Operating Procedure for Horiba Raman Microscope

SAFETY

Be aware of Laser radiation at all times!

Do not remove the covers of the instrument.

Components are supplied with 110V electric source. Do not touch cables or boards.

Do not touch objectives.

The optical table is floated with nitrogen gas. Please Do Not apply forces on the table.

System Specifications

Laser: 532nm, 633nm

Gratings: 150g/mm (available but not installed), 600g/mm, 1800g/mm

Objectives: 10x, 50x, 100x

Manuals

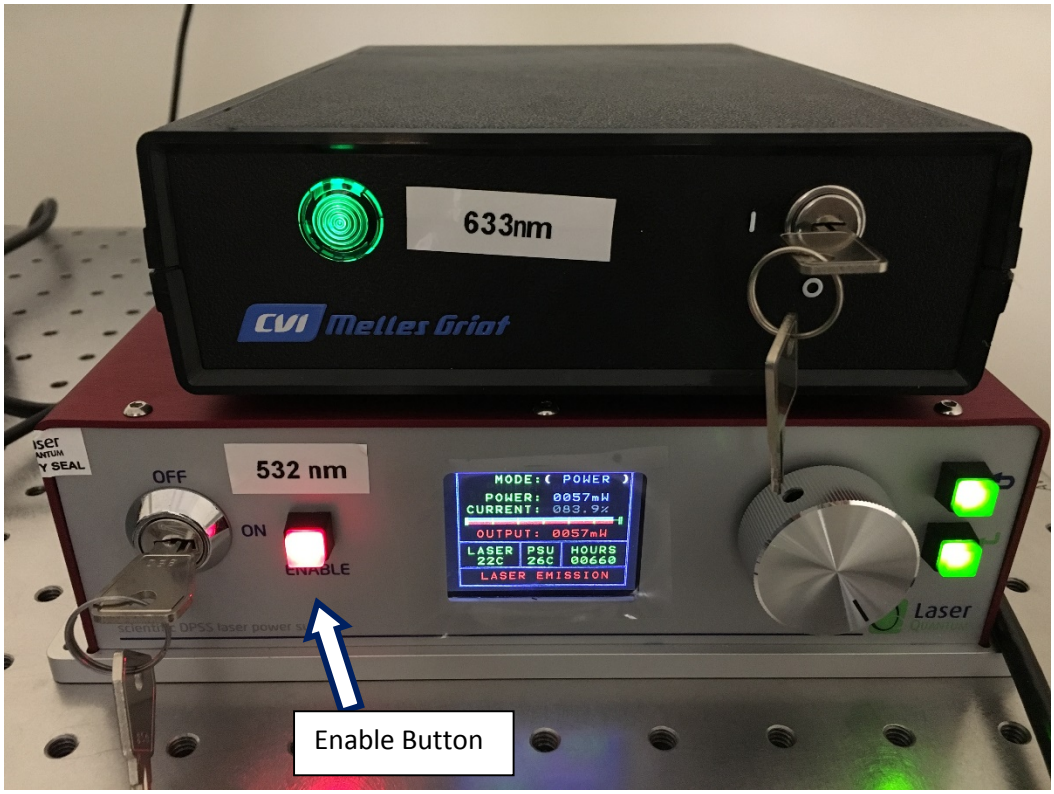
Digital manuals can be found in the folder named 'Manuals' on desktop of computer specifically used for Raman Microscope. Printed copies of User Manual (info and specifications about instrument, maintenance, *etc*) and Reference Manual (Detailed introduction of LabSpec6 functions and interface) are placed near computer.

Initial check

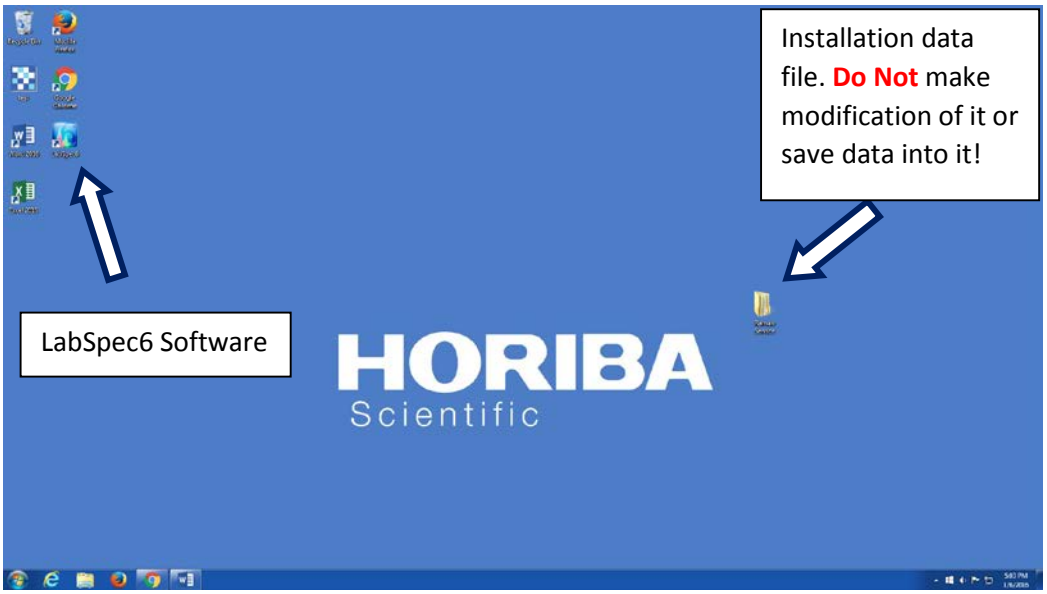
Make sure all controllers are turned on (as shown in the two pictures below). If the light indicator is off, switch on the controllers manually from the rear panel. (*Note: For Scanning Controller, since there's only one CCD camera, CCD1 should always be chosen. If you are not to do DuoScan, XY Stage Scanning mode should be chosen.*)

Start up

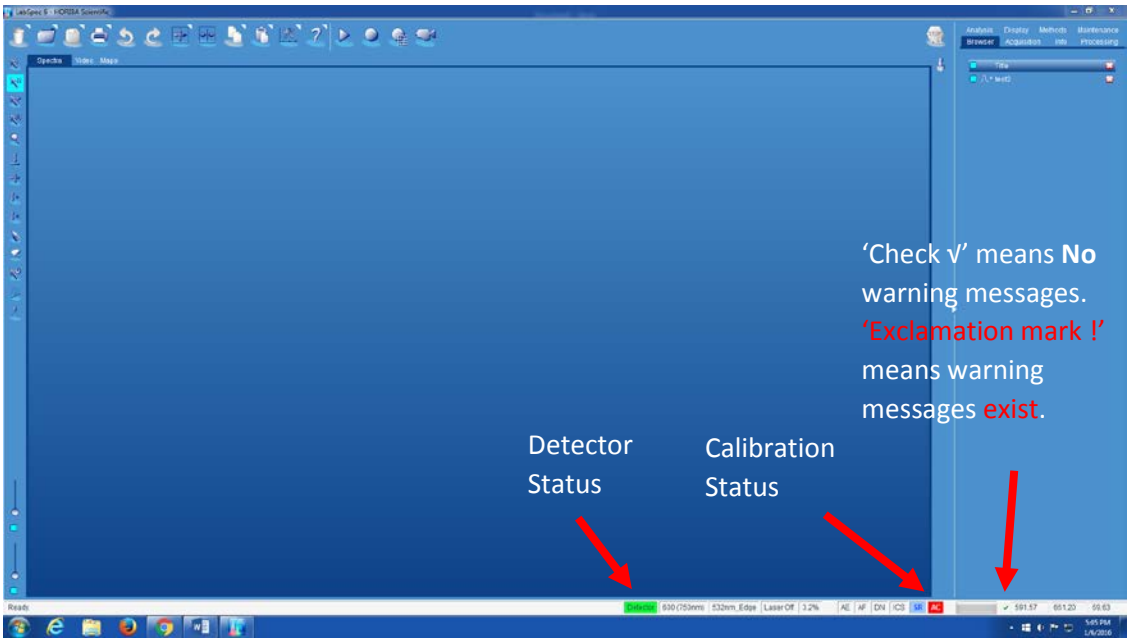
1. Turn on the Laser that you will use by turning the key to '1' or 'ON' positon. For 532nm Laser, after turning the key to 'ON' position, you would also need to press the 'Enable' button (indicated by an arrow in the picture below). You would hear 'Beep' sound after successfully enabling the Laser. The LED panel on 532 Laser controller displays power information of the Laser. The Laser usually works in Power Mode with 57mW power.



2. Double-click on the LabSpec6 software icon to open it.



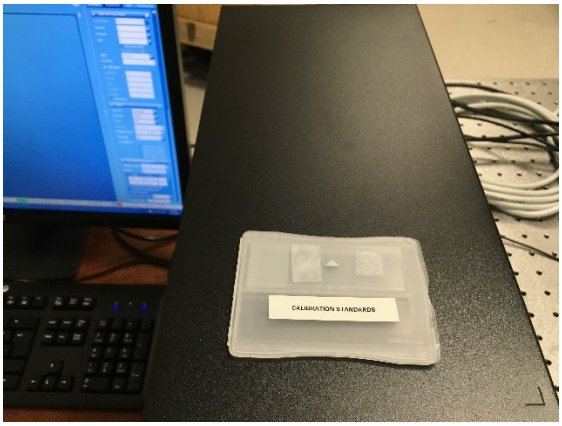
3. Check the bottom of the software interface to see if there's any warning message. If any warning message pops up, ask instrument coordinator for help. (*Note: If CCD camera controller were just turned on, a warning message about 'Detector Cooling down' would appear. Wait for 20-30min for the detector to cool down before any further operation. The warning message would automatically disappear after the detector cools down to desired temperature.*)

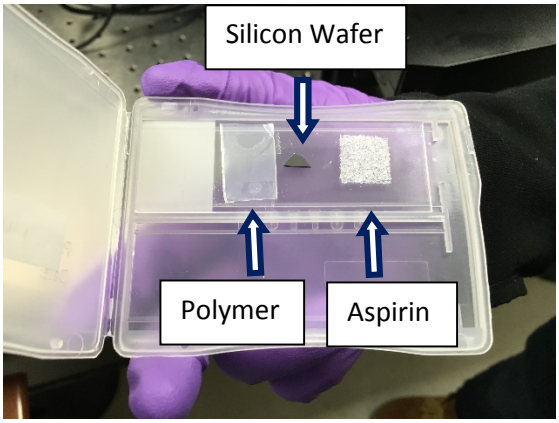


4. Check whether the '**AC**'(abbreviation of Auto-Calibration) icon at the bottom of the software interface is in green color (Calibrated status) or not. If it is in green color, proceed to Sample Measurement step. If it is in red color, calibration of the system is needed. Follow the Auto Calibration procedure to calibrate the system.

Auto Calibration (AC)

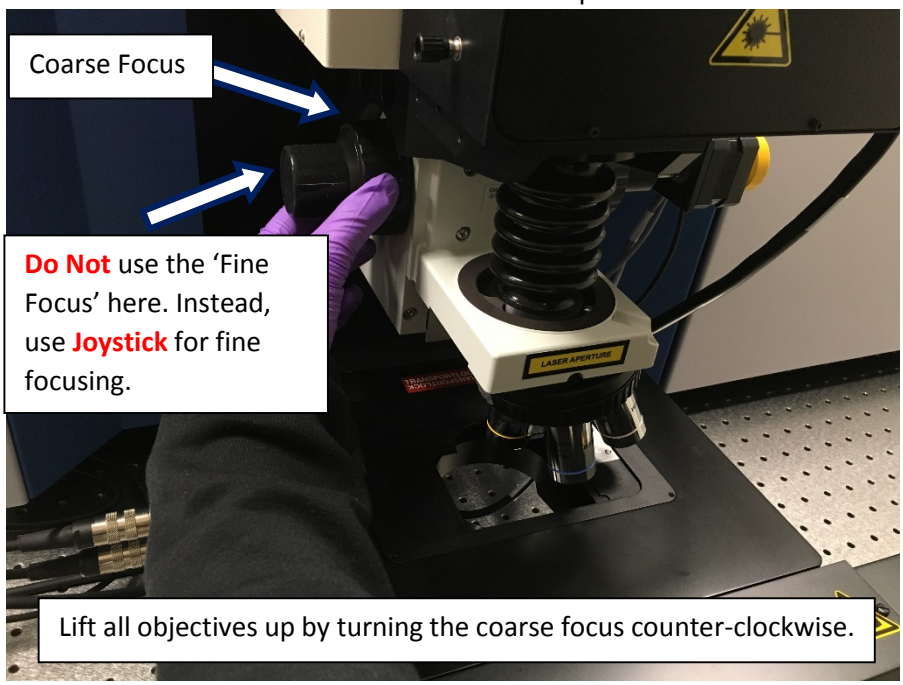
1. Get calibration standards

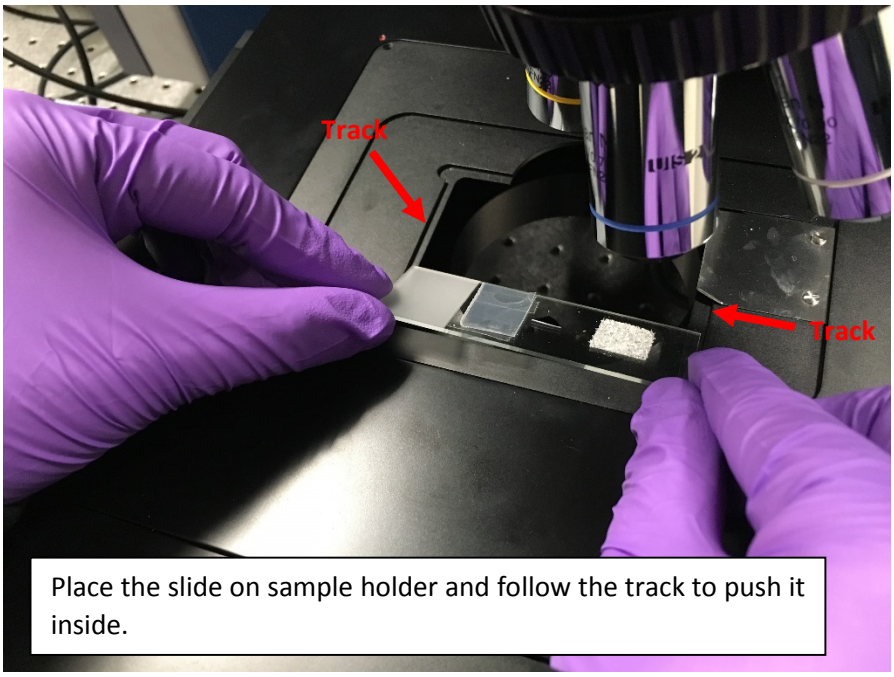




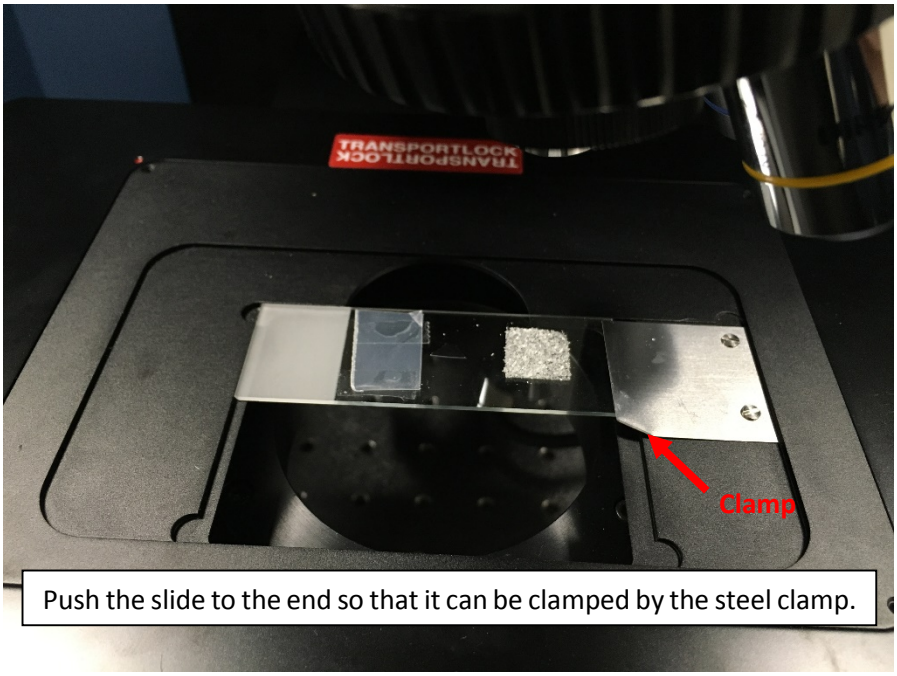
Calibration standards are placed on top of computer. There are three calibration standards: Polymer, Silicon Wafer and Aspirin. Only Silicon wafer would be used for auto-calibration.

2. Load the slide of calibration standards onto sample holder



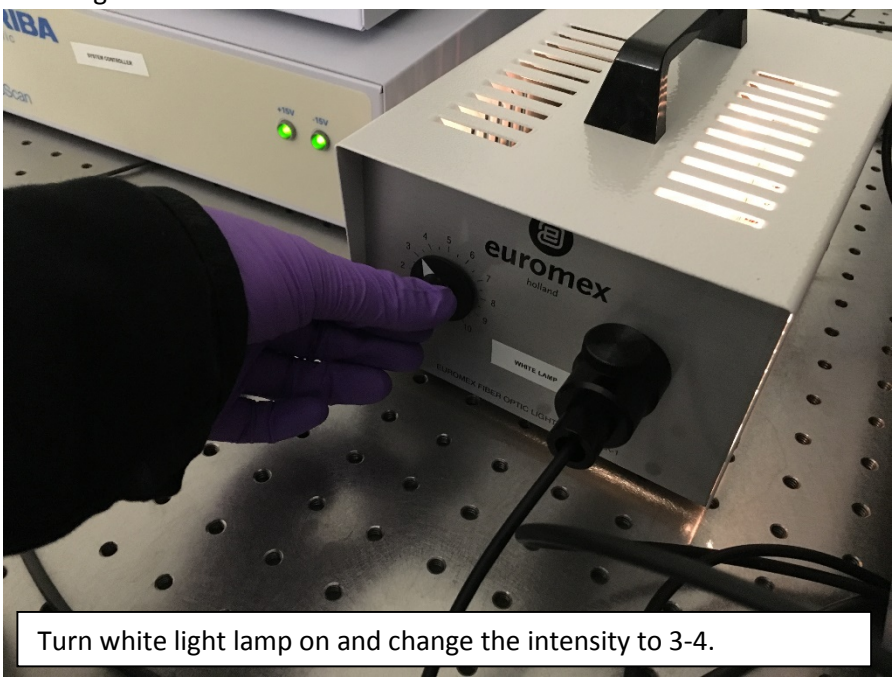


Place the slide on sample holder and follow the track to push it inside.

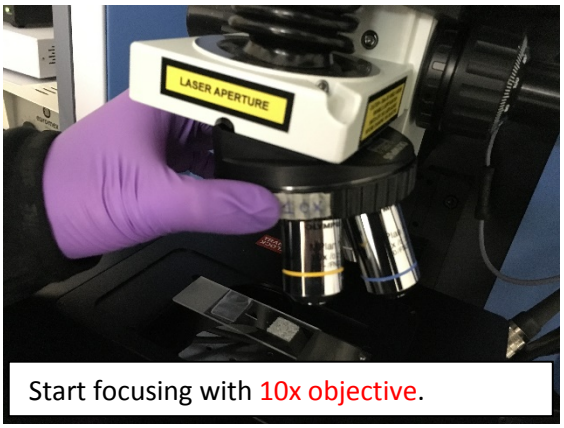


Push the slide to the end so that it can be clamped by the steel clamp.

3. Focusing

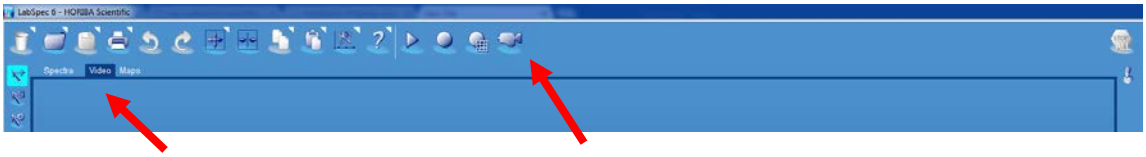


Turn white light lamp on and change the intensity to 3-4.



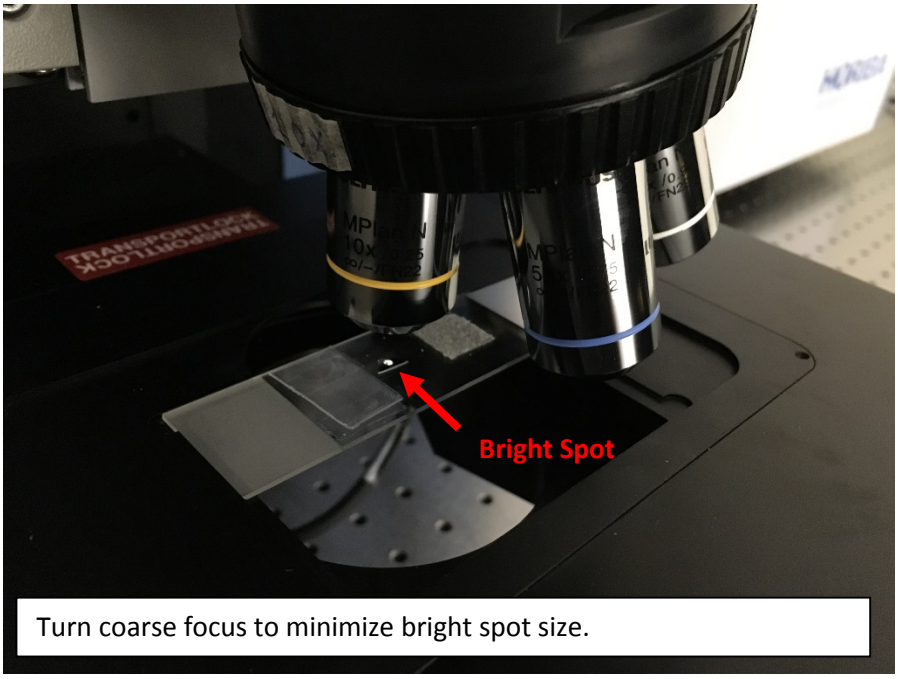
Start focusing with 10x objective.

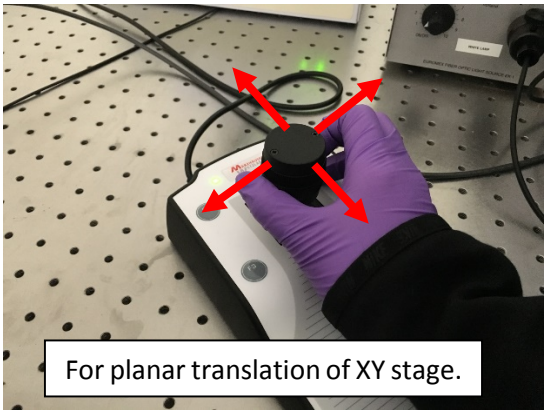
Do Not touch objectives!

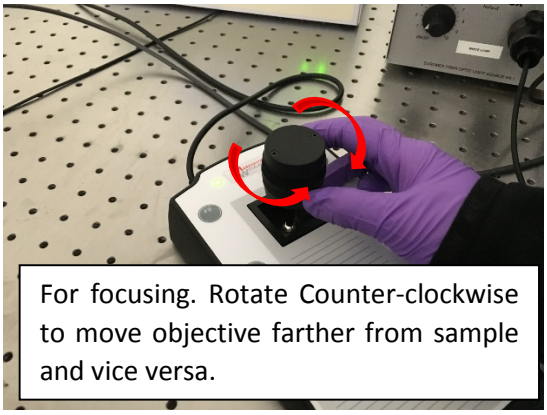


Click on video tab

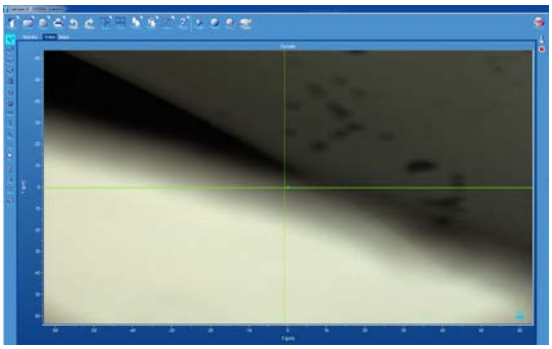
Click the camera icon to turn on video camera

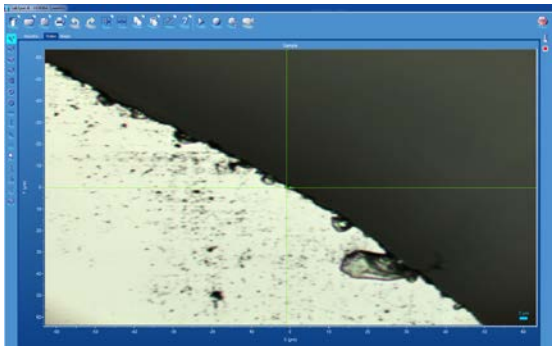






Move Joystick to control planar translation of XY stage and Rotate joystick to change depth of focus.

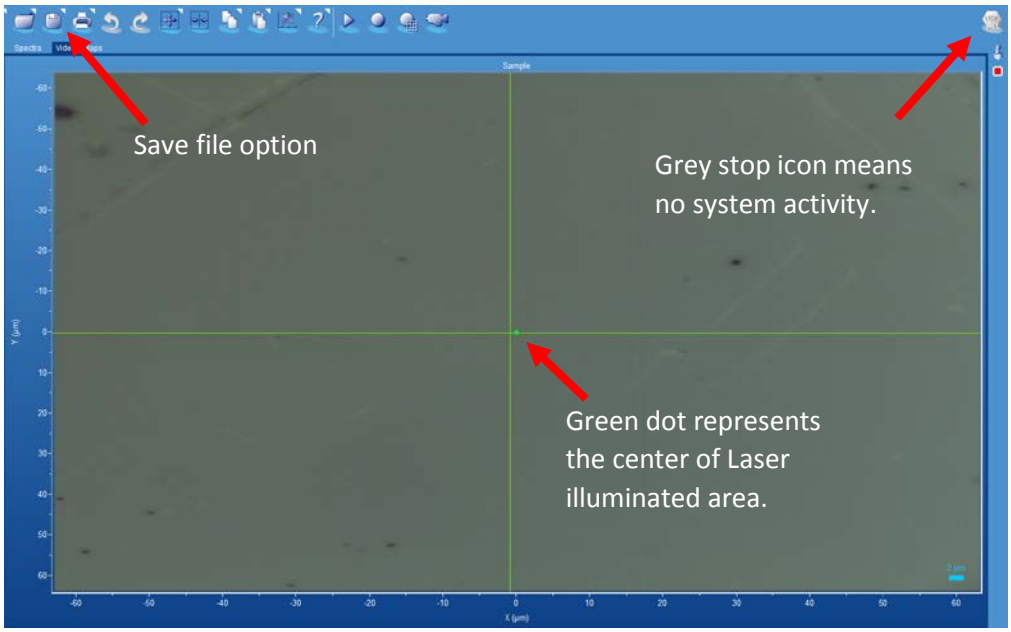




a. Use Joystick to find edge of Si wafer and fine focus it by rotating Joystick.

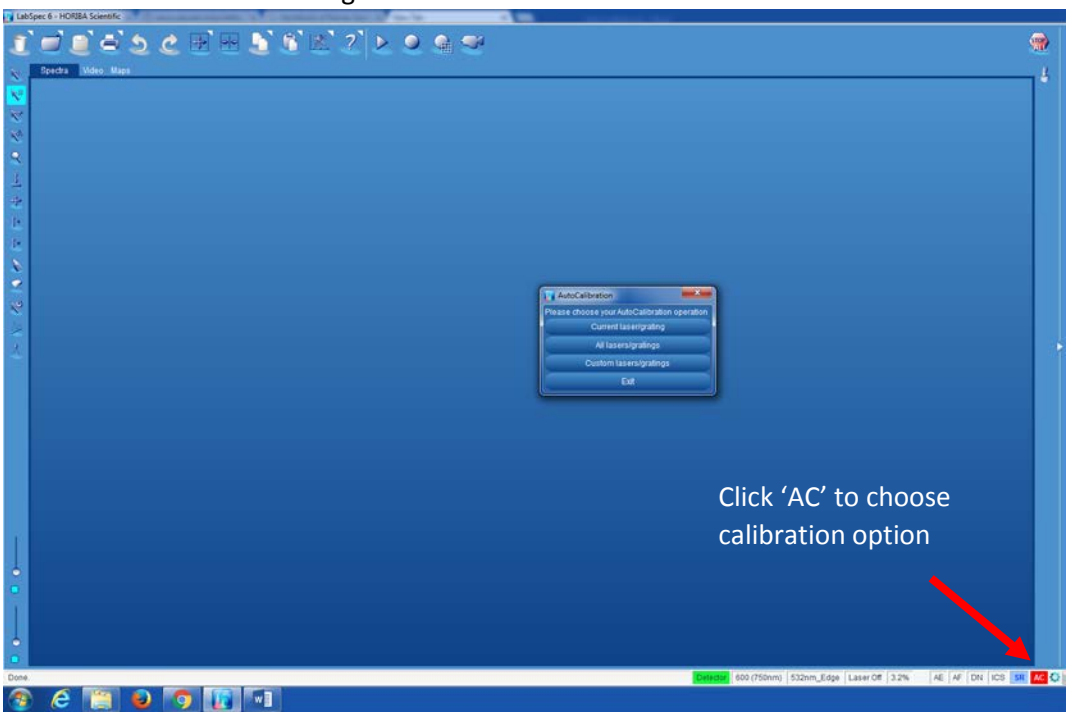
b. After finding the edge, rotate Joystick 1/8 counter-clockwise and change objective to 50X. Find edge and fine focus it. (*You may adjust intensity of white light to change brightness and contrast.*)
c. After finding the edge, rotate Joystick 1/8 counter-clockwise again and change objective to 100X. Find edge and fine focus it. (*You may adjust intensity of white light to change brightness and contrast.*)
d. Move away from edge and find a position on Si wafer to do calibration. (*Fine focusing by rotating joystick would be needed if Si wafer tilts slightly from slide.*)

Never let the objective touch sample!

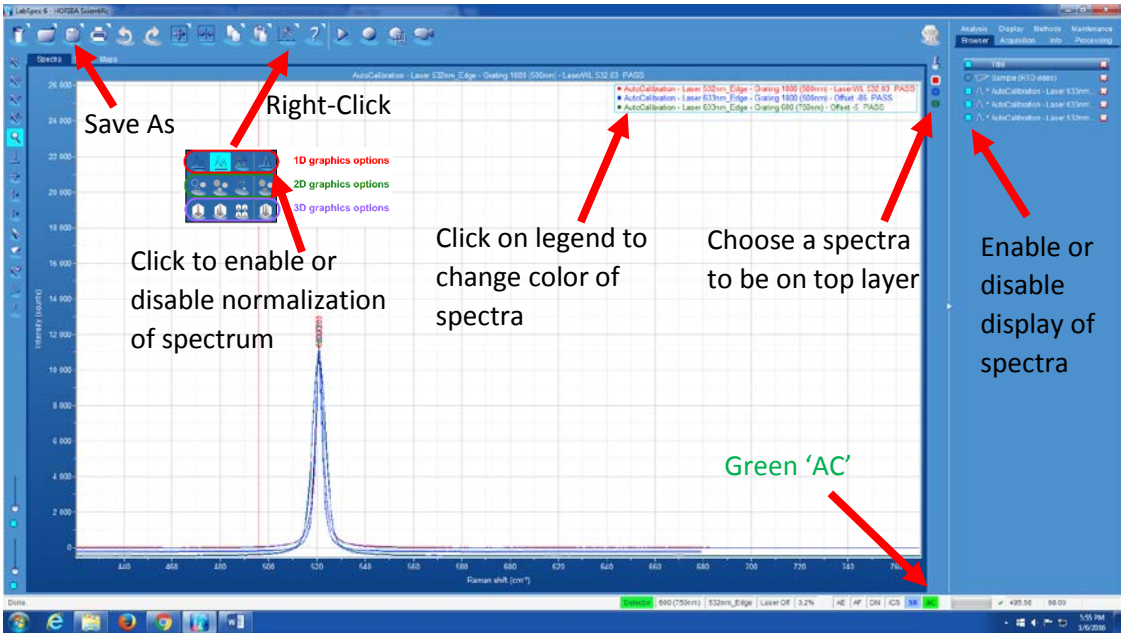


After choosing the position for calibration, click the red 'Stop' icon to turn off video camera (*Note: At this time, the 'Stop' icon would turn grey, indicating no system activity exists, such as imaging, mapping or scanning*). The image you saw before clicking `Stop` would be captured and kept. You can save the frozen image by clicking on 'Save File' button. (*The frozen image is temporarily stored in volatile memory. If it is not saved, it will be replaced by new image once you re-open the video camera.*)

4. Calibration of Laser & Grating

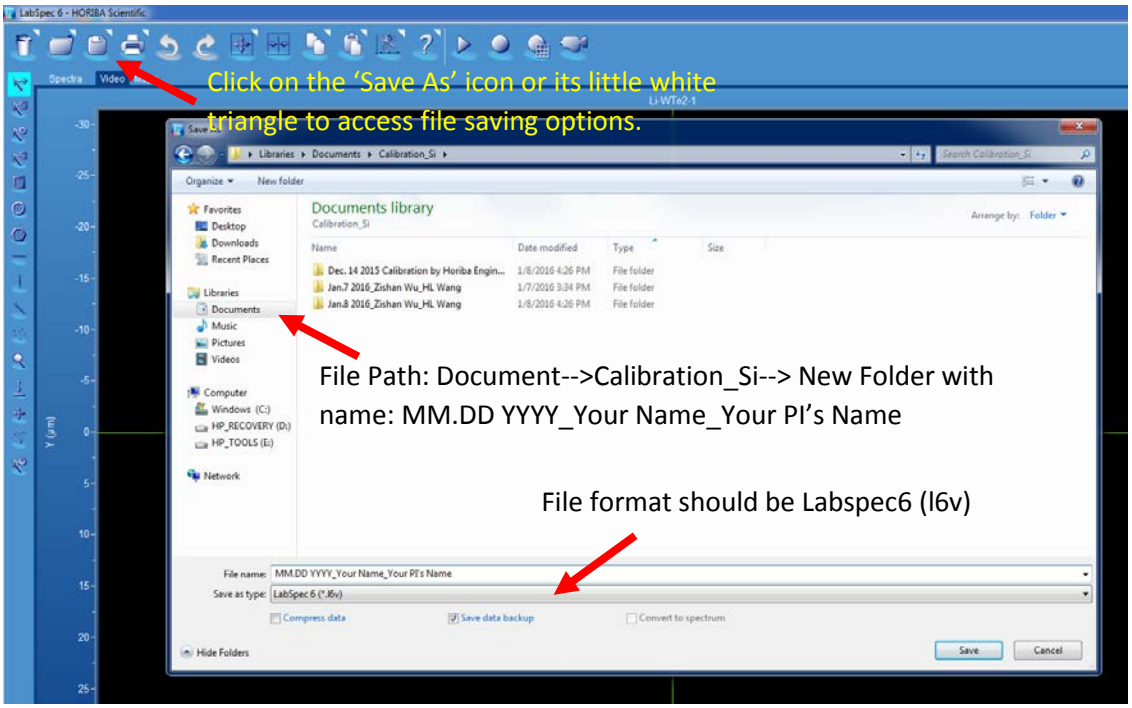


Click on the red 'AC' icon. A window for you to choose the type calibration will pop up. Choose the one you need. Be sure the corresponding Laser is turned on!



After successful calibration, the 'AC' icon will turn green. If any of the calibration fails, ask instrument coordinator for help.

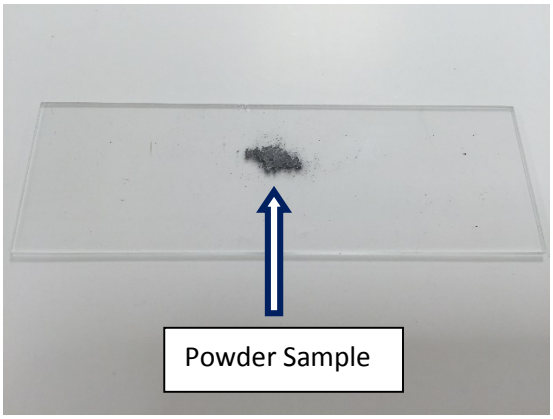
Please save all calibration files in LabSpec6 (.l6v) format by clicking on the 'Save As' icon. All calibration files should be saved in a new folder with name in 'MM. DD YYYY_Your Name_Your PI's Name' format under 'Document-->Calibration_Si' path.

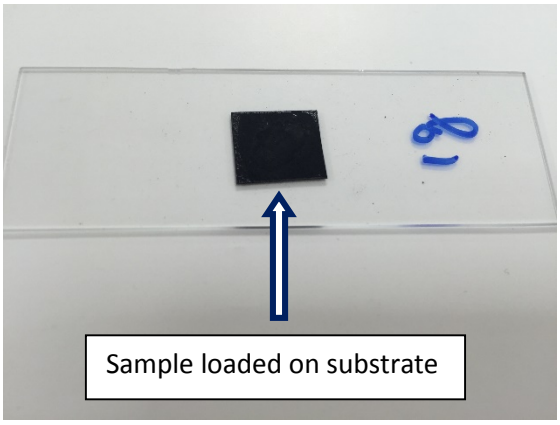


For detailed graphic options, please refer to Chapter 4 in the LabSpec6 reference manual placed near computer.

Sample Measurement—Simple Raman Spectrum Acquisition

1. Put your sample on slide(eg: flat film of powder sample, sample loaded on silicon or other substrates)

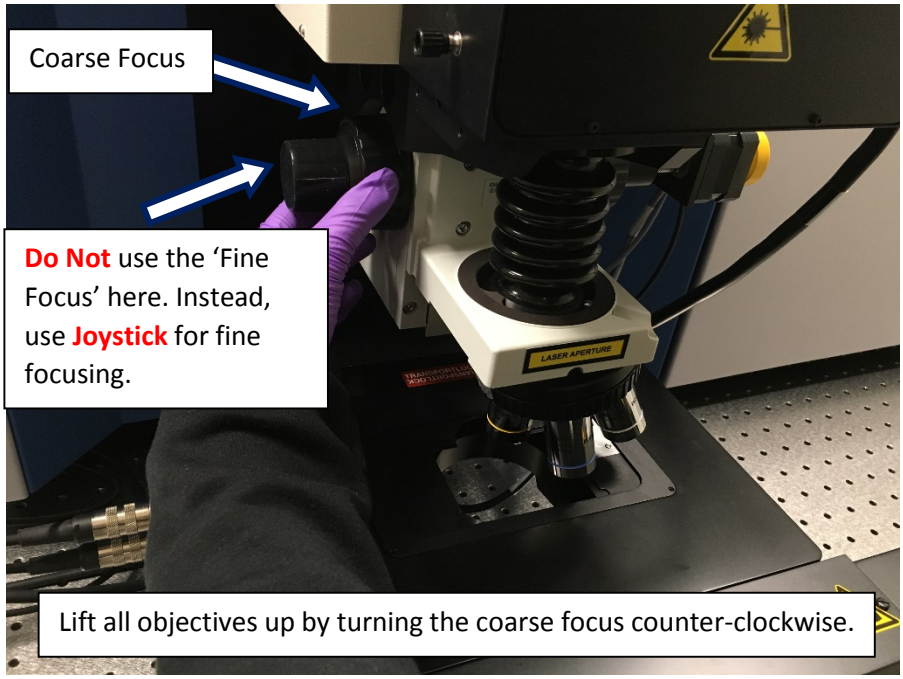


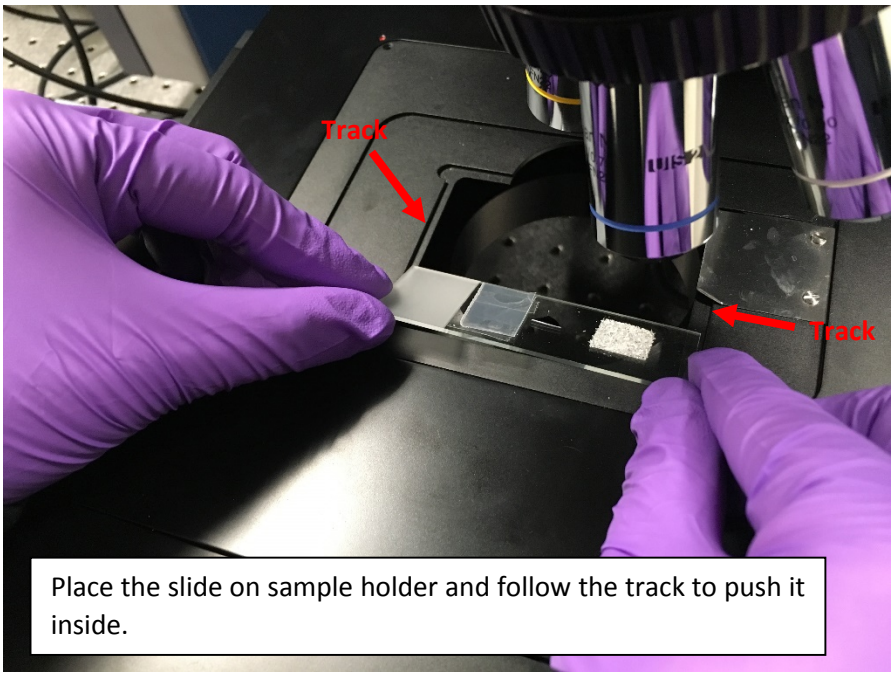


Sample surface should be flat enough to avoid crashing into objective lens.

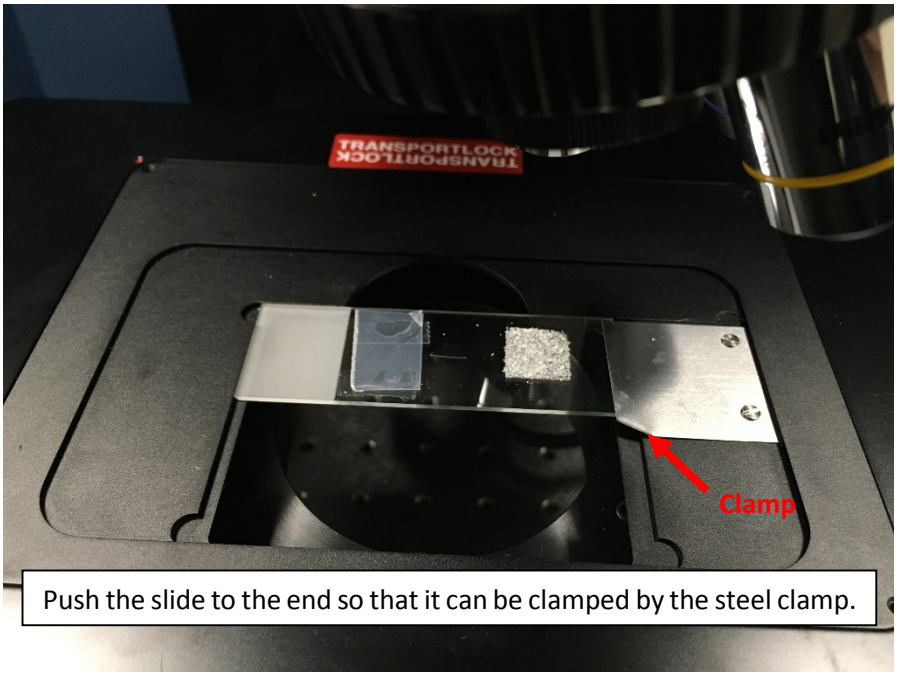
(*The following is an example of acquiring Raman spectrum of Si wafer*)

2. Load the slide of sample onto sample holder



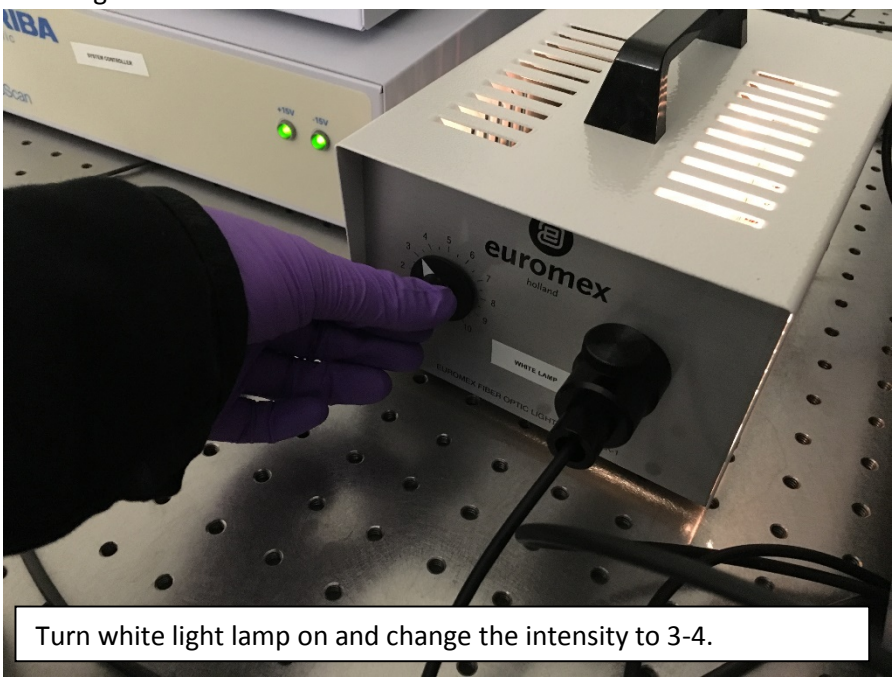


Place the slide on sample holder and follow the track to push it inside.

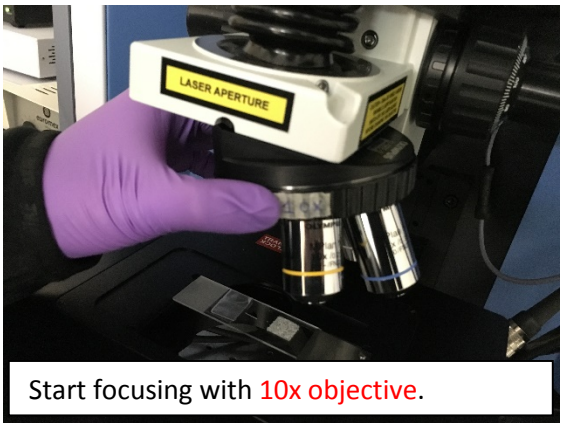


Push the slide to the end so that it can be clamped by the steel clamp.

3. Focusing

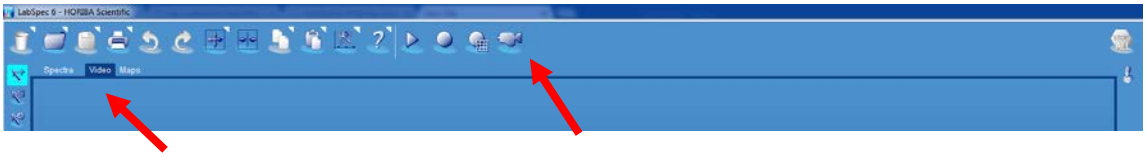


Turn white light lamp on and change the intensity to 3-4.



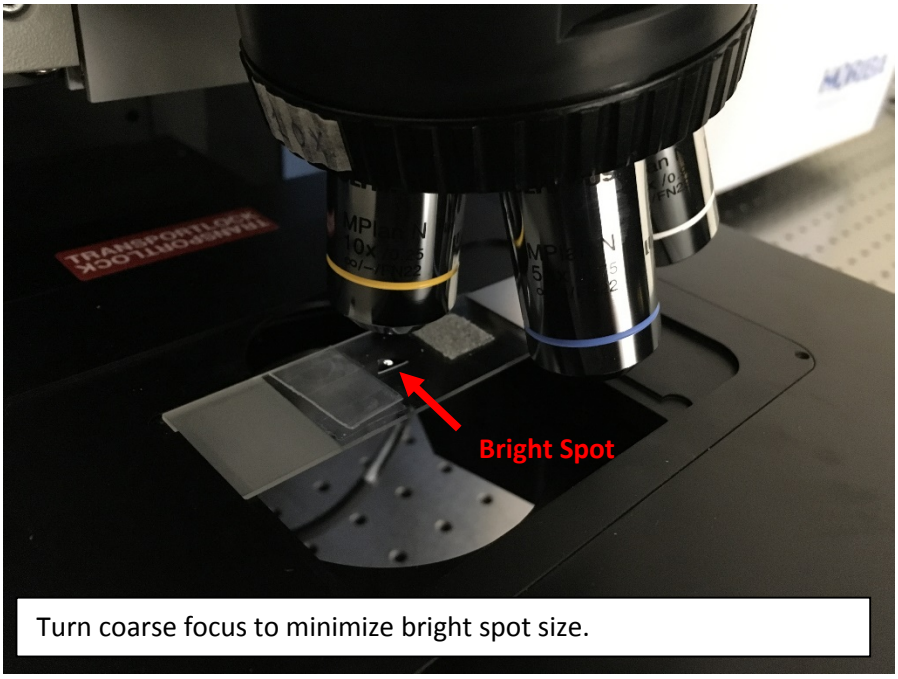
Start focusing with 10x objective.

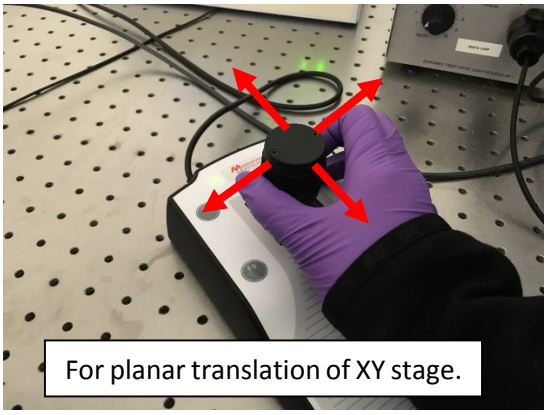
Do Not touch objectives!

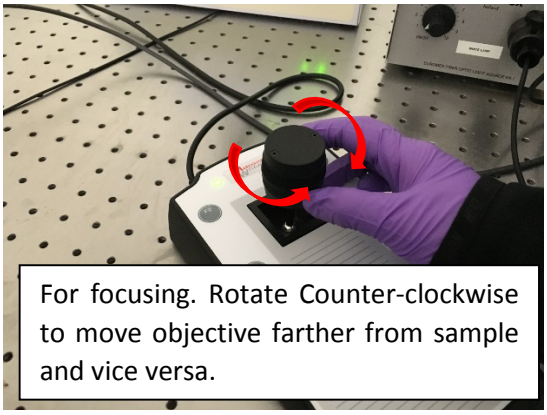


Click on video tab

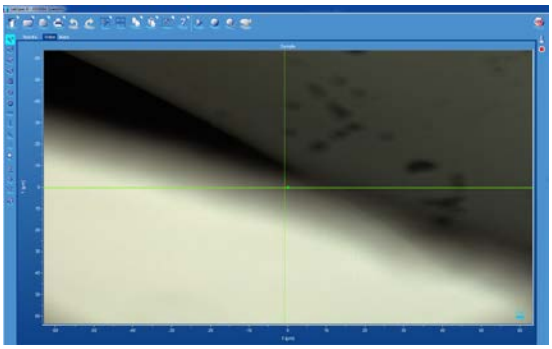
Click the camera icon to turn on video camera

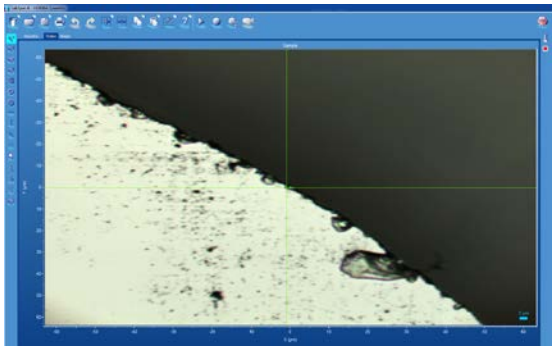






Move Joystick to control planar translation of XY stage and Rotate joystick to change depth of focus.

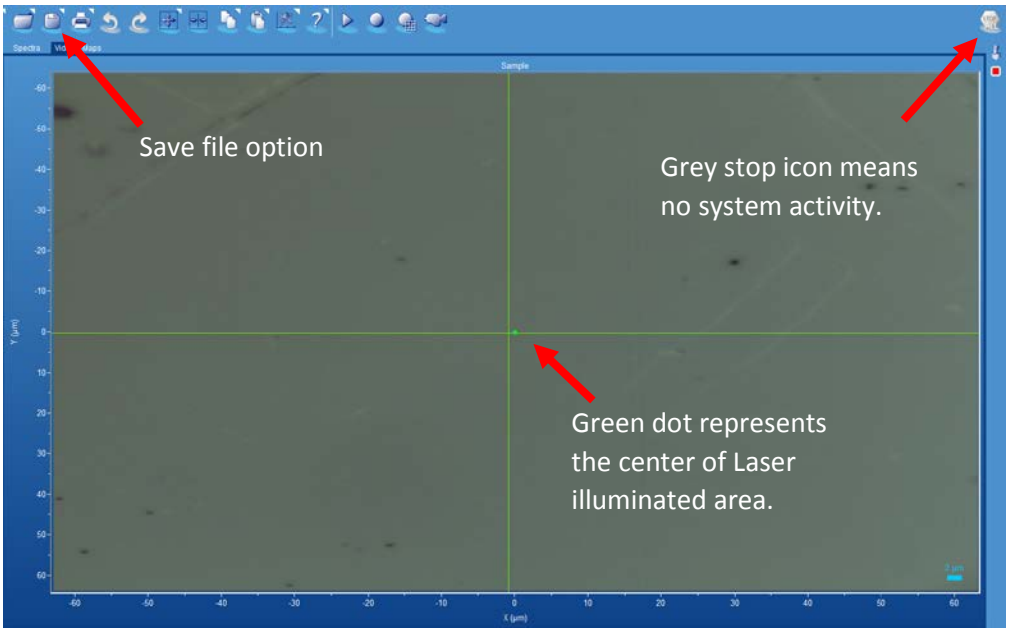




a. Use Joystick to find edge of Si wafer and fine focus it by rotating Joystick.

b. After finding the edge, rotate Joystick 1/8 counter-clockwise and change objective to 50X. Find edge and fine focus it. (*You may adjust intensity of white light to change brightness and contrast.*)
c. (If 100X objective is used) After finding the edge, rotate Joystick 1/8 counter-clockwise again and change objective to 100X. Find edge and fine focus it. (*You may adjust intensity of white light to change brightness and contrast.*)
d. Move away from edge and find a position on Si wafer to do Raman measurement. (*Fine focusing by rotating joystick would be needed if Si wafer tilts slightly from slide.*)

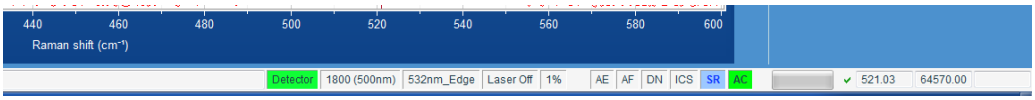
Never let the objective touch sample!



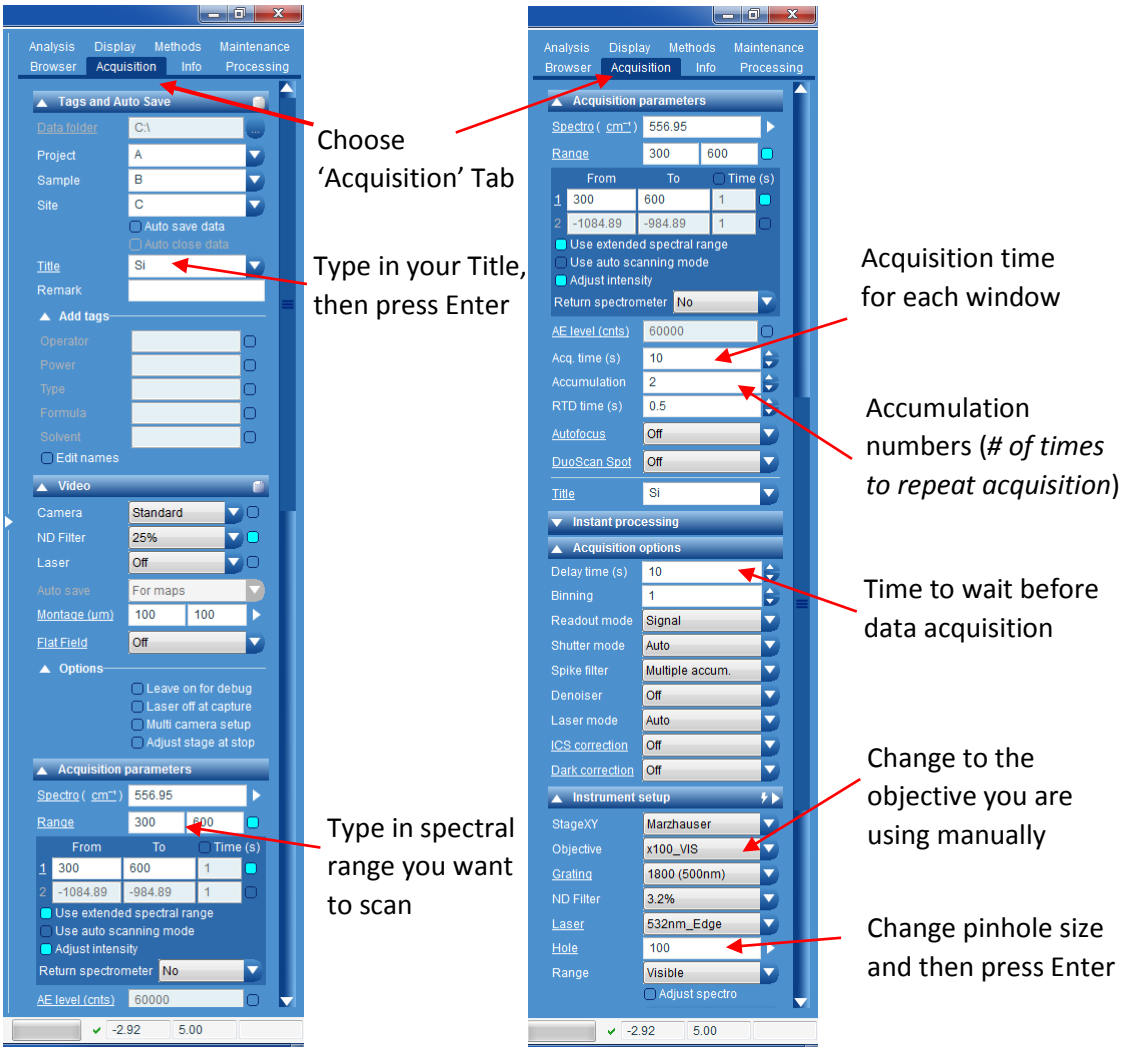
After choosing the position for calibration, click the red 'Stop' icon to turn off video camera (*Note: At this time, the 'Stop' icon would turn grey, indicating no system activity exists, such as imaging, mapping or scanning*). The image you saw before clicking `Stop` would be captured and kept. You can save the frozen image by clicking on 'Save File' button. (*The frozen image is temporarily stored in volatile memory. If it is not saved, it will be replaced by new image once you re-open the video camera.*)

4. Acquisition Parameters

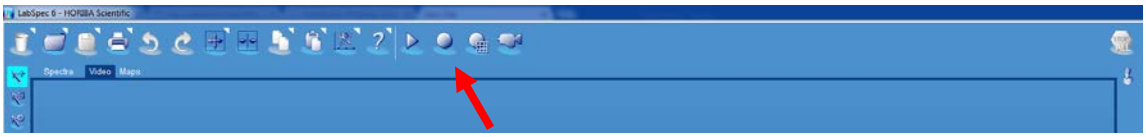
At the bottom of LabSpec6 interface, you can choose grating (1800g/mm or 600g/mm), Laser (532nm or 633nm, Ensure the one you choose is turned on.) and filter (vary from 0.01% to 100%. The number represents the amount of light transmitted. 100% means no filtering.)



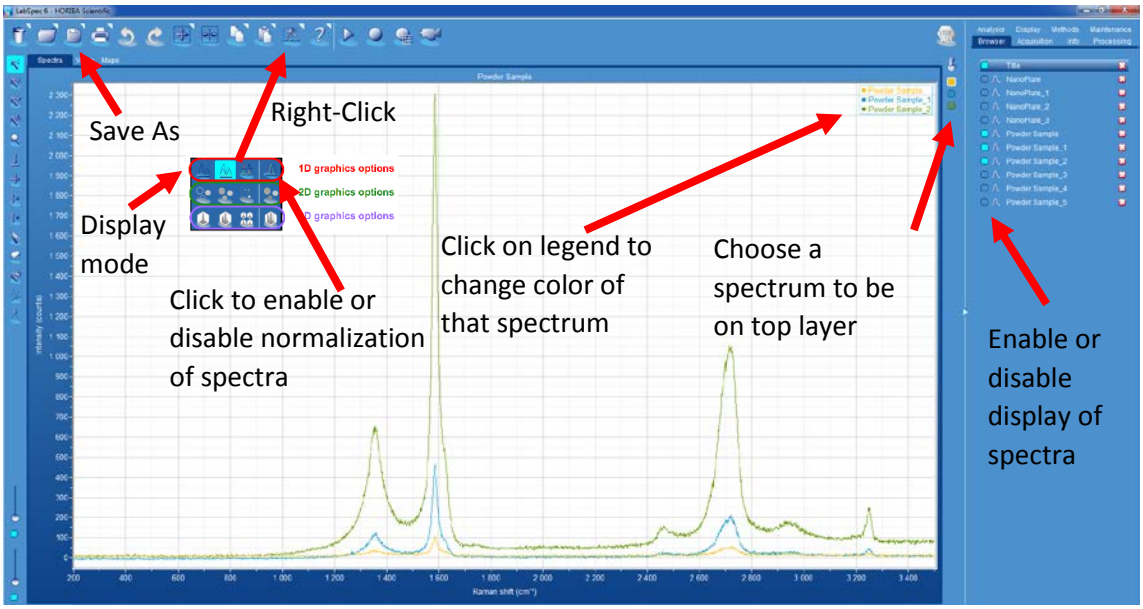
On the right hand side of LabSpec6 interface, you can choose 'Acquisition' tab to manage acquisition settings.



5. After giving Title, choosing Laser, grating and filter, setting acquisition parameters such as spectral range, objective, acquisition time, accumulation numbers, you would be able to run the measurement.



Click the circular icon to start acquisition

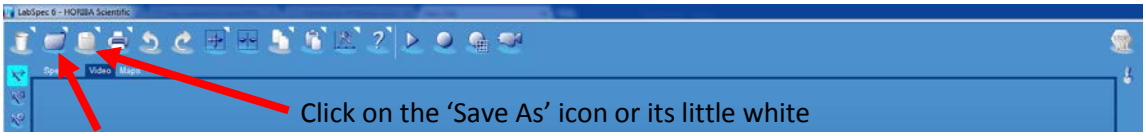


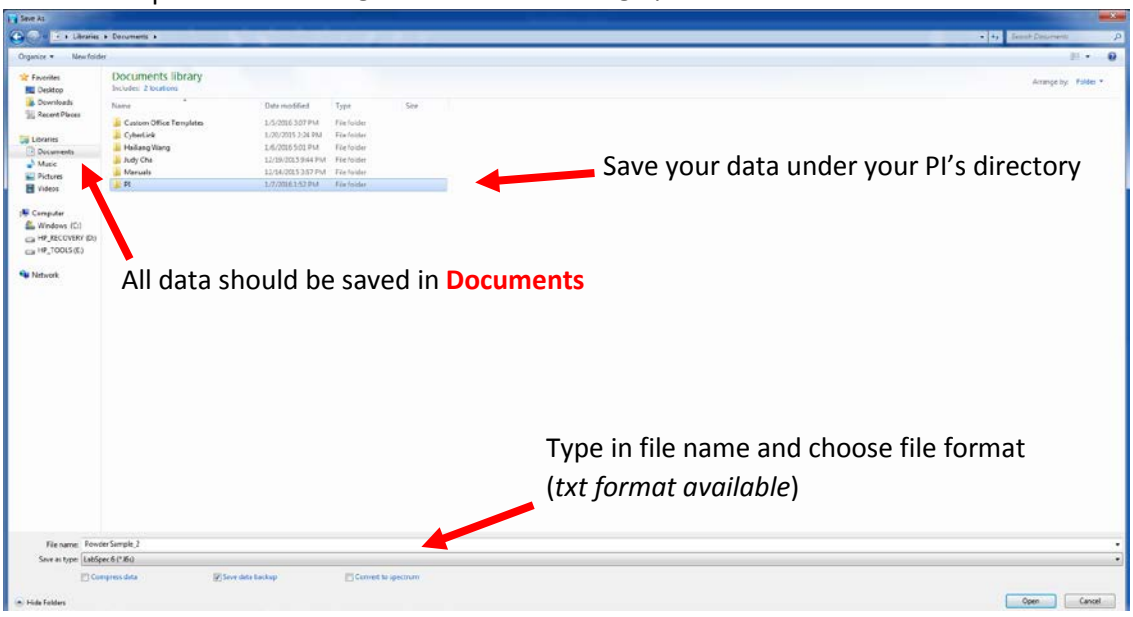
The above Si Raman spectra were acquired with different acquisition time (2s, 10s, 50s). Spectra can be displayed in two modes, single-spectrum or multi-spectra modes. For multi-spectra mode, spectra can be normalized or dis-normalized. The above result is displayed in multi-spectra mode without normalization of spectra.

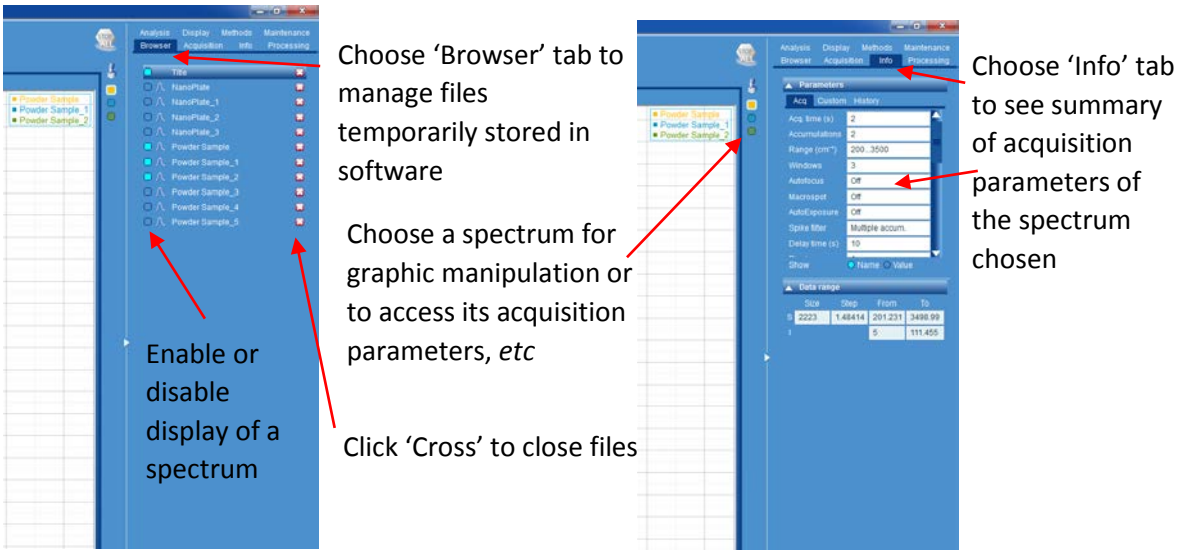
For detailed graphic options, please refer to Chapter 4 in the LabSpec6 reference manual placed near computer.

6. File Management

To save or open files, Click on 'Save As' icon or 'Open File' icon on the top of software interface.



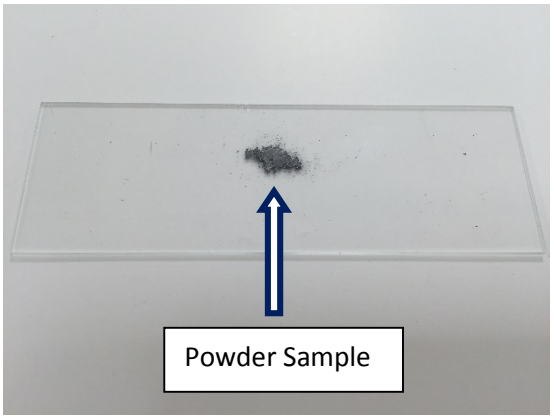


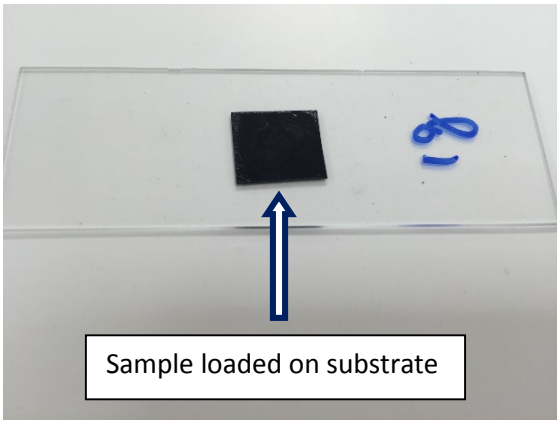


7. Shutting down
    a. After saving your data, close all of the files by clicking on the ‘Cross’ following file names under ‘Browser’ tab.
    b. Close LabSpec6 software by clicking on the ‘Cross’ at top left corner.
    c. Lift objectives and take away your samples.
    d. Clean the working area.

Sample Measurement—Mapping

1. Put your sample on slide(eg: flat film of powder sample, sample loaded on silicon or other substrates)

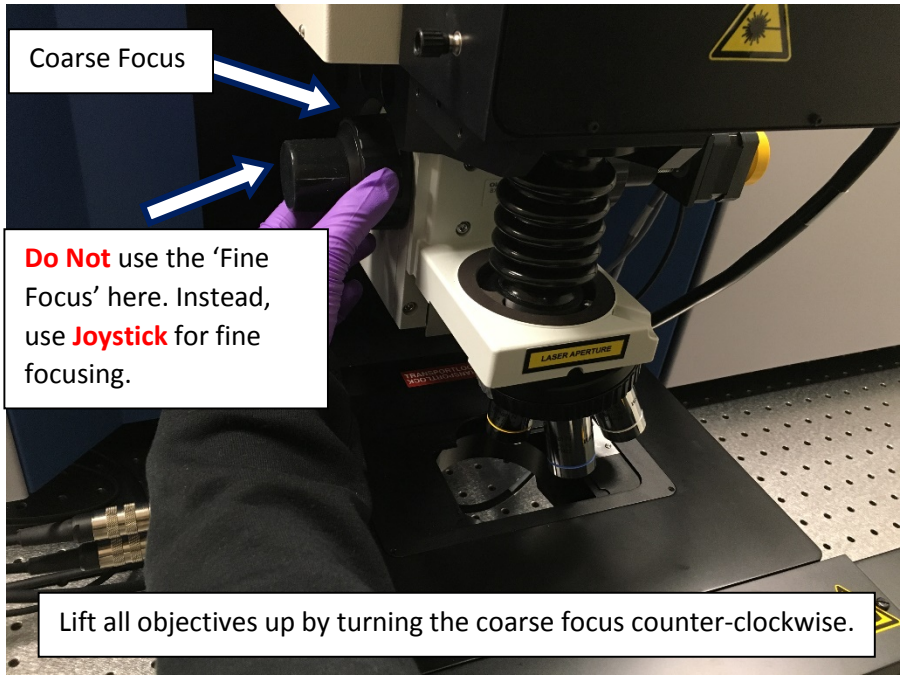


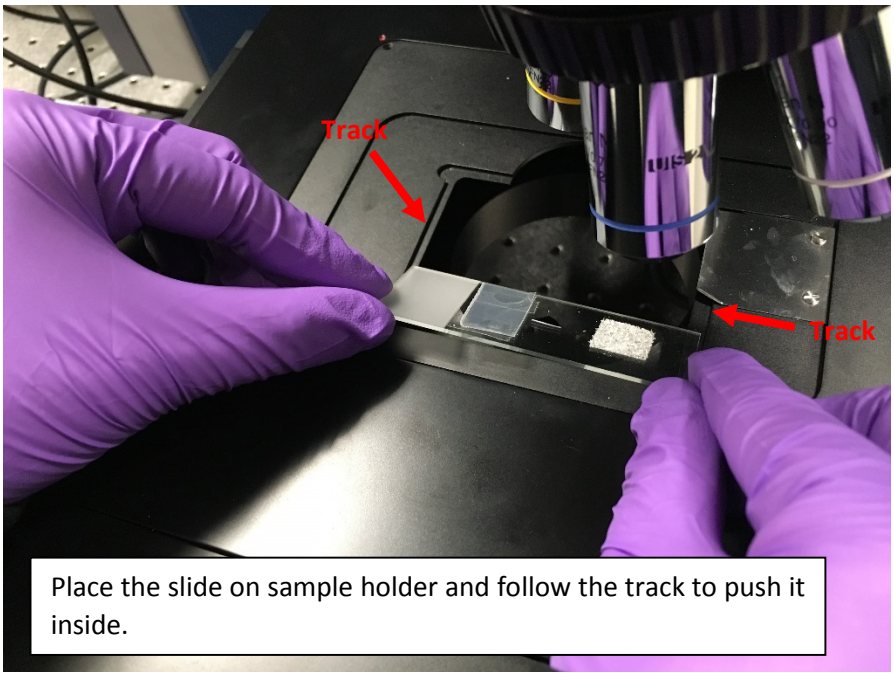


Sample surface should be flat enough to avoid crashing into objective lens.

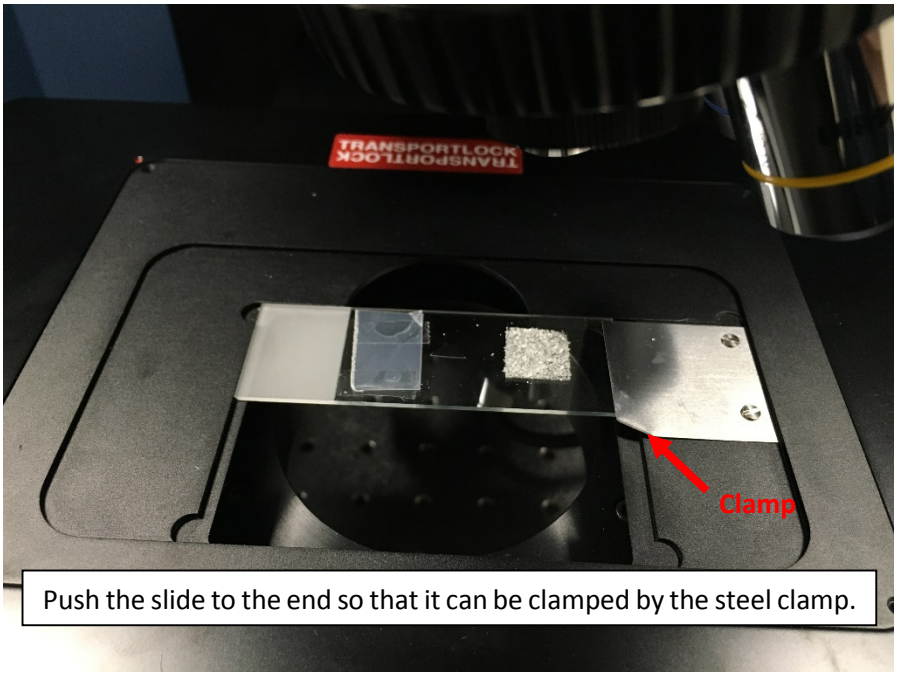
(*The following is an example of Raman mapping around Si wafer edge*)

2. Load the slide of sample onto sample holder



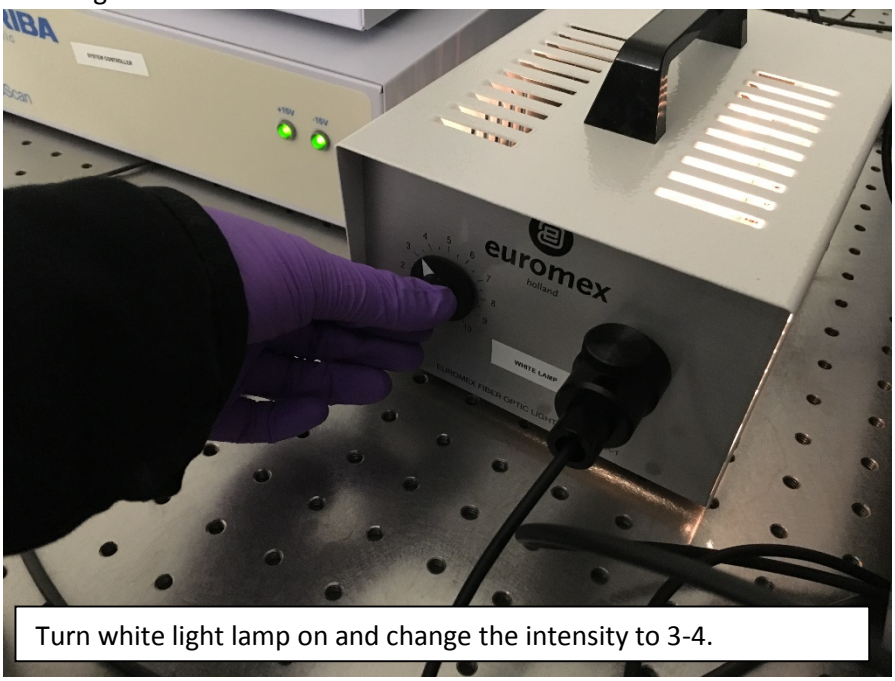


Place the slide on sample holder and follow the track to push it inside.

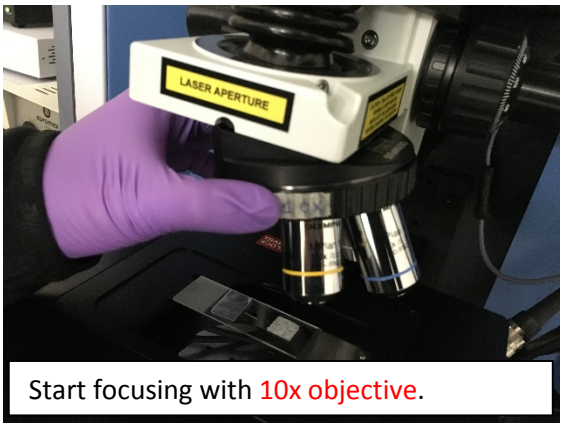


Push the slide to the end so that it can be clamped by the steel clamp.

3. Focusing

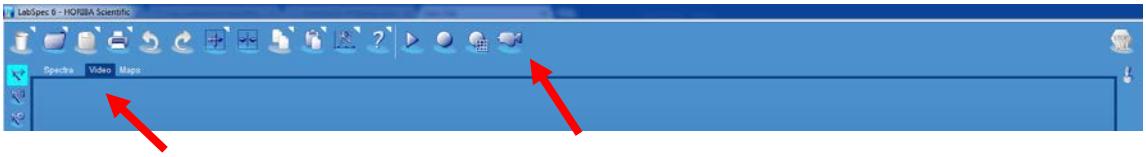


Turn white light lamp on and change the intensity to 3-4.



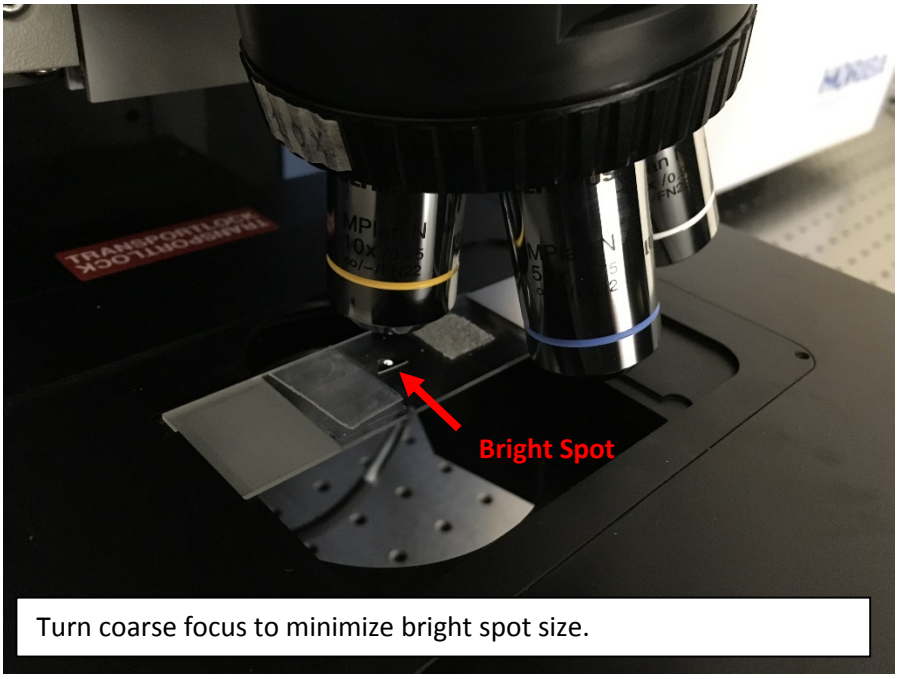
Start focusing with 10x objective.

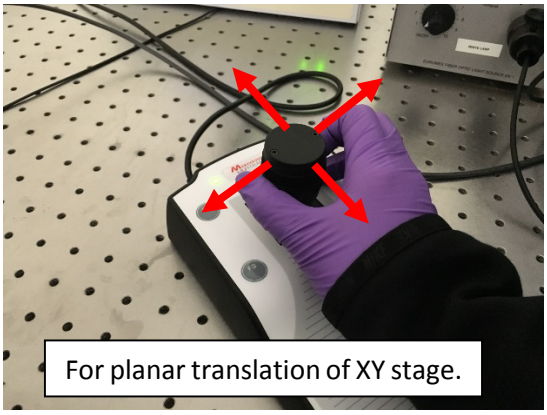
Do Not touch objectives!

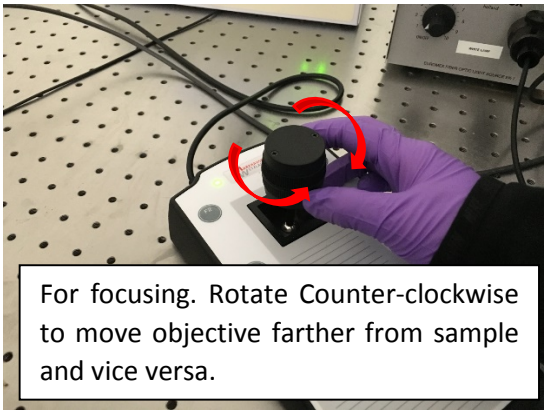


Click on video tab

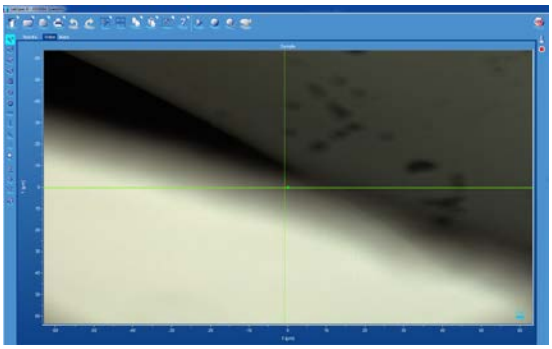
Click the camera icon to turn on video camera

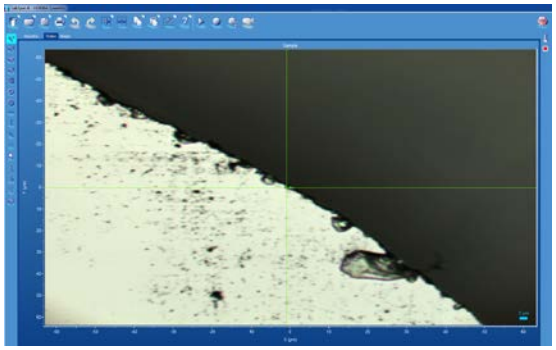






Move Joystick to control planar translation of XY stage and Rotate joystick to change depth of focus.



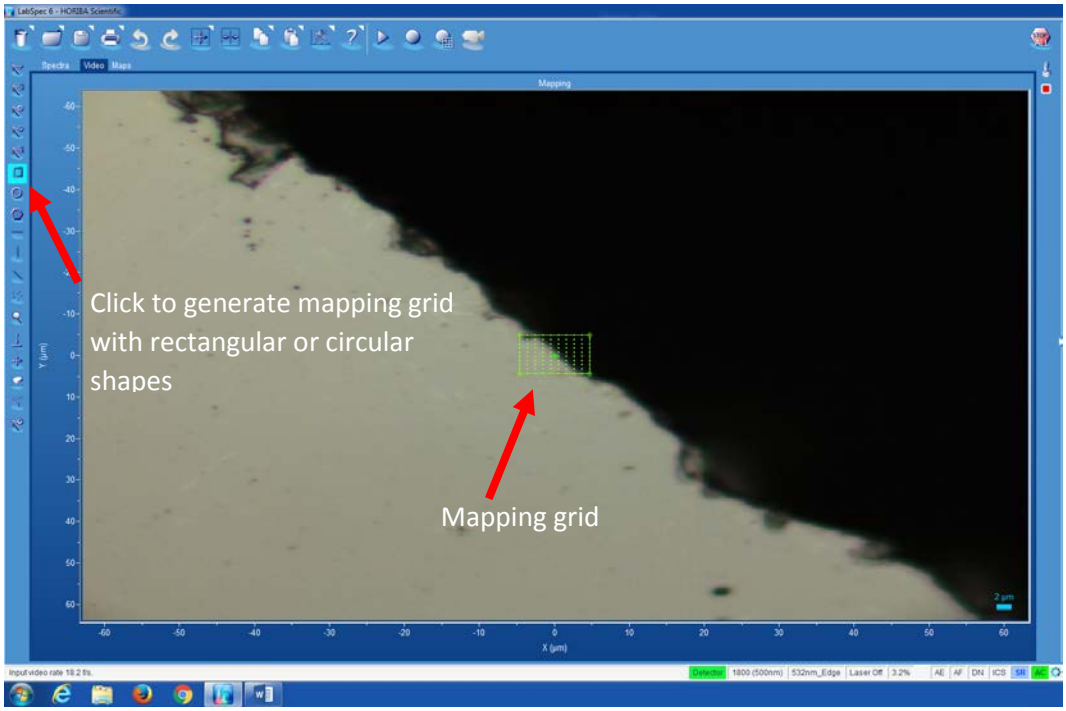


a. Use Joystick to find edge of Si wafer and fine focus it by rotating Joystick.

b. After finding the edge, rotate Joystick 1/8 counter-clockwise and change objective to 50X. Find edge and fine focus it. (*You may adjust intensity of white light to change brightness and contrast.*)

c. (If 100X objective is used) After finding the edge, rotate Joystick 1/8 counter-clockwise again and change objective to 100X. Find edge and fine focus it. (*You may adjust intensity of white light to change brightness and contrast.*)

Never let the objective touch sample!

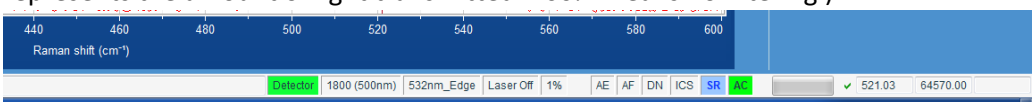


d. Click on the mapping grid options on the left hand side of LabSpec6 interface.

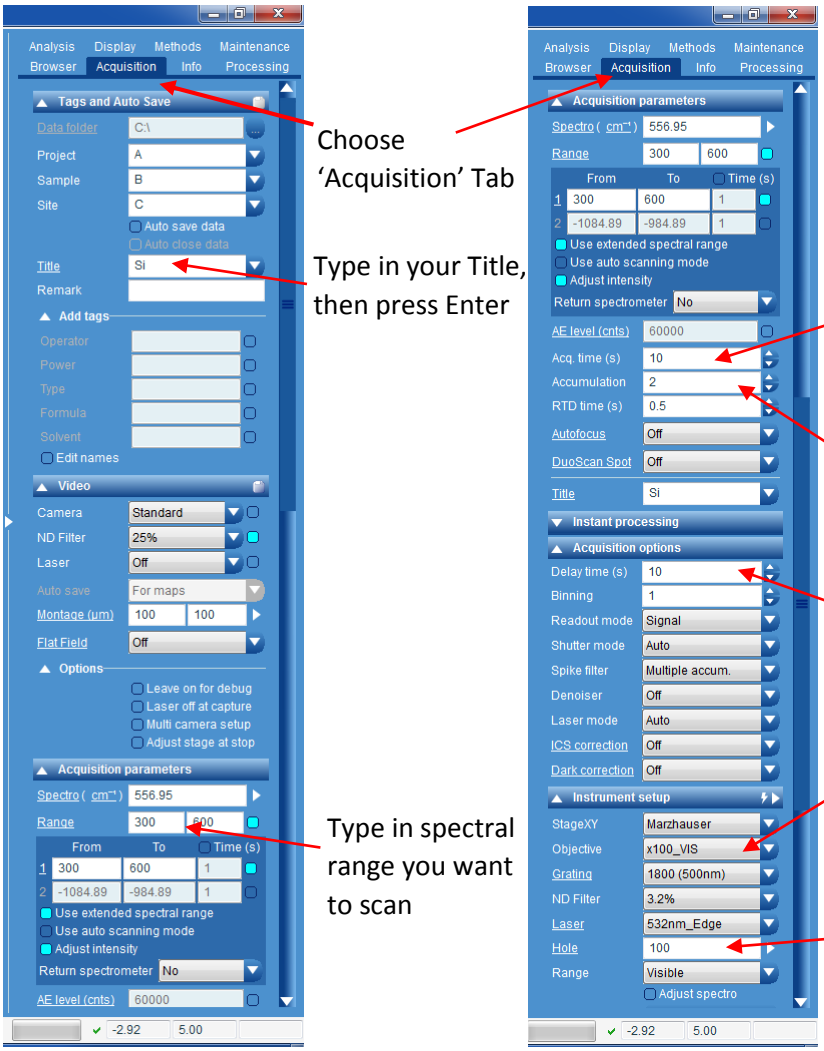
e. You can either select the grid to move it or drag at its corners to change its size. Resolution setting can be done manually by inputting number of points per row or column. (*See Step 4 Acquisition Parameters*)
(*Note: Since the screen ratio is not 1:1. A square mapping grid would look like a rectangle.*)

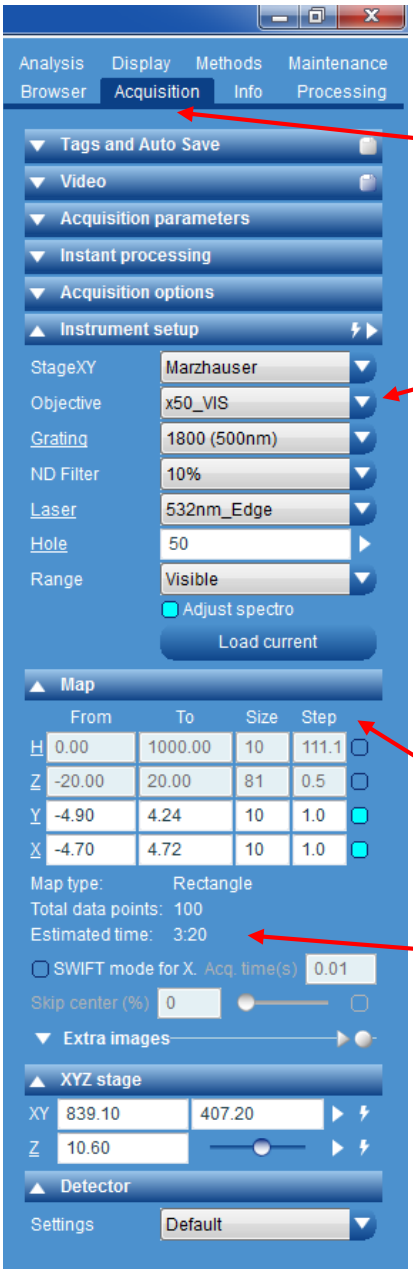
4. Acquisition Parameters

At the bottom of interface, you can choose grating (1800g/mm or 600g/mm), Laser (532nm or 633nm, Ensure the one you choose is turned on.) and filter (vary from 0.01% to 100%. The number represents the amount of light transmitted. 100% means no filtering.)



On the right hand side, you can choose 'Acquisition' tab to manage acquisition parameters.


Choose 'Acquisition' Tab
Type in your Title, then press Enter
Type in spectral range you want to scan
Acquisition time for each window
Accumulation numbers (# of times to repeat acquisition)
Time to wait after Laser on and before data acquisition
Change to objective you are using manually
Change pinhole size and then press Enter



Choose 'Acquisition' Tab

Manually choose the objective to in use!

The real size of mapping grid is related to objectives. Wrong objective selection will lead to incorrect movement of XY stage.

'Size' is number of points per row or column.

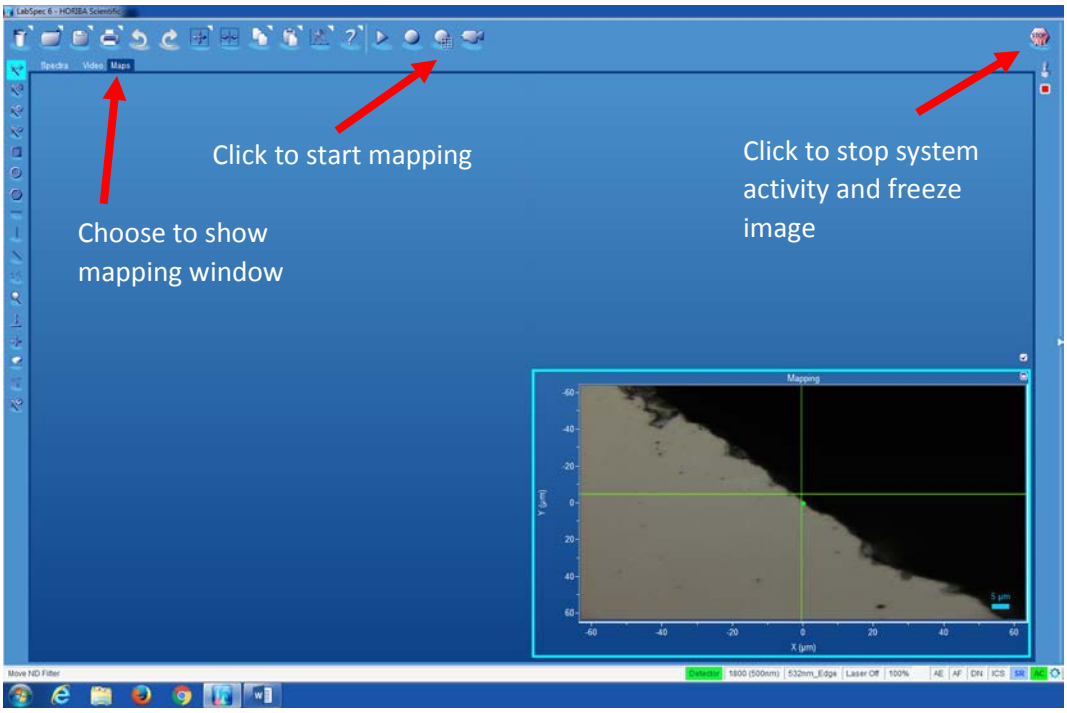
'Step' is step size in micrometer unit.

Estimated time for mapping.

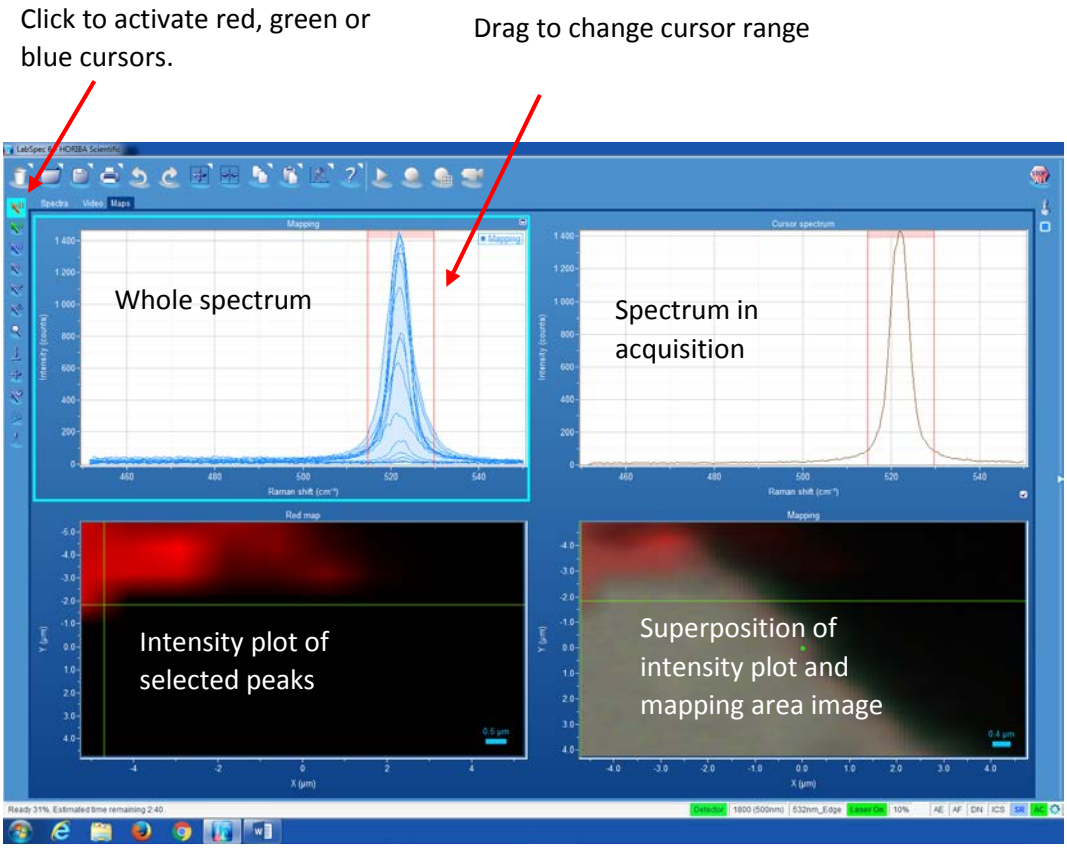
5. Start Mapping

   After giving Title, choosing Laser, grating and filter, setting acquisition parameters such as spectral range, objective, acquisition time, accumulation numbers, you would be able to run the measurement.

   a. Click on 'Stop' icon to freeze image.
   b. Wait until 'Stop' icon turns grey.
   c. Choose 'Mapping' tab to show mapping window.
   d. Click on 'Mapping' icon to start mapping process.

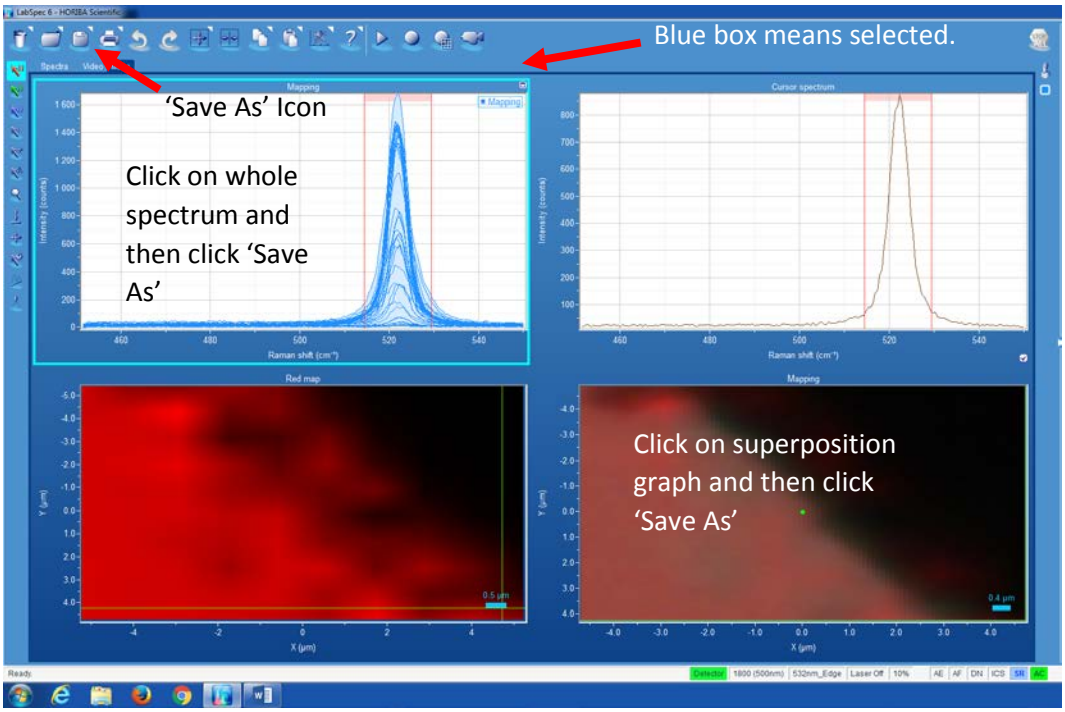


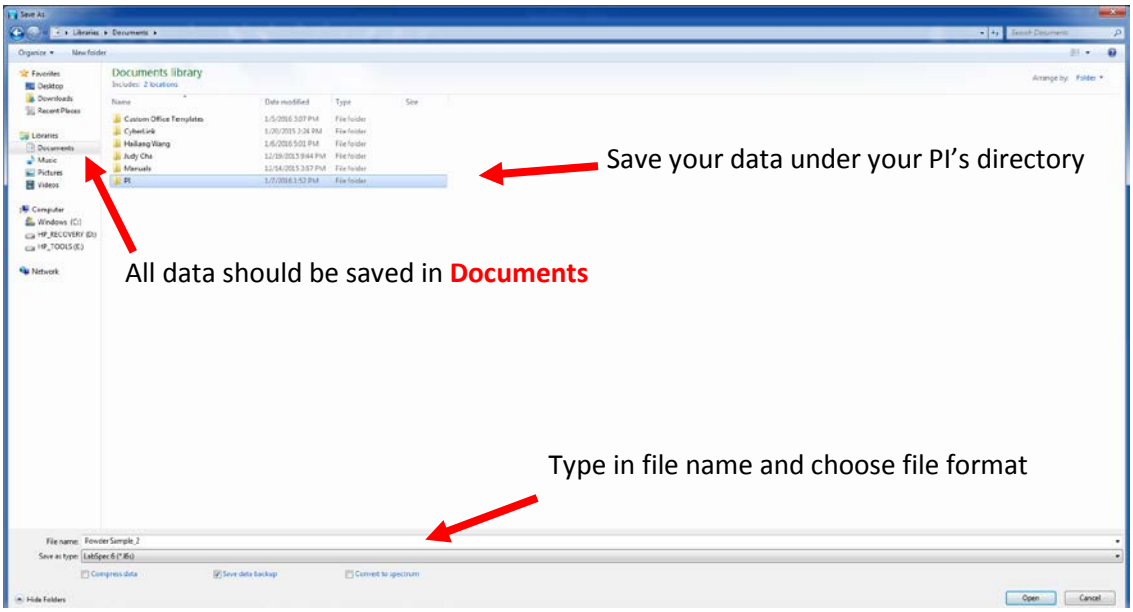
6. During or after mapping, you can choose peaks (up to three) of interest to be displayed in intensity graph at bottom left and superposition graph at bottom right.



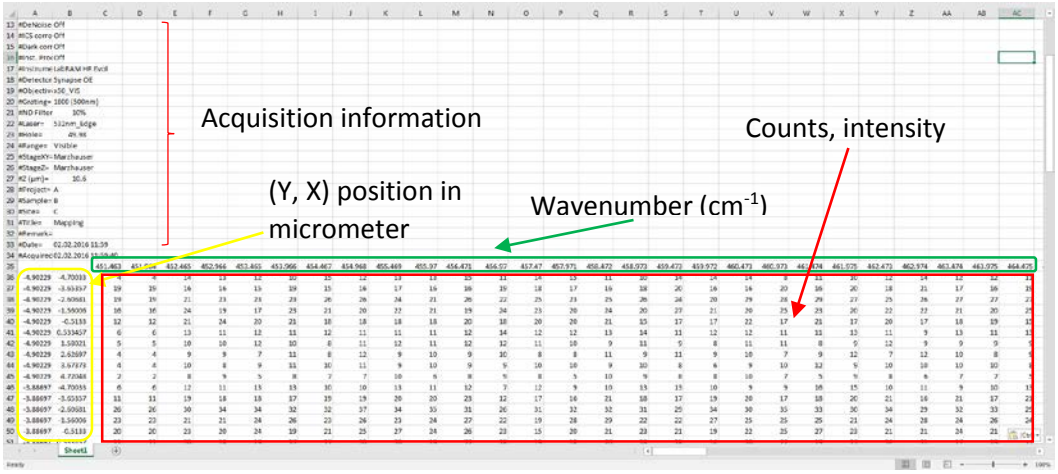
7. Save Data

To save full information of mapping data, whole spectrum (top left) and superposition graph (bottom right) must be saved. Click on whole spectrum so that a blue box appears around it. Then click on 'Save As' icon to save data. Also, click on superposition graph so that a blue box appears around it. Then click on 'Save As' icon to save data.

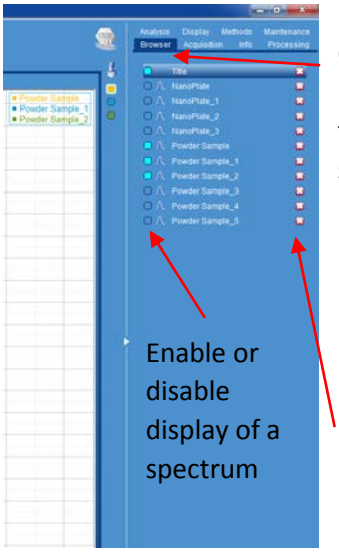




If the whole spectrum is save into txt format, it will have the following data arrangement:



8. File Management

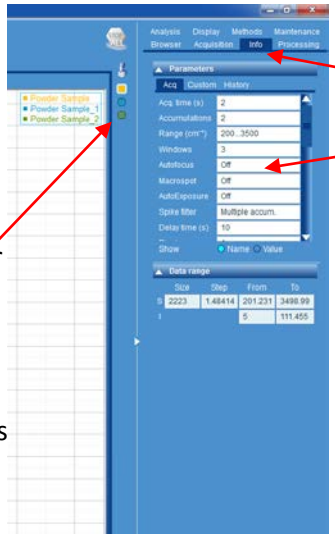


Choose 'Browser' tab to manage files temporarily stored in software

Choose a spectrum for graphic manipulation or to access its acquisition parameters, *etc*

Click 'Cross' to close files

Enable or disable display of a spectrum



Choose 'Info' tab to see summary of acquisition parameters of the spectrum chosen

9. Shutting down
    a. After saving your data, close all of the files by clicking on the 'Cross' following file names under 'Browser' tab.
    b. Close LabSpec6 software by clicking on the 'Cross' at top left corner.
    c. Lift objectives and take away your samples.
    d. Clean the working area.
    e. Logbook sign out

Troubleshooting

1. Florescence
   If florescence of the sample interfere with Raman signal, you may try switching to a different laser.
2. Unstable sample
   For unstable sample which may decompose under intense light, try using Laser filter (Smaller number means less transmitted light. 100% represents fully transmitted light.) to decrease the intensity of Laser.
3. Communication between computer and system
   If any issues occur during data acquisition process, you may try clicking on the 'STOP' icon to stop all system activities. You can either restart acquisition or close LabSpec6 software and restart it. Usually restarting software would reinitialize communication between computer and instrument. **Do Not** restart computer!