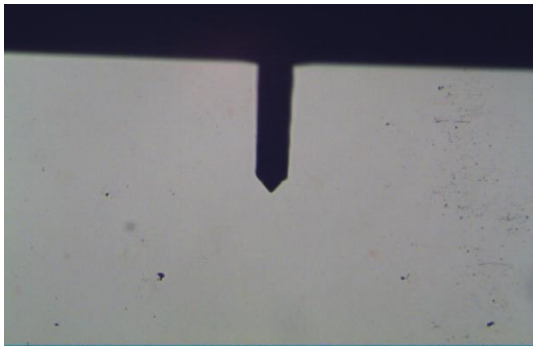
Instruction Manual on the Use of the AFM-based TERS Probes. Type SB-G, SB-S.

This instruction relates to the use of the gold (SB-G) or silver (SB-S) coated TERS active AFM probes exclusively available from AIST-NT Inc. TERS probes are based on standard silicon AFM cantilevers with the following properties of the lever: k=7.5 N/m; f=150 kHz; length:150µm, width:30µm; backside coating: Al. The probes can be used for high resolution TERS imaging in AIST-NT OmegaScope-R, OmegaScope-T and TRIOS platforms. TERS performance in any alternative equipment is not guaranteed. TERS measurements with these probes can be performed either in the side or the bottom access illumination /collection using the excitation lasers 633-785 nm for BS-G type of the probes and 532-785 nm for BS-S type. Top access TERS measurements are NOT possible with these probes. If used properly, the tips can maintain high amplification factors and very decent spatial resolution both in AFM and Raman(TERS) channels for 1-2 weeks of active everyday use.

Operation with the Side Optical Access

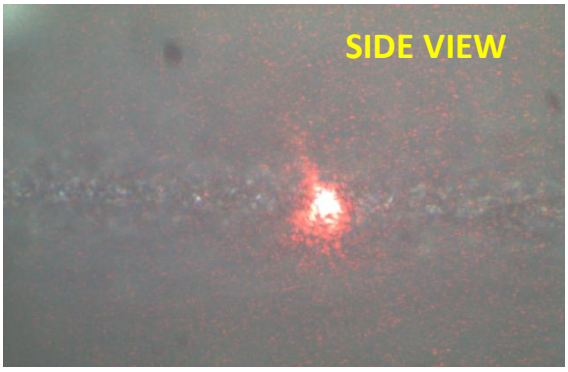
Marking the position of the focus of the side objective in the top and the side videochannels. This operation is preparatory and needs to be done only one time per 1-2 weeks.

- Install any (not necessarily a high quality) AFM probe and use a sample holder metal disk (supplied with every AFM or AFM-Raman system) as the sample.
- Approach and land on the sample, for the convenience and speed you can use "Auto" button in the software.
- Make sure that the flipping mirror is in the "Side" illumination position and the turret of the top channel is set such that you can see cantilever in the top

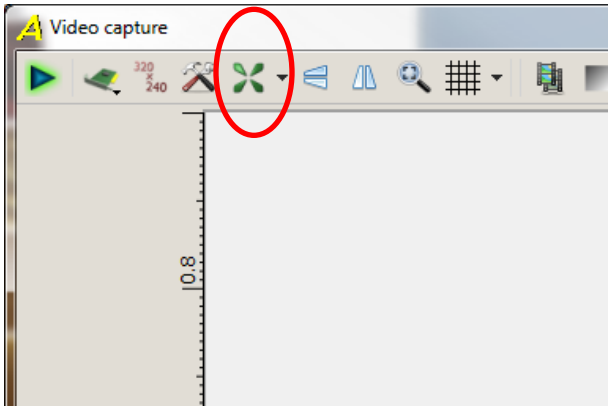


video window.

- In LabSpec Software set the ND filter to 0.1% (or even stronger attenuation, if available). Turn the laser on.
- Open the side video window. Activate the head light LED control (MacroExecutor- head-light.lua) and turn it on.
- Adjust the fine Z screw that moves AFM up and down relative to the optical input-output system so that the focus of the side laser is sharp, in both the top and the side video windows, making sure that the narrow line of the in-focus portion of the sample seen in the side video channel crosses the position of the laser focus.



- Mark the position of the focus in both windows using corresponding tool in the video tool panel.

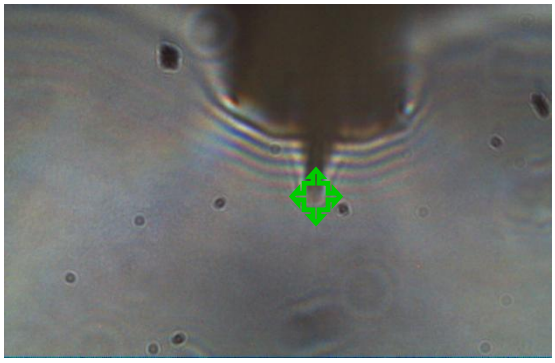


- Write down the value of the optical zoom in top video channel for which you marked the side laser focus position. We recommend to use x20. **It's very important to set the video zoom on the optical input-output system to the same value at which the laser position was marked.**

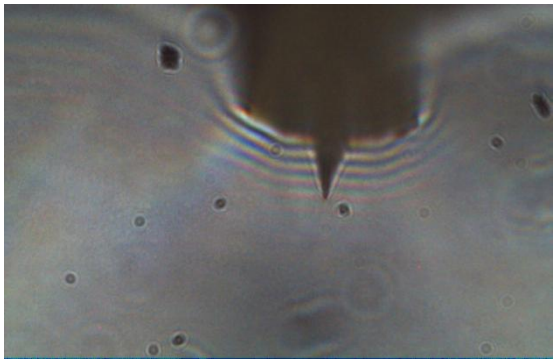
Alignment of TERS probes

Preliminary alignment of the focus of Raman laser and the apex of the TERS probe should be performed when the probe is far away (at least 200-300 microns) from the sample in order to avoid confusing situation inevitably occurring in the vicinity of the mirror-like sample when we see both the direct Raman image of the tip and its reflection in the sample.

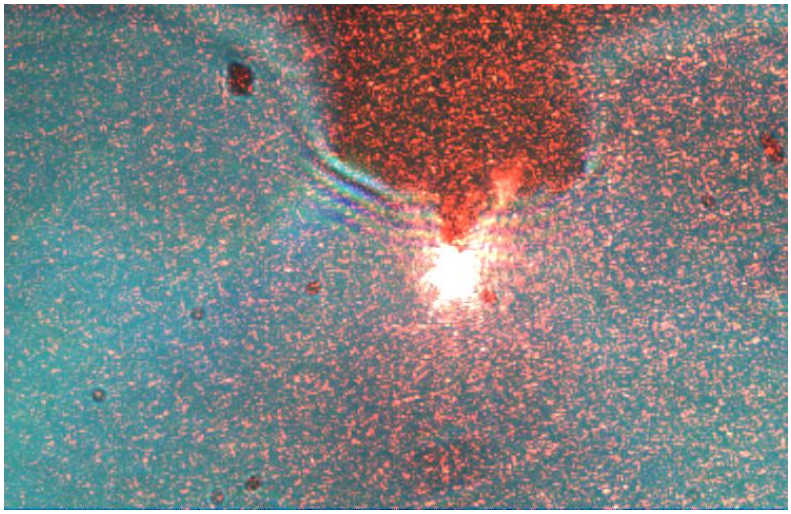
- Install the probe in the probe holder, insert the probe holder into HE002 AFM head of SmartSPM, being careful not to touch the top or the bottom of the probe holder opening in the AFM head.
- Make sure that the sample is at least 2mm below the AFM imaging position.
- Perform the AFM feedback laser-to-probe-to photodiode alignment. Set the amplitude to 10 nm.
- Move the side video unit into the beam path and open the side channel video window. Turn the head light ON.
- Move X and Y screws positioning Smart SPM so that in the side video window the tip of the probe is on the same vertical line as the laser mark. Adjust fine Z screw so that the tip of the probe coincides with the laser mark, not necessarily in focus.



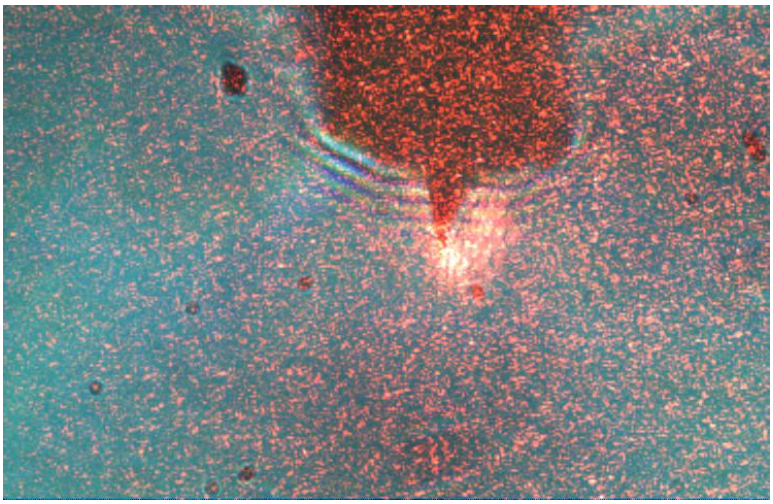
- Move the X and Y screws keeping the position of the apex of the tip in the side video window to bring it in sharp focus.



- Set the laser power to minimum (0.1%) and turn the laser on.
- In AFM -Raman software open SpecScan window (Rainbow icon in the very bottom) and choose XY Objective scanning option. Click on the "Full available scan area" button, then click on the scanner position control (green cross) and move the red marker around 3-5 microns until you see strong scattering from the apex of the tip.

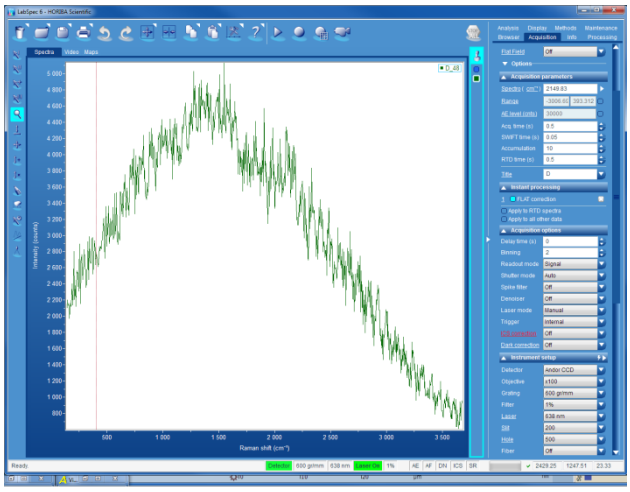


Move the marker down 3-4 microns to make sure that the laser comes off the tip

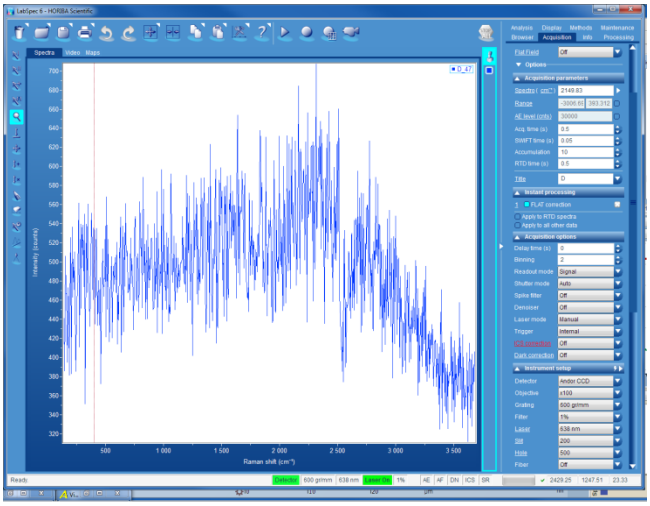


Return the marker to the position of the strongest scattering. Now we are ready to map the tip scanning the objective around.

- **Move the side video unit out of the beam path - it is VERY important not to forget this step**.
- Turn the head light off, close the side video window and turn the top light off. In the SpecScan window you should still have XY Objective scanning option. Set preliminary scan area 4x6 microns, choose 20 x30 pixels (longer scan in Y direction). Set the laser power to 1% (150-200 µW on the sample for red laser, it may be less for the green). Switch laser to manual mode in LabSpec software. Turn the laser on. Run RTD with 0.3s integration time. The spectrum should look like this:

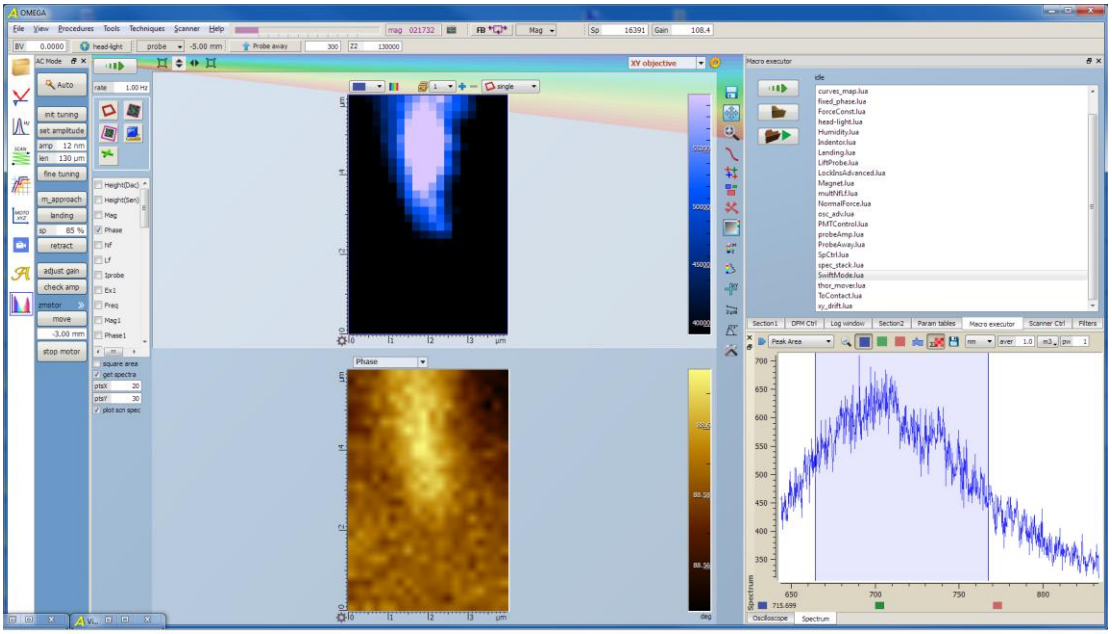


- Move the position of the red cursor in the scanning field to the bottom of chosen 4X6 scan area, the spectrum in LabSpec should change:



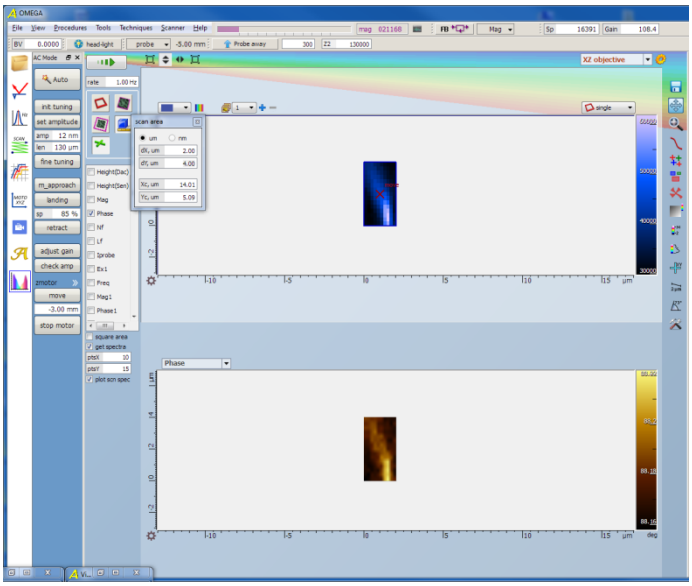
Such behavior guarantees that we'll map the very apex of the probe. Stop RTD acquisition in LabSpec (if it was running) and run the XY Objective map collecting the Phase signal along with the spectra. Make sure that the laser is turned ON in LabSpec software.

- The result of the mapping should look like this:

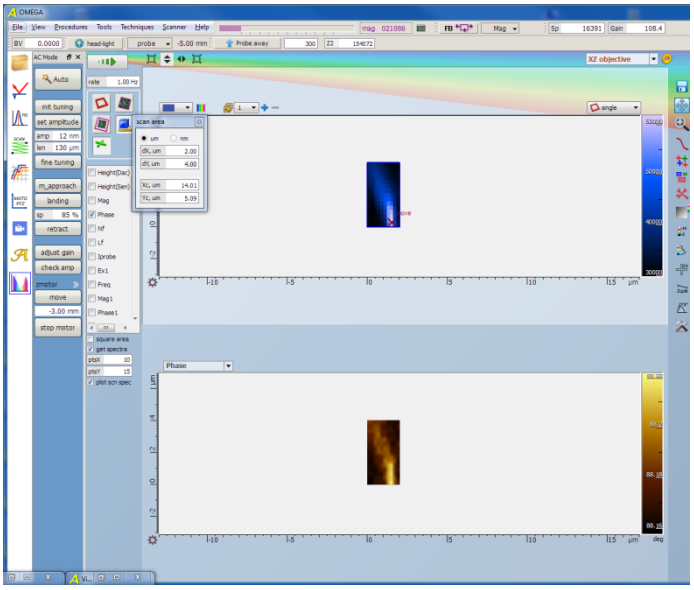


- If the apex of the probe does not look very sharp (the apex is over 500nm across) in both the spectral and the phase shift maps, it means that we are not perfectly focused. In order to adjust Z focusing, we need to position the laser at the apex of the probe and run XZ mapping.

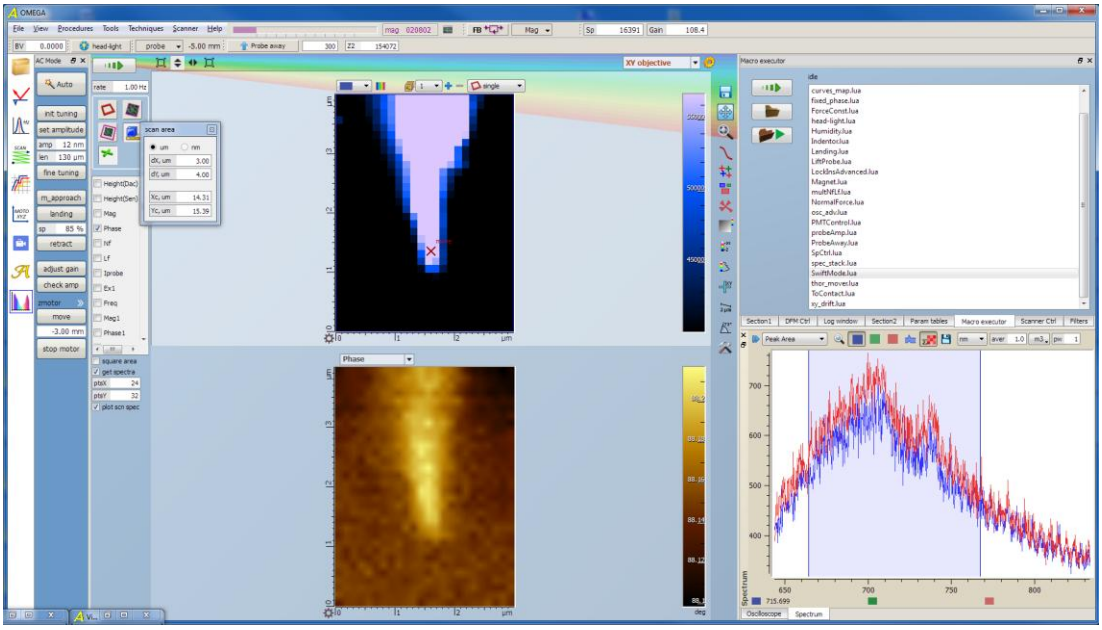
Maximize the XZ Objective scanning area, click on current position button (green cross) and choose area of 2x4 (or more) microns with the center coinciding with current position of the laser. Set the number of pixels in the map to 10x15. Run the map.



Position the laser to the strongest signal / highest contrast location.

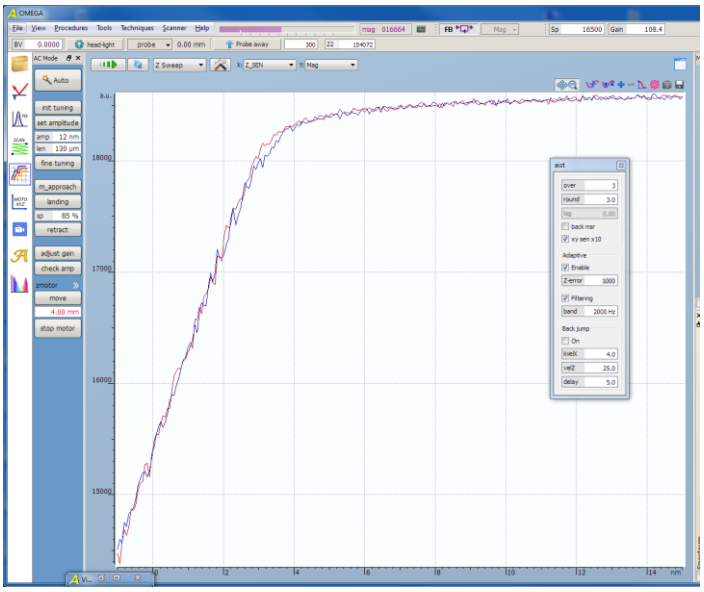


- After the laser focus in Z direction is adjusted, we need to re-run XY map, this time the scan size may be decreased to 3x4 microns.

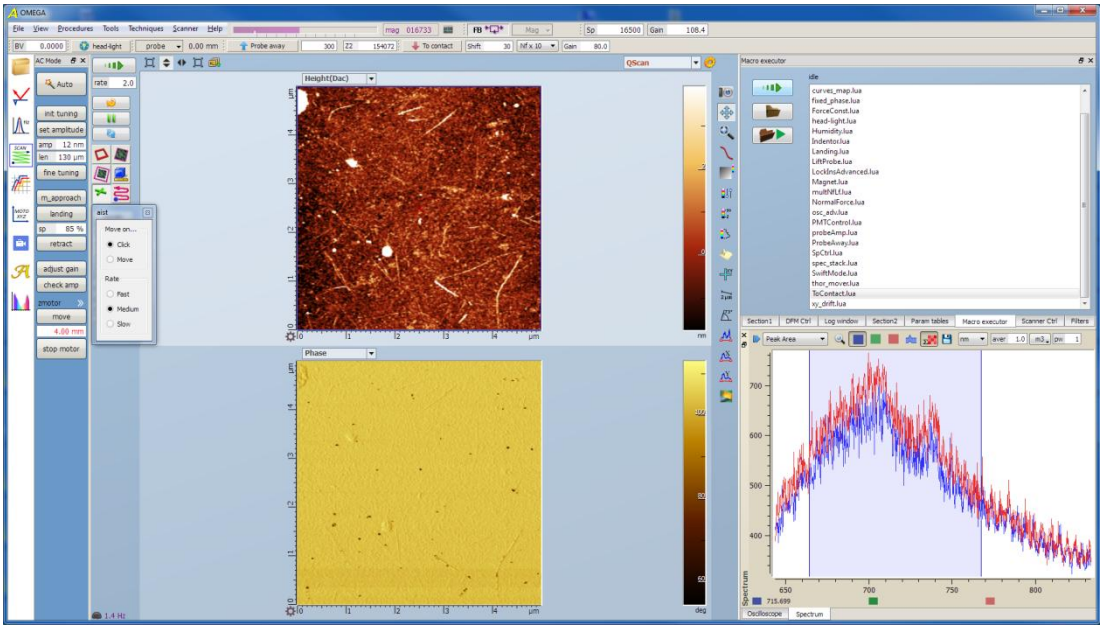


If the apex of the probe looks sharper than half a micron, and the red cross corresponding to the laser position is placed at the apex of the tip image, we can consider preliminary alignment to be successfully completed.

- As the following step, we need to scan the sample, find a feature that can produce TERS signal and slightly adjust the position of the laser relative to the tip based on the TERS signal itself. Approach the sample using "Auto" procedure. Sample scanning should be performed using QScan (default) procedure with adaptive scanning option enabled- this is the safest option that minimizes the risk of damaging or contaminating the tip. In order to choose rationally the value of the Z-Error for adaptive scanning you'll need to run the amplitude vs distance curve with a sweep range from -1nm to 15nm. If attraction-repulsion crossover is observed in the amplitude vs distance curve, make sure that the set point is chosen far away from the crossover amplitude, preferably in the attractive part of the curve. Write down the value of the difference between the setpoint and the amplitude corresponding to the plateau portion of the curve (free amplitude). The value of the Z-Error field in Adaptive scanning procedure should be set to 50-75% of the difference between the setpoint and the free amplitude.



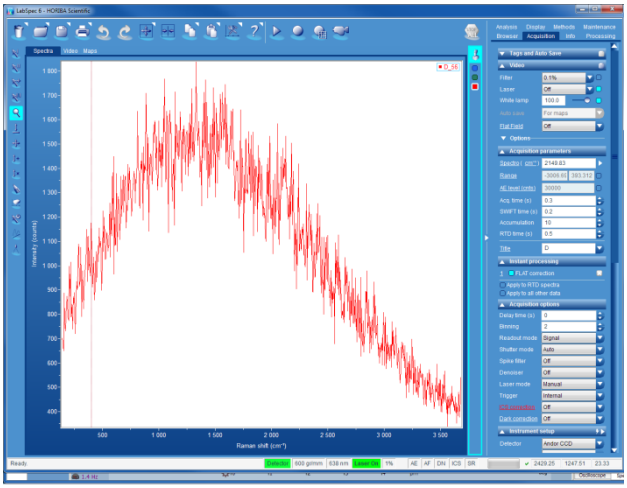
- Take a 4x4 micron scan using Q Scan mode, adaptive scanning option enabled.

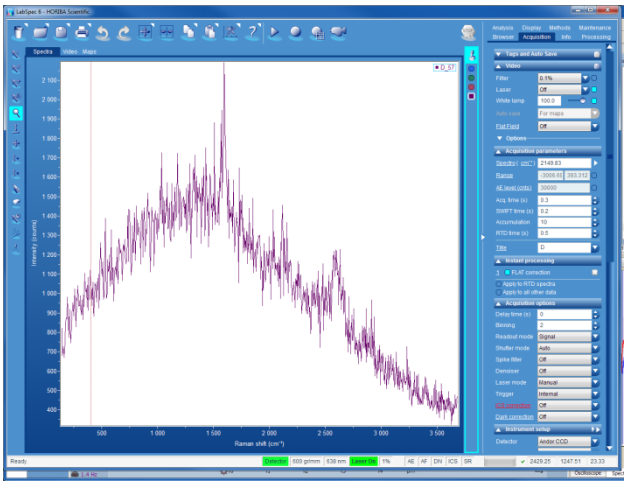


Position the probe over a feature you'd expect to be TERS active and launch "To Contact" macros.

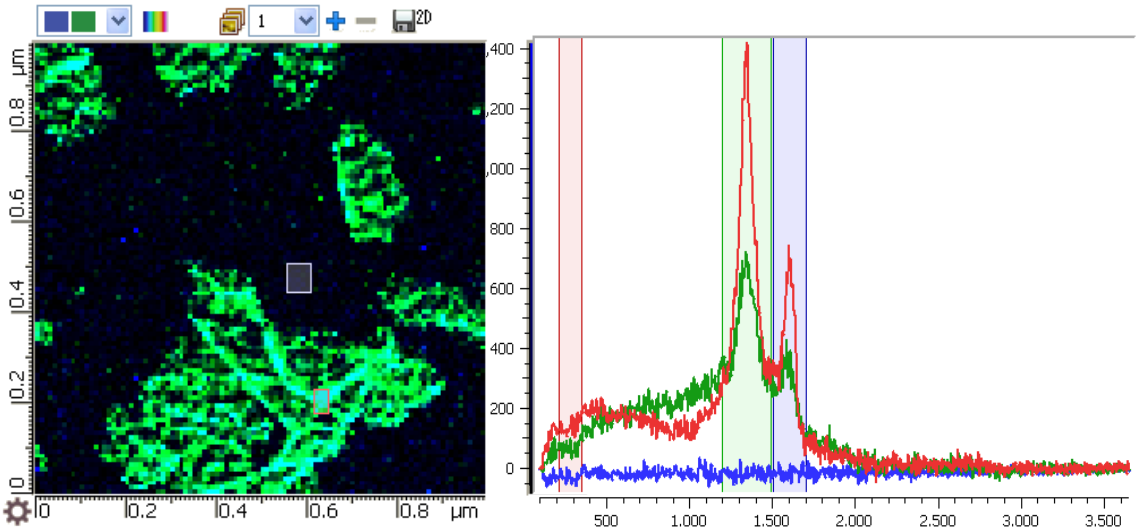
- Make sure that the NFx10 amplification is chosen, run RTD with 1% - 2.5% attenuation and integration time of 200-400 ms, then press "To Contact" button and observe if there is a change in the Raman spectrum. Open XY Objective mapping window and vary the position of the laser by 100-300 nm around the apex of the tip trying to maximize the TERS signal. If no TERS is observed, keep increasing the force (NF shift)

with 20 units increments. Usually the TERS activity appears within 30-150 units range (with NFx10 multiplication).





- Collect TERS map using Spec Top mode setting the number of pixels per line to 100-200 depending on the TERS signal level and the integration time chosen. Try to keep expected mapping time under 30 minutes. Set the value of the NF shift in SpecTop mode matching the value at which optimal TERS activity was observed. You might increase the value of the gain in SpecTop settings to 200 (default value is 80). Run TERS map collecting at least the topography along with the spectral map. The result should look like this map of graphene oxide:


1
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