Standard Operating Procedure
- Renishaw inVia Micro-Raman Microscope

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Log on the Raman Microscope via FOM in any networked computer.

I. System Start-up

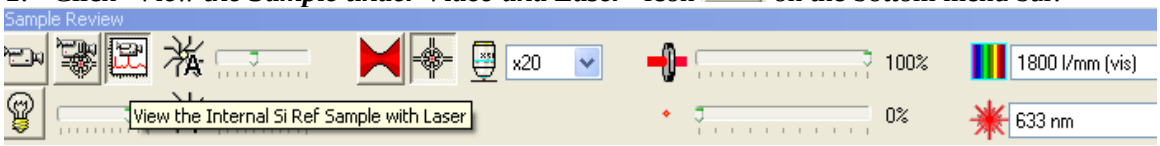
1. Check and make sure the main system unit (orange spectrometer) is ON.
2. Power on the PRIOR stage controller for the motorized xyz stage.
3. Power on the desired laser(s):
   a. 229 nm and 488 nm lasers: ***only started by NFCF staff, indicate your need when you reserve the instrument time***.
   b. 633 nm: switch on the key on the laser unit
   c. 785 nm laser: switch on the key on the laser unit
4. Start the Renishaw **WiRE 3.0** software.
   a. The software will prompt for a position check of the relevant motors.
   b. Choose the '***Reference un-referenced motors only***' option, and click on '**OK**'.

II. Quick Calibration on Si (100) Reference Sample

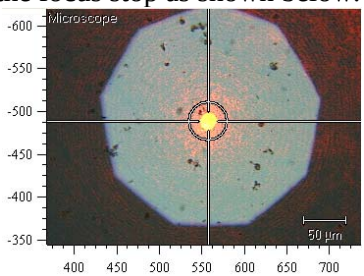
1. Open **Measurement>New Measurement**.
2. Select "***Internal Si Reference Measurement***" (with the same laser you use) and then click **OK**.
3. Use **Measurement>Run** to collect static Raman spectra of Si(100) at 520 cm^{-1}.
4. Zoom into the peak at 520 cm^{-1}. Right click to bring up options to check peak position. If it is not in the 520-521 cm^{-1} range, calibrate the system by clicking **Tools>Calibration>Quick Calibration**. *Alternatively you can calibrate the Raman shift by clicking **Tools>Calibration>Offset** and input the offset value (positive if the Si peak is greater than 521 cm^{-1}).*
5. Repeat steps 3-4, if necessary.

III. Collecting Spectra on Samples

1. Click "***View the Sample under Video and Laser***" icon on the bottom menu bar.



2. Load your sample (*on microscope slide*) to the stage and focus on the surface.
3. Focus your specimen using the microscope focus knobs. If your specimen is featureless, you may reduce the field stop and focus with the focus stop as shown below.



4. Move the Region of Interest (ROI) to the center of cross hair to which laser is positioned.

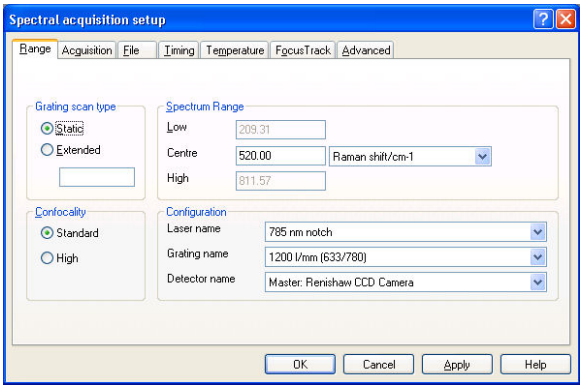
5. Use ***Measurement>New>Spectral acquisition*** or  to start a new spectral measurement. Modify the "Spectral acquisition Setup" for your experiment following the setup guidelines:

5.1. Range tab:

Grating Scan Type: Extended.

Spectrum Range: Low 100; High 3200 and Raman shift should appear in the white box to the right.

Configuration: (no lenses change is needed for visible wavelength).



Laser Name	Grating Name
785 nm (300 mW)	1200 l/mm
633 nm (17 mW)	1800 l/mm
488 nm	1800 l/mm
229 nm	3600 l/mm, UV optics

5.2. Acquisition tab:

Exposure time: 10s

Accumulations: 3

Laser Power: 10% (sample dependent)

5.3. File tab:

File name: -Browse >Desktop >User Data > (Your folder) > Enter a file name to which the Raman data will automatically be saved.

Auto Increment: Selected (This option will automatically save each subsequent run to the entered file name incrementally. (i.e. If your file name you entered is called "Raman" then each subsequent run will be saved as "Raman1", "Raman2", "Raman3" etc.)

5.4. Unless the sample has some pre-determined needs, no adjustments need to be made under any of the other headings. To apply your settings, click either "Apply" then "OK" or simply click "OK"

6. Use ***Measurement>Run*** or the ***Run*** button to collect spectra.

6. 1. Change the "***Grating scan type***" to ***static*** with the spectrum centered at a strong peak.

6.2. Go to **Measurement>Cycle** to collect static LIVE Raman spectra of your sample and use Leica focus knob to optimize signal to maximum counts.

7. Use ***Measurement>Run*** or the ***Run*** button to collect spectra.

8. Save the spectrum if you haven't set the automatic save.

IV. Mapping on Samples

1. Follow the same as in Section III.

2. Go to ***Measurement>New>Map acquisition*** to start a new mapping measurement and to setup acquisition parameters for your experiment.

   a. Under the Image Source section, ensure that "Video Viewer" is selected.

   b. In the top tool bar, left click once on the leftmost icon to display a drop-down menu. Using this menu, you may select the shape of the area you wish to map.

      Note: The term "filled" in the menu indicates that the instrument will scan points both on the border of the shape and inside the shape. For a shape that does not specify "filled", only the outline of the shape will be scanned.

   c. When you have selected your shape, then move the mouse to the video viewing section of the screen and draw the shape on the image by left clicking on the mouse, holding, and dragging to make the shape the direction and dimensions that you want.

      Note: If you are unsatisfied with the placement or size of the shape, you may remove it and try again by clicking the "**X**" icon in the top toolbar of the "Map Points" dialog box.

      Note: The units for the step size are not specified in the dialog box, but they are microns.

Note: Between the "Area Properties" section and the "Image Source" section, there is an information section that will indicate the number of points in the scan. The information in Table 1 may be used as a guide for setting up mapping runs.

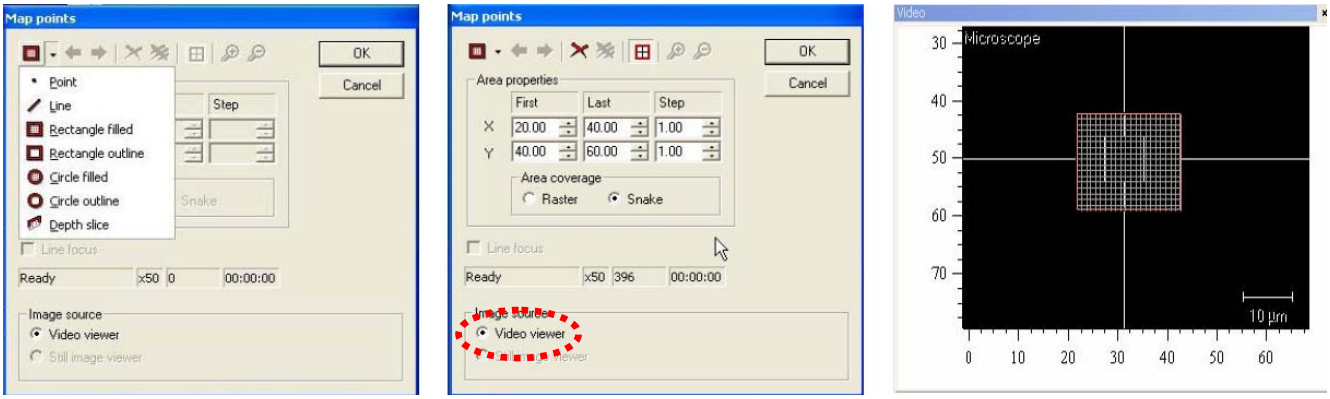


Table 1. Run time information based on Area and Step Size.

Time (hours)	Area (microns)	Step Size (microns)
2	50x50	5
7	50x50	2.5
11	50x50	2
28	100x100	2.5
7	100x100	5

3. When you are satisfied with the parameters, click "**OK**" and the "**Map Measurement Setup**" dialog box will automatically appear. You may now set up the parameters for the spectral acquisition as described in the Spectral Acquisition Setup.
4. Use ***Measurement>Run*** or the ***Run*** button to collect mapping spectra.

V. Mapping Analysis

1. After you have finished the map acquisition, open the map data file by going to **File > Open** or **File > Open in a New Window**, or by clicking on the **Open** icon in the toolbar.
2. In the Navigator window click on the **Data** tab and make sure that the data file you wish to analyze is highlighted.

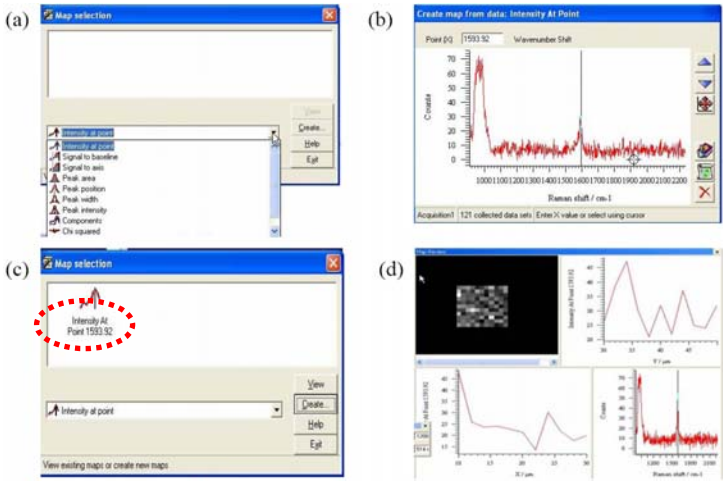


Figure. Location of “Data” tab.

3. Go to **Analysis > Mapping Review** and the "**Map Selection**" dialog box will appear. Select the type of map you wish to create. Then click on the "**Create**..." button.

Note: The most common type of map is the "**Intensity at a Point**". This map allows you to select a given wavenumber and generate a map that displays the relative intensity of that peak at each point. This is the kind of map that will be used as an example for this guide, however, the setup for the other types of maps is very similar.

4. After hitting "**Create**", a dialog box will appear. You may use the mouse to left click (once) on the vertical black line and drag it to any point in the spectrum. If necessary, you can zoom in on an area as you would normally. When you have selected the desired point, click on the "Create New Map" icon (second from the bottom on the right-hand side).
5. You will return to the "Map Selection" dialog box automatically. To view the map you have created, click "View"
   a. While viewing the map, you can click on any point to display the complete spectrum at that point (Bottom right), the Intensity at selected wavenumber compared to other points in the same Y-line (Upper right) and the Intensity at selected wavenumber compared to other points in the same X-line (Bottom left).
   b. You may save this data by going to **File > Save As**
   c. You may save the view on the screen by going to **File > Save View As**

VI. Shutting down

1. Close the WiRE 3.0 Software.
2. Shut down the **PRIOR** xyz stage controller.
3. Turn off the lasers if nobody else reserved the Raman immediately after you.
5. Turn off power of the spectrometer
6. **Leave computer ON and turn off the monitor.**
7. **Sign off the instrument via FOM.**