Confocal Raman Microscopy (WITec Alpha 300R)

Please refer to Witec Alpha300R Confocal Raman Microscope User Manual for the details of the operating procedure.

Sample preparation

1. Attach your sample on a microscope slide.

Mounting samples on the stage

1. The objective in position to start with should be 10x. Laser should be turned off. (Important Note: Since the WITec microscopes use various laser sources during operation, all users, and everyone near this system should be aware that direct eye contact with the laser beam can cause serious eye damage and possible blindness.)

2. Place the microscope slide on scan stage, secure the slide with clips.

System Preparation

1. Start WITec Control Program by clicking on the icon in the desktop. On the window, you will see many subwindows, the top left is to control the operation parameters, top middle is the messaging window, top right shows the data and measurement files that are created, bottom middle window is the camera view.

2. The program takes ~5-8 mins to check the status of hardware and cool the CCD, and gives the messages in the “Message” window. Wait until the CCD is cooled down on the operation temperature (-60 °C). The messaging window will inform you when it is reached.
3. In the ‘Control’ window, switch on the illumination, and set the illumination to the required level (3 for 10x objective). Move the reflector slider to the illumination position, to direct the light to the objective. The beamsplitter is usually mounted in the left chamber of the three position slider. Use the pushrod (push in), and slide the prism to direct the beam to the video camera.
4. Focus on the sample by raising the stage in z-direction, this movement is controlled by the graphic control window. Start with a low speed and click on the red arrows to move in +z or –z directions. The speed of microscope on z-direction can be adjusted by selecting speed on right side black circle. The focusing speed can be adjusted between about 0.01 µm/s (potentiometer fully counter-clockwise) and 500 µm/s (potentiometer fully clockwise).

(Important: Always keep watching the distance between the sample and the objective lens. Never move the microscope too fast and hit the sample surface.)

5. If you want to switch to 100X objective lens: Increase the illumination level to 20. When you can get the focused image on Video Control window, switch to 100X objective by turning objective turret. Get the focus image of sample surface. At this time, select low speed of microscope z on the graphic control, e.g. less than 2 µm/s, and be careful not to touch the sample by objective.

Realtime Raman spectral observation

1. Turn on the laser source and wait for 5-10 minutes to see the green LED light.

2. The laser power is adjusted by the knobs. (The top view is shown on the right)

3. To start measurements, slide Reflector Slider (Black rectangular box in the middle of microscope body) to center position and pull the Pushrod out.

2. Check “Spectrograph”, and make sure the wavelength is set at “531.95nm”, and grating is at “600g/mm”. You may change the grating to “1800g/mm” if you want to get higher resolution of the spectrum (with smaller spectral range). Check the Center Wavelength e.g., 568nm.

3. Start the "Oscilloscope" to look at the realtime Raman spectra of the focal plane. Increasing the "Integration Time", or increasing the laser power may help to achieve an optimum signal. Ensure that you have adjusted the y-axis to view all spectra. When the background is minimum, adjusting the focus (+Z or -Z on the remote control) may enhance the spectrum.
Collect a Single Raman Spectrum

1. Stop the "Oscilloscope".
2. Go to "Control → Single Spectrum". Set desired "Integration Time" and "Accumulations" number (e.g. 0.5s integration time and 200 accumulations will need 100 seconds to collect a single spectrum).
3. Click "Acc. Single Spectrum". Wait until the measurement is done.

Take a Z Image Scan of the Sample

1. Go to "Image Scan" → Scan details → scan mode → depth.
2. Go to "Image Scan", set the scan dimension under "Geometry"
3. Set the resolution of the image: "Points per Line" and "Lines per Image". (Note the x or y resolution is ~250nm, and the z resolution is ~0.5um. More points will only increase your collection time, but not the resolution).
4. Go to "Microscope Z Control", click "Set Zero" to change the current Z position to 0. Come back to "Geometry", click "Center at Current Pos." Set "Center (Z) [um]" to a center coordinate of z (e.g. if you are scanning 2 um in z, and the current setting is 0. Then set it to -1um)
5. Set a proper "Int. Time (Trace) [s]", and click "Start Scan". Click on Scan1 in Data menu to Display the image.
6. Right Click on the image -> Export -> Bitmap to File. Save the data after scan is finished.

Turning Off

1. Turn off the Laser by turning the on/off switch on the Laser and then turn off the key.
2. Move the three-position slider to the left most position and push in the pushrod.
3. Close "WITecControl_1_38", wait until the CCD is warmed up.
4. Log off the computer. Log in your time.
Basic Analysis

1. Subtract background from the spectra: drag spectra data to “background”. It popups two windows: a prompt window and background preview. Click on “listen”.

2. Click on select an area button on the graph tools window. Select the peaks.

3. Click to clear area on the fit mask on the prompt window, the figure shows the peaks and purple region is the background.

4. Change the order in the prompt window, and click extract. You’ll obtain a new set of spectra with all background subtracted (spec.data(Sub BG)).