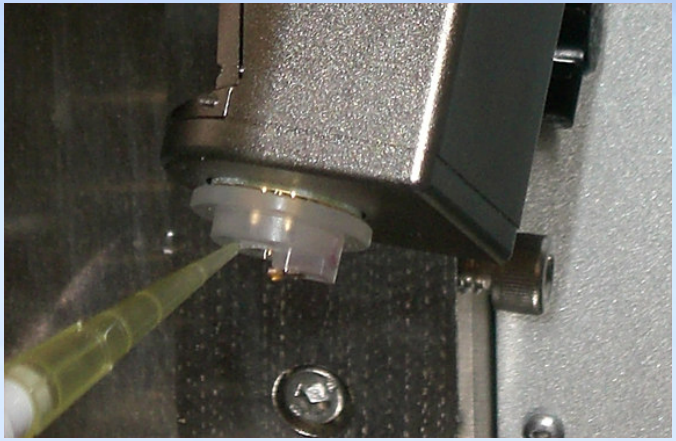
1. Place probe in fluid cell, mount on scanner and put scanner splashguard in place.
   (NOTE: For AFM studies in fluid, it is very important that the fluid cell is clean. See the accompanying document; ***'Cleaning Procedures for Tips and Cantilever Holders – BioScope II'*** for recommended cleaning methods.)

2. **HINT!** Align laser and set up the tip-sample separation while DRY, if possible.
   i. Bring probe close to dry sample surface
   ii. Align (locate tip)
   iii. Navigate (focus surface)

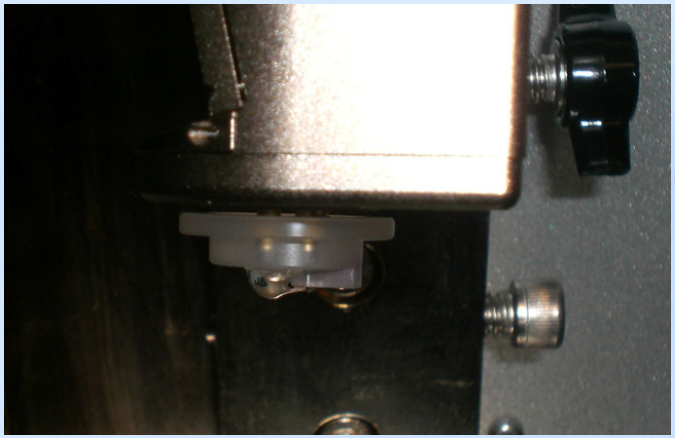
3. Remove scanner, place a droplet of imaging fluid on the sample and on the cantilever
   – Why on cantilever?
      • Avoids air bubbles
      • Minimizes problems with cantilever interaction with the top of a drop



Placing Droplet of Fluid on Cantilever

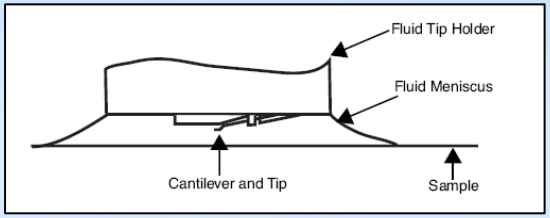


droplet
of fluid



Icon Setup for Fluid Imaging (continued)

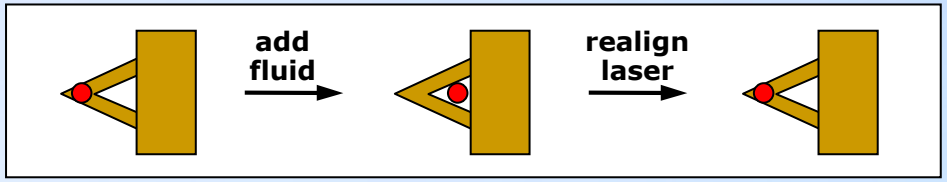
4. Put the scanner back in place. This will produce a meniscus of fluid between the sample and fluid cell. Add more fluid, if needed, to form an adequate-sized fluid droplet. Remember: the fluid will evaporate over time.



5. Realign the laser position on the cantilever.

   Due to the change in the refractive index of the imaging fluid vs. air, the laser spot will now be focused at a point behind the end of the cantilever. The change in refractive index will also alter the focus of the camera optics and you will no longer see your cantilever clearly.

   DO NOT refocus the camera to see the cantilever and the sample. This will affect the tip-sample distance (as they will appear further away in fluid) and the system will false engage. Simply rotate the horizontal laser positioning knob COUNTERCLOCKWISE until your maximum sum signal is regained. Note: The sum signal will be higher in fluid than in air.



6. Adjust the photodetector, tune the cantilever (if operating in TappingMode™), and engage the tip on the surface.

Icon Setup for Fluid Imaging (continued)

IMPORTANT: There are samples that will need to be prepared and maintained under wet conditions for AFM imaging. This will prevent you from setting up the Icon system in air (i.e. tip-sample distance) prior to adding fluid.

Use the following setup procedure for these samples:

1. Place probe in fluid cell, mount on scanner and put scanner splashguard in place.

2. Align the laser on the cantilever and place a droplet of fluid on the cantilever.

3. Mount the scanner and lower it until the droplet of fluid on the cantilever joins and forms a meniscus with the fluid on the sample.

4. Realign the laser position on the cantilever (same as previously described on Slide #7, Step #5).

5. Refocus the camera on the tip and the sample surface.
    i. Align (locate tip)
    ii. Navigate (focus surface)

Icon Setup for Fluid Imaging (continued)

6. As mentioned the change in the refractive index due to the presence of the fluid alters the optical path. As such, the tip and the sample will appear further apart than they actually are. To keep the system from false engaging upon approach, offset the focus position in 'Navigate' by ~300 µm to a point below the surface when focusing in fluid.

   Example: if the sample surface is in focus at a Z-motor position of -5000 µm, then you must move the Z-motor down to -5300 µm.

7. Adjust the photodetector, tune the cantilever (if operating in TappingMode™), and engage the tip on the surface.

Quick Start: Contact Mode in Fluid

- Contact Mode in fluid.
  1. Install cantilever substrate; ensure it is seated at the correct angle.
  2. Align the laser on the cantilever in air.
  3. Insert liquid, readjust laser spot on cantilever and photodector position. If starting with wet sample, remember to offset the focus position by ~300 µm below the sample surface.
  4. Set gains low to avoid feedback oscillation before and after the engage. Operating in liquid may be susceptible to feedback oscillations at lower gains than imaging in air usually is, especially in TappingMode.
  5. Set Deflection Setpoint
  6. Engage
  7. Optimize imaging parameters (setpoint, scan rate, gains)

Quick Start: TappingMode™ in Fluid

- TappingMode in liquid.
  1. Install cantilever substrate; ensure it is seated at the correct angle.
  2. Align the laser on the cantilever in air.
  3. Insert liquid, readjust laser spot on cantilever and photodector position. If starting with wet sample, remember to offset the focus position by ~300 µm below the sample surface.
  4. If using the Z modulation fluid cell, make sure to enable “Z Modulation” (Show All > Feedback > Z Modulation) . IMPORTANT: The default channel in the ‘TappingMode™ in Fluid’ Experiment is ‘Height Sensor’. Change this to ‘Height’ if using the Z modulation fluid cell.
  5. Tune Cantilever.
     - Direct Drive Fluid Cell: typical frequency is 11-13 kHz
     - Z Modulation Fluid Cell: typical frequency is 8-10 kHz
  6. RMS amplitude is set depending on the sample (0.3V for fragile samples).
  7. After engage, tune again on the surface (better coupling and frequency shift).
  8. Optimize scan parameters (scan rate, setpoint, gains)

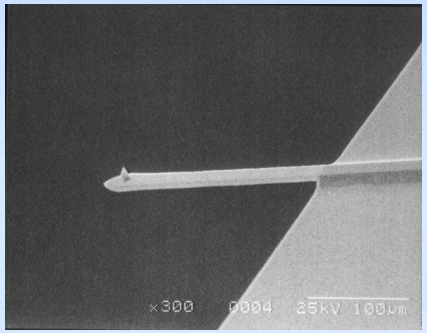
Fluid Imaging: Optimization

- Optimization of the imaging force is an interplay between:
  - Setpoint
    - The setpoint is the relative position of the tip with respect to the surface.
    - In contact mode, raising the setpoint brings the tip closer to the surface.
    - In TappingMode, lowering the setpoint brings the tip closer to the surface.
  - Scan Rate
  - Gains
    - The integral and proportional gains help to optimize the feedback loop response. The higher the gains, the faster the response. The lower the gains, the slower the response.

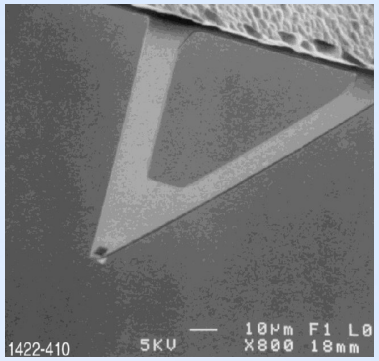
Troubleshooting

- Cantilever not held well, changes angle to photodiode, also the sample may come into contact with the cantilever substrate before the tip has a chance to reach the sample.
- Bend in the cantilever is accentuated by operation in liquid, reflected laser light does not go to photodiode.
- Change in angle of Liquid cell can disrupt laser/optical path if cantilever is positioned too far forward.
- Thermal drift due to liquid at different temperature from the microscope.
- Liquid can wick between liquid cell and latex protective skirt and short pins or scanner, check periodically.
- Change in index of refraction causes system to shift to slow part of engage too soon, can get error message.
- Air bubbles can disrupt laser path or bend the lever.
- Contaminated tips.

AFM Probe Selection For Fluid Studies



Etched Silicon Cantilever
5-10 nm



Silicon Nitride Cantilever
10 - 40 nm

			Imaging Environment		AFM Mode		
Sample Type	Probe Family/Model		Liquid	Air	Tapping	Contact	Force Curves
Biomolecules (nucleic acids, proteins, lipids, carbohydrates, etc)	Silicon	OTESPA	-	X	X	-	-
		RTESP	-	X	X	-	-
		TESP	-	X	X	-	-
Biomolecules (nucleic acids, proteins, lipids, carbohydrates, etc)	Silicon Nitride	DNP-S	X	-	X	X	X
		MSCT	X	-	X	X	X
		NP-STT	X	-	X	X	-
		OTR4	X	-	-	X	X
Cells	Silicon Nitride	DNP	X	-	X	X	X
		MLCT	X	-	X	X	X
Tissues	Silicon	TESP	-	X	X	-	-
Tissues	Silicon Nitride	DNP	X	-	X	X	X
		MLCT	X	-	X	X	X
		DNP-S	X	-	X	X	X
		MSCT	X	-	X	X	X

- ESP and FESP probes are also often used to image polymer samples in fluid.
- Resonance frequency of probes in fluid drops to 1/2 -1/3 of the resonance frequency of the probe in air.
- Resonance frequency in fluid easily identified through use of thermal tune.