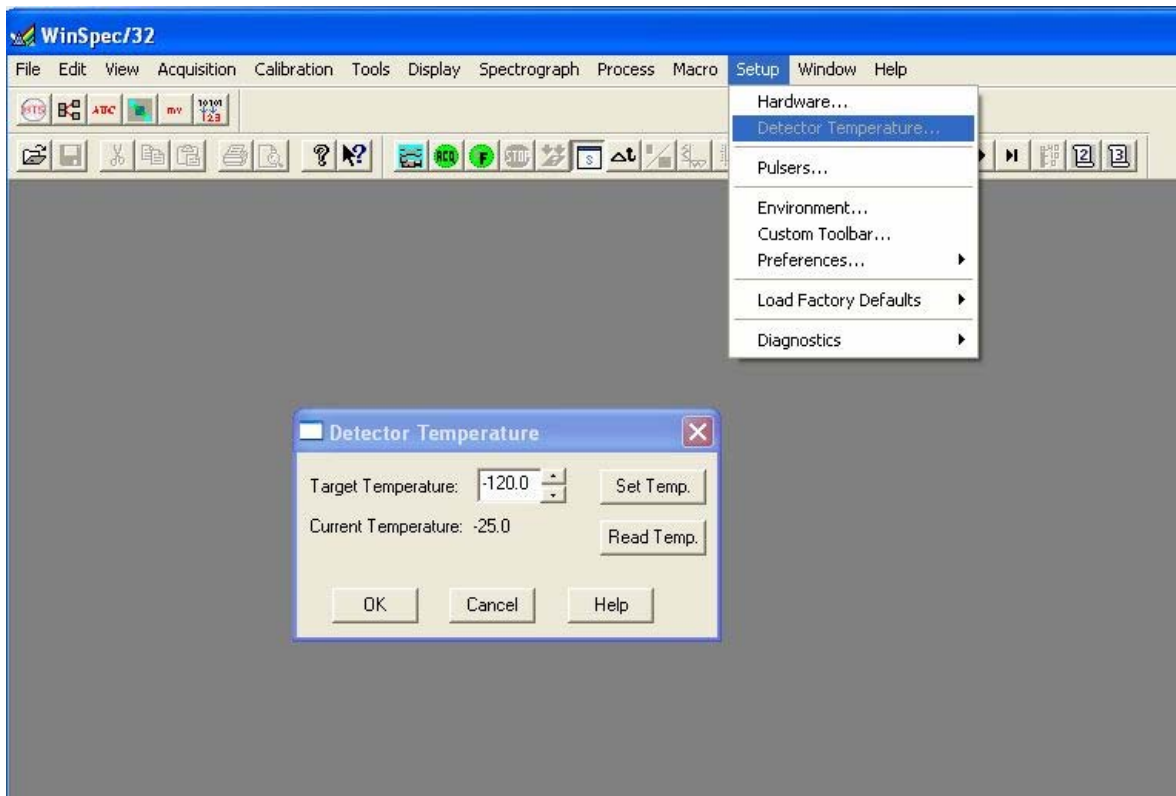


Brief Operation Manual of Confocal Raman Spectroscopy

Turning on the system

1. Fill LN₂ to the CCD detector till full at least one hour before your session.
2. Log on the FOM.
3. Start S&I software and WinSpec.
4. Check the detector temperature from WinSpec – Setup - Detector Temperature menu (see below picture).
5. Open the dialog box of “Detector temperature window” from the pull-down “Setup” menu of WinSpec.
6. The target temperature should be -120 and wait till the current temperature is locked (It takes ~1 hour cooling down from the room temperature.)
7. Make sure “LN locked” green light is lid on the detector controller.



Switching on the laser

1. Turn on the switch key on the remote control and increase the current to 40mA.
2. Check the aperture (it should be 10.) and the shutter (it should be open.) at the laser head.
3. Tune the coarse VERT at the back of the laser to select the appropriate wavelength. Rotate the coarse VERT clockwise to increase the wavelength and counterclockwise to decrease the wavelength.

- Slightly adjust to maximize the power of the laser.
- Take the power meter away.
- Adjust the grating scale to let the laser beam go through the grating and point to the center of the mirror. (**tip**: the scale should not be zero, it should be close to “5”. The shorter wavelength corresponds to a small value.)

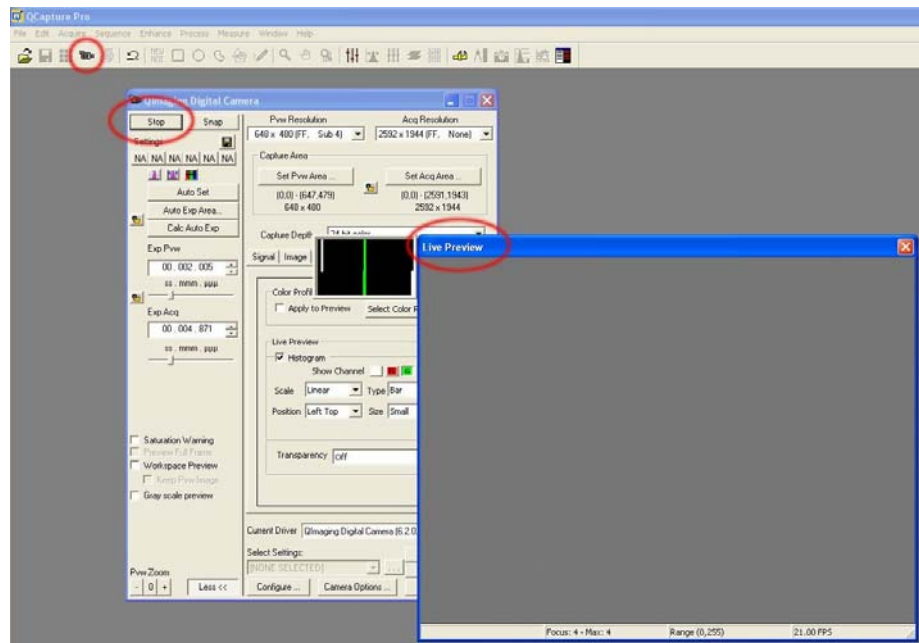


Check the wavelength of the laser

- Open the “OOIBase32” software from the desktop.
- Point the probe to the laser and collect a spectrum.
- Put the cursor at the peak and obtain the wavelength of the laser.

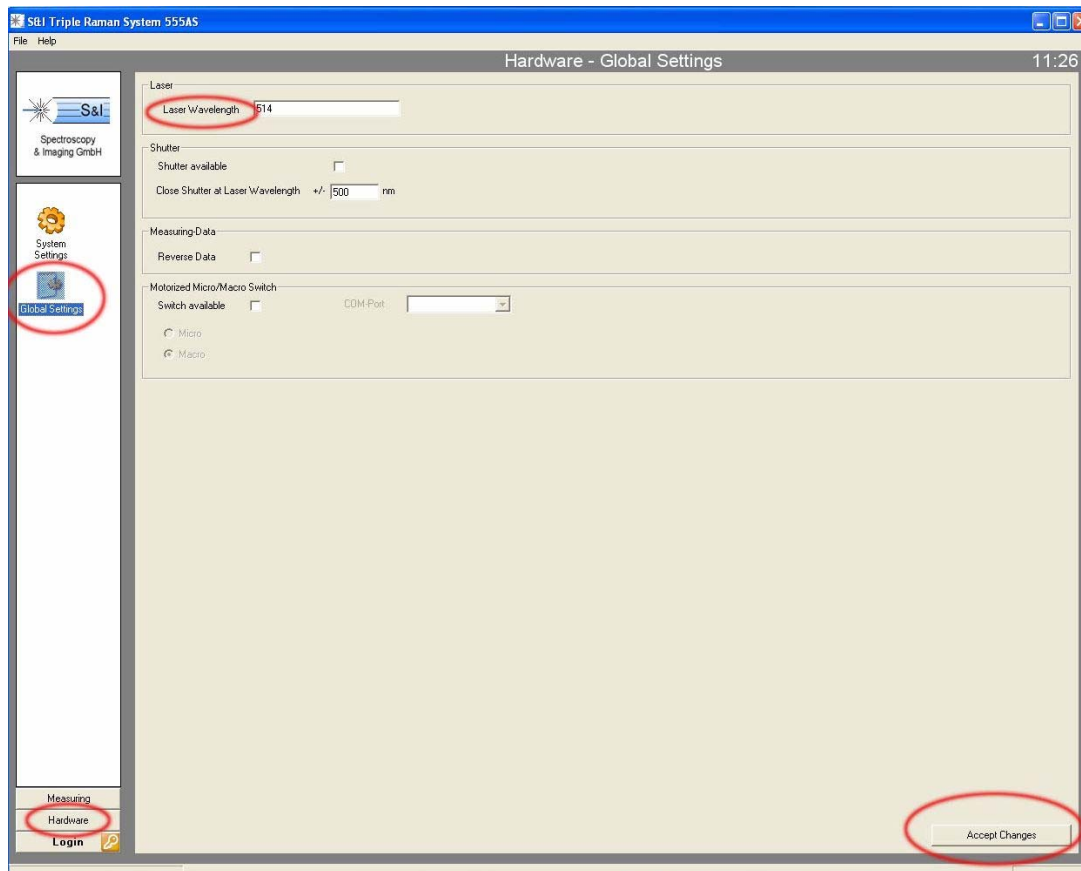
Focus on the sample

- Open the “QCapture Pro” software from the desktop.
- Click the video button to pop up “QImaging Digital Camera” window (see below picture).
- Click the “Preview” to open the “Live Preview” window.
- Turn on the illumination of the optical microscope.
- Open the shutter and push in the bar and change into video view.
- Select the wheel filter position 5.
- Block the laser beam.
- Move the region of the interest with the joystick under the objective 10x.
- Focus the sample and adjust the illumination.
- Change to objective 100x and increase the illumination intensity and then focus.
- Unblock the laser beam and rotate the filter to a very small fraction like 0.0003.
- The reduced laser beam will appear on the image and move the interested area under the laser spot.
- Select the wheel filter position 4 and pull out the bar.
- Rotate the filter from small fraction to 1.



Data acquisition

1. Click on the "Hardware" button on the left column of S&I software and then click "Global Settings" to open the "Hardware - Global Settings" window.
2. Input the laser wavelength and then click "Accept changes" on the right bottom. (see below picture).



Option 1 (for small range scan)

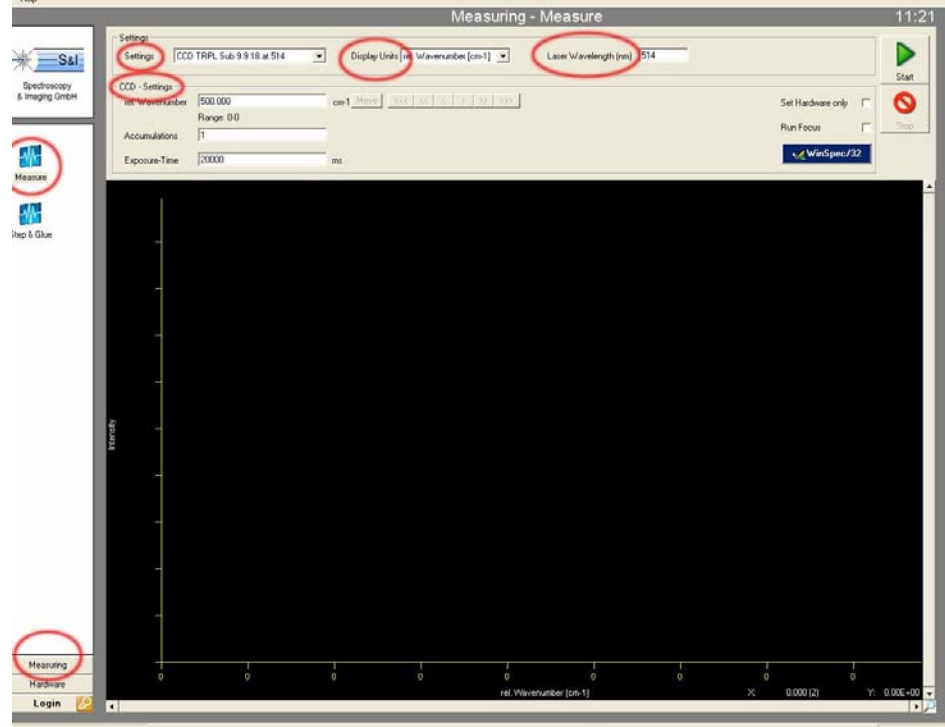
1. Click on the "Measuring" button on the left column and then click "Measure" to open the "Measuring – Measure" window (see below picture).
2. At first choose one of the configurations that have been generated in "System Settings". The list of the configurations will appear by clicking the arrow at the first combo box in the "Settings" region.
3. Select "CCD TRPL Sub 9 9 18 at 488" if you use 488 nm laser or others corresponding to the laser wavelength that you use.
4. Define the way the units of the spectra are displayed at the bottom of the measuring window such as "rel. wavenumber".

- If the selected units are either "Relative Wavenumbers" or "Energy", you also have to enter the used laser wavelength at the third field within "Settings" so the software can perform a correct calculation of the dispersion with reference to the stimulation energy or wavelength.
- Input the center peak position and the exposition time.
- Open "Experiment Setup" window from WinSpec – Acquisition menu.
- Click "Data Correction" tab.
- Find the "Cosmic Ray Removal" and check "spatial" radio button.
- Input 50 at sensitivity.

11. **To start a measurement,** click on the "Start" button from S&I software. While the measurement is in progress, you can interrupt the measurement by clicking on the "Stop" button.

12. To save an acquired spectrum, click "File" and "Save File". Measurements can also be stored through "WinSpec".

Please save your file as .spe format in C/users/your folder.

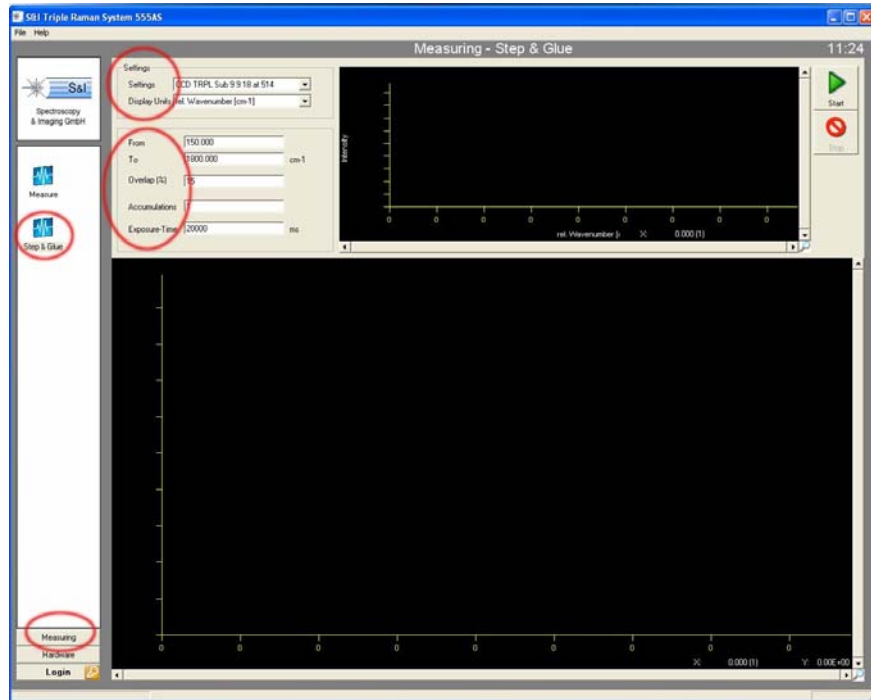


13. You can convert the spectrum to ASCII through WinSpec - Tools - Convert to ASCII menu.

Option 2 (for wide range scan)

- Click on "Step & Glue" to open the "Measuring – Step & Glue" window.
- Choose one of the configurations that have been generated in "System Settings". The list of the configurations will appear by clicking the arrow at the first combo box in the "Settings" region.
- Select "CCD TRPL Sub 9 9 18 at 488" if you use 488 nm laser or others corresponding to the laser wavelength that you use.
- Define the way the units of the spectra are displayed at the bottom of the measuring window.
- Input spectrum range and exposure time.
- Open "Experiment Setup" window from WinSpec – Acquisition menu.
- Click "Data Correction" tab.

8. Find the "Cosmic Ray Removal" and check "spatial" radio button.
9. Input 50 at sensitivity.
10. **To start a measurement**, click on the "Start" button from S&I software. While the measurement is in progress, you can interrupt the measurement by clicking on the "Stop" button.
11. To save an acquired spectrum, click "File" and "Save File". Measurements with a CCD camera can also be stored through "WinSpec". Please save your file as .spe format in C/users/your folder.
12. You can convert the spectrum to ASCII through WinSpec - Tools - Convert to ASCII menu.



Turning off the system

1. Stop data acquisition.
2. Turn off the laser (minimize the current and switch the key off)
3. Close S&I software.
4. Close WinSpec.
5. Turn the microscope illumination off.
6. Log off from FOM.