Short Instructions for Raman Microscope Horiba Xplora
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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. Anti-Stokes lines could be interesting if Stokes lines are masked with fluorescence.
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.

Check if lasers are powered on.

Figure 1: Construction of Raman microscope.
**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:

- **Analysis**
- **Display**
- **Methods**
- **Maintenance**

**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

**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 [ ] at the bottom of the screen autocalibration is required.

**Figure 2**: Basic commands on LabSpec 6.
Place reference silica plate with marked lens at 100X.

Sharpen.

Press AC to activate the process of autocalibration.

**Video acquisition**

Set filter wheel on position 2.

Activate camera.

Set objective in use.

Real Time Display.

Set filter wheel on position 1.

*Figure 3*: Video acquisition.
Set the spectrometer

Set the RTD time

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 into 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”

**Figure 5:** Options for viewing the spectrum.
Figure 6: Overlay view of Raman spectra.