SOP for the Renishaw confocal Raman Microscope.

Be aware of the basic safety rules:

- Lasers are dangerous.

- You can burn your sample. Don't leave the instrument unattended.

- To open the microscope door press the door release button. Never open the microscope door when the lights active or laser are on. The interlock light should be always green.

- Don't damage the microscope objectives or the eyepieces, sample must never be in contact with the lenses.

- Lasers can't be left on for long period of times. Switch off the lasers when you finish. Please talk with Pedro for continuous analysis (more than 30 minutes).

1. System start up.

From Tuesday to Friday the instrument and the PC should be on, so you only need to turn on the lasers you want to use 30 minutes before starting. You can open the Wire 4 and start immediately.

(532 laser you may need to turn it on several times) 3.4

If you are working on Monday or during the weekend you may need to switch on the instrument and then the lasers. Please see the manual in the red binder: system start-up, TM002-02-A page 7.

(785 laser : all lights are on)

2. Select the correct sample viewing and configuration.

Please see red binder manual, TM003 page 1 for the meanings of the main viewing and configuration controls.

Then follow the configuration change protocol (TM003-02-A, page 5): please be aware that the only configuration options installed are:

1. Laser: 633/785 edge, grating: 1200 l/mm (633/780).

2. Laser: 532 edge, grating: 2400 l/mm (vis).

x20

3. Silicon (internal standard) verification.

New measurement, select desktop, silicon reference folder and the laser template you are using.

Select run

To see the Raman Shift select peak pick icon. Record the results in the instrument notebook.

only the main peak

If you need to apply and offset (the value must be 520 cm^{-1}) go to tools, calibration, offset and run the analysis again.

519 → offset = -1   *519.5 → offset z-0.5*
521 → offset z-1   *520.5 → '' ≥ +0.5*

4. Focusing the sample. *1. SELECT VIEW THE SAMPLE UNDER VIDEO AND EYE PIECES.*

Don't damage the microscope objectives or the eyepieces, sample must never be in contact with the lenses.

1. Coarse adjustment with the microscope eyepieces.

2. Fine adjustment with the joystick (minimum speed) and looking at the life window (camera).

3. Close the microscope door and follow TM003-02-A focusing the sample section page 2 (red binder). *→ Close the P → ↺ & clear widow*

4. Take a picture of your sample before and after the analysis. *↘ LIVE VIDEO, SAVE IMAGE AS.*

5. Sample analysis.

Select measurement, new, spectral acquisition.

Set-up options, see red binder, TM004, Measurement set-up and data acquisition, page 1-8. Check also Renishaw ppt data acquisition 1. *// TO SAVE THE SET-UP SELECT APPLY AND OKAY.* *↳ TO MODIFY THE METHOD GO TO SET UP MEASUREMENT TOP.*

For mapping please see the additional SOP: it is usually wise to run single Spectral Acquisition measurements on the sample to determine optimal acquisition settings.

File: Save in Disk E, supervisor's folder, your folder.
Name: SAMPLE NOME ___ laser λ ─ power W ─ exposure time ─ ⌊ 0 ─ autoincrement ⌋

6. Shutdown procedure.

Check that your data have been saved. You can also save your data as .txt (save file as). Use Snipping tool to save a picture.

Close Wire software.

Log off

Switch off lasers

The instrument and the PC should be left on.
*REMOVE YOUR SAMPLE AND CLEAN THE STAGE.*

Before 6: data analysis: see workflow. // ASK PEDRU.

A mapping measurement is a measurent that produces a single dataset for each defined collection point in a region. Follow the following steps to configure a basic mapping measurement.

1. Decide on the type of map data that is required and locate a suitable region over which to perform a map. Maps can be performed on single non-uniformly spaced points, at regular intervals along a line, or over a regular area in a regular array (X and Y intervals may differ). Choose the smallest region that satisfies the purpose of the map. This will allow better data to be collected, either by achieving a higher signal to noise or by improved spatial resolution, or both. With the region in the field of view of the video camera, zero the co-ordinates using the 🔳 button. Ensure that the correct objective magnification is shown in the Sample Review tool (this reflects the scale bar shown in the Video viewer).

2. Select *Measurement > New > Map Acquisition* to initiate the *Map Points window*
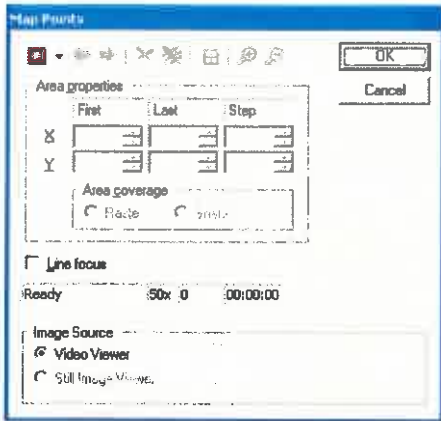
A map can be performed over a larger area, and with a corresponding white light video image, by creating a montaged image. To create a montaged image see White light montaging. If a montage has been created, the Map Points window will now have the *'Still image viewer'* button active under Image Source available. Select it to use the montaged area for mapping. Montage the white light image first, then create the new map acquisition.

3. With the base image selected, decide on the map type from the Map Points window:
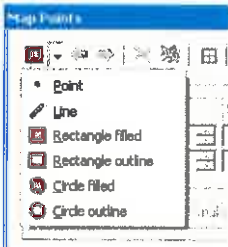
Point – the user can select numerous points on the base image and analyses will be performed sequentially. The X and Y co-ordinates are reported in the Map Points window. The blue arrows toggle between the points.

Line – the user defines a straight line on the base image using the left mouse button.

Rectangle – either filled or perimeter. The user drags a rectangular box using the left mouse cursor.

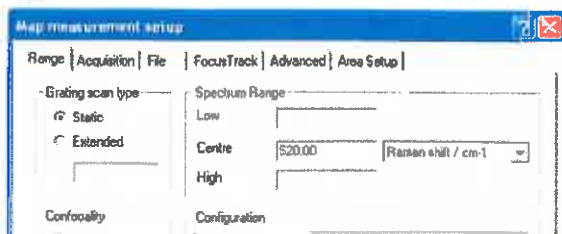**Circle** - either filled or perimeter. The user drags a rectangular box using the left mouse cursor.

The ✖ button removes the set co-ordinates and allows the user to redefine the map. The ⊞ button toggles the grid on and off.

The X and Y step sizes (for areas and rectangles) can be altered in the Map Points window. *Raster* and *Snake* refer to the direction pattern in which analyses are performed. This is usually only important when FocusTrack (autofocus function) is used, and should be set to *Snake* in this case.
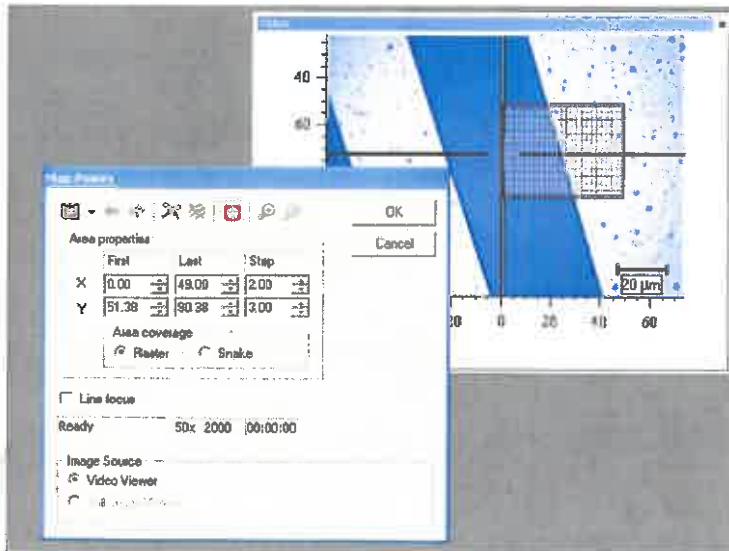
Typical + raster

LD PLEASE DO NOT USE FOCUS TRACK / AUTO FOCUS

Once the map area has been defined press *OK* to continue on to the Main Measurement Setup window. The *Map measurment setup* dialog is very similar to the Standard measurement setup dialog, but with an additional Area Setup tab.

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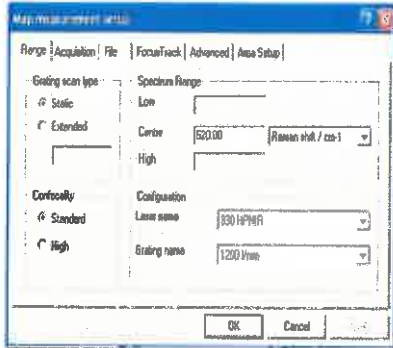
4. Before setting up a mapping measurement it is usually wise to run single Spectral Acquisition measurements on the sample to determine optimal acquisition settings, bearing in mind the total available time for mapping. You can improve the signal to noise ratio by either increasing the number of accumulations at each analysis point or by using longer exposure times. It may be useful to perform Static Grating measurements, as this requires less time than a Continuous Grating Scan. The Area Setup tab allows modification of the defined map, including the co-ordinates and step sizes. Press OK to exit the Map measurement setup dialog.

5. The mapping measurement can be started using the ⊳ button or selecting *Measurement > Run* from the menu.

6. Once all the data has been collected and the map has finished, you can survey the mapped region and the spectra taken at each point without creating a map (to regain this view on loading a saved mapping experiment Select *View > View map data*). A *Map Review* window opens showing the base white light image at the top and the spectrum corresponding to the position of the cursor at the bottom of the screen.

Note: unless the map has been autosaved through the File tab, the map is only locally saved. It is recommended that a map be saved on completion.

7. See Mapping review for help on viewing maps based upon the collected mapping data.

HOW TO SAVE AN INDIVIDUAL SPECTRA FROM THE MAP: Go to data tree (next dropdown navigation), select the acquisition/spectra you want. Right click, and select save data set as. You have created a file with this individual spectra that you can label and work with (correct format: e.g. Wxd, spcon txt). (Morerspfo wave 3.4 TM (78) DATAE.

8. To return to the origin, go to the coordinates and select (0, 0, 0). It is advisable to check if you have burnt your sample.