Standard Operating Procedure

Horiba LabRAM HR Evolution

Confocal Raman Microscope

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Safety notices

1. High power laser irradiation may lead to **serious eye damage**.

In normal operations, laser beam is focused by the objective lens so that the beam becomes diverged after passing the focal point. Therefore, it is generally safe to operate without special eye protection.

Extra caution must be taken when dealing with highly reflective samples e.g. Si wafer, polished metals, etc. Never look at the sample during Raman measurements when laser is ON.

Verify that laser is OFF before removing/installing objectives.

2. Improper operations can cause damage to the objective lenses.

Objectives are fragile and must not be rammed into samples. The 50X and 100X objective lenses operate at less than 0.4 mm of working distance, and cover glass is not allowed. Always proceed to higher magnifications step-by-step and do not skip adjusting the focus with 50X objective before using 100X. For these two objective lenses, Focus must be slowly controlled via the joystick-coupled fine adjustment knob, and the coarse adjustment knob is NOT allowed. After finishing measurements with these two lenses, first switch to 10X before lifting up the objectives.

All 5 objective lenses are securely mounted on the nosepiece and must not be removed without permission. When installing objectives, verify that the three 10X, 50X, 100X lenses are mounted in a sequential way (clockwise from top view) and all lenses are secured hand-tightly.

3. Other potential safety hazards.

The instrument is powered by 110 V electricity from wall outlets. Do not touch any plugs.

The whole table is stabilized by compressed air. Do not exert extra force on the table.

Instrument specifications

1. Model name: LabRam HR Evolution

2. Laser information

Two laser sources are available: **532 nm** and **633 nm**. Both rated at 50 mW source power.

Laser intensity can be reduced via software-controlled ND filters. These percentages are available: 100%, 50%, 25%, 10%, 5%, 3.2%, 1%, 0.1%, 0.01%.

An ultra-low frequency (ULF) filter is installed for 633 nm laser to allow measuring down to 10 cm^{-1} Raman shift; however, this ULF filter poses an artificial negative peak at 2999 cm^{-1} in the Raman spectrum when using 532 nm laser. This ULF filter belongs to Professor Judy Cha group, and cannot be removed from the optical pathway. Please contact the manager and request for permission before using this ULF filter.

3. Objective information

Five Olympus objectives are available; the three 10X, 50X, 100X are open to general users.

The 50X-LWD and 60X-W objectives belong to specific research groups. Please request for permission before using them: 50X-LWD – Professor Judy Cha group; 60X-W – Professor Hailiang Wang group.

Below is the summary of the objectives. Working distance is the distance between sample surface and objective; samples with rough or non-planar surface should only be operated with 10X or 50X-LWD to prevent potential objective damage. Numeric aperture determines the objective's ability to gather light, therefore the Raman signal intensity is positively correlated with larger NA objectives.

OBJECTIVES	MODEL NAME	WORKING DISTANCE	NUMERIC APERTURE
10X	MPLN10X	10.6 mm	0.25
50X	MPLN50X	0.38 mm	0.75
100X	MPLN100X	0.21 mm	0.9
50X-LWD Long Working Distance	LMPLFLN50X	10.6 mm	0.5
60X-W Water	LUMPLFLN60XW	2 mm (in water)	1

4. Spatial and spectral resolution

The spatial resolution of the microscope is determined by the optical components, namely the laser wavelength and objective numeric aperture: **Spatial resolution = 0.61 λ / NA**. This equation can also be used to estimate the size of focused laser spot.

The spectral resolution of the Raman spectrometer is mostly determined by the grating and laser wavelength. Two gratings, 600 and 1800 [groves/mm], are available, and 1800grating offers higher spectral resolution but at the compromise of lower signal intensity and increased acquisition time. For the two lasers, 633nm intrinsically offers higher spectral resolution than 532nm.

5. Mapping function

The mapping function enables Raman chemical mapping in x, y, z directions as well as time and pinhole. Details are introduced in the section ‘Performing Raman mapping’.

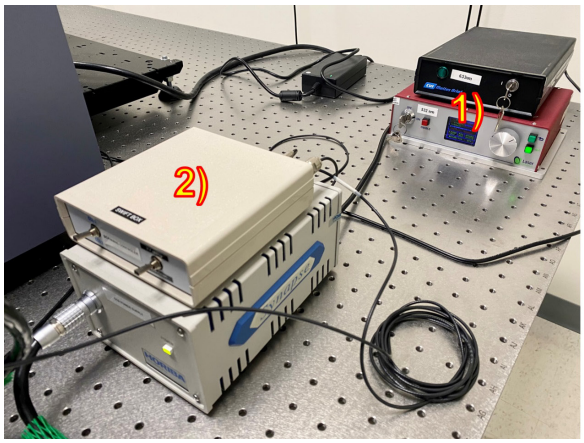
6. DuoScan function

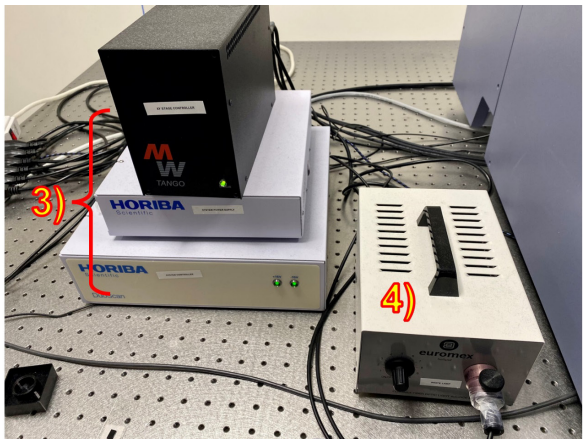
The DuoScan accessory can be utilized for Raman mapping by scanning the laser beam across X-Y plane (stepping mode) instead of moving the sample stage, or creating an equivalent ‘macro laser spot’ to lower the power density and mitigate photo damage (averaging mode). It also enables full coverage of Raman mapping by adapting macro laser spot to standard mapping (macro mapping mode). Details of operating in DuoScan mode are introduced in the section ‘Activating DuoScan’.

Users are required to set back the software parameters when they finish operation in DuoScan mode. Violation is subject to access suspension.

Hardware Control: Overview and Inspections

1. Overview of the hardware





1) **532 nm** and **633 nm** laser control switches.

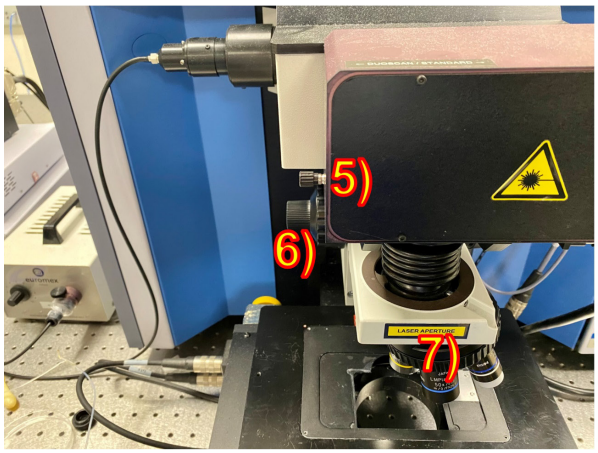
2) Scanning controller.

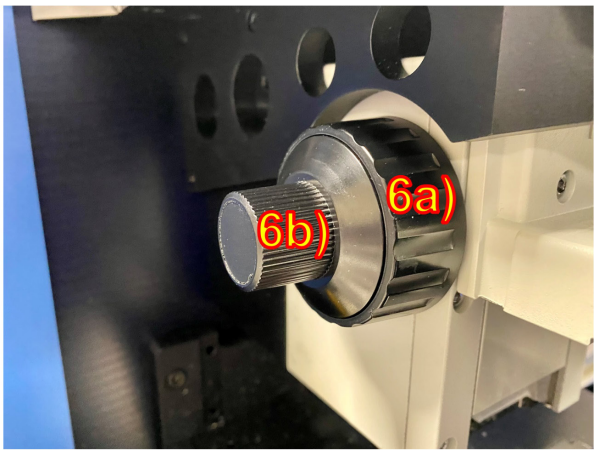
These are located at the right side of the Raman microscope.

3) System power controllers.

4) White lamp controller.

These are located at the left side of the Raman microscope.





5) DuoScan push/pull rod.

6) Adjustment knobs. a) coarse; b) fine. **Warning:** never touch the fine adjustment knob.

7) Nosepiece and the objectives.

2. Inspection of the hardware

Inspect laser control switches: both switches should be OFF. Notice: report to the instrument manager if they were left ON by the last user.

For 633nm laser, turn the key by 90° to ON position, and the green light becomes on.

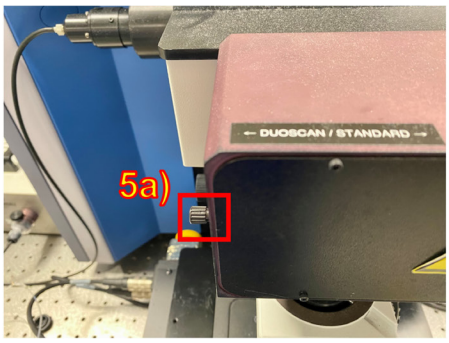
For 532nm laser, turn the key by 90° to ON position, then push the flashing red button once.

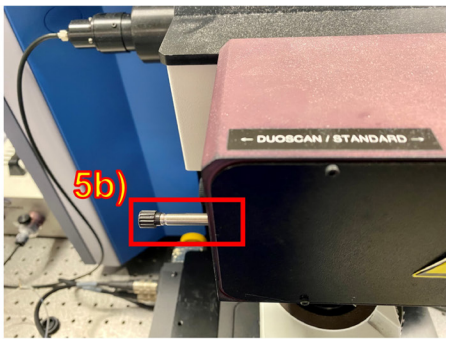
Inspect the scanning controller. The left switch should be CCD1; the right switch should be XY stage.

Inspect system power controllers: all indicating lights should be on and green. If not, stop proceeding and report to the instrument manager immediately.

Inspect the white lamp controller. The adjusting knob should be OFF. Notice: report to the instrument manager if it was left ON by the last user.

Inspect the DuoScan push/pull rod. It should be 5)a Standard mode instead of 5)b DuoScan mode. Otherwise, gently push the rod into 5)a position. Caution: delicate optical elements; do not exert extra forces.



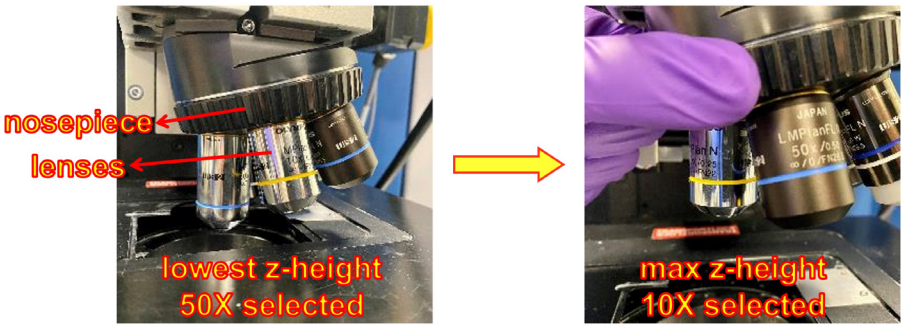


Inspect the Z height of objectives. **Warning:** never touch the fine adjustment knob 6)b. Rotate the coarse adjustment knob 6)a counter-clockwise to increase Z height and move the objective away from the sample; clockwise to decrease Z height and move the objective towards the sample.

Rotate the coarse adjustment knob counter-clockwise to the maximum Z height.

Inspect the nosepiece and objectives, Notice: report to the instrument manager immediately if there are missing objective lenses on the nosepiece.

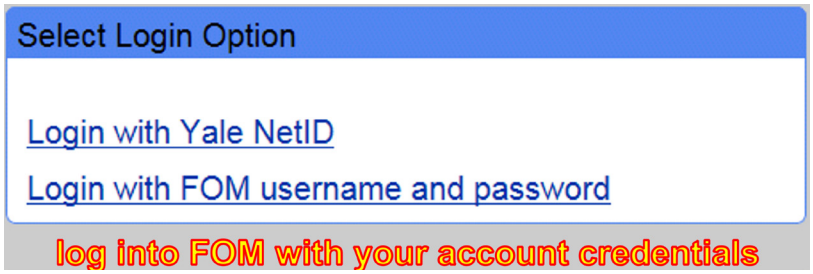
Rotate the nosepiece to select the 10X objective. Notice: a resistance is sensible when the objective lens is rotated in position. **Warning:** do not touch lenses; handle the nosepiece only.



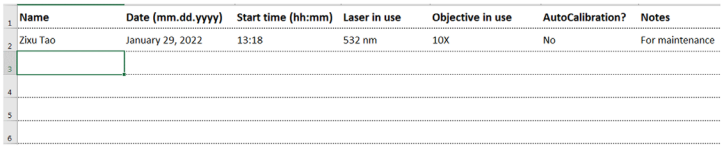
Software Control: Overview and Inspections

The computer log-on is controlled by FOM system.

Select appropriate identity and type-in account credentials to log in. The actual usage will be calculated by the FOM system from log-in to log-off.



Open the Raman Sign-on Book sheet on the desktop, and enter required information. This includes your name, date, start time, laser in use (633 nm or 532 nm), objective in use, AutoCalibration? (Yes or No), notes (note down abnormal events), sample info. Notice: we rely on users' sign-on information to track the instrument status and maintain it in good condition.



Name	Date (mm.dd.yyyy)	Start time (hh:mm)	Laser in use	Objective in use	AutoCalibration?	Notes
Zixu Tao	January 29, 2022	13:18	532 nm	10X	No	For maintenance

Open the LabSpec6 on the desktop. The image shown below indicates the initialization of the instrument and should only exist for a few seconds before entering the software interface.

1. Overview of LabSpec6 software

a) File operation commands. From left to right: Close, Open, Save, Print.

b) Data editing commands. From left to right: Undo, Redo, Rescale, Initialize, Copy, Paste, Config, Help.

c) Data acquisition commands. From left to right: Start real-time display RTD, Start spectrum acquisition, Start map acquisition, Start video acquisition.

d) STOP command. This command stops any currently running acquisitions.

e) Function panel. Most instrument and software functions are controlled under each individual tabs e.g. Browser, Acquisition, Info, and Display.

f) Data manipulating commands. Depending on which tab is selected in **g)** Data panel, various cursors, manipulations, stackings, mapping areas, etc. will be available.

g) Data display panel. The Raman spectra, image, or mapping data files are displayed here depending on the tab being selected at up-left position. Active data file is highlighted at top-right position.

h) Current instrument status. Proceed any new command with 'Ready' or blank status.

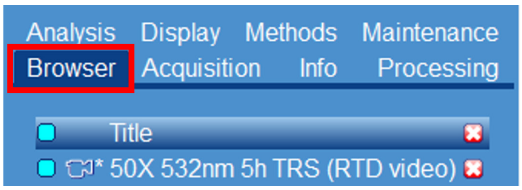
i) Instrument parameter control. Click to change parameters. Notice: this also automatically changes the parameters under **e)** Function panel – Acquisition tab. From left to right: detector status, grating status, laser status, laser switch, ND filter status, AE—Auto Exposure, AF—Auto Focus, DN—Denoiser, ICS—Intensity Correction, SR—Spectral Range, AC—Auto Calibration, Progress bar.

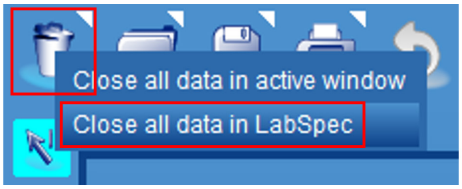
j) Cursor information. Depending on which cursor is selected in **6)** Data manipulating commands and which tab is selected in **7)** Data panel, information of the cursor e.g. Raman shift and intensity, x and y position will be displayed here.

2. Initialization of LabSpec6

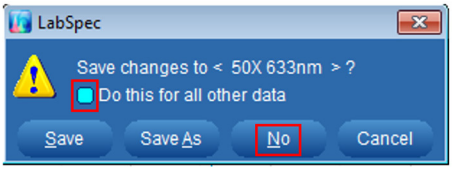
In area **e)**, select 'Browser' tab to see all the files opened/collected in LabSpec6.

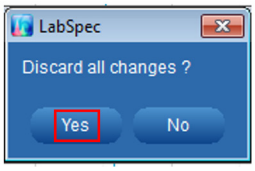
If there are files open, in area **a)**, right-click 'Close' icon and select 'Close all data in LabSpec'.





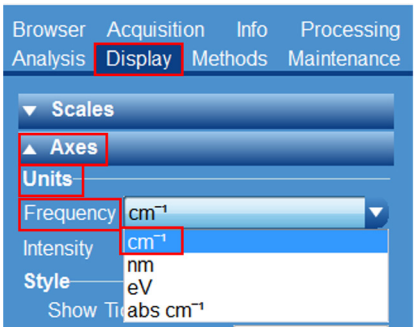
If a check window pops out, check 'Do this for all other data', then click 'No', then 'Yes'.





In area **e)**, select 'Display' tab, 'Axes', 'Units', 'Frequency', verify that '**cm^{-1}**' (Raman shift) is selected.

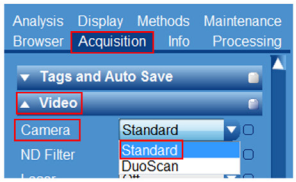
Notice: other frequency units can interfere Auto Calibration.

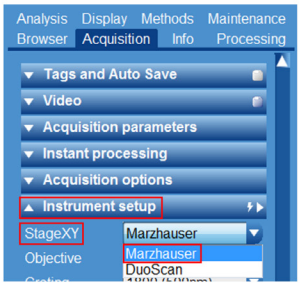


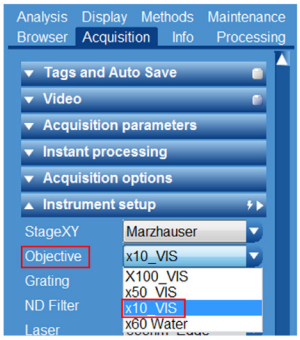
Select 'Acquisition' tab, proceed to 'Video', 'Camera', verify that '**Standard**' is selected.

Proceed to 'Instrument setup', 'StageXY', verify that '**Marzhauser**' is selected.

Proceed to 'Instrument setup', 'Objective', verify that '**x10_VIS**' is selected. Notice: always update the objective selection here to match with the current objective lens in use, which updates the scale bar of microscopic image and affects the flat-field correction.







In area **i)**, verify that 'Detector' is green. **Report to the instrument manager immediately** if it is orange or red, which indicates detector errors and the instrument must be inspected.

Verify that 'AE', 'AF', 'DN', 'ICS' are not highlighted, and 'SR' is highlighted, as shown below. Click the corresponding area to change them accordingly.

Auto calibration

During auto calibration, a **silicon (Si) wafer** is used as standard reference for calibrating all later measurements. Users can choose not to perform auto calibration if peak position accuracy isn't concerned.

In a 5-hour monitoring of Si 520cm^{-1} peak, the $\Delta_{\text{Raman shift}}$ is 0.63 cm^{-1} for 633nm laser and 0.99 cm^{-1} for 532nm laser. Both correspond to **2 pixels** of the CCD detector, and behave as only random shifts.

Users are suggested to select the laser and calibration interval based on their needs.

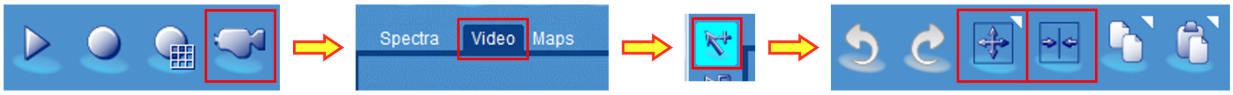
1. Operating the microscope to focus on Si wafer

In area **c)**, click 'Start video acquisition' icon, wait to hear a low-frequency motorizing noise.

In area **g)**, verify that 'Video' tab is selected to show live image.

In area **f)**, select the topmost 'point spectrum cursor'.

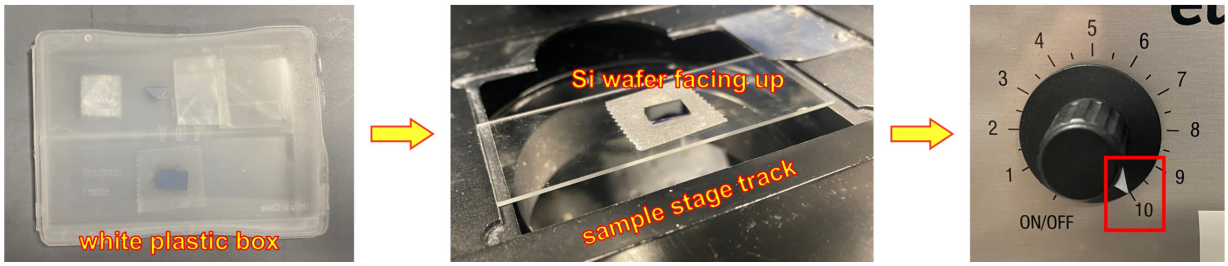
In area **b)**, click 'Rescale' icon, then click 'Initialize cursors' icon.



Find the microscope slide attached with standard Si wafer in a white plastic box.

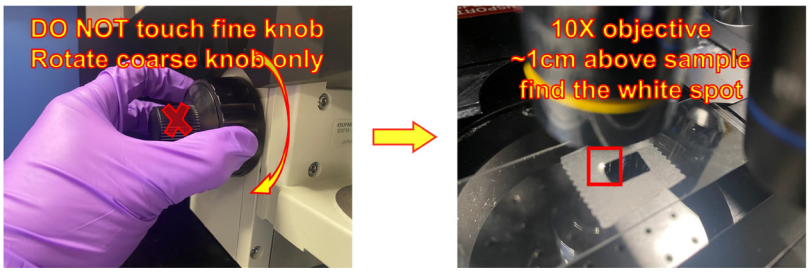
Secure the slide in the sample stage track with the Si wafer facing up.

Turn on the white lamp controller to **10** intensity.



Rotate the coarse adjustment knob clockwise until objective is **~1 cm** above the sample surface.

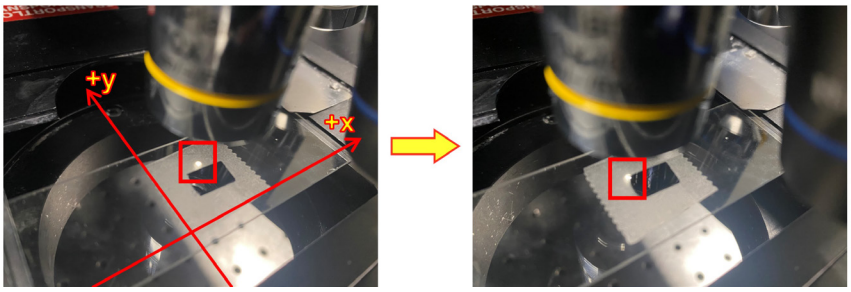
Locate the white spot nearby. **Warning:** DO NOT touch the fine adjustment knob.



Use the joystick controller to move the sample stage by pushing the joystick towards desired directions.

Notice: the joystick always returns to neutral position; its speed is controlled by your input amplitude.

Move the stage so that the white spot is at a corner of the Si wafer.



Adjust the white lamp intensity so that the live imaging is properly exposed. Unclear live image shall be displayed now.

Move the sample stage while looking at the live imaging so that white area (reflective Si surface) and black area (tape surface) are both shown. Notice: steadily control the joystick; **push-then-hold** and see how the stage responses.

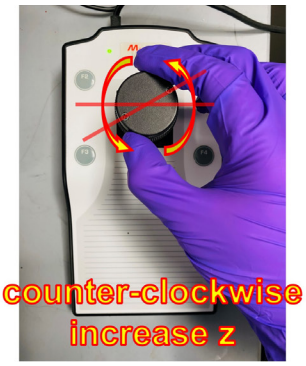
Warning: stop proceeding if the live imaging seems not responsive to the sample stage movement. Refer to Live imaging slow to response section and resolve the issue.

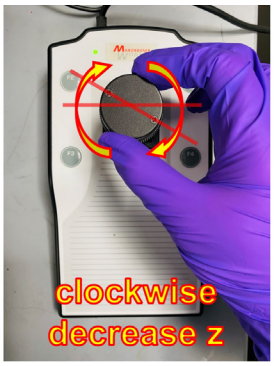
Rotate the coarse adjustment knob while looking at the live image to focus on Si with clear image.

Move the sample stage so that the center of image is on Si.

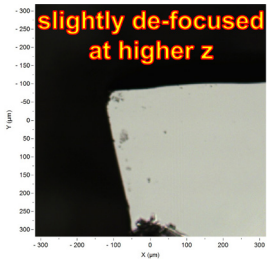
Use the joystick controller to finely control the Z height by rotating the joystick counter-clockwise or clockwise. Notice: the joystick always returns to zero angle; its speed is controlled by your input angle.

Steadily control the joystick; **rotate-then-hold** and see how the stage responses.



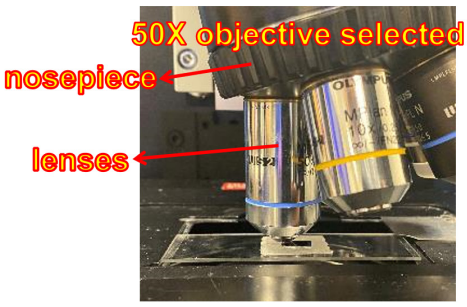


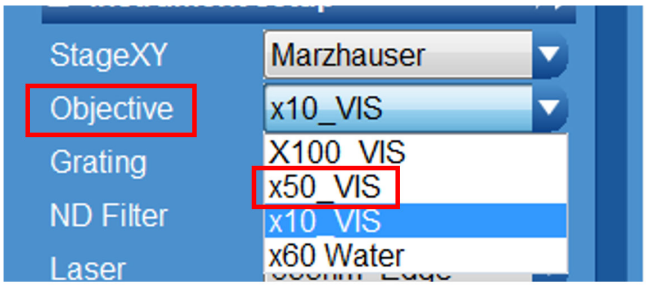
Rotate the joystick counter-clockwise by a small angle (~15°) for ~1 sec from the best focus. This slightly increases Z height to prevent objective damage. The live image appears slightly blurred.



Switch to 50X objective. Notice: **not** 50X-LWD. **Warning:** do not touch lenses; handle the nosepiece only. **Warning**: Stop proceeding with any sign of collision; return to 10X and verify if Si surface is focused.

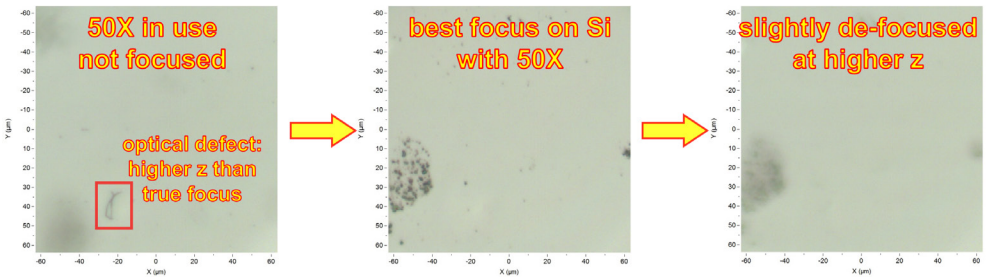
In area **e)**, select 'Acquisition' tab, Instrument setup, Objective, verify that x50 VIS is selected.





Rotate the joystick to focus on Si with clear image. **Warning**: do not adjust focus with the coarse adjustment knob for both 50X and 100X objective lenses. Notice: the optical defect may appear at slightly higher Z height than the true focus, so that it can be used as a reference.

Rotate the joystick counter-clockwise by a small angle (~15°) for ~1 sec from the best focus.



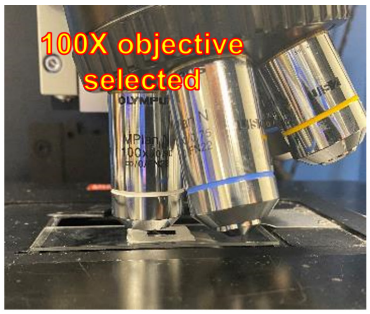
Switch to 100X objective. **Warning:** do not touch lenses; handle the nosepiece only. **Warning**: Stop proceeding with any sign of collision; return to 10X and verify if Si surface is focused.

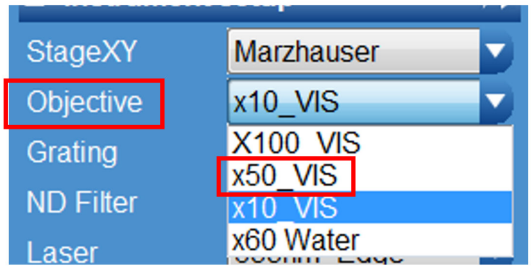
In area **e)**, select 'Acquisition' tab, Instrument setup, Objective, verify that x100_VIS is selected.

Adjust the white lamp intensity so that the live imaging is properly exposed.

Rotate the joystick to focus on Si with clear image.

Move the sample stage so that the center spot of image is on clean area.





2. Using laser spot to assist with focus

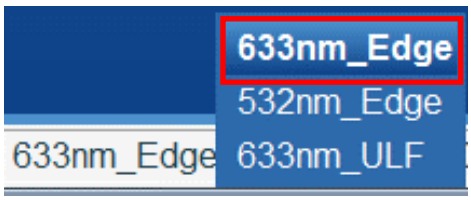
Some samples, e.g. Si, may be so clean and smooth that finding the focal plane becomes difficult with the white lamp. Users may use the laser spot to assist with focus whenever in need during video acquisition.

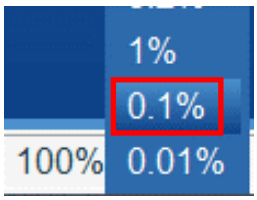
Turn down the white lamp to **~2** intensity.

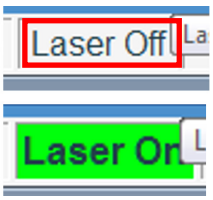
In area **i)**, click 'laser status' to select '**633nm_edge**'.

In area **i)**, click 'ND filter status' to select '**0.1%**'.

In area **i)**, click 'laser switch' and it turns into Laser On.

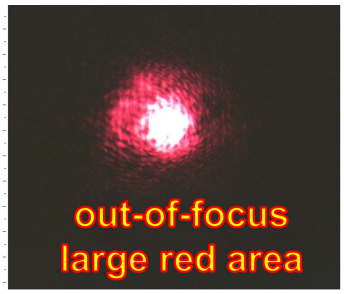


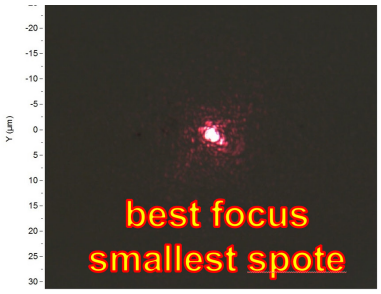


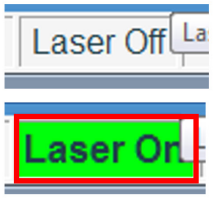


Rotate the joystick to adjust Z height and find the smallest laser spot. which corresponds to the best focus.

In area **i)**, click 'laser ON' and it turns into 'Laser Off'.





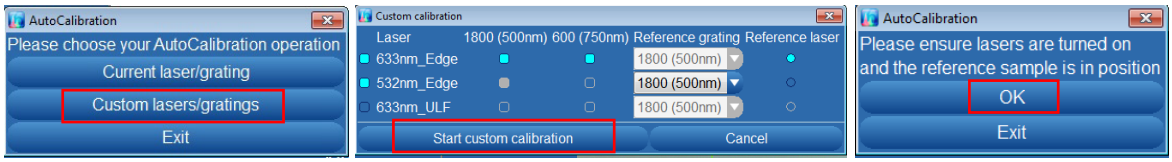


In area **d)**, click 'STOP' icon. In area **g)**, select 'Spectra' tab.

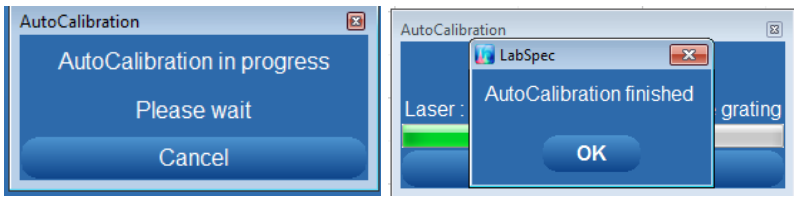
3. Performing Auto Calibration

Notice: users must verify that 100X objective is in use and focus is on Si surface.

In area **i)**, click 'AC'. In the popping window, click 'Custom lasers/gratings', then verify the check boxes are highlighted as shown below. Click 'Start custom calibration', then 'OK'. **Warning**: changing the checkboxes is a serious violation which results in access suspension.



The Auto Calibration program finishes in 7 minutes, and a final window will pop out with finished or failed confirmation. Click 'OK'. Notice: refer to Autocalibration failed section if Auto Calibration fails.

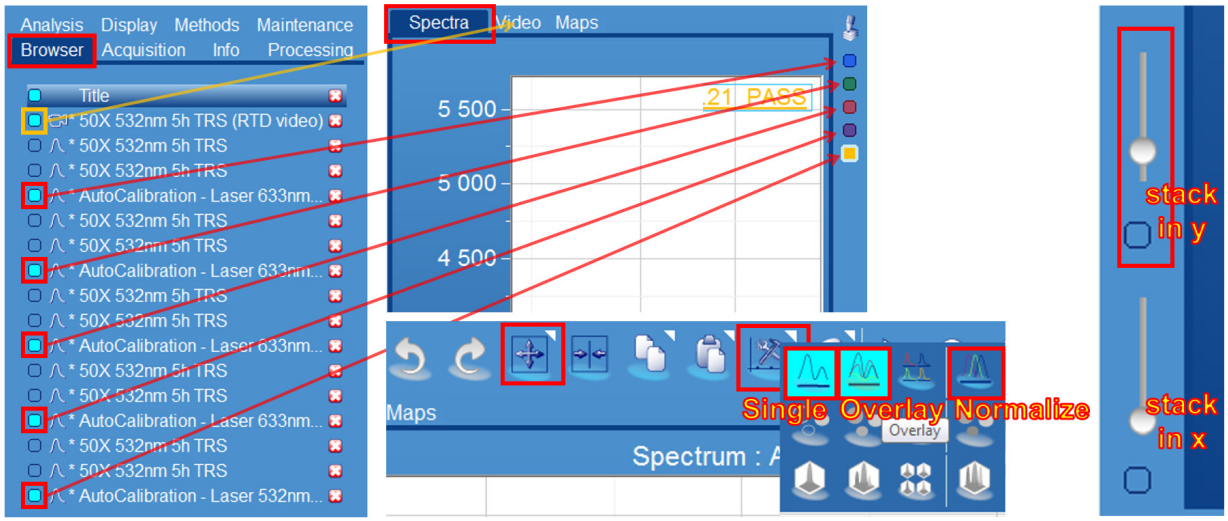


4. Displaying spectra and saving data

Notice: we ask users to save the 5 Auto Calibration data files generated in the Auto Calibration. The operating procedure below also applies to the Raman spectra data collected for your own samples.

In area **e)**, select 'Browser' tab to see all the files opened/collected in LabSpec6. The highlighted files correspond to the displayed spectra in area **g)** 'Spectra' tab shown in top right corner. The first highlighted file is image-type which only shows in area **g)** 'Video' tab.

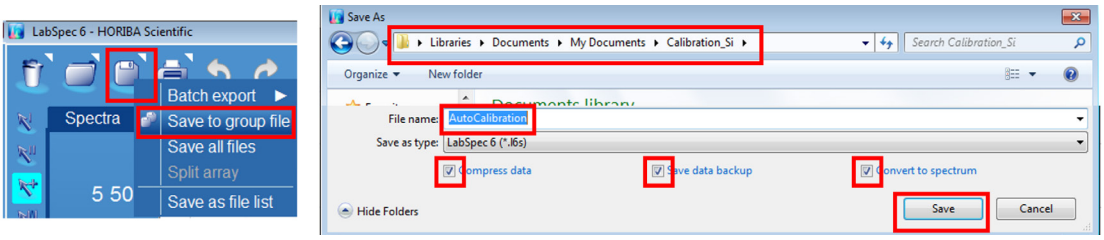
In area **b)**, move the cursor onto 'Config' icon to select between 'Single' or 'Overlay' modes of the displayed spectra. 'Normalize' can be used to normalize all displayed spectra into the same intensity scale; click 'Rescale' to automatically adjust spectral range to fit the currently displayed spectra. In area **f)**, the bottom two checkboxes allow users to manually stack and adjust spectra in x or y directions.



In area **a)**, right-click 'Save' icon and select 'Save to group file'. This saves all the highlighted Raman spectra data files in area **g)** Browser tab into a single Labspec6-compatible file.

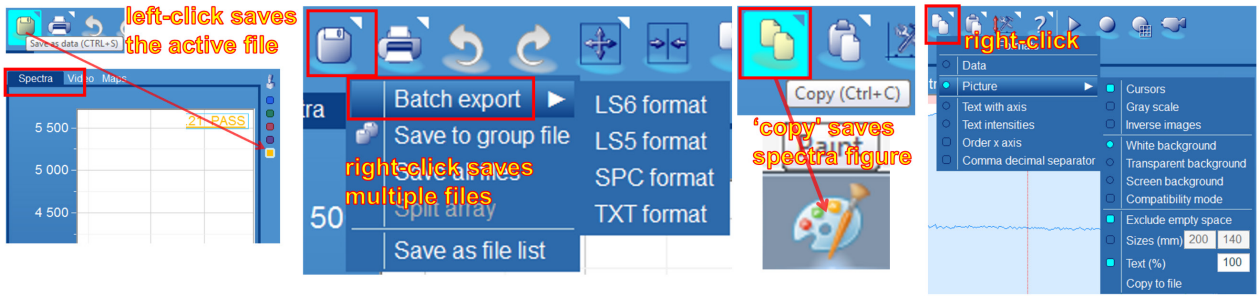
Locate the folder path as 'Documents → Calibration_Si', enter file name in the format as 'yyyymmdd' followed by 'your name initials' then 'your PI's name initials'.

Check the boxes 'Compress data', 'Save data backup', and 'Convert to spectrum', then click 'Save'.

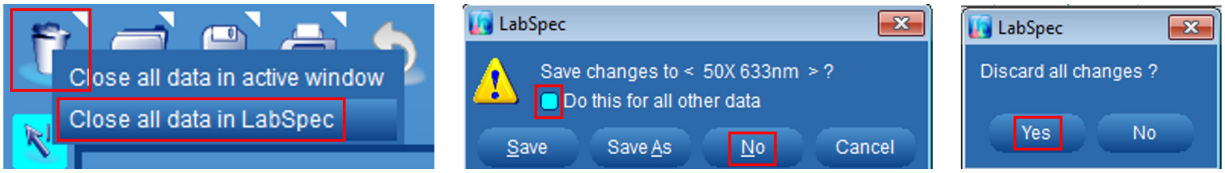


The ‘Save’ icon in area **a)** allows users to save Raman spectra in many different ways. For example, left click to save [the active spectrum] currently highlighted in the top right corner of area **g)** ‘Spectra’ tab; right-click it and select ‘Batch export’ to save [all the highlighted spectra files in ‘Browser’ tab] of area **e)**. Users may select different formats depending on their needs, e.g. ls6 (compatible with LabSpec6 software) or txt (compatible with excel, OriginLab, Prism etc.).

Users can also click ‘Copy’ icon in area **b)** to save the currently displayed area **g)** as a [figure] by pasting into ‘Paint’ software: Raman spectra figure (in ‘Spectra’ tab) or image (in ‘Video’ tab). Notice: right-click on ‘Copy’ icon to specify more format options, e.g. including cursor or not, background, resolution etc.



After saving the Auto Calibration files, <u>remove all the opened files by right-clicking ‘Close’</u> in area **a)**. If a check window pops out, <u>check ‘Do this for all other data’, then click ‘No’, then ‘Yes’.</u>



5. Retrieving the Si sample

Notice: This also applies to your own samples after finishing Raman measurements.

<u>Rotate the nosepiece to select the 10X objective</u>.

<u>Rotate the coarse adjustment knob counter-clockwise to the maximum Z height.</u>

Warning: before switching to the 10X objective, never operate the coarse adjustment knob.

Collecting Raman spectrum

1. Loading solid samples

There is very little requirement for processing the solid samples before measuring it.

The sample's height should be smaller than 2 cm when placed on the motorized sample stage.

The sample's top surface should be relatively smooth and flat to minimize the chance of ramming objective into sample.

It is highly recommended to load the sample onto a piece of microscope slide which has a clean Raman background and fits into the sample stage track in a perfectly horizontal way.

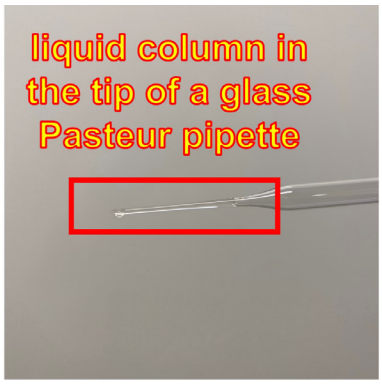
Focus on the top surface of the sample with the desired objective lens by referring to Operating the microscope to focus on Si wafer section. **Warning:** do not use 50X and 100X objectives for bulky samples with a top surface larger than 5×5 mm^2. Only use 10X or 50X-LWD for such samples.

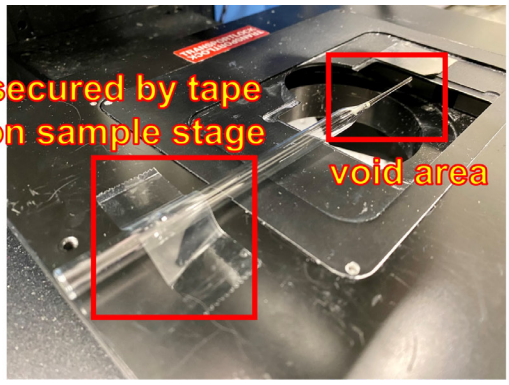
2. Loading liquid samples

Liquid can be measured in a qualitative way by using glass tubes, e.g. Pasteur pipettes. Notice: only use glass material which has a clean Raman background.

Obtain a section of sample liquid column in a glass tube.

Secure the glass tube horizontally on the sample stage, so that the liquid column is in the void area. Warning: do not spill the liquid. Notice: wipe away liquid spills immediately.



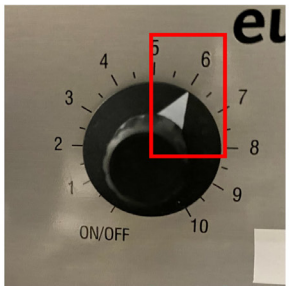


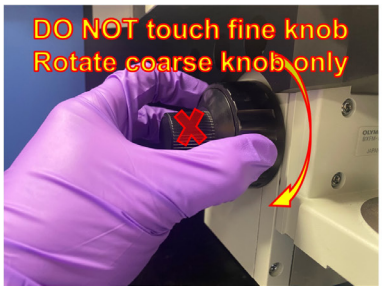
Select 10X objective, and adjust the white lamp intensity to **~6**.

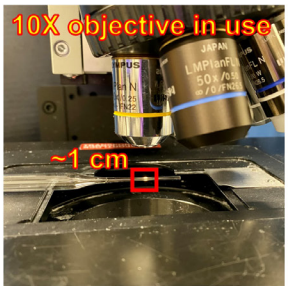
In area **e)**, select 'Acquisition' tab, Instrument setup, Objective, verify that 'x10 VIS' is selected.

Rotate the coarse adjustment knob clockwise until objective is **~1 cm** above the glass tube.

Use the joystick to move the sample stage so that the bright spot falls on the liquid column section.



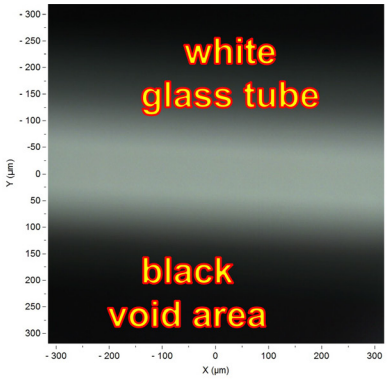


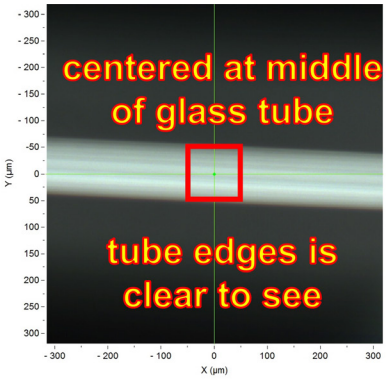


Unclear live image shall be displayed in LabSpec6 – Video now. White areas indicating the glass tube and black areas indicating the void area. If nothing can be observed, use the joystick to further move the sample stage around, and rotate the coarse adjustment knob back and forth while looking at the live image.

Rotate the coarse adjustment knob to focus on the glass tubing edges, i.e. edges are sharp and clear to see.

Use the joystick to center at the middle position of glass tube.





3. Collecting Raman spectrum with selected acquisition parameters

Different samples have different Raman responses. Therefore, users are recommended to optimize the acquisition parameters with an overall consideration of signal intensity, photo damage, and work efficiency.

Notice: signal intensity is severely decreased when de-focused. Always ensure the center of live video, where laser spot hits, is focused. Refer to Activating Auto Focus section if interested in performing Auto Focus.

In area **d)**, click 'STOP' icon. In area **g)**, select 'Spectra' tab.

In area **e)**, select 'Acquisition' tab. Proceed section-by-section as described below.

Warning: do not modify parameters without knowing their meanings.

1) 'Tags and Auto Save'

Enter your sample name in the 'Title' blank. Notice: the instrument will automatically rename newly collected Raman spectra if 'Title' is not changed.

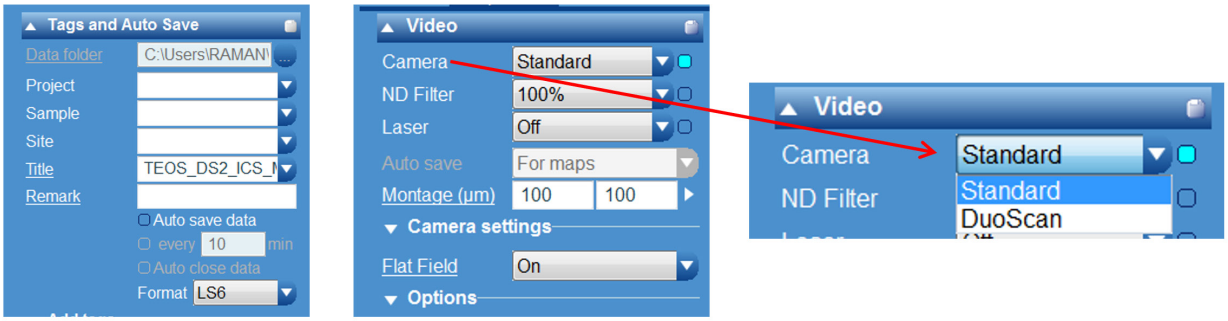
Check the 'Auto save data' box if you prefer to. Specify the saving location to your own folders in 'Data folder', then select the format based on your needs.

Notice: you will be responsible for manually saving the desired data files afterwards if 'Auto save data' box isn't checked. Please refer to Displaying spectra and saving data section.

2) 'Video'

Nothing needs to be changed under this tab.

Notice: 'Camera' selection must be correlated with the DuoScan push/pull rod position described in Hardware Control: Overview and Inspections → Inspection of the hardware section.

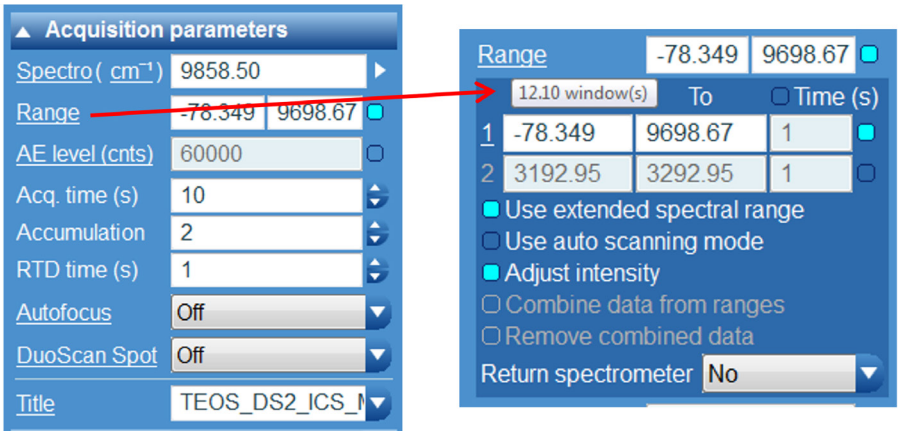


3) 'Acquisition parameters'

Enter the spectral range (cm^{-1}) in the 'Range' blanks. Notice: move the mouse cursor to 'Range' so that it indicates how many windows the measurement will cover. Notice: click 'Range' to open the secondary configuration, which enables multiple spectral ranges.

Enter 'Acq. Time (s)' and 'Accumulation' blanks. Notice: total acquisition time = window number × time × accumulation.

Verify that ‘Autofocus’ and ‘DuoScan Spot’ are OFF.



4) ‘Acquisition options’

Enter ‘Delay time (s)’. Notice: Raman scattering is a fast process upon laser excitation; 0 s applies to most materials unless to avoid thermal fluorescence interferences.

Nothing else needs to be changed under this tab.

5) ‘Instrument setup’

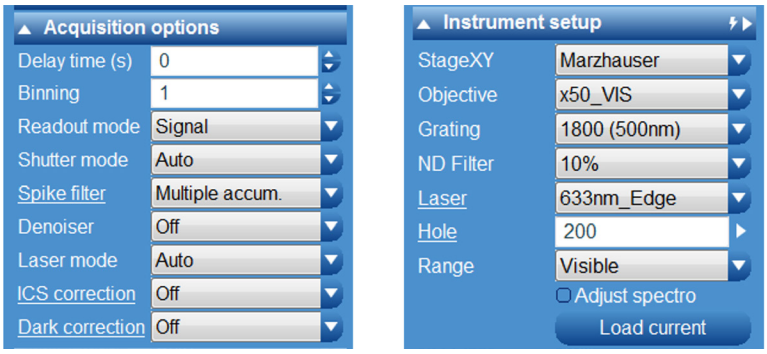
Select ‘StageXY’ as ‘Marzhauser’.

Select ‘Objective’ based on what is currently in use. Notice: this wouldn’t determine anything about measuring Raman spectrum, but it determines the Video image correction and scale bars.

Select ‘Grating’ between ‘600’ and ‘1800’. Notice: always use 600-grating first for the high signal intensity and fast scan time; use 1800-grating only when needed, e.g. to resolve overlapped peaks in a small spectral region.

Select ‘ND Filter’ to determine the laser intensity. Notice: always try laser power as high as possible (without photo damage) to ensure signal intensity and efficiency.

Enter ‘Hole’ from 25 to 1000 (unit: μm) to determine the z-axle depth of field, then click ‘Enter’ key on the keyboard. Notice: Use 200 for general solid samples, 1000 for liquid samples described in Loading liquid samples section. Smaller numbers narrow the z-range of signal collection centered at focal plane, but also reduces the signal intensity.



In area **c)**, click 'Start spectrum acquisition' icon. The Raman spectrum measurement will be conducted.

Notice: the measurement can be stopped by clicking 'STOP' icon in area **d)**. The measured data will still be kept in area **e)** 'Browser' tab.

4. Performing photoluminescence(PL) measurement

In area **e)**, 'Display' tab – 'Axes' – 'Units' – 'Frequency', select 'nm' (wavelength) or 'eV' (photon energy) based on your need.

In area **e)**, 'Acquisition' tab – 'Axes' – 'Acquisition parameters' – 'Range', enter the spectral range in the blanks. Notice: the unit is automatically updated to your selection in the 'Display' tab.

Refer to Collecting Raman spectrum with selected acquisition parameters section for setting up other parameters.

In area **c)**, click 'Start spectrum acquisition' icon. The Raman spectrum measurement will be conducted.

Notice: the measurement can be stopped by clicking 'STOP' icon in area **d)**. The measured data will still be kept in area **e)** 'Browser' tab.

Performing Raman mapping

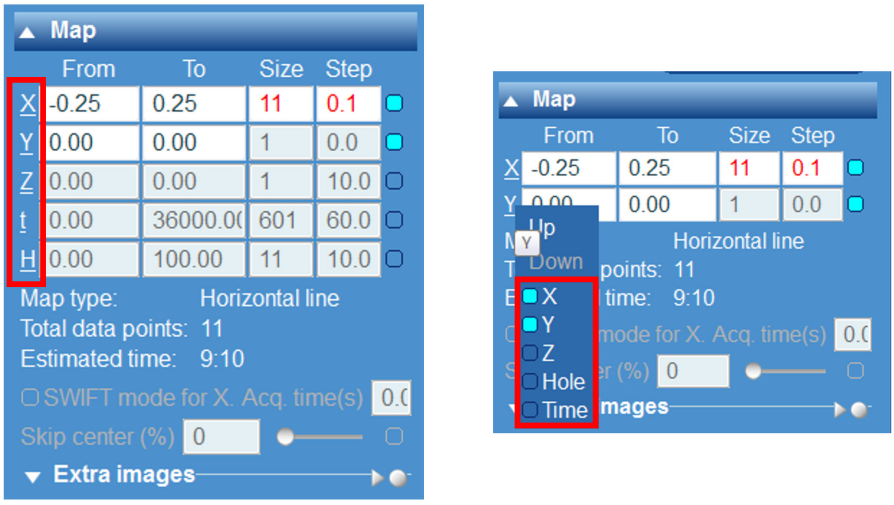
This function enables chemical contrast imaging, depth profiling, or time-based measurements.

Focus on the interested sample area with desired objective by referring to Operating the microscope to focus on Si wafer section. **Warning:** do not use 50X or 100X objective to map tilted samples or rough sample surfaces with more than 100-μm z-variance.

Set up the acquisition parameters by referring to Collecting Raman spectrum with selected acquisition parameters section. Notice: these parameters determine the point-by-point Raman measurements. Always optimize the parameters to save the time of mapping.

In area **e)**, select 'Acquisition' tab, then 'Map'. Notice: there should be 5 dimensions, 'X', 'Y', 'Z', 't'(time), and 'H'(Hole) to define the mapping range, which can be freely combined for multiple-dimension mapping.

Click on any existing dimension to highlight all the dimension boxes if there are missing ones.



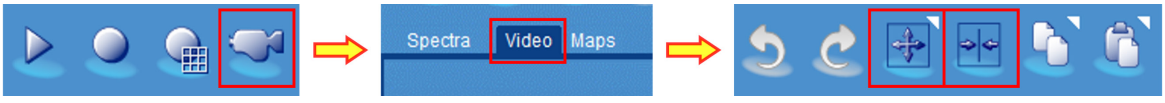
1. 1-D or 2-D mapping on X-Y plane

Notice: it is possible to perform X-Y plane mapping with DuoScan function, which will be described in Activating DuoScan section.

In area **c)**, click 'Start video acquisition' icon.

In area **g)**, select 'Video' tab to show live image.

In area **b)**, click 'Rescale' icon, then click 'Initialize cursors' icon.



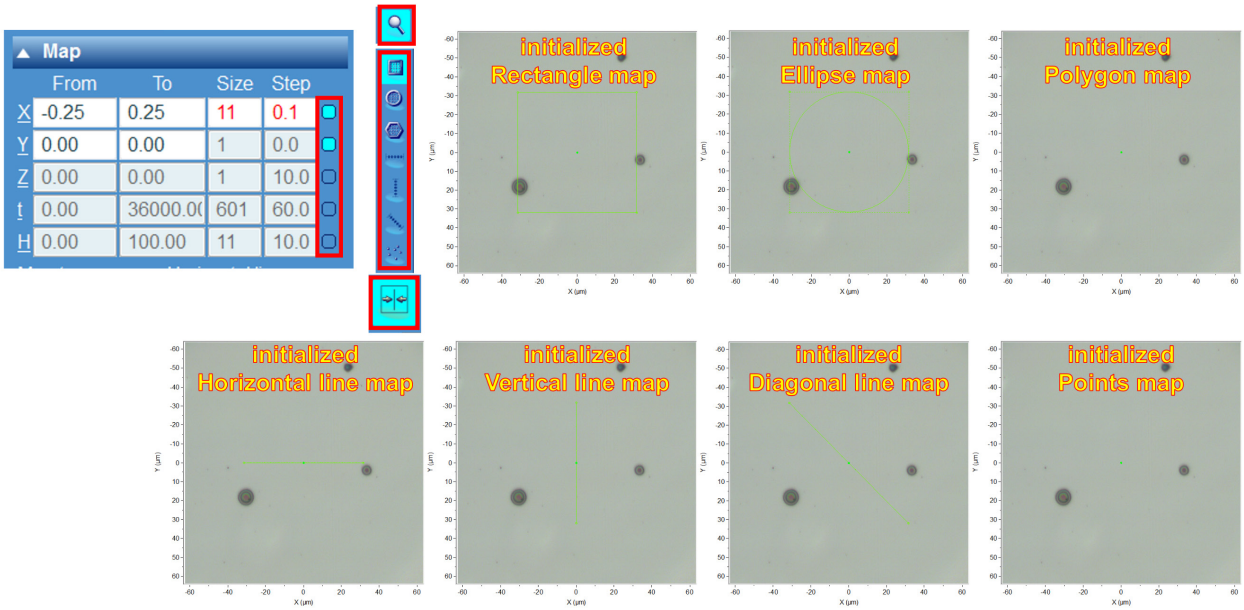
Adjust the white lamp intensity for proper brightness of the live imaging.

In area **e)**, 'Acquisition' – 'Map', select both X and Y dimensions, and uncheck all other dimensions.

In area **f)**, select 'Zoom' command to zoom in the interested area if needed.

In area **f)**, select one of the seven mapping commands. From top to bottom are: 'Rectangle map', 'Ellipse map', 'Polygon map', 'Horizontal line map', 'Vertical line map', 'Horizontal line map', 'Diagonal line map', 'Points map'.

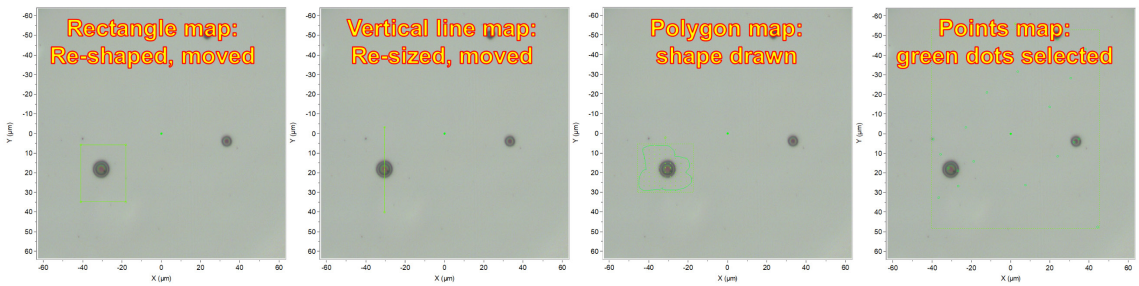
In area **b)**, click 'Initialize cursors' icon. Notice: this initializes the map command cursor based on your zoomed area.



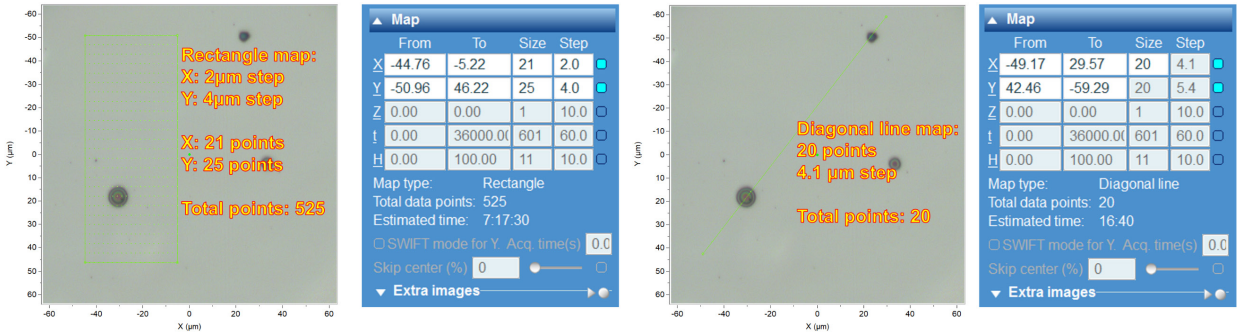
Adjust the mapping range and area accordingly. Notice: drag (click and hold) the corner/edge of the area/line to re-shape it; drag other areas to move the mapping area. This adjustment is also automatically reflected in area **e)**, 'Acquisition' – 'Map' – X and Y ranges.

For 'Polygon map', draw the shape by clicking then holding the mouse across the interested area.

For 'Points map', Manually select the interested positions by clicking on the image.



In area **e)**, 'Acquisition' – 'Map', enter the 'Size' or 'Step' blanks of 'X' and 'Y' dimensions. Notice: these two parameters are mutually determined: Size (how many points on this dimension) × Step (interval in μm between points) = range on this dimension. The smallest step, i.e. highest resolution is 0.1 μm determined by the motorized XY stage.



In area **c)**, click 'Start map acquisition' icon. The Raman mapping measurement will be conducted.

Notice: the measurement can be stopped halfway by clicking 'STOP' icon in area **d)**. The already measured mapping data will be kept.

2. Depth profiling on Z axis

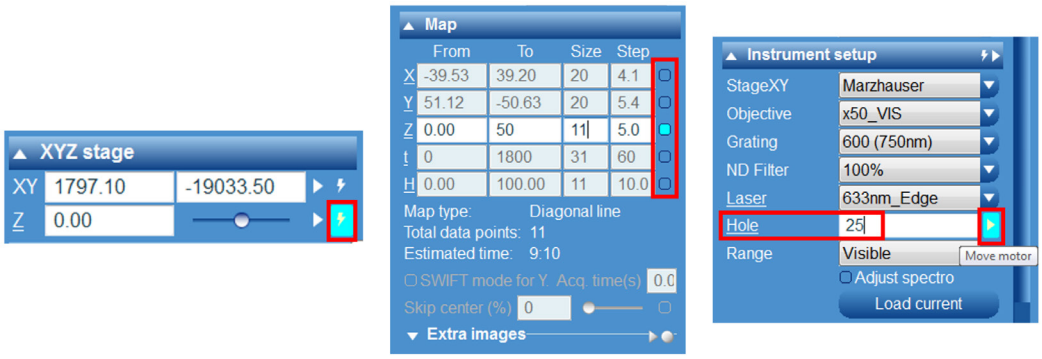
The Raman mapping function enables depth profiling along Z-axis.

Focus on the interested sample area with the desired objective by referring to Operating the microscope to focus on Si wafer section. Notice: depth profiling is only possible for visible-light transparent samples.

In area **e)**, 'Acquisition' – 'XYZ stage', click 'Set Z stage origin'. **Warning**: failure to initialize Z origin may cause damage to the objective lens.

In area **e)**, 'Acquisition' – 'Map', check 'Z' dimension only, then specify the range ('From' and 'To'), 'Size' or 'Step' of 'Z' dimension. Notice: the units are 'μm' for range and 'Step'. Negative value means higher Z height; positive value means lower Z height. **Warning**: to prevent damaging the objective lens, never enter a positive value greater than 280(μm) for 50X, or 110(μm) for 100X objective.

In area **e)**, 'Acquisition' – 'Instrument setup', enter a small number for 'Hole' (25~100 preferred; unit: μm), then click 'Move motor' or 'Enter' key on the keyboard. Notice: smaller Hole values e.g. 25 μm increases axial resolution (to < 1 μm) with the trade-off of signal intensity, which requires longer acquisition.



In area **c)**, click 'Start map acquisition' icon. The depth-profiling measurement will be conducted.

Notice: the measurement can be stopped halfway by clicking 'STOP' icon in area **d)**.

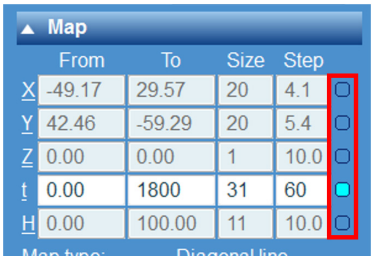
Warning: stop the measurement immediately for any sign of hitting the objective into samples.

3. Time-resolved repeated measurement

The Raman mapping function also enables repeated Raman measurement with fixed time interval.

In area **e)**, 'Acquisition' – 'Map', check 't' dimension only, then specify the range ('From' and 'To'), 'Size' or 'Step' of 't' dimension. Notice: the units are second for range and 'Step'.

Verify that the t step i.e. interval between Raman measurements is larger than the needed time for a single Raman measurement.



In area **c)**, click 'Start map acquisition' icon. The time-resolved Raman measurement will be conducted.

Notice: the measurement can be stopped halfway by clicking 'STOP' icon in area **d)**.

4. Multiple-dimension Raman mapping

The mapping dimensions described above can be freely combined to perform multi-dimensional mapping for samples. For example, combining X, Y, and Z dimensions enables 3-D Raman mapping for the sample; combining X, Y, and t dimensions enables time-resolved Raman monitoring of the X-Y plane; combining Z and H dimensions enables analyzing the effect of Hole size on Depth profiling.

If the 't' dimension is used, verify that the t 'Step' is larger than the needed time for the Raman mapping of all other dimensions.

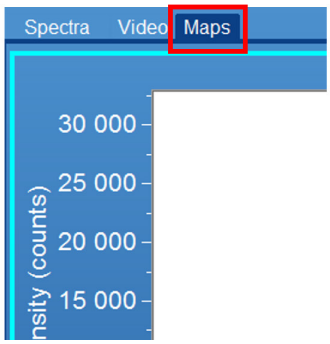
In area **c)**, click 'Start map acquisition' icon. The desired Raman mapping will be conducted.

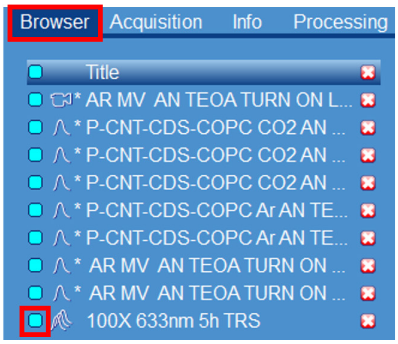
Notice: the measurement can be stopped halfway by clicking 'STOP' icon in area **d)**.

5. Displaying mapping data and basic data processing

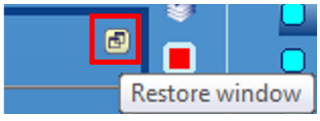
In area **g)**, select 'Map' tab.

In area **e)**, select 'Browser' tab, then highlight the interested map datafile. Notice: only mapping-type datafile will be displayed in 'Map' tab.



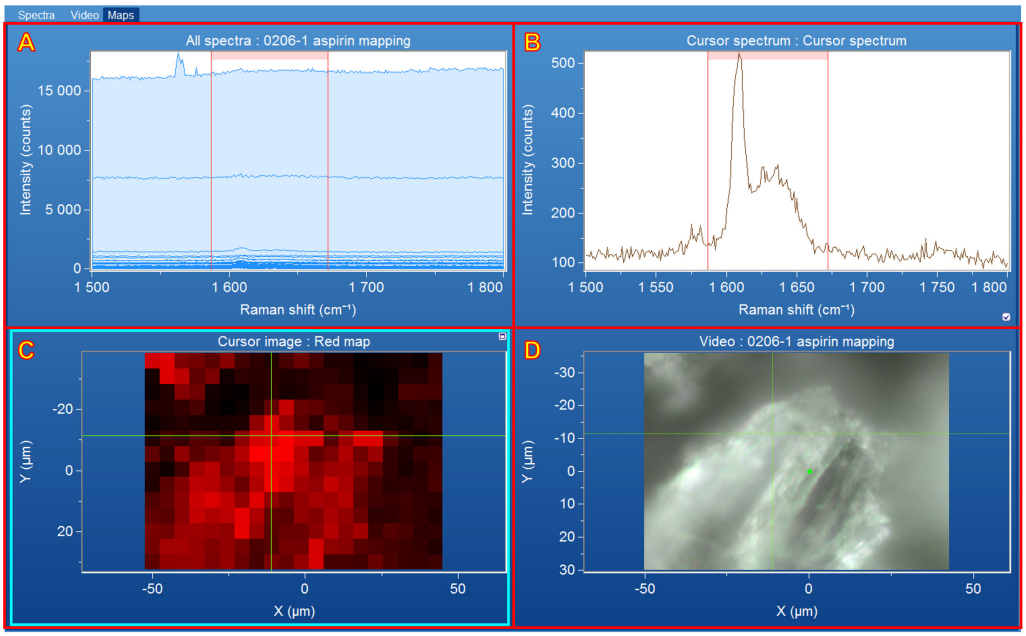


Click on 'Restore window' in the top-right corner of area **g)** if only one window is displayed.



By default, there are 4 windows displayed in the 'Maps' tab of area **g)**.

Click on the specific window to highlight it, which enables respective commands from area **f)**.



Window **A**: all Raman spectra collected in the mapping datafile.

Window **B**: the Raman spectrum (or averaged multiple spectra) from cursor selection in window **C**.

Window **C**: Raman map figure. Various Raman characters can be plotted using three cursors, red, blue, and green in window **A** or **B**.

Window **D**: microscopic image of the mapped area.

In area **g)**, select 'Video' tab.

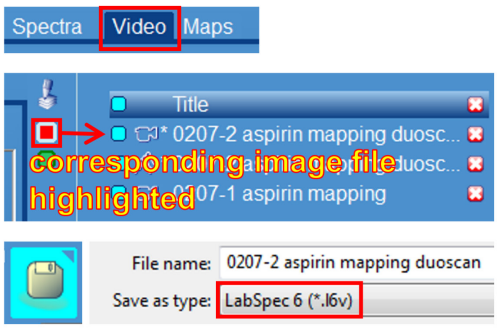
Verify that the corresponding image file is highlighted at the top-right corner in area **g)**.

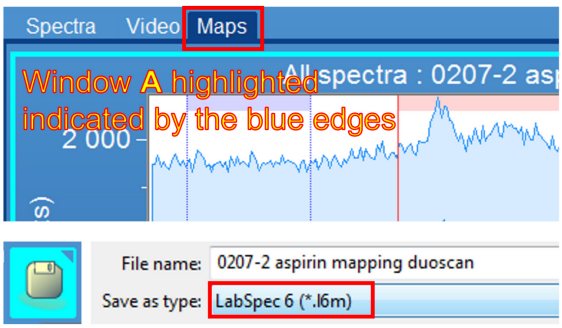
Click 'Save' icon in area **a)** to save the image datafile in.l6v format.

In area **g)**, select 'Map' tab, highlight window **A**.

Click 'Save' icon in area **a)** to save the mapping datafile in.l6m format.

Notice: both the image and mapping datafiles must be saved as described above, to ensure that the mapping data file can be re-opened and processed by LabSpec6 in the future.



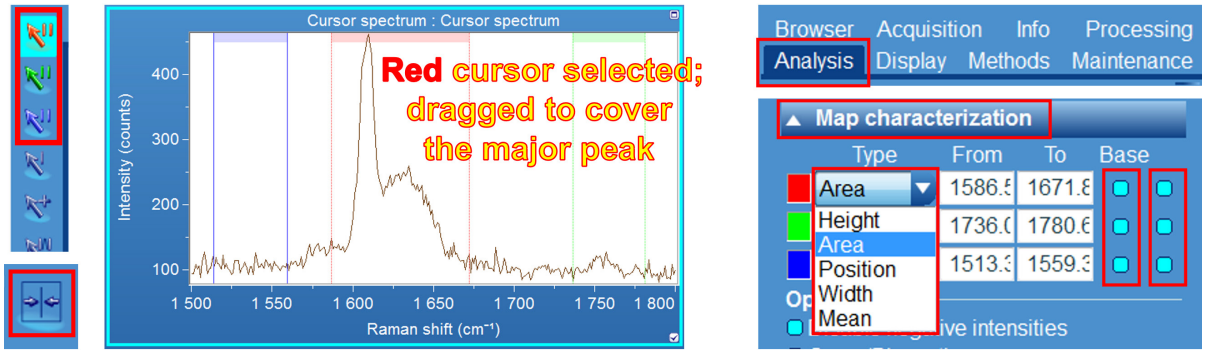


Highlight window **A** or **B**, then select one colored cursor in area **f)**.

Click 'Initialize cursors' icon in area **b)**.

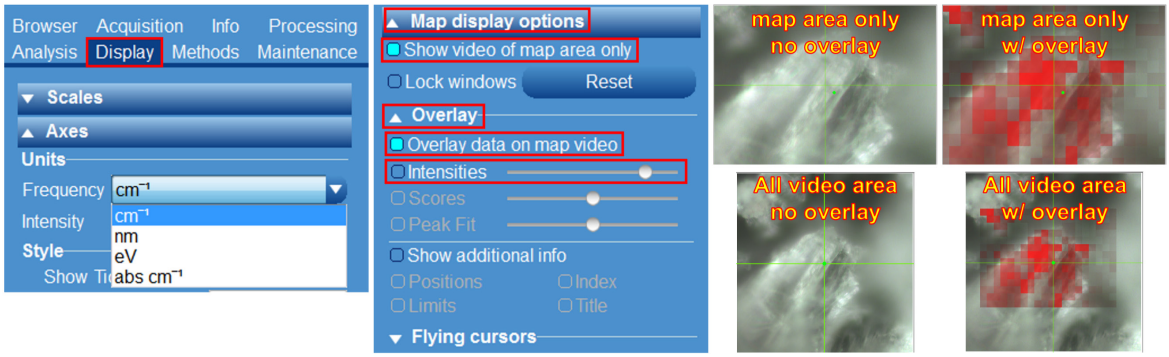
Drag this colored cursor in window **A** or **B** to cover the interested Raman peak area.

In area **e)**, select 'Analysis' tab, 'Map characterization'. Notice: each colored cursor can be specified to analyze 'Height', 'Area', 'Position', 'Width', 'Mean' of the selected range; 'Base' box determines whether a baseline is applied during the analysis; the rightest-row box determines whether this colored cursor is displayed or hidden. The result will be automatically reflected in window **C** (and **D**).



The colored mapping in window **C** (and **D**) is determined by these colored cursors in window **A** or **B**, and they can be displayed in either single or overlay modes, similar to displaying spectra by modifying 'Config' icon in area **b)**.

In area **e)**, select 'Display' tab, 'Map display options'. 'Show video of map area only' box rescales window **D** between full image or mapped area; select 'Overlay' to expand its secondary list: 'Overlay data on map video' and 'Intensity' boxes blend window **C** into **D** at desired intensity.



Activating Auto Focus

The Auto Focus function relies on depth profiling along Z-axis to determine the Z height of highest signal intensity. It can be performed manually or automatically. Notice: Auto Focus is most effective for reflective or Z-defined samples e.g. Si wafer and monolayer 2-D materials. **Warning**: improper use of Auto Focus may lead to objective lens damage.

1. Manually performing Auto Focus

Notice: always ensure a good visual focus with the white lamp before performing Auto Focus.

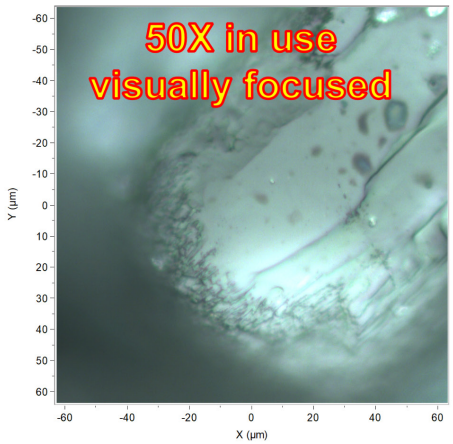
Focus on the interested sample area with desired objective by referring to Operating the microscope to focus on Si wafer section.

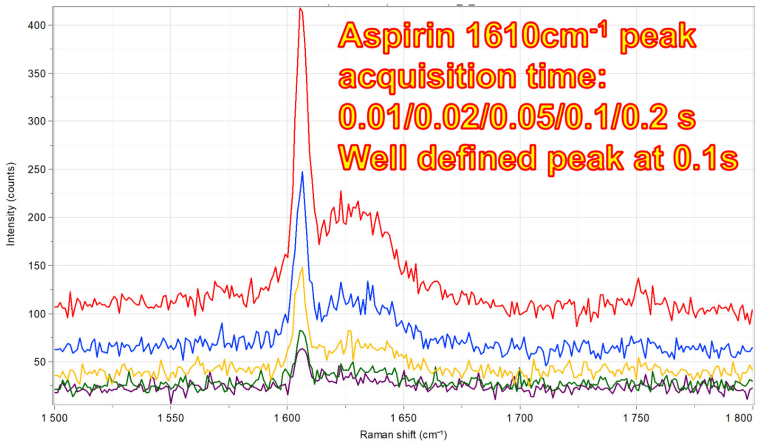
In area **e)**, 'Acquisition' – 'XYZ stage', click 'Set Z stage origin'.

Perform trial Raman measurements by referring to Collecting Raman spectrum with selected acquisition parameters section.

Find the major Raman peak of the sample (e.g. 520cm^{-1} peak of Si, 1610cm^{-1} peak of Aspirin).

Optimize the acquisition parameters, so that the major Raman peak is detected with shortest acquisition time possible. Notice: set 'ND Filter' at 100%, 'Accumulation' at 1, then perform trial spectrum measurements with increasing acquisition time from 0.01(s) until the peak becomes well defined.





In area **e)**, 'Acquisition' – 'Acquisition parameters', click 'Auto Focus' to expand its settings.

Select 'Spectral' in the 'Type' options.

Enter 'Z limits&step' blanks. The three blanks from left to right: 'max Z height', 'min Z height', 'step' in μm unit. Notice: -250, 250, 50 recommended for 10X; -50, 50, 10 recommended for 50X; -25, 25, 5 recommended for 100X. **Warning**: to prevent damaging the objective lens, never enter a positive value greater than 280(μm) for 50X, or 110(μm) for 100X objective.

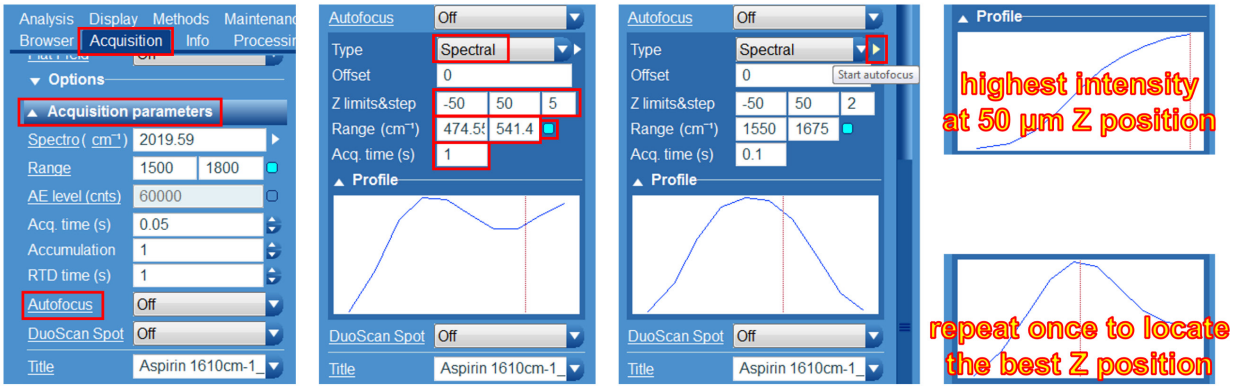
Enter Range (cm^{-1}) blanks to cover the major Raman peak range. Notice: check the box to enable 'Range' if the blanks are deactivated.

Enter Acq. time (s) blank based on the previously optimized results.

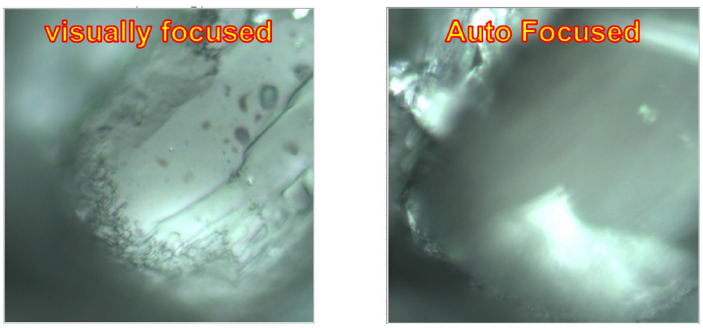
Click the 'Start autofocus' triangle. The Auto Focus is manually performed once.

The Z-profile will shortly be displayed/updated in 'Profile', and Z height will be automatically adjusted to the highest intensity position.

Auto Focus may be repeatedly performed if the highest-intensity position is out of the range. **Warning**: monitor the Z position in area **e)** 'Acquisition' – 'XYZ stage' and never let it become greater than 280(μm) for 50X, or 110(μm) for 100X objective.



The Auto-Focused Z height is not necessarily the same as visual focused Z height.



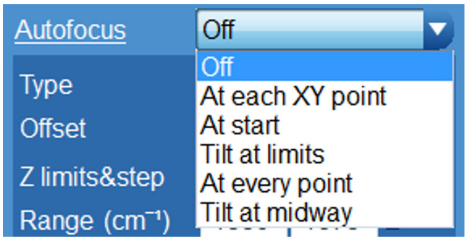
2. Enabling Auto Focus for Raman mapping

The Auto Focus can be coupled with Raman mapping to ensure that the whole X-Y plane is mapped within focus for the best signal intensity.

Refer to the previous Manually performing Auto Focus section to adjust the parameters of 'Auto Focus'.

Notice: The 'Acq. Time' in 'Auto Focus' must be adjusted accordingly to ensure the major Raman peak can be detected under 'ND Filter' parameter set for Raman mapping measurement.

Click 'Auto Focus' to select options based on mapping needs. For example, select 'At each XY point' for Raman mapping of the X-Y plane for improved focus and signal intensity; select 'At every point' for time-resolved repeated measurement for preventing focus drift during long-time measurement.



Activating DuoScan

The DuoScan enables three functions, i.e. Macro Spot, Stepping mode, and Macro Mapping mode.

1. Macro Spot

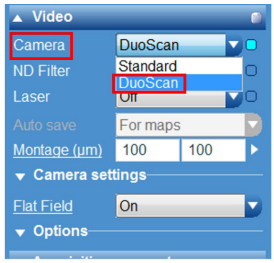
An equivalent large spot is created to lower the power density and cover larger area by continuously scanning the laser beam in the specified area by DuoScan (up to 100×100 μm^2).

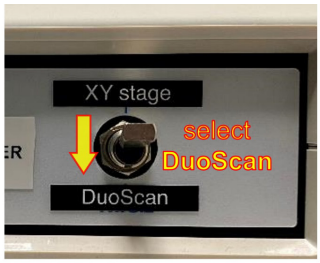
In area **e)**, 'Acquisition' – 'Video', select 'DuoScan' in 'Camera' options.

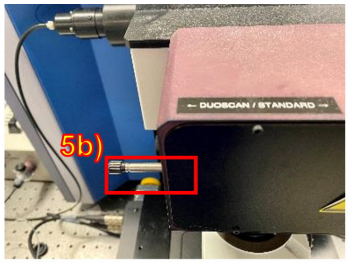
In area **e)**, 'Acquisition' – 'Instrument setup', select 'DuoScan' in 'Stage XY' options.

Push the right-side switch on the scanning controller downwards to select 'DuoScan'.

Gently pull out the DuoScan push/pull rod to 5b) DuoScan mode position. Notice: delicate optical elements; do not exert extra forces.







Focus on the interested sample area with desired objective by referring to Operating the microscope to focus on Si wafer section.

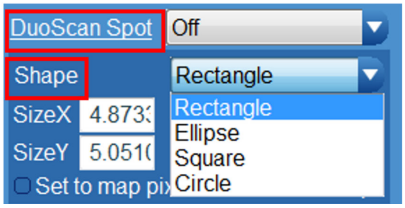
In area **e)**, 'Acquisition' – 'Acquisition parameters', click 'DuoScan Spot' to expand its settings.

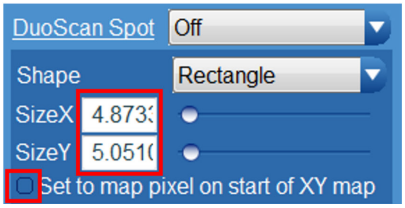
Select the scanning area in 'Shape' options.

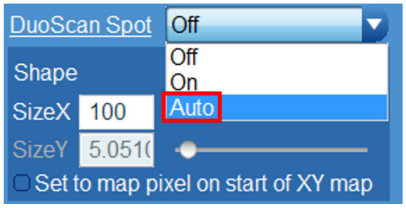
Define the X (and Y) range of the scanning area. Notice: the spot size will be in [-X/2, +X/2] X-dimension, and [-Y/2, +Y/2] in Y-dimension.

De-select the box 'Set to map pixel on start of XY map'.

Click 'DuoScan Spot' to select 'Auto'.







Define other acquisition parameters by referring to Collecting Raman spectrum with selected acquisition parameters section. Notice: use high laser intensity since the beam is dispersed into larger area.

In area **c)**, click 'Start spectrum acquisition' icon. The Raman spectrum measurement will be conducted in the defined 'Macro Spot' area.

2. Stepping mode

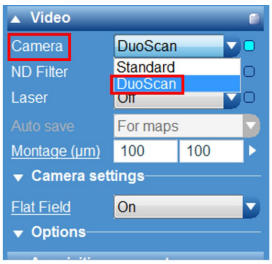
The laser beam moves point-by-point across the defined X-Y plane for Raman mapping, contrary to the standard Raman mapping which moves the sample stage.

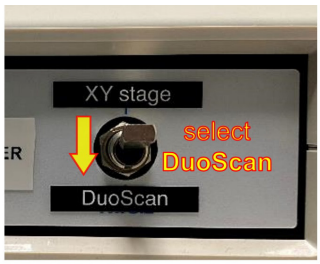
In area **e)**, 'Acquisition' – 'Video', select 'DuoScan' in 'Camera' options.

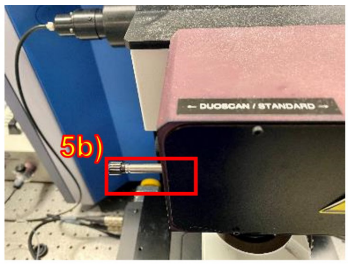
In area **e)**, 'Acquisition' – 'Instrument setup', select 'DuoScan' in 'Stage XY' options.

Push the right-side switch on the scanning controller downwards to select 'DuoScan'.

Gently pull out the DuoScan push/pull rod to 5b) DuoScan mode position. Notice: delicate optical elements; do not exert extra forces.

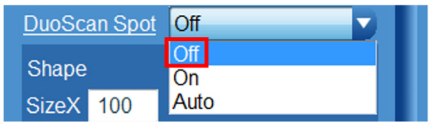






Focus on the interested sample area with desired objective by referring to Operating the microscope to focus on Si wafer section.

Click 'DuoScan Spot' to select 'OFF'.

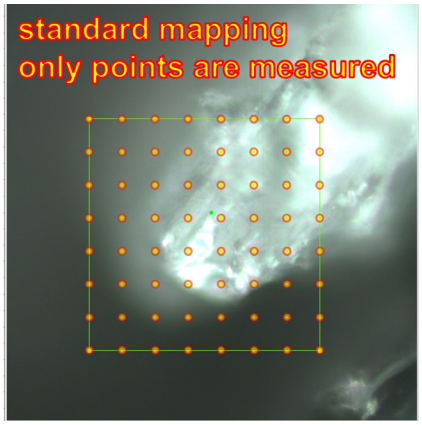


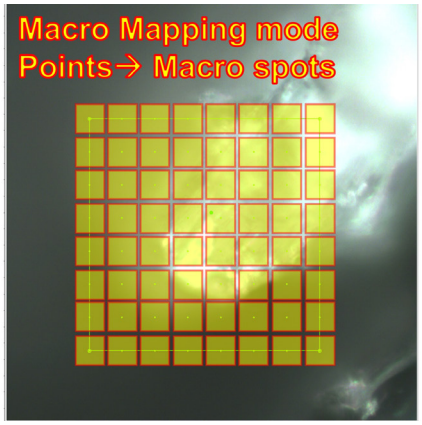
Define other mapping parameters by referring to 1-D or 2-D mapping on X-Y plane section.

In area **c)**, click 'Start map acquisition' icon. The DuoScan stepping mode will be conducted.

3. Macro Mapping mode

In standard Raman mapping of X-Y plane, the sample information between defined points is missing due to the small size of laser spot (usually less than 1 μm^2). This drawback can be mitigated with Macro Mapping mode enabled by DuoScan.



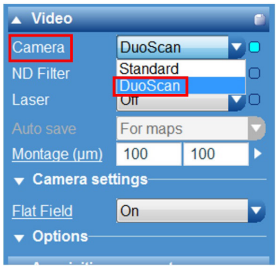


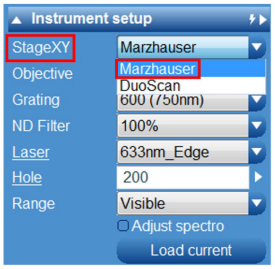
In area **e)**, 'Acquisition' – 'Video', select 'DuoScan' in 'Camera' options.

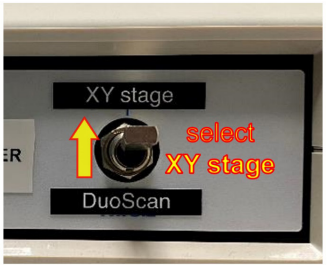
In area **e)**, 'Acquisition' – 'Instrument setup', select 'Marzhauser' in 'Stage XY' options.

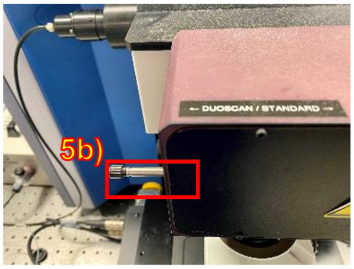
Verify the right-side switch on the scanning controller is selecting 'XY stage'.

Gently pull out the DuoScan push/pull rod to 5b) DuoScan mode position. Notice: delicate optical elements; do not exert extra forces.









Focus on the interested sample area with desired objective by referring to Operating the microscope to focus on Si wafer section.

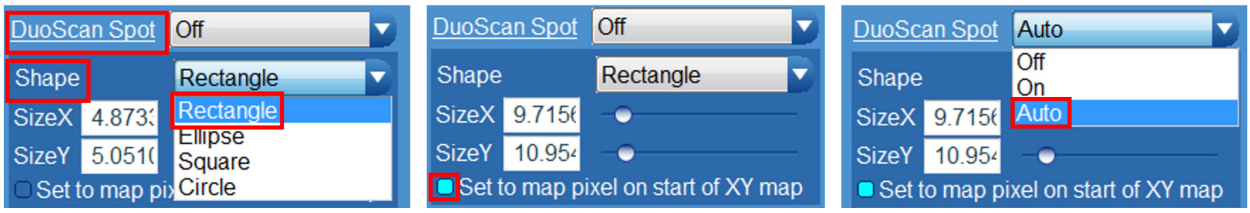
Define other mapping parameters by referring to 1-D or 2-D mapping on X-Y plane section.

In area **e)**, 'Acquisition' – 'Acquisition parameters', click 'DuoScan Spot' to expand its settings.

Select 'Rectangle' in 'Shape' options.

Select the box 'Set to map pixel on start of XY map'. Notice: no need to define 'SizeX' and 'SizeY'.

Click 'DuoScan Spot' to select 'Auto'.



In area **c)**, click 'Start map acquisition' icon. The Macro Mapping mode will be conducted. The 'SizeX' and 'SizeY' will be automatically updated to fit with the mapping points step.

Finishing the operation

Initialize both hardware and software parameters by referring to Inspection of the hardware and Initialization of LabSpec6 sections.

Rotate the key to ‘OFF’ position for both laser control switches.

Turn off the white lamp.

Clean the area for any spills.

Return the microscope slide attached with standard Si wafer into the white plastic box.

Troubleshooting

1. Autocalibration failed

The Auto Calibration may fail. If this happens, go through the checklist below:

Verify that 100X objective is in use. Otherwise the peak intensity cannot meet the requirement.

Verify that both lasers are turned on. Notice: you must push the flashing red button for 532-nm.

Verify that the Si surface is well focused. Notice: do not mistakenly focus on the tape or glass.

Verify that the live imaging center is clean. This is where the laser spot is.

2. Live imaging slow to response

Occasionally, the live imaging feels delayed or frozen, e.g. the image does not move instantly following the sample stage movement or the change of Z height. If this happens, leave a note in the Raman Sign-on Book and go through the re-starting procedure below:

Remove the sample by referring to Retrieving the Si sample section. Clear the sample stage.

Rotate the coarse adjustment knob counter-clockwise to the maximum Z height.

Warning: the sample stage will move violently during initialization. Make sure nothing is on it.

Close LabSpec6.

Shut down the computer. Notice: if the computer is stuck during shutdown, press and hold the power button on the computer case until the computer display turns black.

Turn off the switches on the back side of system power controllers (refer to Overview of the hardware). Notice: each of the three controllers has its own power switch; no sequence for turning on/off them.

Wait for three minutes after verifying that computer is shut down and controllers are turned off.

Turn on the switches on the back side of system power controllers.

Start the computer by pushing the power button on the computer case.

Open LabSpec6, and click 'OK' in the pop-out window of stage initialization. Notice: it takes a few minutes to initialize; do not operate until the area h) shows 'Ready' or blank status.