Instrument Hazards and Best Practices: Raman

This document will cover the inherent hazards when utilizing this piece of equipment as well as the best practices and procedures to avoid danger. These hazards will not include basic things that may be included in the basic safety training document that each user has attested to have reviewed at fom.wwu.edu/documents. Samples are to be prepared in the user's laboratory before being transported to the Optical Microscopy lab. Lab coats are to be provided by the user if needed due to sample type.

Hazards:
- Compressed gases
- Ultraviolet to near infrared laser radiation

1. Required PPE
Appropriate laboratory attire is required at all times in the SciTech laboratories. Whenever chemicals are being used, an additional requirement of a lab coat is required. Lab coats are to be provided by the user. Whenever a user is in the SciTech labs, the minimum requirement for eye protection is wrap around impact glasses. Anytime liquid chemicals are present in the same room as the user without a direct barrier, all users in the lab must wear chemical splash goggles. Splash goggles must be approved by State of Washington Administrative Code (WAC 296-24-078). If chemicals being used are considered toxic, caustic, corrosive, flammable solvents, carcinogenic, mutagenic, or teratogenic, a minimum of disposable nitrile gloves is required. Avoid chemical transfer by taking off gloves when using anything other than the chemical(s).

2. Compressed gases
All users must have completed the compressed gas and cryogenic liquid training before accessing this equipment or touching any compressed gas cylinder used with the Linkam Cell stage accessory. All prudent practices dictated in that training as well as associated training documents must be strictly adhered to at all times.

3. Ultraviolet to near infrared laser radiation
The Raman is equipped with four lasers (405nm, 532nm,633nm, and 785nm). It utilizes a high powered, 213 nm laser to ablate samples. The laser system is designed with many interlocks and safety devices in place to prevent users from ever being exposed to the laser. Under normal operation, the chances of being exposed to the laser are almost zero. If any interlocks are not satisfied and cannot be remedied by the user, the lab manager must be notified, and the user should cease use of the system immediately. At no time should anyone tamper with or attempt to dismantle or repair any part of the instrument. Failure to follow these instructions could lead to serious injury or death.

Table I. Grating Settings.

Wavelength (nm)	Laser Name	Grating Setting (lines/mm)
405	Diode	2,400
532	Nd:YAG	1,800
633	HeNe	1,800
785	Diode	1,200

1. Turning On the Instrument and Laser Warmup

- **Turn instrument on in this order:**
- Turn Spectrometer. Wait 60 seconds for initialization to finish.
- Open WiRE 5.5 software. May take a few minutes to load.
- Turn on lasers with key on back of laser (quarter turn CW)
- Warmup 15-20 minutes

2. Initial Set Up, Sample Insertion, & Focusing

- Turn on microscope illumination (lightbulb icon) and video (camera icon)
- Check that the objective & laser setting in the software corresponds with the objective & desired laser excitation being used. Check that grating setting is correct for the laser wavelength (**Table I**)
- Push the door release button on the microscope enclosure to open door.
- Lower the stage and place a metal slide onto the stage.
- Rotate the turret to the 5x or 50xL objective.
- Focus on the polished metal slide. Move the **stage up past focus**, then move **stage down while looking through binocs to focus**. This prevents hitting the objective.

Table II. Microscope Focusing Using Right Knob.

Knob Turning	Sample Stage Movement
Clockwise	Stage moves **UP**, towards the objective
Counterclockwise	Stage moves **DOWN**, away from the objective

Only use the corresponding objective when controlling the stage with the trackball

3. Laser Alignment

- Set laser power low so the laser spot is within the crosshairs (~0.0001%. for 532nm; 5e-07% for 785nm)
- *Focus* the laser to a small spot.
- Only if necessary: Tools → "Manual beamsteer" Beam steer **LEFT** to center the laser spot in the crosshair. Move laser up/down, then left/right. If the laser needed to be centered, click SAVE and OK. **Please DO NOT change the right beamsteer.**
- Tools → Calibration → Quick Calibration Narrow peak 521 ± 1 cm^{-1} should appear. Right click in window → Tools → Peak Pick. Record the intensity of the 521 ± 1 cm^{-1} peak in the log.

4. Sample Analysis

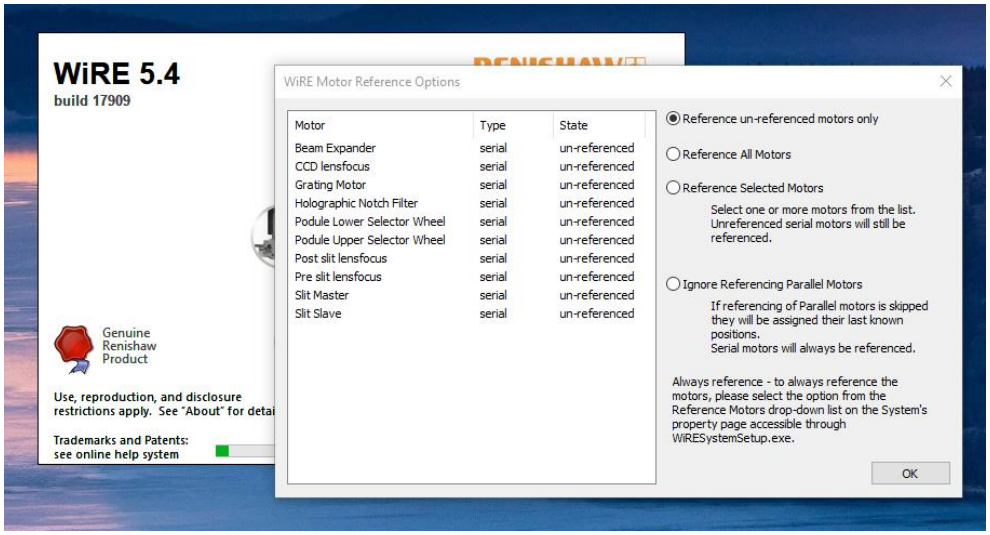
- Confirm that the laser is shuttered, open microscope enclosure, load sample, and focus sample surface.
- Go to Measurement → New → Spectral Acquisition
- Range tab → select either Static or Extended exposure. For static, choose center value.
- Acquisition tab → Set Exposure Time, Laser Power, and Accumulations. Check cosmic ray removal and restore instrument state upon completion
- File Tab → select save location, give a file name, check auto increment and export txt file
- Press "Apply" and "OK." Run sample.
- Processing → Subtract Baseline
- Right click "Accept" to subtract baseline. Save as (*.wdf) and (*.txt)

5. Turning Off the Instrument

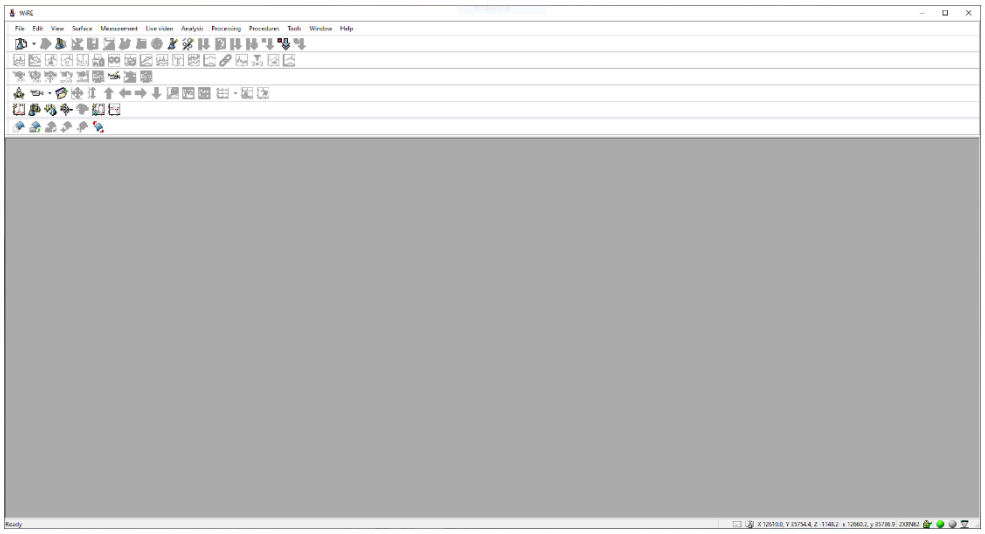
- Switch all lasers off, exit software and logout of FOM, unload sample, & flip the power switch to turn off the Raman
- Replace binocular covers on microscope

A. Turning On the Instrument and Laser Warmup

1. Make sure the microscope enclosure is closed
2. **Turn instrument on in this order:**
3. Turn Spectrometer on [the main power switch is located on the right side of the unit
   Wait 60 seconds for initialization procedure to finish
4. Open Renishaw Raman software, WiRE 5.5, on Desktop. Wait for software to load, may take a few minutes.
5. Turn on lasers to be used [turn key on back of laser, wait until laser light turns on, length of warmup should be 15-20 minutes. If using the 532nm laser, choose edge]
6. If the WiRE Motor Reference Options box appears, select Reference un-referenced motors only [which would be the same as Reference All Motors], then click OK

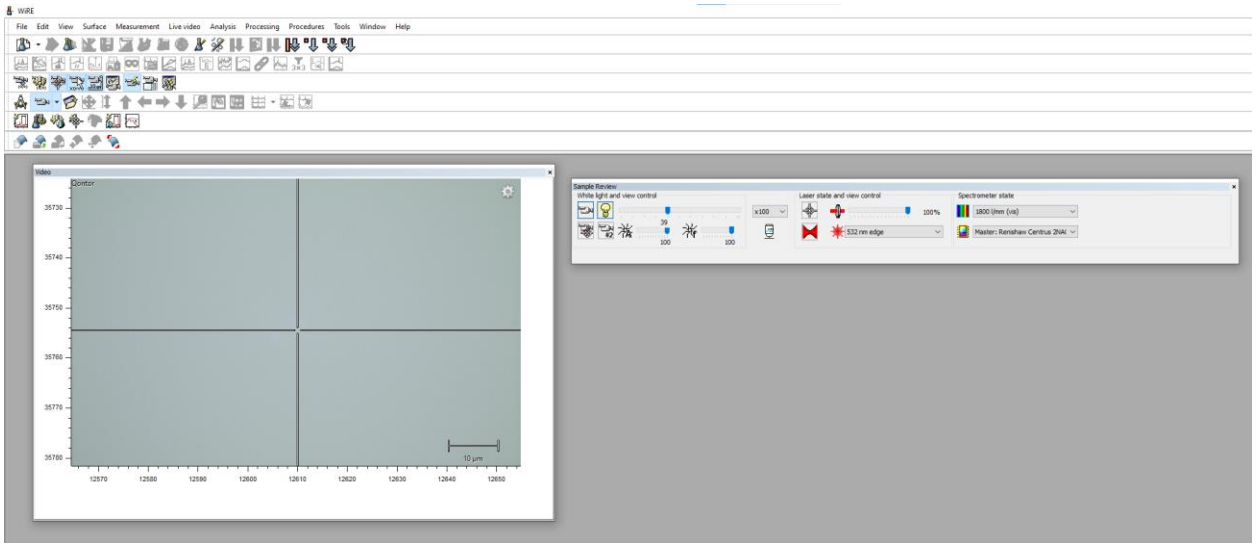


7. If your main WiRE window looks like this:



Open 3 windows: 1) View → Live Video 2) View → Sample Review 3) View → Navigator

[Note: Right click on Video and/or Sample Review window and deselect Docking for the ability to resize/move the window, perhaps to the right-side monitor]



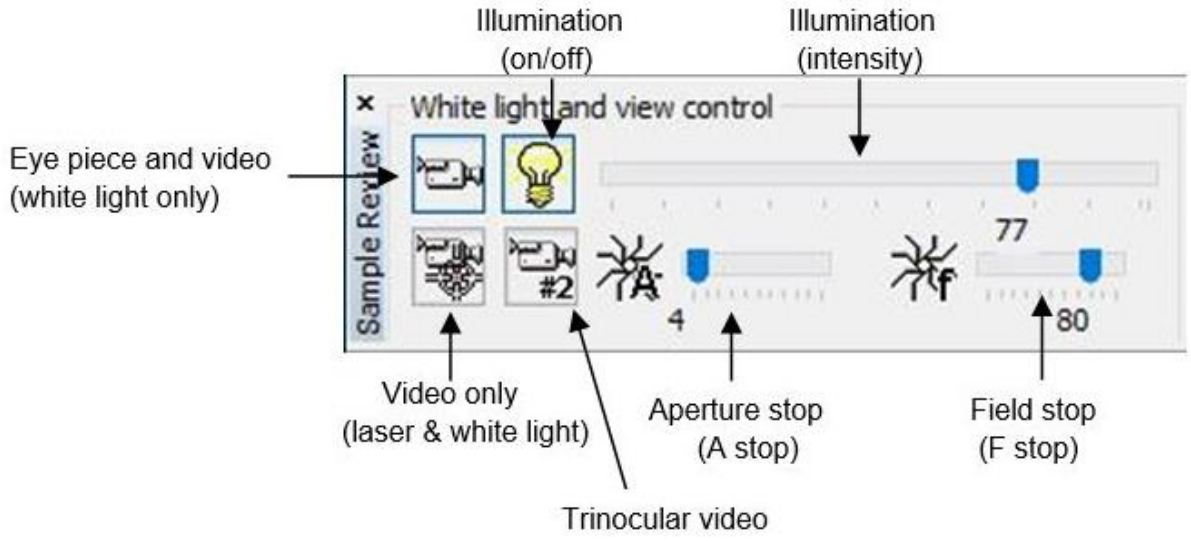
B. Initial Set Up, Sample Insertion, & Focusing

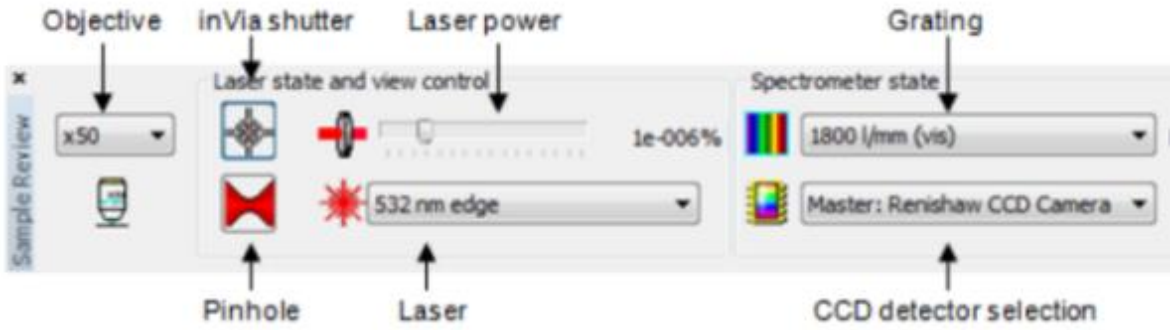
1. Check that grating setting is correct for the laser excitation wavelength (**Table I**). (Note: the microscope has the 1,800 and 1,200 lines/mm gratings loaded. If the 2,400 lines/mm is needed, a ***trained STS staff member*** will insert the grating.)

Table I. Grating Settings.

Wavelength (nm)	Laser Name	Grating Setting (lines/mm)
405	Diode	2,400
532	Nd:YAG	1,800
633	HeNe	1,800
785	Diode	1,200

2. Push the Door Release button on the microscope enclosure and open door
3. Lower the stage by turning the inner big black knob on the right side of the microscope counterclockwise to the lowest setting possible.
4. Place a polished metal slide onto the stage. [polished metal slide up]
5. Rotate the turret to the 5x or 50xL objective into position [change software setting for correct objective first]
6. Turn on microscope illumination (lightbulb icon) and video (camera icon) using the software.
7. General settings in the Sample Review window: Lamp=50, AStop=50, FStop=50





8. Focus on the surface of the polished metal slide. It is recommended that the microscope binocular and focusing knobs be used for initial focusing (**Table II**). Once the surface if found, then the software controls can be used. (Note: the computer trackball moves the stage, and the track ring adjusts the focus.)

Table II. Microscope Focusing Using Right Knob.

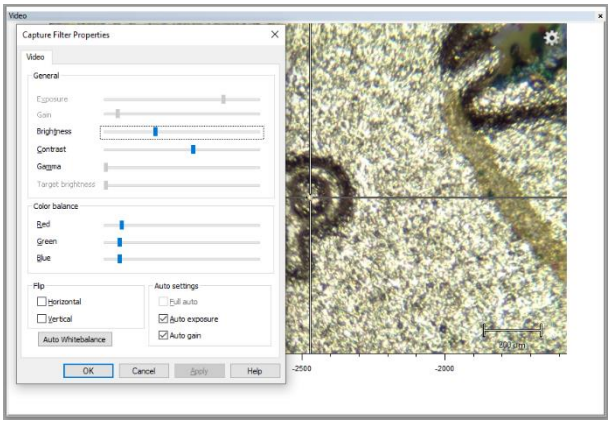
Knob Turning	Sample Stage Movement
Clockwise	Stage moves **UP**, towards the objective
Counterclockwise	Stage moves **DOWN**, away from the objective

WARNING: Care must be taken when using the trackball to avoid collision between the stage (or sample) and the microscope objective.

The XY stage and Z drive are controlled from the trackball as shown to the left. Ball movement (yellow) controls the X and Y motion, and the scroll ring controls Z motion. X and Y motion is disabled while the scroll ring is being rotated. Each of the four buttons selects a different speed of stage movement. The microscope objective in use will influence which speed to use. The suggested speeds are color coded in the diagram above corresponding to microscope objectives 100× [white], 50× [blue], 20× [green] and 5× [red].

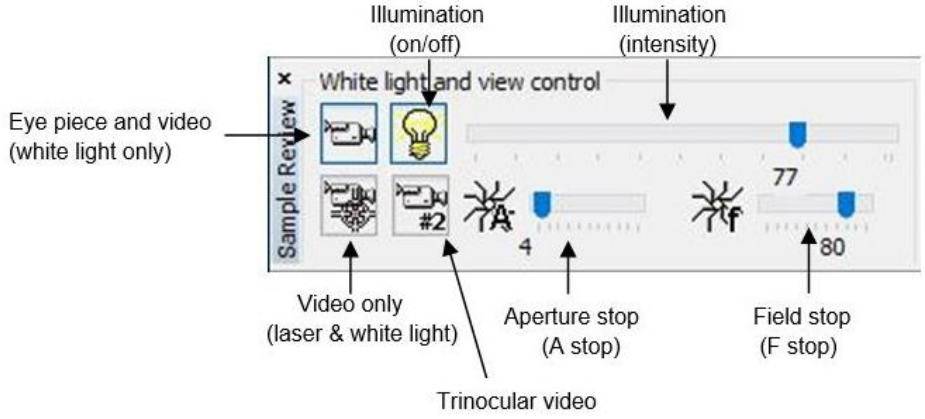
The trackball will be disabled if untouched for 1 minute. This is to prevent unintended stage motion. Pressing one of the four buttons will re-enable the trackball.

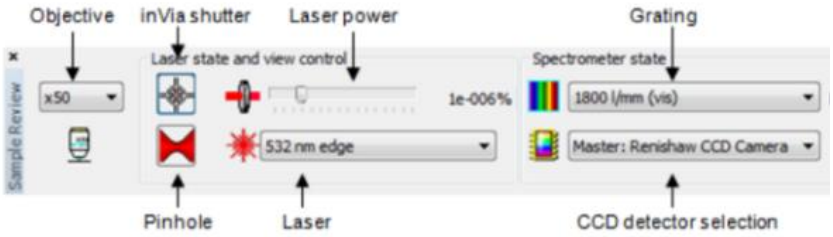
9. To adjust the video camera settings, click on the gear icon (upper right of video image) and select autoexposure and auto gain. Once the appropriate video settings are obtained it is recommended that the user deselect autoexposure and auto gain to avoid image lag time.



C. Laser Alignment

1. Double check in the Sample Review window that the objective and laser setting in the software corresponds with the objective being used and the desired laser excitation.
2. Decrease laser power (~0.0001% for 532nm, ~5e-07% for 785nm). (Note: this is a percentage of the total laser power, not the actual power of the laser on the sample.
3. Press button in the lower left so that the sample is viewed under video and laser.





4. *Focus* the laser to a small spot.
5. OPEN laser shutter (inVia shutter)
6. Go to Tools and select “Manual beamsteer.”
7. Only if necessary, use Beam steer LEFT to *center* the laser spot in the crosshair. When results are satisfactory, click SAVE. Note: in this case the laser spot will follow the direction of the arrows. If you make a mistake, you can simply hit RESTORE.
8. **DO NOT CHANGE RIGHT BEAMSTEER SETTINGS EVER**. If you think the laser is misaligned, contact STS staff
9. After the laser has been aligned go to Tools → Calibration → Quick Calibration. If the calibration is not within a set of limits, an error message will appear. If this occurs, immediately contact a trained STS staff member and do NOT proceed any further.
10. An internal silicon standard is used to calibrate the Raman shift for each laser wavelength being used. A strong and narrow peak 521 ± 1 cm^{-1} should appear if the calibration was successful.
11. Shutter the laser and turn the laser power to 0.5%.
12. Raman microscope is now ready to analyze samples.
13. Laser alignment & calibration needs to be done *only* for lasers that will be used for a given experiment. Changing microscope objectives does NOT affect the calibration, but if changing the microscope objective, change the setting in the Sample Review FIRST.

D. Sample Analysis

1. Confirm that the laser is shuttered. Open microscope enclosure and load sample.
2. Make sure “Sample Illumination On” and “View the sample under Video and Eyepieces” are selected. Turn up lamp intensity if needed.

3. Bring the sample into focus by moving the stage up past focus and then moving **stage down while looking through binocs to focus**. This prevents hitting the objective. It is recommended that the microscope binocular and focusing knobs be used for initial focusing (see **Table II**). Once the surface is found, then the software controls can be used. (Reminder: the computer trackball moves the stage, and the track ring adjusts the focus.)
4. Go to Measurement → New → Spectral Acquisition
5. Adjust the settings as needed.
6. Select either Static or Extended exposure.
7. If using Static scan type, set spectrum range by setting the center value.
8. Go to the Acquisition tab.
9. Set Exposure Time, Laser Power, and Accumulations (select Cosmic Ray Removal if applicable).
10. Press “Apply” and “OK.”
11. Run sample. (Note that if the counts start to get into kilo-counts you run the risk of oversaturating your detector).
12. Go to Processing → Subtract Baseline.
13. Right click “Accept” to subtract baseline.
14. Save as (*.wdf) and (*.txt).
15. Switch samples and repeat steps 2 - 14.
16. To switch lasers, select dropdown menu, switch wavelength, and recalibrate the laser. Make sure correct grating is selected for a given laser (see **Table I**).

E. Turning Off the Instrument

1. Unload sample.
2. Switch all lasers off.
3. Exit the software and logout of FOM.
4. Flip the power switch to turn off the Raman microscope.