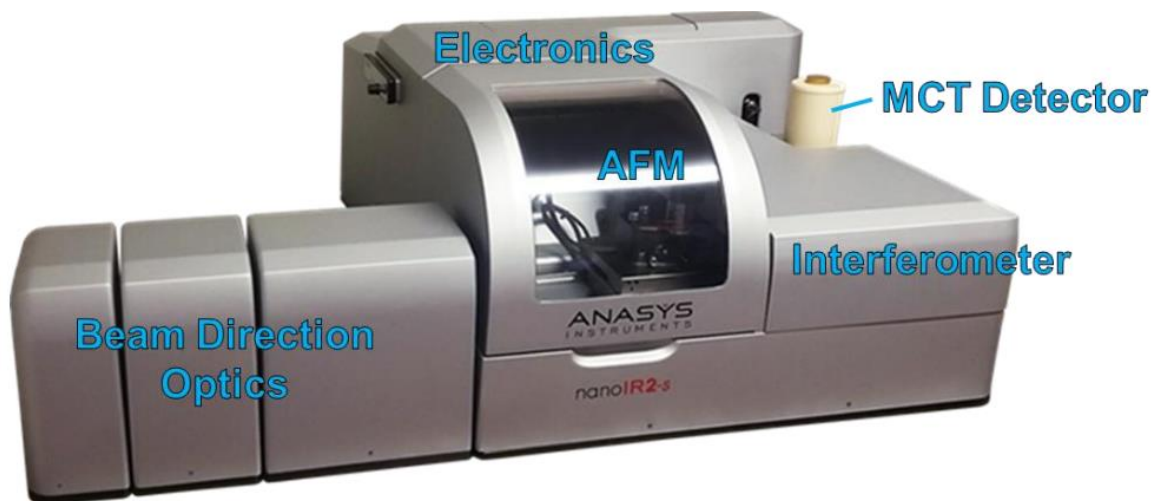


Standard Operating Procedure of nanoIR2-s

The Anasys nanoIR2 system is the AFM-based nanoscale infrared (IR) spectrometer, which has a patented technique based on photothermal induced resonance (PTIR), significantly increases the vertical sensitivity and enables spectroscopic measurements of less than 20nm thick films.



Product specifications

Motorized XY Stage Range: ~8 x 8 mm

Spatial Resolution (XY stage): <1 μm

Z Stage Range: >6 mm

Optical field of view: ~900 x 600 μm low zoom, ~300 x 200 μm high zoom

Spatial Resolution (optics): ~1.5 μm

Max Image Size: 80 x 80 μm

Z Scan Range: >5 μm

Spectral Range: 950-1900 cm^{-1}

Spectral Resolution: ~4 cm^{-1}

Spectral Acquisition Time: ~1 minute per spectrum (or less)

I Sample Preparation

I-1 Sample Placement

- Samples need to be placed or prepared on a metal disk which can be supplied by Anasys. The metal disks are magnetic stainless steel and they are held in place in the afm+ by a magnet in the sample mount.
- The measurable area of the disk is 8 mm by 8 mm, so all areas of interest must be within this region.
- Samples need to be **held down well to the metal disk** either with epoxy or double sided tape.

I-2 Sample Preparation Methods

Most samples can be imaged directly as long as they are flat, dry and rigidly held in place. For some samples, such as polymer blends or multilayer films you may need to section them to access the material of interest. This can be done by using a microtome (or cryo-microtome) to make a flat surface. If you have a sample in solution you can spin coat onto a piece of glass slide or simply deposit a droplet of material to dry (it is hard to control the thickness with this method)

I-3 Sample thickness

The thickness of the sample is limited by the Z travel of the stage which moves the AFM probe down to the sample surface. Typically this is greater than 4 mm.

I-4 Sample roughness

The smoother a sample is, the easier it will be to get good results. Generally it is best to keep the roughness below 50 nm RMS.

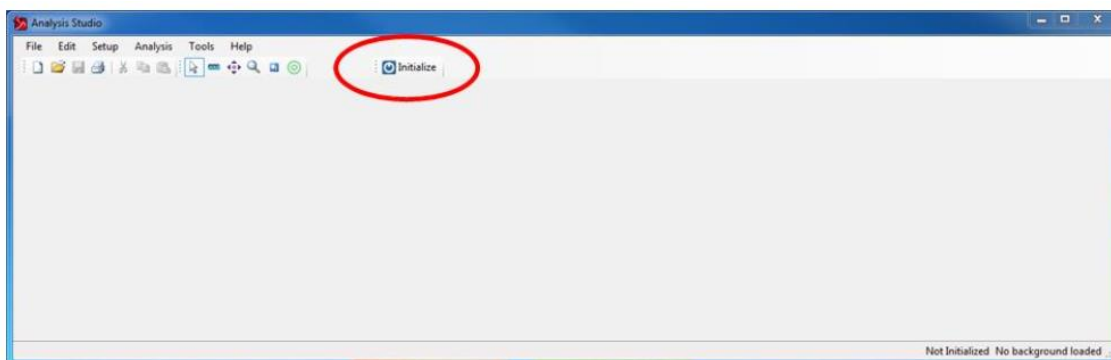
I-5 Handling/Storage

The sample must be kept clean – free of dust, fingerprints, or other contaminants as well as defects such as scratches.

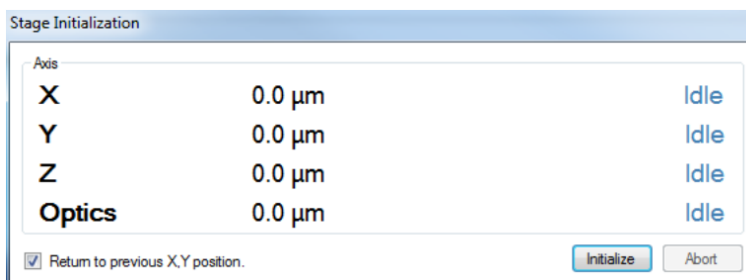
II System Preparation

II-1 Turn on the afm+ electronics via the rocker switch on the back of the Power Supply box. The power indicator lights on the fronts of the Controller and Power Supply boxes should illuminate.

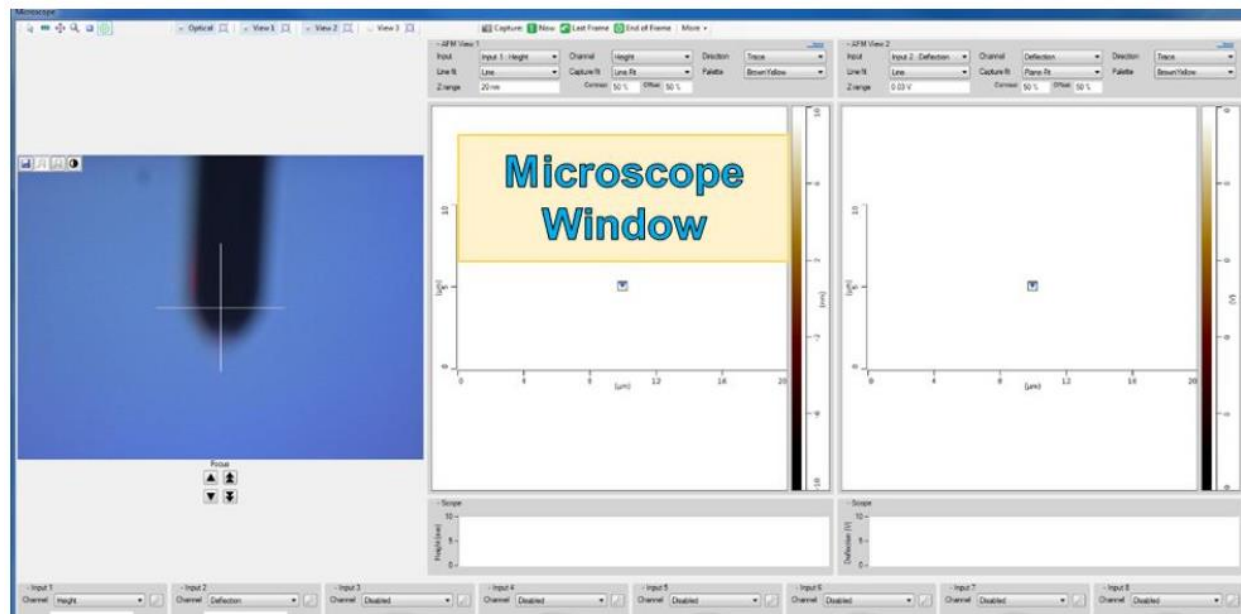
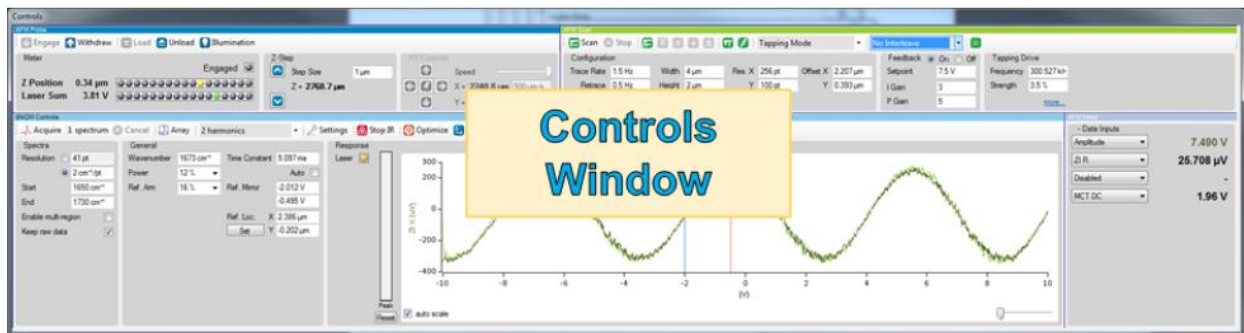
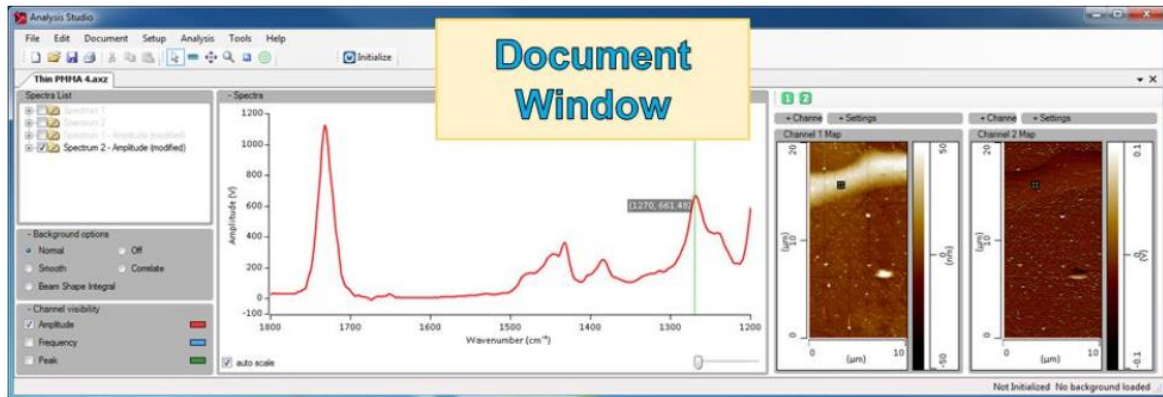
II-2 Open the Analysis Studio software by double-clicking its icon on the computer screen. The software interface should look similar to the screen below.



II-3 Initialize the system by selecting the **Initialize** icon on the top toolbar of the software. This operation verifies communication between all the components and readies the hardware and software for use. The bottom status bar will change from “Not Initialized” to “Idle” when initialization is complete.



After initialization is complete, the 3 main windows of Analysis Studio are open: the Document, Controls, and Microscope windows.

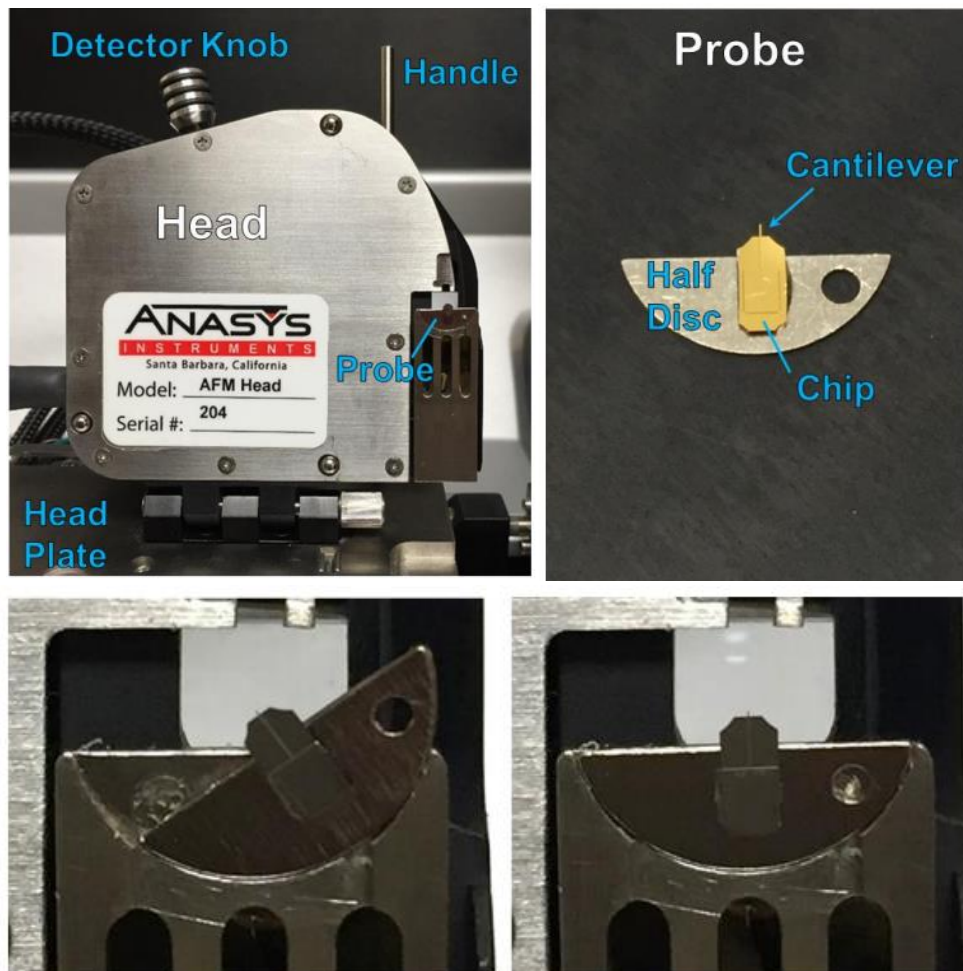


III Probe Installation

Place the probe into the recessed seat on the head.

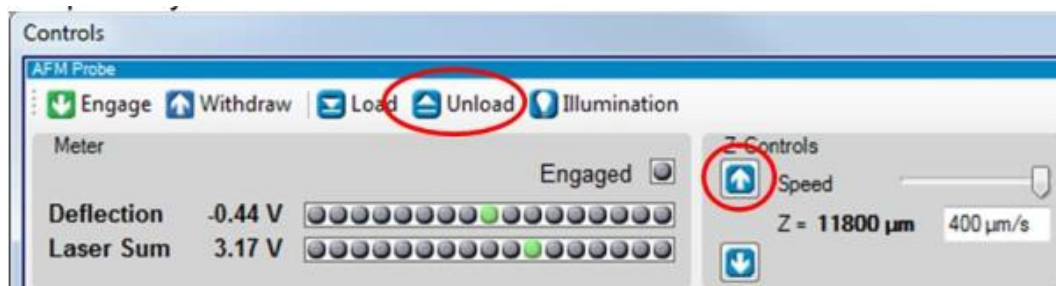
- The seat has magnets to hold the probe in place.
- Place the half disc into the seat at an angle to accommodate where the tweezers are holding the disc, but enough into the seat so that the magnets hold the disc securely.
- Push on the flat edge of the disc near the protruding corner until the disc lines up properly with the seat.

- Be careful **not to touch** the chip and cantilever.
- The **probe must be seated firmly into the holder** at the correct angle



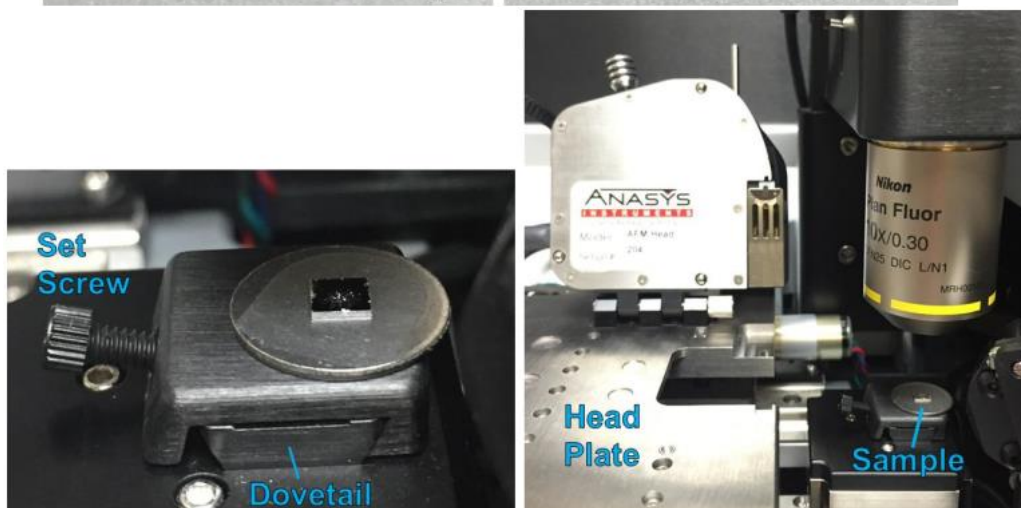
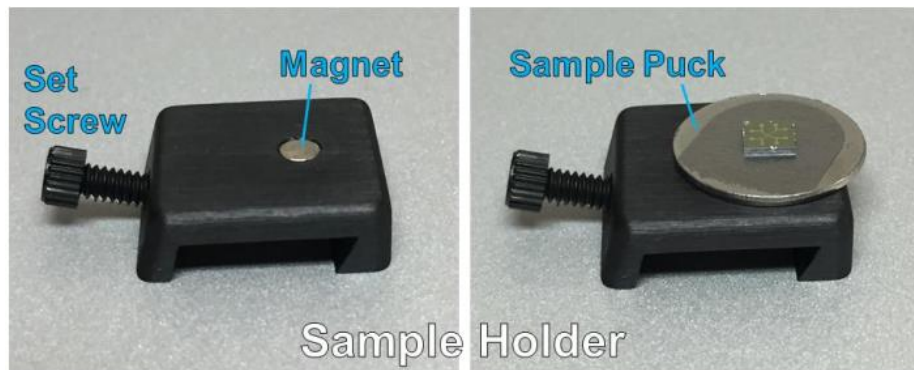
IV Sample Installation

Always remember to use **upload** or **Z-Controls** to move the head and sample away from each other.




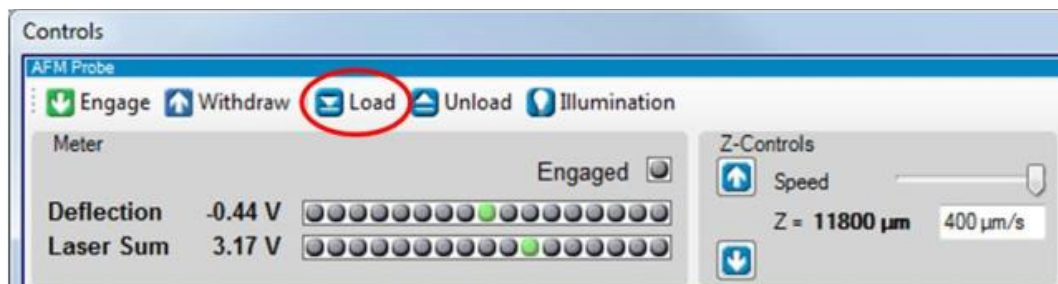
- A sample is typically attached with an adhesive tab or epoxy to a sample puck so it will hold to the magnet in the nanoIR2 sample holder. Attach the sample to a sample puck.
- Place the sample puck on the sample holder.
- Slide the sample holder onto the dovetail mount

- Tighten the set screw to secure the sample holder
- Return the head into operating position while checking for clearance.

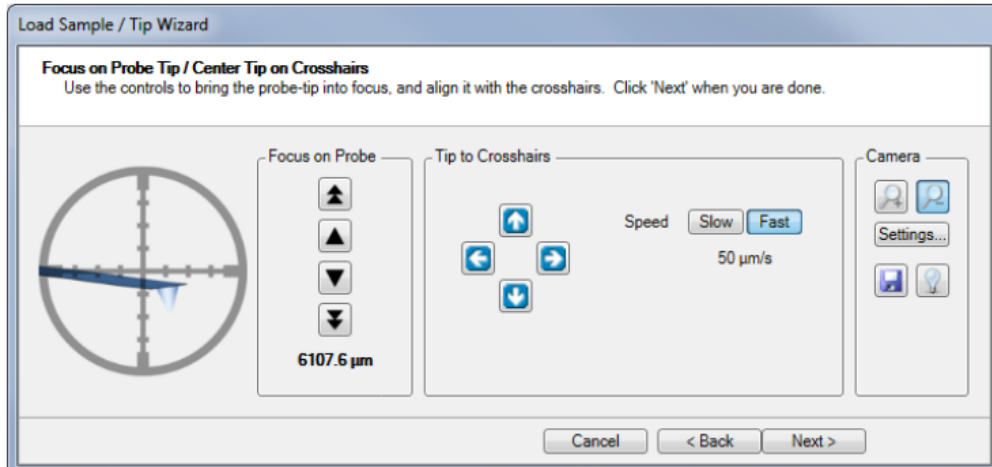


V Load Wizard: Probe and Sample Alignment

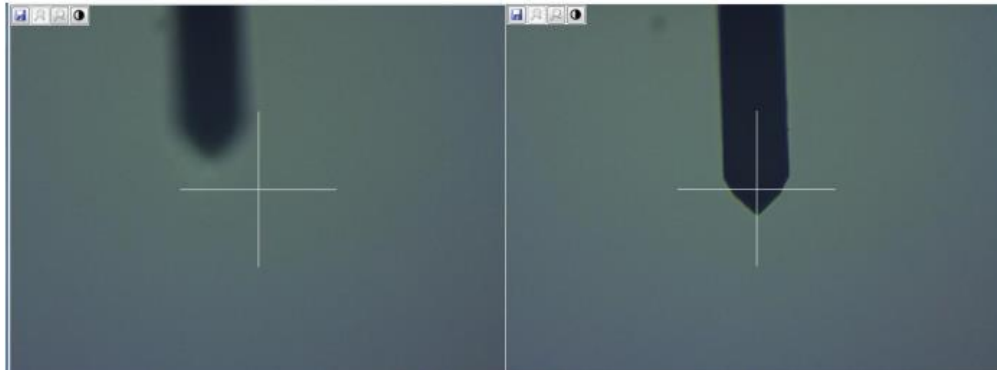
- Align the Probe and Sample using the Optical View. Click next to on the Microscope Window toolbar to make the optical view full screen 
- Click “Load” to launch the Load Wizard.



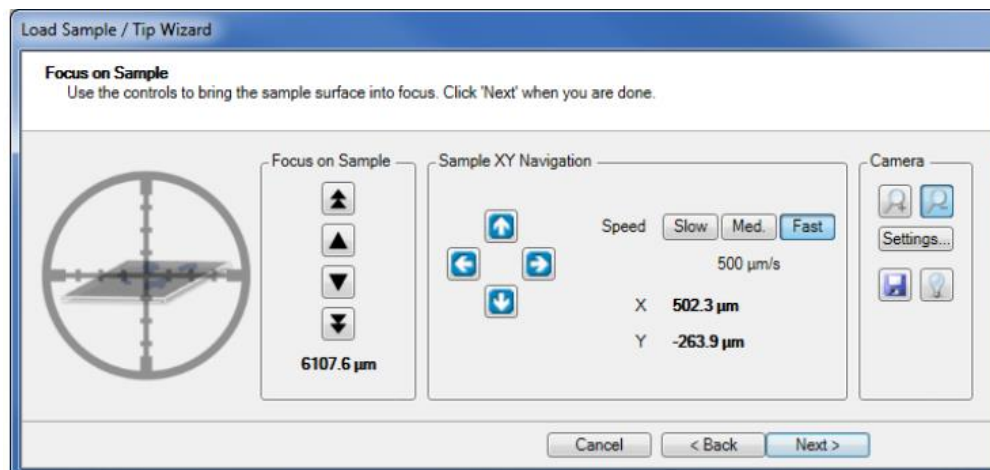
- Use the “Focus” on Probe controls to bring the probe into focus.



- Use the Tip to Crosshairs controls to move the tip **under the crosshairs**.



- Align the Sample, bring the sample into focus and then use the Sample XY Navigation controls to move the sample feature of interest to the crosshairs. First the optical focus moves down. When it reaches the bottom of its range then the sample moves upward to bring it into the optical focal plane. Be careful during this process; **it is possible to crash the tip and sample together. If an image of the probe appears while moving the focus down, stop!** This is a reflection of the probe below the sample surface and the focus has been lowered too far.



- Click “Approach Only”, the sample will be moved very close to the probe (to the Standoff distance) so the sample and probe will almost be in focus at the same time.

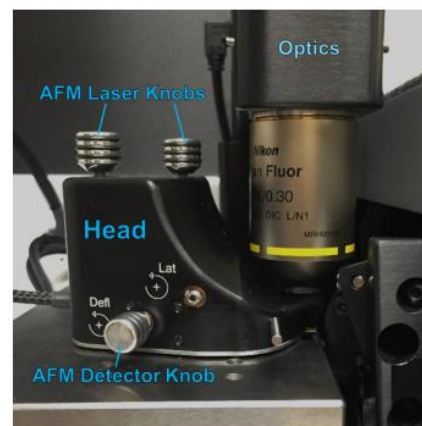


VI AFM Laser and Detector Alignment

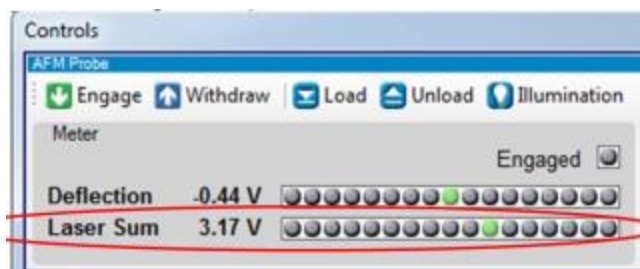
The AFM laser is positioned onto the end of the cantilever. Then the reflected light from the cantilever is centered onto the AFM photodetector.

Align the AFM Laser using the Optical View:

1. Bring the red AFM laser spot into the optical field of view.
2. Position the laser on the end of the cantilever using the AFM Laser Knobs.

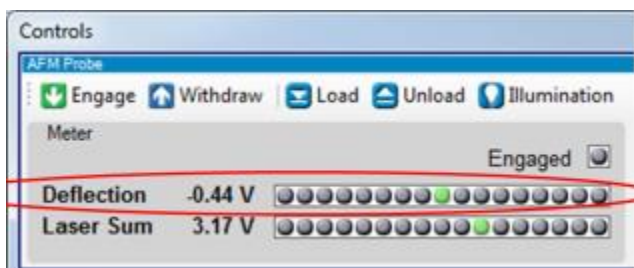


3. Fine-tune the left/right laser position to **maximize the Laser Sum**.

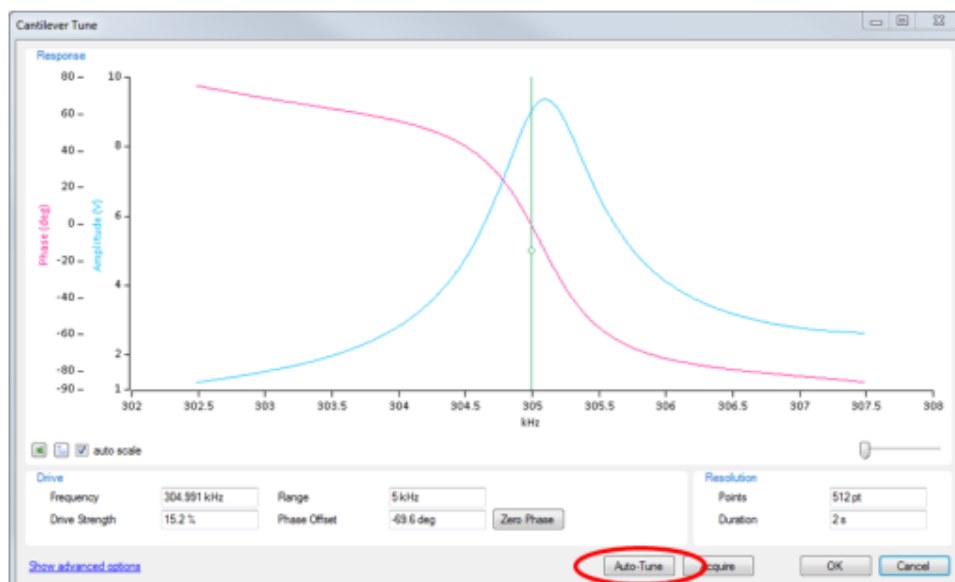


4 Align the AFM Detector, Adjust the Detector Knob on the head until the Deflection indicator light is green. The Deflection is green for a small range of values around a target deflection that is different for different AFM modes:

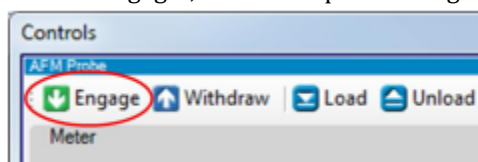
- ◆ Contact Deflection Target = -1 V ◆ Tapping Deflection Target = 0 V



5 Cantilever Tune, only done for tapping mode operation. It identifies the cantilever's resonant frequency and sets the cantilever's initial amplitude. If contact mode is in use, skip to the Engage section.



6 Click “Engage”, the last step is to bring the probe and sample into controlled contact.

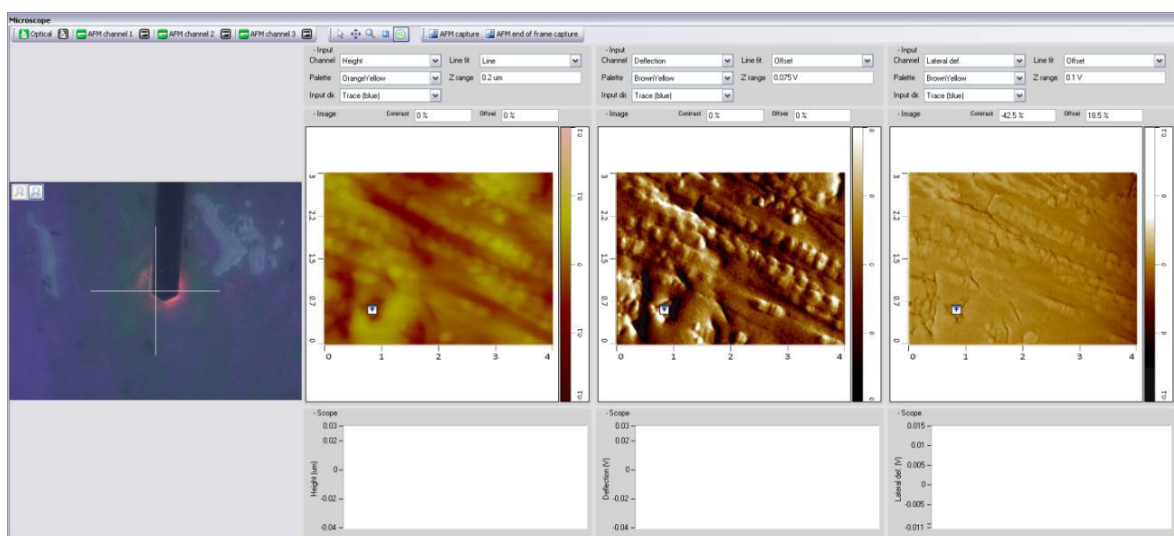




- The probe and sample are slowly brought into contact.
- When complete, the Engaged status button in the AFM Probe panel will flash green and the Feedback status in the AFM Scan panel will be On.

VII AFM Image Acquisition

Once the probe is engaged on the sample surface, the imaging parameters in the AFM Scan panel can be optimized. The first parameter to set is the Setpoint value. This parameter controls the force between the tip and sample. The lower the Setpoint, the smaller the force is. Set the Scan Dimensions for the desired image size. Resolution is the number of data points collected in x and y. The number of scan lines (y resolution) and the scan rate directly affect the amount of time it takes to collect an image. **offset x=0 and offset y=0** A typical scan rate is 1 Hz for regular AFM imaging.

Minimize optical microscope and begin scanning, click the Scan button on the AFM Scan panel toolbar. During the process of scan, z-range can be changed. To collect a height image, set Channel 1 to Height in the Input panel above the left image in the Microscope Window. Up to three channels can be collected simultaneously.



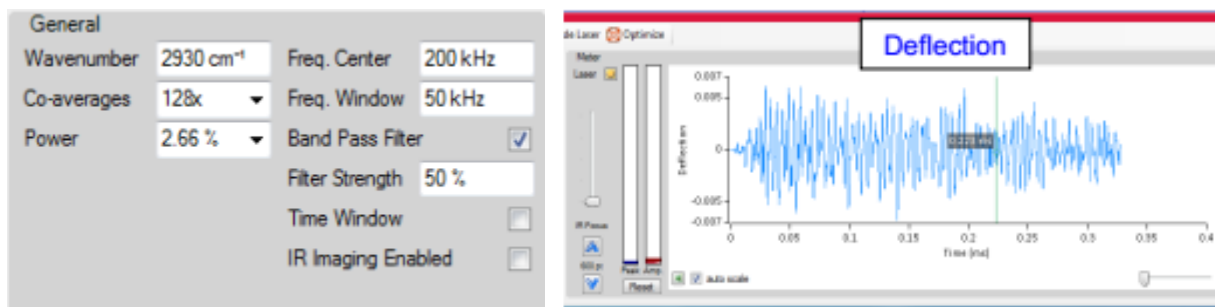
Centering the image at a specific location can be done by putting the cursor in Target mode (select the target button  on the upper toolbar), then clicking on the AFM image at the desired location. This will stop the scanning and move the probe to this location on the sample. To begin scanning centered about this location, click the Target Scan button  in the toolbar of the AFM Scan panel.

AFM data is written to the document and will appear in the Document Window when the “AFM Capture” button in the Microscope Window toolbar is clicked. If the AFM **end of frame capture button is selected**, the data will be moved into the document once the AFM scan reaches the top or bottom of its current frame. **Go to Analysis Studio, and save the document (File/Save)** to save the data beyond this session.

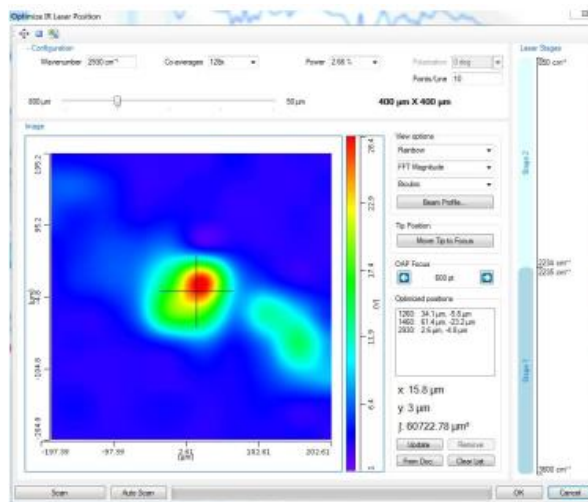
VIII AFM-IR Setup

1 IR Laser Alignment

The probe **must be engaged** on the sample but not scanning. It is easiest to align the IR laser if the probe is on a feature or area on the sample with a known IR absorption peak (wave number). If needed, take an AFM image to locate such a feature. Make sure to stop the scan in an appropriate area or use the target to place the probe before continuing with the IR alignment. The nanoIR meter is configured by the **General parameters**. Set the Wavenumber to a value that corresponds with a large absorption peak for your sample. If it is a totally unknown sample try a common absorption peak such as 2920, 1720, or 1450 cm^{-1} . Set the Co-averages to 128x. Set the Power to 5%. Turn off the Band Pass Filter. Click **“Start IR”** to turn the IR laser on. Now the Laser status button flashes yellow and the nanoIR Meter plots and graphs are active.



When the probe is on a place in the sample that is absorbing IR, the deflection should have a periodic waveform or “ring-down” and the FFT should have one or more clear peaks. This is the signal we are looking for. If there is no signal, you may use the **“Optimize”** function to search for the IR spot. The Optimize function moves motorized mirrors which shifts the position of the IR spot on the sample. Select in the nanoIR panel. Use the slider bar in the Optimize window to select a large search area, 400 μm by 400 μm . Hit the Scan button. Click OK in the dialog box that confirms that the scan is completed. The resulting image is a map of the FFT magnitude signal at each x and y location. The computer will pick the location with the largest signal and center it under the cross hair. If there is no obvious spot in the optimize view the power of the IR source can be increased. To accept this location, click the Add button (or Update button if there are previous offsets already stored). Now rescan at a higher resolution: move the slider to 200 μm by 200 μm then click Scan.

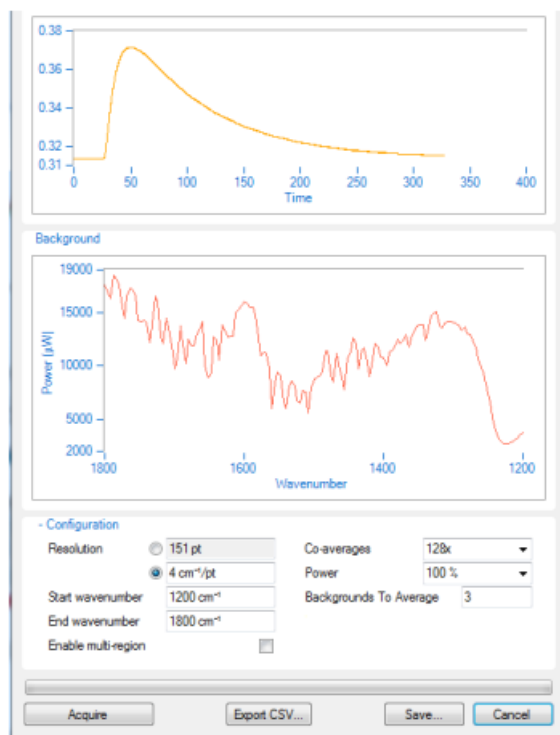


Thickness	Typical Power
Standard (ex. 400 nm)	4 %
Thick (ex. 1 μm)	1 %
Thin (ex. 100 nm)	8 %

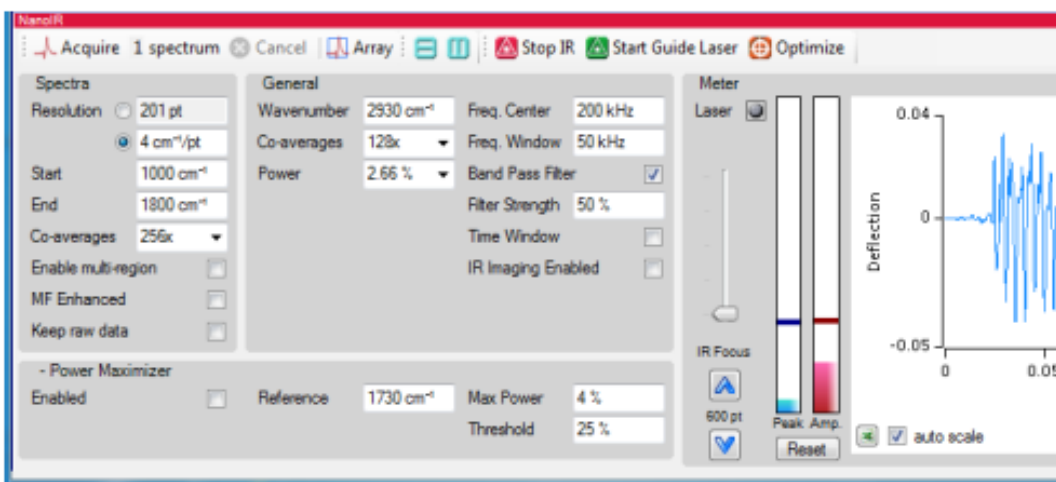
Once the final focus is chosen, the Power may need to be adjusted so that there is not too much sample heating leading to the probe penetrating into the sample and making a hole. The table lists typical values for the Power for the 1200 to 1800 cm^{-1} wave number range as a function of sample thickness.

IX Acquiring AFM-IR Data

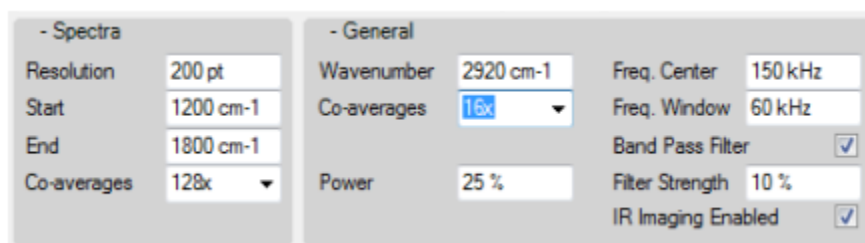
1 IR Background Calibration: An IR Background file must be loaded before any IR data can be collected. The background is the power of the IR laser recorded across a specified range of wave numbers. The background is used to remove the effect of power variation in the IR source at different wave numbers. Under the “Tools” menu, select “IR background calibration” and click on “New”. After changing any necessary parameters in the dialog box, click on “Acquire” and the background spectrum will be collected in the window. Click on “Save” to save the background data into a file (it will have the extension .irb). When saved, it will automatically load as the background file currently in use. When a background file is loaded its name is displayed on the right of the status bar along the bottom of the Document window.




2 AFM-IR Spectrum Acquisition: To begin taking a nanoIR spectrum, click the “Acquire” button. During the spectrum, all the nanoIR controls are grayed out except the Cancel button which may be used to stop the acquisition. The spectrum data is written to the document as it is taken and plotted in the Spectra Graph in the Document window. To save the data beyond the current session, the overall document must be saved (File/Save). Three channels of data are collected: IR-Peak, IR-Amplitude, and Frequency.



3 AFM-IR Image Acquisition: IR imaging maps the relative IR response across the local area of a sample, which is being irradiated at a particular wave number. The contact resonant frequency of the cantilever can be acquired simultaneously when using IR imaging mode.



Check the “IR Imaging Enabled” option in the nanoIR control panel. Determine the desired frequency range from the ring-down’s FFT. Click “Start IR” and set the Wavenumber to a value that has a response for that particular sample. The frequency in kHz is measured by putting the cursor on one of the peaks of the FFT; enter this as the “Freq. Center”. Input a value for the “Freq. Window” of the frequency search. A wider window, such as 50 kHz, should be used for a sample that has components with larger stiffness differential. In the “General” settings, set the Co-averages to 8x, 16x or 32x depending on the scan rate and desired signal to noise. Reduce the scan rate to ~ 0.1 Hz, and the integral gain of the feedback to about one-fifth of the previous value.

When the scanning session is over, click Stop, press the withdraw/Unload button  to pull the probe away from the surface. Right after stop withdraw, **Deinitialize**. Open the door and slide AFM head away and remove the sample.