Renishaw inVia Raman Confocal Microscope System Operating Protocols

Center for Chemical Sensors Development (CCSD) and Chemical Imaging Center and Surface Analysis (CICSA)

Dr. Samuel P. Hernández Rivera

Physical Chemistry Professor

CCS/CICSA
CENTER FOR CHEMICAL SENSORS
Chemical Imaging Center and Surface Analysis

SOCIETY for APPLIED SPECTROSCOPY

System and Optical Setup:

Each Raman System has different lasers sources which are selected via the needs for each one of the analysis. For these systems, there is an optical setup for the pathway of the laser to enter the Raman System.

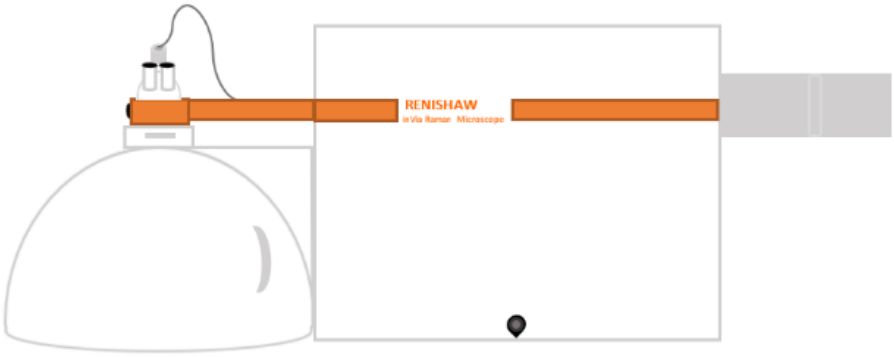
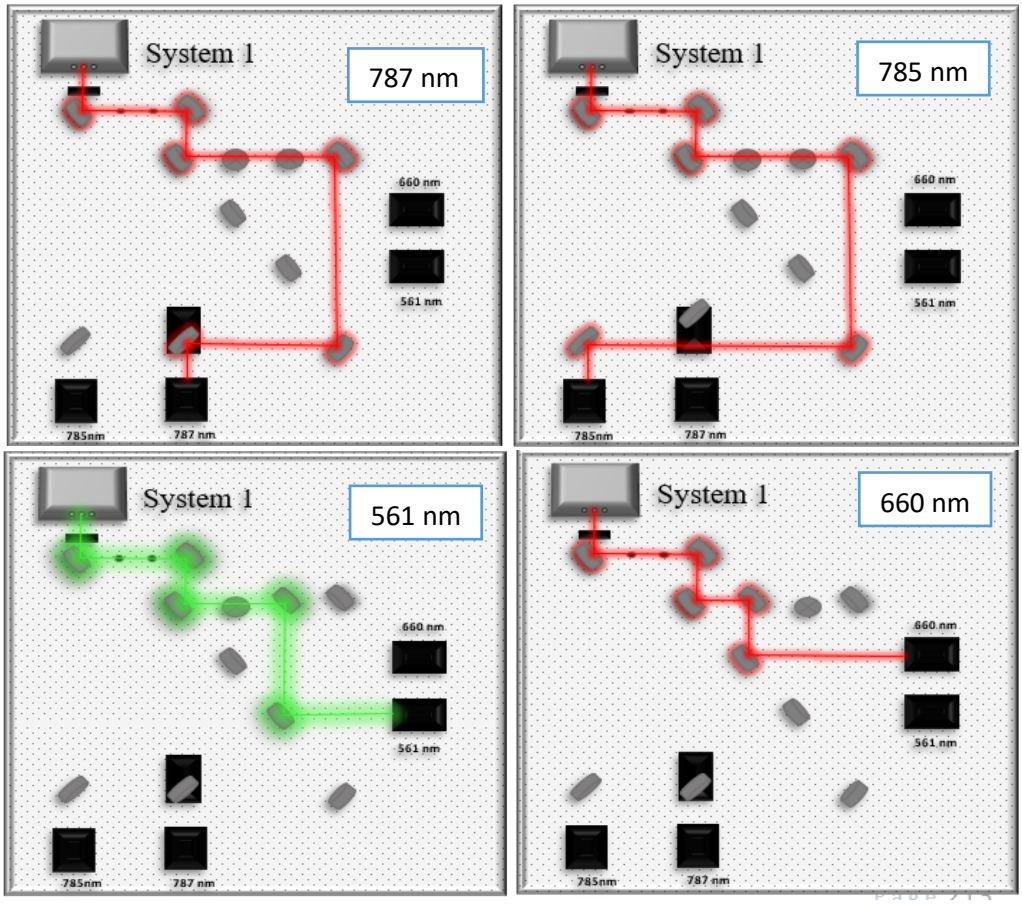


Figure 1: Renishaw inVia Raman Microscope Instrument



For System 1, there are multiple lasers that could be used.

- 561 nm
- 660 nm
- 787 nm
- 785 nm

The laser pathway is show in the figure 2.

Figure 2: Laser pathway for Renishaw inVia Raman Microscope System 1

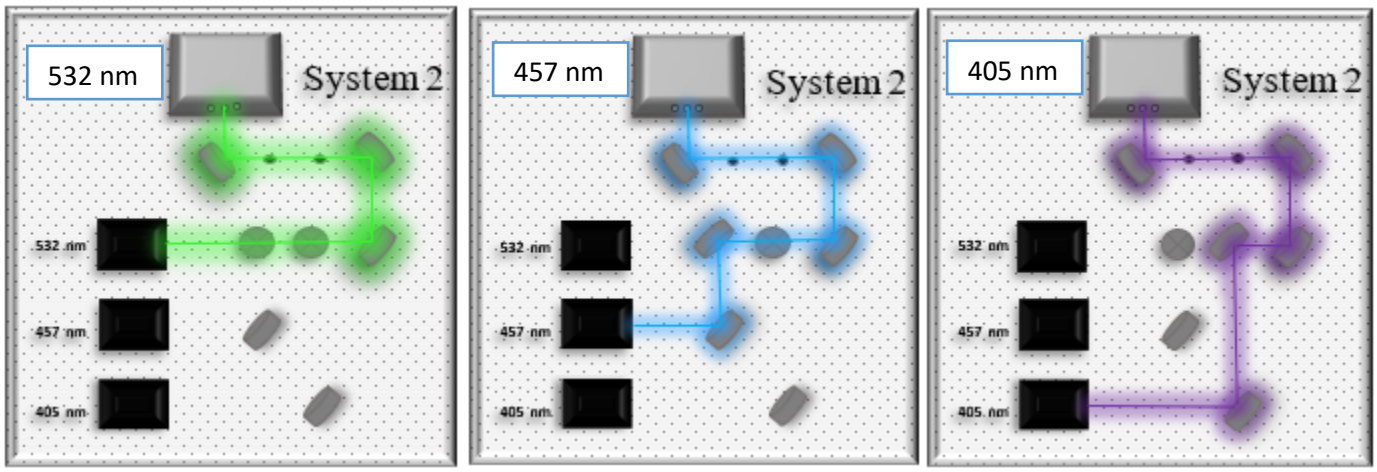


Figure 3: Laser pathway for Renishaw inVia Raman Microscope System 2

For System 2, there are multiple lasers that could be used. The laser pathway is show in the figure 3.

- 532 nm
- 457 nm
- 405 nm

Instrument Startup:

For starting up the system the procedure below must be followed:

1. Verify if the notch filter in the system is appropriate for the laser line to be used.

   Caution: Only authorized personnel can do this part.

2. Uncover Mirrors
   a. The mirrors are covered to minimize the deposition of dust; the cover should be removed.

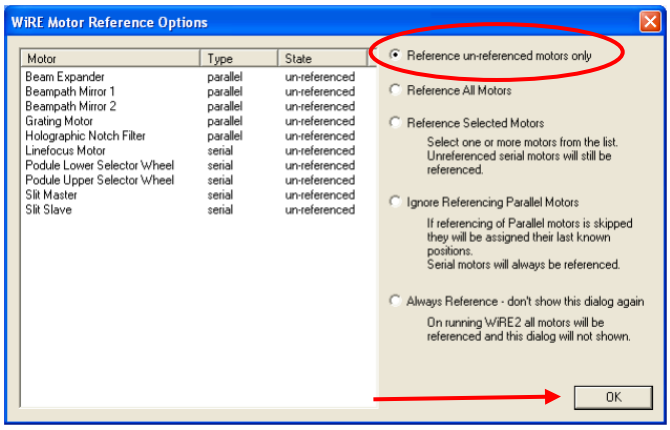
   Caution: Covers must be removed with extra care without touching the mirrors. A rotation or any movements in these mirrors can cause a misalignment in the system.

3. Connect the fan connection to the AC for the lasers that requires it.
4. Turn on the laser line of preference (refer laser user manual for individual laser start-up procedures)
   a. The laser has a key in a horizontal way and a green light indicating that the laser is off.
   b. To turn on the laser you should rotate the key in a clockwise rotation.
   c. The green light will then change to a red light indicating that the laser is on.
   d. "Each laser requires at least 30 minutes to reach optimal pointing and power stability."
5. Turn on the Raman Confocal Microscope System
   a. The System has a main ON/OFF button situated at the far right of the system as shown below.
   b. "CCD camera will take ~ 20 minutes to cool to its operating temperature"

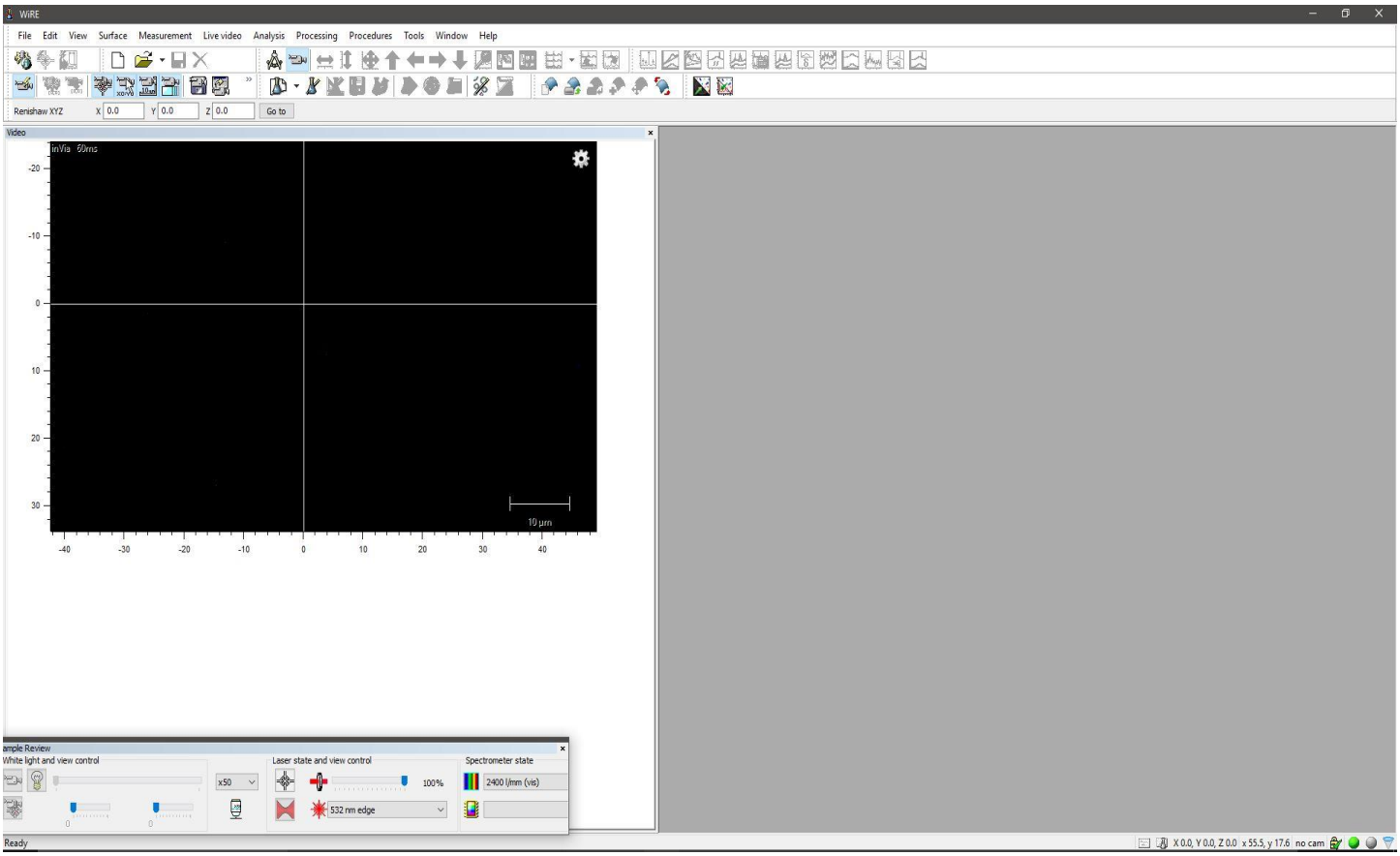
Caution: CCD detector should not be touched under any circumstance. Extra care must be made since misalignment may occur.

6. Start the program
   a. The program used is Wire 4.4.
   b. After opening the program
      i. As it opens:

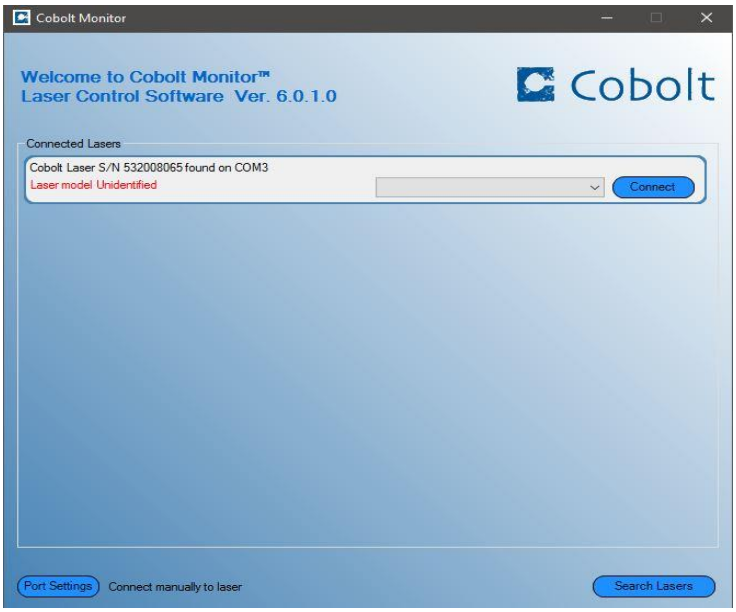
      ii. First: The software will prompt for a position check of the relevant motors. Choose the "Reference un-reference motors only" option.



iii. Second: The software opens as follows:



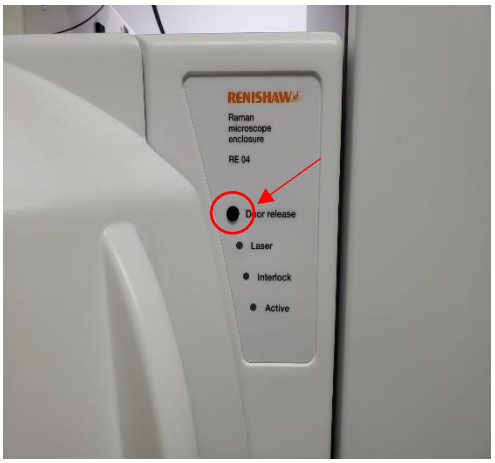
7. Start Cobolt Software to adjust the voltage (mW) for your analysis.



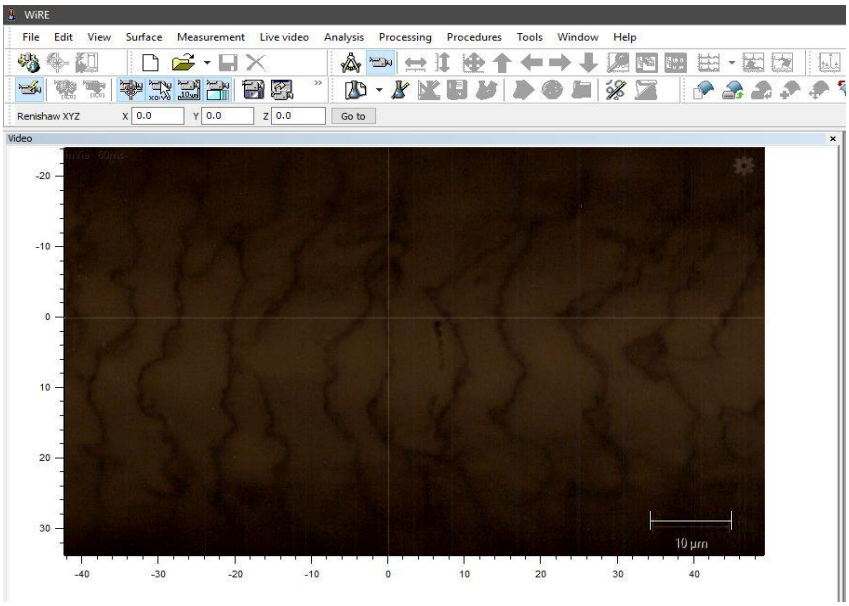
8. Having completed all these steps (laser, instrument, program) the system is ready to be configured.

Calibration Sample/Sample Placement

1. To start a Raman Analysis the system must be calibrated.
    a. For solid samples use Silicon standard
    b. For liquid samples use Cyclohexane standard
2. The following steps must be done before the calibration/sample measurement.
3. Open de Raman door to uncover the sample placement setup as shown below:
    a. Press the black button on the front of the instrument
    b. A small sound will be heard
    c. Proceed to open the door.



4. On the inside; look for the light source button on the left to turn on the visible light (green colored). This will allow the user to see the sample surface on the computer.
5. On the same side, there is a rotation knob that adjusts the brightness.
6. Place the sample on the Raman platform and from a low objective to a high objective carefully focus the sample. (Until desired objective). (Like using a common microscope - adjusting macro a micro knob)



Example: Black Human Hair strand. Focused with a 50x objective.

7. Once the sample is focused, close the Raman door carefully and rotate the following part from 1 to 4 indicating that there is no visible light passing and the laser can pass through.
8. On the left side the Raman System, there is a knob that also must be rotated clockwise for the laser light and counterclockwise to return to the visible light.

II. Rotate clockwise (visible to laser)

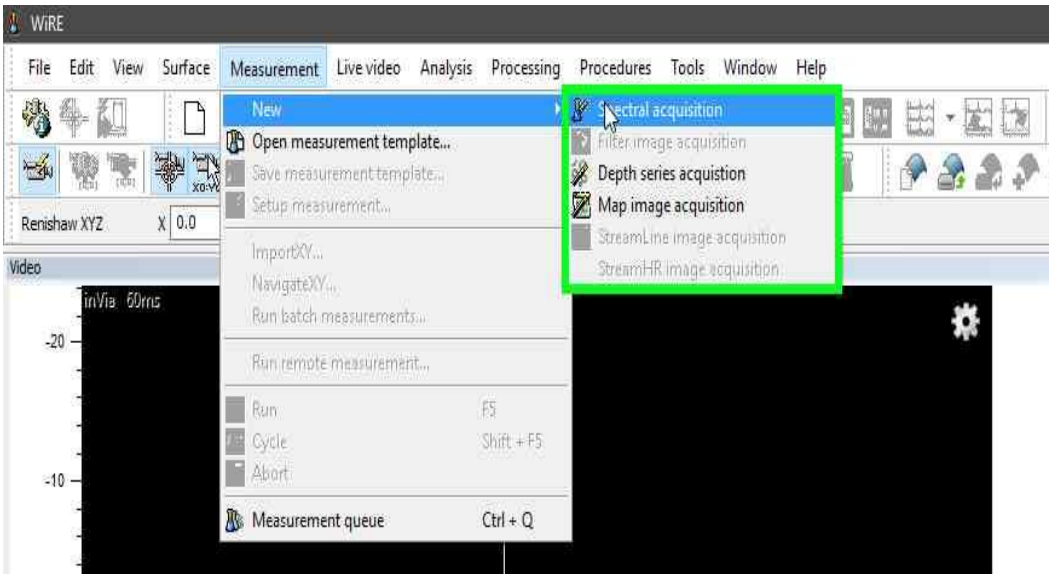
I. Rotate 1 to 4

Caution: Must be extra careful with the knob's rotation.

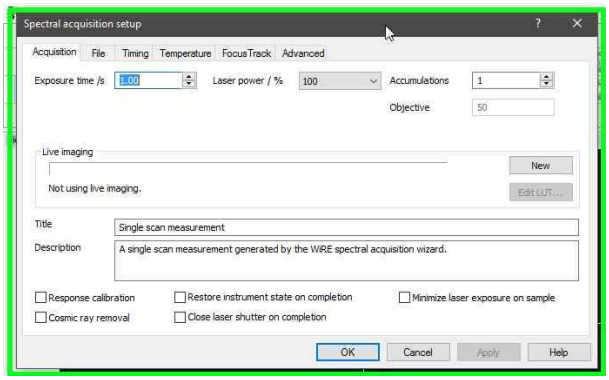
9. Both knobs' must be positioned on laser light to continue with the analysis.
10. Once you set up this part you may proceed to make changes to the program.

Software Operation

1. On the software, the parameters must be selected.
2. Before you set up your sample, you must create a new spectral acquisition measurement.
3. Open Measurements → New → Spectral acquisition

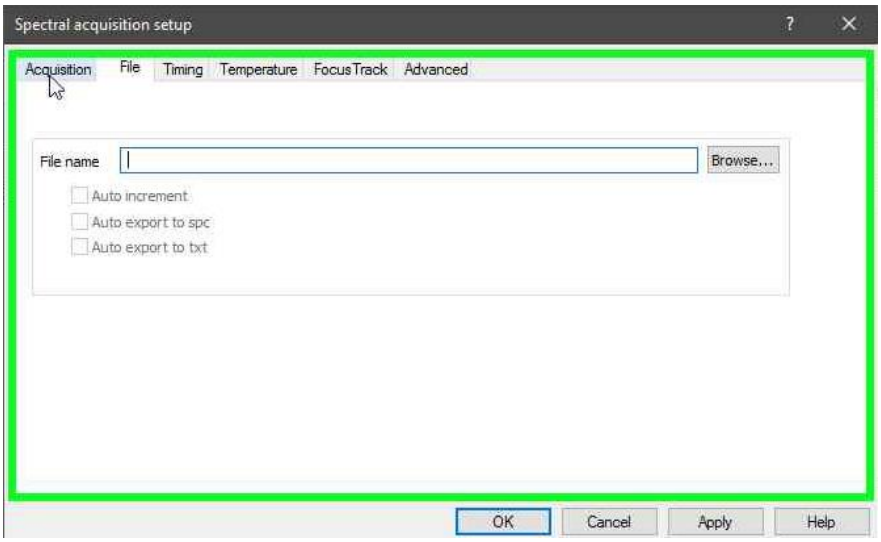


4. A tab must open:



   a. Range: Select the range you want for the analysis indicating one of the following:
      i. Static: Select the center of the wavenumber, this automatically selects the initial and final range.
         1. Example: Center selection: 1200 cm^{-1}; Range will go from 297.78 cm^{-1} – 1200 cm^{-1} – 1987.42 cm^{-1} (It varies)
         2. This will allow the user to use a minimum exposure time of 1 second.
         3. Decrease time of analysis.
      ii. Extended: Select the initial and final wavenumber for a wider spectral range.
         1. Example: From 0 cm^{-1} to 3800 cm^{-1} (maximum for this system)
         2. This will allow the user to use a minimum exposure time of 10 seconds.
   b. Acquisition:
      i. Exposure time (ET): It will allow to establish the time, in seconds, that irradiates the sample.
      ii. Laser power (LP): Portion of the laser irradiating the sample.
         1. For a 30mW voltage, if you let 100% of de LP pass through, approximately all 30mW from the source is passing to irradiate the sample.
         2. If the LP is reduced to 50%, it should allow pass to approximately 15mW from the source.
      iii. Accumulations (AC): Number of spectra that takes on an assigned point.
         1. Usually, the more accumulations specified, the better S/N.
         2. It also increases the time for your analysis.
      iv. Title: Specify the parameters on the file name.
         1. Example:
            a. Silicon_ET10_LP100_AC3_30mW_50x_Extended_Range200-1000
            b. Silicon_ET1_LP100_AC3_30mW_50x_Static_Center1000

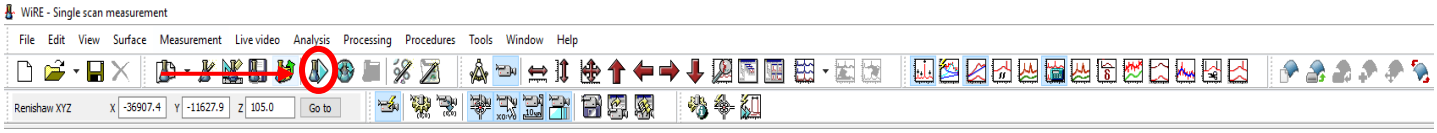
c. File



- Browse: Select the folder to save your measurement.
- Paste the same name as the title on the acquisition tab.
- Select the format type to save the data.
  - Usually all are selected

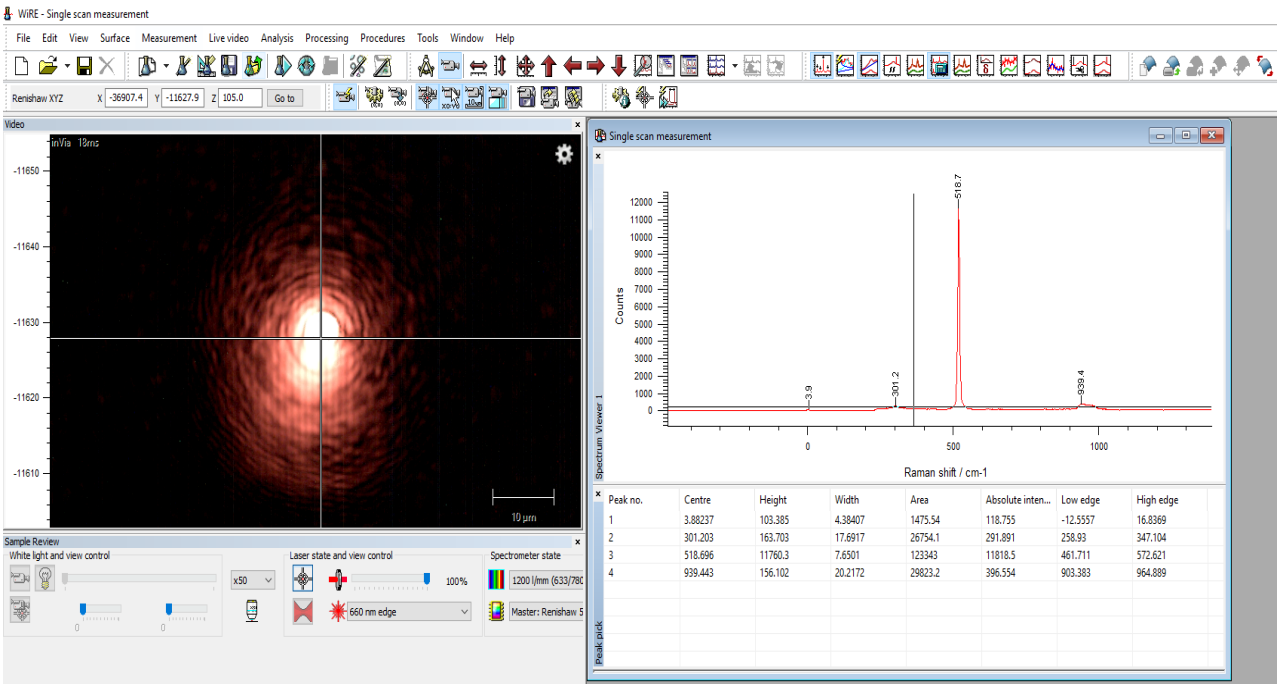
5. Press Apply once the parameters are set and then "ok".
6. Be sure the system is letting laser light pass (procedure explained in ***Calibration Sample***)

7. Press the Run button, a blue bar should come up at the bottom indicating the time left for the spectra.

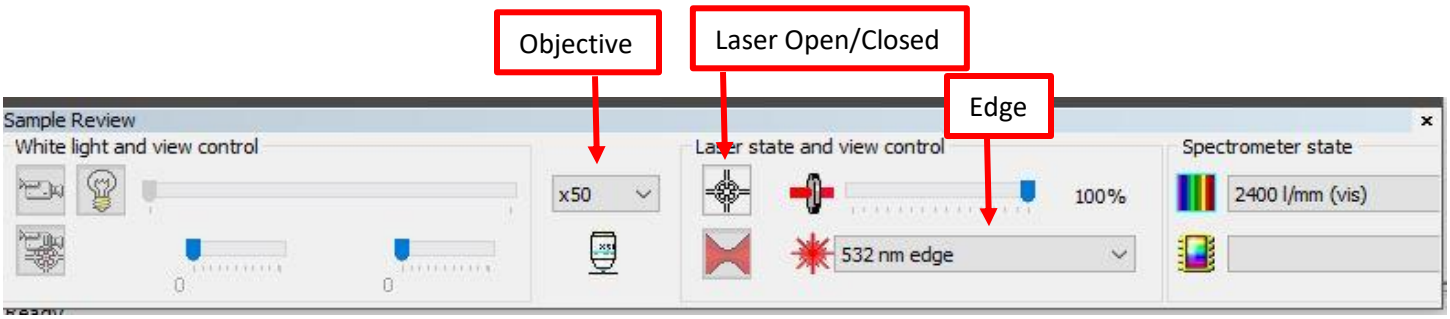


To calibrate the system:

1. Once the run button is pressed the software will show the spectra for the calibration sample as shown below.

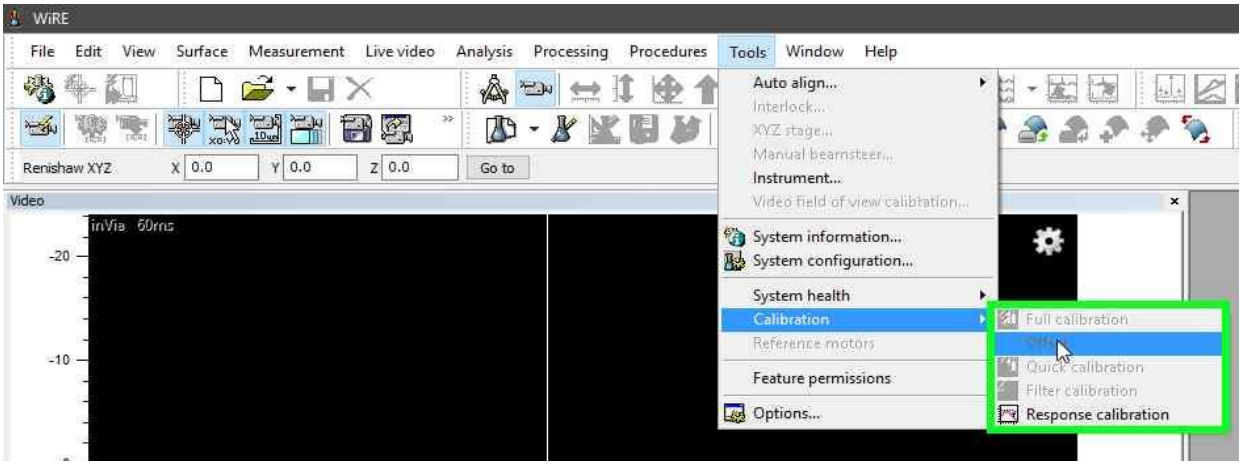


2. Once the measure is finished, if the data is acquired from a static range, then the laser should be closed pressing the Laser Open/Closed button.



3. Verify that the peak for this calibration sample always show the same Raman peak value.
    a. Silicon: 520.8 cm-1
    b. Cyclohexane: 801.3 cm-1

4. If not proceed to the following:
    a. Tools → Calibration → Offset



5. A tab should appear: the shift of the band should be placed to calibrate
    a. Always do little increments at the time.
    b. Example: By pressing the Run and the Silicon peak appeared at 520.4 cm^{-1}. The peak for silicon should be on 520.8cm^{-1}, for that reason a shift of 0.4 cm^{-1} is required.
        i. The approach will be 0.2cm^{-1} at the time.
        ii. Follow the above procedure, in the tab I indicate the negative version of what is needed for example 0.2cm^{-1}, indicate -0.2cm^{-1}.
        iii. Press OK and then Run again.
        iv. Repeat the same procedure if needed until reaching the desired value.
6. After the completion of the calibration place the sample to be analyzed. Follow the same procedure of handling the equipment and software.

Instrument Power down

1. Same steps as shown on the system startup must be done in reverse: Close Program → Turn off Instrument → Turn off Laser → plug off Fan (If Any).