

Short Instructions for Raman Microscope Horiba Xplora

Dejan Lazić

When laser light hits the sample most of the light is reflected with no shift in the frequency. Part of photons transfer energy to the sample which irradiates it with the shifted frequency. Fluorescence occurs when the sample absorbs energy and releases it slowly. Instantaneous changes in frequency are Raman scattering. Shift in frequency, Raman effect correlates to the energy difference between ground and virtual state. Emitted light of lower energy than the energy of laser are called Stokes lines and ones of higher energy are called anti-Stokes lines. They are formed if electron was excited before light hit it. Considering the fact that more molecules are in the ground state, it is expected to see more Stokes than anti Stokes lines. Antistokes lines could be interesting if Stokes lines are masked with fluorescence.

How to cite this book chapter:

Lazić, D. 2019. Short Instructions for Raman Microscope Horiba Xplora. In: Vucelić Radović, B., Lazić, D. and Nikšić, M. (eds.) *Application of Molecular Methods and Raman Microscopy/Spectroscopy in Agricultural Sciences and Food Technology*, Pp. 199–206. London: Ubiquity Press. DOI: <https://doi.org/10.5334/bbj.q>. License: CC-BY 4.0

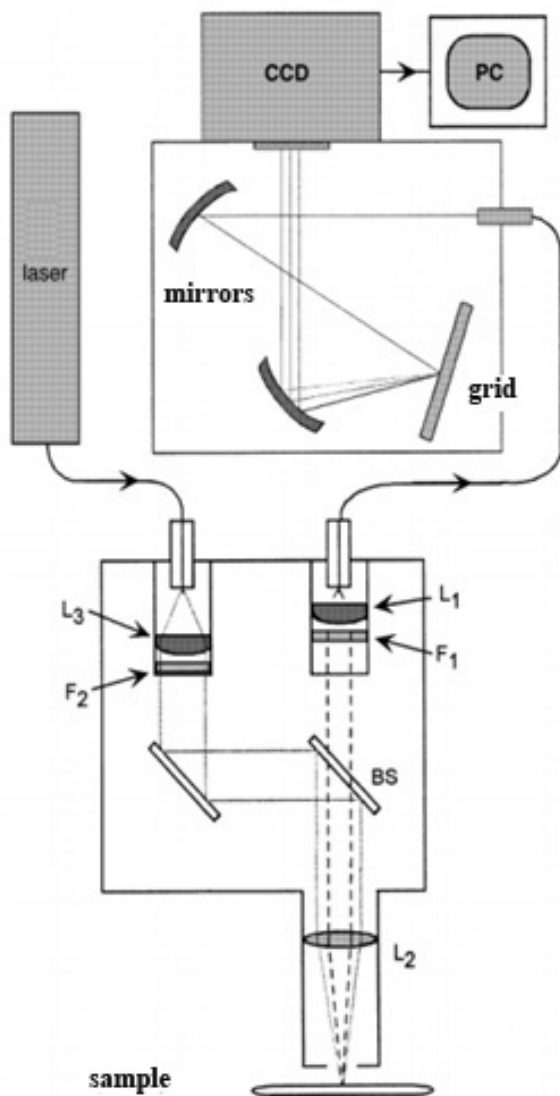
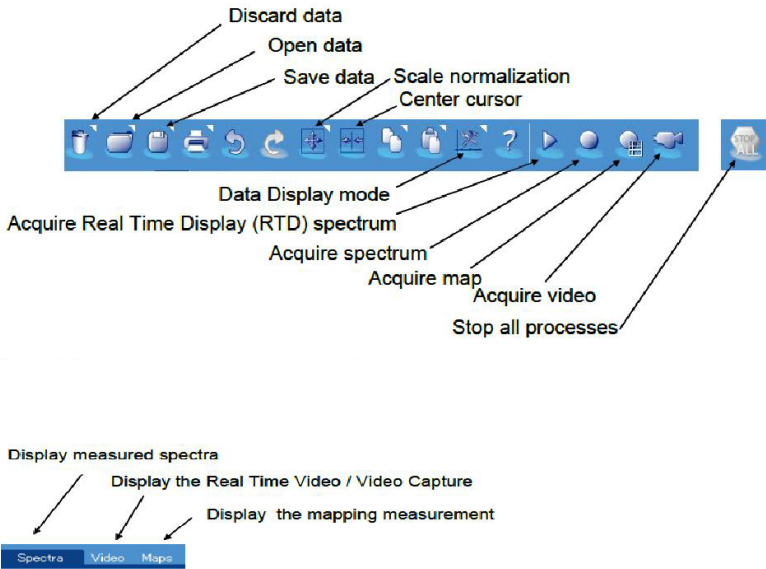


Figure 1: Construction of Raman microscope.

As Horiba Xplora instrument is the only Raman microscope at the Faculty of Agriculture further short instructions are aimed to facilitate its use by newly trained staff and PhD students in their research.

Turn on by pressing a switch on the extension cord. Switch on the computer, start LabSpec 6, icon on the middle of the screen.

Chech if lasers are powered on.



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 LabSpec6.

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

Methods: create customized multi-step one click sequences, data acquisition and analysis.

Maintenance: system calibration, Auto Calibration and Auto Alignment

Autocalibration

If you see a red **AC** at the bottom of the screen autocalibration is required.

Figure 2: Basic commands on LabSpec 6.



Place reference silica plate with marked lends 100X

Sharpen

Press **AC**, activates process of autocalibration

Video acquisition

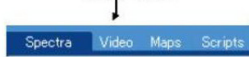
Set filter wheel on position 2



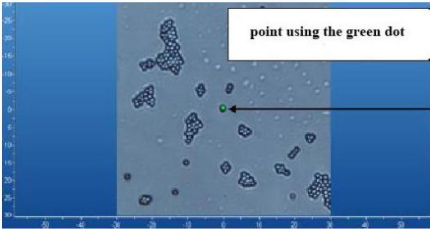
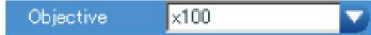
Activate camera



show video



Set objective in use



Stop.



Real Time Display

Set filter wheel on position 1

Figure 3: Video acquisition.



Set the spectrometer

Objective	x100
Grating	1800 gr/mm
ND Filter	100%
Laser	633 nm
Slit	150
Hole	70

Set the RTD time

▲ Acquisition parameters	
Spectra (cm ⁻¹)	4099.23
Range	0 4000
AE level (cnts)	30000
Acq. time (s)	1
Accumulation	2
RTD time (s)	2
Autofocus	At start
DuoScan Spot	Off
Title	0

Set objective

Greater number of lines gives better resolution but a narrower spectrum

Set laser intensity.

Set laser wavelength.

Less gives better resolution but lower intensity

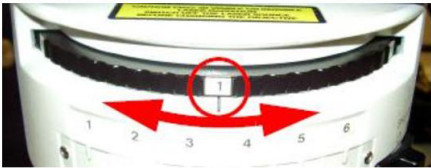
Smaller hole, better resolution, lower intensity

Activate RTD  . Stop at 

Spectrum acquisition

Set the filter wheel to 1

Figure 4: Acquisition parameters.



activate extended spectral range


input necessary spectral range


Set acquisition time
Set the accumulation


Record spectrum , save it 

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  in task bar, or right click on the spectrum and select "Rescale"

Single view : 1 spectrum is displayed in window

Overlay view : all the recorded spectra are displayed in window

Normalize: show all displayed spectra with normalized intensities

Tile view: all recorded spectra are displayed in individual tiled windows

Figure 5: Options for viewing the spectrum.

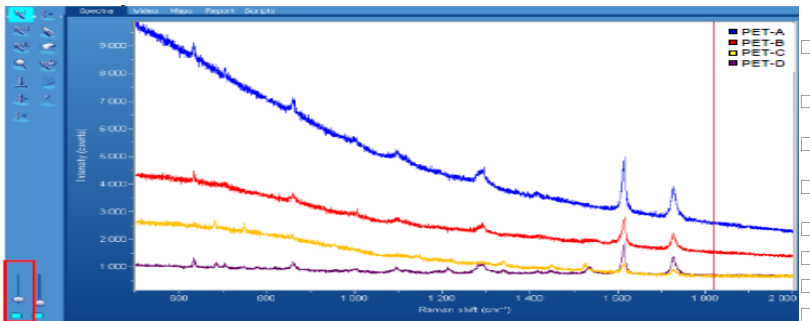


Figure 6: Overlay view of Raman spectra.

