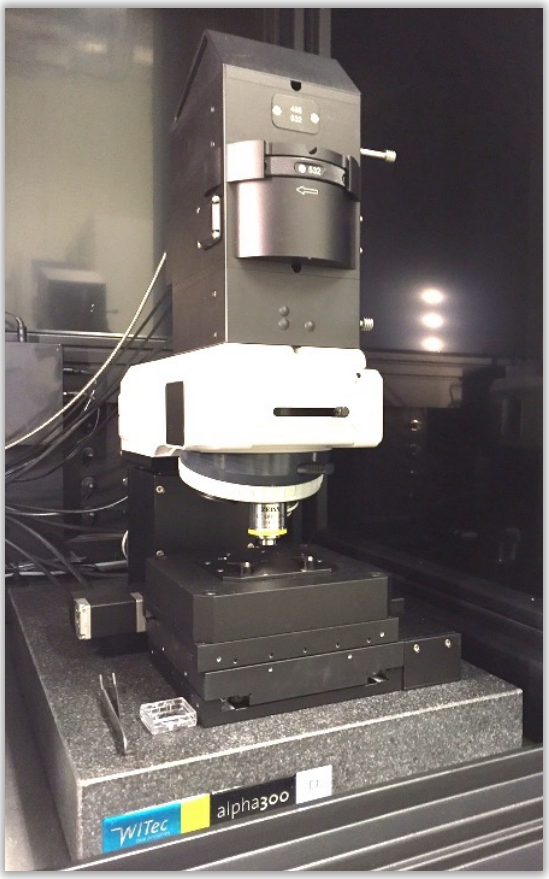
User’s manual

WITec alpha300 R



2023-04-13

This manual is intended for all users of the WITec alpha300 R, particularly new users learning how to operate the instrument. The manual consists of seven short sections that will take you through the basic steps you need to know in order to safely operate the instrument:

1. General instructions
2. Start the session
3. Check the alignment
4. Measurements
5. Data analysis
6. Some tips and tricks
7. End the session

Main responsible person for the instrument and research engineer at CMAL

- Katarina Logg, Email: katarina.logg@chalmers.se; Phone: 031 772 3280

WITec alpha300 R User's Manual

1. General instructions

- Don't hesitate to ask the CMAL staff for assistance if needed. We are here to help you!
- You must report any encountered problem/error with the instrument to the CMAL staff.
- All samples must be removed from the instrument/desk and taken care of by the user when finished.
- Never insert a USB drive into the instrument computer! For instructions on how to get your data, see section 7.

2. Start the session

2.1. Start the computer.

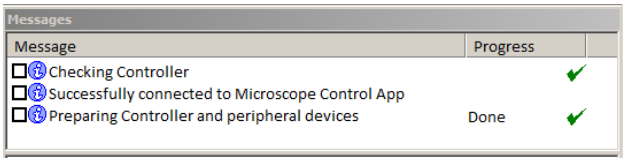
2.2. Turn on the multiple socket on the table.

2.3. Wait for a minute.

2.4. Open the WITec Service monitor. It is found on the lower right on the screen . Check that you have no error message. If the SpectralCamera1 is Not Available, click on the Initialize All Cameras button.

2.5. Click the the Control FIVE 5.3. icon on the desktop to start the software.

2.6. Check that there is no error message in the Messages window. This is how it should look like if there were no errors with the startup:



In case of any error message:

2.6.1 First try to shut off the software, wait for some minute and start it again. If there is no error message after this restart, continue to 2.7.

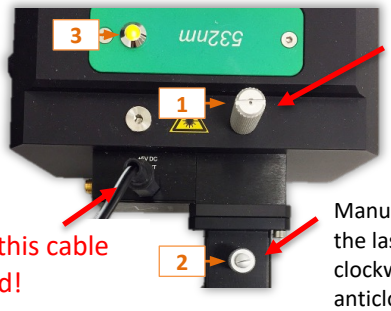
2.6.2. If 2.6.1. did not work, turn off the software, then the computer and the multiple socket on the table. Restart from 2.1. If there is no error message after this restart, continue to 2.7.

2.6.3. If 2.6.2. did not work, turn off software, then the computer and the multiple socket in the table. Then turn off the multiple socket on the floor, with the text "FOR CONTROLLER BOX. DO NOT TURN OFF". Wait some minute, then turn on the multiple socket on the floor again. Then restart from 2.1. If there is no error message after this restart, continue to 2.7.

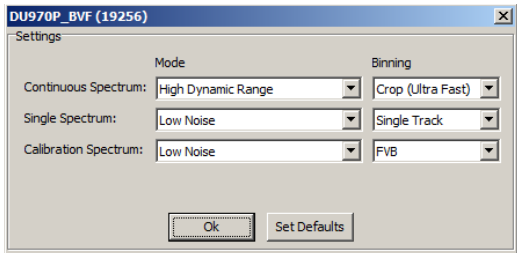
2.6.4. If 2.6.3. did not help, contact CMAL staff.

2.7. Start the EasyLink controller by pressing the on button on the controller.

2.8. Start the laser that you want to use. First make sure that the manual shutter, **1**, is blocking the beam and that the knob that regulates the laser intensity, **2**, is at its minimum (it should not be possible to turn clockwise.) Then, start the laser by turning the key on the back of the laser. The laser is on when the key is parallel to the table and the indicator lamp, **3**, is lit.



2.9. Check the setting of the detector. Go to Configurations/User Mode and make sure that you are in the Expert mode. Go to SpecCamera1 in the Control window and select Options. Make sure that the Continous Spectrum is set to High Dynamic Range and that Single Spectrum is at Low noice. These settings should be the default and is used for the calibration check. For large area scans with a good signal to noice the select Electron Multiplying. More information about the options is found in the help (press F1 key when the window DU970P_BVF(19256) is active.)

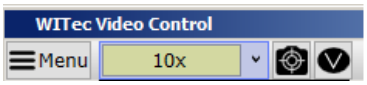


2.10. Go to Configurations/User Mode again and make sure that you are in the Beginner mode. (If you are not an Expert user.)

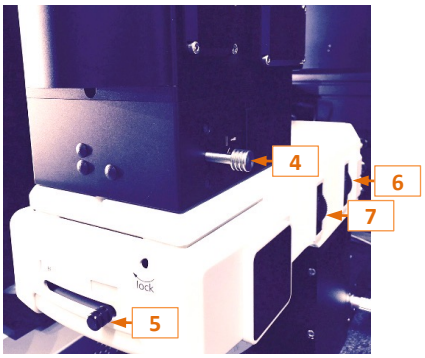
3. Check the alignment

3.1. To check the alignment use the Si sample next to the instrument. Turn the objective turret so that you have an empty slot above the sample position before putting the Si sample on the XY table.

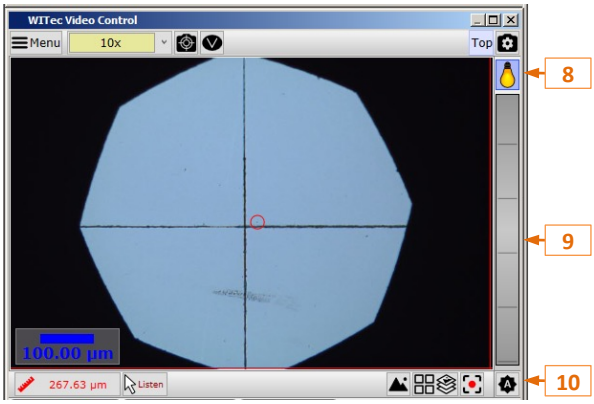
3.2. Turn the objective turret to use the 10X objective (the one with the yellow ring). Center the Si sample roughly under objective. Make sure that the 10X objective is selected in the Video Control window. If you need to change to the 10X objective, select 10X in the dropdown menu and then click the Finish button in the pop up window Turn Objective Turret.



3.3. Enable bright field mode by gently pulling the pin on the side of the microscope, **4**, to its the outer position and move the switch on in the front of the microscope, **5**, to the BF position. Minimize the size of the two diaphragms, **6** and **7**, on the right side of the instrument. The optical design for bright field illumination is according to Köhler. The aperture diaphragm, **6**, controls the numerical aperture and hence the contrast in the sample. Closing the diaphragm will result in higher contrast. The field diaphragm, **7**, controls the width of the illuminated area in the sample and is placed in the conjugate plane of the image plane. That means that you will see a sharp image of the diaphragm when in focus. Closing the diaphragm makes it easier to find the focus by observing the appearance of the hexagon shape while focusing.



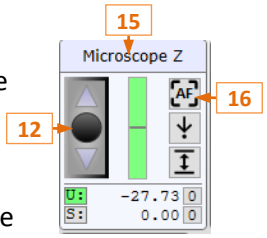
3.4. Check that the bright field lamp is on. The lamp symbol, **8**, in the upper right corner in the Video Control window should be lit yellow. If not click on the lamp symbol. Adjust the intensity with the slider, **9**, until you see some light in the image or use the auto brightness option, **10**. You can also adjust the brightness by pressing the up and down side of the cross key on the Easy Link controller or use the Y button for auto brightness.



3.5. Focus on the sample. When in focus the field diaphragm should be sharp as in the Video Control window above. NOTE: Always start by moving the objective upwards in order not to risk damaging the objective and your sample.

There are three options for how to focus.

1) Use the Focus Up/Down stick on the EasyLink controller, **11**. Push the stick sideways upwards moves the objective away from the sample, pushing it sideways downwards moves the objective closer.
To change the step size, push the stick down (not sideways). In this way you can choose between a step size of 2% or 8%. To change to any step size between 1 and 100 % right click and hold the stick button under Sample Position, **12**, in the Video Control window using the mouse and and drag the mouse to the left or right. To move continuously hold the lower right side Continues movement button, **13**, while pushing the stick sideways. To go really fast, hold the Turbo button, **14**, instead of the Continuous movement button. NOTE: If you have samples that are not flat, do not use the the Turbo button using the 50X or 100X objective.



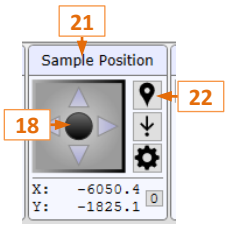
2) Use the Microscope Z buttons, **15**, in the software. Click on the arrows in the direction that you want to move. To move the objective away from the sample click the arrow pointing upwards. To move closer to the sample, click the arrow pointing downward. To change the step size, hold the stick button, **12**, under Sample Position with the right mouse button and drag the mouse to the left or right. To move continuously start at the center of the arrows, hold the left mouse button and move the mouse in the direction you want to move.

3) Use the Video Auto Focus button, **16**, in the software. NOTE: do not use this unless you have a good contrast in your sample and being reasonable close to focus.

3.6. Move the sample in X and Y so that you see the cross in the Si sample clearly, as in the Video Control window on the former page.

There are three ways how move the sample in the X and Y direction

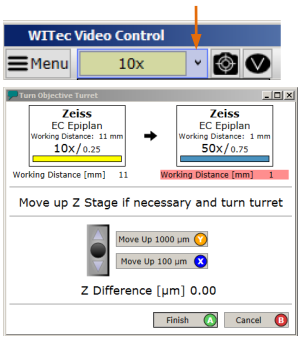
1) Use the Sample Positioning stick on the EasyLink controller, **17**. Push the stick sideways in the direction you want to move. To change the step size, push the stick downwards (not sideways). In this way you can choose between a step size of 2% or 8%. To change to any step size between 1 and 100 % right click and hold the stick button under Sample Position, **18**, and drag the mouse to the left or right. To move continuously hold the lower right side Continues movement button, **19**, while pushing the stick sideways. To go really fast, hold the Turbo button, **20**, instead of the Continuous movement button. NOTE: If you have samples that are not flat, do not use the the Turbo button using the 50X or 100X objective.



2) Use the Sample Position buttons, **21**, in the software. Click on the arrows in the direction that you want to move. To change the step size, hold the stick button, **18**, under Sample Position and drag the mouse to the left or right. To move continuously start at the center of the arrows, hold the left mouse button and move the mouse in the direction you want to move.

3) Use the Move Sample to Mouse Position option. Activate this option by clicking the Move Sample to Mouse Position button, **22**. The cross that appears when you drag the mouse over the video image will now be orange instead of green. To move, click on the position of your sample in the Video Control window that you want to move to.

3.7. Switch to the 50X objective.



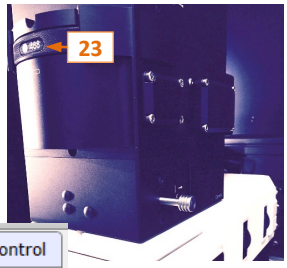
3.7.1. First click on the drop down menu, in the Video Control window to select the 50X objective. The Turn Objective Turret window will appear. If you have a sample that is not flat move up the objective using either the Move Up 100 um or Move up 100 um option. Click the Finish button.

3.7.2. Go up a bit in Z, just to be sure you are above the focus with the objective. Turn the objective turret to use the 50X objective. Focus.

3.8. Repeat the procedure form 3.7. to change from the 50X objective to the 100X objective. NOTE: The 100X objective has a working distance of 0.31 mm. When finding the focus you must start with moving the objective up from the sample otherwise you risk damaging the objective (and your sample). Never use the turbo option using the 100X objective.

3.9. Find the cross in the Si sample to verify that you are in focus and move slightly in the XY plane in order not to measure on the cross (the red circle indicates where you measure).

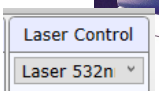
3.10. Switch from bright field mode to Raman mode. Gently push the pin, **4**, on the side of the microscope and move the switch in the front of the microscope, **5**, to the Raman position.



3.11. Make sure that the wavelength selection turret on the microscope, **23**, matches the laser that you want to use. Use the filled circle position (● 488 or ● 532).

3.12. Close the door, otherwise the mechanical shutters of the lasers won't open.

3.13. Make sure that you have the correct wavelength in the Video Control window.



3.14. Open the Spectrograph 1 section in the Control window and select the 600 g/mm grating.

3.15. In the Spectrograph 1 part of the Control window make sure that the Spectral Center is about 2300 for the 488 nm laser and 1950 for the 532 nm laser.

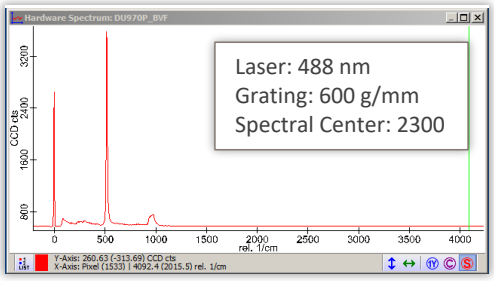
3.16. Open the Oscilloscope section in the Control window and make sure the Integration Time is 0.05 s. Start the live spectra viewing by clicking the Start Oscilloscope button in the Control window.

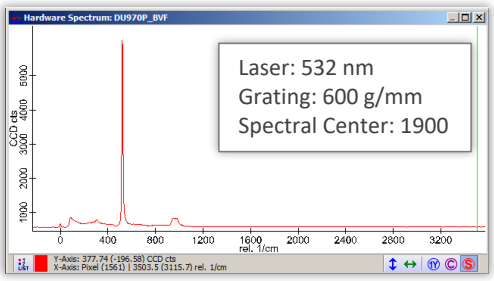
3.17. Open the manual shutter of the laser, **1**. Increase the intensity by turning the intensity knob, **2**, anticlockwise. Observe the live spectra in the Hardware Spectrum window. Turn the intensity knob until you see that the Si peaks do not increase anymore. The spectrum should appear as in the corresponding spectrum below. Use the arrows at the bottom of the Hardware Spectrum window ↕ ↔ to automatically set the axes.

3.18. Check the alignment by measuring the counts of the second order peak.

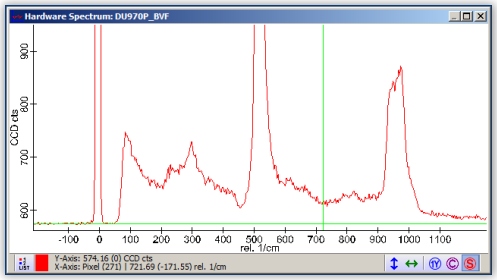
- Zoom in so that the baseline (negative cm^{-1}) and the second order peak are well seen in the Hardware Spectrum window (the live spectra window). To zoom hold the Control button on the keyboard and drag with the mouse over the area that you want to see.

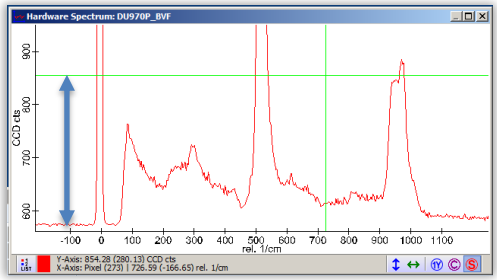
- <u>Do not forget to optimize the focus by observing the live signal while focusing.</u>





- Place the green cursor with the vertical line on the baseline level (see left spectrum below) and click with the left mouse button. Check that the relative Y-Axis value became 0 (the value within parenthesis). Place the cursor so that the vertical line goes through the top values of the second order peak (see right spectrum below). The relative counts should be about 140-150 counts or more for the 600 g/mm grating and about 35 or more for the 1800 g/mm grating.





3.19. Note what laser wavelength that you have used and the relative intensity that you measured in the log book.

3.20. Measure the 1st order peak position and note also that in the log book.

3.21. Stop the oscilloscope.

3.22. Decrease the laser intensity to its minimum and block the laser with the manual shutter.

3.23. Remove the Si sample. To do this first move the objective up, away from the sample, using the hand held controller. Turn the objective wheel so that you have an empty slot above the sample. Remove the Si sample and put it back in the box next to the instrument.

4. Measurements

4.1. Insert the name of the sample/measurement

4.1.1. Go to Sample Name in the Control window and insert the name of your sample/measurement.

4.2. Find focus, adjust the laser intensity and acquire a bright field image

4.2.1. Mount your sample on the XY table. Find the focus as described above by first using the 10X objective and then the objective that you want to use in bright field mode. NOTE: always move away from the sample first when focusing.

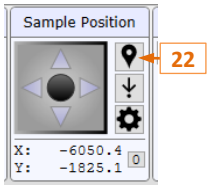
4.2.2. The 600 g/mm will cover a spectrum of up to about 4300 cm^{-1} for the 488 nm laser line and 3700 cm^{-1} for the 532 nm laser line. The 1800 g/mm will cover a spectrum of 1400 cm^{-1} for the 488 nm and 1100 cm^{-1} for the 532 nm laser line, however with better resolution than with the 600 g/mm grating. If you want to change grating to the 1800 g/mm grating, open the Spectrograph 1 section in the Control window and select the 1800 g/mm grating. Make sure that the Spectral Center is about 600 for the 488 nm laser and 550 for the 532 nm laser.

4.2.3. Find a position in the sample that you want to analyze and move slightly away to find the focus using the Raman signal as a measure. To get a live view of the acquired spectra, set the microscope to Raman mode and go to Oscilloscope. Set the integration time that you want to use. 0.5 s is a good starting time. Start the oscilloscope and adjust the focus my maximizing the counts in the live spectrum.

4.2.4. Move slightly again and adjust the intensity of the laser to achieve the signal to noise level that you find reasonable. Try to minimize the laser intensity as it might harm your sample as well as the objective. If you think the intensity is too high, increase the integration time if possible. When finished, close the manual laser shutter and stop the oscilloscope.

4.2.5. Move back to the position that you are interested in. Acquire a bright field image by clicking the icon  below the image in the Video Control window. You can also press the B button on the EasyLink controller. The acquired image will appear in the Project Manager window. To open the acquired bright field image, double click on the image name in the Project Manager window. This image can now be used to select positions or areas for Raman measurements using something called "Listening" as will explained below. It is possible to acquire larger images that the field of view (the manual will be updated with instructions how to soon).

4.3. Single spectrum measurements



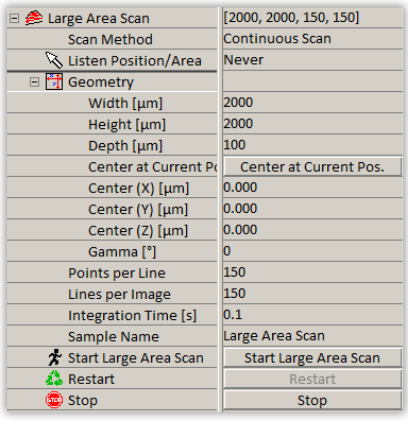
4.3.1. To set a position for a Raman measurement activate "listen position" by clicking on the Move Sample to Mouse Position button, **22**, in the Video Control window. If listening is on then the cross in the video image is red, if not it is green.

4.3.2. Click in the acquired image on the position where you want measure. With the listening activated the XY stage will automatically move to measure at the selected position.

4.3.3. Open the Single Spectrum part of the Control panel and select the integration time and number of accumulations that you want to use. Accumulations are recommended if you have one strong peak but are interested in the small peaks.

4.3.4. Open the manual shutter on the laser and start the measurement by clicking Acc. Single Spectrum in the right panel of the Control window. The acquired spectrum (and the acquisition settings) will appear in the Project Manager when the measurement is finished. NOTE: When the measurement is finished, remember to close the manual shutter of the laser in order not to risk damaging your sample.

4.4 Large area scan



4.4.1. Open the acquired bright field (4.2.5) image by double clicking the image name in the Project Manager window.

4.4.2. Open the Large Area Scan part and then the Geometry part in the Control window.

4.4.3. Select Scan Method. Area is recommended for XY scans. For an area scan in the Z direction select Depth Scan.

4.4.4. Select Listening Position/Area. For XY scans: Area(Once) or Area(Multiple) and for depth scans: Depth by Line (Once) or Depth by Line (Multiple) and draw an area or line in the acquired bright field image. Check that the input data in the Geometry have been automatically changed according to your selection. If you want to, adjust the values manually. If you are acquiring a depth scan enter the height of your measurement.
NOTE: If you are acquiring a depth scan make sure not to go too far deep, risking the objective to touch the sample. The working distances of the objectives are: 10X: 11 mm, 50X: 1.1 mm, 100X: 0.31 mm.

4.4.5. Enter the number of Points per Line, and Lines per Image that you want to have. The best achievable resolution is approximatively 300 nm in XY and 900 nm in Z.

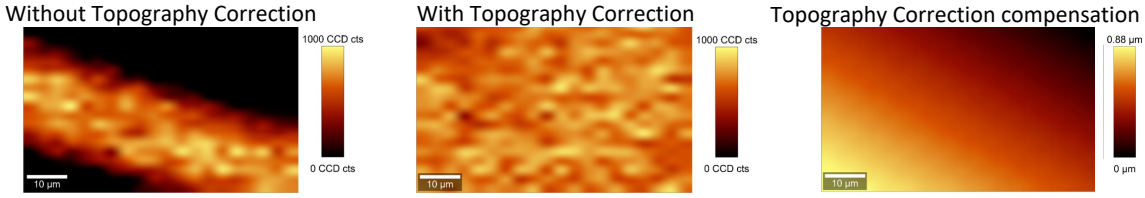
4.4.6. Choose Integration Time. When acquiring area scans the signal to noise can be allowed to be rather poor. The large number of spectra that will be collected will produce statistics that will help to distinguish between different species within the sample. Hence, the integration time could possibly be lower than what you selected in 4.2.3. and 4.2.4.

4.4.7. Open the manual shutter of the laser and click the Start Large Area Scan button. In the Message window you can see the progress of your measurement. NOTE: When the measurement is finished, remember to close the manual shutter of the laser in order not to risk damaging your sample.

4.5. Topography correction for flat samples during Large area scan

NOTE: For Expert users only.

When you scan large areas and your sample is flat but tilted in respect to the image plane, the sample will likely not be in focus over the whole measurement area, leading to variations in the Raman signal intensity. The autofocus feature of the software allows you select three points of the desired scanning area and then the software adjust the image plane to those points. Obviously, this correction works just for samples that consist of a flat plane which is lying at a tilted angle under the microscope, e.g. a Si wafer. Here is an example of the benefit of Topography Correction activated for a sample that was slightly tilted:



4.5.1. Go to Configurations/User Mode and select the Expert mode.

4.5.2. Acquire a bright field image.

4.5.3. Adjust the microscope for Raman measurements and make sure you have optimized your settings for the measurement.

4.5.4. Activate Large Area Scan/Geometry/Listen position/Area/Area(Multiple) and define the area that you want to measure over.

4.5.5. In the Large Area Scan menu, define the number of Points per Line and Lines per Image.

4.5.6. In the Large Area Scan menu, change Topography Correction to On.

4.5.7. Under menu Topography Correction/Manual Learning choose Learn Plane (3 pts). The oscilloscope will now start and the microscope will move to the the first of the defined image plane points. Inspect the Raman signal while optimizing the focus. When the optimal Z position is found, click the Next Step button in the True Surface menu. Repeat this for the other two positions.

4.5.8. Start the measurement by clicking the Large Area Scan button in the Control window. In the Status window below the Graphic Control window you can follow the automatic adjustment of the Z position during the measurement. The software will provide a map with the Z positions in the Project Manager.

4.5.9. When you are finished with your measurement make sure to change Topography Correction to Off.

4.6. Signal stabilization to compensate for focus drifts

NOTE: For Expert users only.
The purpose of the signal stabilization is the compensate for focus drifts over long term (several hours) measurements, e.g. due to fluctuations of the ambient temperature.

The reference point should be flat (a slight drift in XY will affect focus) and also stable for selected power.

4.6.1. Go to Configurations/User Mode and select the Expert mode.

4.6.2. Take a bright field image.

4.6.3. Adjust the microscope for Raman measurements and make sure you have optimized your settings for the measurement.

4.6.4. In the Large Area Scan menu, define the area that you want to measure over (Listen position/Area(Multiple)).

4.6.5. In the Large Area Scan menu, define the number of Points per Line and Lines per Image.

4.6.6. Open the Signal Stabilization part of the the Large Area Scan menu and select Yes for Stabilization Enable.

4.6.7. At Stabilization Mode, select the mode that you prefer. Peak are better for sample giving a signal at the surface only (e.g. silicon), Positive Edge for samples giving a signal also under the surface (e.g. adhesive strip).

4.6.8. Choose Actuator for Compensation. Scan Table is recommended.

4.6.9. Leave the Step Size Multiplier at 1.

4.6.10. For Number of Accumulations 3 is a good value.

4.6.11. Use Listen Stabilization Position to define a reference position.

4.6.12. Set Listen Mask to Multiple. In the oscilloscope Hardware spectrum window, select the spectral range that will be used for the stabilization (red).

4.6.13. Click the Start Stabilization button to check the performance and success in the Messages window. If the stabilization failed, focus on maximum spectral intensity for peak mode or on half of maximum for edge mode using the Oscilloscope. Repeat clicking on the Start stabilization several times until the stabilization height is nearly zero.

4.6.14. Switch to software controlled Z positioning by clicking S: in the Microscope Z area of the Video Control window. NOTE: You must switch back to U: when finished for manual z control.

4.6.15. Adjust the focus for your measurement using one of the following options:

- Optimize the within your measurement area and change the Center(Z) value to the current Software-z value.
- Optimize the focus in the center of the measurement area and click in the Center at Current pos.
- If you are using Topography Correction as described above you do not need to do something additionally.

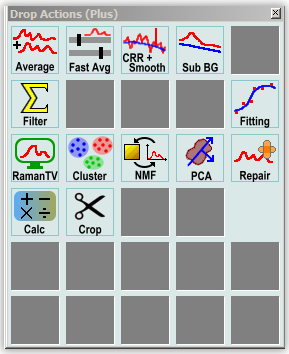
4.6.16. Click the Start Large Area Scan or Start Image Scan to to start the measurement.

4.6.17. You can observe the progress of the Signal Stabilization in the Messages box. If it fails at one time it will try again at each following stabilization step. (Pointing with the mouse at the message gets you a hint, why it did not work.)

4.6.18. NOTE: When finished, remember to:

- Switch back to U: when the measurement is done for manual z control
- Switch back Listen Mask to Never

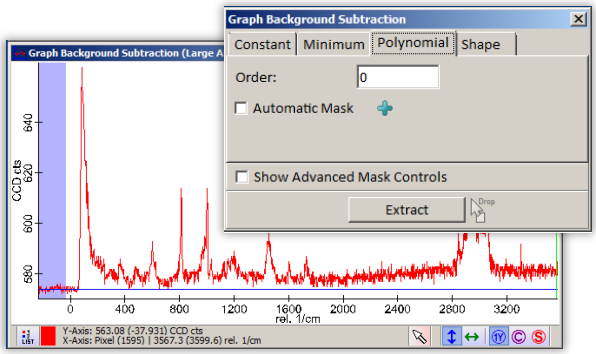
5. Data analysis

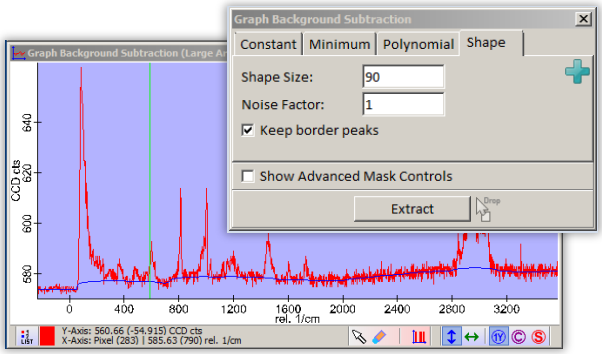


5.1 Background subtraction and cosmic ray removal

5.1.1. To do a background subtraction, click, hold and drag the dataset in the Project Manager window in any direction. The Drop Actions window will appear. Drag the data to the Sub BG icon. This action will generate two new windows, one for settings, named Graph Background Subtraction and one showing the spectra.

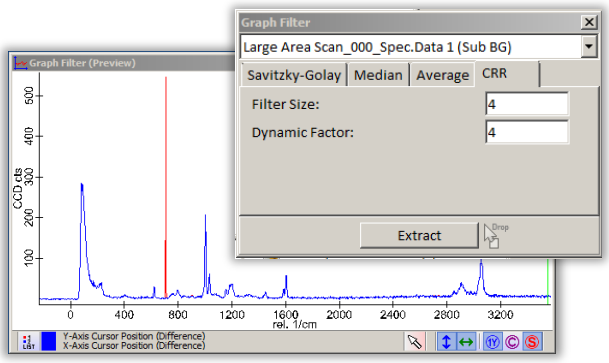
5.1.2. It is recommended to use either the Polynomial or Shape option in the Graph Background Subtraction. The purple area in the graph indicates the part of the spectra that is used in the estimation of the background. To remove parts of the spectra that you do not think should be included, hold the Shift key on the keyboard and drag with the mouse over the area while holding the left mouse button. If using the Polynomial use the 0 order and use only the negative part of the spectra to find the background level. If you have more than one spectra in your data use the up and down keys on the keyboard to scroll through the whole data set to to make sure that the background subtraction looks reasonable. When satisfied with the background click the Extract button in the Graph Background Subtraction window. The corrected data will appear in the Project Manager window with the name extension (Sub BG).





5.1.3 To do a cosmic ray removal, drag the background subtracted data in the Project Manager to the CRR+Smooth icon in the Drop Actions window. As for the background subtraction this action will generate two new windows. One for selecting cosmic ray removal parameters called Graph Filter and one spectra preview window.

5.1.4. In the CRR tab in the Graph Filter window select the Filter Size and Dynamic Factor that seem to work with your data. Filter Size: 4 and Dynamic factor 4-20 is a good starting point. If you have more than one spectra in your data make sure to scroll through the whole data set to see if the result looks reasonable. When satisfied click the Extract button in the Graph Background Subtraction window. The corrected data will appear in the Project Manager window with the name extension (Sub BG) (CRR).

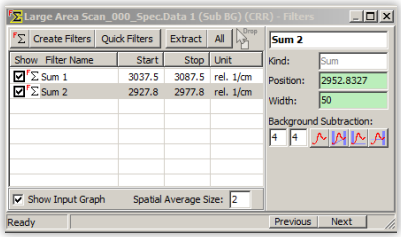


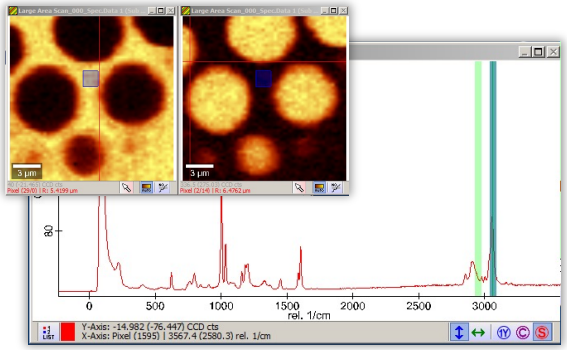
5.1.5. To do a background subtraction, click, hold and drag the dataset in the Project Manager window in any direction. The Drop Actions window will appear. Drag the data to the Sub BG icon. This action will generate two new windows, one for settings, named Graph Background Subtraction and one showing the spectra.

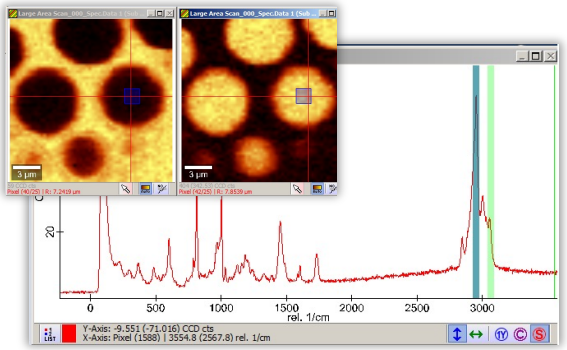
5.2 Image generation by filtering

5.2.1. For manual image generation drag the corrected data to the Filter icon in the Drop Actions window. This action will generate two new windows. One for setting the filter parameters and one showing the spectra, both called the name of the data with the extension –Filters.

5.2.2. Click on the sum symbol button  in the filter settings window to create a filter. This action will generate a colored stripe in the spectra window and a third window with the generated image. You can either move the filter by dragging the colored stripe over the spectra use the inputs Position and Width in the settings window. The image will be continuously updated with the sum for the corresponding spectra window over the whole area. You can add more filters by clicking the sum symbol. Each filter will generate a new image.







5.2.3. When you are satisfied with your filters mark the filter, not with the tick box, but by clicking on the file names so that they get blue and then click the Extract button. This will generate two images in the Project Manager.

5.2.4. To combine the images, mark the generated images in the Project Manger and drag them to the Combine icon in the Drop Actions window.

5.3 Image generation by True Component Analysis

5.2.1. Click, hold and drag the sub background and cosmic rays removed data in the Project Manager window in any direction. The Drop Actions window will appear. Drag the data to the Analyze icon. This action will generate three windows, one settings window, called True Component Analysis, and one called Fit Preview.

5.3.8. In the Fit preview window showing, remove the parts of the spectra that you don't want to include in the basis analysis. Hold the Shift key and mark the spectral parts in the mask preview window that you want to leave out. The Rayleigh area should be removed as well as parts where there are no peaks.

5.3.9. Click the 1 Auto button. A second spectra window appeared showing a spectra of the new component. In the Fit preview window you can compare the original spectrum in red and the component in blue.

5.3.10. If the residual image shows areas with high intensity click the 1 Auto button again and continue to do so until the residual image has a more or less even intensity distribution.

5.3.11. If you want to do a combined image of the different components, click the Extract & Next Step button, else the Extract button.

5.4 Peak analysis, Fitting

5.4.1. Do a cosmic ray removal and background subtraction of your data (see 5.1.).

5.4.2. Drag the corrected data ((CCR)(SUB BG) data) to the Fitting icon in the Drop Actions window.

5.4.3. In the Fit Function tab in the Advanced Fitting Tool pop up window

- Tick the Advanced Mode option in the top right corner.
- Under Category: select Peak Functions.
- Under Function: select Lorenz.
- Under Options:, for Number of Functions, select how many peaks you want to fit.

5.4.4. In the Fit Mask tab in the Advanced Fitting Tool pop up window deselect the parts of the spectra that should not be included by holding the Shift key drag the mouse over the areas that should not be included while you are pressing the left mouse button.

5.4.5. In the Fit Parameters tab in the Advanced Fitting Tool pop up window

- Select Define Start Values.
- For the background level, y0, tick the Auto option.
- For the Raman shift, x0, double click in the window of the x0 number in the Value column. The window gets red. Use the mouse to select the Raman shift of the peak in the spectra
- For the FWHM of the peak, w, double click in the window of the w number in the Value column. The window gets red. Define the FWHM by holding the left mouse button down while dragging over the peak at half maximum.

- For the area of the peak, A, double click in the window of the A number in the Value column. Hold the mouse button down, while dragfing the mouse from the bottom to the top of the peak.
- Click the Fit Once button.
- Click the << button to transfer start values to the Start Value column.
- Select the Fit on Change option.
- Make sure the Vary options are ticked for all settings.
- Click the Fit and Extract All button.
- Close the Advanced Fitting Tool window.

5.4.6. In the Project Manager you now should have

- Images for each of the four measured parameters: the peak position (x0) the FWHM of the peak (w) and area of the peak (A).
- An image called Fit Fail Mask. Check this image to see if there was ant part of the image where there fit failed.
- A file with the fitted peaks.

5.4.7. Examples of analysis of fitted peaks.

- Histogram: drag for example x0 data to Stat in the Drop Action window.
- Scatter plot: Mark for example the x0 and w and drag them together to the Correlate option in the DropAction window. To change min and max values of the plot, click on the LIST button and the left bottom corner of the plot window.
- Calculatations: Mark for example the x0 and w drag to the Calc icon in the Drop Action window. In the middle lower window type the calculation that you want to do.

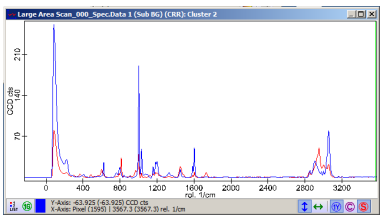
TIP: To get color scale for the images, right click on the image, hold the right mouse button down and select the floppy disc (Export). Click the Preview to see the scalebar and select the floppy disc icon in the upper left of the image of the scalebar to save it.

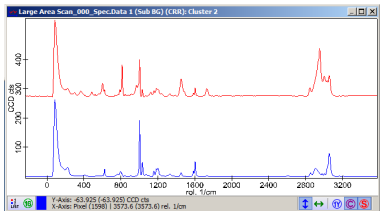
6. Some tips and tricks

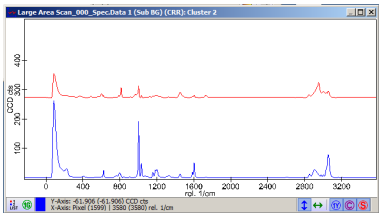
- The detector saturated at 60 000 counts.
- To learn more about a function click on the window of the function and press the F1 key on the keyboard. For the general manual go to Help/WITec Control Help in the main menu.

- Click either of the EasyLink control help button, **25**, to open the EasyLink Controller Help.

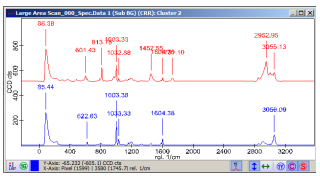
- You can compare spectra by dragging the data you want to add from the Project Manager to the plot window of the other spectra. If the 1Y button in the lower right corner it not activated the two spectra will be normalized to the highest intensity of two spectra. Activating the C button the spectra will be cascaded, meaning that one of them will have an offset in intesity as seen below.





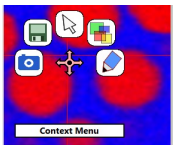


- To get peak positions, activate the window of the data and click the F button on the keyboard.

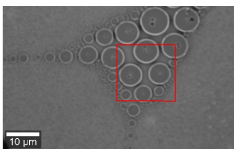


- To scroll through a dataset of several spectra, activate the window of the raw data and use the up and down arrow keys on the keyboard

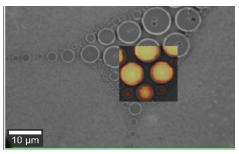
- To modify your image click and hold the right mouse button on the image. The Context Menu will appear.



- If you want see where in the bright field image you did your Raman measurement drag the Raman file from the Control Manager to the open bright field image and select Show Position. This can done both for acquired single spectra or area scans data.



- You can do and overlay of your Raman image with the bright field image. To do so, mark the two images in the Project Manager window and drag them to the Overlay Transform icon in the Drop Actions window.



- To extract one spectra from a stack: Go to the spectra you want to extract, right click and select the floppy disk icon. Select Extract.

7. End the session

7.1. If you have been using the 1800 g/mm grating, switch back to the 600 g/mm grating and then change the Spectral Center to 1950.

7.2. Save your data in your personal folder. Go to File/Save Project As… and save the files in your personal folder. To save data as Matlab or Ascii mark the data, right click and select Export. Select the format you wish to have. It is recommended to use the Use Data Object Name option.

7.3. Move the objective somewhat up and turn the objective turret so that there is no objective over the sample. Remove the sample. Make sure to leave the tweezers in the cabinet.

7.4. Switch to the 10X objective in the the Video Control window.

7.5. Move the XY table back to a central position (aligned with the base).

7.6. Shut down the software by selecting File/Exit.

7.7. Leave the microscope in bright field mode by gently pulling the pin on the side of the microscope, **4**, to its the outer position and move the switch on in the front of the microscope, **5**, to the BF position.

7.8. Turn the smaller knob on the laser, **2**, clockwise until it stops. Make sure the manual shutter, **1**, is shut. Turn the laser off with the key on the back side of the laser.

7.9. Fill in the instrument logbook.

7.10. Turn off the multiple power socket on the table. NOTE: You are not allowed to turn off the multiple power sockets on the floor.

7.11. To transfer you data to a USB drive, copy your data from your folder to the folder:
Desktop/Raman microscope folder on Mac transfer.
You can now transfer you data from the folder:
Desktop/Raman microscope
to an USB drive on the Mac transfer computer. Remove your data from the transfer folder.
NOTE: NEVER dock a USB memory stick on the instrument computer.
NOTE: it is OK to save the data on the instrument computer until you have safely transferred your data to your own computer. Preferably, erase your data from the previous session when you are using the instrument the next time. Data stored on the instrument computer for more than 6 months will be erased.

7.12. Shut down the computer.

7.13. Make sure to clean after yourself and bring all your samples with you.

REMEMBER: always report to the CMAL staff if something is wrong with the instrument or software.

Always clean after yourself!

All samples must be removed from the instrument and desks.

All table areas should be kept clean.