



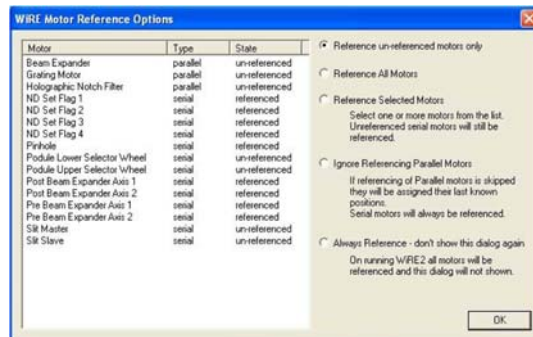
# Rosencrantz and Guildenstern - Dark Field / Raman / fluorescence microscopy system

## Operation instructions

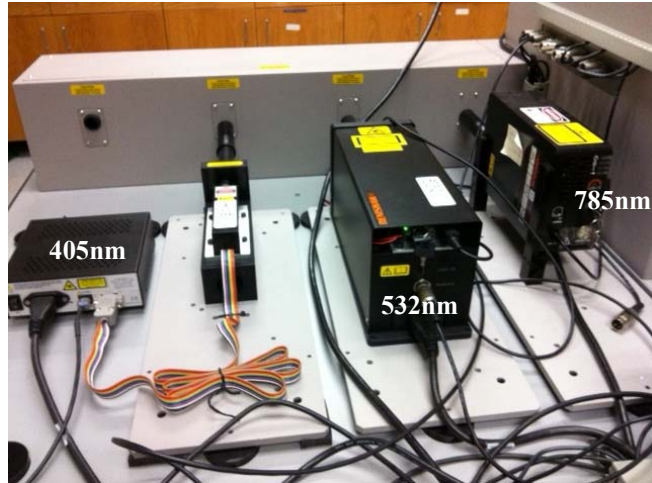
1. Turn On the power switch, system power switch is located towards the back of the system on the right hand side. Wait ~10 min for CCD to achieve operating temperature.



2. Start the software by double clicking on the **WiRE 3.4** icon, select Reference unreferenced motors only, Ok



3. There are three lasers: 785, 532 and 405nm. Decide which laser wavelength(s) will be best suited to the intended application, and power up the laser(s) required:
  - 405 nm laser: power switch at the back of the power supply on on position, then the key at the front of the power supply onto the on position.
  - 532 nm laser: power key at the back of the laser. 1 min initialization time.
  - 785 nm laser: power key at the back of the laser. 3 minutes initialization time.



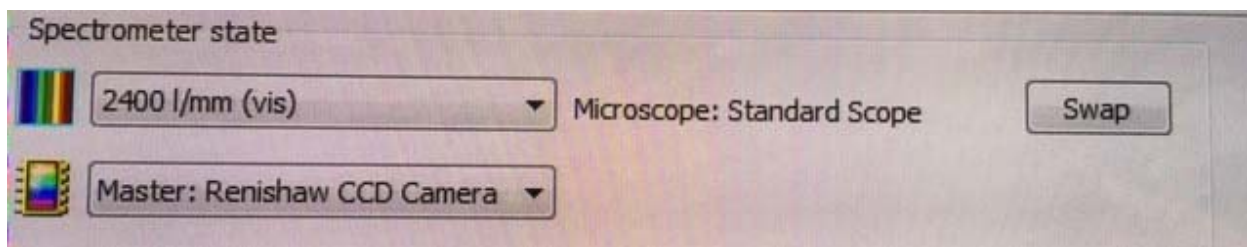
4. Lower the microscope stage using the Z-position adjustment knob on the side of the microscope. Pull back the sample clip on the stage, and place your sample slide, and secure it in place with the spring-loaded stage clip. Care should be taken to ensure a sufficient clearance to allow the required objective to be selected without colliding with the sample. You can move the stage by using either the manual controls or the motorized XYZ stage



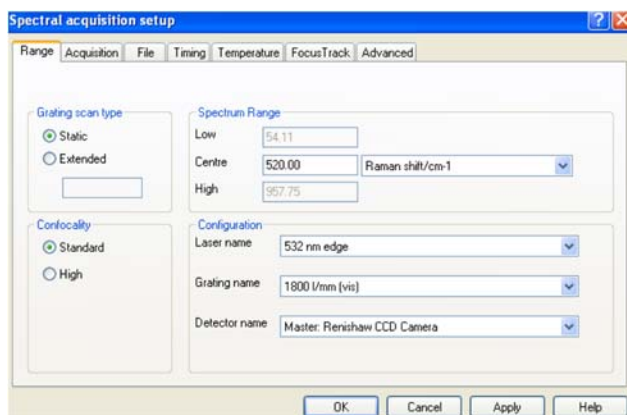
5. On the microscope move the 20x objective into position. Focus on the surface of the sample using the stage height adjustment knob. You may need to adjust sample illumination by using A and F controls. Once the sample is in focus, move the objective 50x into position and carefully adjust the stage height. **BE CAREFUL NOT TO CRASH THE OBJECTIVE ONTO THE SAMPLE.**
6. Whenever the sample objective on the microscope is changed, then the objective setting needs to be changed in the Sample Review Tool located at bottom of screen. In Sample Review Tool change the value of the objective setting to x50, this will adjust the scale on the video window to match the objective value. Use the video viewer in Sample Review tool to view the sample using white light. You can still view the sample using the microscope eyepieces while under white light illumination.



7. Make sure the system is in Standard Scope mode for conventional Raman measurements:



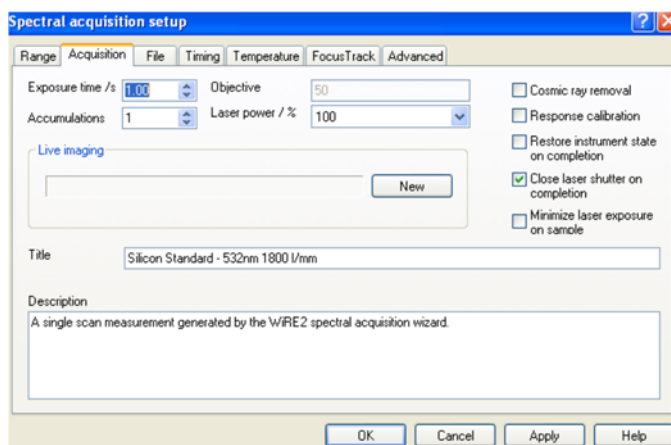
8. In Sample Review select a laser and the corresponding grating: for 532nm or 405nm lasers use 2400 grating, for 785nm use 1200 grating.
9. To setup a spectral acquisition, from the main menu, Select **Measurement, NEW, Spectral Acquisition**. The Spectral Acquisition Setup window appears:
10. To input your scan parameters:
  - a. Range tab:



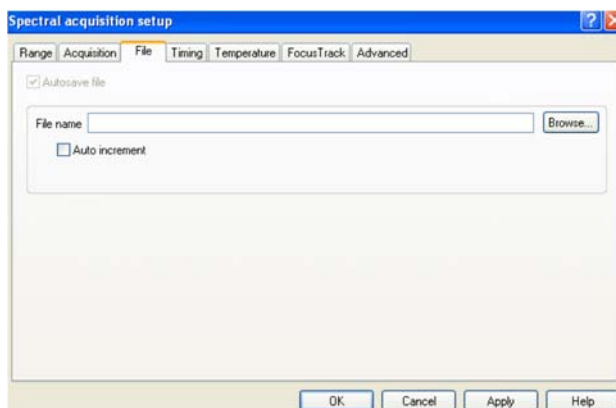
- Grating Scan, select either static or extended scan. In the Static Scan you can select the center point of the Spectrum Range and the system will set the scan range 500 cm-1 around the center point with an exposure time of less than one second per accumulation. In the Extended Scan you can select the upper and lower limit of the Spectrum Range and the spectrometer will continuously scan over the range with an exposure time of 10 seconds or greater.
- Confocality: select either Standard or High. The Standard confocality uses a larger sample volume which increases signal. High confocality reduces the volume and signal strength but increases the depth resolution.

- Configuration: select your laser to use and the proper grating (for 532nm and 405nm use 2400 grating).

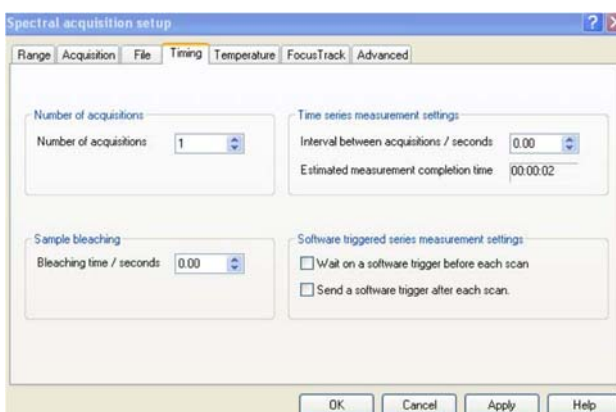
b. Acquisition tab:



- Exposure Time: longer exposure time at detector improves the signal-to-noise ratio
  - Accumulations: is the number of scan repetitions, more scans improves signal-to-noise ratios. If the cosmic ray removal box is selected, two additional scans will be processed in addition to the number of accumulations
  - Objective: the objective selected in the Sample Review window and must be changed every time the user changes the objective manually on the microscope.
  - Laser Power: the percentage of laser power being used for the scan. The higher the power the better signal-noise ratios. If sample is susceptible to being damaged by the laser start with lower laser power
  - Title and Description.
  - Cosmic Ray Removal: the spectrometer takes two additional scans to remove random cosmic peaks
  - Close Shutter on Completion: closes the laser at the end of the acquisition and limit laser exposure on the sample.
- c. File tab: enter a unique filename. Select Auto Increment to save every acquisition with a sequential numbered filename.



d. Timing tab:

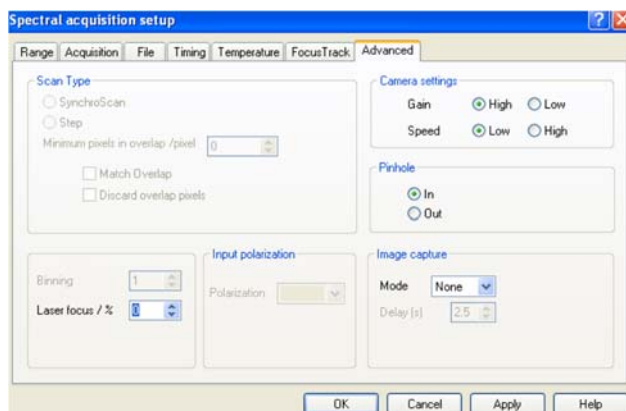


- Number of acquisitions:
- Sample Bleaching: is used to decrease fluorescence from the sample by exposing the sample to the laser prior to taking a measurement.
- Time Series Measurements: to set a specified time in between scans.

e. Temperature tab: N/A

f. Focus Track tab: is used to maintain the laser focus position for mapping acquisitions.

g. Advanced tab:



- **Scan Type:** if the Extended is selected in the Range tab, the user can select a Step Scan or a Synchroscan (Continuous Scan, 10 seconds is recommended). If saturation of the detector is occurring, then reduce exposure time under 10s.
- **Camera Gain:** Select the HIGH sensitivity option.
- **Pinhole:** this is a motorized pin-hole assembly and is used alignment purposes. Select “out”.
- **Laser Focus:** at 100% the laser beam is completely defocused, at 0% the laser beam is a highly focused. Use highly defocused beam if sample is subject to damage by the laser
- **Image Capture:** can be used to have white light images of the sample taken before or after an acquisition.

11. Click Apply. Ok

12. To start the measurement click on the Run button:

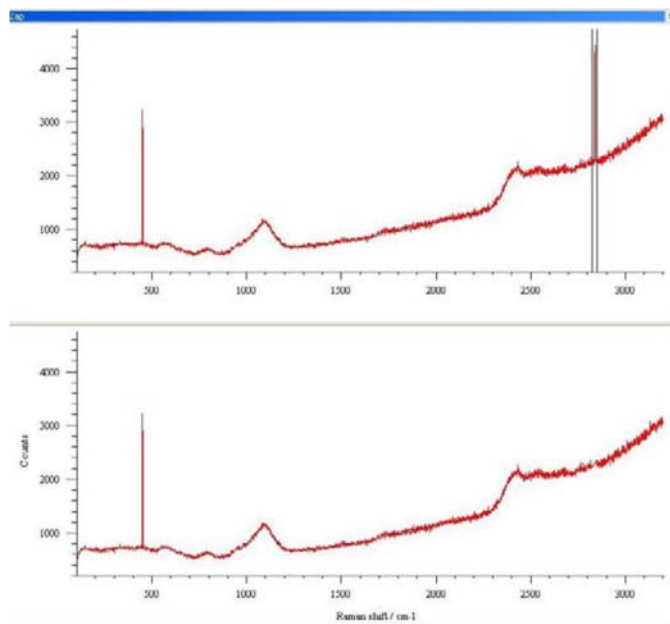


13. To modify your acquisition settings click on

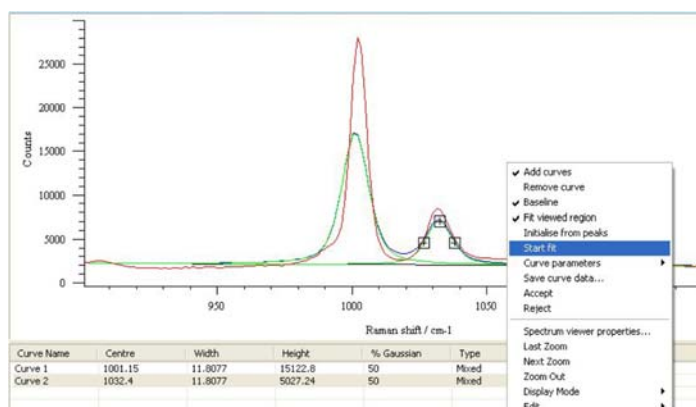


14. To analyze your data:

- To remove stray bands or cosmic rays: while spectrum is open, select **Processing**, then select **Zap**. A new viewer will open below the original one. The top spectrum has a zap region between two vertical lines; adjust the zap lines positions around the peak to be removed. Right click and select remove. Select accept.

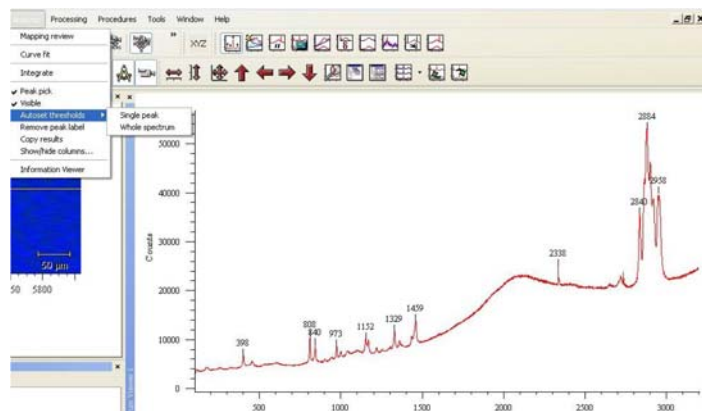


- b. If you need to curve fit a specific peak select **Analysis, Curve Fit**. Highlight then **Zoom** into the peak that you want to fit, use the mouse to position the peak center. You can adjust the peak location and width markers. Right click and select **Start Fit**



- c. To assign peaks: click on **Peak Pick** tab , If you need to change threshold, select **Analysis**, then **Autoset** threshold

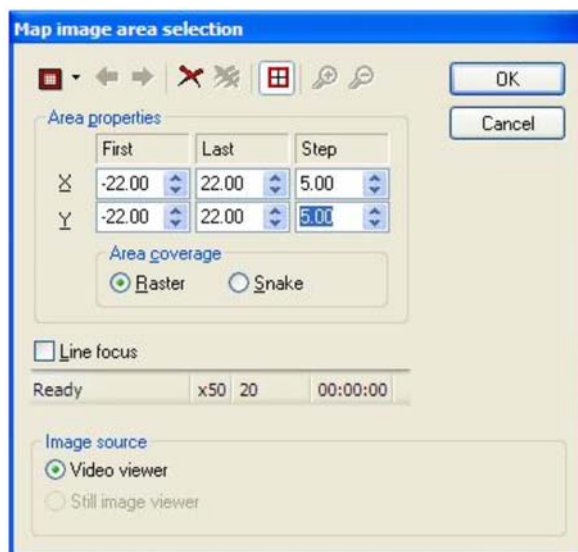




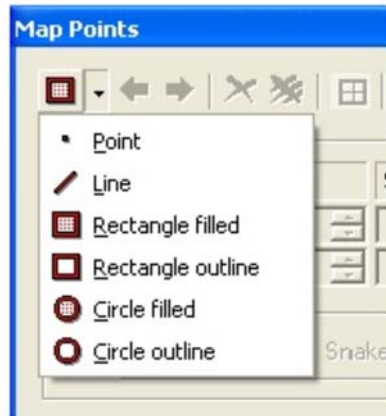
## Mapping procedure

### 1. Points mapping:

- Display the area to be mapped on the video viewer
- Capture the area to an image viewer by clicking on the **Snap Video** tab
- From **Measurements** select **New** then select **Map image acquisition**; this opens the Map image area selection.



- Use the upper left corner button and select **Point**



- Under Image source select Still image viewer
- Move cursor inside the image viewer and click to select the points to be mapped, click Ok
- The Map measurement setup dialogue box appears, verify parameters, click Ok.
- Once mapping is done you can survey the individual spectra at each selected point
- Save your file.

## 2. Streamline mapping:

- Display the area to be mapped on the video viewer
- Capture the area to an image viewer by clicking on the Snap Video tab
- From **Measurements** select **New** then select **Streamline image acquisition**; this opens the Map image area selection; this opens the streamline image area selection.
- Under Image source select Still image viewer
- Select the area to be mapped by drawing a rectangular box around it, click Ok.
- In Map measurement setup dialogue window only static scan can be done, so make sure to select the proper wavenumber. Click Ok
- Run your scan
- Save your file

## Shut down Procedure:

**Please, note that the switching off procedure is extremely important. The failure to follow this steps or interchanging the steps sequence may result in severe equipment damage.**

1. Turn off the lasers power. For 405 nm laser the key first need to be switched off, then the power switch at the back of power supply.
2. Close the WIRE 3.4 software.
3. Turn off the System Power switch
4. Clean the work area and properly dispose used materials.