

Acquiring Raman Spectra with Renishaw inVia Reflex Microspectrometer



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SOP-01	Francheska Colón, Annette Colón, Maria Villarreal, Edwin Caballero		University of Puerto Rico at Mayagüez
Effectivity: June/11/2022	Acquiring Raman Spectra with Renishaw inVia Reflex Microspectrometer		Revised by:
Revised:			Approved by:

This SOP uses the following:

- Instrument: Renishaw inVia Reflex Microspectrometer
- Laser

Company	Product	Wavelength	Laser Power
Hubner Photonics	Cobolt Flamenco™	660 nm	$\leq 500 \text{ mW}$
Hubner Photonics	Cobolt Jive™	561 nm	$\leq 500 \text{ mW}$
Hubner Photonics	Cobolt Samba™	532 nm	$\leq 1500 \text{ mW}$
Hubner Photonics	Cobolt Twist™	457 nm	$\leq 300 \text{ mW}$
		405 nm	

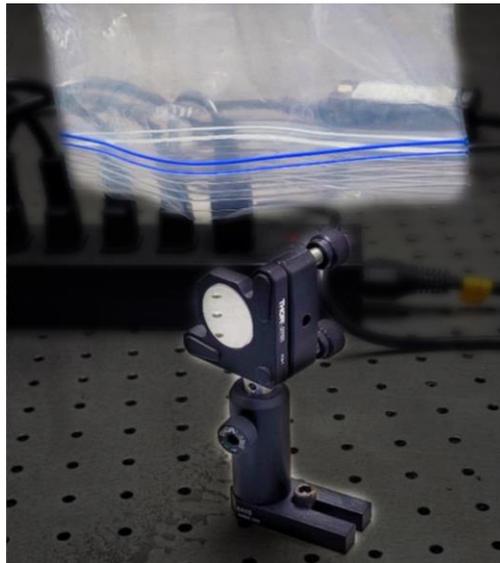
- Filter: Vary
- Program: Cobolt, WiRE

TURNING ON MICROSPECTROMETER

1. Turn on the power outlet to turn on fan (only for system 01).



2. Remove the zip-locks on the mirrors used for the laser. Extra care to avoid touching or moving/rotating the mirrors.

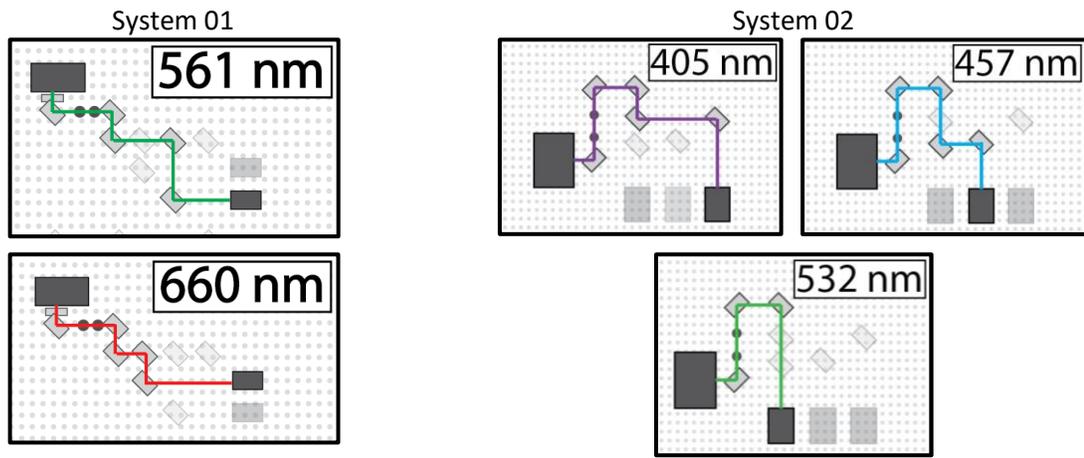


3. Choose the desired laser.

System 01 660 nm 561 nm
System 02 405 nm 457 nm 532 nm



4. **Determine** which mirrors must be active for the laser to pass through the microspectrometer.



The optical setups utilize a fused silica (SiO_2) [broadband dielectric 400-750 nm mirror](#) placed on a [kinematic mirror mount](#) with 3 adjusters. All mirrors are fixed in position with an [optical post](#), [post holder](#), and a [swivel base adapter](#). However, some mirrors contain, in addition, a [flip mount adapter](#). The flip mount adapter allows the user to either place the mirror upwards and reflect the incoming light source or downwards so that the source continues towards the next mirror.

5. **Activate** the necessary mirrors by flipping their kinematic mount vertically upwards (perpendicular to the table).



6. **De-activate** the necessary mirrors by flipping their kinematic mount horizontally (parallel to the table).



7. **Open** inVia spectrometer door with key.



8. **Verify** that the correct edge filter is placed (varies per laser excitation line).

Edge filter =

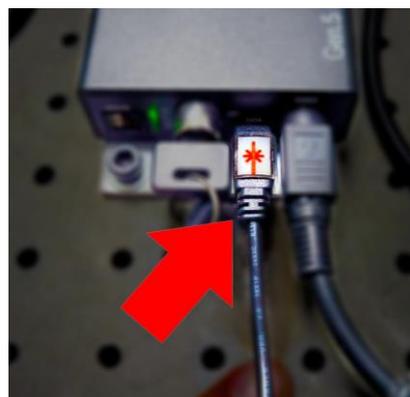




9. Close inVia spectrometer door.



10. Connect the following cable to the Cobolt controller of the desired laser.



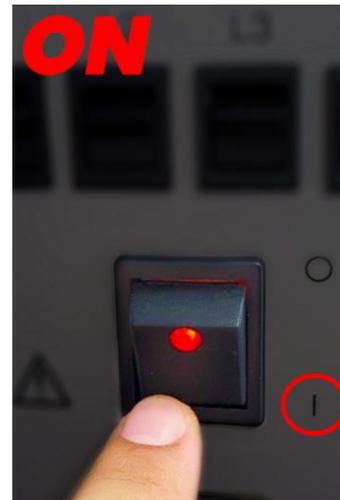
11. **Turn on** the desired laser. System 1 and 2 contain Cobolt controllers that need the following cable to be plugged when in use.

Laser has a key parallel to the table with green light indicates that the laser is off. Rotate key vertically to turn on laser.



WAIT 30 MIN BEFORE USING THE LASER THAT WAS TURNED ON

12. Turn on spectrometer AT THE SAME TIME AS THE LASER.



**WAIT 20 MIN BEFORE USING THE CCD
DETECTOR THAT WAS TURNED ON**

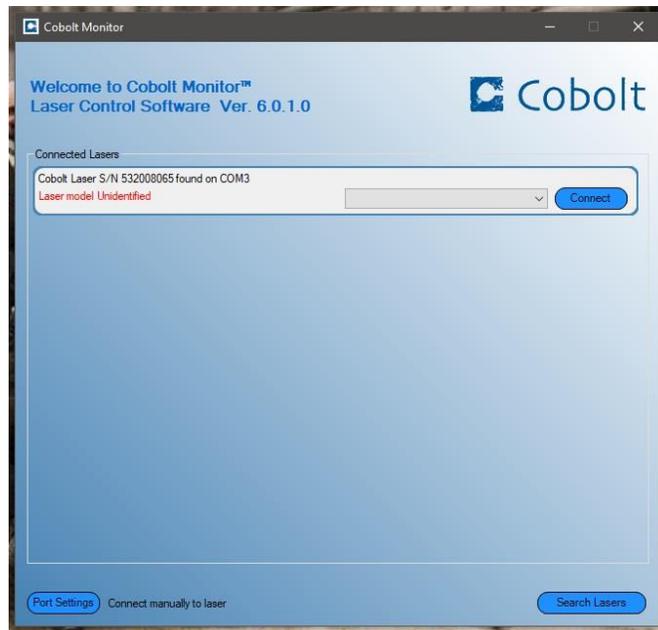
**BOTH THE LASER AND INSTRUMENT
CAN BE TURNED ON
SIMULTANEOUSLY**

**(i.e. you do not have to wait the initial 30 min to
start the spectrometer and wait other 20 min)**

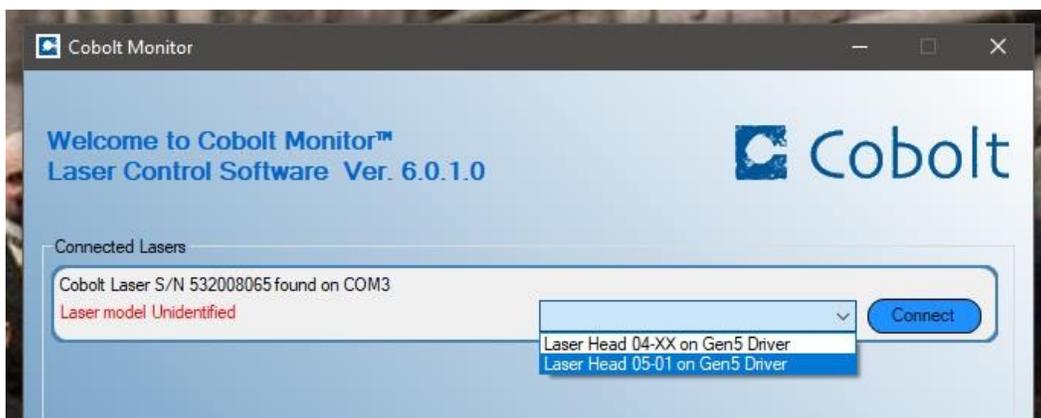
13. Turn on the tower of the computer.

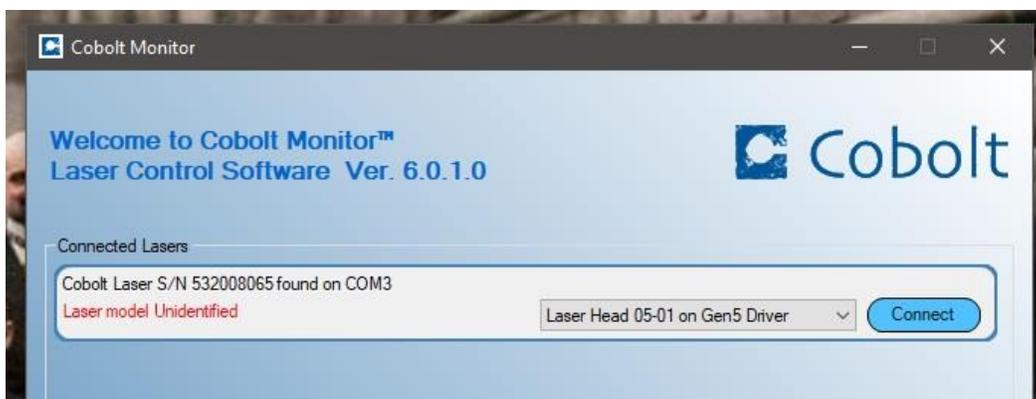


14. Open Cobolt program.

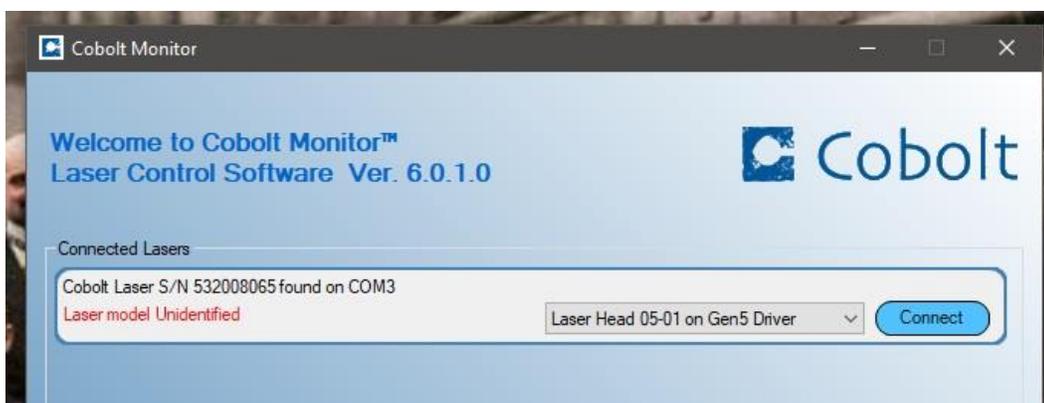


15. Select desired laser.

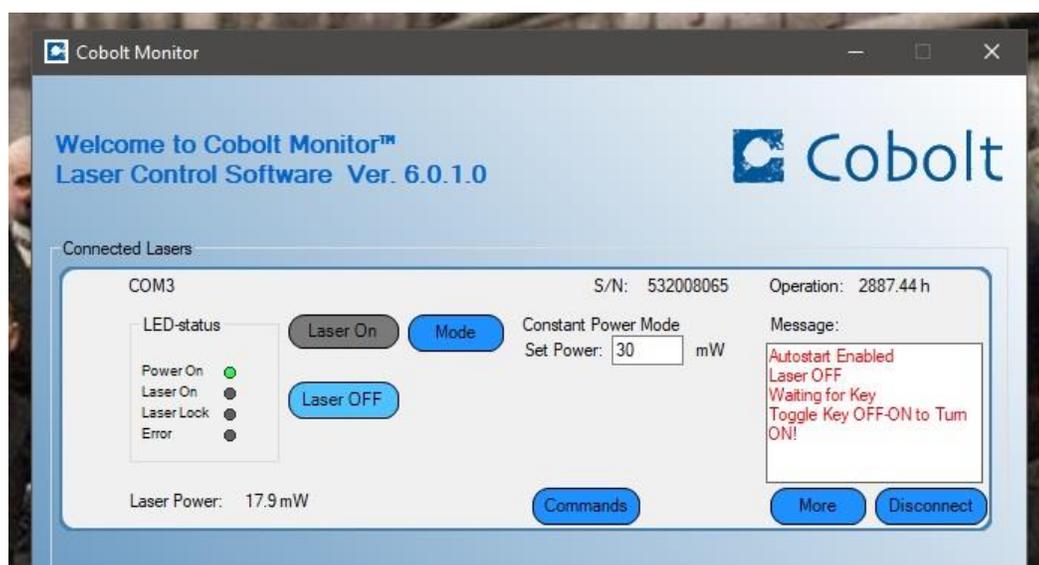




16. Select second option and click “Connect”.

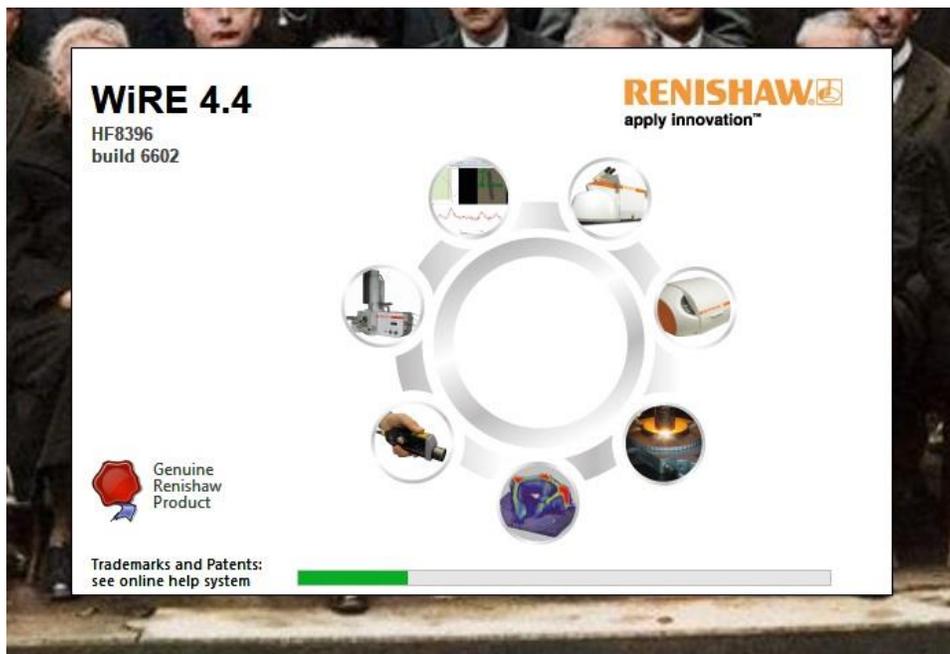
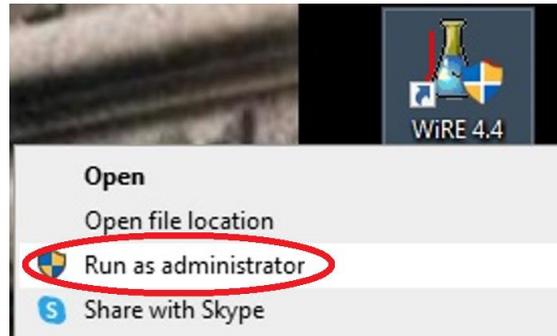


17. Place 30-50 mW power and readjust. Laser power depends on the analyte being utilized.

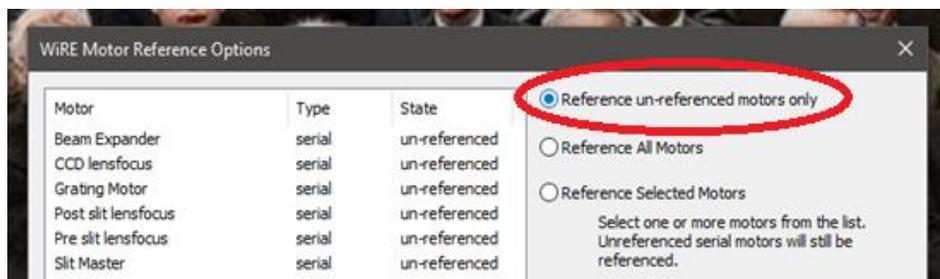


18. Press “ENTER” key after placing the laser power. There is no button, you press ENTER.

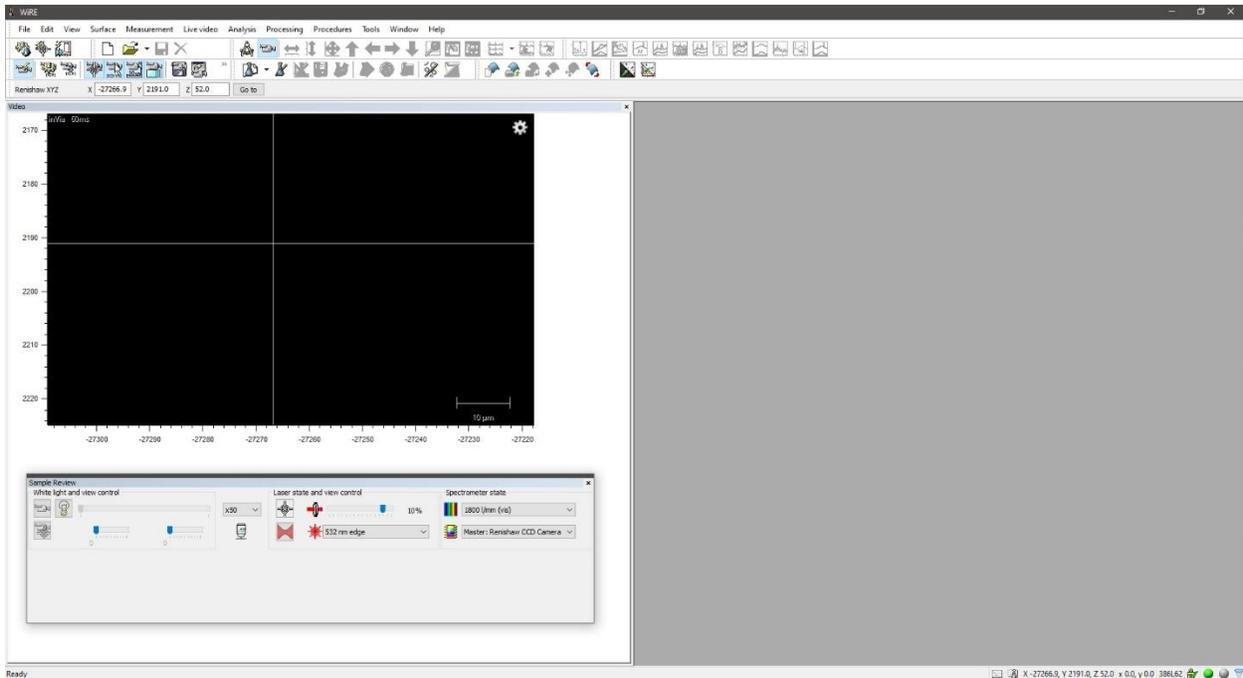
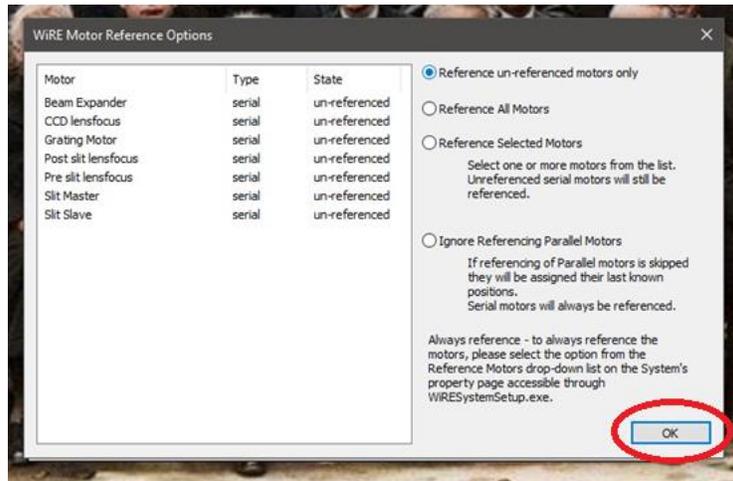
19. Open the WiRE 4.4 program.



20. Select “Reference un-referenced motors only” on the “WiRE Motor Reference Options” window.



21. Click "OK".



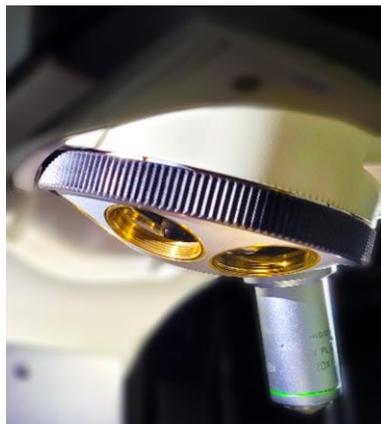
22. **Open** Raman microscope enclosure door by pushing the “door release” button.



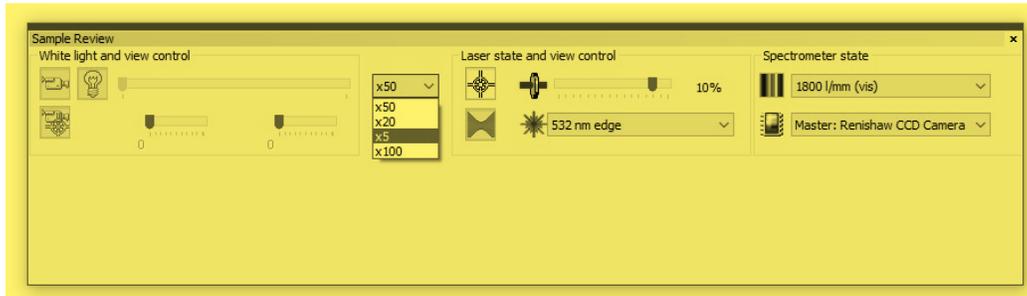
23. **Turn on** microscope light.



24. **Set** microscope objective of 100X for calibrating samples. **WEAR GLOVES.**



25. Set optical magnification on the WiRE software.



CALIBRATING FOR SOLID SAMPLES

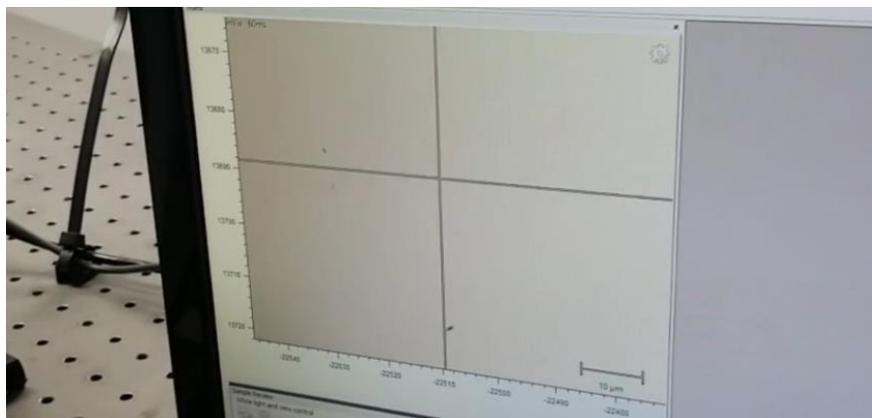
1. **Place** standard sample to calibrate the microspectrometer.



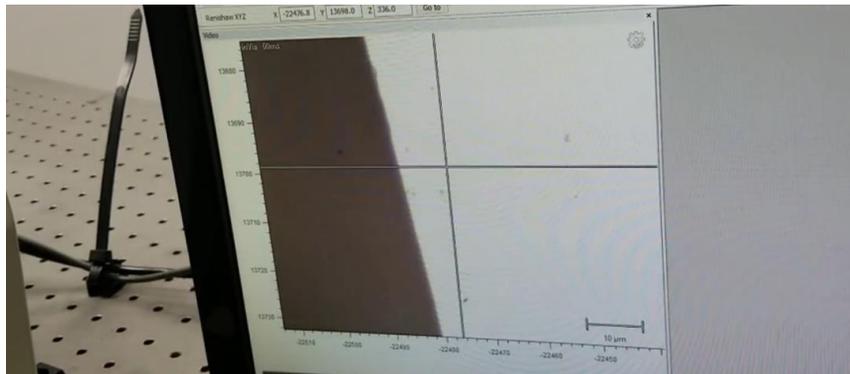
2. **Find** the optimal distance for the focal point.



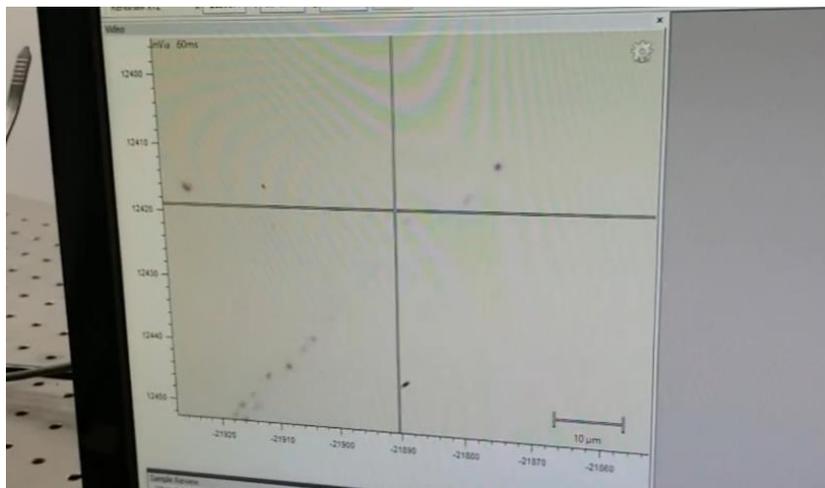
3. **Move** sample until the light is placed between the corner of the plate and sample. **Move** montage vertically to observe a gradient between corner of the plate and sample.



4. **Focus** until the line between the plate and sample is seen.



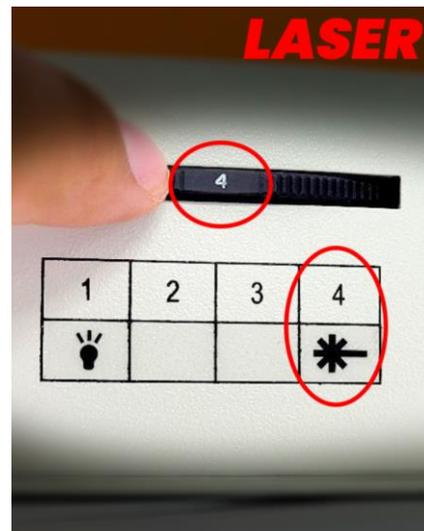
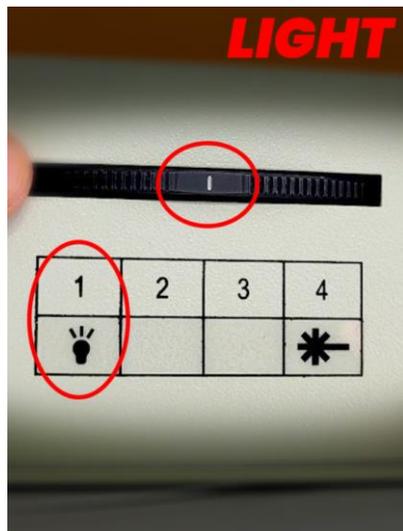
5. **Place** microscope on the place where there are stains.



6. **Close** microscope door.



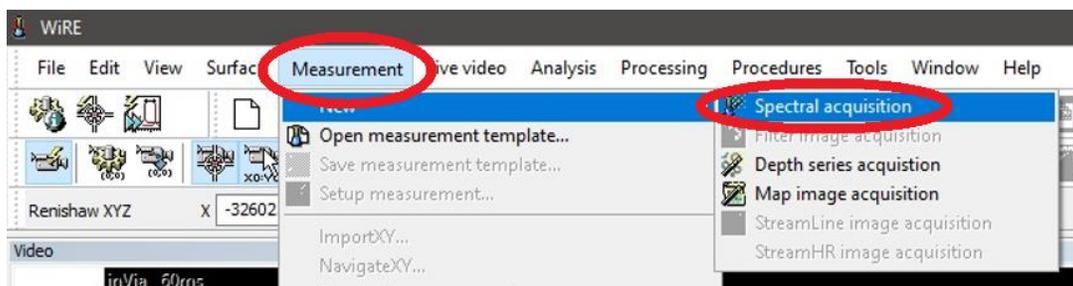
7. **Change** visible light option (1) to a laser (4).



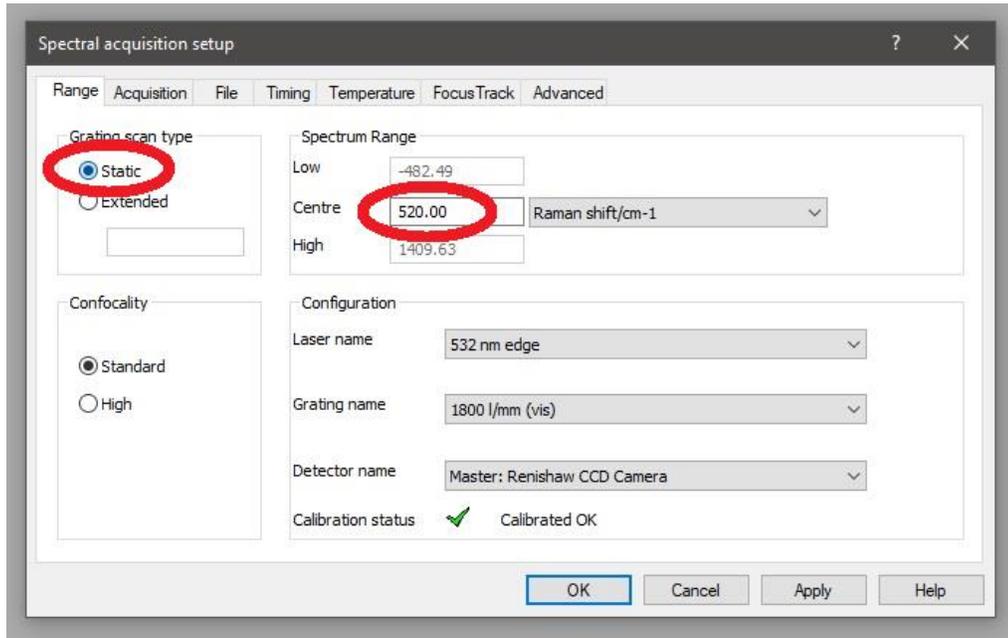
8. **Change** de light to laser rotating clockwise (CW). There are two clicks.



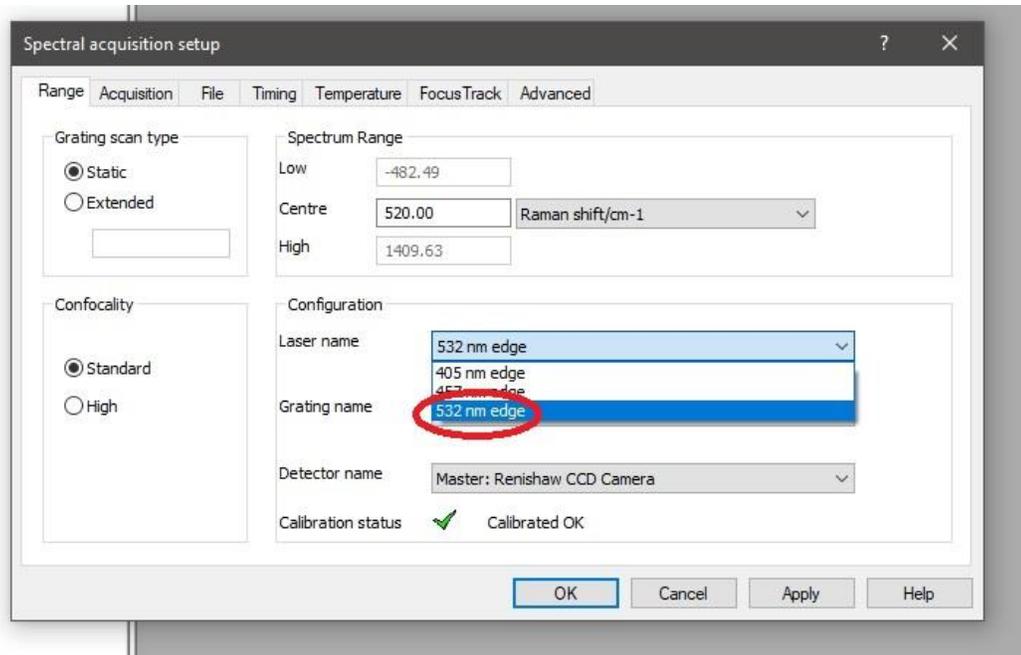
9. **Go to** Measurement → New → Spectral Acquisition in the WiRE program.



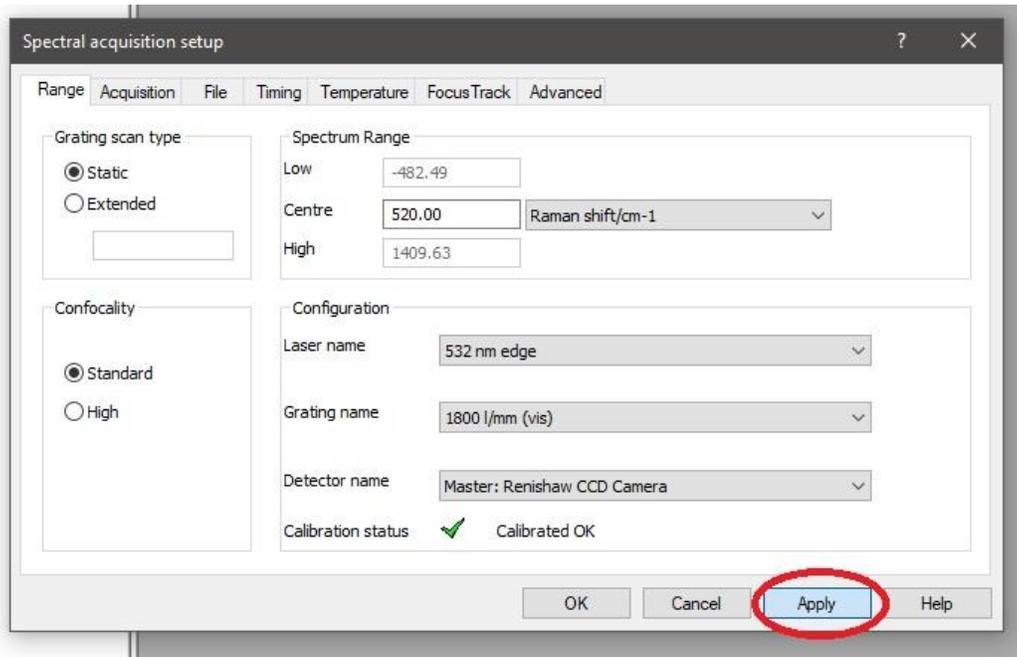
10. Change the Grating scan type to “Static” mode from the “Range” tab in the “Spectral acquisition setup” window.



11. Choose the Edge filter placing previously from Configuration → Laser name.

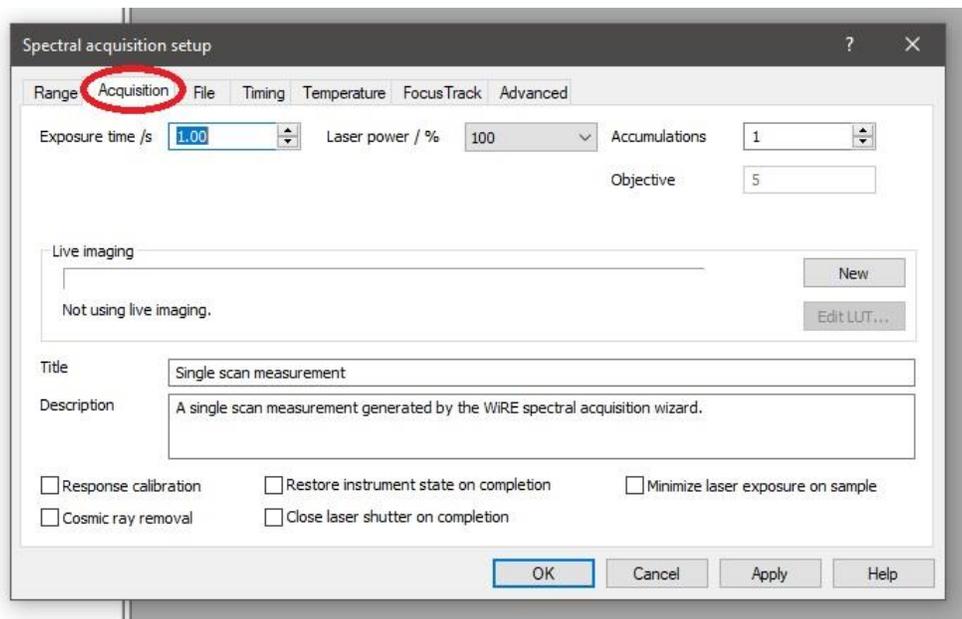


12. Click “Apply” and go to “Acquisition” tab.

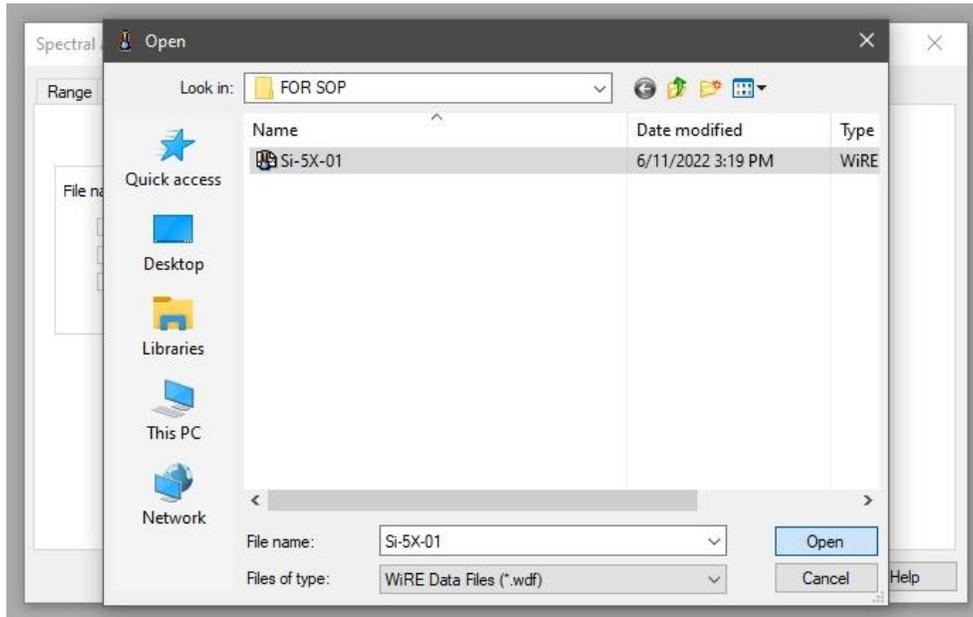


13. Adjust laser power, exposure time, and acquisitions.

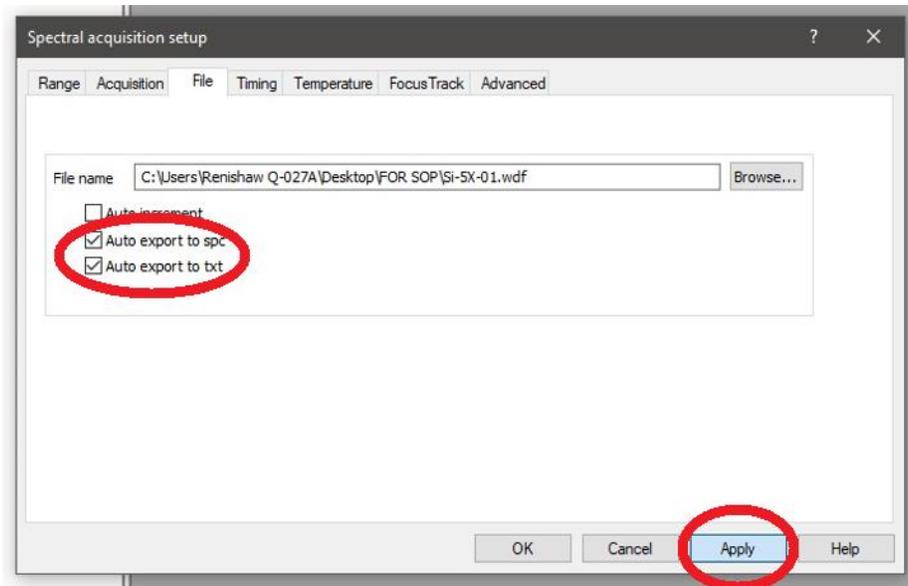
Laser power (LP) [mW]	
Exposure time (ET) [s]	
Accumulation number (ACC)	
Objective of Magnification (OBJ)	
Signal-to-noise Ratio (SNR)	



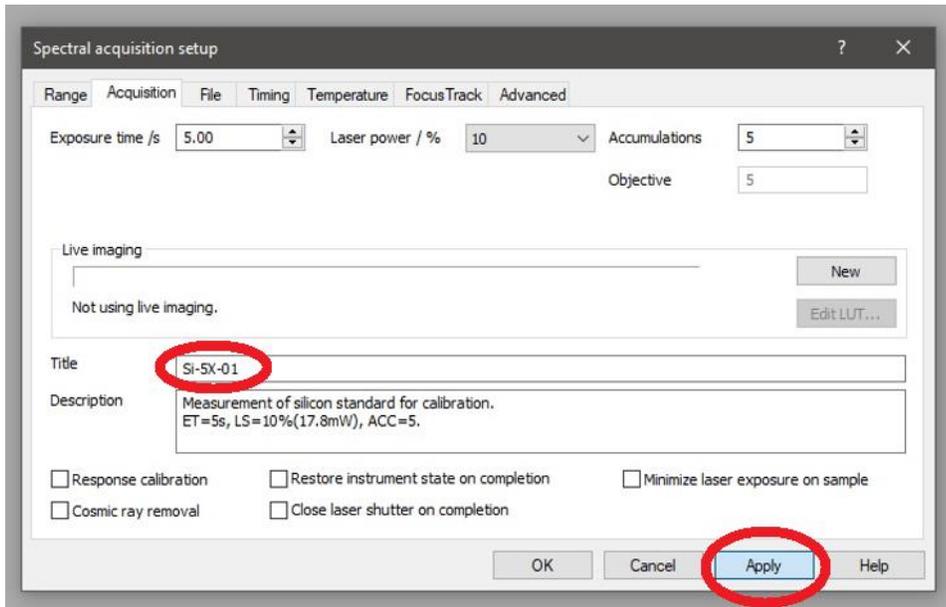
14. **Search or Create** folder where spectra will be recorded by going to the “File” tab and clicking “Browse”.



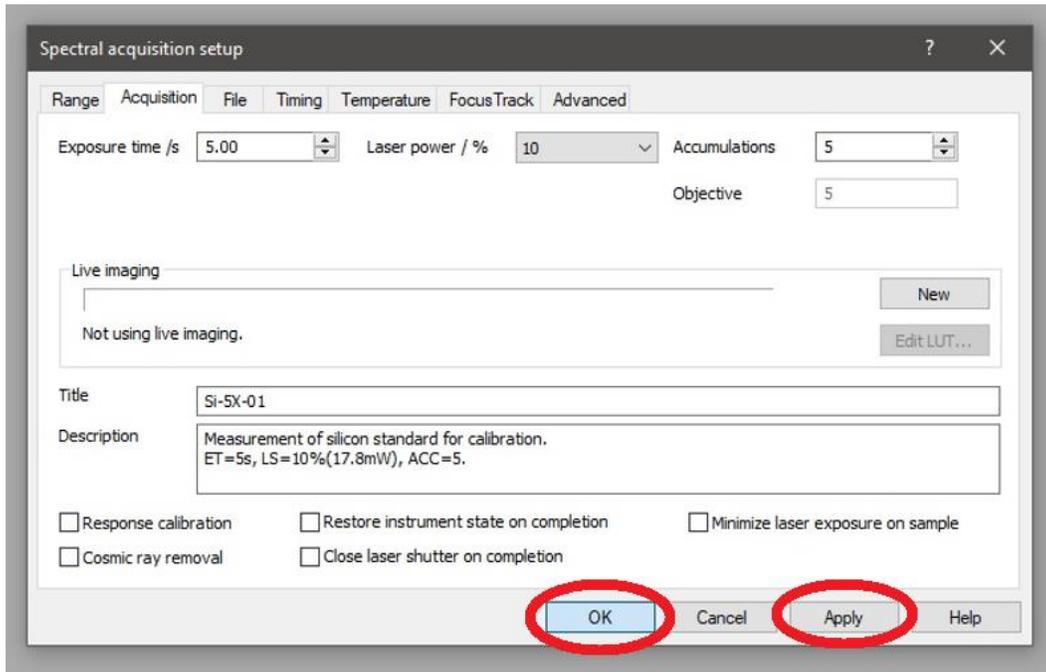
15. **Record** record spectra in *.spc and *.txt formats and click “Apply”.



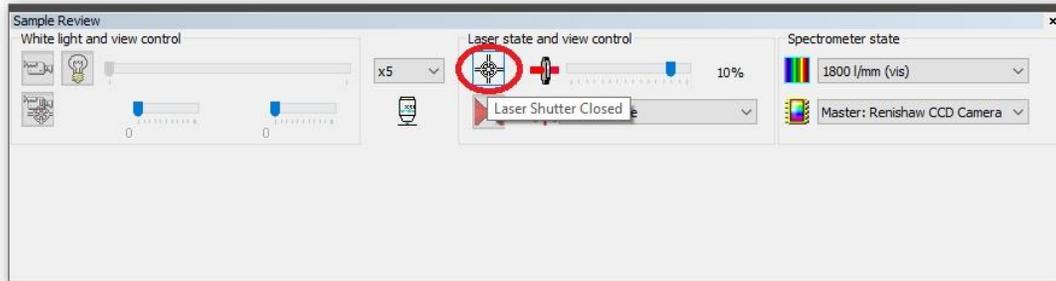
16. Place title on "Title" and click "Apply". E.g., Si-LP10-ET2-ACC-1-OBJ20X.



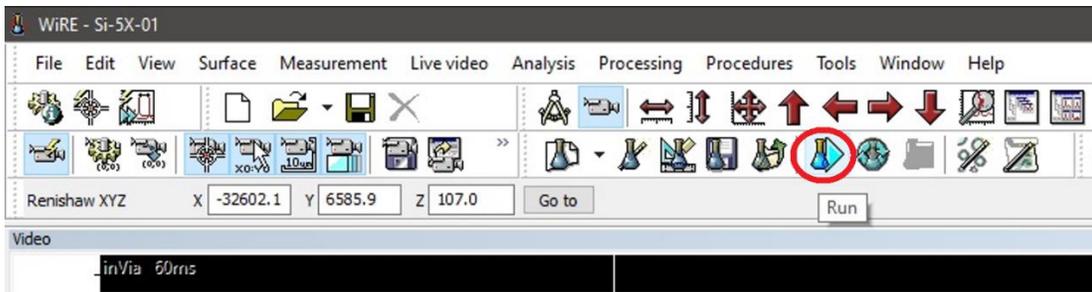
17. Click "Apply" and then "OK".



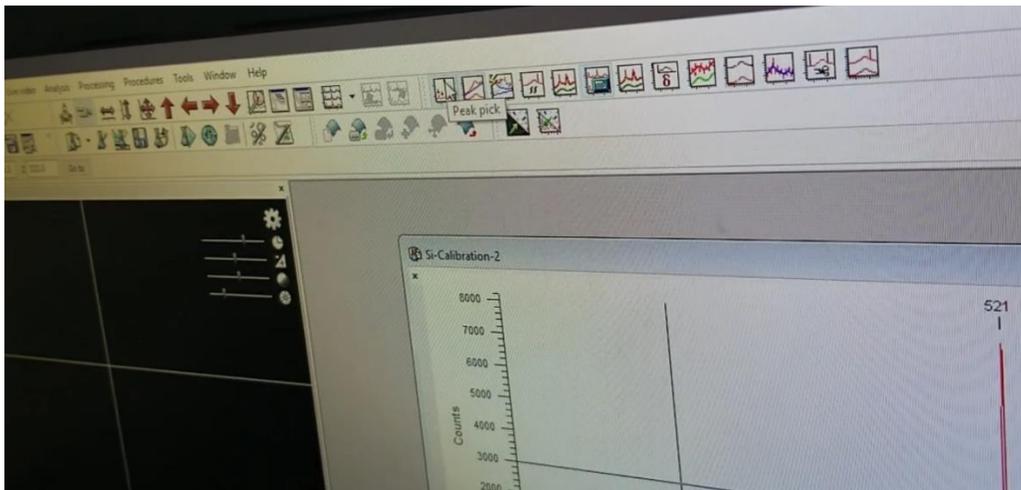
18. If it runs in “Static” mode, **turn off** laser from the “Sample Review” window.



19. Click “Run” to acquire spectra of the sample.

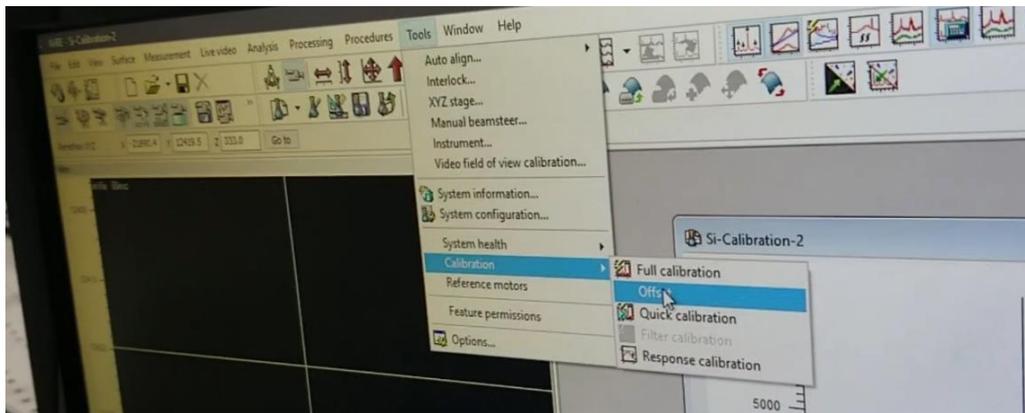
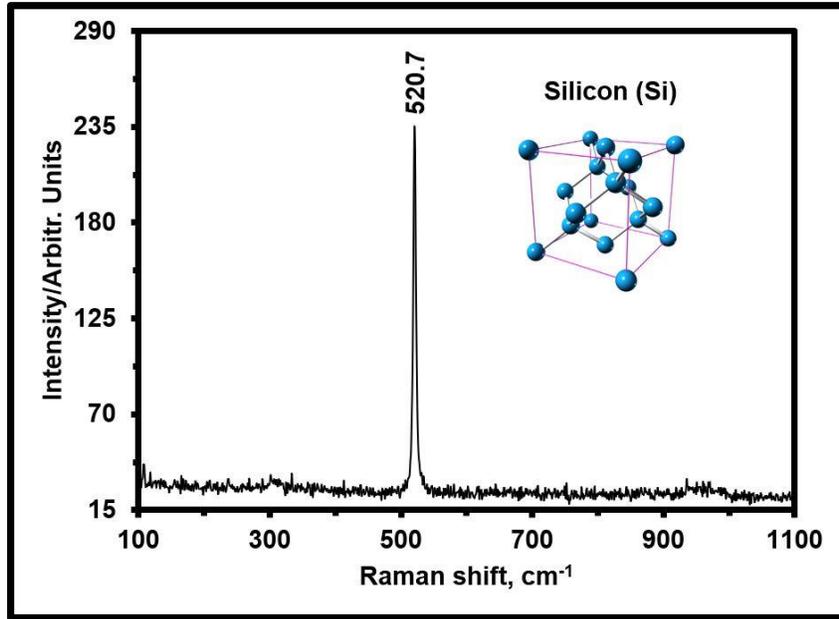


20. Identify peaks from the “Peak Pick” option.

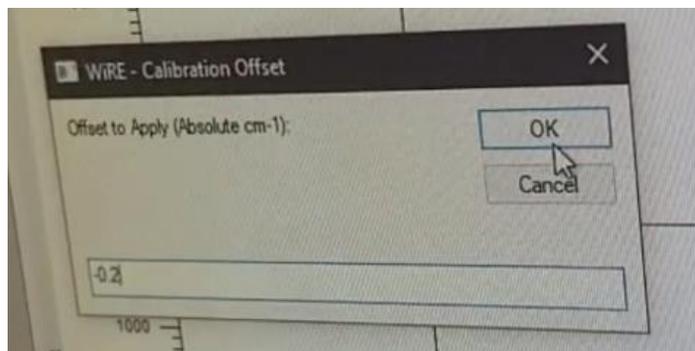


21. Fix peak shift from Tools → Calibration → Offset.

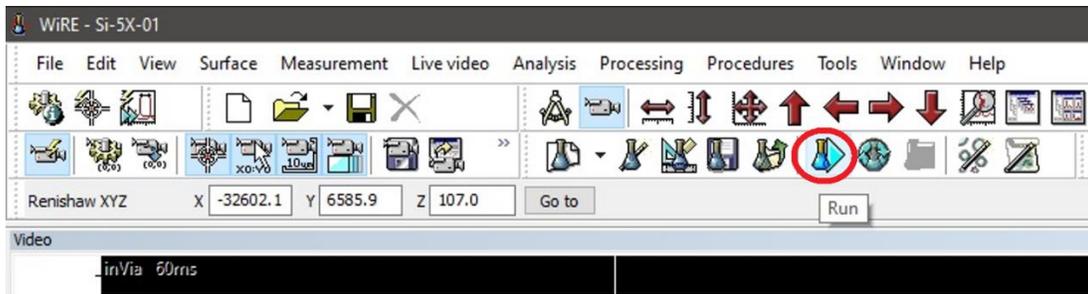
Solid Samples -> Silicon (520.744 cm⁻¹)



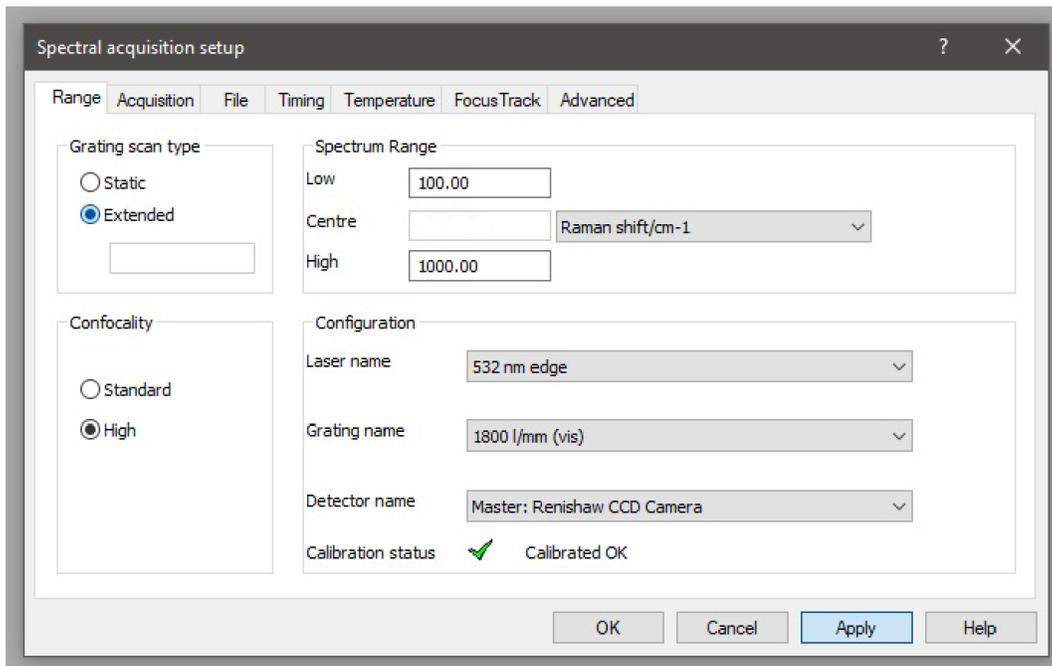
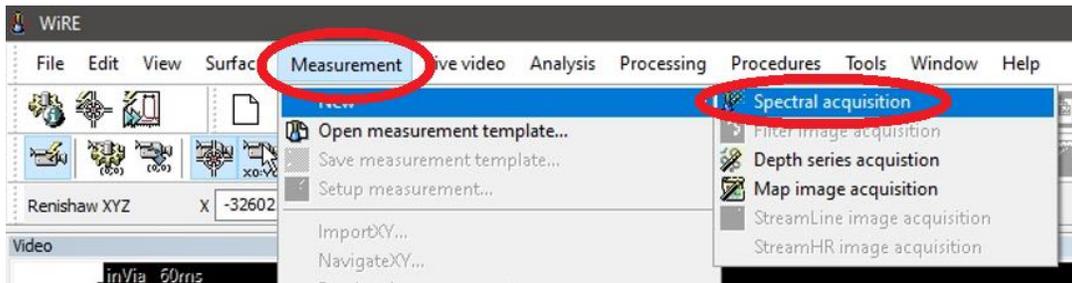
22. Place value to fix offset (positive values are subtracted and negative values are added) and click "OK".



23. Click "Run" to acquire a new spectrum.



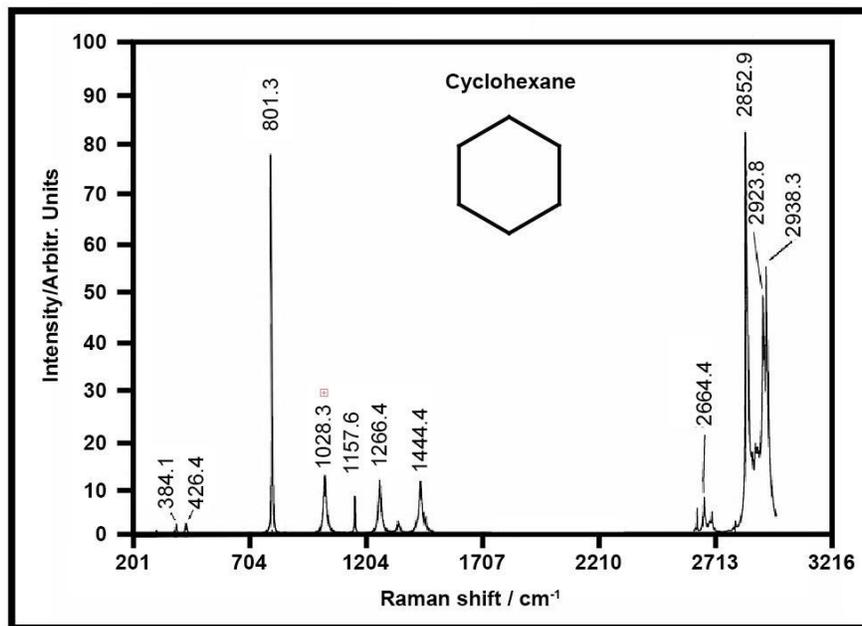
24. [OPTIONAL] Run again in “Extended” mode from 100 to 1000 cm^{-1} to validate offset.



CALIBRATING FOR LIQUID SAMPLES

1. **Place** standard sample to calibrate the microspectrometer.

Liquid Samples -> Cyclohexane (801.484 cm^{-1})



ACQUIRING SAMPLE SPECTRUM

1. **Open** microscope door.



2. **Place** sample to analyze inside.



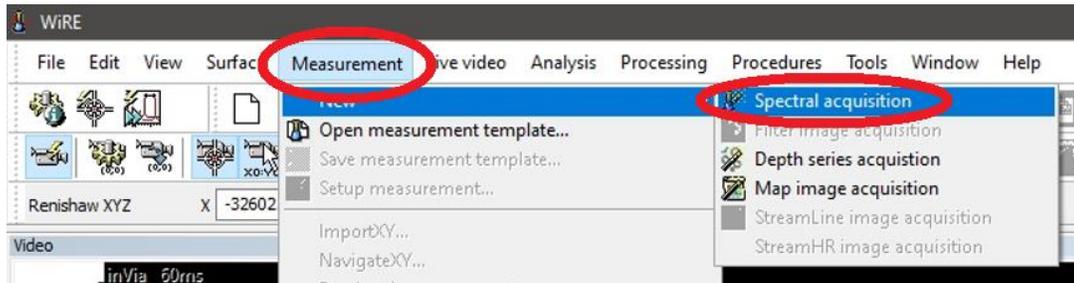
3. **Focus** the sample.



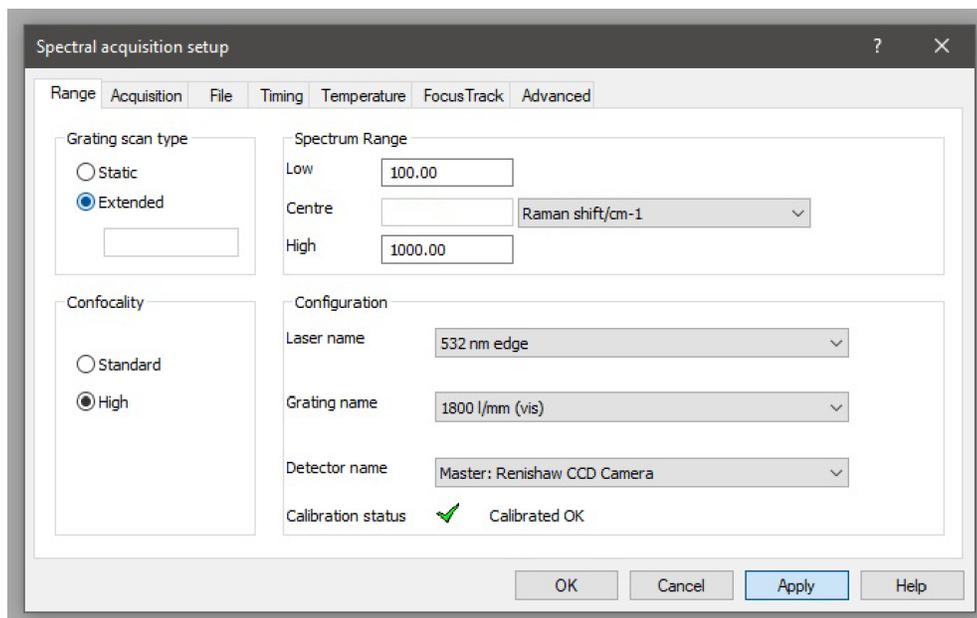
4. Close door.



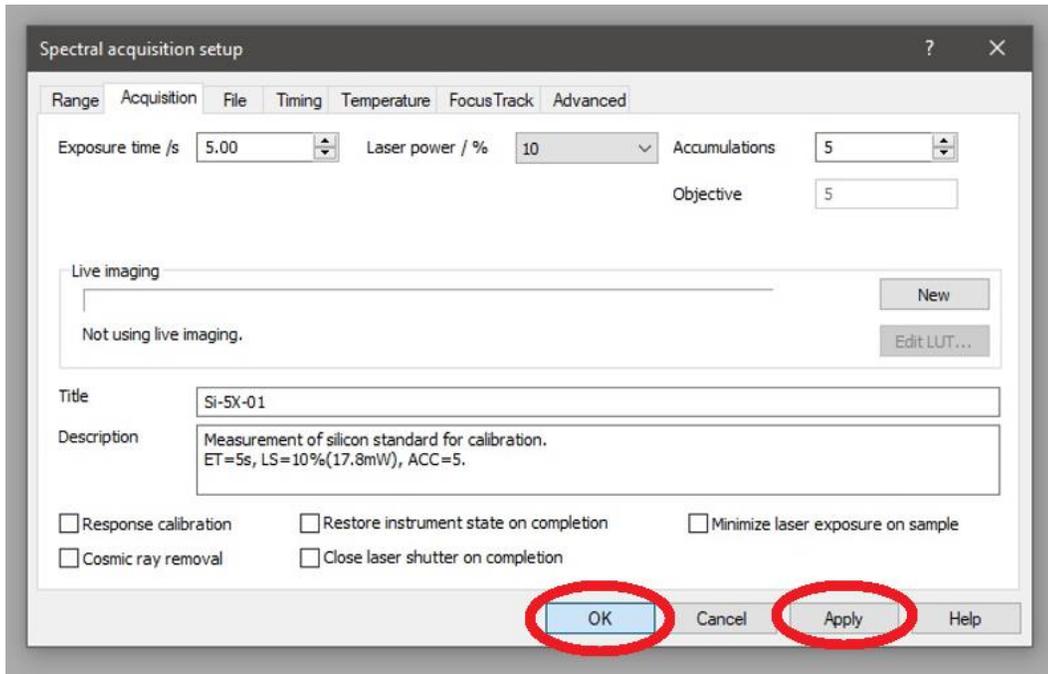
5. Go to Measurement → New → Spectral Acquisition.



6. Place “Extended” mode from the “Range” tab and the “Spectral acquisition Setup” window with the desired spectrum range.



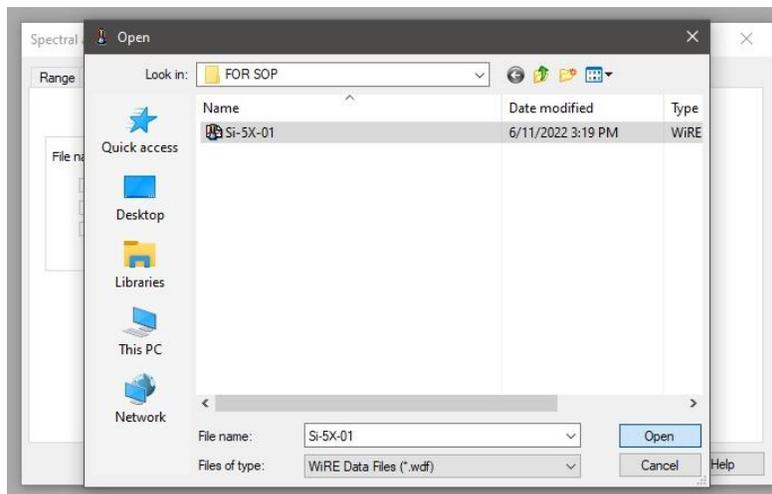
7. Click “Apply” and go to the “Acquisition” tab and then click “OK”.



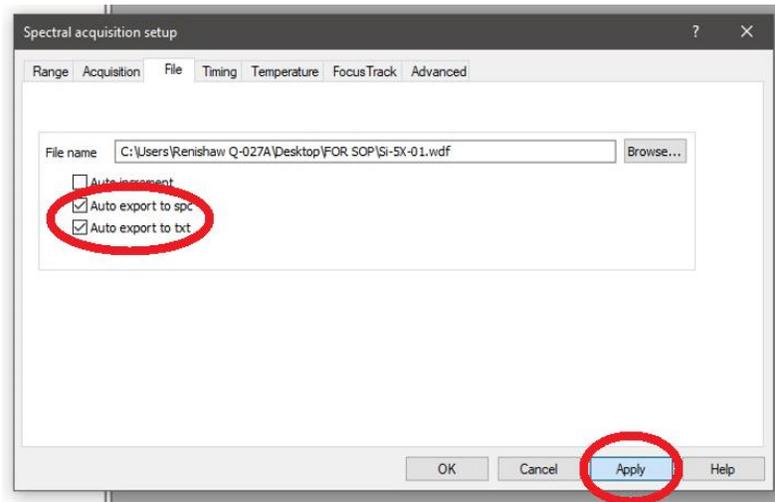
8. Adjust laser power, exposure time, and accumulations.

Laser power (LP) [mW]	
Exposure time (ET) [s]	
Accumulation number (ACC)	
Objective of Magnification (OBJ)	
Signal-to-noise Ratio (SNR)	

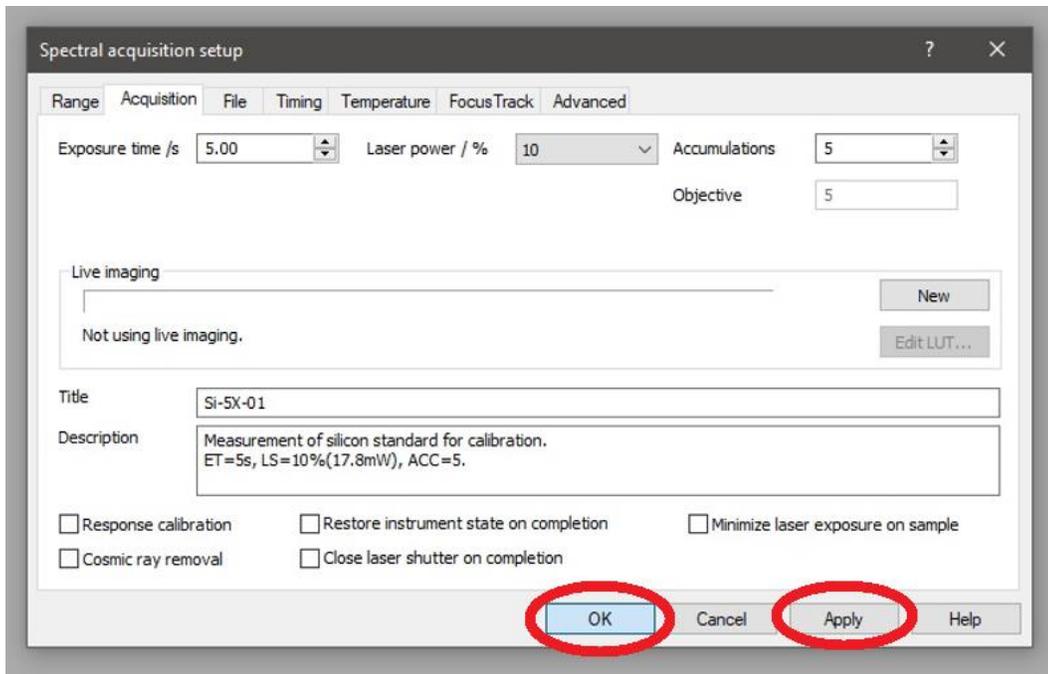
9. Search or Create folder where spectra will be recorded by going to the “File” tab and clicking “Browse”.



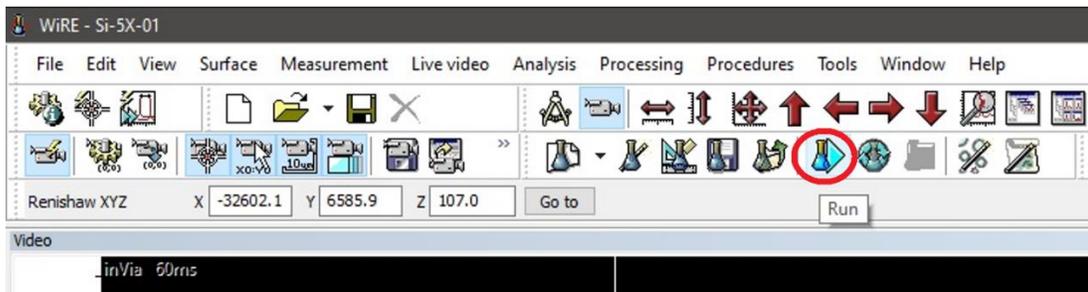
10. Record spectra in *.spc and *.txt formats.



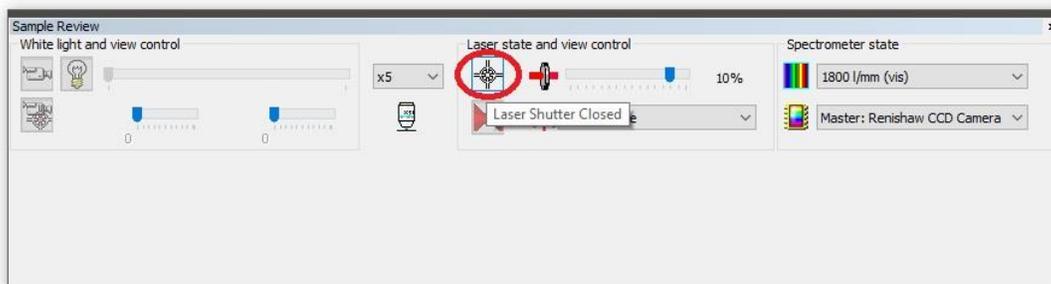
11. Place title on "Title", click "Apply" and then "OK".



12. Click “Run” to acquire spectra of the sample.



13. If “Static” mode ran, **turn off** laser from the “Sample Review” window.



TROUBLESHOOT

1. Optical setup is misaligned.

DO NOT TOUCH contact authorized personnel for help.

If authorized personnel cannot be contacted, move the mirror closest to the laser.

Advisor Signature

Co-Advisor Signature
