

WITec Confocal Raman Microscope *alpha300R*

THIS MANUAL IS TO BE SERVED AS A GUIDELINE FOR GENERAL USE OF THE WITEC CONFOCAL RAMAN MICROSCOPE. **YOU ARE EXPECTED TO TAKE NOTES DURING TRAINING SESSION** AND KEEP UPDATING YOUR NOTE BOOK AS YOU PROGRESS THROUGH PRACTISE SESSIONS. **PLEASE ALWAYS BRING THIS MANUAL AND YOUR NOTE BOOK WITH YOU EACH TIME YOU USE WITEC CONFOCAL RAMAN MICROSCOPE.**

PLEASE ALWAYS REMEMBER TO **ENABLE** AND **DISABLE** WITEC CONFOCAL RAMAN MICROSCOPE IN THE BADGER SYSTEM BEFORE AND AFTER YOU USE THE INSTRUMENT.

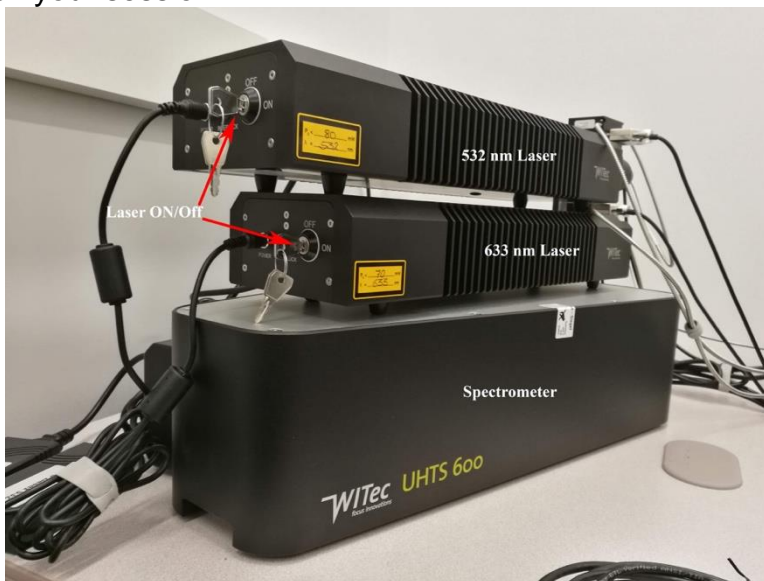
Safety

- DPSS laser, 532 nm, 75 mW, laser class 3B
- Diode/solid state laser, 633 nm, 50 mW, laser class 3B

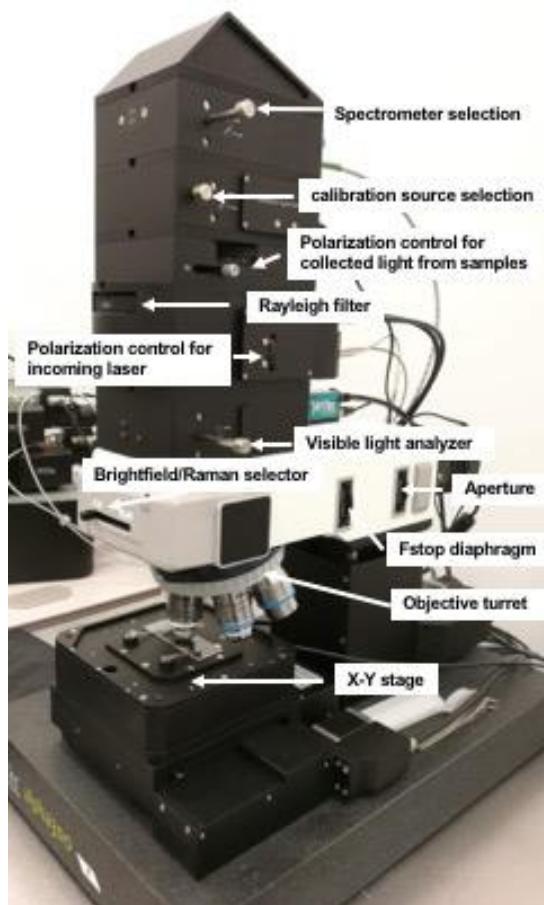
Laser safety eyewear is not required, but laser shield with the X-Y stage is recommended.

Lasers: Both lasers and spectrometer are located on the table to the left of the microscope. Fibers are used to connect laser sources to microscope and back to spectrometer. Pay attention to those fibers, DO NOT BEND/FOLD FIBERS. There is no hardware operation with spectrometer; there is only one operation that user can perform on laser control box, the laser power keys:

- Turn the key 90° clockwise turns on laser and 90° counterclockwise turns off laser. For stable power laser should be given 3-5 mins to warm up. Both lasers must be off at the time you finish your session.



Microscope: see the picture below for the microscope parts and functions



Spectrometer selection: Always pull out.

Calibration source: it is only used for spectrum calibration with external calibration source (pull out). Always push in for regular use.

Polarization control: use to change polarization angles of incoming and collected light.

Rayleigh filter: laser wavelength selection, always turn the wheel following the marked direction.

Visible light analyzer: white light selector for viewing samples in video mode. Pull out for WL video; push in for Raman spectrum/imaging.

Brightfield/Raman selector: left position is for BF imaging and right position is for Raman spectrum/imaging

F-stop and aperture: aperture is to control optical density and BF imaging contrast, F-stop is to help focus on difficult samples.

Objective turret: select one of the six objective lenses, turn the turret following the marked direction.

X-Y stage: automated stage is controlled by software or Xbox controller in XY directions. Automated Z movement is controlled by software or Xbox controller that moves the whole microscope head (objective lens move in Z direction).

Objective lenses:

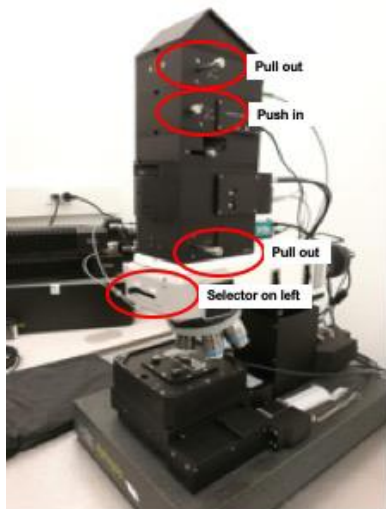
1. 10X: Zeiss EC Epiplan-Neofluar, WD9.3mm, NA 0.25 DIC
2. 50X: Zeiss EC Epiplan, NA 0.75 HD (darkfield only)
3. 50X: Zeiss EC Epiplan-Neofluar, WD0.58mm, NA 0.8 DIC
4. 100X: Zeiss EC Epiplan-Neofluar, WD0.31mm, NA 0.9 DIC
5. Empty (compatible with Zeiss water-dip and glass-corrected lens)
6. 50X: Nikon DF Plan ELWD, WD8.7mm, NA 0.55 (heating stage only)

Software: Microscope is controlled by Control FIVE, which can be started by double click on the desktop icon. Control FIVE contains four major windows: Control, Message, WITec Video Control, Project Manager (part of Project FIVE)

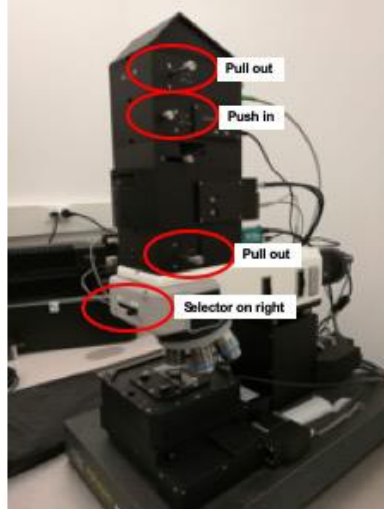
Microscope Settings:

Microscope has three major settings that user is able to choose depending on applications (see figure below):

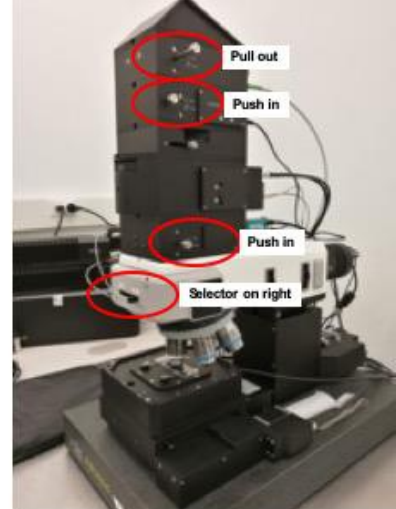
Video imaging, Laser imaging, Raman spectrum/mapping



WL video imaging setting



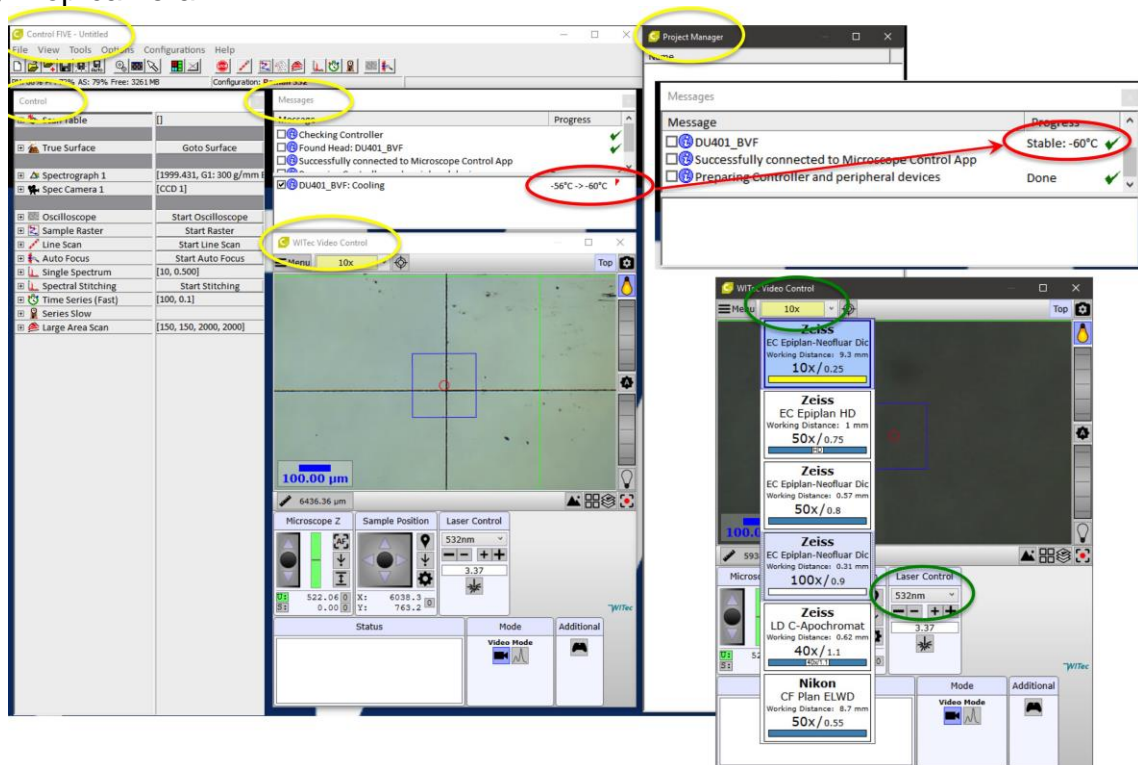
Laser imaging setting



Raman setting

System power on:

- 1) Log in Badger and enable WITec Raman in IS in Badger.
- 2) Turn on microscope control computer, wait 30s.
- 3) Turn on both lasers, wait 2-3 mins. If you are confident that you only need one laser you can just turn on the one you need.
- 4) Click on “Service Monitor” at the quick load zone of Windows (lower right corner) and make sure the following two items are checked (green).
 - a) Microscope Controller
 - b) Top camera



- 5) Double click on “Control FIVE” on the desktop to start microscope control software. It contains five parts (see figure above): Main Menu, Control, Messages. Video Control, Project Manager (yellow circles).
- 6) Control FIVE also starts Project FIVE that is used to process the data.
- 7) Watch the message window of Control FIVE and wait for hardware to cool down to -60°C (red circle in figure above).

Note: “Project FIVE” is data processing software.

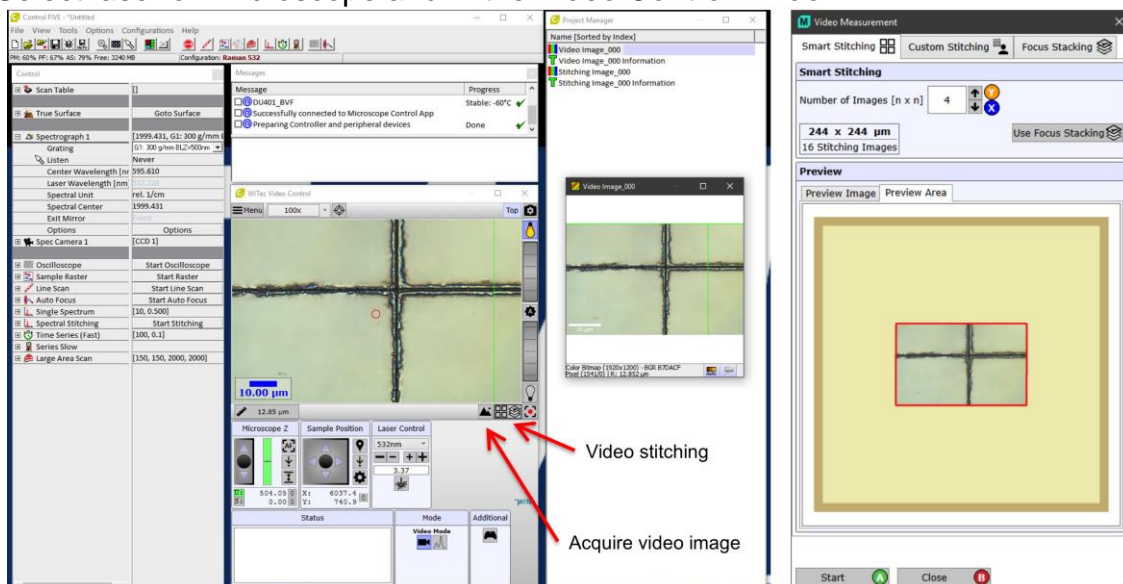
Note: steps to solve problems with the microscope and control software.

- a) Shutdown Control FIVE
- b) Shutdown lasers
- c) Shutdown power strip (controller power included), wait 2-3 mins
- d) Turn on all in reversed order of shutting-down

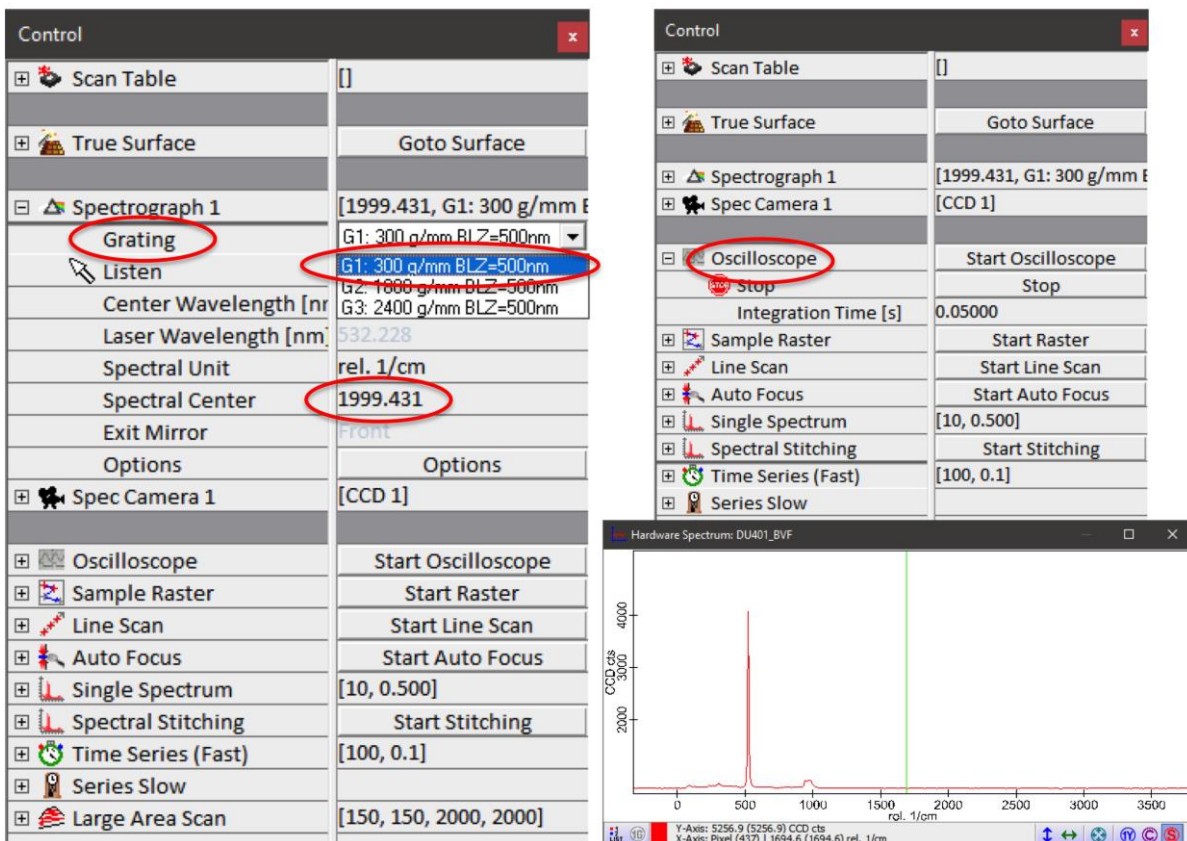
Standard Operation:

We now have objective lens that can only image dry samples without any cover glass. We recommend using 1” x 3” glass slide to prepare your samples. Please discuss with facility staffs before scheduling training if you have solution samples or if you need to use cover glass for your sample.

- 1) Choose 10x objective lens by rotating turret (following the direction) since this lens has the largest working distance.
- 2) Load glass slide (with sample) on the X-Y stage and use the two springs to secure slide.
- 3) Make sure to select the 10x obj lens in Video Control window (lower right of figure above), find an easily recognizable feature on the sample, and do focus using the Xbox controller.
- 4) Switch lens to 50x or 100x by rotating turret along directions indicated. Remember to change lens in Video Control window. Focus again on the sample. If the sample has no clear feature, using field-stop diaphragm as focusing reference.
- 5) Take either video image or video stitching image or both (figure below). Double click image name in project manager window to open video images.
- 6) Select laser on microscope and in the Video Control window.



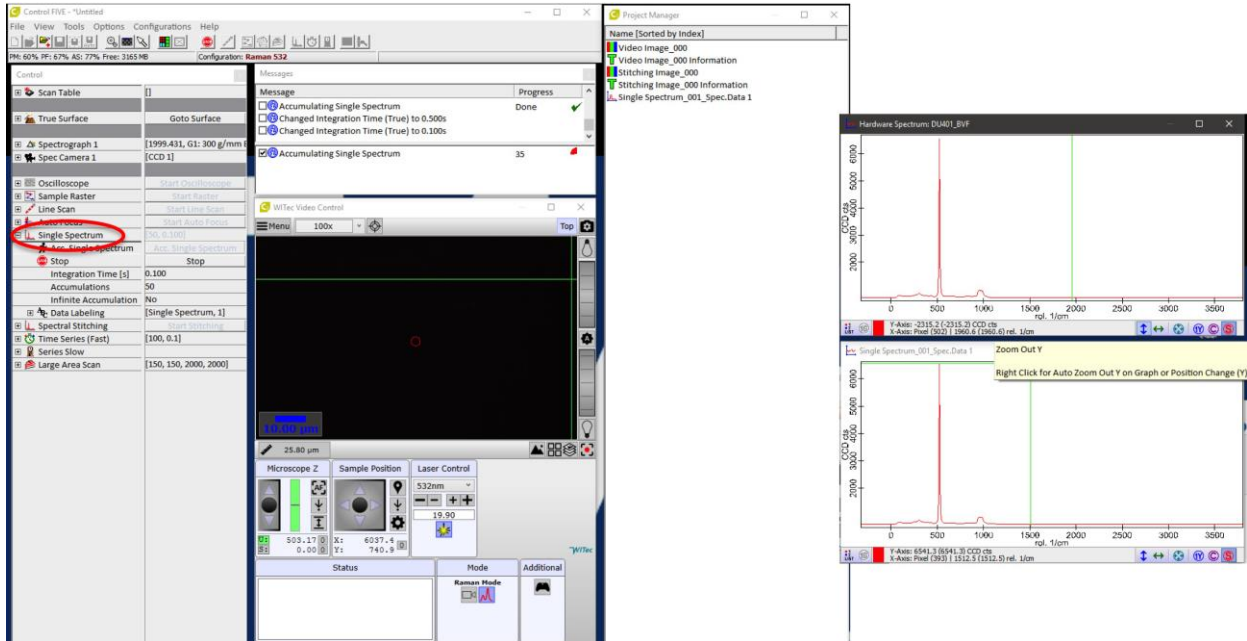
- 7) Check the grating in Spectrograph 1 in Control window (left figure below). There are three gratings for this instrument, G1:300g/mm; G2:1800g/mm; G3:2400g/mm. Only G1 give the full spectrum range 0-4000 wavenumber (1/cm). if you are not sure please select G1 for grating and choose 2000 for Spectral Center.



- 8) Expand **Oscilloscope** in Control window (up-right figure above) and select parameter for Integration time (depending on samples and laser power). If you are not sure, starting with short time (0.05s-0.2s) and low laser power (<5 mW). Fine adjust focus to maximize spectral signal by Xbox controller while watching live Raman spectrum (low-right figure above). The laser power range is 0-60 mW for both lasers.

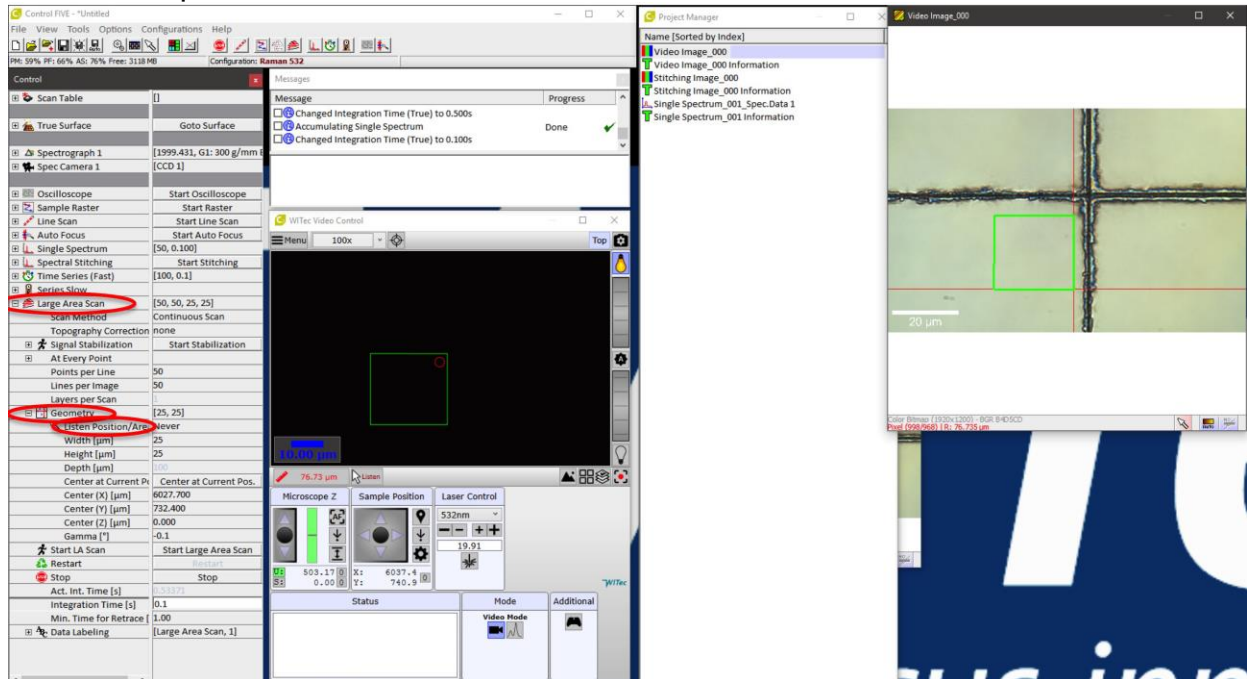
Note: the main function of oscilloscope is to fine focusing on sample and find proper parameter for integration time and laser power. All parameters are chosen to get decent Raman signal while not damaging the sample.

- 9) Expand **Single Spectrum** in Control window, set parameter based on the oscilloscope testing: integration time, laser power and accumulation. Accumulation could be set to smaller numbers (10-30) for samples with strong Raman signals and larger numbers (100-150) for sample with weak Raman signals, e.g. protein/peptide. Click Acc. Single Spectrum to start. The up-right spectrum (figure below) is live single acquisition and the low-right one is accumulated spectrum.



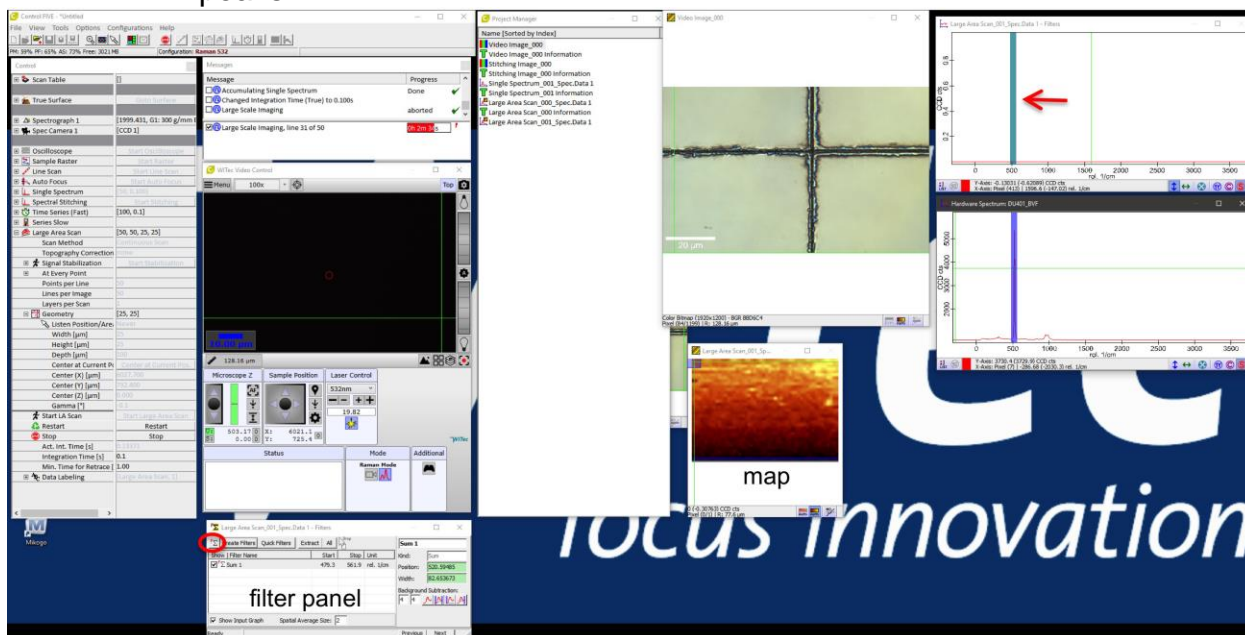
10) Expand **Large Area Scan** and **Geometry** in Control window, select the type of Listen Position/Area (I suggest Area Multiple) and draw an area in the opened video image (green rectangle in the right figure below), you can draw multiple times until you are satisfied. You can also adjust width and height, number of pixels in the Control window.

Note: remember there is no live video in the video control window when the microscope is in Raman mode.



11) Once you are satisfied with all settings, click Start Large Area Scan to start data collection. Four new windows will appear (figure below):

- Filter panel window is used to add filter band to spectrum to select peaks for the map panel to display maps depending on the peak you selected.
- Map window is to display maps depending on the filter selected.
- Live spectrum window. You can only move the green band (red arrow) in this window to select peaks in large area spectra window. The width and center of such band can be manually set in filter window.
- Large area spectra window. Purple band in this window is coupled to the green band in live spectrum window and works as indicator to select peaks.



Finish:

- 1) Save the project file. This is important.
- 2) Close the Control FIVE program and wait for the spectrometer to warm up.
- 3) Turn off laser (**IMPORTANT!!**). Turn off computer. Login Badger and disable WITec Raman in IS in Badger.
- 4) Clean up computer desk and sample prep area.
- 5) Take all samples and glass slides with you.