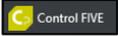


CONFOCAL RAMAN MICROSCOPE – ALPHA 300 R

Performing Raman Spectroscopy Analysis

Starting the System

- Switch the computer **ON**.
- Wait until the system status is ready. Check it via **WiTec Service Monitor** on the right side of the **main taskbar**:
 - Click on the icon with a left mouse button.
 - All controllers must be checked green. 
 - If not, please, restart the computer.
- Select proper laser for the measurement – the wavelength is written on the top of the laser body:
 - 355 nm laser (0 to 4 mW power range)
 - 532 nm laser (0 to 53 mW power range)
 - 633 nm laser (0 to 30 mW power range)
- Turn **ON** required laser with the key to the **ON** position prior measurement (to stabilize the laser electronic).
- Start **Control FIVE**  software for data acquisition.
- Wait until the detectors are cooled down to the required temperature of -60°C.



Inserting Sample

- Turn the microscope objective turret to the empty position.
- Put the laboratory glass on the holder and fix it with metal clamps.
- Use the tweezers to insert the sample on the laboratory glass.
- Check if the objective 10x is already set in the software. If not, please follow the step 34.
- Move the slider above the objective turret to **BF** position and pull the rod out of the **Camera Coupler**.
- Adjust the contrast with **AutoBrightness** icon / click on “Y” button on **EasyLink Controller**.
- Use the **EasyLink Controller** to focus the sample surface:
 - Use the focus **Up / Down** button
 - With holding **Continuous Movement** button for smooth movement and rough focusing (direction towards the user is moving the objective down, opposite is moving up).
 - Without this button, for small step movement and precise focusing.
 - With right joystick move the sample to the desired position (red circle represent the laser beam position).
- For spectra measurement, select objective 100x (for measurement with a UV laser, objective 40x is recommended):
 - In **WiTec Video Control** window, select **100x** objective from roll-up menu.
 - Turn the turret to the position with Zeiss objective 100x.
 - Confirm exchange in **Turn Objective Turret** window with **Finish** button.
- In small steps, adjust sample surface focusing and positioning.
- Acquire **Video Image**  or **Stitch Video Image**  of the sample for future selection of measured spot.



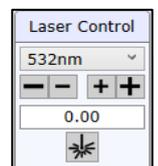
System Adjustments

- Select desired laser and spectrometer with CCD camera – in the **Main Menu** select **Configurations /Raman/**
 - Raman CCD1** – 355 nm laser and UV spectrometer
 - Raman CCD2** – 532 nm laser and VIS spectrometer
 - Raman CCD3** – 633 nm laser and NIR spectrometer

18. In **Control** menu, check the number of chosen **Spectrograph**. According to the sample material, select proper **Grating** (for the beginning of measurement usually the lowest number), and **Spectral centre**.
19. Concerning selected spectrometer, laser source can be changed in the **Laser Control** menu. Laser power has to be set concerning the sample material – too high laser power may damage the sample!!! Minimum reachable spot size is approximately 250 nm (X, Y) and 700 nm (Z).
20. Adjust microscope tower configuration to be able to analyze the sample with selected spectrometer and laser:
 - a. Move the slider above the objective turret to **empty** position and push the rod into the **Camera Coupler** to direct scattered light from the sample up to the microscope tower.
 - b. Rotate **Raman Filter** wheel, placed on the **Laser Coupler**, and select the filter wavelength according to the laser source wavelength to filter primary laser beam energy out of the measured spectra.
 - c. Send the signal to the desired spectrometer for the analysis by pulling the rod out of the proper **Output Coupler**. Check that rods belonging to two other **Output Couplers** are pushed in.

Laser Signal Optimization

21. With **EasyLink Controller**, move to the homogeneous area on the sample. The laser signal is best optimized using detection of silicon peak (520 cm^{-1}).
22. In **Laser Control** window, select **532 nm** laser and adjust laser power to **25 mW**.
23. In **Control** menu / **Spectrograph2**, select the lowest **Grating / G1 = 600 g/mm** and **Spectral Center** approximately **1200 cm^{-1}** .
24. Start **Oscilloscope** mode. The real-time silicon spectrum appears.
25. Use the **EasyLink Controller** and **Z-joystick** to focus until you get maximum intensity (=height) of the silicon peak.



Oscilloscope	Start Oscilloscope
Stop	Stop
Integration Time [s]	0.05000

Single Spectra Acquisition

26. Go to **Single Spectrum** tab, where you can define the **Integration Time** and **Accumulations**. The spectrum is recorded from the spot marked by a red circle displayed in the **WITec Video Control** window.

Single Spectrum	[10, 0.500]
Acc. Single Spectrum	Acc. Single Spectrum
Stop	Stop
Integration Time [s]	0.500
Accumulations	10
Infinite Accumulation	No

Spectral Map Acquisition

27. Open **Large Area Scan** tab. In the subtab **Geometry / Listen Position/Area** select from the roll-up menu **Area (Once)** possibility and in **WITec Video Control** window define the area on the sample for measurement.
28. Modify **Width** and **Height** dimensions of the defined area to get rounded values.
29. **Points per Line** and **Lines per Image** parameters set the point resolution of the map.
30. Finally, set the **Integration Time** per one spectrum (one point).
31. **Start Large Area Scan**.
32. The **Large Area Scan Filter** window appears. Filter(s) for the peak intensity mapping can be selected here. This filter function enables to visualize immediately a distribution of different Raman peaks across the defined sample area.
33. Post-processing of acquired data can be also done after finishing the map.

Large Area Scan	[150, 150, 2000, 2000]
Scan Method	Area
Topography Correction	none
Signal Stabilization	Start Stabilization
At Every Point	
Points per Line	150
Lines per Image	150
Layers per Scan	1
Geometry	[2000, 2000]
Listen Position/Area	Area (Once)
Width [μm]	2000
Height [μm]	2000
Depth [μm]	100
Center at Current Pos.	Center at Current Pos.
Center (X) [μm]	0.000
Center (Y) [μm]	0.000
Center (Z) [μm]	0.000
Gamma [°]	0
Start LA Scan	Start Large Area Scan
Restart	Restart
Stop	Stop
Act. Int. Time [s]	0.1
Integration Time [s]	0.1
Min. Time for Retrace	1.00

System Shut Down

34. Before finishing your measurement, please change to objective 10x:
 - a. In **WITec Video Control** window, select **10x** objective from roll-up menu.
 - b. Turn the turret to the position with Zeiss objective 10x.
 - c. Confirm exchange in **Turn Objective Turret** window with **Finish** button.
35. Turn the turret to empty position and take your sample out.
36. Switch off the laser(s).
37. Close the **Control FIVE** software and wait until the cameras are heated up to room temperature. Or the option **Shutdown Computer after WITec Control finished** can be checked to shutdown PC after the heating procedure.

