User guide to Renishaw InVia Raman microscope

Open the PC (should always be on) Username: Shd-Raman, Psw: inVia2014, Remote access (via Remote Desktop on Windows): shd-raman.nfit.au.dk

0. Fill out the logbook on the desktop of the PC.

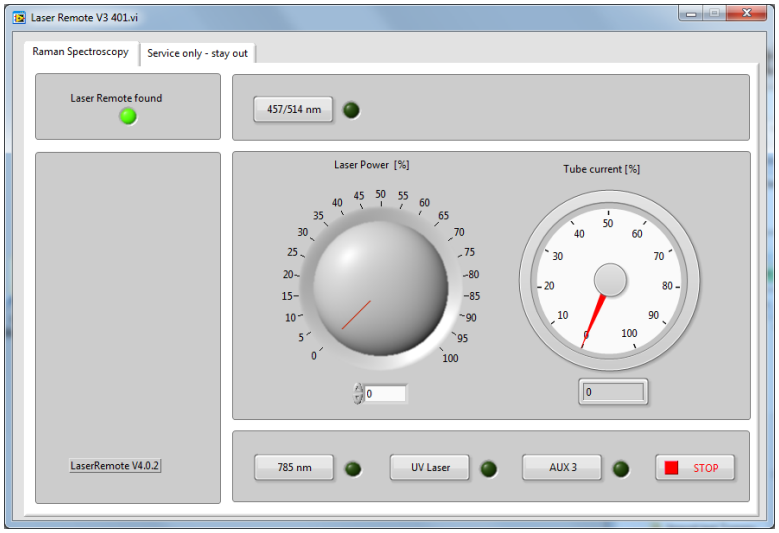
1. Make sure to book the instrument in the Outlook Calendar (1590-063 Renishaw inVia Raman Microscope).
2. Fill in the logbook with name, group. Please comment here if anything is wrong with the instrument.
3. Open the program "Laser Remote" and identify yourself with name and group (use droplist). If your group is not registered and you have been trained and allowed to use the instrument then give the name of the group leader as user.

The "Laser Remote found" diode should be green indicating that the interface to the lasers is operating well (if it fails then remove the power to the interface box placed on top of the PC (this is power cable to the left on the back) and retry Laser Remote. We will use the amount of Laser time to charge your group for using the instrument. The charge in 2018 is 25 kr/laser hour and will be collected in January each year. The lasers should always be operated through "LaserRemote" – never operated manually!

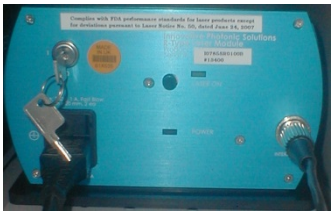
1. Start-up of Instrument

The laser needs 15-30 min to warm up, so startup the laser as the first thing. The Ar-Ion laser at 514 nm is the most powerful and would normally be considered as the standard wavelength for initial investigations.

a) Start laser(s)



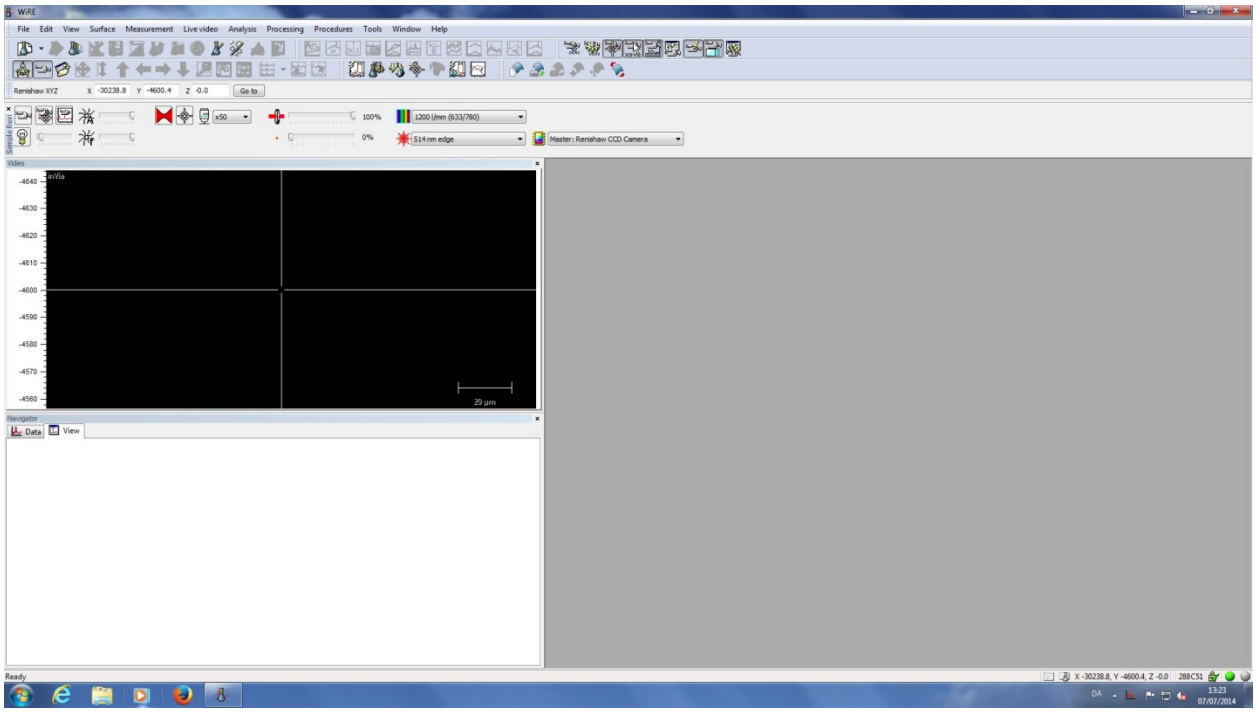
Ar-ion laser (457 nm and 514 nm): **First ensure that the switch on the laser unit is on and the key on the laser is in (1) position**. The laser is activated/ignited via LaserRemote by the button “Laser 457/514”. The neighboring diode will become green showing that the laser is ignited (the cooling fan can be heard after a few seconds and the tube current increase to 40-50%). Laser power is adjusted on the knob to the left. It should normally be set at 33 % (line on the old manual adjuster) unless a special application should require higher laser power. You may also use value below the knob so the same value can be used each time. If you increase the laser power **always** remember to set it back to 50% when the high power is not necessary anymore. The “Tube current” knob is only for reading. After switch on it will take some seconds before a laser/tube current flow. A current will run even at zero laser power (idle current) and the Tube current is not linearly related to the laser power. Notice, that you will be charged by the time the laser is on even if the laser power is zero. Therefor switch off the laser in Laser Remote when you do not need it anymore. **Switching off the laser** is done by pressing the button “Laser”. The green light beside the button should go off. The ventilation of the laser will proceed even after switching off the laser for some minutes but the tube current should go to zero. Leave the switch “on” and the key in “1” position on the Ar-ion laser (do not use these).



He-laser (785 nm): Switch on the laser by pressing the button “785 nm” (diode gets green). The key on the Helium laser (blue box) should always be at (1). Use the ignition button on the blue box to ignite the beam. **Switch off the laser** by pressing the “785 nm” button again. For the moment you will not be charged by using the He-laser.

b) Check that the instrument is switched on (normally not necessary to do, since the instrument should be left switched on!). Power button is found at the right side.
The four small switches above the power button are interlock switches and should ALWAYS be on! If these are not on, do NOT use the instrument.

c) Start the program “Wire 4.2” on the desktop or in the Program menu "Renishaw"

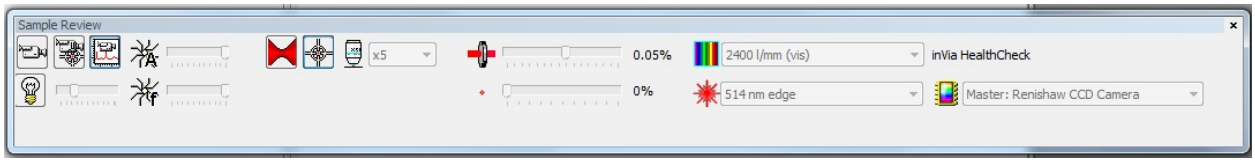


2. Sample in/out of Microscope and change of Objective.

a) Open the Microscope by pressing the small ”open” button above the door, and immediately open the door. Start by turning the objective turret to the x5 objective. Use the riffled ring to turn the turret – do not touch the objectives. Lower the stage by turning the knob on the right side towards you. If this is not enough, the whole stage can be mechanically lowered. See appendix A for this.

b) Insert the sample (normally lying flat on a standard 5x3 cm microscope glass plate) and fasten it with the lock (move lock to the right to insert the glass plate and release lock gently to fasten glass plate)

3. Focus on the sample



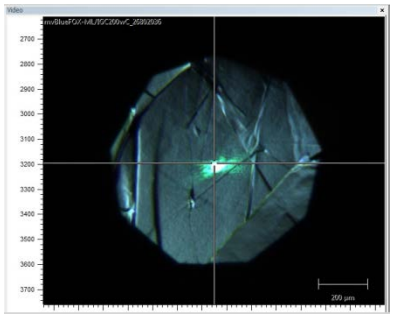
a) Make sure that you are using the 5x objective for the initial adjustment of focus.

b) Press on the Video icon and switch on the light in the microscopy, and adjust the adjacent slider for the light.

c) Carefully focus the sample in the eyepiece or video view by slowly adjusting the z (height) of the stage either via the "height knob" or use the track ball ring. (To use the track ball activate it by pressing the correct objective magnification - see appendix B for further details). The working distance (WD) for x5 objective is >2 mm.

d) When the sample is in focus, the objective turret can be turned (use always the riffled ring to handle the objectives – never touch the objectives) stepwise to higher magnifications with focus adjustment for each magnification. Be careful not to touch the sample with the objectives! **WD's for x50, x100 and x150 are only between 0.37 and 0.33 mm.**

Focusing blank samples



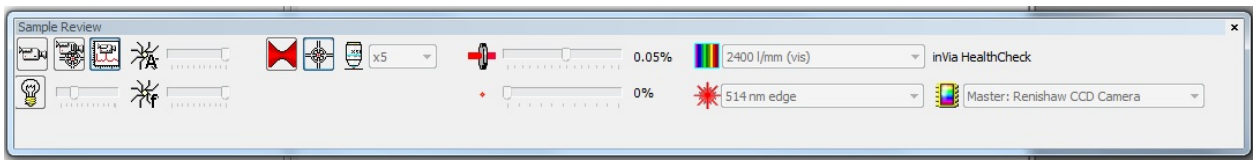
If the sample has obvious features these can easily be focused using white light and Eyepiece/Video. However, for blank samples it is more difficult. Closing (slider to the left) the F stop will reduce the field of view and show an octagonal ring in the video/eyepiece. For blank samples, adjust focus until the edge of this ring is sharp.

Fine adjustment of laser focus

When the sample is visually in focus at the wanted magnification (recommended objectives for measurements are the 50x or 100x objectives), then close the microscope door and remember to **set the current working objective in the Wire program** .

You can now optimize the focus of the laser. To do this switch on the laser/video combination . Switch off the white light . Open the laser shutter, and adjust the laser power 0.05% so the bright spot made by the laser is small but bright. Adjust the focus using the Z-ring on the track ball so that the laser spot is as small as possible.

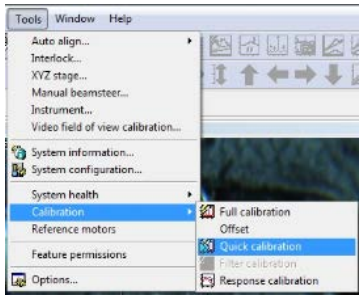
4. Select laser and grating



Now select the laser (457, 514* or 785 nm) and grating you are going to use for your measurements in the sample review bar. For maximum spectral resolution use 2400 grating for 457 and 514 nm and 1200 grating for 785 nm. The combination 514nm/1200 lines is also possible and very useful for a static scan over a wide spectral range of ~2400 cm^{-1}. The combinations 457/1200 and 785/2400 do not work.

When choosing the laser for your measurement be aware that photoluminescence from the sample might cause problems with the baseline at small wavelengths. Furthermore high laser intensity and power (low wavelength) may damage the sample.

5. Calibration



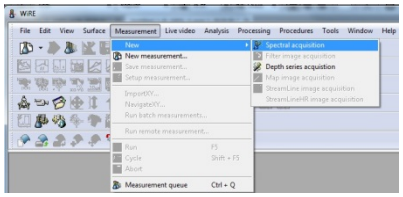
Once the system has loaded, the most commonly required action is a quick calibration. To run the quick calibration, go to Tools -> Calibration ->Quick Calibration. The calibration will run automatically. The quick calibration should ideally be done for every combination of laser/grating you are going to use.

If quick calibration fails, the usual problem is too high laser power. In this case adjust the laser power on the remote unit of the Ar laser.

If you suspect that the system suffers a malfunction then before anything else go to Tools -> System Health -> Health Check. When the “Silicon Calibration Reference” box appears, ensure that “Regular” is selected then click “OK”. This tool checks the alignment and calibration of the instrument. When the health check is finished, if any action is recommended, click “OK” to close the dialog and perform any actions as stated in the system health viewer.

6. Recording one Raman spectrum for a spot/sample

If you have used the laser to focus on your sample, move the sample in xy plane using the track ball to inspect for damages in the surface of the sample from the laser. If the selected laser damages your sample, lower the laser intensity or use the weaker NIR (785 nm) laser.

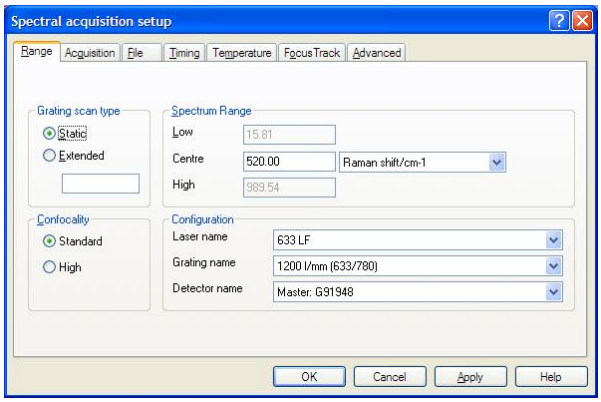


Use the menu "Measurement" → "New" → "Spectral acquisition" or use to setup a new measurement. This will open the “Spectral acquisition setup” dialog.

Range tab

a) First select the grating scan type.

Laser/Grating	Low/cm^{-1}	Centre/cm^{-1}	High/cm^{-1}
514/2400	85	800	1447
	851	1500	2087
	1942	2500	3007
	2486	3000	3467
514/1200	-28	1500	2799
	561	2000	3229
	1147	2500	3650
	1730	3000	4092
785/1200	400	1000	1539
	957	1500	1991
	1511	2000	2443
	2062	2500	2898
	2611	3000	3354
457/2400	66	1000	1840
	617	1500	2295
	1717	2500	3206
	2266	3000	3664



"Static" recording mode covers only a fixed spectral range of ~1200 cm^{-1} or ~2400 cm^{-1} (see table below). It is however very fast (seconds) and should be your preferred choice whenever possible.

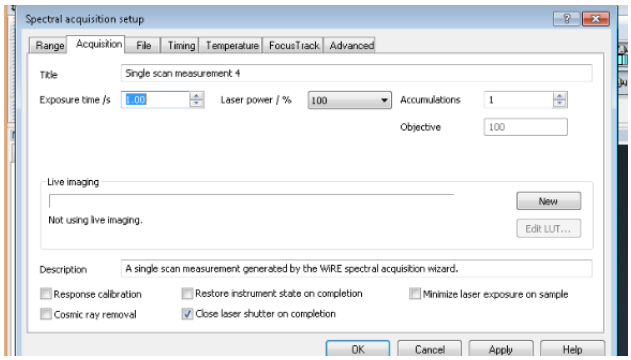
"Extended" recording is slow (5-10 minutes) but can cover any spectral range in one continues recording.

Use "Standard" "Confocality" unless the sampled volume should be sharply z-limited, which is important for depth profiling and might be useful for recording the Raman spectrum of a liquid film.

"Acquisition" tab.

For “Static” recording the "Exposure time" can be almost freely adjusted whereas in Extended recording the minimum "Exposure time" is 10 s. The risk of saturating the detector with long “Exposure time” may dictate a substantial lowering of the “Laser power”. In static recording the smaller “Exposure time” may allow higher “Laser power” and may therefore provide a faster and more intense recording. Remember however that the sample might be damaged by the intense laser light. If more than one accumulation is selected, the

measurement will be repeated to the number of accumulations set and the data co-added, giving an improved signal-to-noise ratio.



Further options(often irrelevant).

In the bottom of the Acquisition tab, there are a few options you might use.

- "Cosmic ray removal" will repeat your acqusition an extra 2 times, to remove any pixel failures due to cosmic rays.
- "Response calibration" – require advanced calibration of the instrument, please refer to the help files.
- "Restore instrument state on completion" could be used in the case where you would like the instrument to go back to the settings you used for focusing on your sample immediately after finishing your measurements. This is often useful as the camera will automatically turn back on after a spectral acquisition.
- "Close laser shutter on completion" is useful to minimize the laser exposure of a sensitive sample.
- "Minimize laser exposure on sample" will turn of the laser between accumulations to further minimize the laser exposure of a sensitive sample.

File tab

If you are doing important measurements, it is a good idea to put in a filename in the "File" tab. This will make the software save your measurement immediately.

Timing tab

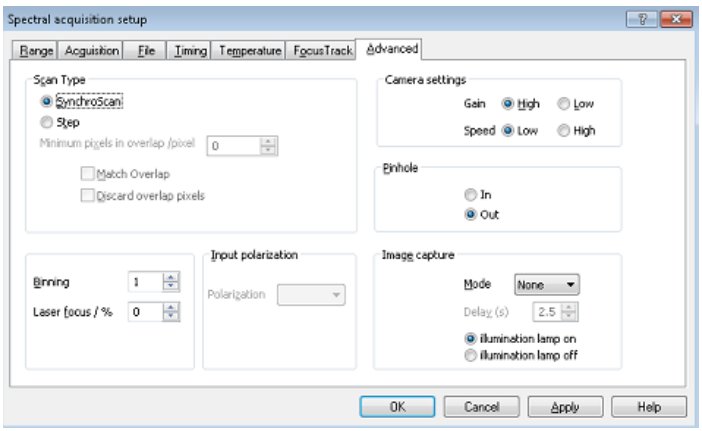
Allow advanced timing of serial measurements with laser bleaching etc. Please refer to the help files.

Temperature tab

Please refer to the help files.

Focus track tab

Is only used for maps, see section 9.3.



Advanced tab

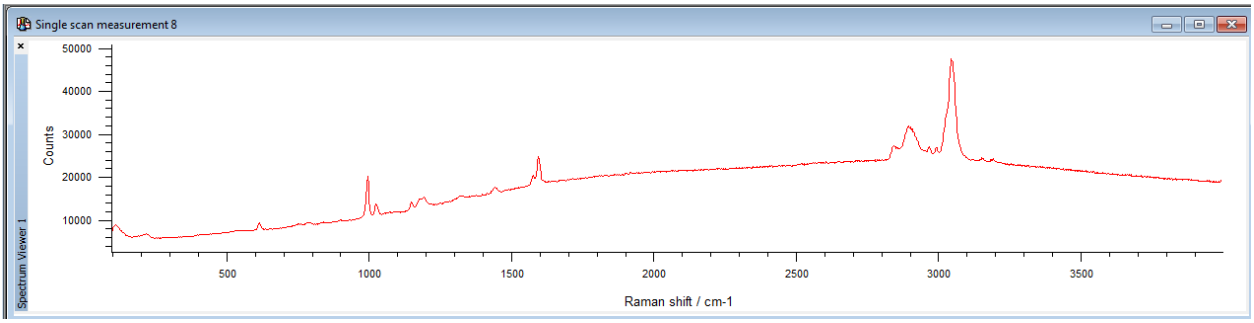
Generally the settings in this tab should not be changed, except for extended scans. If you are making such an extended scan across a broad range of wavenumber, you have two options:

- "Synchroscan" will continuously move the grating during measurement, essentially measuring one single wavelength at a time. This is a really slow process, but results in a continuous spectrum.
- "Step" will take several "static" measurements with a range as in the table above and stitch these spectra together. This will result in a "kink" wherever the spectra are stitched together, but it will be significantly faster.

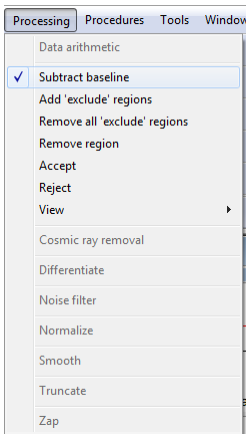
After adjusting the measurements, just click on "Ok". The measurement is started by selecting "Measurement" → "Run" or the Run button, . This will record one spectrum. Alternatively, the measurement may be run repeatedly by selecting Measurement > Cycle, or the Cycle button, . Pressing the button again ends the cycling again.

7. Single Spectrum Processing and Analysis, Manipulation and Export

Before doing any spectrum manipulation, ***save a backup of your data*** as the Wire software will overwrite your data during manipulation!!!



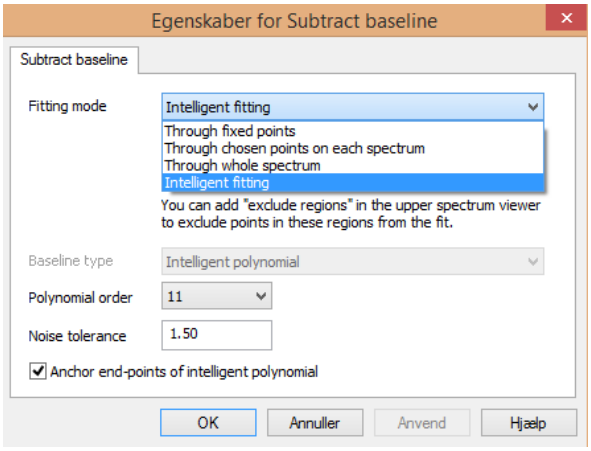
Prior to using data analysis functions, the spectrum to be analyzed must be loaded into a Spectrum Viewer. This happen automatically following data collection (recording). Already saved data may also be loaded into a spectrum viewer using the "File" → "Open".



7.a Baseline Correct and Exclusion of Regions.

Use "Processing" → "Subtract Baseline" to eliminate any background from the spectrum.

Right click with the mouse in the "Spectrum Viewer" to see the context menu. Selecting "properties" will open the "subtract baseline properties" dialog. This allows you to change the method for baseline subtraction.

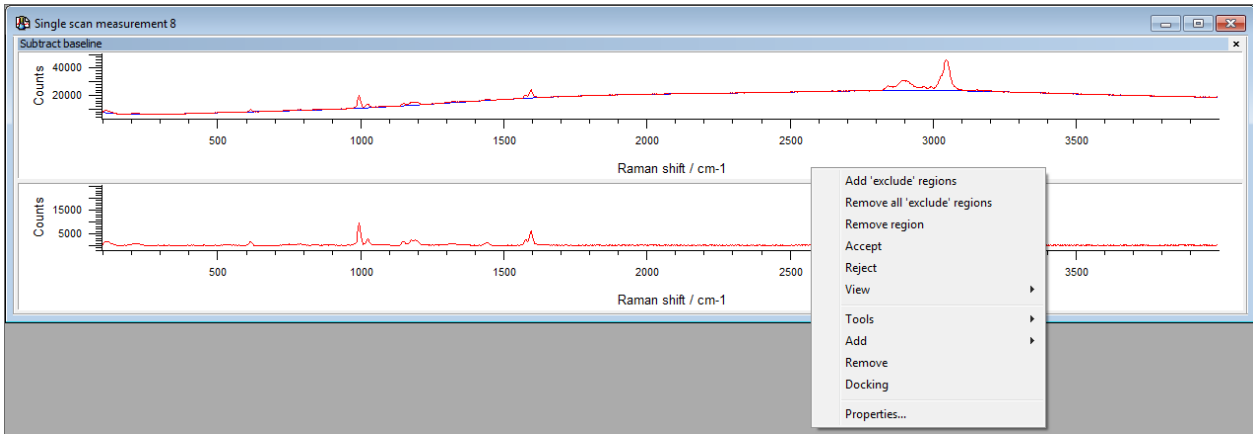


You have four different methods to choose between. The default is the automatic “Intelligent fitting”, which works great for most spectra. However in a number of cases you may want a better control of your baseline fitting.

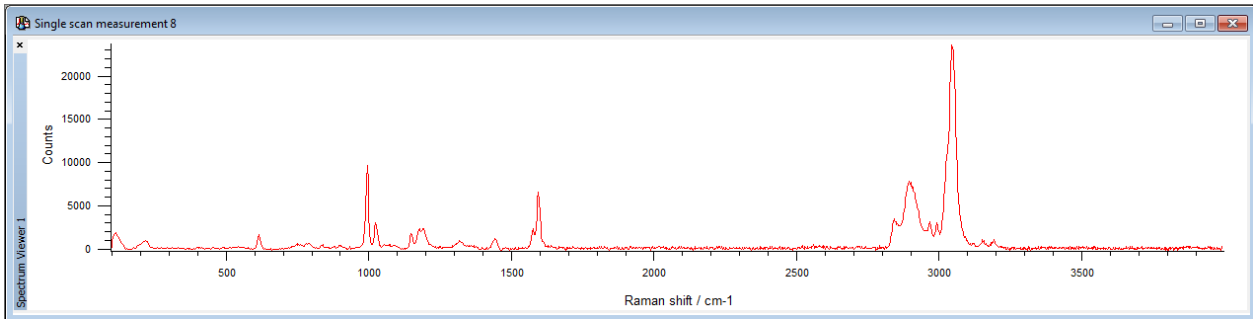
- "Through fixed points” will allow you to freely select points that your baseline should go through, independent of your spectrum.
- "Through chosen points on each spectrum” will allow you to select specific wavenumbers on your spectrum for baseline fitting.
- “Through the whole spectrum” will fit a baseline to the entire spectrum, including any signals.

After selecting either "Through fixed points” or "Through chosen points on each spectrum” you will press ok. Then click on the spectrum to select points. Then right click and open the properties dialog again to select the polynomial order of the baseline.

When you have an acceptable baseline, right click on the spectrum again to open the context menu:

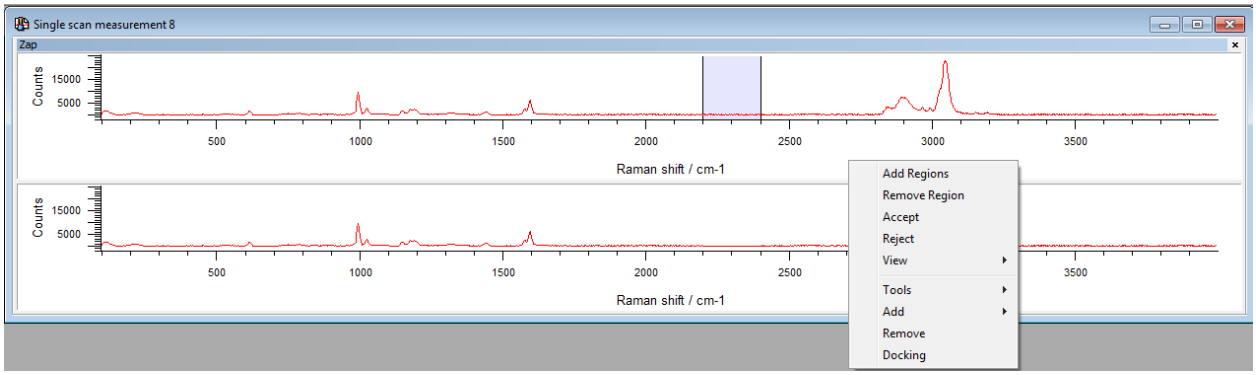


Accept: modifies the original data to that displayed as the current result and exits the viewer.
Reject: exits the viewer without modifying the original data.

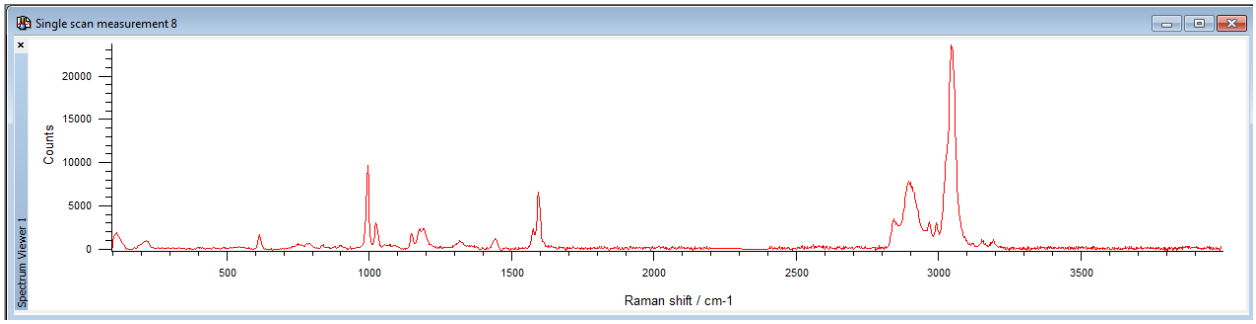


Zap regions from the spectrum (remove regions)

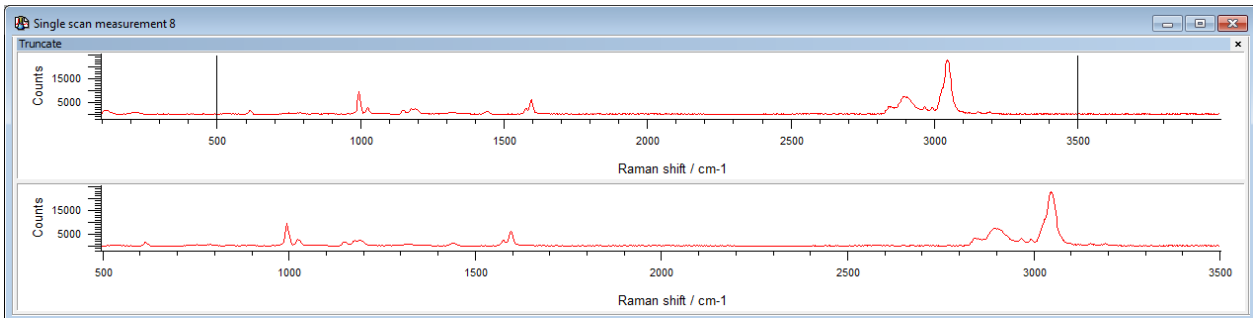
Select “Processing”->”Zap” to zap away data in certain regions of your spectrum.



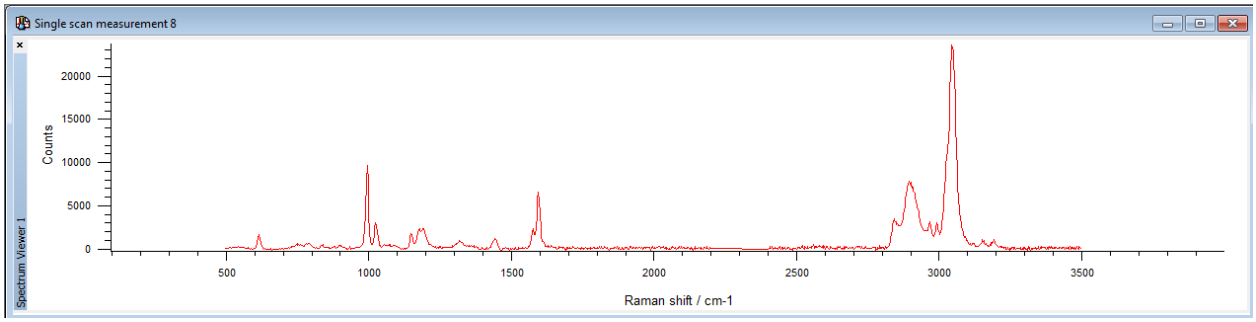
After accept:



"Process" → "Truncate" the spectrum to limit the displayed region by moving the two vertical lines in both ends of the spectrum:

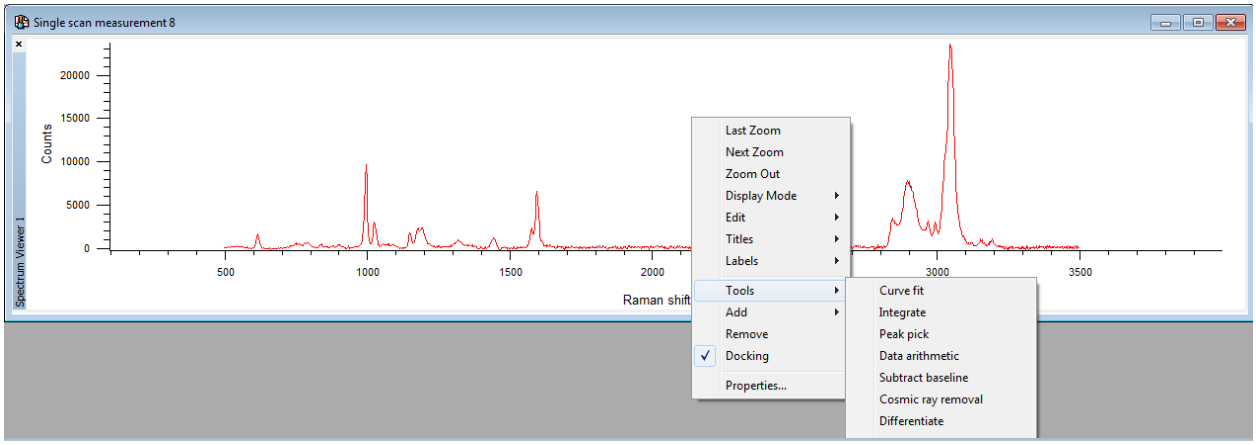


After right click and "Accept" (remember that "accept" changes the original data):

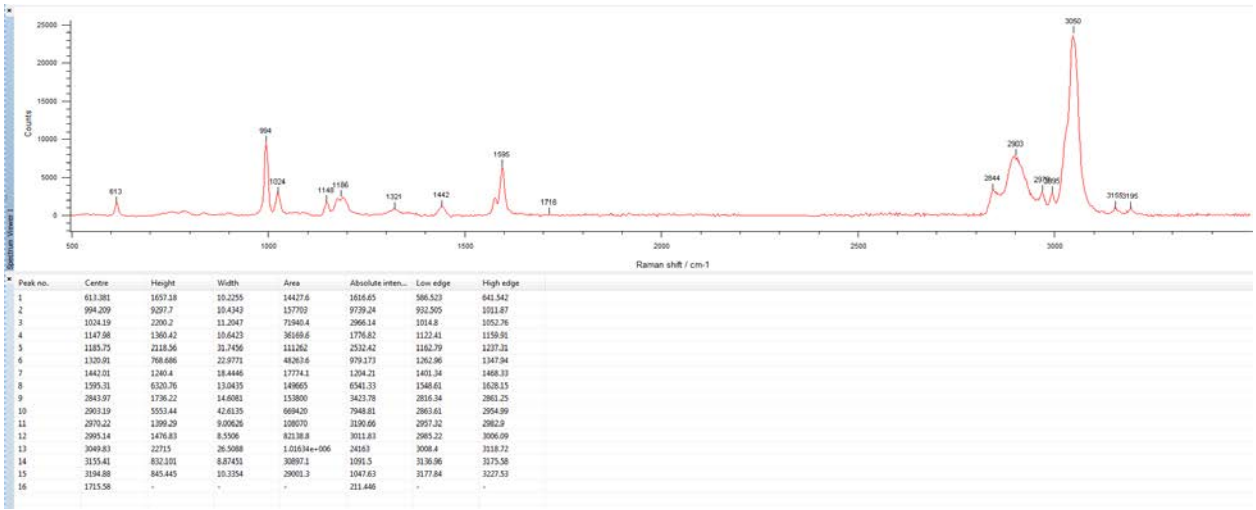


7.b Peak detect and label spectrum

The already processed spectrum can now be analysed directly by right click in the "Spectrum viewer" and use "Tools" or the menu "Analysis" can be used:

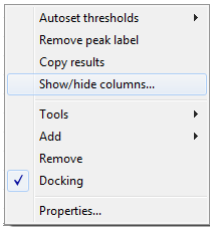


Use "Peak Pick" to label the peaks and to create a table:

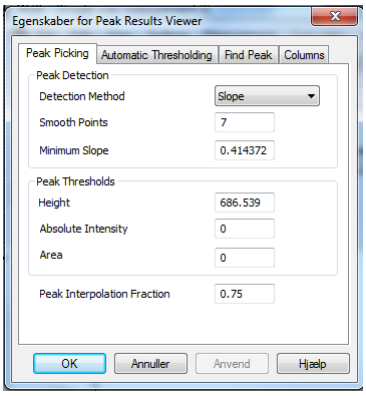


Peak no.	Centre	Height	Width	Area	Absolute inten...	Low edge	High edge
1	613.381	1657.18	10.2255	14427.6	1616.65	586.523	641.542
2	994.209	9297.7	10.4343	157703	9739.24	932.505	1011.87
3	1024.19	2200.2	11.2047	71940.4	2966.14	1014.8	1052.76
4	1147.98	1360.42	10.6423	36169.6	1776.82	1122.41	1159.91
5	1185.75	2118.56	31.7456	111262	2532.42	1162.79	1237.31
6	1320.01	768.686	22.9771	48263.6	979.173	1262.96	1347.94
7	1442.01	1240.4	18.4446	17774.1	1204.21	1401.34	1468.33
8	1595.31	6320.76	13.0435	149665	6541.33	1548.61	1628.15
9	2843.97	1736.22	14.6081	153800	3423.78	2816.34	2861.25
10	2903.19	5553.44	42.6135	669420	7948.81	2863.61	2954.99
11	2970.22	1399.29	9.00626	108070	3190.66	2957.32	2982.9
12	2995.14	1476.83	8.5506	82138.8	3011.83	2985.22	3006.09
13	3049.83	22715	26.5088	1.01634e+006	24163	3008.4	3118.72
14	3155.41	832.101	8.87451	30897.1	1091.5	3136.96	3175.58
15	3194.88	845.445	10.3354	29001.3	1047.63	3177.84	3227.53
16	1715.58	-	-	-	211.446	-	-

To see the table requires sometimes that you limits the "Spectrum Viewer" window by taking the lower limit (⇳) with the mouse and move it up. The number of labels can be changed by changing the threshold. The latter can be done automatic by "Analysis" → "Automatic Thresholds". Choose either "Whole Spectrum" that gives a limited number of peaks or choose "Single Peak" and zoom in on the smallest peak that should be included.



The actual threshold can be adjusted by right click in the table and choose "Show/hide columns". A new window "Properties for Peak Results Viewer" will open where all parameters for Peak detection can be adjusted. In the tab "Automatic Thresholding" the "Maximum Peaks" can be set.



The spectrum is now ready to be printed or saved as a picture.

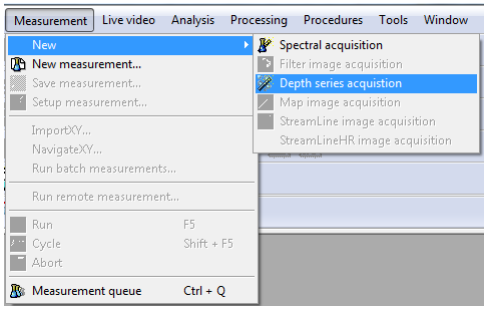
You can print to pdf file. Choose Print setup and "horizontal". After this Choose Print and the spectrum and Table will both be printet to a pdf-file. If only the spectrum should be printed then use "Analysis" → "Visible" to eliminate the table. Maximize then the Spectrum Viewer window with the mouse and print the spectrum.

8. Recording Raman Depth Profiles

This facility allows the user to examine the Raman spectrum of a substrate at varying depths inside the sample by varying the focus height (z). Typically the depth profile will be initiated above (+z) the surface (z=0) of a substrate and Raman spectra are then recorded while the z-parameter is lowered to a negative value in order to inspect the composition of the substrate beneath the surface.

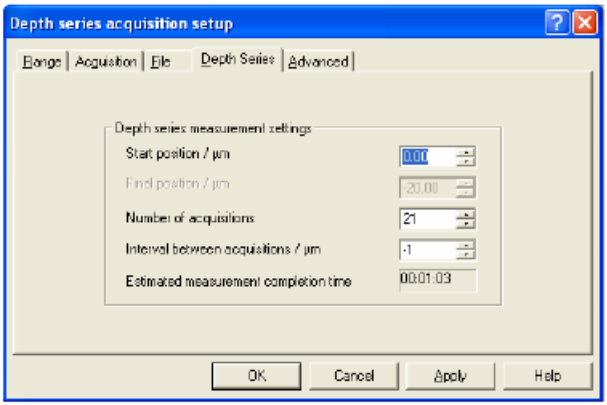
Before making a "Depth Series" measurement, you should set your current position to origo, to make sure that the focus is at z=0. This is done by pressing the “set origo” button or if this is already done then use “go to origin” .

To set up a depth profile measurement use the menu "Measurement" → "New" → "Depth series acquisition" or use . Use the Setup dialog to adjust Range, Acquisition and Depth Series. The Range and Acquisition pages are the same as in paragraph 6 for Spectral acquisition. Be aware that as a depth profile require several measurements, the time it takes to make one measurement becomes important.



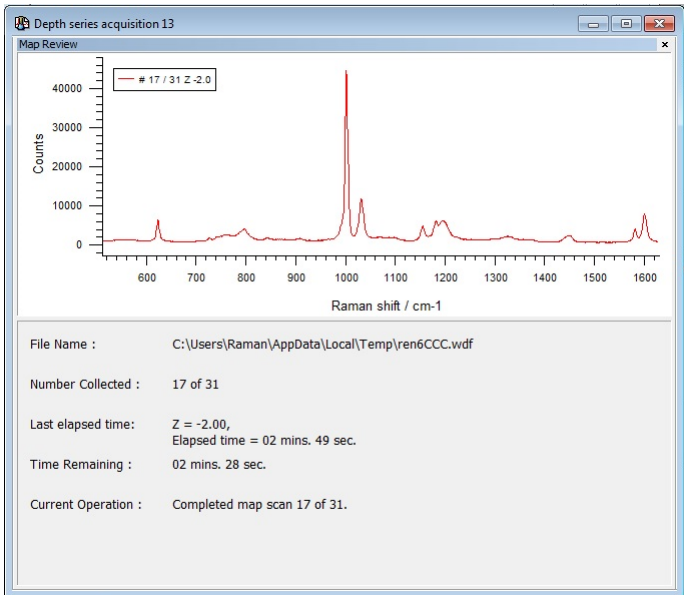
Therefore, in the Range tab choose "Static" recording if possible (e.g. 514 nm/1200 lines). "Extended scan" can also be chosen but consider that ~2-5 min will then be used for each z-value.

Important: Remember to change the



"Confocality" to "High", as depth profile measurements require a narrow focal plane. Also be aware that this will result in a lower signal and you should compensate for this in laser power, integration time or number of acquisitions. In Acquisition use similar considerations as in paragraph 6.

Normally the depth profile will start above the surface (+10 to +30 μm). The Confocal depth resolution is for 514/457 nm about 1.35 μm and about 2.1 μm for 785 nm. There is no reason to use an "Interval between acquisitions" which is numerically much lower than the Confocal depth resolution. Perhaps an interval of 0.5 μm should be optimal. Use negative values for the interval to indicate that the z-position is lowered down towards the sample and into the material. Input "Number of acquisitions" so that the "Final position" (μm) is the last negative position beneath the surface in μm. Be aware that in principle the real depth into the substrate has to be corrected with the refractive index, n, of the substrate. If "Final position" (μm) is -10 μm and n = 1.5 then the real final position will be -15 μm. **Important:** *Be aware of the working distance of the objective to make sure that you do not crash the objective into the sample! WD for x50 and x100 is only 370 and 330 μm respectively.*

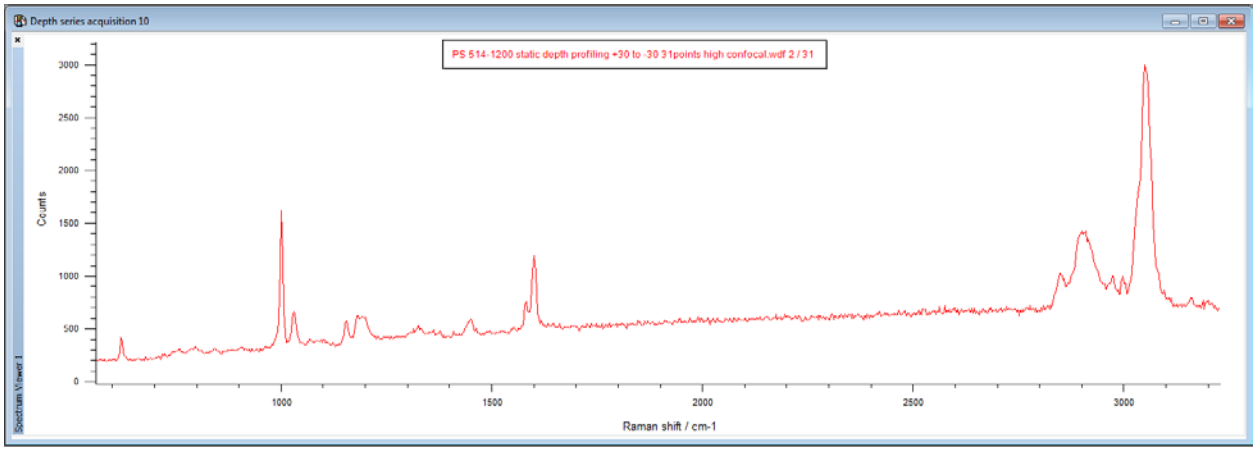


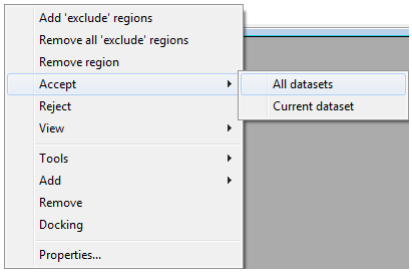
Press "Ok" and go to "Measurement" → "Run" or the Run button, to start the measurement. It is currently not possible to depth profile a map (volume imaging) but if you want depth profiling of several points then "queue" the measurements by setting up several depth profiles and use on each of them. Measurements are then processed by the system in the order in which they were submitted to the queue. To view all the measurements in the queue, select Measurement > Queue, or click the Queue button on the Measurement toolbar.

8.b Data Analysis of Depth Profiles

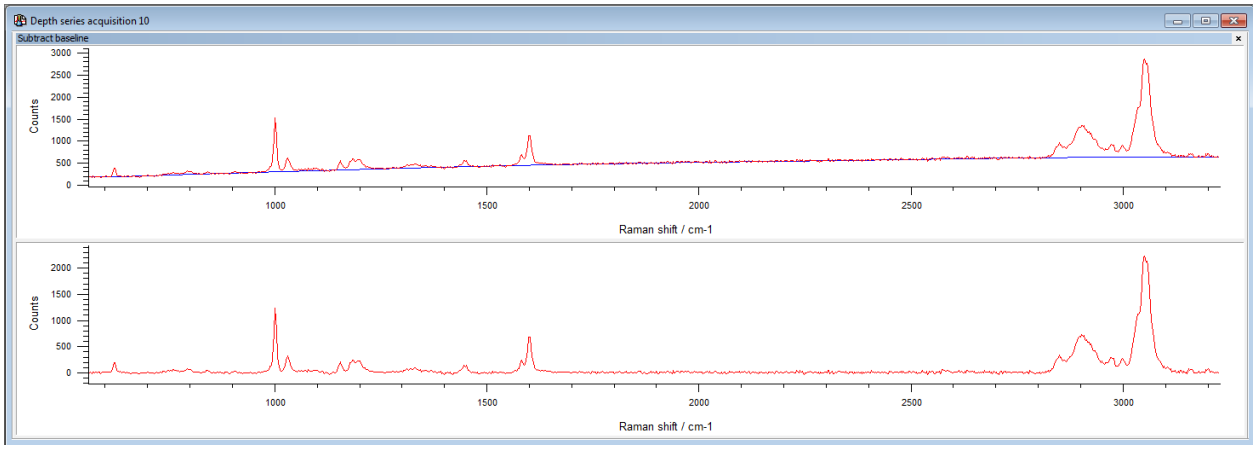
Before doing any spectrum manipulation, ***save a backup of your data*** as the Wire software will overwrite your data during manipulation!!!

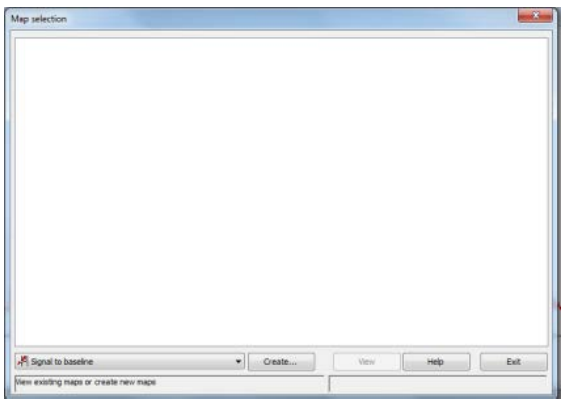
Prior to using data analysis functions, the depth profile to be analyzed must be loaded into a Spectrum Viewer. This will happen automatically following data collection (recording). Already saved data may also be loaded into a spectrum viewer using the "File" → "Open".

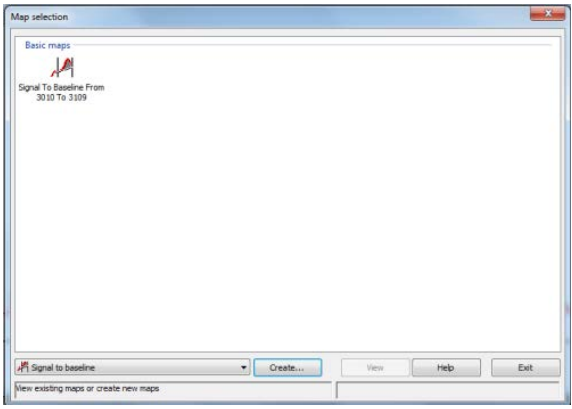




It is possible to step through the depth profile by or the menu "View". You may perform "Subtract Baseline" in "Processing" and choose to do it on "All datasets" (all spectra). Similar processing can be used to truncate and remove regions in the spectra.

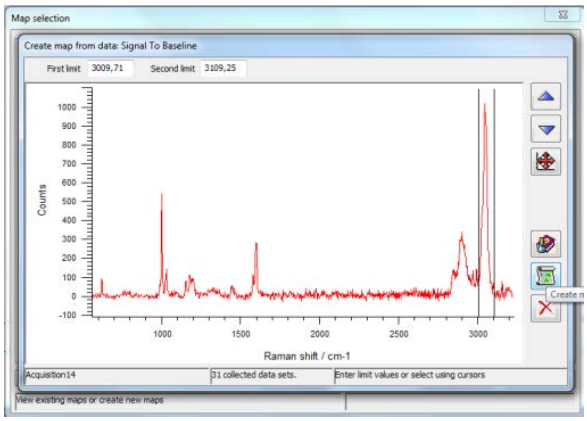






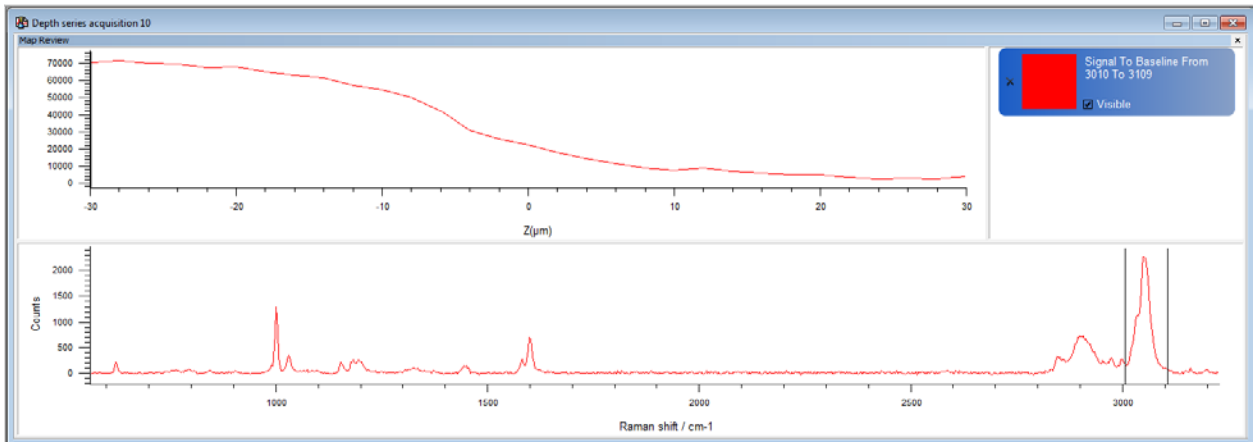
When finished processing then go to "Analysis" → "Mapping review". First you should pick the data to map. A simple "Signal to baseline" plot will be used as illustration. Click "Create"

and move the two vertical black lines to define the peak of interest and click in "Crate new map",  . In "Map selection" appears now a "Basic Map". Highlight this map (or maps if several) with the mouse and click "View".



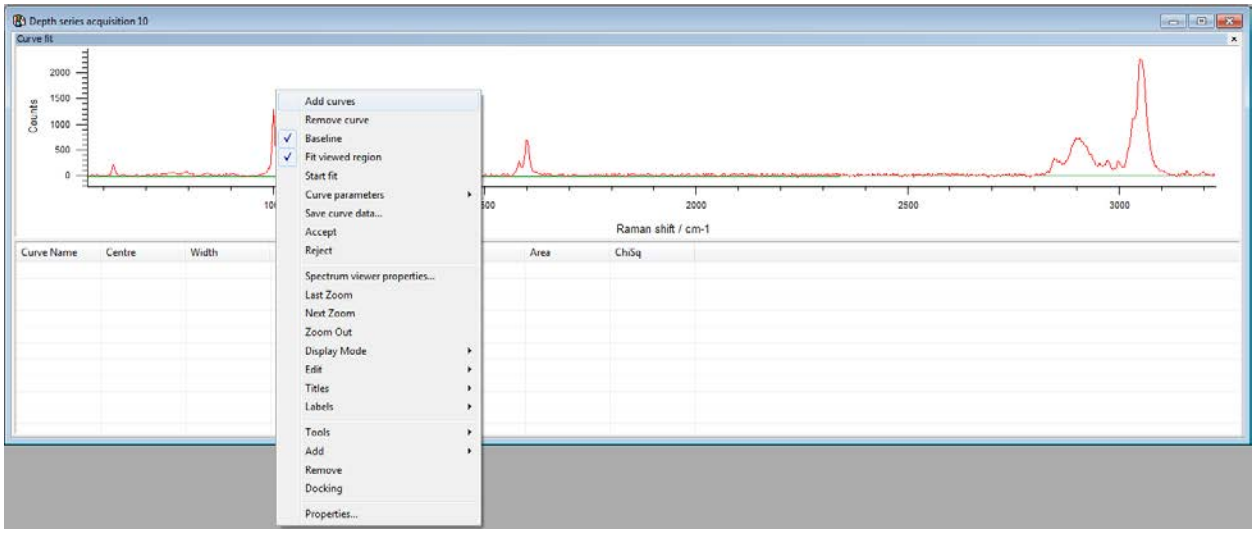
If asked click Ok to save data.

The map is now generated together with the first spectrum in the profile. The latter can be changed by moving with the mouse the verical black line (initially together with the y-axis) in the depth profile plot.



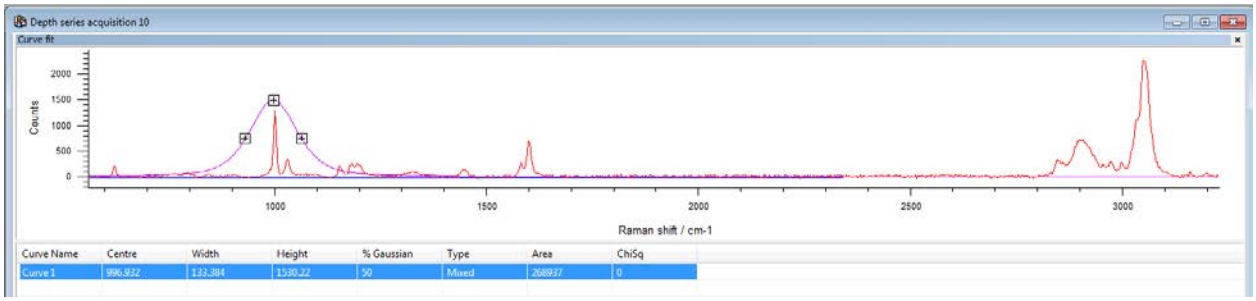
In many cases, the data of interest will be the peak height, peak width or similar data requiring the data to be fitted.

The best way to generate maps based on curve fitted functions is to first fit a single spectrum to optimize the fit parameters. Go to "Analysis" → "Curve Fit" or use  .

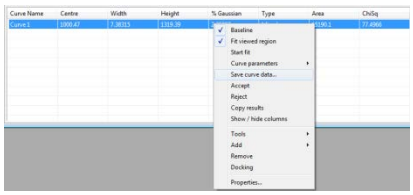


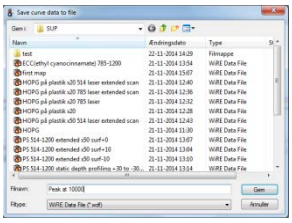
Right click in the "Curve Fit" spectrum and choose "Add curves". Click in the spectrum to add new peaks.

Roughly adjust the shape to the peak in the spectrum and right click again and pick "Start fit".

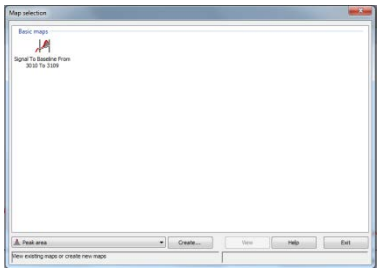


If you are not satisfied with the fitting, the detailed parameters for the fitted background, peak shapes etc. can be adjusted by right clicking the spectrum and selecting properties.
The result of the fit is shown in the table below the spectrum. Now right click on the highlighted line in the table and use "Curve parameters" → "Save".

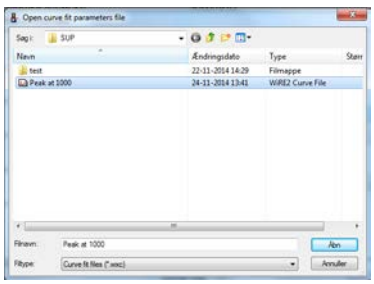


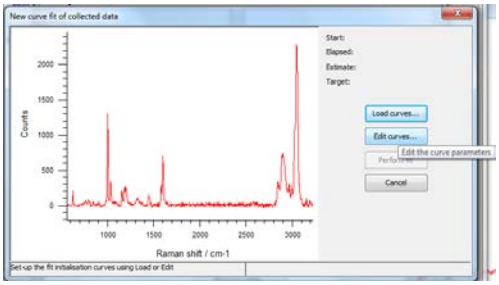


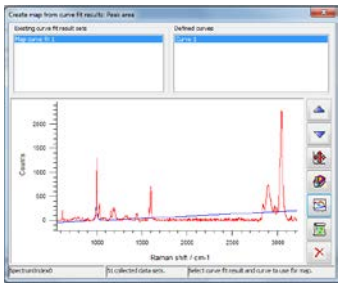
Remove the "Curve fit" view by closing the window in top right corner. Now go to



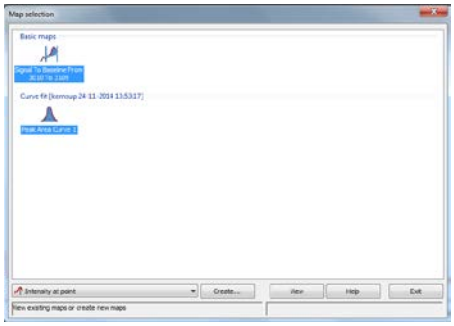
"Analysis" → "Mapping review". Select a map type based on the curve fitted function for the peak (area, position width, intensity) and click "Create" to enter the "Create map from curve fit results: Peak area". Use  to enter "New curve fit of collected data". Use "Load curves..." to find the file containing the fitted curve parameters. Adjust the detailes parameters for the fit if nessecary. Back in "Create map from curve fit results: Peak area" click in "Create new map"  .

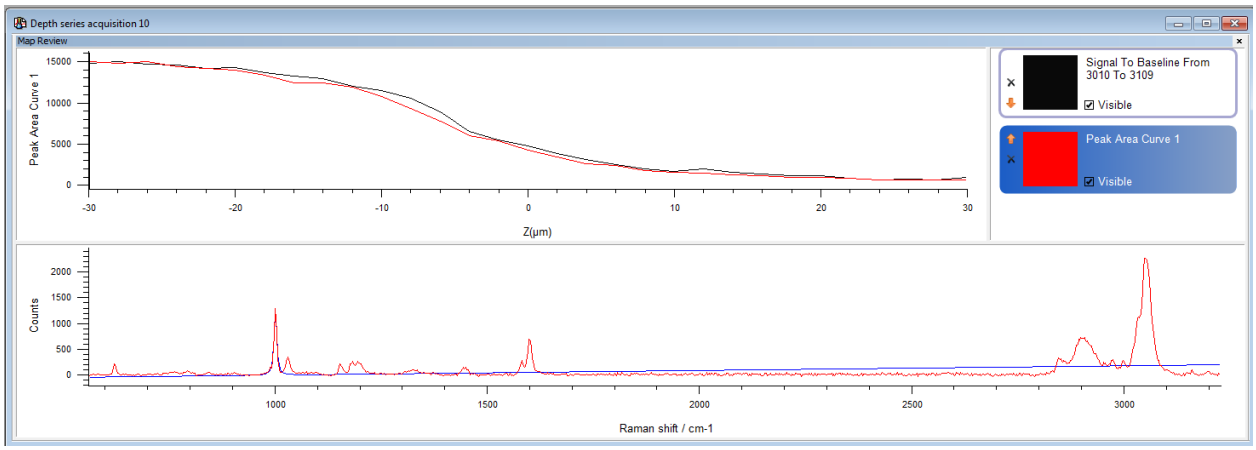






More Maps can be shown by highlighting more maps and pressing View:



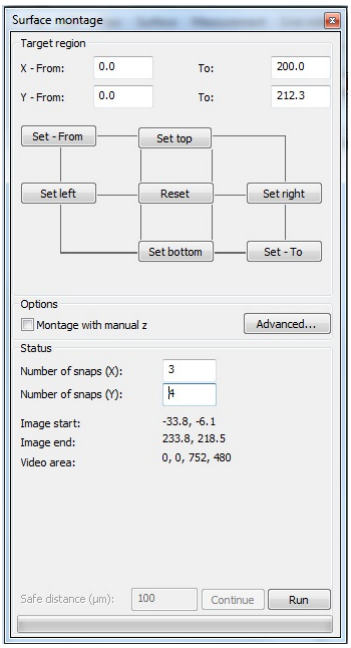


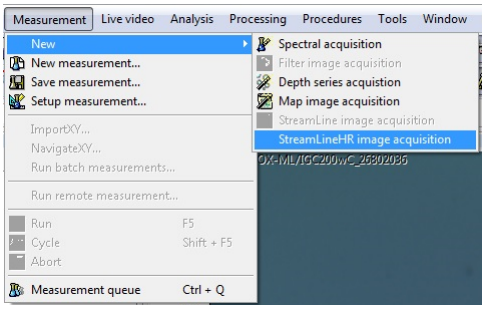
9.0 Area Maps

Area maps can be recorded in several ways depending on the state of the sample and how focus can be achieved during the mapping. First, a method to record a map for planar substrate that might be tilted a bit compared to the sample stage will be described. The method is shortly to manually create a map of the focus distances (z position) in each corner of the sample. During recording, the best focus is calculated automatically by interpolation.

For this method the first step is to start by defining a "Surface" (your focus map), then make a visual map "Montaging" and finally setup the Raman mapping.

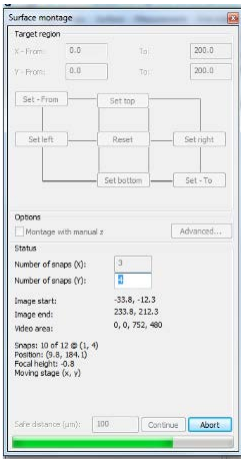
9.1. Mapping a planar substrate (tilted or not tilted)



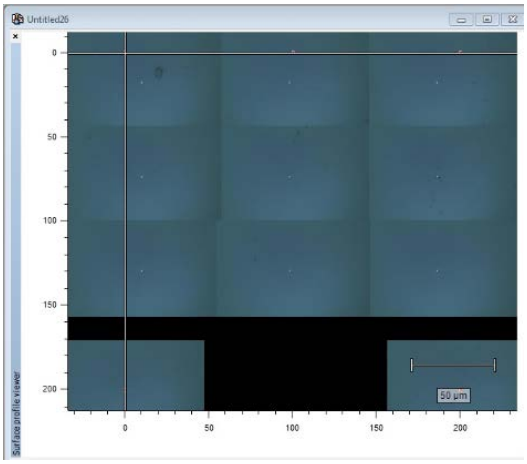


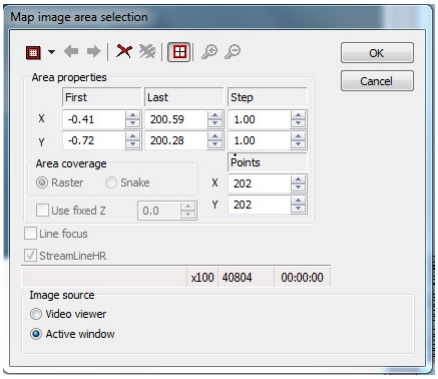
Double check that you have selected the correct active objective in the "Sample review" bar. First, move the sample and set the focus in the left upper corner of the area you plan to map. Define this as origo (or "Live Video" → "Set Origin"). Then go to either "Surface" → "New" or click on to setup your focus surface. Now add the origin point to the surface by "Surface" → "Add point" or use . Now move the stage to the upper right corner by adjusting the x coordinate in 

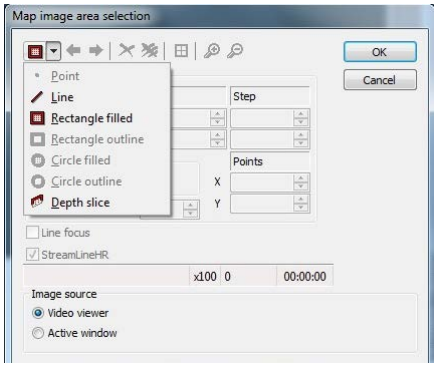
After this you adjust the focus (z) on the track ball (be careful not to move the x,y position. Add this point to the Surface, . Repeat the process for at least the two lower corners as well.



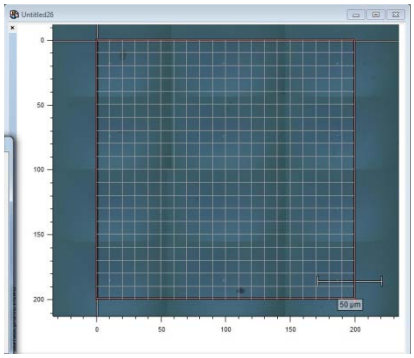
To obtain a full optical image of the area of interest use "Live video" → "Snap" → "Montage". If the surface is still active in the "Surface profile viewer", then the x,y-coordinates of the corners should already define the "Target region" in the "Surface Montage". Everything is ready for "Run" and the full picture is now put together by combining several photos. The progress of the montage can be followed in the "Surface montage" or in the "Surface profile viewer".

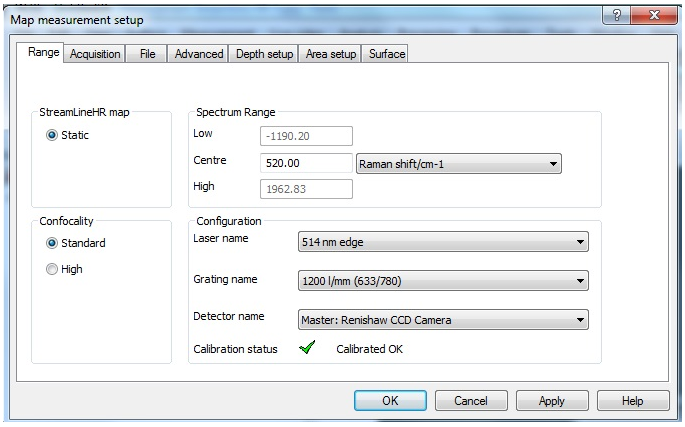






When the montage is finished, keep the montage as the active Window. Go to “Measurement” → "New" → "StreamlineHR image acquisition". Define the shape of the map at .Typically this will be “Rectangle filled”. Set the “Image source” to “Active window” (the montage). The dimensions of the montage are automatically used as the defined “Area properties”. The default value for “Step” is 1 µm, indicating 1 µm between each measurement. Be aware, that depending on the size of the map this might result in excessive number of points and indeed very time consuming measurements. The actual map with the measurement pattern can be seen overlaid the montage. In the shown example the step was changed to 10 µm consisting of 441 points to be recorded. Click “Ok” when you have defined the desired map.

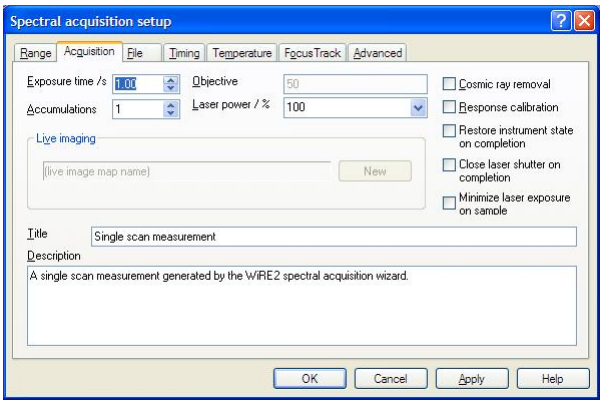


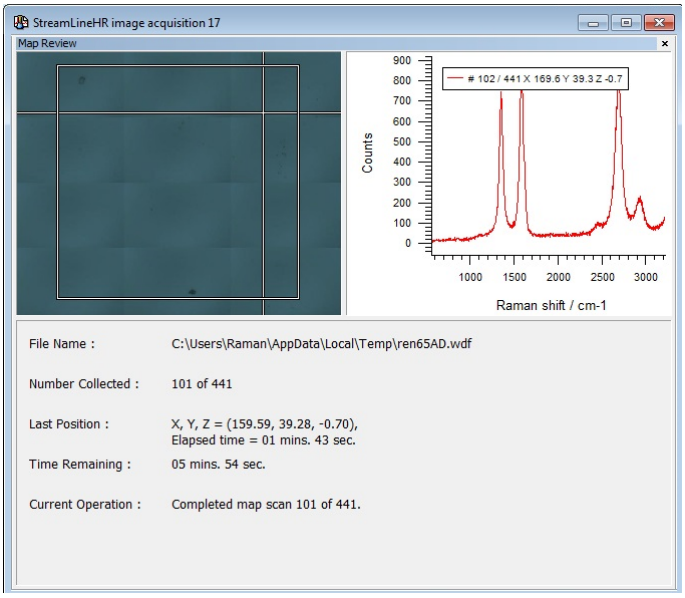


The standard page for measurements settings automatically open. The Range and Acquisition tabs are the same as in paragraph 6 for Spectral acquisition. Since a map includes many recordings time becomes a very important issue. For “streamline” mapping only “Static” recording is possible. In order to cover the largest spectral range the combination 514 nm and 1200 lines grating is particularly useful since it allows a total range of ~2500 cm^{-1}. Use Standard “Confocality” to maximize the signal output and adjust the

“Spectrum Range” by adjusting the “Centre”. For graphene “centre” equal to ~2300 should provide a spectrum with D, G and 2D peaks covered.

The tab “Acquisition” should also be adjusted as described in paragraph 6. It might be worth to test the right combination of “Exposure time” and “Laser power” before attempting the large mapping. It is most time efficient to use 100% Laser power and then adjust the Exposure time. The latter has a minimum value of 0.05 s (50 ms). Be aware that a high Laser power may damage the sample. For graphene 100% laser power can normally be used and it may be advantageous to adjust on the laser to full power. Remember to return the knob to 30% again after the mapping. Use “Ok” save the mapping setup. go to "Measurement" → "Run" or the Run button, to start the measurement. The progress of the mapping can be followed in “Map review”. In the shown example the recording of one spectrum takes 1 sec. The area 21 x 21 points takes 7,5 minutes. With a step of 1 µm the same size of map would take 750 minutes or 12,5 hours to record. A facility will soon be implemented that allow you to control the powering of the lasers from the PC. This may be used to power down the laser from remote.





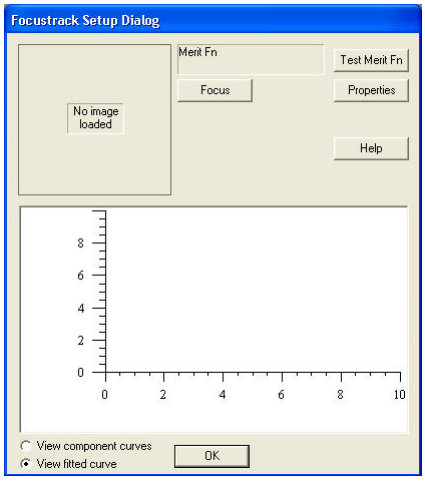
9.2 Mapping with extended scan

It is possible in “Measure” → “Map Image Acquisition” to image a sample point by point and record the extended spectrum for each point however the procedure will be very time consuming and should normally not be attempted. The recording of one simple extended spectrum takes alone ~5 min and a similar map of 21 x 21 points would take 35 hours. The StreamlineHRImaging is therefore 280 times faster. The setup of the experiment is quite similar to mapping with StreamlineHRImaging except that ”Extended” scanning can be selected.

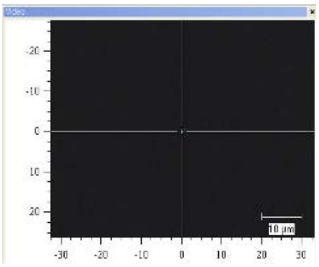
9.3 Mapping of rough sample with focus track (Automatic focus during mapping)

If the sample is almost flat but include areas with valleys and protrusions then definition of a "Surface" with manual focus might still work but it would require a lot more points to define the surface than just the four corners. It becomes important to provide a true surface with many focus points in the rough areas. By doing so, the sample can still be imaged using the fast StreamlineHRImaging.

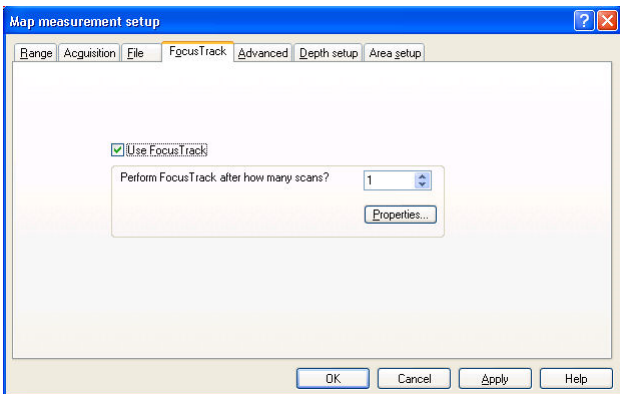
If the sample cannot be defined properly as a surface then another, but much slower method is available. Unfortunately this only works together with the point by point imaging. The method relies on an automatic focusing during mapping. Initially the FocusTrack should be set-up to verify that the parameters to be used during the mapping experiment enable the laser focus to be adequately maintained for the extremes of the change in sample depth.



Focus on the sample with the objective to be used for the mapping experiment, set the position to origo with  . Ensure that the correct objective magnification is shown and the video displays a black background (white light off), and highly attenuated laser so that the spot or line can be just observed without saturating the video camera. Select Live Video > FocusTrack > Set-up



The 'test merit function' button takes a snap of the video, and calculates the merit function value. It is suggested that a value of between 1000 and 1500 is achieved where possible. The attenuation can be varied with the 'FocusTrack Setup Dialog' window still open. Adjust the attenuation and recalculate the merit function. The optimum attenuation to be used with focus track can then be determined. In properties many details can be adjusted and here you should look in the on-line help function for further help.



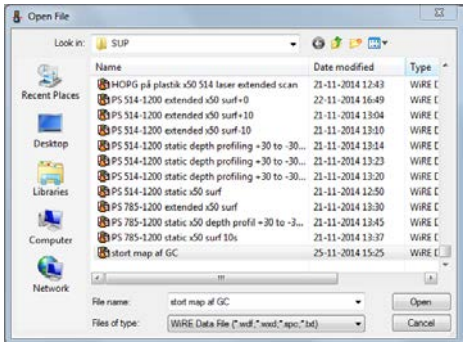
A trial FocusTrack can be performed to ascertain the performance. This is achieved by selecting Focus, or the button  The 'Detail' button will display a graph on completion of the FocusTrack to allow a visual representation of the change in merit function (spot

intensity) with sample depth. The optimum focus position is determined by an automatic curve fit of this graph. It is therefore clear that a meaningful change in merit function as the focus position is approached is required. For lower magnification objectives (where small focus changes do not significantly affect the spot size) larger FocusTrack step sizes are therefore required.

The use of FocusTrack within maps can be easily set from the FocusTrack tab, available from the Map image acquisition set-up. To use FocusTrack simply tick the box.

In addition the periodicity (FocusTrack per scans) of the FocusTrack use in the map can be set. This value should relate to the rate of change of the sample surface position with mapping step size. Where the sample is generally quite flat, the FocusTrack is required infrequently, whereas rapidly changing sample surfaces require a frequent FocusTrack.

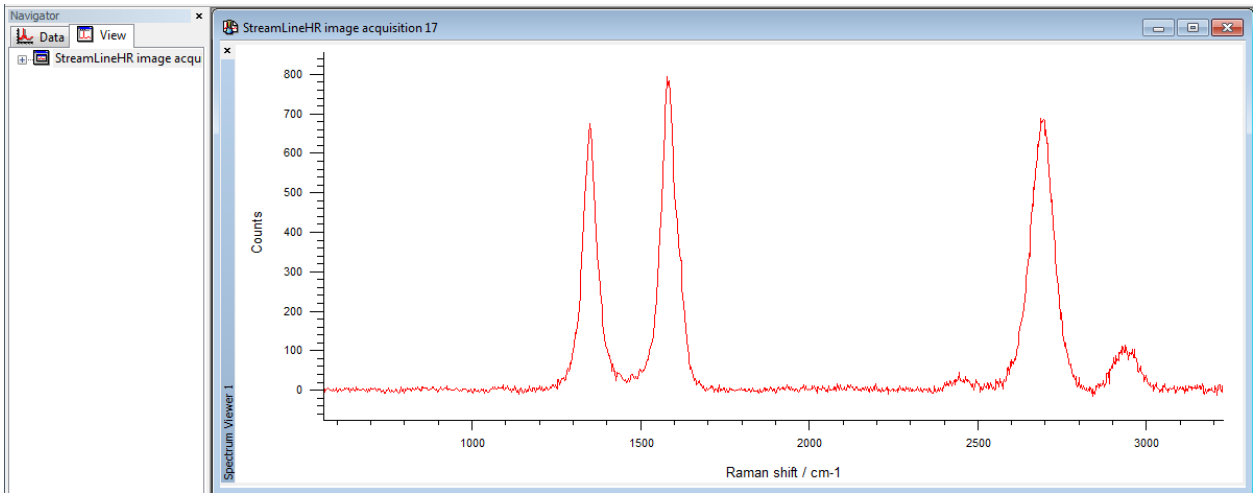
10. Data manipulations

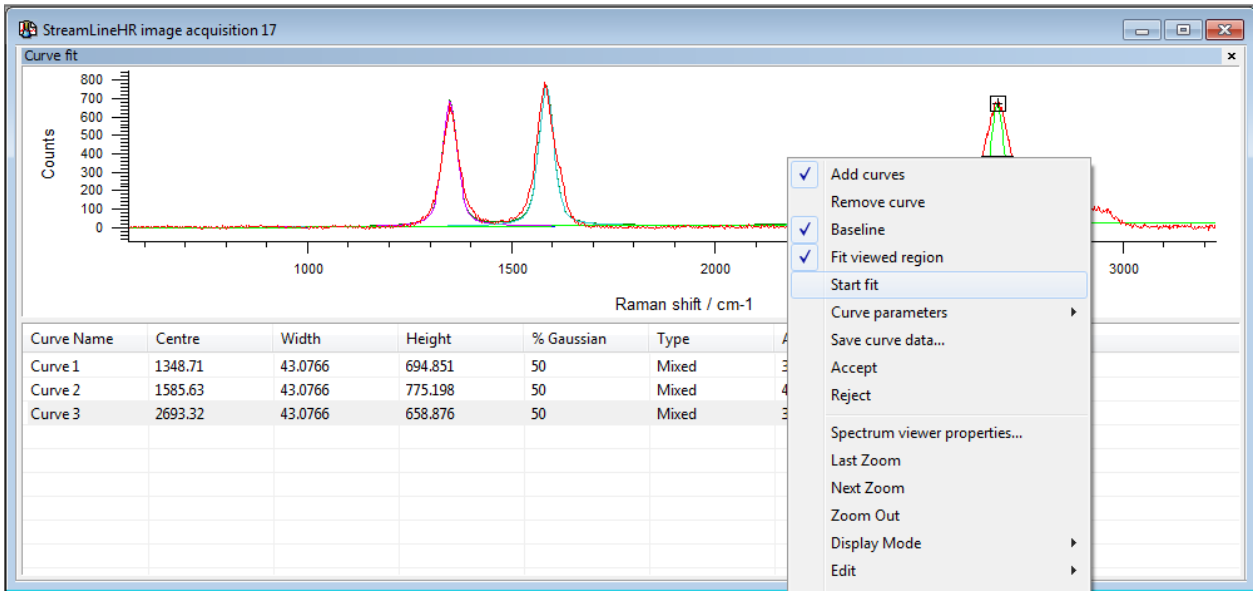


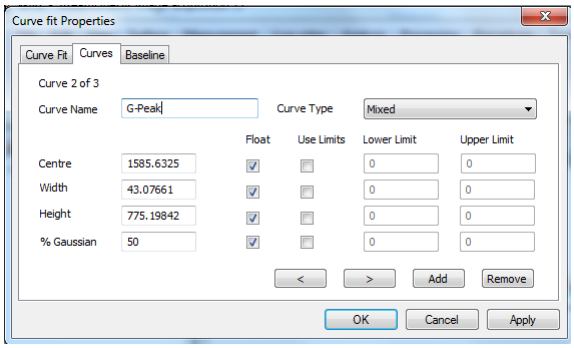
10.1 Manipulating maps

Open a map-file. First do the processing needed (substract background etc). Second, go to "Analyse" → "Curve fit"

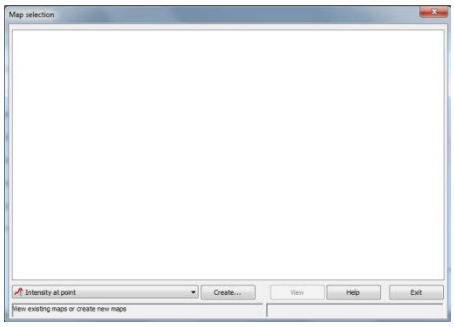
Use the peak marking tool (if not active already, then chose right click and "Add curves") to mark the three peaks and with the mouse adjust roughly the width of the peaks. Rename the peaks by right clicking to enable "Properties".





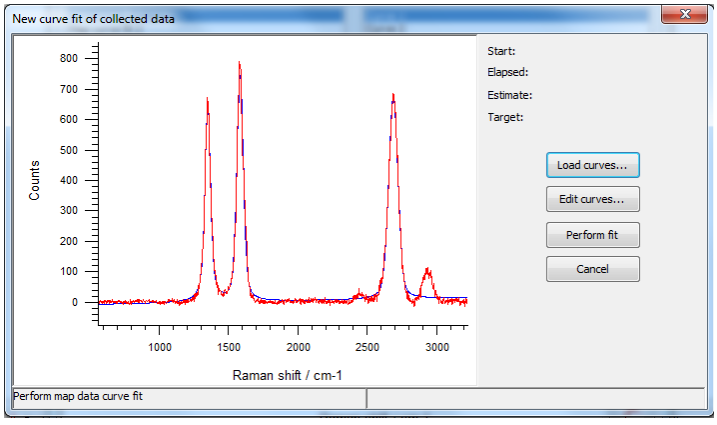


Rename all three curves by using < > to step between the curves. Now right click in the "Curve Fit" window and chose "Start fit". The program will now fit a Lorentzian-Gaussian curve and display the characteristics in the table below the spectrum. Right click and use "Curve parameters" to "Save" the characteristics for the three peaks. You can now close the "Curve fit" window. Go to "Analysis" → "Mapping review". There are two main types of maps. A) Maps created from direct analysis of the data including 1) Intensity at point, 2) Signal to baseline, 3) Signal to axis. B) Maps created from curve fit results including



1) Peak area, 2) Peak position, 3) Peak width, 4) Peak intensity, 5) Percent Gaussian, 6) Chi squared. Here will be shown maps of type B-4 (Peak intensity). Pick the right type of map 

and press "Create". In "New curve fit of collected data" use the "Load curves" and find the file containing the saved Curve Parameters (here "graphene") and by "Perform Fit". Chose the new "Curve Fit.

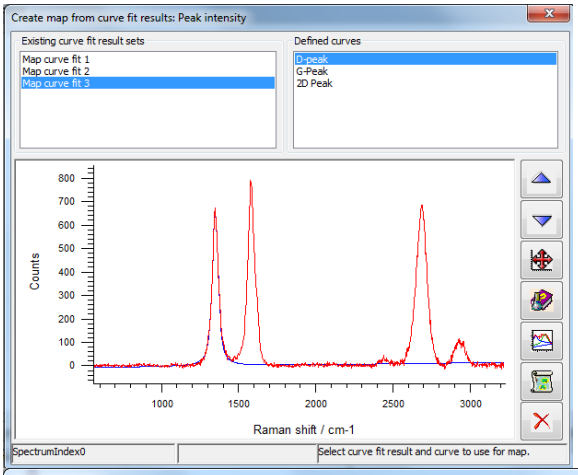


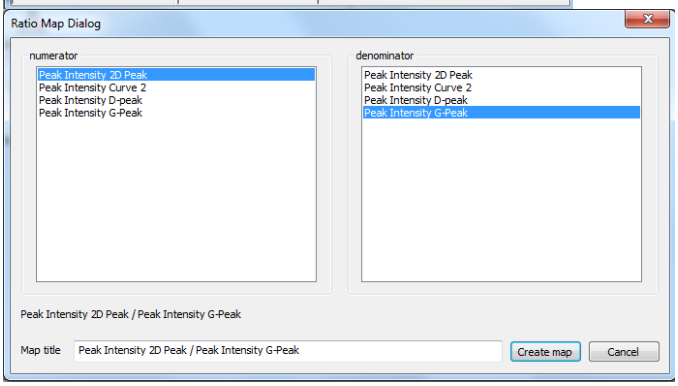
Back in "Create map from Curve fit" Create the maps one by one by highlighting the "Map curve fit" and the "Defined curves" and press  .

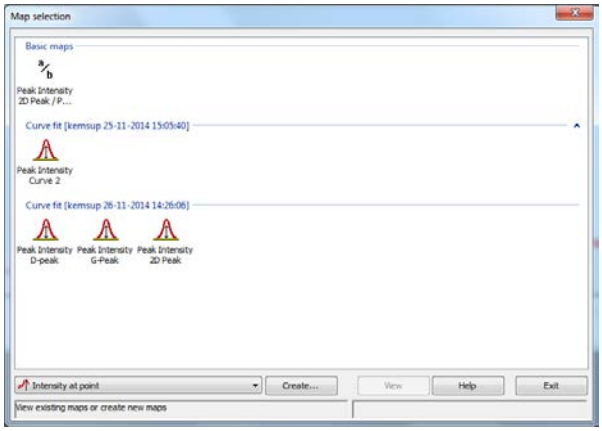
In this way we used the “Graphene” file with curve parameters for D, G and 2D to generate three maps of peak intensity. Now it could be interesting to get a map of I_{2D}/I_G. to do this go again to “Map selection” and choose 

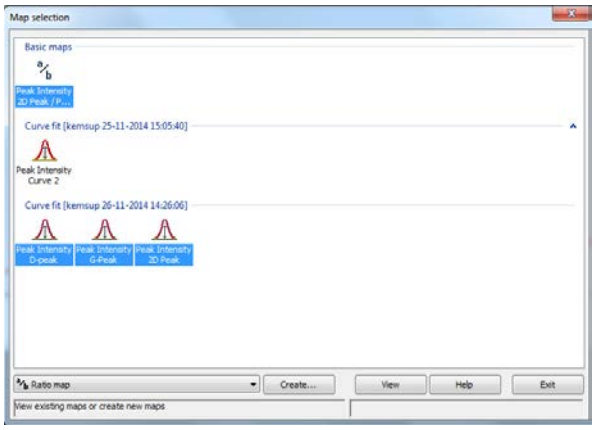
and “Create”. You obtain now a chance to combine the curve fitted function by division. Perform “Create map” and the Wire 4.1 program will very frequently crash here. However, when you start the program again and load the same data file the ratio map will be found in the list of maps.

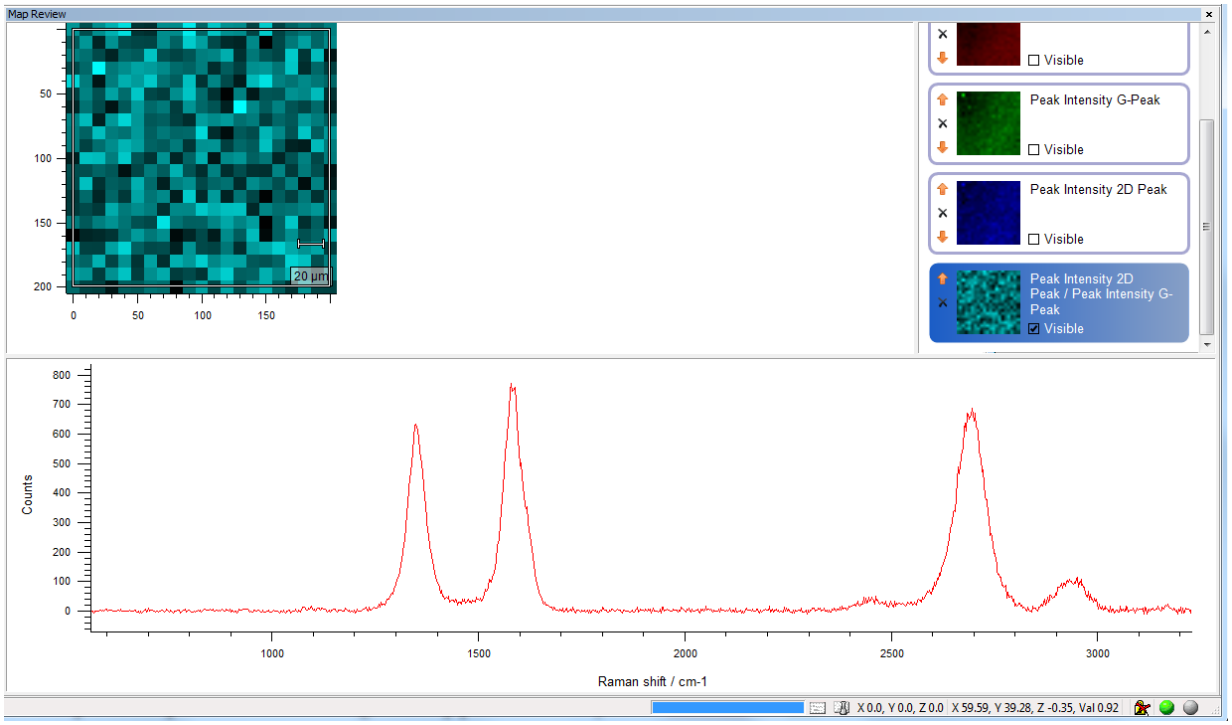
Highlight the maps you want to see and press “View”. You now see four maps, D, G, 2D and 2D/G but they will be shown on top of each other. You can remove visually a map by unselect “Visible”. In this way you can display one map at the time. The colorscale of the maps can be adjusted by right-clicking on the colored map and choosing LUT Control. Use the sliders to change the upper and lower limits, manually put in numbers of interest or use either the “5%-95%” or “Auto” buttons to adjust.


Create map from curve fit results: Peak intensity
Existing curve fit result sets
Map curve fit 1
Map curve fit 2
Map curve fit 3
Defined curves
D-peak
G-Peak
2D Peak
Counts
Raman shift / cm-1
SpectrumIndex0
Select curve fit result and curve to use for map.


Ratio Map Dialog
numerator
Peak Intensity 2D Peak
Peak Intensity Curve 2
Peak Intensity D-peak
Peak Intensity G-Peak
denominator
Peak Intensity 2D Peak
Peak Intensity Curve 2
Peak Intensity D-peak
Peak Intensity G-Peak
Peak Intensity 2D Peak / Peak Intensity G-Peak
Map title
Peak Intensity 2D Peak / Peak Intensity G-Peak
Create map
Cancel

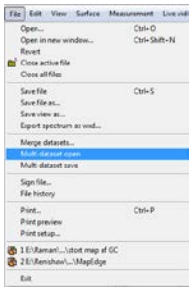

Map selection
Basic maps
Curve fit [kemsup 25-11-2014 15:05:40]
Curve fit [kemsup 26-11-2014 14:26:06]
Intensity at point
Create...
View
Help
Exit
View existing maps or create new maps


Map selection
Basic maps
Curve fit [kemsup 25-11-2014 15:05:40]
Curve fit [kemsup 26-11-2014 14:26:06]
Ratio map
Create...
View
Help
Exit
View existing maps or create new maps

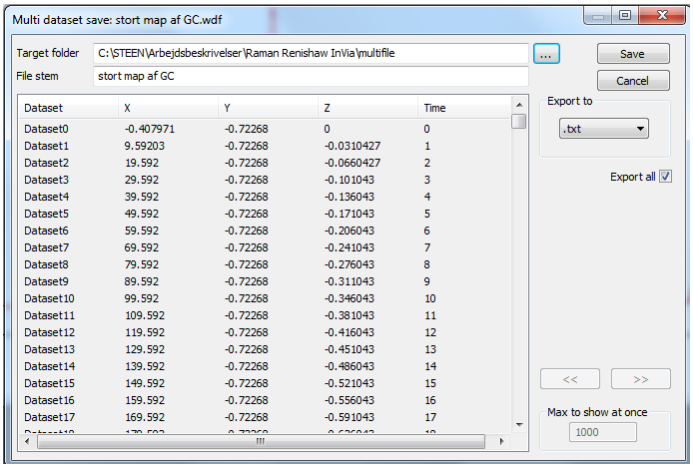


11. Export and Print

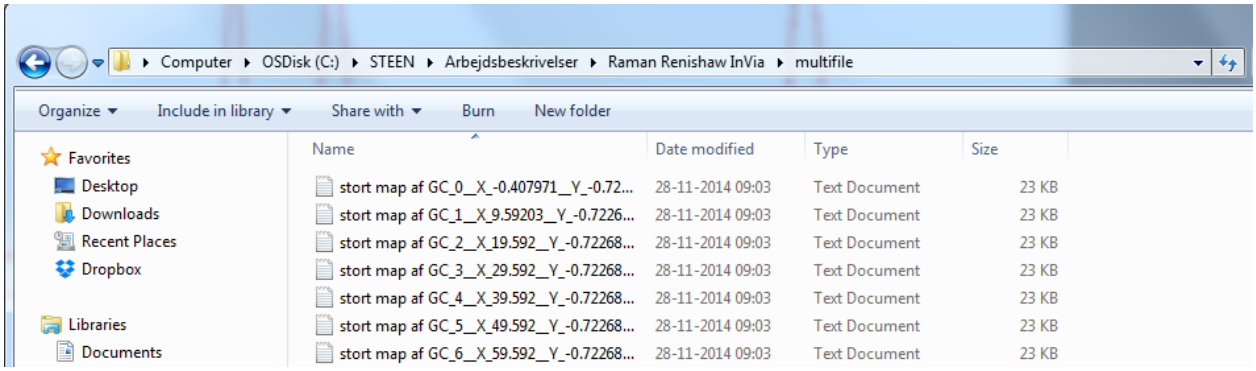
11.1 Export of Multi dataset (large maps of spectra)

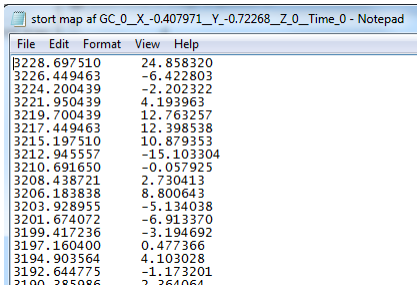


Maps are essentially a big dataset of full spectra. You can save this Multi dataset as text files, one file per spectrum for a specific position (x, y, z). Go to "File" → "Multi dataset save" and insert the folder you want to use and the format in "Export to" as ".txt" and either "Export all" or highlight the Dataset you want to save.



The name of the individual text files start with the map file name followed by spectrum number, x-coordinate, y-coordinate, z-coordinate and finally time (which is just an integer). The spectrum files can be opened with any text editing program like "Notepad" and will show lines with wavenumber and intensity.

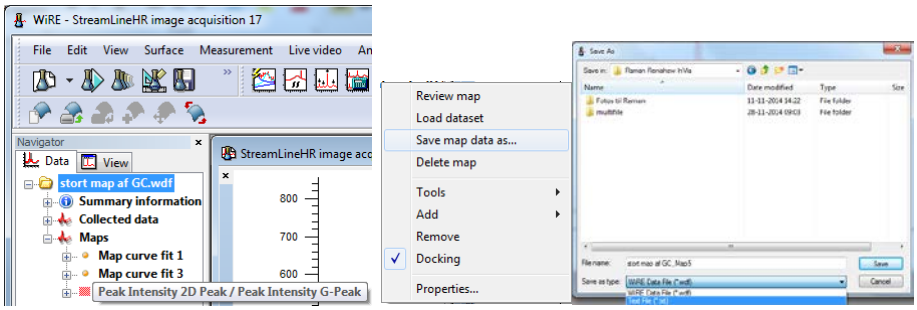


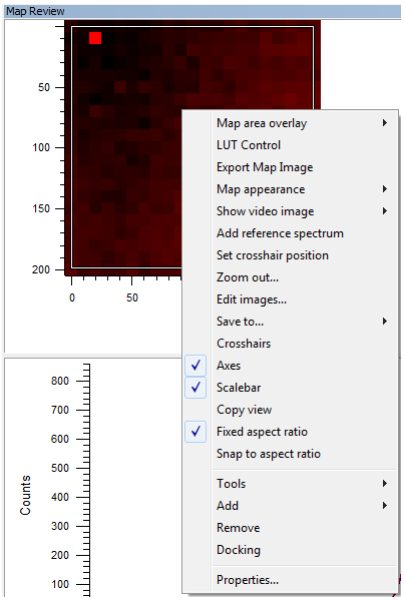


The text files can be read (imported) as spectra by Origin or Sigmaplot but the tricky part is to decode from the file name the x, y and z coordinates. I believe that we in the near future will make a small program that can read the text files and import them into Origin or Sigmaplot.

11.2 Export of Maps as text files.

A map can also be saved as a text file. Use “Navigator” and “Data”. Expand by clicking stort map af GC.wdf. Expand “Maps” by Maps. Highlight the map you want to save, right click and on the context menu click on “Save map data as”. Save the file as “Text file (*.txt).





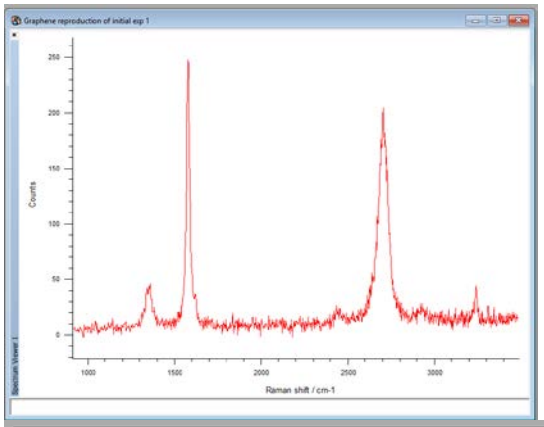
Maps can also be saved as jpg or bmp files. In “Map review” right click in the Map and choose “Export Map Image” and save it where you want as jpg or bmp file. The resolution of the image is however rather bad and essentially the same as taking a screen shot. Perhaps this facility will be improved in the next version of Wire.

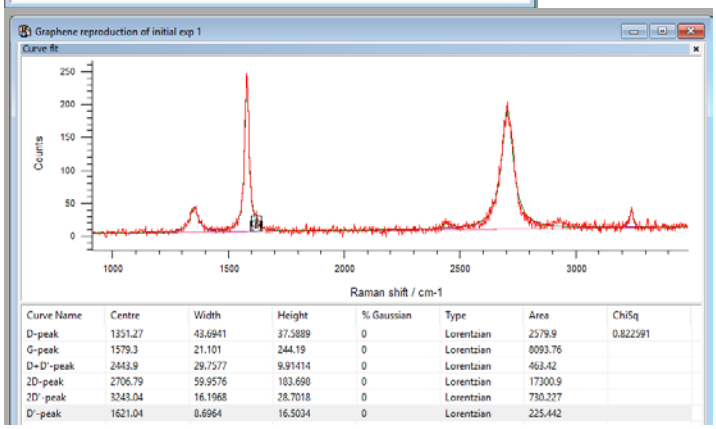
11.3 Export of Curve fits

In some cases it is useful to export a curve fit, so that it can be plotted in other software. There are two approaches: A) Use the curve parameters to plot the fit function (if it is Lorentzian) or B) Export the single fitted peaks.

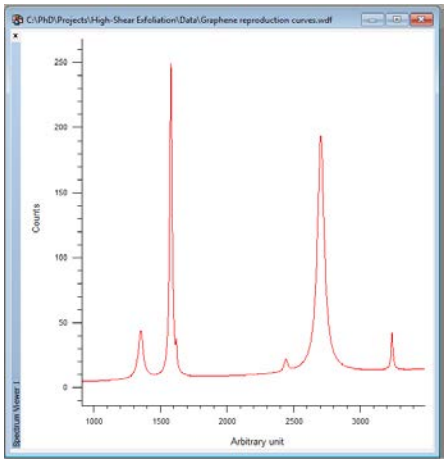
For Method A) one needs the area, width and position of each peak which can be saved by the method described in 11.2. These can then be plotted in other software as the Lorentzian function $(x) = \frac{A}{\pi} \frac{\frac{1}{2}\Gamma}{(x-x_0)^2+\left(\frac{1}{2}\Gamma\right)^2}$, where Γ is the peak width (FWHM), x_0 is the position and A is the area of the peak, which is used for scaling.

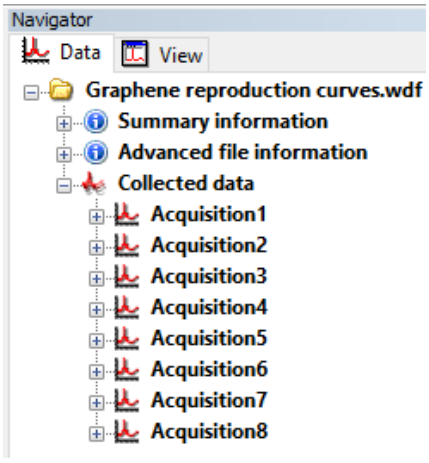
One can also export the individual curves from a Curve Parameters file (i.e. the curve fit file generated in section 10.1). To do this open your map or spectrum file. Then go to “Analysis” and “Curve fit”. Right click on the spectrum and go to “Curve parameters” > “Load”. Select your fit file (i.e. the “.wxc” file you saved in section 10.1) and press Open. Right click on the spectrum and press Start fit. As an alternative to loading a curve fit file, a new curve fit can also just be made as previously described. Now right click on the spectrum and select “Save curve data” as a .wdf file. Open this file and go to the Data tab in the Navigator window. If you open the “Collected data” menu that you will see a series of “Acquisitions”. The first one will be the full spectrum, number two is the baseline and the rest are the individual peaks. Right click each of them and go “Save dataset as..” and save as a .txt file. This gives a simple file with wavenumber and intensity of each peak, which can be plotted in any software.





Curve Name	Centre	Width	Height	% Gaussian	Type	Area	ChiSq
D-peak	1351.27	43.6941	37.5889	0	Lorentzian	2579.9	0.822591
G-peak	1579.3	21.101	244.19	0	Lorentzian	8093.76	
D+D'-peak	2443.9	29.7577	9.91414	0	Lorentzian	463.42	
2D-peak	2706.79	59.9576	183.698	0	Lorentzian	17300.9	
2D'-peak	3243.04	16.1968	28.7018	0	Lorentzian	730.227	
D'-peak	1621.04	8.6964	16.5034	0	Lorentzian	225.442	





Graphene reproduction curves_Acc

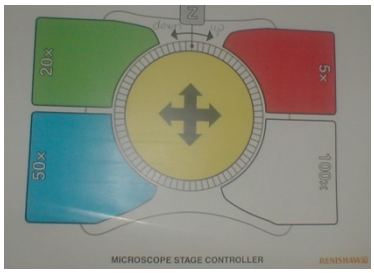
Filer Rediger Formater Vis Hjæ

3486.843018	0.007470
3484.668213	0.007487
3482.492432	0.007504
3480.316650	0.007521
3478.139893	0.007538
3475.963135	0.007556
3473.784424	0.007573
3471.606689	0.007591
3469.427002	0.007608

Appendix A – Adjusting the stage height

First, it is advised that the nosepiece of the microscope is rotated to a position that does not contain an objective, as this will greatly reduce the risk of damaging the objective. It is also helpful to position the stage in approximately the centre of its' focus travel. Then, whilst supporting the weight of the stage, unlock the stage by disengaging the locking lever (located just above the right-hand focus controls). Carefully raise or lower the stage until it is approximately at the correct height and re-engage the locking lever. The focus controls can now be used as before to make smaller adjustments to the stage height.

Appendix B – Using the track ball



First activate the track ball by pressing the button with the correct objective magnification. The sensitivity of the trackball and movements of the stage in x,y and z directions are balanced with the magnification. After activation the ball can be used to move the stage/sample in x,y directions and the z-direction by turning the knurled ring (Clockwise for diminishing the distance between sample and objective (up) . The track ball goes to a sleep mode after being inactive for at few seconds, and then needs to be activated again by pressing one of the magnification buttons.

C. Specifications:

Slit width 20 um and integration time 100 ms

sensitivity tests were performed with 100% laser power, while rest of the tests were performed using 5% laser power.

Laser 785 nm

Sensitivity (signal/noise) = 12 (laser power 50 mW)

confocal depth resolution = 2.17 um

lateral resolution = 0.73 um

spectral resolution = 0.69 - 0.76 cm-1

Laser 514 nm

Sensitivity (signal/noise) = 27 (laser power 9.5 mW)

confocal depth resolution = 1.37 um

lateral resolution = 0.62 um

spectral resolution = 0.69 - 0.89 cm-1

Laser 457 nm

Sensitivity (signal/noise) = 18.6 (laser power 11.5 mW)

confocal depth resolution = 1.33 um

lateral resolution = 0.57 um

spectral resolution = 1.1 cm-1

Mapping speed 2 min 23 secs for 2500 data points

Appendix D – Installation of Wire 4.1 program

Important: it is only allowed to have Wire 4.1 installed on your computer as long as you are affiliated with Aarhus University.

Step 1. Download the software from the network drive
Copy the folder \\uni.au.dk\dfs\ST_RamanPC\Software and Manuals\Wire 4.1 to your own computer.

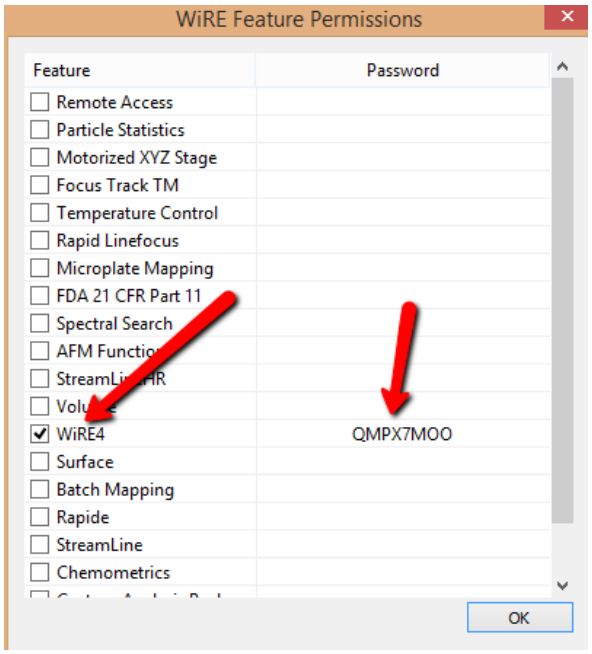
Step 2. Install Wire
Go to the subfolder \WiRE4_4308-r4.1\WiRE4_4308-r4.1 and execute the file "Setup.exe" and follow the setup procedure.

Once that is done go back to the Wire 4.1 folder and execute the hot-fix file "HF7656.exe". This fixes a lot of small bugs in Wire.

Step 3. Load registry files
After installing wire you will see an icon called "Regedit (32 bit)" on your desktop:

Find the two registry files "288C51-28_11_2014_sys.reg" and "288C51-28_11_2014_usr.reg" in the Wire4.1 folder you downloaded and drag them one by one onto the Regedit icon. This will load the necessary registry changes for Wire4.1 into your computer.

Step 4. Start the program and put in the license code
As you start the Wire software you may be asked to put in a "password". To do this you need to put a mark in front of "Wire 4" and put in the code "QMPX7OO".



Appendix E – Access to Raman data

Contact Emil (emilbp@inano.au.dk) with your full name, department, NFIT username and AU e-mail to get access to the Raman network drive. Once you have access the data can be downloaded from \\uni.au.dk\dfs\ST_RamanPC

Appendix F – Links to Raman

http://www.doitpoms.ac.uk/tlplib/raman/printall.php

Appendix G – Relation between laser wavelength, Stokes wavelength and Wavenumber

$$\frac{1}{\lambda_{Stoke}(nm)} = \frac{1}{\lambda_{laser}(nm)} - \frac{\bar{\nu}}{10^7}$$

	Laser Excitation		
ν	457	514	785
4000	559	649	1149
1600	493	562	900
100	459	518	794
Δλ	100	121	355
Lines	2400	2400 or 1200	1200