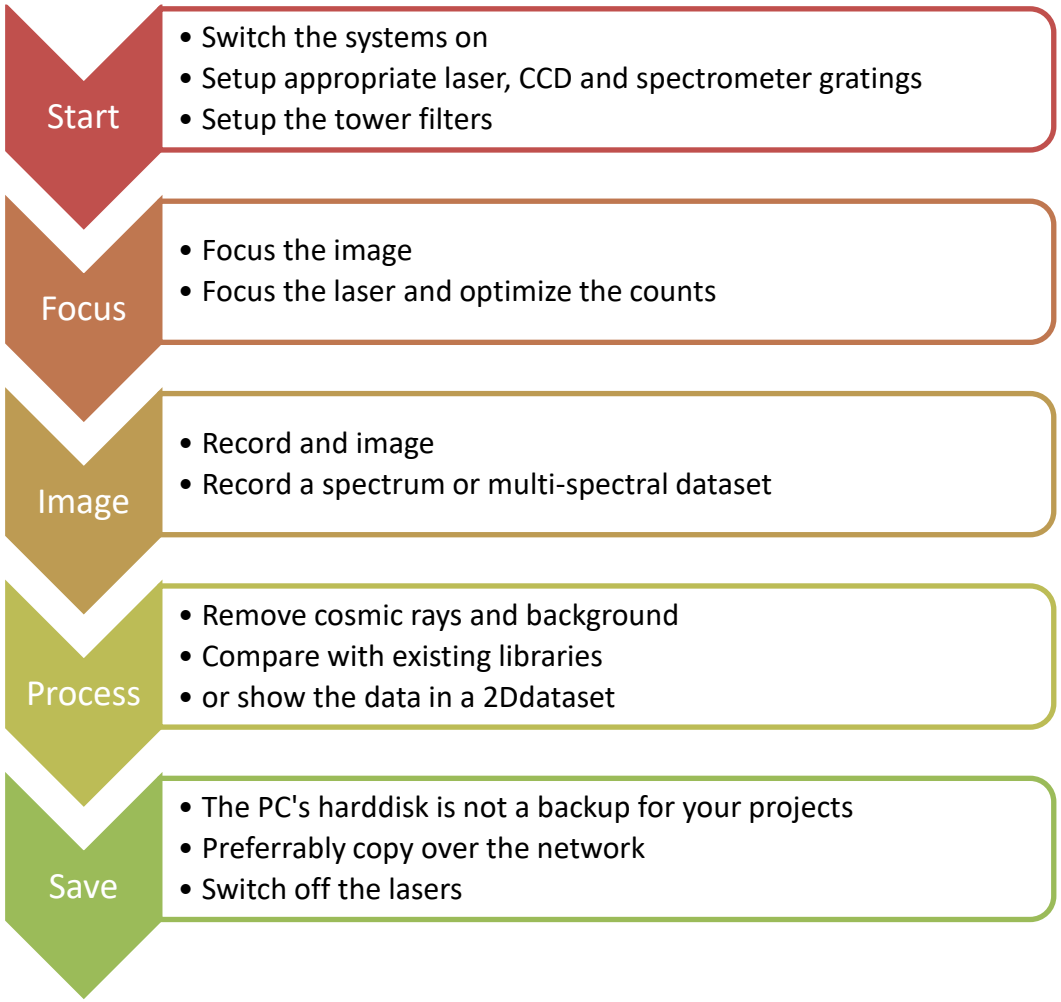
Quick summary



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Witec Raman Alpha 300 R

Introduction

Version 4 – February 2026

Theory, Hardware, Acquisition and Analysis

Universal rules

Rule 1: don't touch a control if you are not sure of the outcome of that action

Rule 2: never, ever force anything beyond finger strength

Rule 3: if in doubt ask for help

Demonstration: Switching the system off

Prerequisites:

Data recorded and analyzed

Stop the system

This is the order that need to be performed:

1. Save or export your data
2. Make sure the light is switched off in the system
3. Switch the turret to position 6 (empty)
4. Remove your sample and clean up
5. Place the light protector on the stage
6. Close the software, do not logout
7. Cover the microscope with the plastic cover
8. Turn the key(s) to switch off the laser(s)

Peak Width Filter (FWHM)

Starting from the F(pos of max) the algorithm searches to the right and
pixels of these two positions are used in a linear model to calculate th

Minimum Filter

$$F_{min} = min\{S_i : n_l \leq i \leq n_r\}$$

Maximum Filter

$$F_{max} = max\{S_i : n_l \leq i \leq n_r\}$$

Position of Minimum Filter

$$F_{pos\ of\ min} = first\{x_i : S_i = F_{min}, n_l \leq i \leq n_r\}$$

Position of Maximum Filter

$$F_{pos\ of\ max} = First\{x_i : S_i = F_{max}, n_l \leq i \leq n_r\}$$

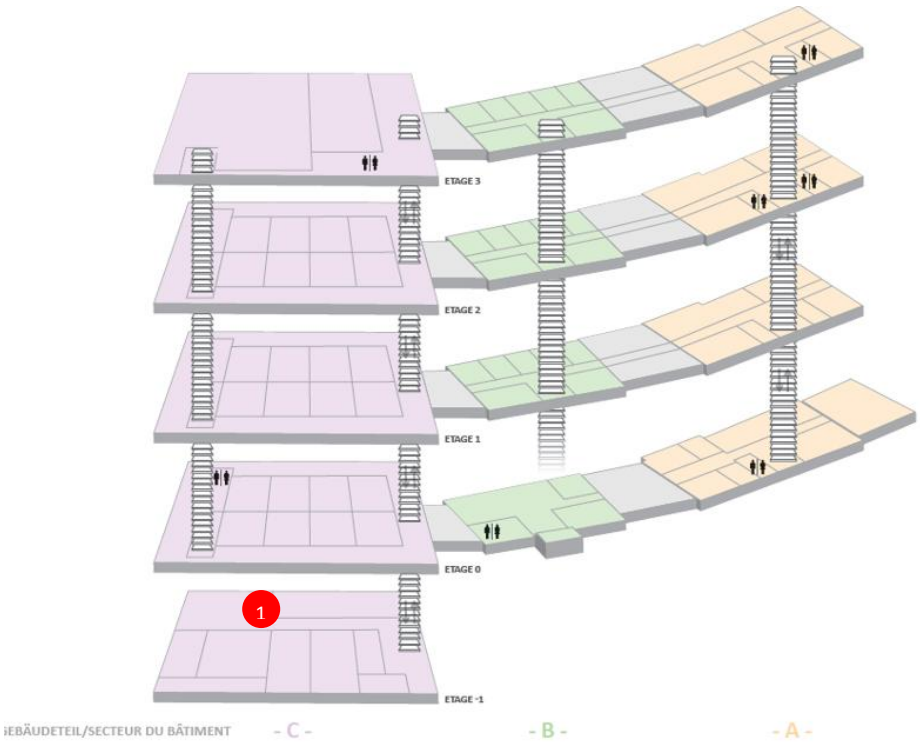
Calculates the maximum of the range. Shows the position of the maximum intensity in the result image. Can also be used e.g. to find a specific cosmic ray that couldn't be removed by the Cosmic Ray Removal tool. For this, calculate the total average of all spectra in a spectral image, then do the maximum filter on the area of the "small cosmic ray peak" that could be visible in this total average spectrum. The result image then could show the position of the cosmic ray.

Starting from the F(pos of max) the algorithm searches to the right and the left direction at which the signal falls below 0.5*F(max). The neighbor pixels of these two positions are used in a linear model to calculate the FWHM.

Locations

The instrument can be found here:

- Witec Raman Alpha 300R (room C0141)



Information: Theory

Prerequisites:

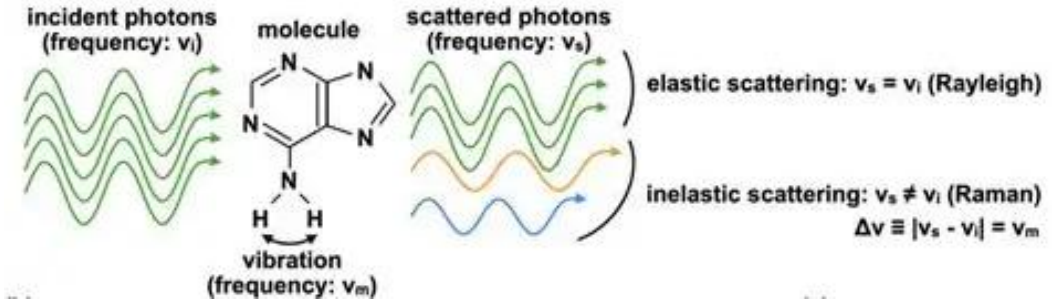
System completely switched off

Understand the signal generation in Raman spectroscopy

1. What is Raman spectroscopy

Raman spectroscopy probes molecular vibrations through inelastic scattering of monochromatic light, typically from a laser, providing a vibrational fingerprint complementary to infrared spectroscopy. This technique reveals information on molecular structure, symmetry, and phase by detecting **shifts** in scattered photon energy corresponding to rovibrational transitions.

Rayleigh scattering is elastic light scattering where the scattered photon's energy equals the incident photon's, while Raman scattering is inelastic, with scattered photons gaining or losing energy corresponding to molecular vibrational or rotational transitions.



Most interactions yield elastic Rayleigh scattering at the incident frequency, but about 1 in 10^7 photons undergo inelastic Raman scattering, producing Stokes (energy loss, lower frequency) or anti-Stokes (energy gain, higher frequency) shifts.

Calculates the overall intensity of the selected range.Is typically used to make structures visible, that are described by a (Raman) peak.Can also be used to suggest the overall number of electrons/photons.

Average

Can be used e.g. to show the background (if the filter is set to a range where there is no raman signal).

Average minus Minimum

The minimum of the selected range is subtracted from the average. Can be used e.g. to do some kind of "background subtraction" if a Raman peak is on a fluorescence offset.

Sum Filter

$$F_{sum} = \sum_{i=n_l}^{n_r} S_i$$

Average Filter

$$F_{ave} = \frac{1}{n_r - n_l + 1} \sum_{i=n_l}^{n_r} S_i$$

Average minus Minimum Filter

$$F_{ave-min} = F_{ave} - F_{min}$$

Standard Deviation Filter

$$F_{\sigma} = \sqrt{\frac{1}{n_r - n_l + 1} \sum_{i=n_l}^{n_r} (S_i - F_{ave})^2}$$

Center of Mass Filter

$$F_{CoM} = \frac{1}{F_{sum}} \sum_{i=n_l}^{n_r} x_i S_i$$

Standard Deviation

Shows the changes within a range. Can be used to determine the amount of noise or to show variations in the spectral range.

Center of Mass (Weighted Position)

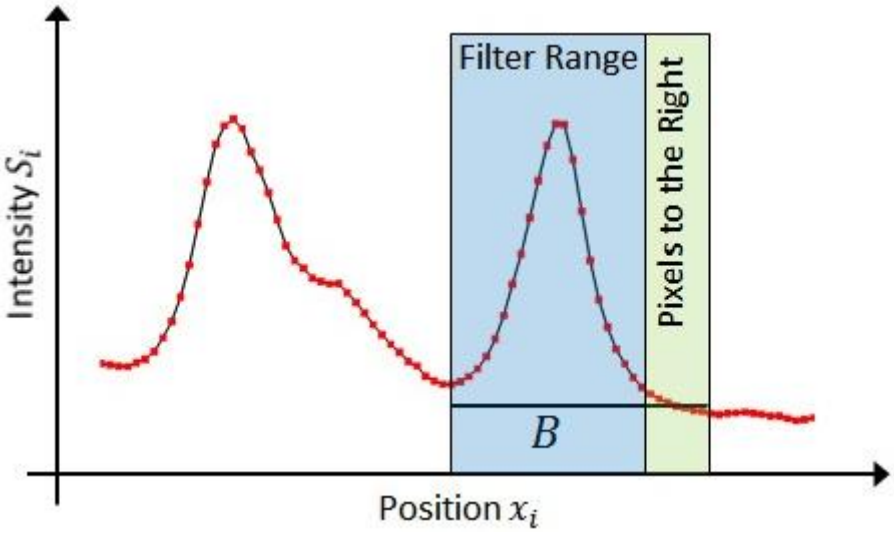
Calculates the peak position (roughly). Use peak fitting tools for more accurate peak position fitting.

Peak Width

Calculates the peak width (roughly). Use peak fitting tools for more accurate peak width fitting.

Maximum

If the number of pixels is zero on one side a horizontal line is used as background estimation. It is calculated by the intensity average of the other side.



If both number of pixels are 0, no background subtraction is performed.

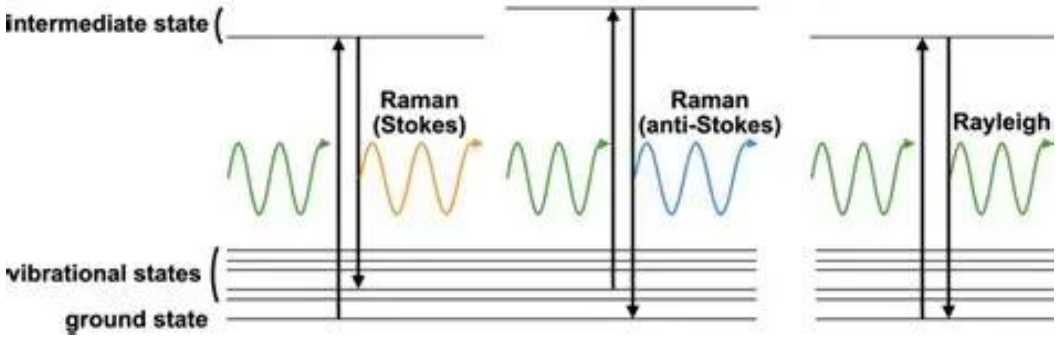
12. Filter calculation

The following notation is used for the filter calculations.

n_l : *leftmost pixel inside the filter range*
n_r : *rightmost pixel inside the filter range*

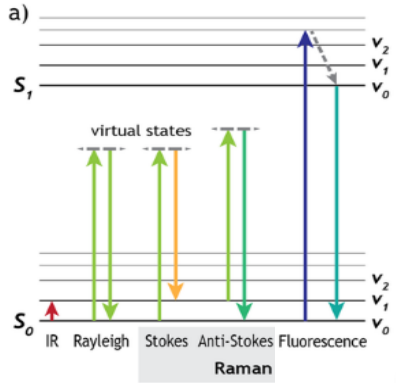
13. Filters

Sum



2. Fundamental Principles

The Raman effect arises when incident photons interact with the electron cloud of a molecule, inducing a dipole moment proportional to the molecular polarizability (α). Unlike fluorescence, which involves real electronic excitations, Raman scattering promotes the molecule to a virtual intermediate state before relaxation to a different vibrational or rotational level, conserving energy overall. These virtual electronic states are energetically very far away from fluorescence states, when electrons in the cloud are excited: Raman vibrational state energies are in the range of 100-4000 cm^{-1} (0.01-0.5 eV), vastly smaller than fluorescence emissions in the visual spectrum (~15,000-25,000 cm^{-1} or 2-3 eV).



- In Rayleigh scattering, the molecule returns to the exact initial vibrational/rotational state $|i\rangle = |f\rangle$, re-emitting a photon at ν_i (also written as ν_0) with no net energy transfer to the molecule.
- In Raman scattering instead the molecule relaxes to a different vibrational state ($\nu_i \pm 1$), producing Stokes ($\nu_0 - \nu_m$, energy loss) or anti-Stokes ($\nu_0 + \nu_m$, energy gain) shifts, where ν_m matches molecular modes.

3. Stokes vs anti-stokes Raman

Stokes shifts dominate in Raman spectroscopy at room temperature because most molecules sit at their lowest vibrational energy level (the "ground state"). In other words: the Boltzmann population favoring the vibrational ground state. This makes it easier for the molecules to lose a bit of energy to that level than gain some.

Simple explanation: Imagine each molecule like a ball on a ladder of energy steps (vibrational levels). At room temperature (~300 K), the Boltzmann distribution acts like gravity: far more balls (~95-99% for typical modes) rest at the bottom step (v=0, ground state) than higher ones (v=1,2,...), since higher steps need extra thermal energy to reach. When a laser hits, Raman scattering can drop the ball down (Stokes: molecule loses energy, scattered light shifts red by about 100 to 4000 cm^{-1}) or kick it up (anti-Stokes: gains energy, shifts blue). But dropping from v=1 to v=0 is rare because very few balls are at the V=1 energy state there, while nearly all can drop from v=0 to v=-1 (effectively exciting a vibration). Thus Stokes shifts dominate at room temperature

Mathematically, the distribution follows the equation $\frac{I_{anti-Stokes}}{I_{stokes}} \approx e^{-\Delta E/kT}$

Where:
ΔE is the vibrational energy gap (~0.1-0.5 eV),
k is Boltzmann's constant, 1.380649×10^{-23} J/K,
T is about 300 K;
this results in kT~0.025 eV (1 J = 6.242×10^{18} eV)

Example: For a 1000 cm^{-1} (~0.12 eV) Raman peak, $e^{-0.12/0.025} \approx e^{-4.6} \approx 0.01$, so anti-Stokes Raman is only ~1% as strong as the stokes Raman. Heat up to 2000 K, and it evens out; cool to 77 K (liquid nitrogen) and Stokes utterly dominates.

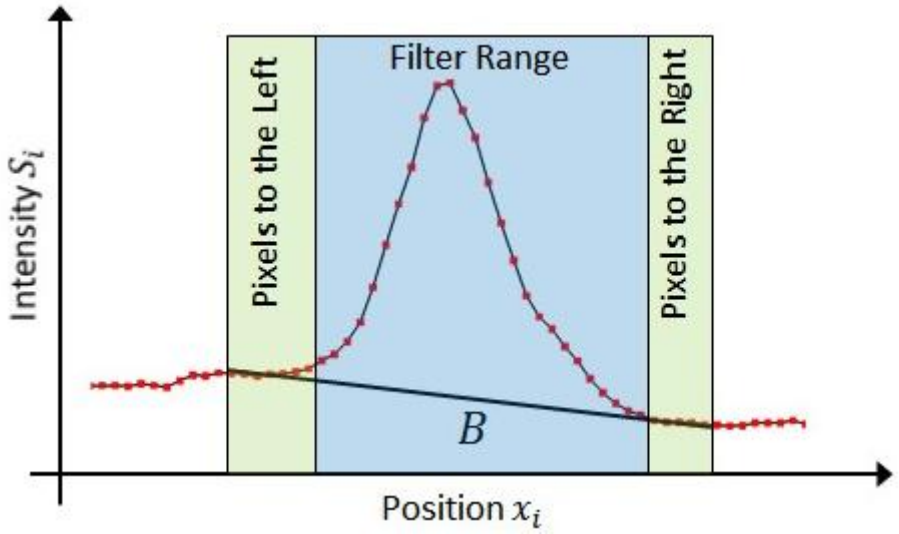
Create Filters (Button): You can simply create a new filter by pressing "Create Filters" and selecting one of the available filters.See Filter List for a short description for each filter and its purpose.

11. Background estimation

$$S_i = \tilde{S}_i - B(x_i)$$

$\tilde{S}_i$: *Spectrum with background*
S_i : *Spectrum without background*
$B(x_i)$: *Background estimation at position* x_i
x_i : *Spectral position at pixel i*

The background B is calculated using the neighbor pixels of the filter range. The number of pixels to the right and to the left can be defined in the Filter Viewer.



If the number of pixels to both sides is larger than 0 a slope is calculated. The average intensity and the average positions on both sides define the coordinate for the slope calculation.

Analysis: Producing images

Prerequisites:

Spectra recorded in map, depth or layers

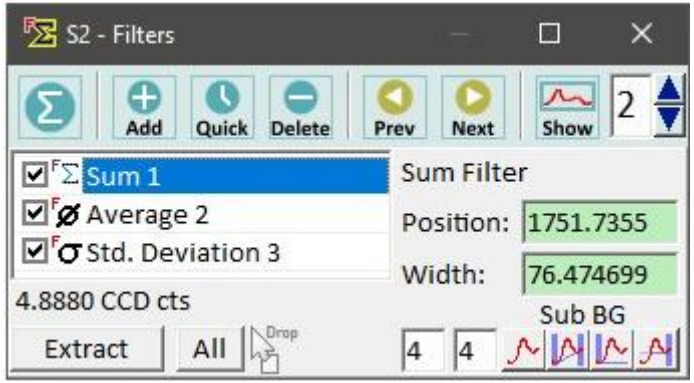
Learn about the Filter viewer

The Filter Viewer creates images from image-graph objects (or graph objects from line-graph objects or single result values from single spectra) using defined filters, e.g. a sum filter. The Filter Viewer helps you finding the interesting Raman bands and creates images which show structures of your components.

Through the drop action, open a filter window.

Input: Use can use any graph object with this dialog.

Results:The result is depending on the dimensionality of the input graph object.The Filter Viewer creates the following results for each defined filter:image objects for image graph input data objectssingle graph objects for line graph input data objectstext object containing result as text for single graph input data objects,



Create Sum Filter (Button): This creates a new sum filter.

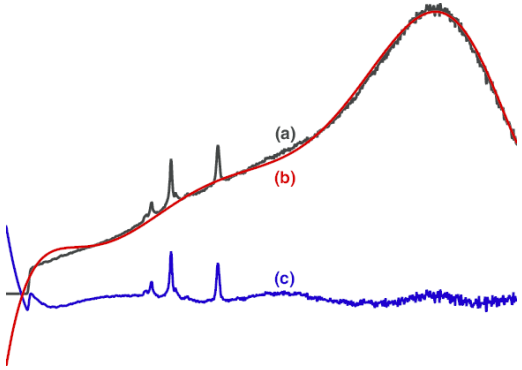
4. Scattering intensity and fluorescence trade off

Raman scattering arises from the induced dipole moment in a molecule interacting with incident light of frequency ν_0. Quantum theory shows the transition rate (intensity) follows $I \propto \nu^4$. This mirrors Rayleigh scattering's wavelength dependence $\frac{1}{\lambda^4} \propto \nu^4$, where electric field strength and energy density both scale with ν^2, yielding the fourth power overall. Shorter wavelengths (higher ν_0) thus boost signal dramatically, aiding weak Raman detection.

Example: A 532 nm laser (ν_0 corresponding to ~18800 cm^{-1}) yields Raman signals 2 times stronger than a 633 nm laser (~15800 cm^{-1}) for the same vibrational mode, and nearly 5 times more than a 785 nm laser (~12800 cm^{-1}). A 633 nm laser yields about 2.4 times more signal than a 785 nm laser.

Rough calculation: $\left(\frac{633}{532}\right)^4 \approx 2.0$, $\left(\frac{785}{532}\right)^4 \approx 4.75$ and $\left(\frac{785}{633}\right)^4 \approx 2.4$

Fluorescence Trade-off: Visible lasers often trigger fluorescence (b) in organic samples, swamping the ~10^{-6} weak Raman signal (a) with broad emission bands.



Fluorescence occurs when excitation energy matches electronic transitions, far stronger than Raman (~10^6 times), and peaks near the laser wavelength for short λ.

Near-IR lasers (e.g., 785 nm) minimize this by avoiding electronic absorption bands, sacrificing ν_0^4 boost for cleaner spectra in fluorescent samples. Background subtraction (c) can help to remove the fluorescence signal.

5. Units conversions

A Raman spectroscope measures a **shift in energy of scattered light**, not absolute wavelengths. Energy of photons is reported in wavenumbers (in eV or cm^{-1}, which is directly proportional to photon energy) even though lasers light emission is typically reported in wavelength (nanometers). Wavenumber, expressing shifts in wavenumbers conveniently quantifies vibrational energies independent of the laser wavelength.

- The laser provides monochromatic excitation light at a known wavelength λ_0 (e.g., 532 nm).
- The Raman scattered light is collected, with wavelengths slightly shifted up or down by vibrational energies in the sample (see above), resulting in scattered wavelengthsλ_S.
- The Raman shift is defined as the difference in wavenumber between the laser excitation and the scattered light: $\nu_0 - \nu_S = \frac{1}{\lambda_0} - \frac{1}{\lambda_S}$

Conversion from cm^{-1} to electron volt (eV)

To convert a Stokes shift given in wavenumbers (cm^{-1}) to electron volts (eV), use:

$$E(eV) = \frac{hc}{e} \times \tilde{\nu}$$

where:

v is the Stokes shift in cm^{-1},
h is Planck's constant ($6.62607015 \times 10^{-34}$ m^2 kg/s)
c is the speed of light (299 792 458 m/s)
e is the elementary charge ($4.35516763 \times 10^{-19}$ coulombs)

Numerically, this simplifies to:

$E(eV) = 1.2389 \times 10^{-4} \times \nu\ (in\ cm^{-1})$ or $E(eV) = \frac{\nu}{8065}$

For example, a 1000 cm^{-1} shift corresponds to about $\frac{1000}{8065} \approx 0.124$ eV.

Analysis: Compare with libraries

Prerequisites:

Spectra recorded and post processed

Compare the data to database datasets

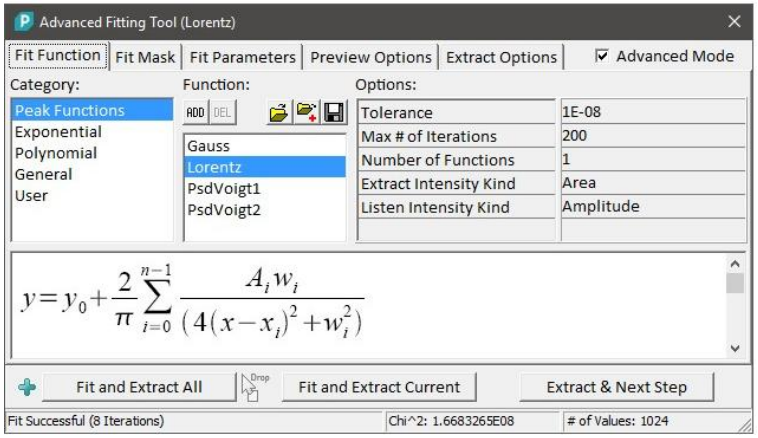
WiTec Truematch software allows the comparison of measured spectra with reference libraries to accurately identify materials. We do not have commercial libraries, so you must build your own library.

Open the drop action Database.

Your spectrum will be loaded (red).

In the search and Options tab, select the number of components in your material. Note that with 2 or 3 materials, the chance of a good hit quickly drops.

WITec's TrueComponent Analysis module in Control 7.0 software automates chemical imaging by identifying and separating distinct sample components from hyperspectral Raman datasets. It employs advanced clustering algorithms to locate up to dozens of unique spectral signatures simultaneously, generating false-color maps where each color represents a pure component spectrum.



6. Polarizability and Depolarization

The induced dipole $\vec{p} = \alpha\vec{E}$ oscillates at ν_0, but vibrational modulation yields sidebands at $\nu_0 \pm \nu_m$. Polarizability in Raman scattering is how easily a molecule's electron cloud gets stretched or squished by the laser's electric field, like deforming a soft balloon with your hand. If the molecule deforms the same way no matter the direction (isotropic), the scattered light keeps the laser's original polarization. Anisotropic molecules deform differently by direction, scrambling the polarization so scattered light becomes depolarized or rotated. If the polarizability is the same in all directions (isotropic), the molecule scatters light that keeps the same polarization as the incoming laser light. But if the polarizability varies with direction (anisotropic polarizability), the scattered light polarization changes or becomes "depolarized."

Isotropic Example: Carbon Tetrachloride (CCl_4)

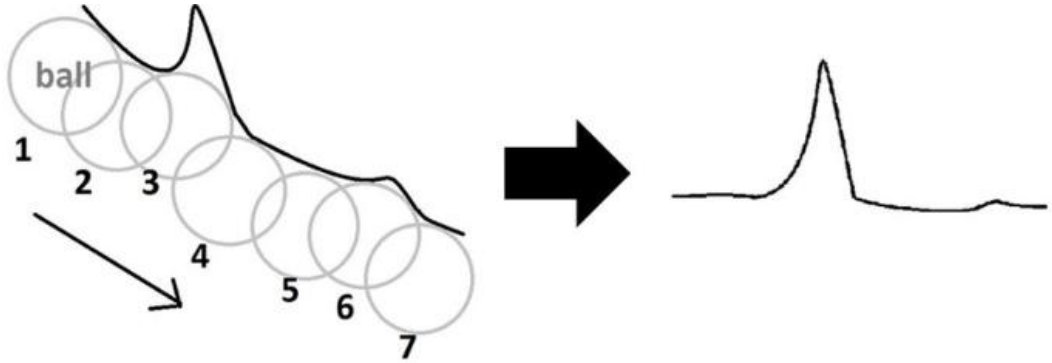
CCl_4 is a symmetric, tetrahedral molecule where the electron cloud distorts equally in all directions for certain vibrations, like the symmetric C-Cl stretch.
The laser's polarized light induces a dipole that scatters with matching polarization, giving a depolarization ratio ρ near 0 ($\frac{I_\perp}{I_\parallel} \approx 0$), meaning almost no light in the perpendicular direction. This confirms totally symmetric vibrations with no polarization change.

Anisotropic Example: Benzene (C_6H_6)

Benzene has a flat ring shape, so vibrations like the in-plane C-H stretch deform the electron cloud more along the ring plane than perpendicular to it.
An incoming vertically polarized laser produces scattered light with mixed polarization, yielding ρ ≈ 0.75 (strong perpendicular component), showing depolarization due to directional differences.

Other examples are distinguishes mineral polymorphs and crystal symmetries, stress/orientation in crystals, Protein secondary structure: Low ρ for α-helix amide I (symmetric) vs. higher ρ for random coil/β-sheet, mapping folding in cells/tissues, Lipid orientation, Chain tacticity/symmetry in polymers, Stress birefringence, Nanoparticle aggregation (Shifts in ρ reveal shape changes or plasmonic coupling in Au/Ag assemblies)

the background is subtracted using a rounded shape (“rolling ball”) which is rolling from left to right.



The shape method is very effective for subtracting fluorescence areas and is easy to use.
Other tabs/methods can be used, but the shape usually gives the best results.

8. Fitting (maps only, see below)

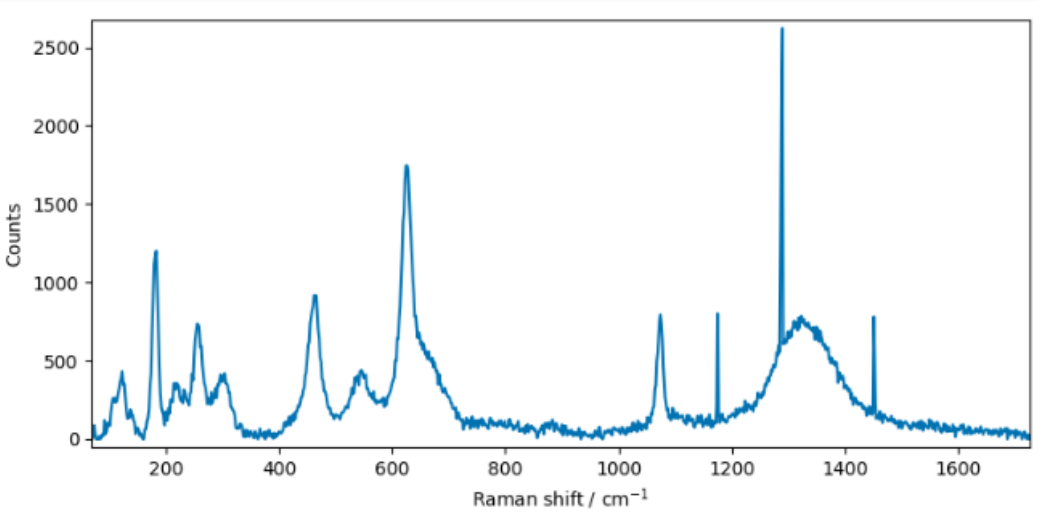
Usually, fitting of single graphs will provide poor results.

9. Smoothing

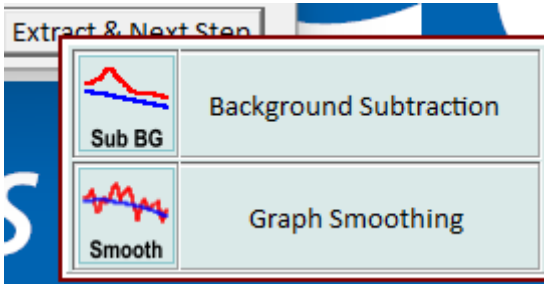
Savizky-Golay (preferred): Adjacent points are fitted using low-degree polynomials / linear least square. The lower the Left and Tight settings, the more moderate the smoothing. Order: 2-4 (to keep the polynomials low) and derivative usually 0.
Median: median kernel filtering. The higher the filter setting, the more smoothing
Average: using an average filter for adjacent values smoothing

10. Analyse / TrueComponent analysis (maps only)

Input: all data types, cosmic rays removed and background corrected.
Output: False-color maps of pure component spectra



Press Extract & Next step



Choose background subtraction

7. Background subtraction

Input: all data types, cosmic rays removed.
Output: Background removed from spectra

Select the "Shape" tab.

Information: Hardware overview

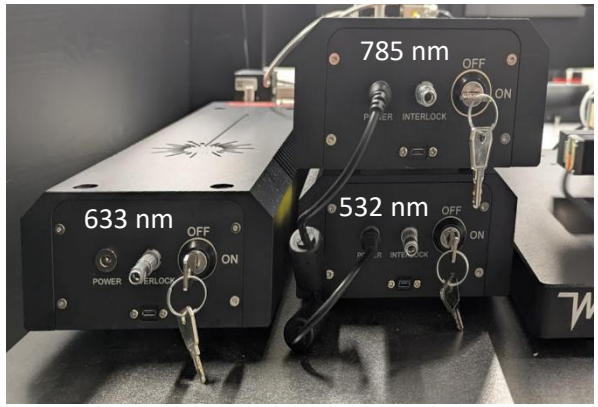
Prerequisites:

Understanding the hardware that produces Raman data

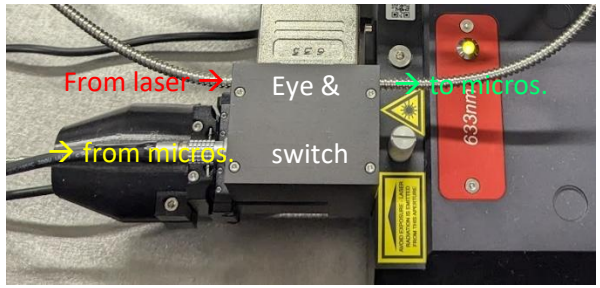
Learn about the hardware setup

Never ever look directly in a laser line!!

1. The lasers



Turn the key to ON of the laser(s) you want to use

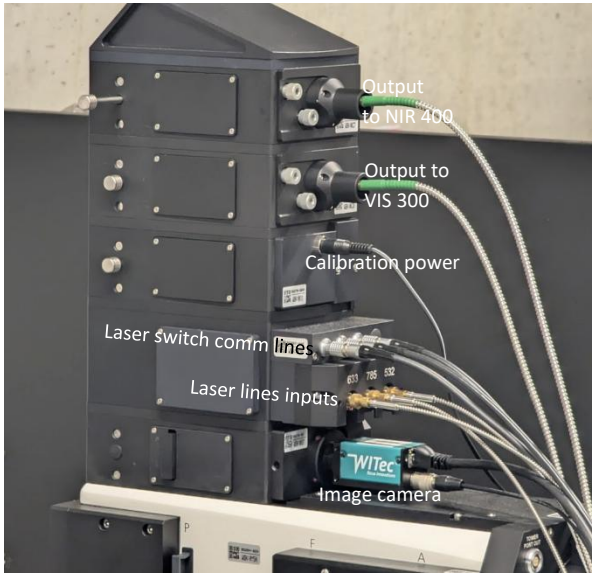


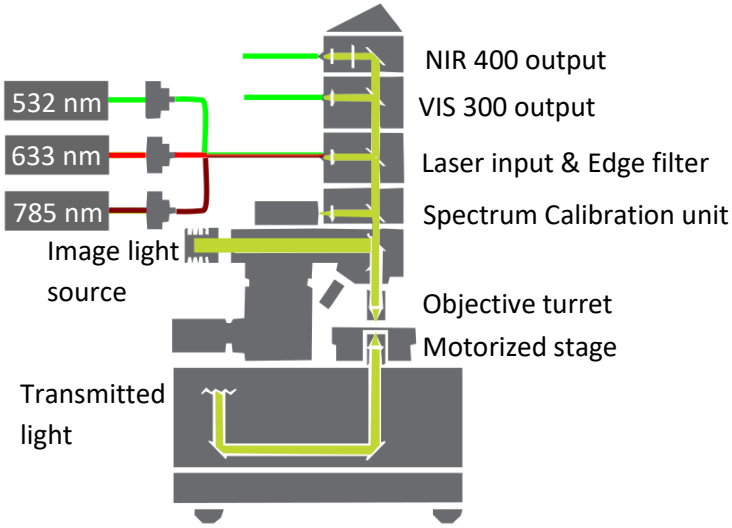
Eye and switch

Fibre optics from Laser to Eye: measure and adjust correct output, then send it to microscope

Switch: comm line from microscope to switch off laser when not in use.

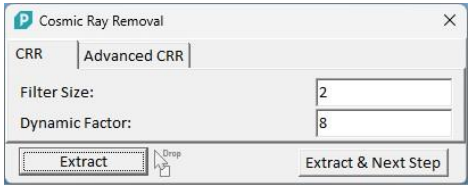
2. The tower





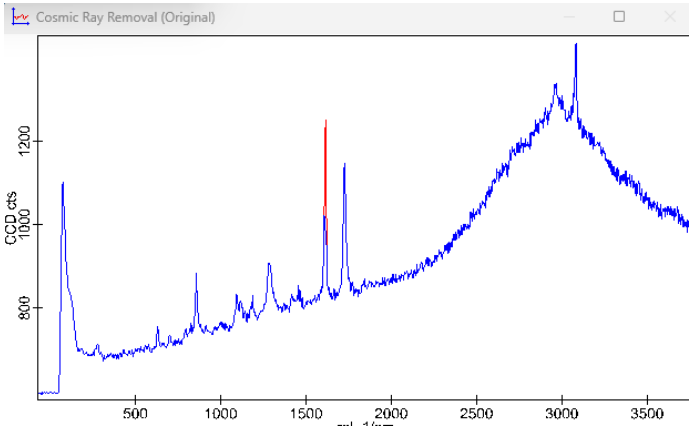
Edge Filter

An edge filter in a is a sharp long-pass optical filter that blocks the intense Rayleigh-scattered laser light (same wavelength as the excitation) while transmitting the weaker Stokes Raman-shifted light at longer wavelengths.



Filter Size: Filter size of the cosmic ray detection algorithm. The total window size is 2*<Filter Size> + 1.

Dynamic Factor: This factor changes the sensitivity of the cosmic ray detection algorithm (a smaller value means a higher sensitivity).



Check visibly if the red lines are indeed cosmic rays. Adjust the Filter size of dynamic factor accordingly. Cosmic rays are visible as very narrow peaks at 'impossible' positions

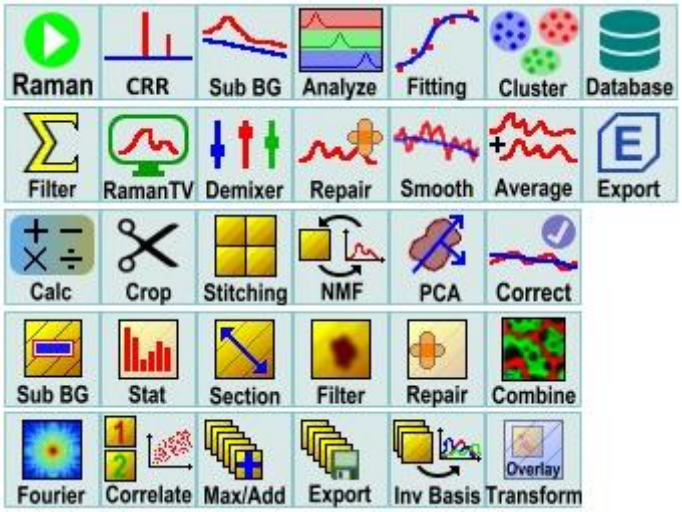
Analysis: Raman analysis

Prerequisites:

Spectra recorded

Run basic post treatment to improve analysis

The drop actions window is the portal for all data analysis. To open the drop actions window, LMB click and hold on a data entry and move the mouse.



Not all actions will be available for all datasets.

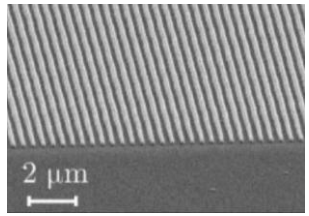
5. Start Raman analysis

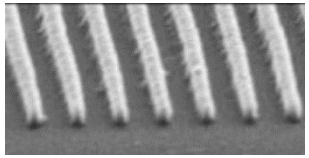
: dummy, will start the CRR

6. Cosmic ray removal

Input: all data types. Advanced CRR works only with image-spectrum .
Output: cosmic ray (or a cosmic ray map) removed from the data

3. The spectrometers



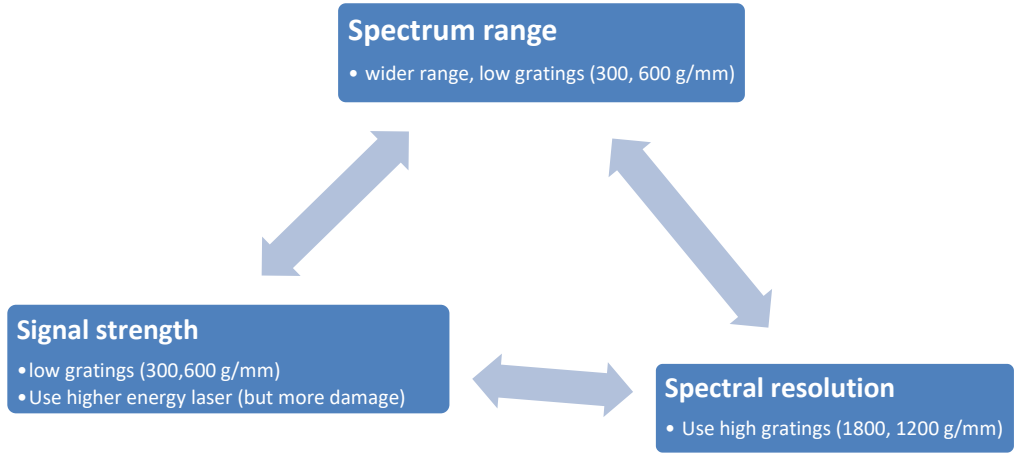


There are two spectrometers: VIS 300 and NIR 400.

VIS300: for 532 nm laser, outputs to CCD1
Gratings: 600 and 1800 g/mm (lower spacing distance means higher spectral range)

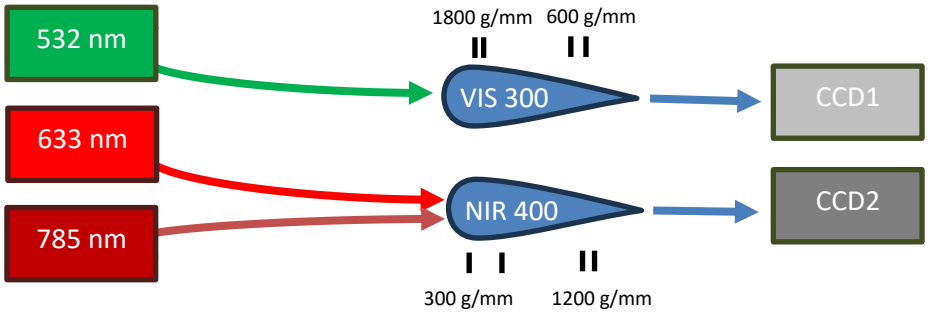
NIR300: for 633 nm and 785 nm laser, outputs to CCD2
Gratings: 300 and 1200 g/mm (lower spacing distance means higher spectral range)

Choose the grating based on:



4. Summary: Which system settings to choose

There are 3 laser: 532 nm, 633 nm and 785 nm.
There are 2 spectrometers: VIS 300 and NIR 400. Each spectrometer has 2 gratings
There are 2 cameras: CCD1 and CCD2



5. The controller

The game controller enables intuitive, hands-on control of the sample stage in X-Y-Z directions, fine focus adjustments, and real-time optimization while viewing live video or Raman spectra, speeding up setup and alignment.

4. Stack (“Layers”)

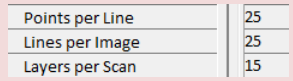
Finally, Layers, which is the most complex data type. The system scans areas at different confocal layers through the object. It is therefore a combination of the area and depth approach.

Experiment: Stack

1. In the Large Area scan, select Stack 

2. Using Geometry > listener Area/position, select an area where the layers will be recorded, or set the position and size of the layers.

3. For volume size, set the Depth to be imaged, where depth is understood as follows: the current z plane is the center, 20 µm depth means 10 µm above and 10 µm below the current Z plane.

4. Resolution: there are now three entries possible In At every point >
Points per line = number of single points in the X direction
Points per image = number of single points in the Y direction


Layers per scan = number of Areas (= layers). This value should be odd if you want an eqzual amount of layers above and below the current Z layer.

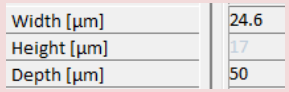
3. Cross section (“Depth”)

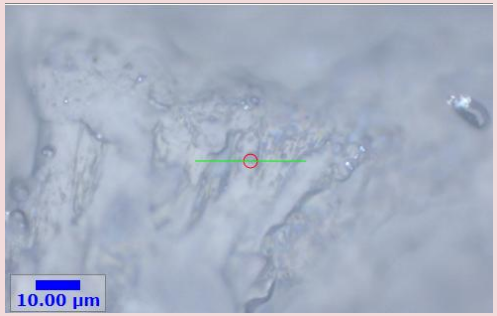
This will scan a map (“Area”) of a defined field of view, perpendicular to the surface, as a virtual cross section.

Experiment: Cross section (“Depth”)

1. Under Large area scan, In the Scan method, choose **depth**

2. In Geometry > listener Area/position, select an area. Note that the Scan method will change back to “area”. Change it back to depth. Two things happen:
   - The blue area becomes a green line (where the cross section will happen)
   - In Geometry, height becomes not enabled, while depth becomes enabled[2].

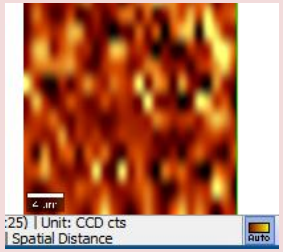




3. Resolution: In At every point >
- Points per line = perpendicular to the surface of your object
- Points per image = in Z direction, number of lines scanned in depth

4. The expected time shown in

Start the area scan by clicking the icon in the top menu:



Open the data in the same way as explained for the map (Area)

[2] Note that the cross section depth will center around the current z value. E.g. if you set a value of 20 µm, then 10 µm will be above the current Z plane, while another 10 µm will be below, i.e. inside the object. Therefore, the first scan lines usually will be very noisy, as there is no signal, or only background fluorescence signal

Demonstration: Switching the system on

Prerequisites:

System switched off

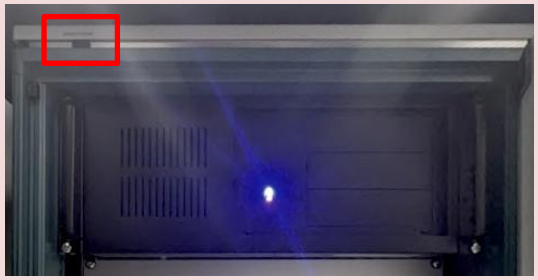
Start the system

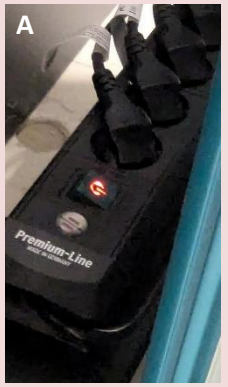
Upon arrival, check the following:

Switching on the system

1. In the Witec cabinet, if the PC is switched on (blue LED). If not, flip the switch at the top left.
2. Left of the cabinet, check if the multiplug A is on (it should be)

3. Right of the cabinet, if the multiplug B is on (it should be)





You should now have a functional system with a switch on PC.

Demonstration: Starting up the hardware and software

Prerequisites:

System switched on

Start up all the needed components

This is the order of switches and alignments that need to be performed:

1. Switching on the appropriate laser
2. Set up the tower
3. Start the software
4. Choose the correct spectrometer in the software
5. Choose the correct laser in the software
6. Choose the correct grating in the software

1. Switching on the appropriate laser

1. At the far end of the instrument near the wall, there are three long blocks (lasers)

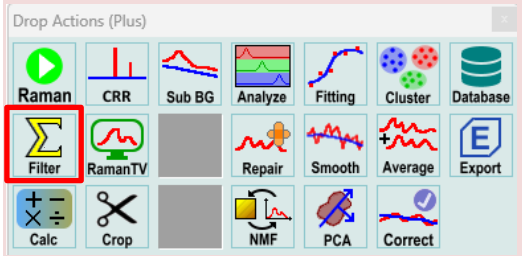
2. Choose the correct laser (532 nm, 633 nm and 785 nm) by turning the key at the relevant laser from OFF to ON.

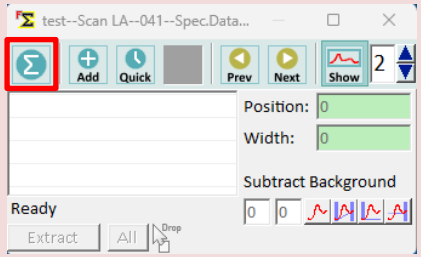
3. A warning LED at the end of the laser informs about the laser being on.

Immediately after start, a new entry will appear in the project manager. During scanning, you can check the progress by opening a filtered map.

Experiment: View the map

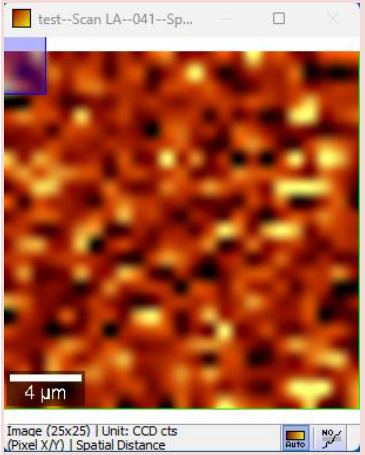
1. LMB and hold the mouse on the resulting entry in the Project manager. Move the mouse until you see the drop actions:





2. Select the filter. A new window under the Project manager opens.

3. Press the  button. 2 windows open: a map and a graph window

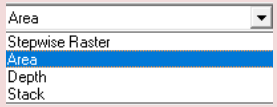


2. Map area

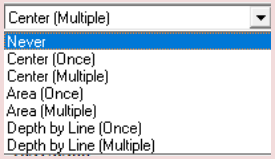
This will scan a map (“Area”) of a defined field of view

Experiment: record a map

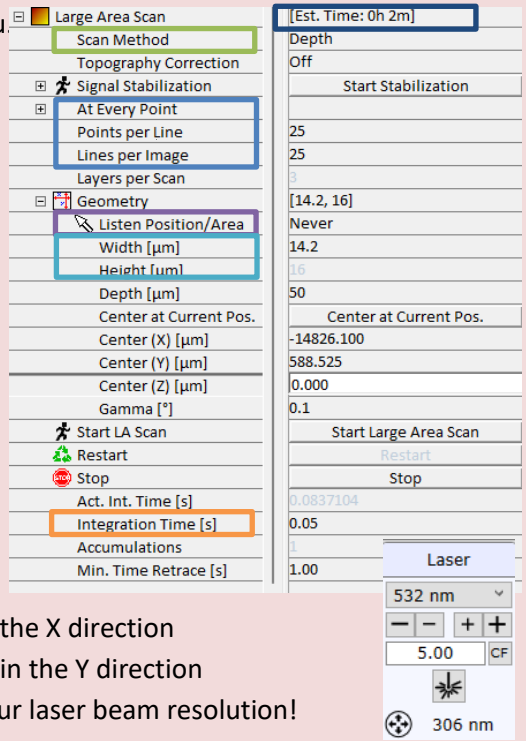
1. In control window: Large Area scan menu
2. In the Scan method, select Area



3. In Geometry > listener Area/position, either draw a rectangle on the image or select a center:



4. Set the size of the area (Width & height)



5. Resolution: In At every point >
Points per line = number of single points in the X direction
Points per image = number of single points in the Y direction
Set these number of points according to your laser beam resolution!

6. Set the integration time

7. The expected time to complete will show up in the Large Area Scan entry

8. Start the area scan by clicking the icon in the top menu:

2. Setup the tower

1. Set the Rayleigh filter wheel (orange box, rotating) to the appropriate laser length

2. Set the mirrors correctly by setting the correct Pins (OUT means sticking out):

532 nm	**633 nm**	**785 nm**
NIR 400: OUT	NIR 400: OUT	NIR 400: OUT
VIS 300: OUT	VIS 300: IN	VIS 300: IN
Calibration: IN	Calibration: IN	Calibration: IN

Watch out: guide the pins gently.

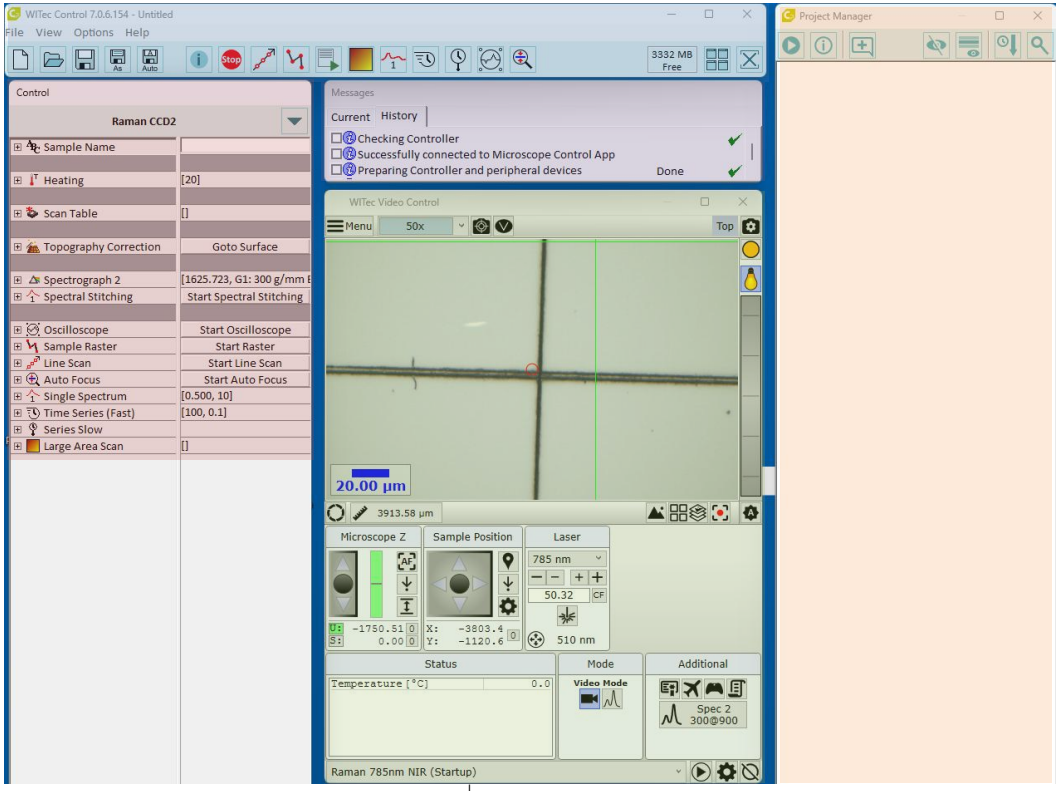
3. Start the software

With the hardware properly set up, start the control software.

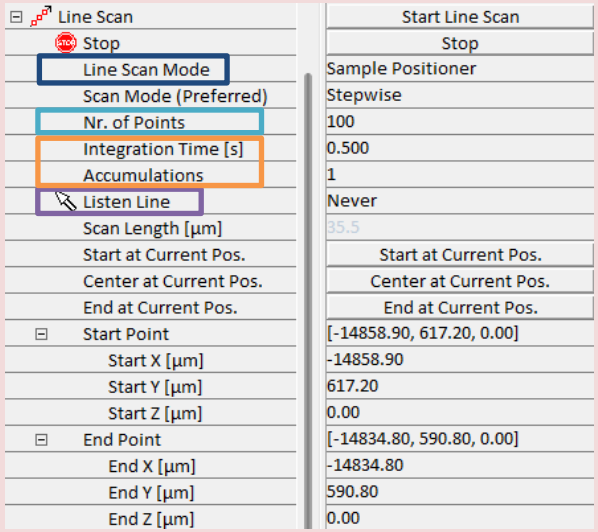
On the desktop, find the WITec Control 7.0 icon and start it.
The startup takes about 30 seconds. You will hear noises from the motors inside the microscope.

Workspace overview

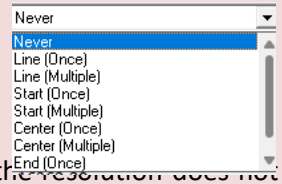
The software has – for now – 5 different windows:
Main window, Control, Messages, Video Control and project manager



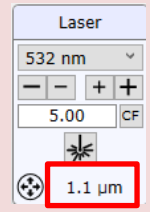
Once = the feature will stop and the listener will fall back to never.
Multiple = the listener does not fall back and the listener must be closed manually



Line: draw a line on the image in the WiTec video window
Start: Set the starting position of the line on the image in the WiTec video
Center: Set the center position of the line on the image in the WiTec video
End: Set the end position of the line on the image in the WiTec video window



Step 2: Nr. of points
the resolution of the single points along the line. Keep the resolution of your current lens in mind: more points than half the resolution does not make sense (Nyquist-Shannon)



Step 3: set the integration time and accumulations

Step 4: set the stage movement in the line scan mode

- **Scan Table.** piezo stage movement, not available
- **Sample Positioner** uses the motorized stage for the movement.
- **Sample Pos. + Topo. Cor**. uses the motorized stage for the movement in combination with a recorded topography (if available) for the z position.

Step 5: Start the line scan by clicking the icon in the top menu:

Demonstration: higher dimension spectra

Prerequisites:

Components correctly setup

Laser focused

Sample loaded

Record 2D, 3D and 4D spectra

There are 5 types of datasets you can record:

Name	# dim	Dimensions
Single spectrum	1	spectral
Line scan	2	1 spectral and 1D spatial
Map (“area”)	3	1 spectral and 2D (XY) spatial
Cross section (“Depth”)	3	1 spectral and 2D (XZ) spatial
Stack	4	1 spectral, 2D area (XY) and maps spatial

For single spectrum, see above

1. Line scan

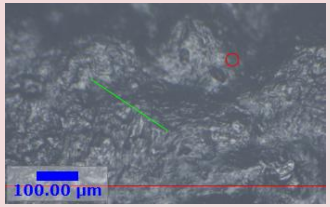
The line scan will scan single spectra along a line. This is ideal to show interfaces between two materials.

Experiment: Line scan

In the control window, expand the line scan menu.

In the Listen Line entry, choose **Line (once)**

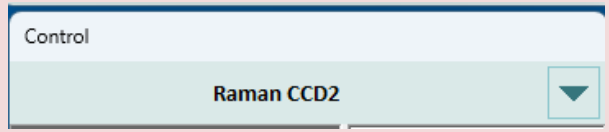
Step 1: On the image in the WiTec video control window, draw a line along which single points will be plotted



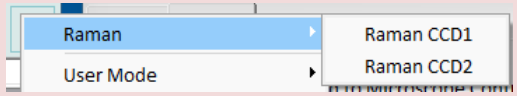
4. Choose the correct spectrometer in the software

In the Control panel window, the header will display the currently selected spectrometer (in this case, the CCD2). Choose:

- CCD1 → 532 nm
- CCD2 → 633 nm and 785 nm



To change the spectrometer, click the triangle at the end of the header ( )
In the menu that is popping up, follow Raman > Raman CCD1 or Raman CCD2



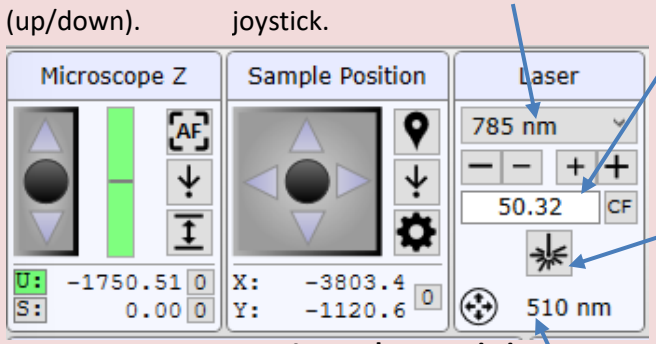
5. Choose the correct laser in the software

In the window Video Control, below the image, there are three setting tabs:

Focus Adjustable with the top left joystick (up/down).

XY position Adjustable with the bottom right joystick.

Laser to select the appropriate laser. Use dropdown box to match the laser you switched on.



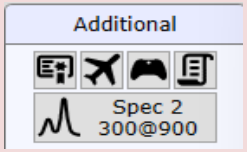
Laser Power
Use – or +, or set it manually (for Raman, not for imaging!)

Laser safety
Block the laser beam.
Beam open:

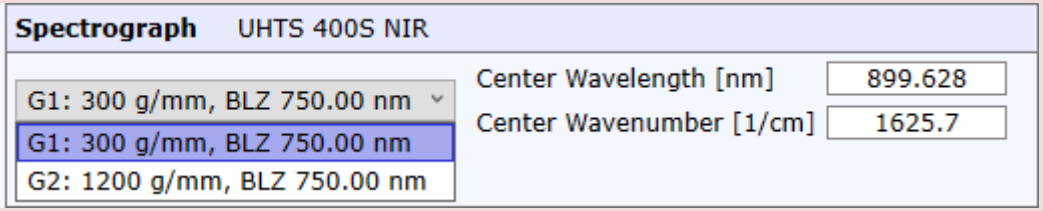
Laser characteristics
toggle between lateral resolution (XY) or axial resolution (Z)

6. Choose the correct grating in the software

In the window Video Control, at the bottom right, there is a box called “additional”. It contains the spectroscopic and grating settings. Click the button with the spectrum icon.



A new dialogue box opens with the settings for the selected spectrometer (in this case, the NIR 400). Under the Spectrograph heading, the grating can be selected:



A higher number of gratings per mm allows better peak resolution, but within a narrower spectral range.

It is possible to choose between 2 different gratings for each laser:

- 600 and 1800 g/mm for 532 nm laser + Raman CCD 1 + VIS 300
- 300 and 1200 g/mm for 633/785 nm laser + Raman CCD2 + NIR 400

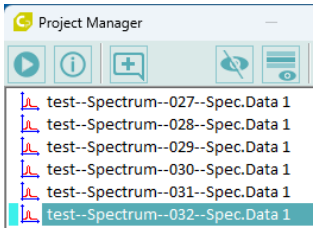
Demonstration: Metadata

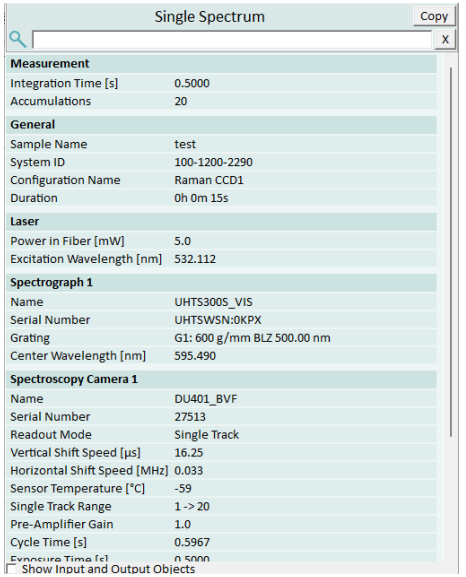
Prerequisites:

Data recorded

Access all the metadata that comes with your recordings

In the project manager, there is a hidden button before every spectrum entry (shown here in cyan blue). LMB and a window will open will all metadata.





Demonstration: Single spectrum

Prerequisites:

Components correctly setup
Laser focused
Sample loaded

Record a single Raman spectrum

Now, the illumination system is switched from the white light to the laser.

Experiment: record a single spectrum

1. After deciding on the laser (see p. 21 and grating (see p. 22), repeat
   - the focusing of your object in the WiTec video window (see p. 25)
   - Repeat the laser focusing, but only the Z plane (p. 26, point 3). Do not touch the screws on the back of the tower anymore (point 4)!
2. Adjust (lower) the laser strength if needed.
3. Increase the accumulation settings (e.g. to 100), if needed
4. Click the single spectrum button in the top row menu to start the spectrum

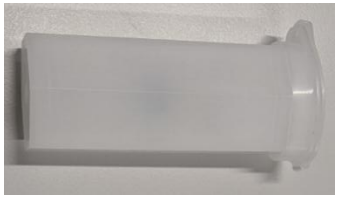
Demonstration: Load a sample

Prerequisites:

System switched on
Components correctly setup
Calibration not performed yet

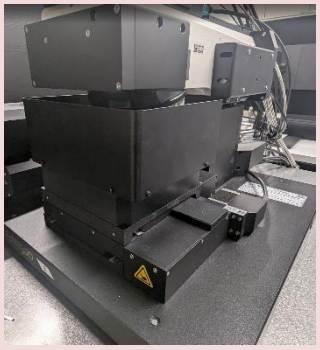
Load the Si test wafer for calibration and alignment. This is mandatory!

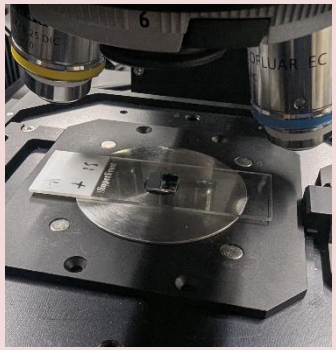
Beside the screen on the right, in the white protective tube, find the **Si wafer calibration** slide.

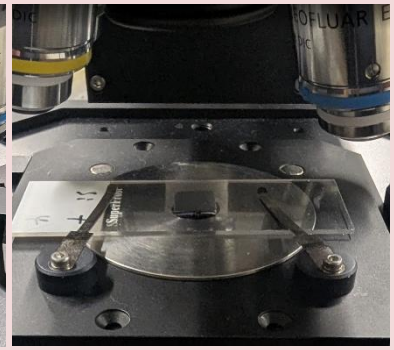


ounting a glass slide

At the microscope, remove the black protection box to reveal the lenses. The selected slot will the number 6, which does not contain a lens.





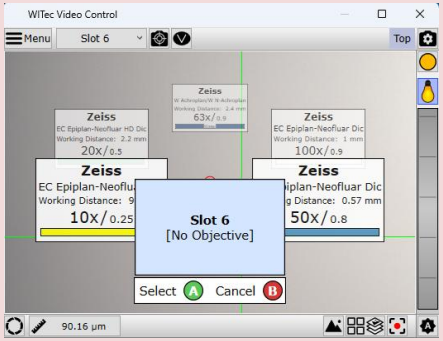


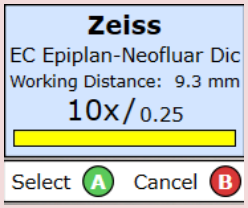
Place the calibration slide on the stage. You can use the magnetic clamps for fixation.

In the Video Control, check the live image.

- If the image is black, switch on the top illumination light 
- Run the auto brightness 

On the controller, press A. An overlay with boxes will appear over the live feed. Use the bottom right joystick (left / right) to select the 10X lens





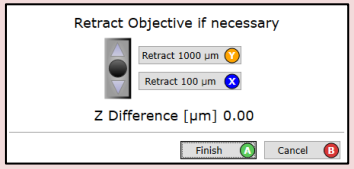
Press A on the controller to select the lens.

Retract the turret or you will destroy very expensive lenses!

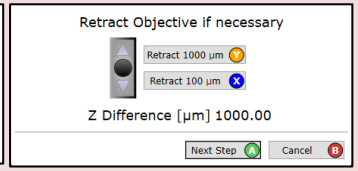
A new Dialog box pops up, telling you to retract the Objective if necessary.

It is necessary!

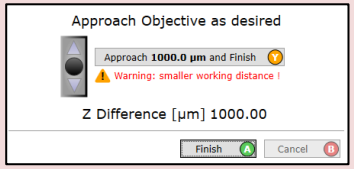
1. Press the Y button
The stage will retract over 1000 µm.



2. Press the A button
The turret rotates to the 10X lens.



3. Press the Y button
The lens approaches to 0 and the window closes



3. Correction of focus differences (focus stacking)

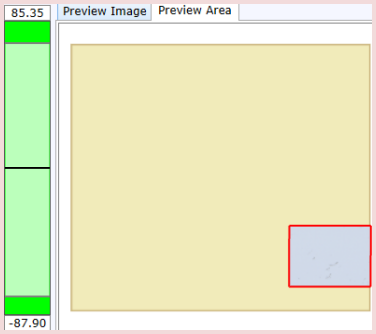
Especially with high magnification lenses or large objects, not the entire object will be in focus in a stitched image. To overcome, use the Focus stacking option.

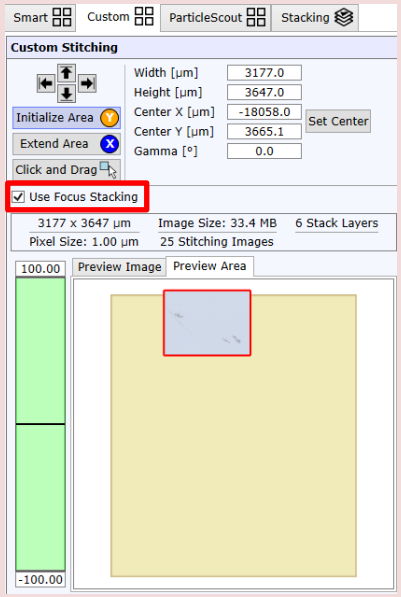
Experiment: focus stacking

1. Ticking the “Use focus stacking” option adds a green focus bar to the preview area

2. Reinitialize and set the preview area borders. Grab the middle black line in the focal green box. Use the mouse to extend the focal range. (max 100 µm up & down)

3. Click start (A)

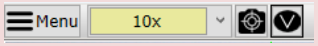




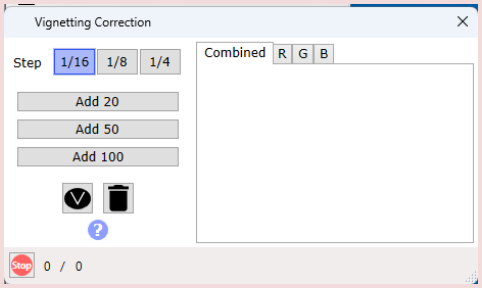
2. Correcting for vignetting

Vignetting is edge darkening: image borders are dimmer than the center, resulting in seams in stitched images. I the object is reasonably flat, Vignetting correcting can solve this.

Experiment: vignetting

1. Make sure you are in focus 

2. RMB (right click) on the in the WiTec video window. A window pops up



Step (1/16, 1/8, 1/4): Defines the step size for the automatic movement (e.g. 1/16 video image size). 1/16 is usually good.

Add 20/50/100: Automatically moves the sample positioner and adds video images to the current Vignetting Correction. 50 is usually good

3. After the correction is recorded, the vignetting icon turns green 

4. Notice a black gradient towards the borders in the vignetting correction window. Now Repeating the stitching (see p. 31) will remove the vignetting in the stitch:

Without vignetting

With vignetting correction

Demonstration: Focus the sample

Prerequisites:

System switched on

Components correctly setup

Focus the image

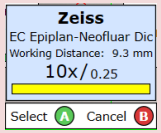
There are two illumination systems:

- The white light for imaging
- The laser for the Raman spectrum

6. Focus the imaging system

Focus the imaging system

1. Select the 10X lens. Assure to retract



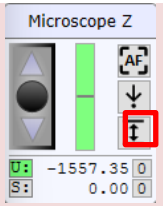
2. Press menu + Y combination
This closes the condenser aperture, edges are helpful to find the object focus plane.

3. Press X for autofocus.
Note: autofocus will search in a range of 200 um of the current focus plane. If the focus is off by more, it will not be found, and the box edges will turn red.

Running the autofocus iteratively will find a focus as far as 300 µm off (in 2-3 steps).

When focus is found, the box edges turn green. If the focus is further than 300 µm, you will need to use the Z button and focus manually.

For reference, reset the relative z position by clicking the top 0



4. Open the condenser (menu + Y) again

Repeat for the 20X and 50X lens.

7. Focus the laser

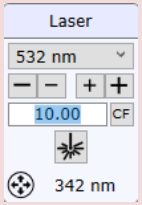
The laser does not necessarily have the same focal plane (but the planes are close)!

Prerequisites:

- 50X lens
- The imaging system is in focus

For the 532 nm laser: place the protective tool around the stage

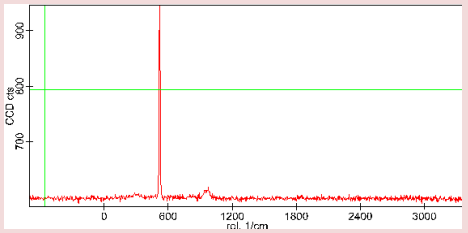
Experiment: focus the laser beam



1. Set the laser to its appropriate strength:
   - 532 nm laser: 10 mW, primary peak above 3500 counts/0.5 s
   - 633 nm laser: 10 mW, primary peak above XXX counts/0.5 s
   - 785 nm laser: 50 mW, primary peak above XXX counts/0.5 s

2. With the Si wafer sample in focus, switch on the oscillator in the top menu:

(You will hear a sliding noise, the image turns black, and a new graph window opens (Hardware spectrum, =live). A spectrum appears with a primary peak around 525 cm^{-1} and a secondary, smaller peak around 950 cm^{-1}.)



3. Focus (Z) toggle (do not use the back toggles!) to maximize the values in the Y axis

Demonstration: Record stitched field of view

Prerequisites:

Components correctly setup

Laser focused

Sample loaded

Recording a stitched field of view of many images

1. Simple stitching

With your object in focus and brightness properly set, select the video stitching and focus stacking button.

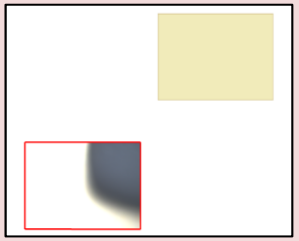
Experiment: simple stitching

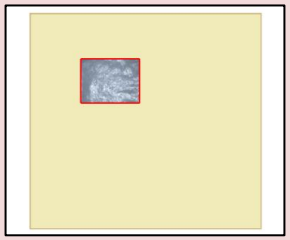
1. Select the “Custom” tab (the other tabs will not be explained here)

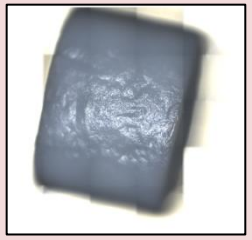
2. click “initialize the area” (Y) and the same image as in the WiTec video control window.

3. Use the XY toggle (bottom right) to move to an edge of your object. The yellow preview area extends to include the new position

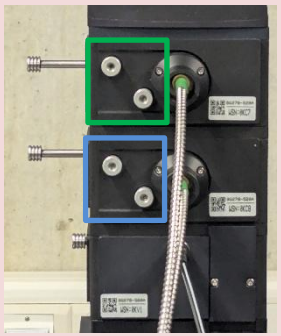
4. Repeat: move to edges of your object & **Press X**. The position is incorporated into the yellow preview area. When finished, press start (A) to start the stitching. Note: Your object may not be entirely in focus, depending on its topography and lens used.


Before pressing “X”


After pressing “X”


After stitching

4. Adjust the crews on the back of the tower to maximize the values of the Y axis in the hardware spectrum window[1]. Use:
   - VIS 300 (lower ones) for the 535 nm laser
   - NIR 400 (upper ones) for the 633 and 785 nm laser.



5. Repeat step 3 & 4 until the primary peak is maximized. E.g.
   - 532 nm laser: primary peak above 3500 counts/0.5 s
   - 633 nm laser: primary peak above XXX counts/0.5 s
   - 785 nm laser: primary peak above XXX counts/0.5 s

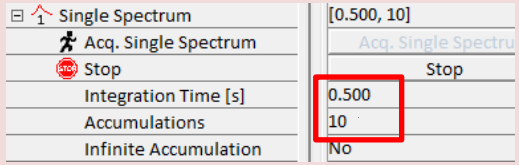
5. Stop the oscillator

Step 3 is especially important if you switch lasers on the same spectrometer (e.g. from 532 nm to 633 nm on the VIS 300)

You have now maxed out the signal transduction from the sample to the detector. Now doublecheck these setting by measuring the height of the **secondary peak**

Experiment: measure the height of the secondary Si peak

1. In the window Control panel, open the menu "Single Spectrum" and set:
   - the integration time to 0.5 s
   - Accumulations to 10



2. Click the single spectrum button in the top row menu to start the spectrum

[1] If you slightly tap the tower on top and the spectrum jumps down (only down!), you have centered the screws perfectly. If the spectrum jumps up and down, you can still improve

Demonstration: Record a single image

Prerequisites:

Components correctly setup

Laser focused

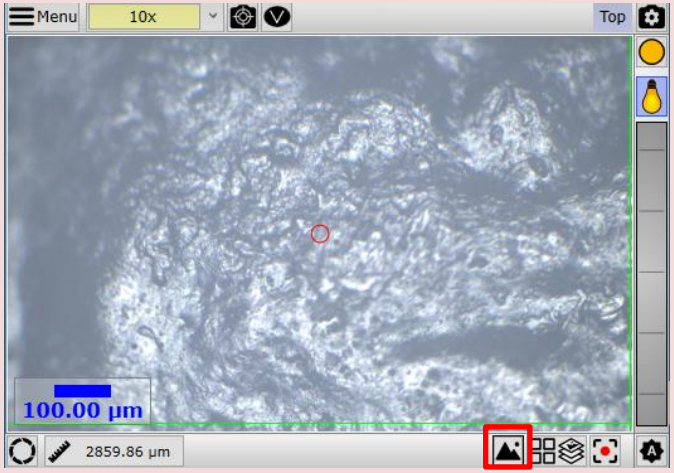
Sample loaded

Recording images

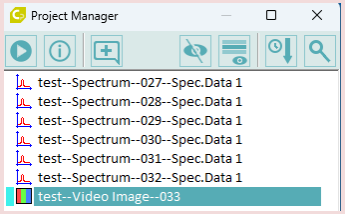
Here, we explain how to record images. Therefore, the white light illumination is used, not the laser systems.

Demonstration: Record an image

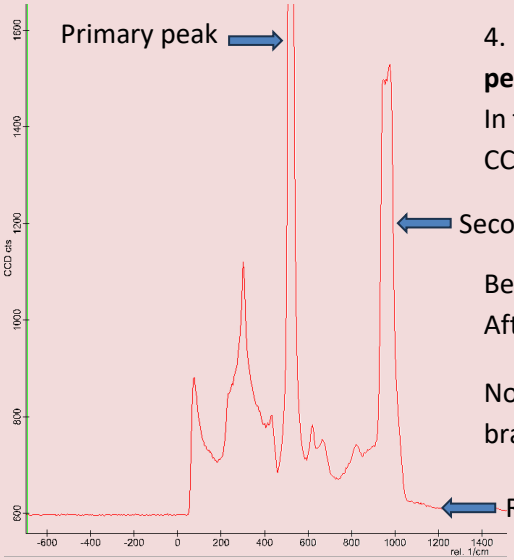
With a properly focused image in the WiTec video control



Click  to record an image. It will appear in your project window as Video image (RGB icon).



3. In the resulting spectrum (not the hardware spectrum!), zoom in on the secondary peak at 950 cm^{-1}.
Mouse scroll button = zoom in, and scroll button click = move the spectrum.



4. Click the **right baseline** of the **secondary peak** at about 1050 cm^{-1}.
In the bottom left of the window, the relative CCD cts is set to 0 (in the brackets of X-axis)

Secondary peak (zoomed in)

Before click: Y-Axis: 546.89 (-472.46) CCD cts
X-Axis: Pixel (297) | 709.57 (116.11) rel. 1/cm

After click: Y-Axis: 627.82 (0) CCD cts
X-Axis: Pixel (377) | 1052.5 (0) rel. 1/cm

Note how the relative counts of the Y-axis (in brackets) turn zero!

Right baseline of secondary peak

4. Move the mouse pointer (without clicking) to the top of the secondary peak at around 980 cm^{-1} and read the relative counts (in the brackets of the Y-axis). **This value must be higher than 800**.

Y-Axis: 1525.[illegible] (897.74) CCD cts
X-Axis: Pixel (958) | 979.93 (-76.58) rel. 1/cm

If not, repeat steps 3-4 of **focus the laser beam**.

You are now ready to record Raman data.

Some troubleshooting:

- Are you on a clean patch of the wafer?
- Is the laser strength correct (10, 10 or 50 mW)?
- Are the integration time and accumulation settings correct
- Try to improve focus of the laser (not of the image)
- The values are off when you are touching the tower

Demonstration: Load your sample

Prerequisites:

Components correctly setup
Laser focused
Sample loaded

Remove the calibration slide and load your sample

1. Save the Si wafer calibration inside your project
2. Retract the 50X lens (A) and switch to position 6
3. Remove the Si calibration slide and place it back in the plastic pocket.
4. Place your sample on the stage
5. For the 532 nm laser: place the protective tool around the stage
6. Retract the lens, if needed – assess your sample height in comparison to the Si wafer – retract the lenses over several mm if needed.
7. Select a proper lens, grating, etc...
8. Focus your image (see p 25)