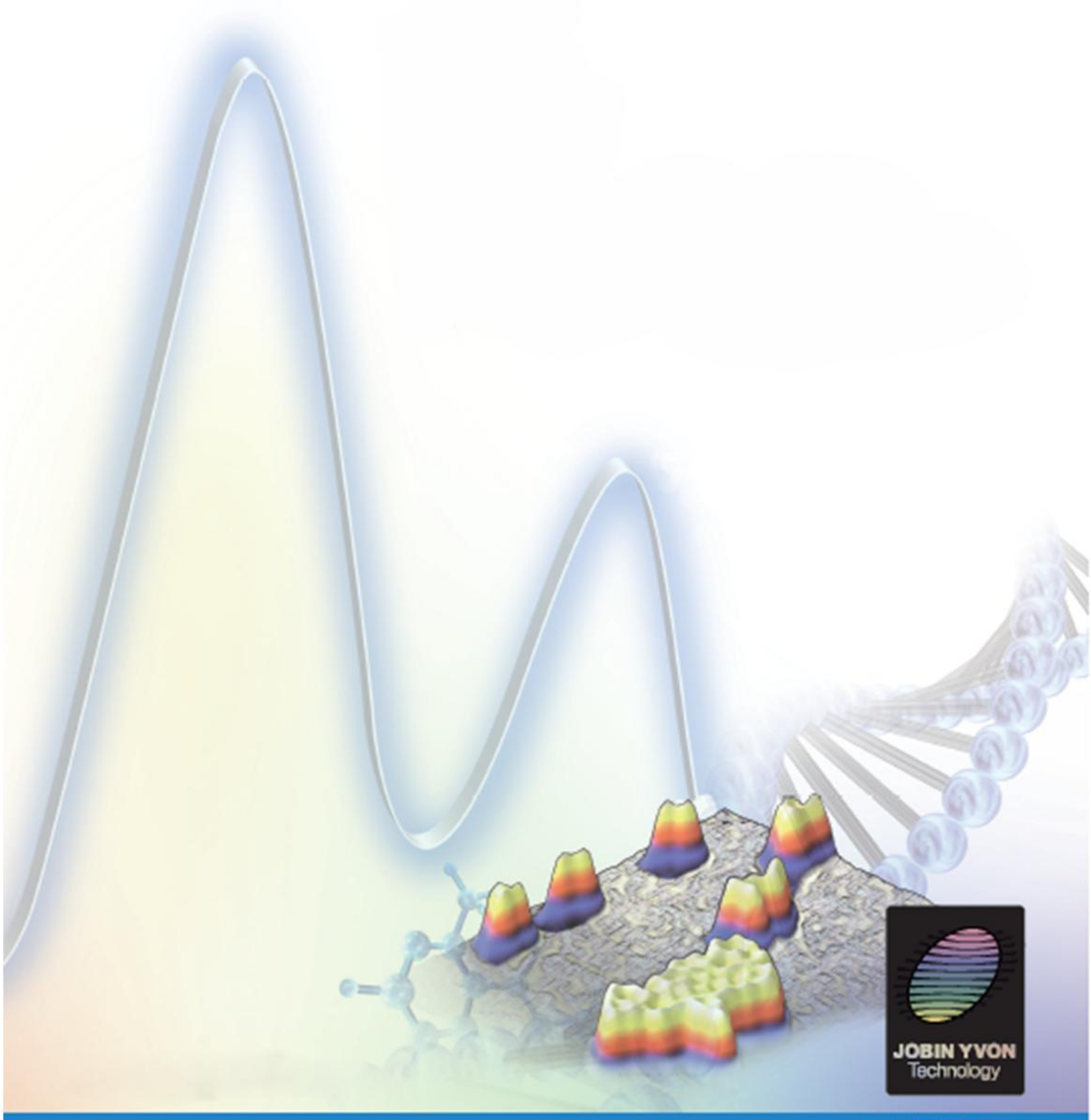
HORIBA Scientific

Quick Start Guide

LabSpec 6



Quick Start Guide

This Quick Start Guide is intended to provide a basic introductory user guide to the general structure of LabSpec 6, and a brief step by step guide to acquiring data. It is not exhaustive in content, and should only be used in conjunction with user training and other guidance provided by your HORIBA Scientific service engineer.

Contents

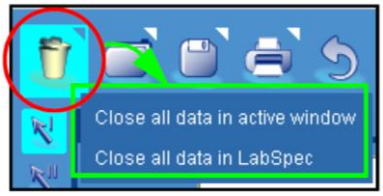
1, Switch on the system

・Switch on the laser(s) 30 minutes before measurement.
・Switch on the PC and double click the LabSpec 6 icon  on the desktop.

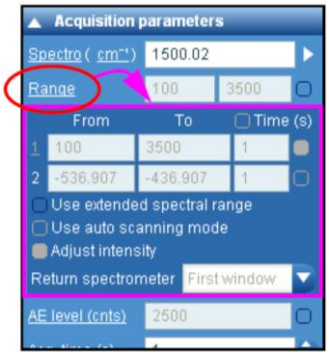
※ CCD detector and main instrument power supply are recommended to be always switched on.

2, LabSpec 6 main interface

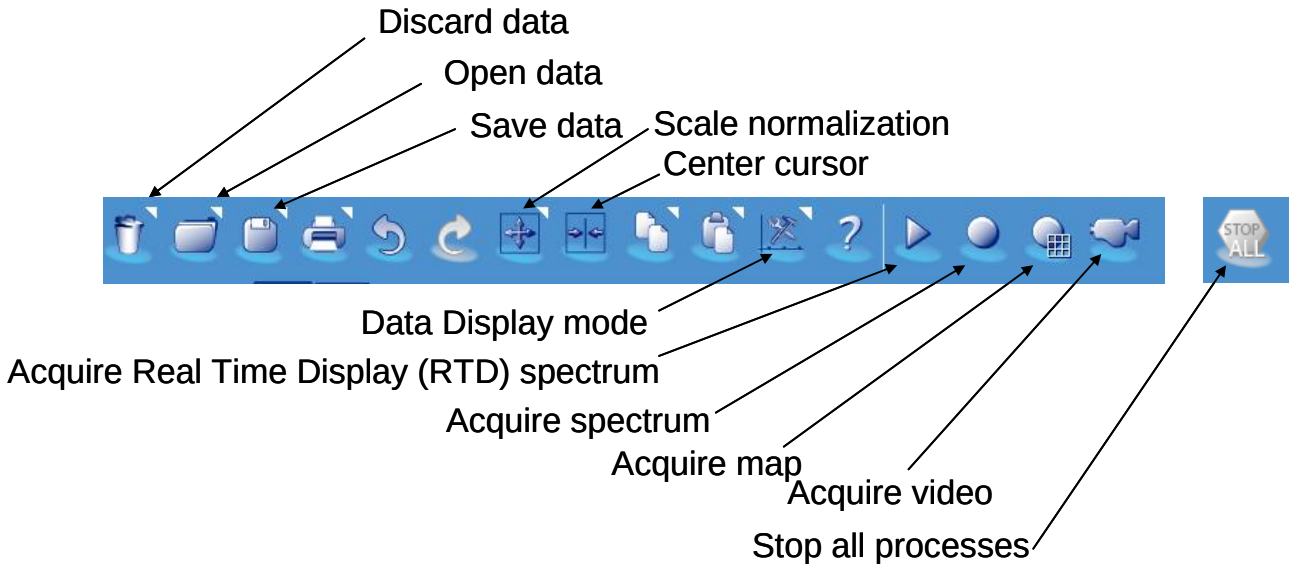
Any icon in the icon bar marked with a white triangle in its top right hand corner has a right click menu which offers further functionality and controls.



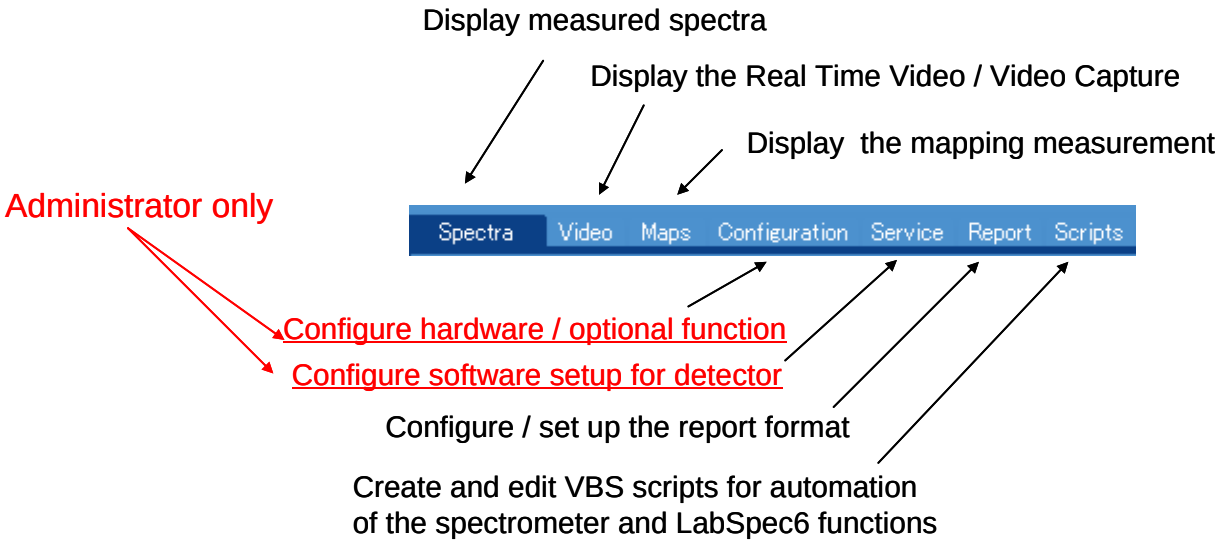
Any text in the Control Panel which is underlined acts like a hyperlink. Left click on the text to access additional menus or controls.



2-1, Icon bar



2-2, Data tab



Right click on the Data Tab names to add/remove tabs.

2-3, Control panel

The Control Panel on the right hand side includes all functionality required in LabSpec 6 to acquire, process, analyze and display data. The individual tabs of the Control Panel are organized as follows:

Browser: view a list of all data opened in LabSpec 6.

Acquisition: set up all acquisition parameters including hardware settings.

Info: view the information shell for each data file

Processing: data processing functions to modify raw data
(including smoothing, baseline correction and math functions)

Analysis : data analysis functions to obtain information from the data
(including peak fitting, map characterization and multivariate methods).

Display : configure the display of spectra / video / mapping window

Methods: create customized multi-step one click sequences
(including data acquisition and analysis).

Maintenance : access maintenance functions
including system calibration, AutoCalibration and AutoAlignment.

3, System calibration

It is necessary to calibrate the system using the HORIBA Reference crystalline Silicon sample. HORIBA's fully automated "AutoCalibration" function provides a fast and easy facility for this.

To learn more about how to work with the AutoCalibration module, please refer to the quick start guide for AutoCalibration.

4, Video acquisition

- Click  to see the real time video image
  On manually operated systems, set the video optics to the "video" or "viewing" position. Fully automated systems will do this step whenever the camera is started.

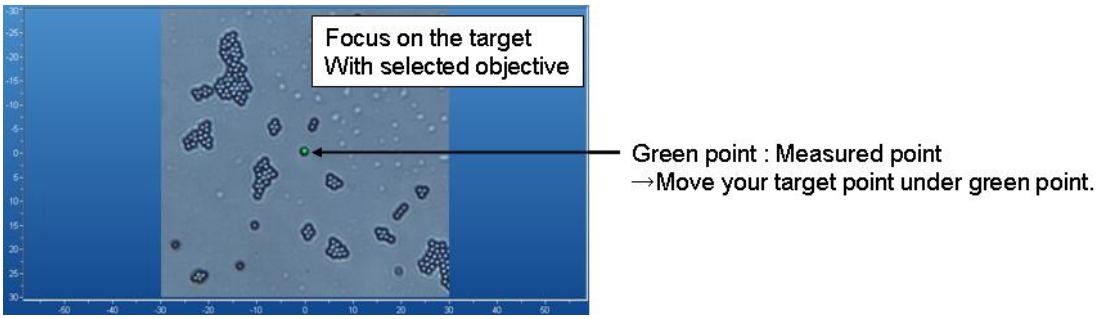
- Position the sample on the stage

- Choose the desired objective and focus on the sample

- In Acquisition > Instrument setup section, select the objective currently being used.
  

  It is important that the objective selected in the software matches the objective used on the microscope

- Choose the analysis position on the sample.
  The green marker on the video indicates the analysis point on the sample.



- Click  to stop the camera read out and capture the video picture

- Click "save data" icon  to save the image.
  - ".l6v" is the standard extension for a LabSpec6 video file, which retains all scale information.
  - Other image formats such as jpeg/tiff are also available, but these will not retain the video scale information.

5, Single point spectrum acquisition

5-1, Real time display

The Real Time Display (RTD) acquisition constantly reads out a spectrum with the selected acquisition time. There is no accumulation (averaging) of the spectra, and only a single window detector read out is possible (no extended spectral range).

The purpose of the RTD acquisition is to check that the measurement settings (including spectral range / laser selection / confocal pin hole) are properly selected and optimized.

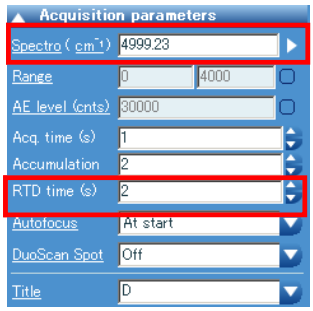
5-1-1, Set up

Set up the experiment configuration as needed, using particularly the Acquisition Parameters and Instrument Setup sections in the Acquisition tab.

Tip *RTD is best used with a short acquisition time (for example, <1 s), since this allows fast update of the spectrum display.*

Tip *RTD can only be used with a single window spectral range. Take care to select the spectro position where you expect to observe Raman peaks.*

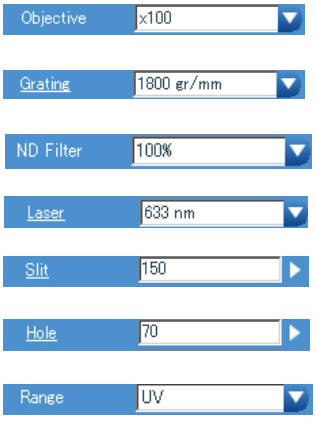
Acquisition parameters



・Set the spectro: for example 1000cm-1
Spectro is the center position of the measured spectrum.
Input the desired position.

・Set the RTD time : for example 2 sec
RTD time is acquisition time for RTD measurement.

Instrument setup



・Select the objective currently being used.

・Select the desired grating.

・Select the desired ND filter.

・Select the desired laser.

・Input the desired slit size: For example 100um.

・Input the desired Hole size: For example 200um.

・Select the Optical system for Visible / UV according to your measurement.

Tips Useful information

Laser selection

In general, a Raman spectrum can be obtained with any laser. However, interference from fluorescence and sample damage (laser induced burning or chemical modification) can restrict which laser should be used.

The lower the laser wavelength (for example, blue/green lasers) the stronger will be the Raman scattering (assuming the same laser power). However, there is increased chance of competing fluorescence emission which may interfere with the Raman spectrum.

High laser wavelengths (for example near infra-red NIR lasers) are generally less susceptible to fluorescence interference, but Raman scattering is inherently weaker.

Acquisition time

In general, the longer the acquisition time the better the spectrum quality will be. The following points may be useful to help decide what acquisition time to set.

- The achieved signal to noise should be sufficient to view the peaks of interest.
- The maximum intensity in the spectrum should be less than 65,000 counts.
- The spectrum is stable (i.e., it does not continuously change, due to the sample being burnt or modified by exposure to the laser beam).

Grating

The user must select the required grating from the selection available on the system. Each instrument will have different gratings according the analytical requirements and included laser wavelengths.

Gratings are characterized by their dispersion (in grooves per mm, gr/mm) and optimized wavelength range. In general, the following characteristics are typical:

	300gr/mm	600gr/mm	1200gr/mm	1800gr/mm
intensity	high	←	→	low
measured range	wide	←	→	narrow
spectral resolution	low	←	→	high

In addition, it is particularly important that the user selects the grating which is optimized for the chosen laser. For example, a UV laser should be used with a UV optimized grating; similarly Visible and near Infra-Red (NIR) lasers should be used with visible and NIR optimized gratings, respectively.

Confocal pinhole

The confocal pinhole affects the sample analysis volume, and the confocal performance of the system. Running a system in confocal mode provides the very best spatial resolution in XY and Z dimensions. Generally the confocality should be adjusted to match the analysis required; it is strongly dependent on the chosen objective too (see below).

Large value: low spatial resolution and non-confocal analysis; higher signal due to large sample analysis volume. Useful for macro measurements and bulk analysis.

Small value: high spatial resolution and confocal analysis; lower signal due to small confocal sample analysis volume. Useful for high spatial resolution of microscopic particles and features.

Objectives

Standard objectives for the visible range are x10, x50, x100 objectives.

With high magnification objectives (such as x50, x100) the achievable spatial resolution is high, and laser power density on the sample is also high. These objectives are particularly well suited for working with opaque samples and microscopic features.

With low magnification objectives (such as x10, x20) the achievable spatial resolution is low, and laser power density on the sample is also low. These objectives are particularly well suited for working with transparent samples, liquid samples, and macroscopic features.

5-1-2, Start measurement

・check the video is stopped (click 

if necessary)

・On manually operated systems, set the video optics to the “Raman” position. Fully automated systems will do this step whenever the camera is stopped.

・Click  and confirm spectrum quality as discussed above.

5-2, Spectrum acquisition

The full spectrum acquisition mode acquires the spectrum of the sample with multiple accumulations (for averaging) and over a full spectrum range, if desired.

In general, the settings used can be the same as Real Time Display (RTD) with the exception of spectral range, acquisition time and number of accumulations.

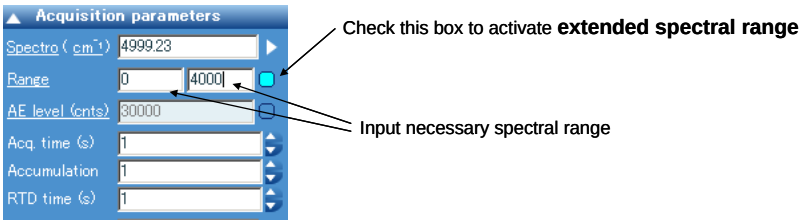
5-2-1, Selection of spectral range

Set the spectro position which will be the center of the detector read out window.

Tip *hover the mouse over the spectro text box to view the width of the current spectral window.*

Alternatively, if an extended range measurement is required, tick the "Range" box, and input the start and stop values for the range. The software will automatically move the spectrometer to acquire the full range.

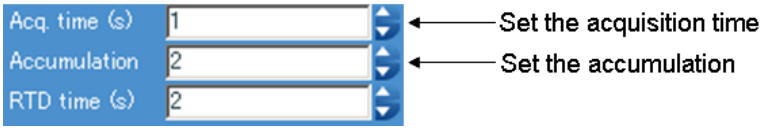
Tip *hover the mouse over the Range label to view the number of windows needed to cover the range.*



5-2-2, Setting of acquisition time and number of accumulations

In Acquisition parameters set the acquisition time, and the number of accumulations.

Tip *as the acquisition time and number of accumulations increase, the spectrum quality will also improve.*



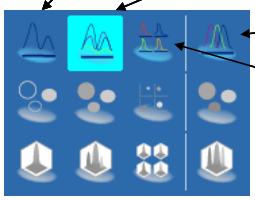
5-2-3, Start measurement

Click  to acquire the spectrum.

5-2-4, Display mode of the spectra

Recorded spectra are displayed in the Spectra data tab.

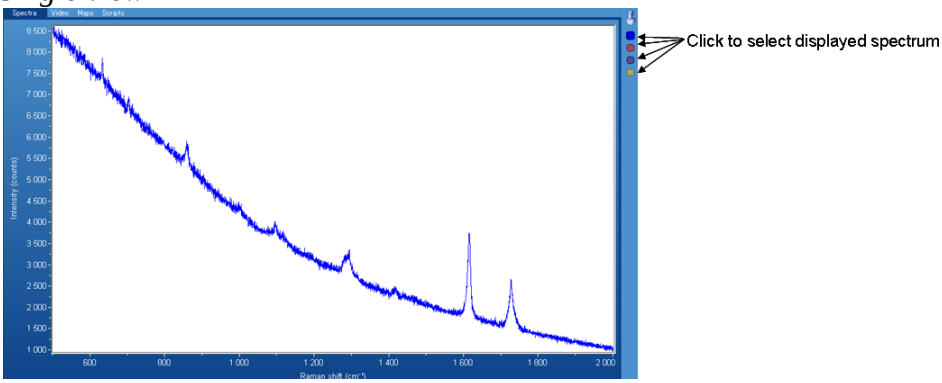
The display mode interface  allows a user to change the Display mode of the spectra tab



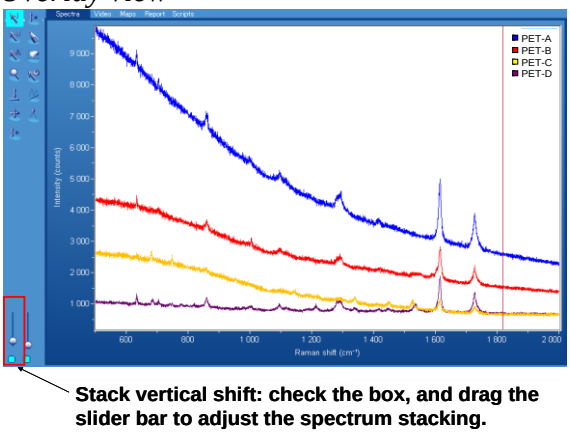
In order to zoom in to a specific spectral region, click  in the graphical manipulation tool bar and drag the target region of the spectrum with the cursor

In order to rescale the spectral view (i.e., remove the zoom), click  in the Icon bar, or right click on the spectrum and select “Rescale”.

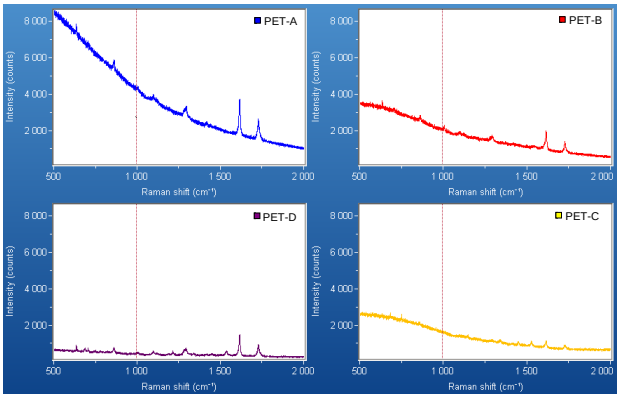
Single view



Overlay view



Tile view



5-2-5, Save data

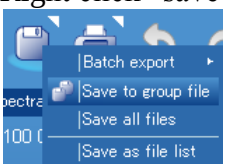
- **Save the selected spectrum**

  Click "save data" icon 

  - ".l6s" is the standard extension for LabSpec6. Data saved in this format will retain the full information shell, including acquisition parameters, custom information, and file history
  - Other formats (such as .spc, .txt, .ngs etc) can be used for compatibility with other softwares, but note that the information shell will be reduced or removed. It will not be possible to restore the lost information.

- **Save all spectra in one group file**

  Right click "save data" icon  and choose "Save to group file".



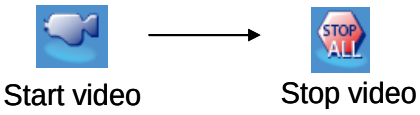
- **Save each spectra in separate files**

  Right click "save data" icon  and choose "Save all files".

6, Mapping measurement

- **Set sample and acquire a video image of the target position**

Acquire a single video image

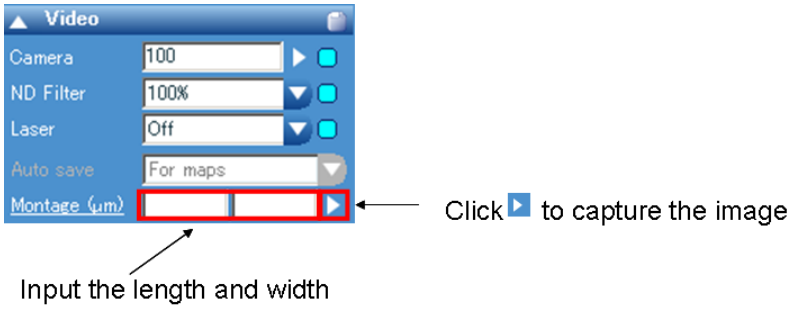


If a larger field of view is required, then the video *Montage* function can be used (only available on systems equipped with an automated XY sample stage).

The montage function is only available when the video is not active. If necessary, click  to stop the current video read out.

Input the required length (µm) and width (µm) of the montage area.

Click  to capture the image

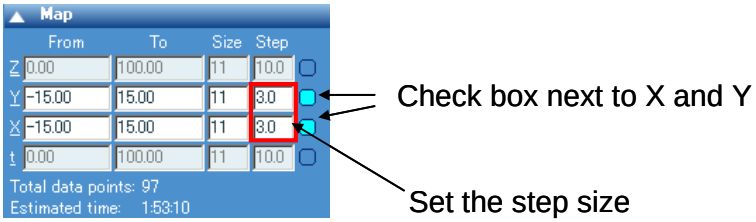


- **Set up mapping parameters**

In the Map section of the Acquisition tab, select the variable that you want to map:

X, Y, Z = spatial dimension [requires automated sample stage]
T = temperature [requires heating/cooling stage]
t = time

For example: XY mapping



- **Set up mapping shape for XY mapping and select area**

Select the map shape form graphical manipulation toolbar in the left side of the main window. The cursor of the selected shape appears.

Use the cursor adjust the map area position and size

 : rectangle area

 : circle area

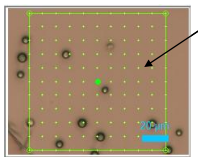
 : hexagon area

 : vertical line

 : horizontal line

 : free line

 : multiple point selection



- **Set acquisition and instrument parameters**

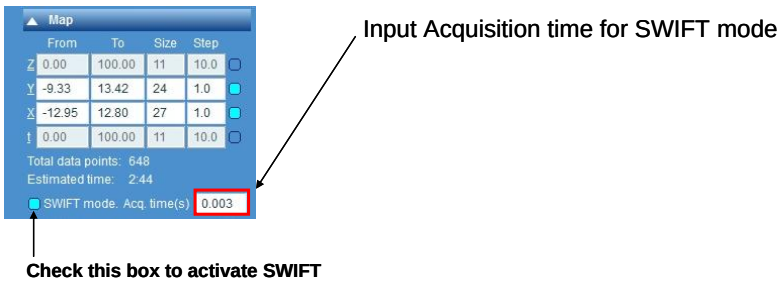
In general the spectrum acquisition at each point of the map can be done using similar parameters to those for a single point measurement. However, note that a map may require the acquisition of many hundreds or thousands of spectra, so it is important to consider the total time required for the map.

It is often advisable to do map acquisition with only a single spectral window (i.e., do not use extended spectral range settings) and with faster acquisition times and less accumulations to save time. Of course, appropriate settings must be chosen for the experiment, even if this means spending a long time to acquire the data!

- **Choose "Point by Point" or "SWIFT" mapping mode (for XYZ mapping only)**

The optional SWIFT mode (not available on all systems) can be used to acquire very fast map data, and should be used for acquisition times <0.5s.

The setting is in Acquisition > Map section.



Note that SWIFT mapping can only be used for single window spectrum acquisition (extended spectral range is not possible) with a single accumulation.

- **Start map acquisition**

Click  to start the mapping acquisition

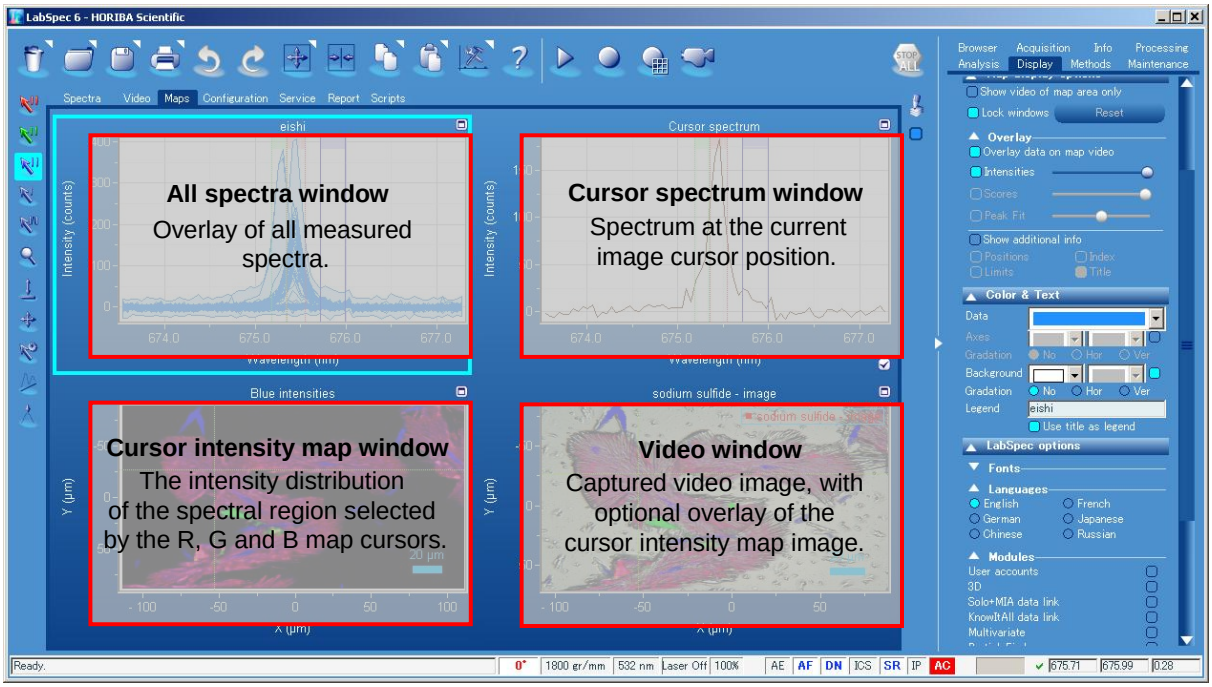
- **Save the map data**

Click “save data” icon 

- “.l6m” is the standard extension for LabSpec6 map files. Data saved in this format will retain the full information shell, including acquisition parameters, custom information, and file history
- Other formats (such as .spc, .txt, .ngc etc) can be used for compatibility with other softwares, but note that the information shell will be reduced or removed. It will not be possible to restore the lost information.

- **Mapping data window**

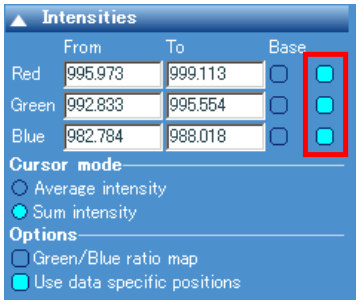
The acquired map data will be presented in the Maps data tab:



- **Create an intensity map**

Select a cursor from the graphcial manipulation toolbar  . The double line cursor can be adjusted to bracket a specific peak or spectral range.

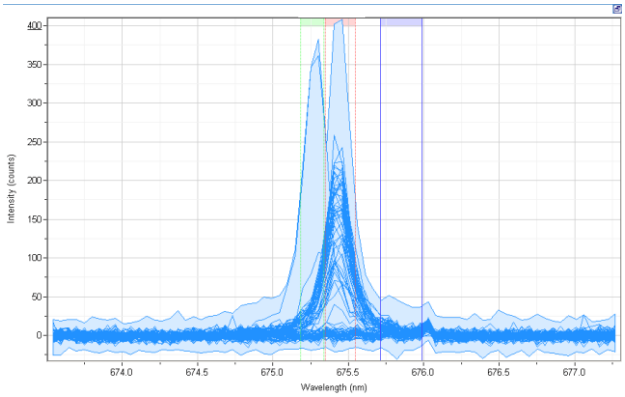
Check/uncheck the box in Analysis > Intensities section to turn on/off each cursor.



Check / uncheck these boxes to choose the cursor to use.

The sum or average intensity between the cursor lines will be used to create an intensity distribution in map window.

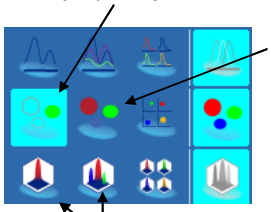
3 ranges are selected simultaneously.
User can select range to use



Right click in map window and select Display mode

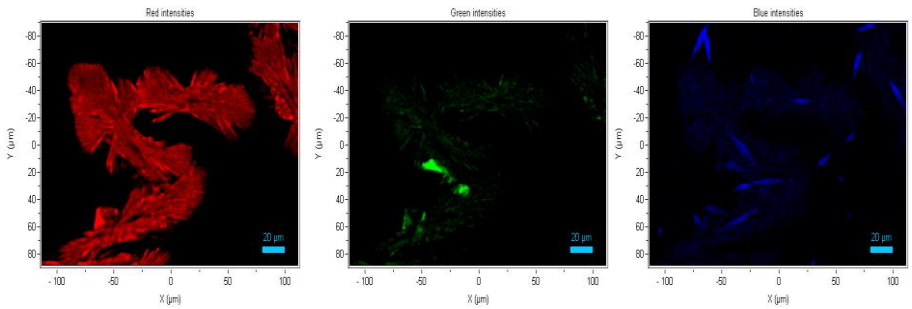
Display single cursor intensity image

Display overlaid cursor intensity images

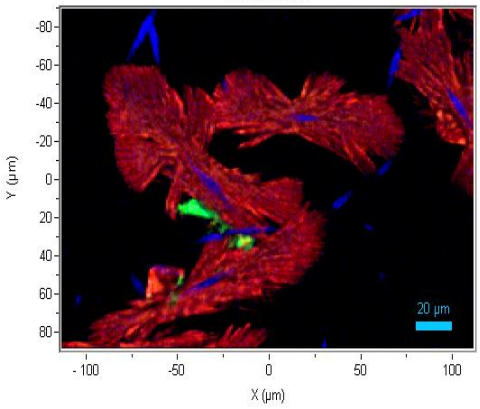


3D display

Single intensity images:



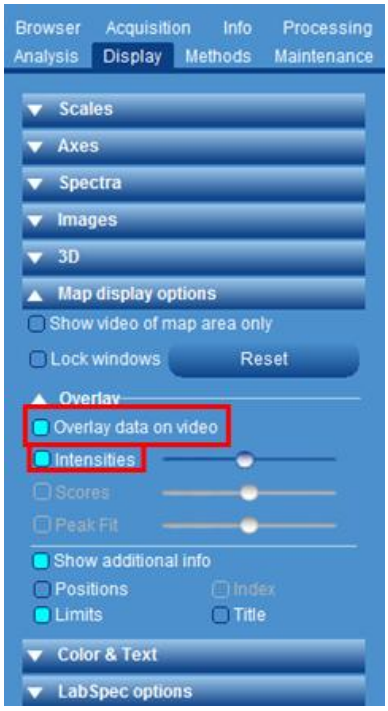
Overlaid multiple intensity images :

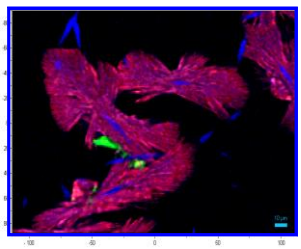


- **Overlay cursor intensity map on the video**

  In Display > Map display options section, check “Overlay data on video” and “ Intensities”.

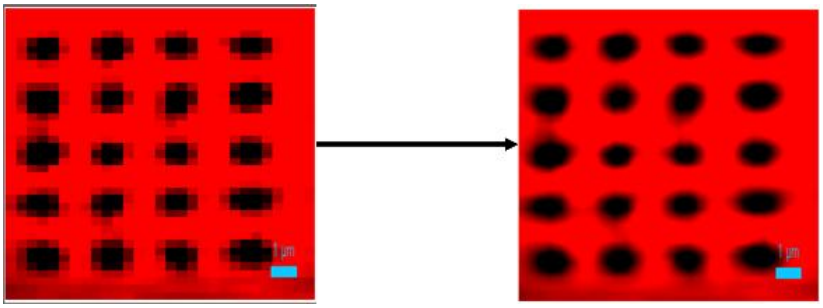
  Use the slider to adjust the transparency of the overlay.





  In order to display only the video of the map area, check tick box “Show video of map area only”.

- **Smooth the image for improved display**

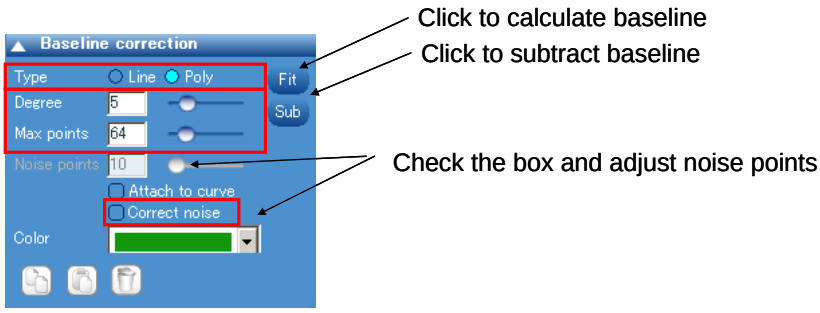


Right click the intensity map image window, and then select Smoothing. Select Smoothed to interpolate data points in the display and give a smoother rendering.

The degree of smoothing can be adjusted in Display > Image section, using the smoothing slider.

7, Data processing

7-1, Baseline correction



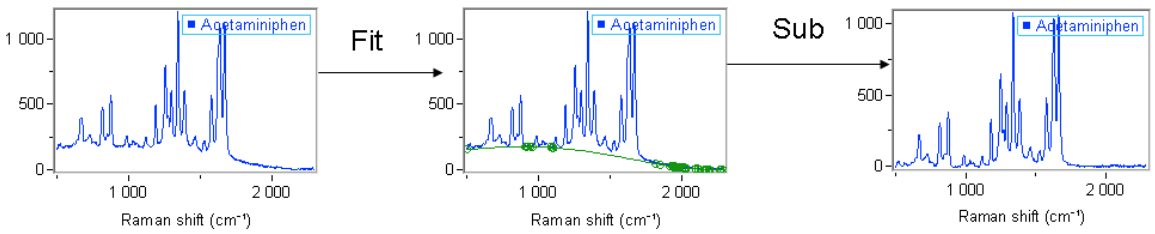
Type: Choose Baseline type of function: Line or Polynomial.
Degree: Input the degree of the baseline polynomial function. The larger the degree, the more curved/flexible will be the baseline.
Max points: Input the maximum anchor point for calculating baseline.
Correct noise: Improved baseline fitting on low signal to noise spectra. Tick "Correct noise" and adjust the noise points.

Click [Fit] to automatically create a baseline curve for the data.

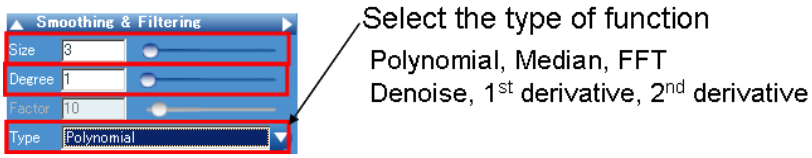
It is possible to create the baseline curve by hand.

Select "add / remove baseline point" icon  in graphical manipulation bar, and place the baseline points on the spectrum by hand.

Click [Sub] to subtract the baseline curve from the original spectrum



7-2, Smoothing and filtering



Size: Size of the moving window used for the calculation. Larger number, stronger effect.
Degree: Degree of the function used for calculation. Smaller number, stronger effect.
Factor: Factor applied to the calculation. Larger number, stronger effect.

The operation will be made as soon as one of the sliders is adjusted. Alternatively, click to run the smoothing/filtering operation.

8, Data analysis

8-1, Peak labelling and peak fitting

For **Peak Labelling**, adjust "Ampl" and "Size". As soon as the sliders are adjusted, the peak searching will occur. Alternatively click on [Find] to run the peak search.

It is possible to add peak position by hand.

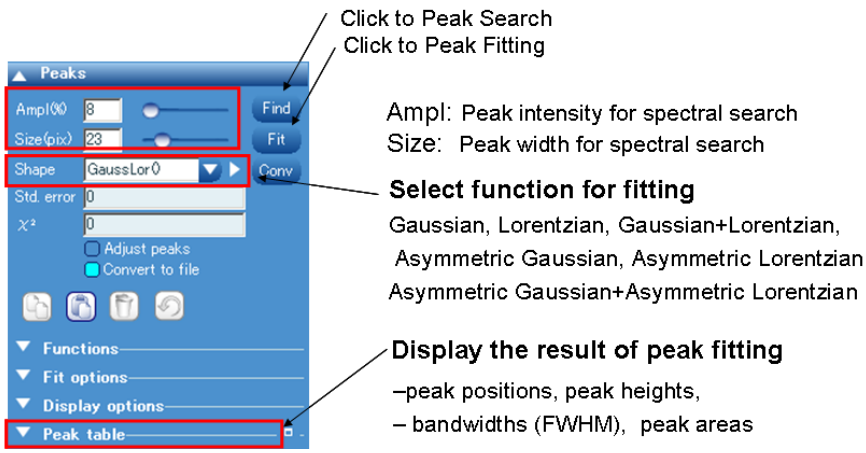
Select "add / remove / edit peaks" icon  in graphical manipulation tab, and manually place peaks on the spectrum by hand.

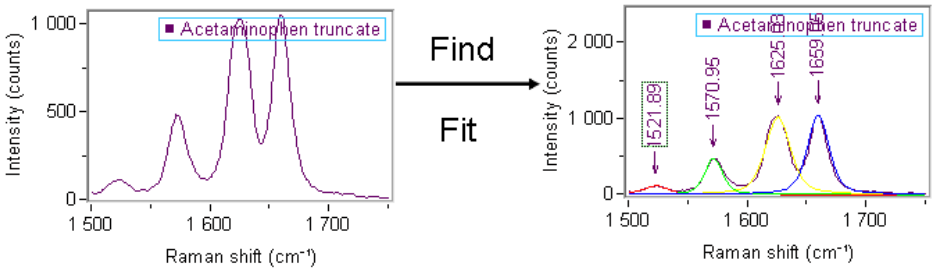
Use the "Display options" section to control what shapes/parameters are displayed on the data (for example, peak label, peak shape, sum of all peaks etc).

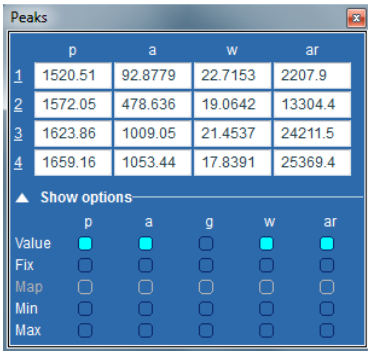
For **Peak Fitting**, select the desired peak shape function (e.g., Gaussian, Lorentzian) and click [Fit].

Fitting parameters (including number of iterations) can be adjusted in the "Fit Options" section.

The fitting results (peak position, peak intensity, peak width FWHM etc) are displayed in the Peak table.







Display peak table

p: peak position
a: peak height
w: FWHM
ar: peak area