NT-MDT Spectra

Raman Manual

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Contents

Loading Sample

- The piezo stage is magnetic as well as the 3mm spacers (Figure 1)
- Samples should be mounted on the steel discs in the lab using double sided carbon tape or some other adhesion method
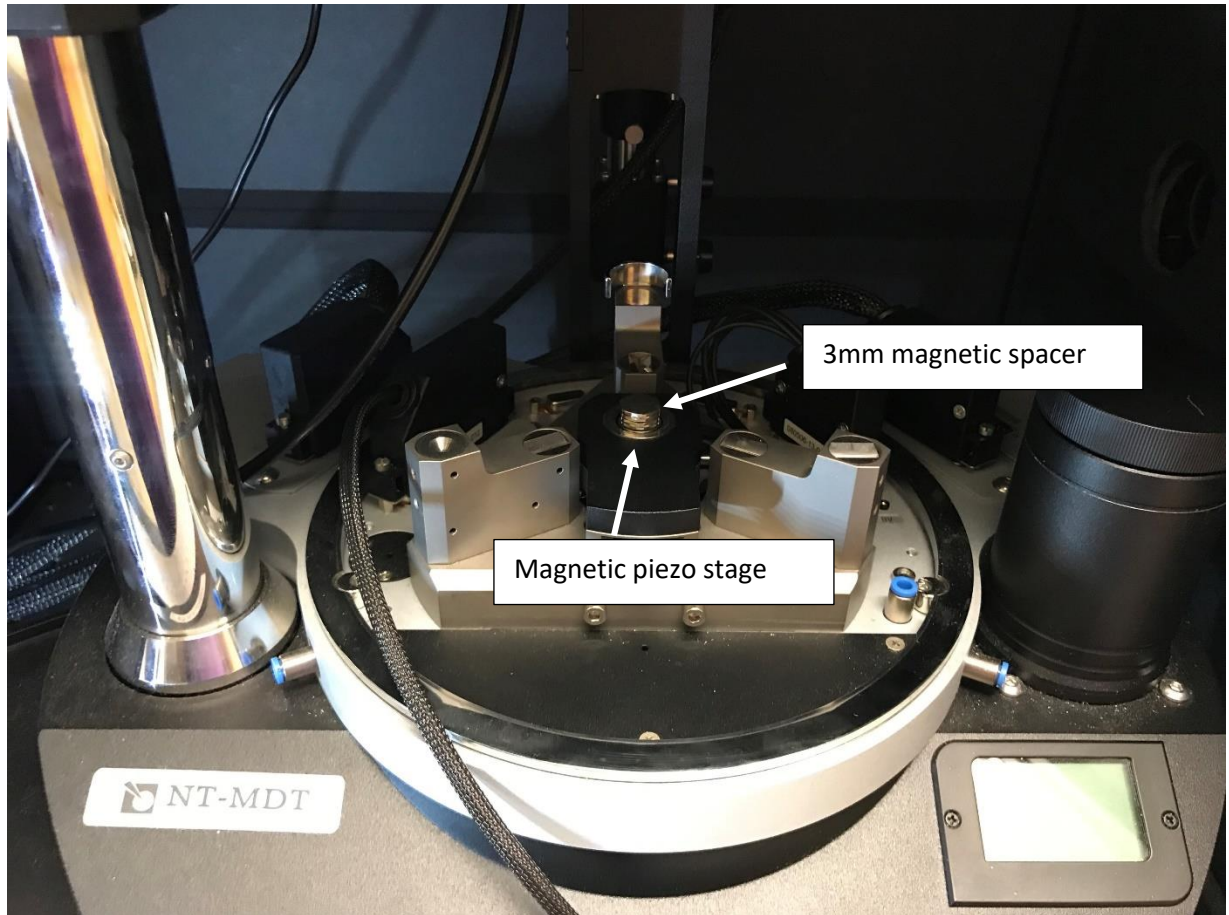

Figure 1: Magnetic spacer and magnetic piezo stage

- To insert the sample, the optical head must be removed. **This must be done carefully or the instrument could be damaged.**
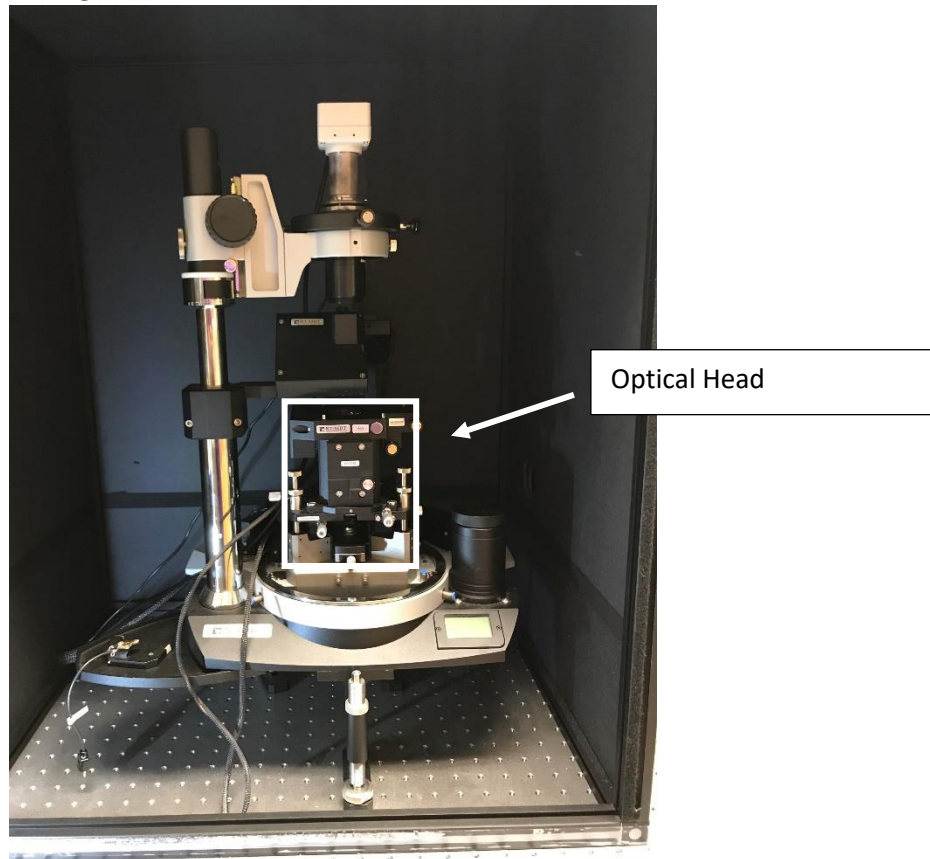

Figure 2: Optical head

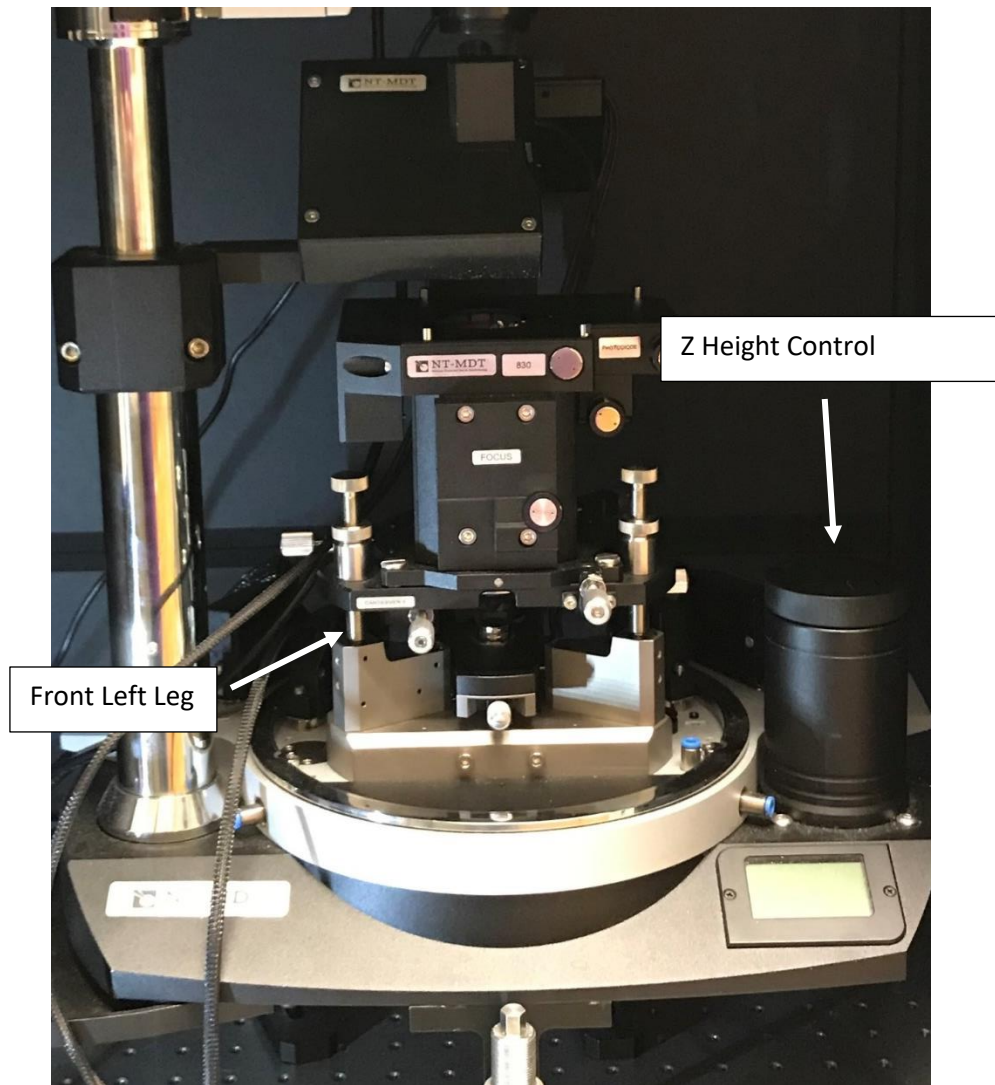- Lower the stage to the lowest point using the z control (Figure 3)

Figure 3: Z height control and optical head legs

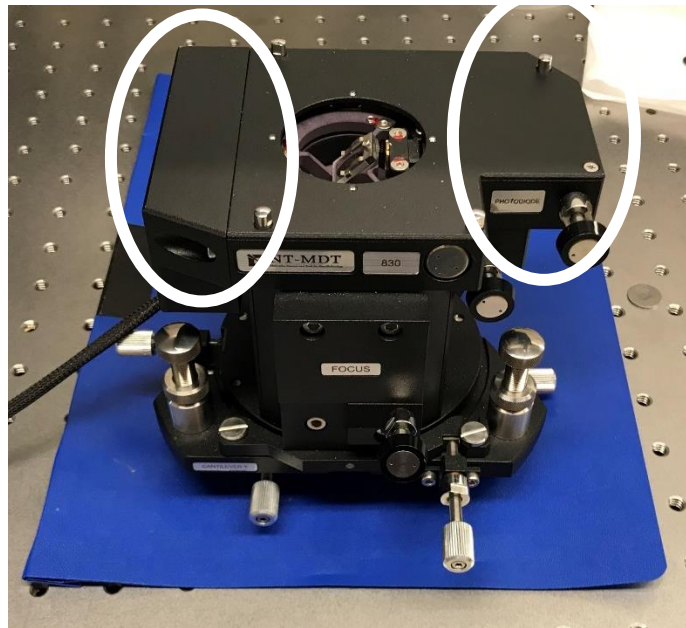- Grab the optical head by the upper sides (Figure 4)

Figure 4: Optical head sides circled in white

- Pivot the optical head on the front left leg (Figure 3)
- Rotate the optical head 90 degrees
- Carefully place on table
- With the optical head off, the sample can be carefully placed on the 3mm magnetic spacer (Figure 1), **it is important to slide the sample from the side and not set directly down on the spacer**. Since it is magnetic, the hard downward force could damage the piezo ceramic in the stage.
- To put the optical head back place the front left leg back on its post (Figure 3) and carefully rotate the head back in place. Make sure to have a good view of the distance between the sample and the optical head to make sure that it clears the sample.

Starting Nova PX for Raman Analysis
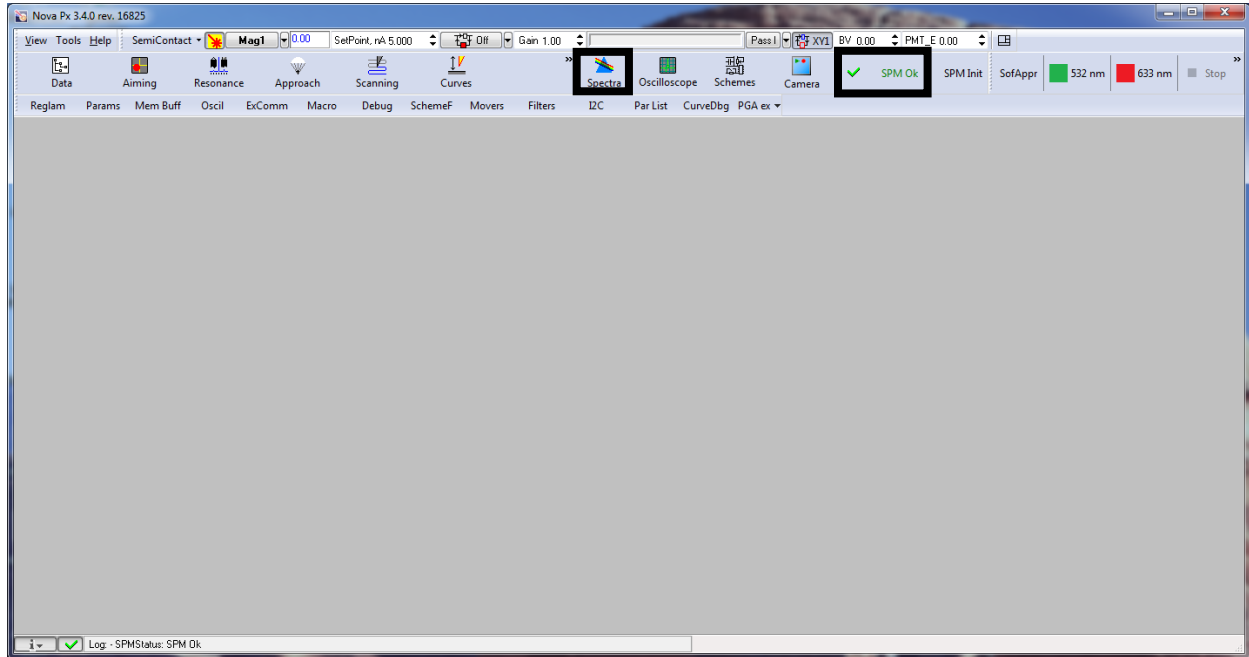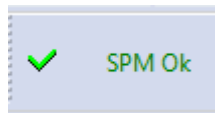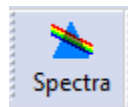
- Start Nova PX

Figure 1: Nova PX start up screen

- Make sure SPM is Ok (Figure 1)

- Click Spectra (Figure 1)
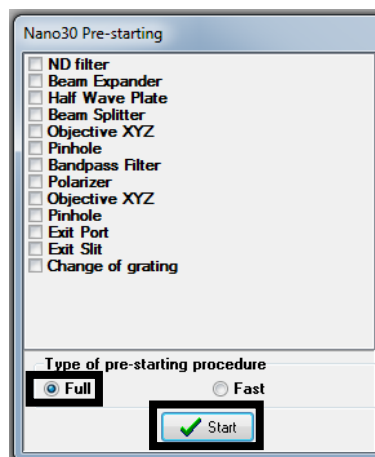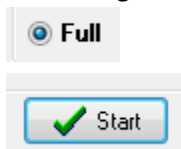
The following menu should pop up

Figure 2: Start up precheck menu

- Click Full (Figure 2)

- Click Start (Figure 2)

When finished, it should look like Figure 3, keep in mind this will take a while
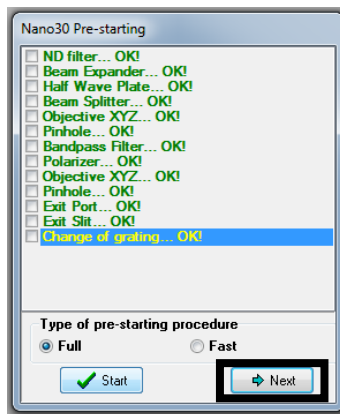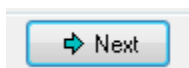

Figure 3: Precheck finished, instrument is ready

If anything comes up red, please contact me and don't use the instrument

- Click Next (Figure 3)

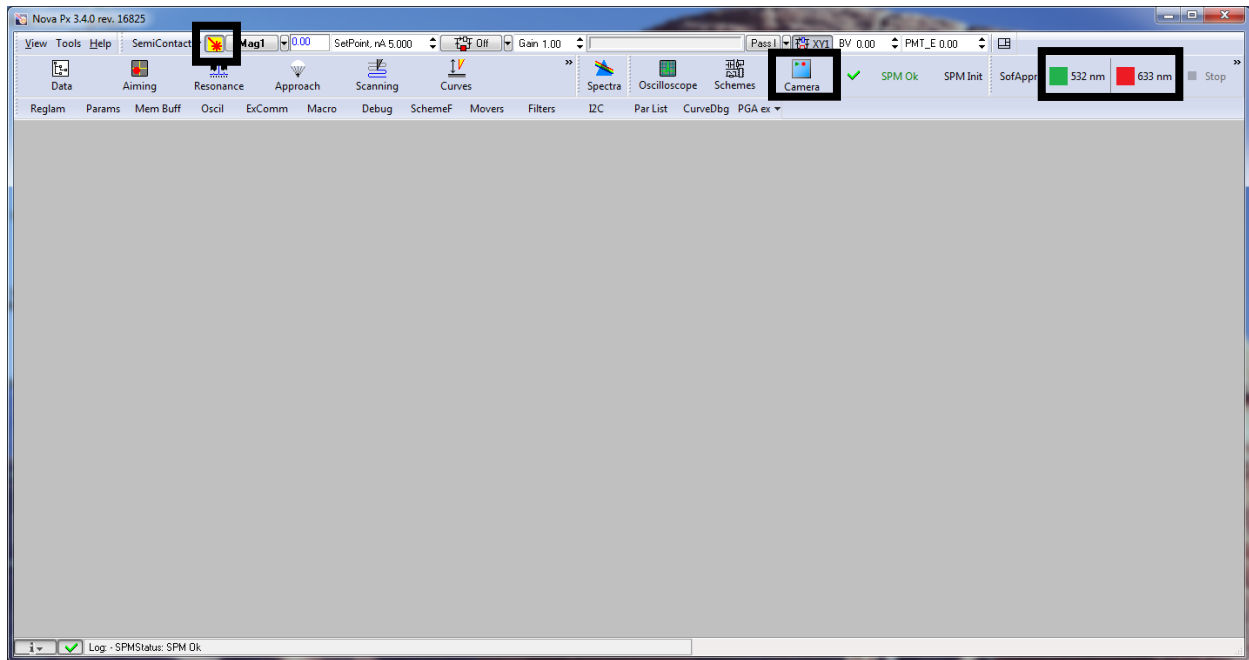# Spectra Acquisition

Figure 1: Nova Px main screen

- Select desired laser for analysis (Figure 1).

    - Click on wavelength desired and follow the prompts.

    - Enter a value in wavenumbers to center the spectral window

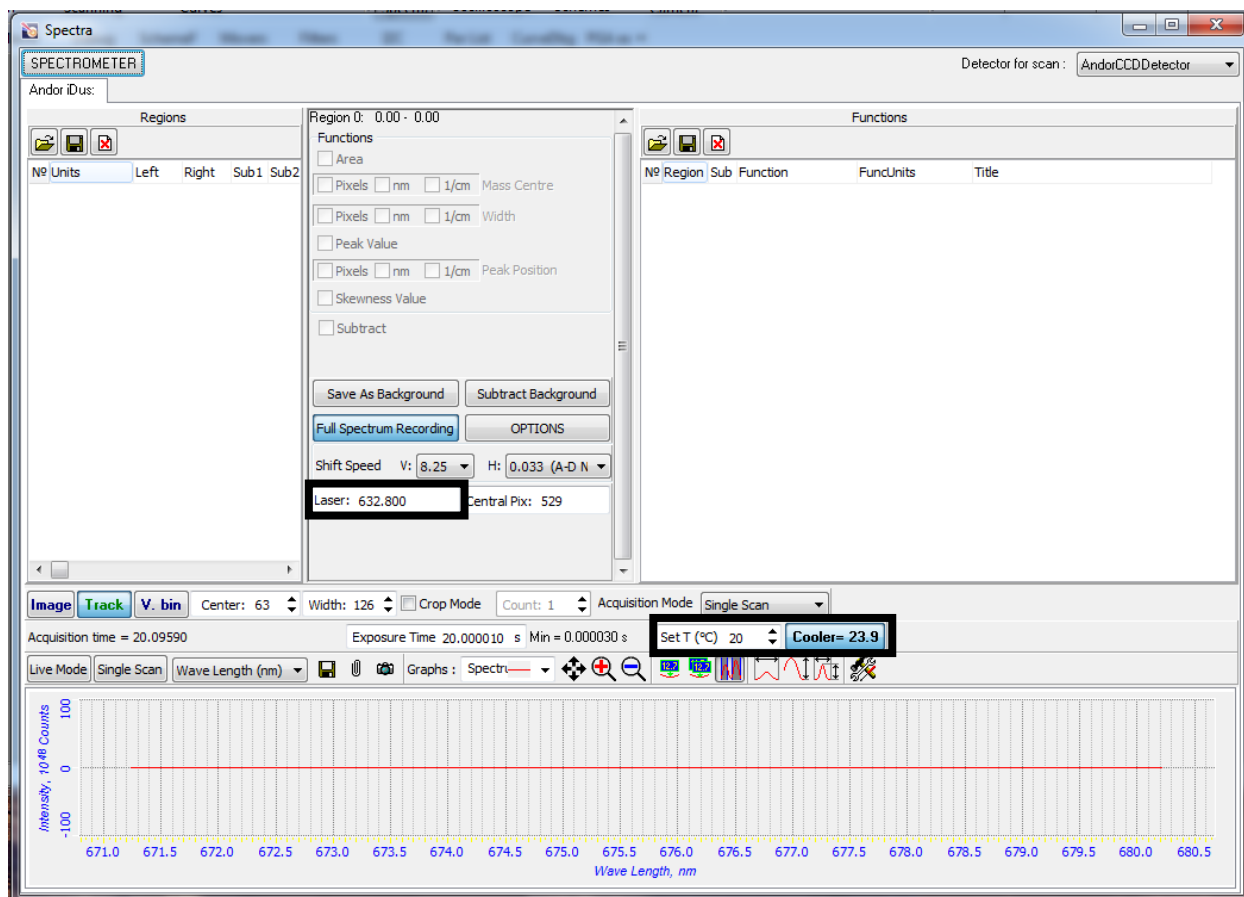   - Click yes or no to open the laser shutter

Figure 2: Main Spectra menu with acquisition parameters

- The CCD detector needs to cool to a range of (-60 to -65) enter -65 in the Set T box (Figure 2).

- Enter the proper laser wavelength in nm in the Laser box (Figure 2).

  - For the 532nm green laser the correct value is 532.2nm
  - For the 633nm red laser the correct value is 632.8nm

- Turn on optical camera (Figure 1).

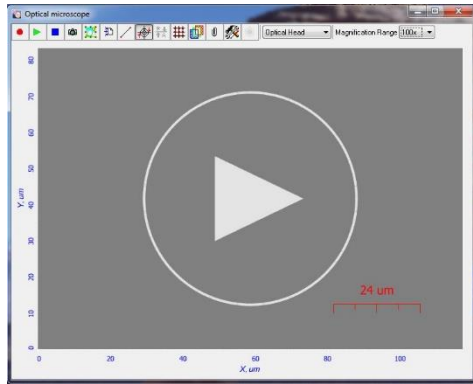- Click the triangle in the center of the screen (Figure 3).

Figure 3: Optical camera

- To view the sample, set the mirror wheel (Figure 5) to "None". There is a tube coming off the back of the mirror box, on the back of the that tube is a switch that turns on the optical light. Make sure the light is on.

- If the sample is homogenous and spatial reference is not important, its ok to skip to step 10

- For samples that are reflective, it should be easy to see the surface of the sample, for samples that are less reflective, increase the brightness by selecting this button (Figure 4).

- Make sure the AFM laser is off (Figure 1). If it is on it will look like (Figure 4).
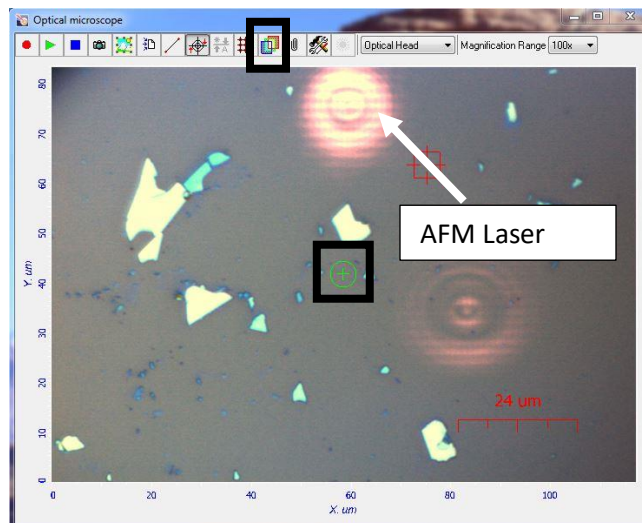

Figure 4: Example of a reflective sample

- There are two ways to center the laser on a specific spot on a sample

- Move the X and Y controls for the stage (Figure 5) to position a spot under the cross hairs. (Figure 4)
- On the back of the mirror box there are two black knobs. That can be used to adjust the mirror position and move the laser without having to move the sample.
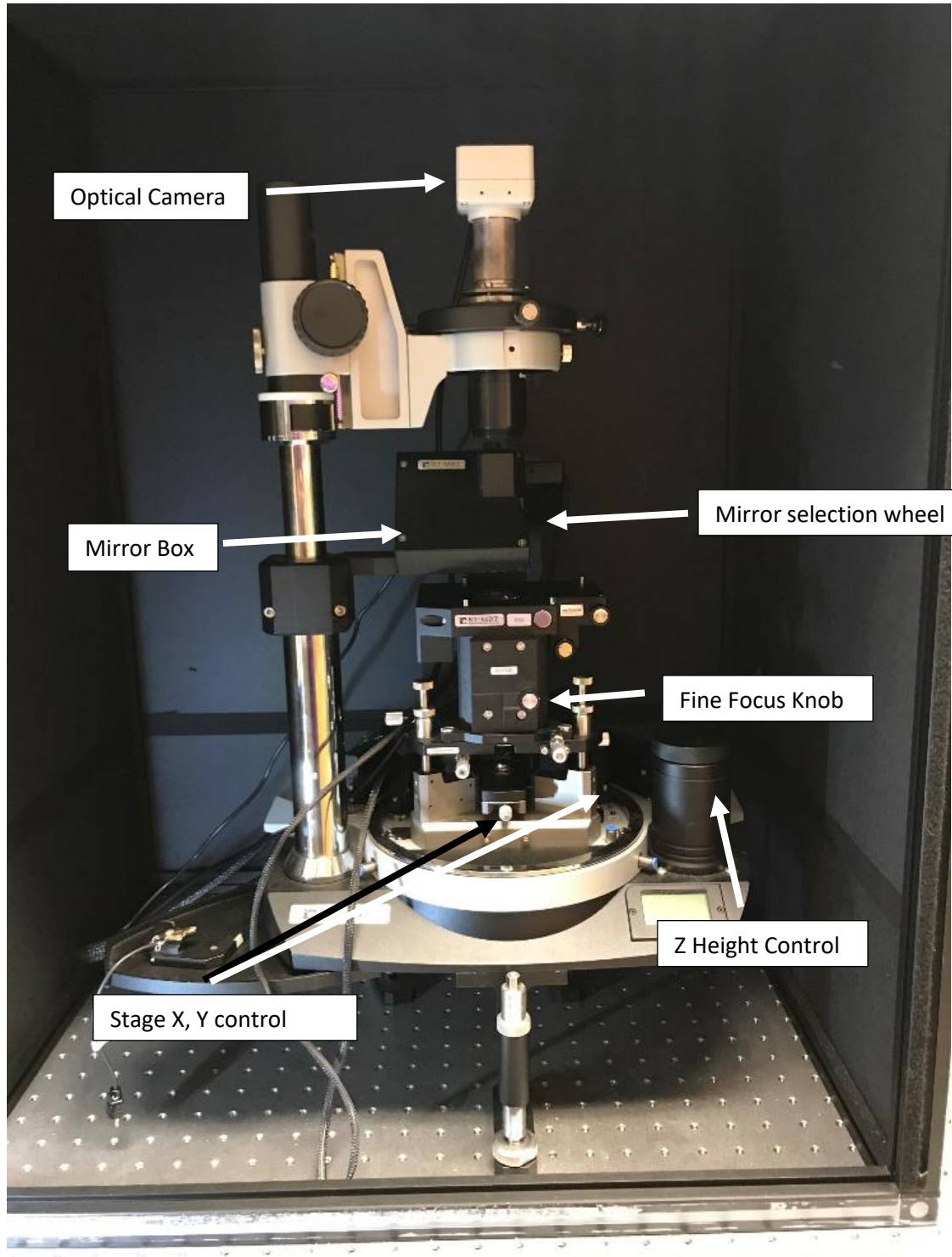

Figure 5: Overview of raman setup

- Set the mirror to 100%. Coarsely focus the laser on the sample starting with the Z height control (Figure 5). It should look like Figure 6 and then using the fine focus on the optical head (Figure 5) focus the laser to a point (Figure 7).
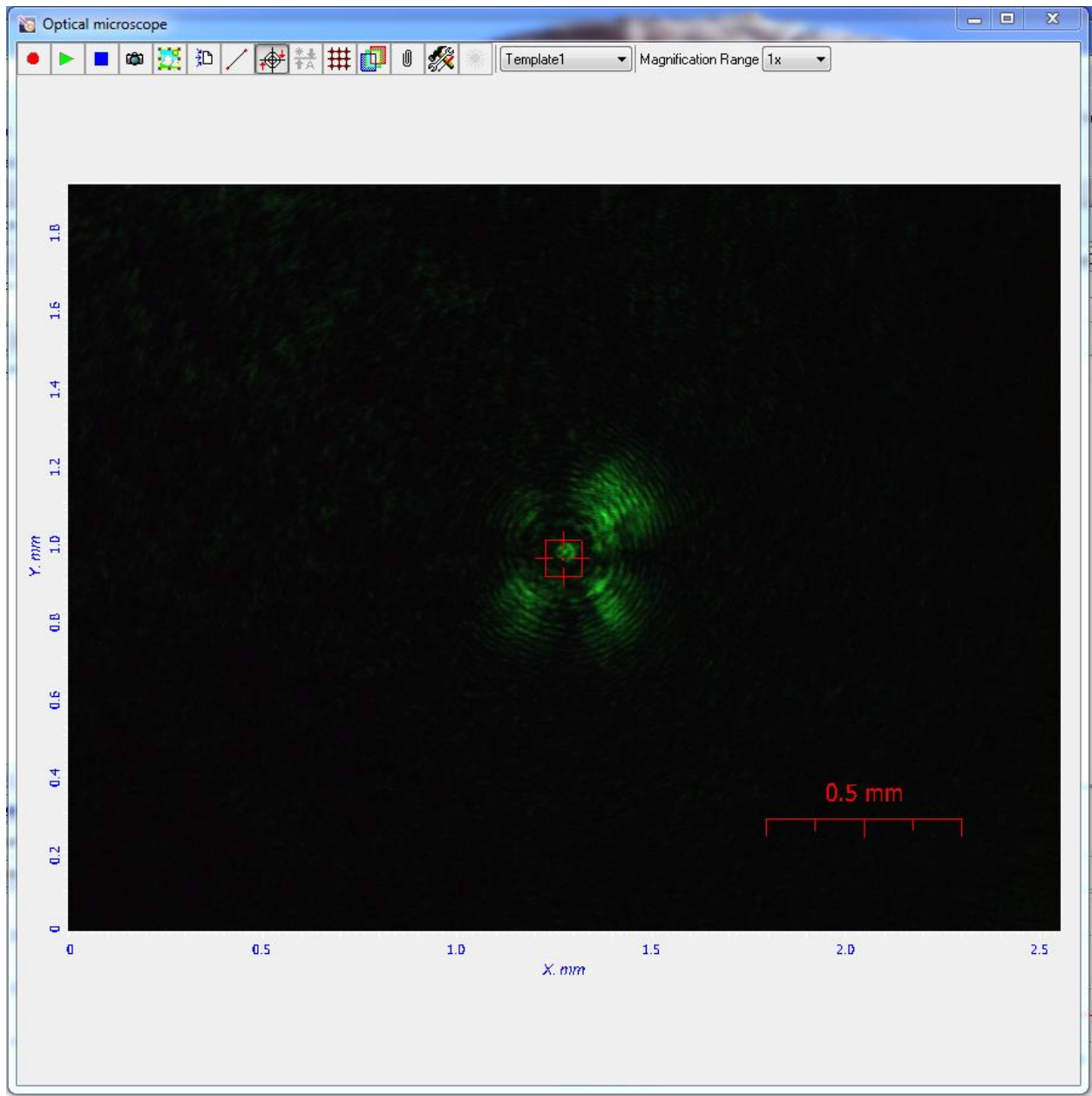

Figure 6: Laser is out of focus but close enough to switch from Z height control to fine focus
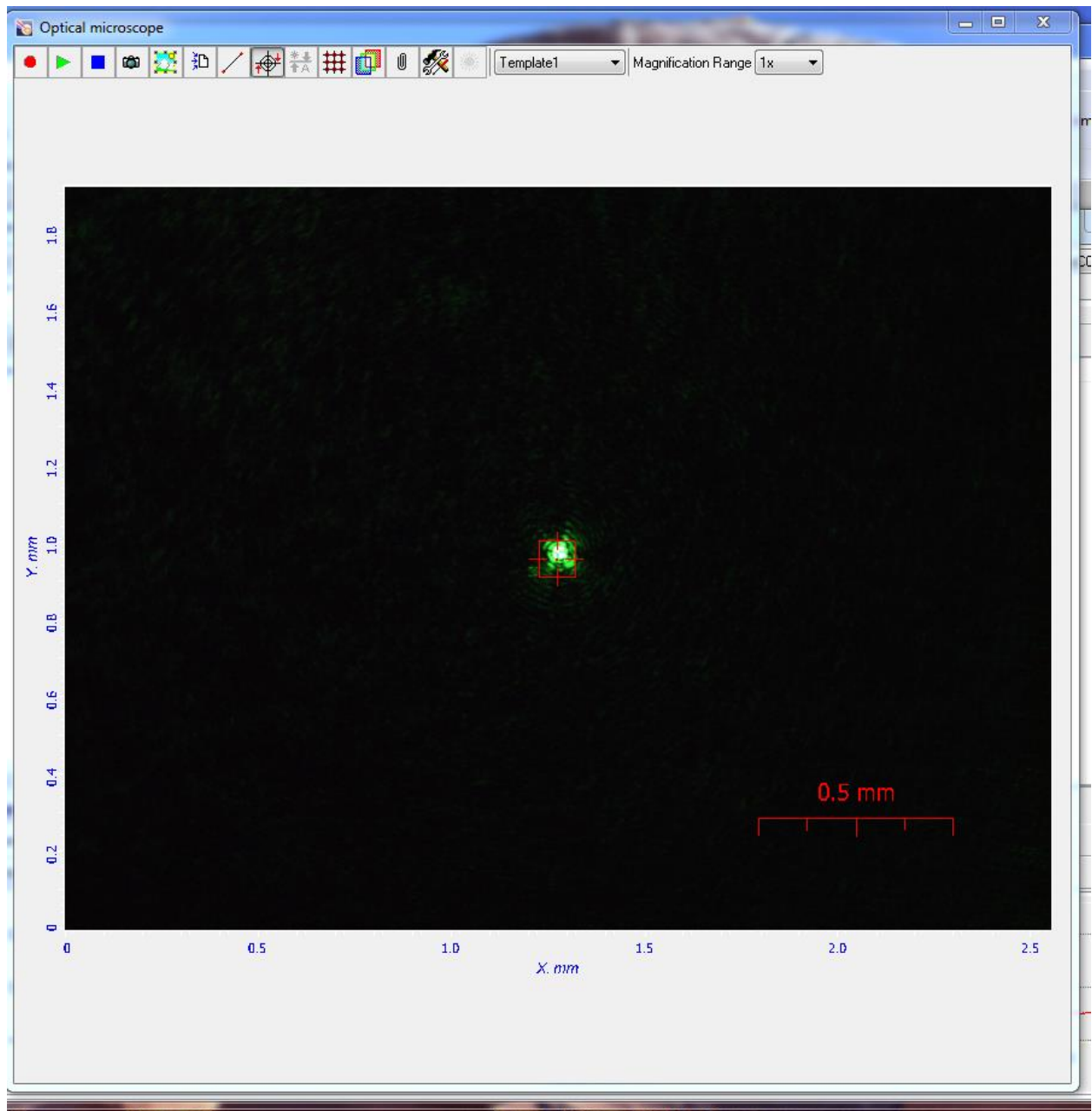
Figure 7: This is the laser focused on the sample

- Make sure spectrometer shutter is open (Figure 8).

- Make sure the desired grating is selected (Figure 8).

- **Once the desired wavelength and grating have been selected the instrument must be calibrated, this must happen every time the laser or grating is changed.** (See Calibration section for more detailed instructions)
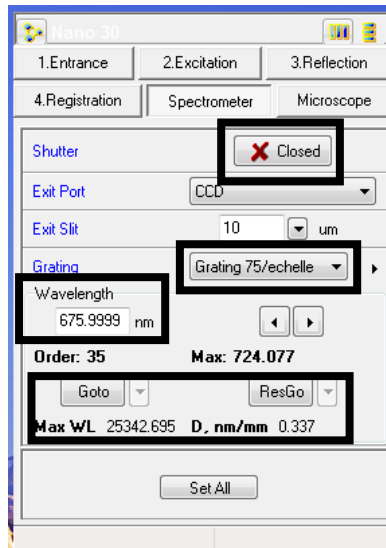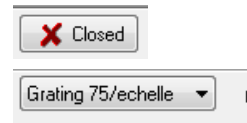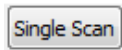

Figure 8: Spectrometer settings

- Select the wavelength to center the spectral window on, this is done by entering a value under "Wavelength" and clicking "Goto" or "ResGo" (Figure 8).

- Set the desired instrument settings for analysis (for more detail see analysis settings).
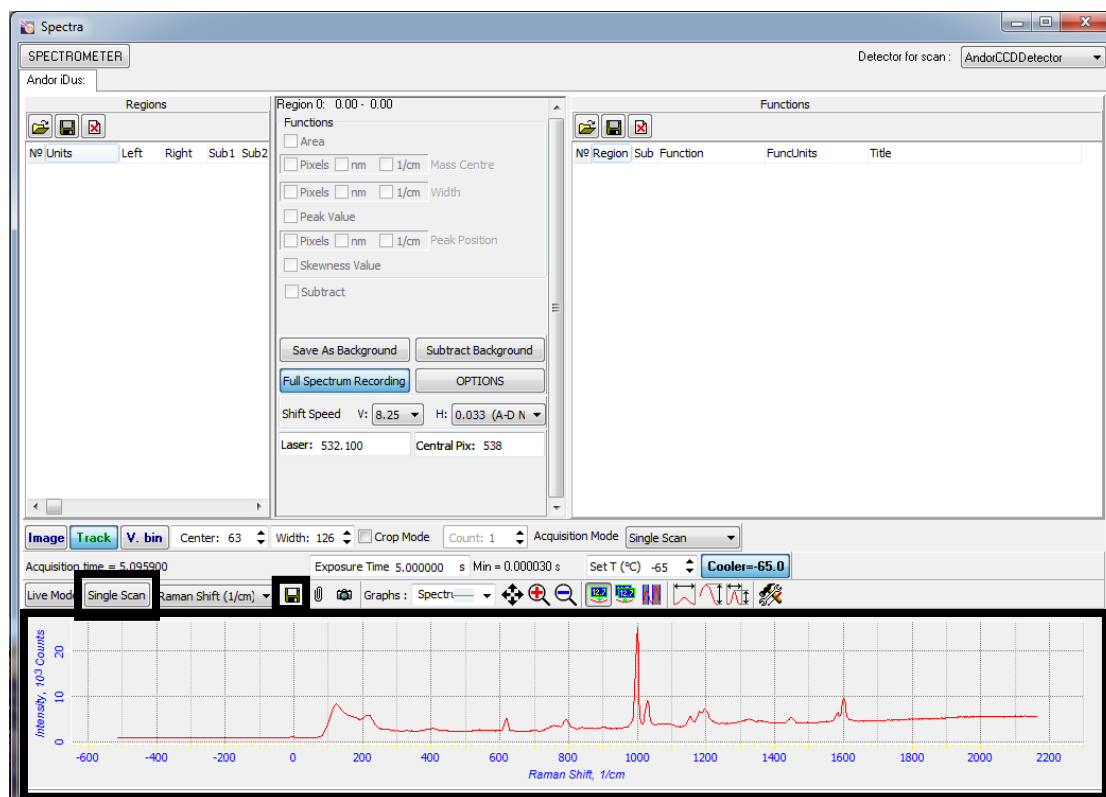
- Click "Single Scan" (Figure 9).

Figure 9: Highlighting single scan and save spectrum icons

- If there is no spectrum, then make sure that the camera shutter is **open.**

- To save the spectrum, click the floppy disk icon (Figure 9).

Shut Down Procedure

1. Close shutter to the camera
2. Warm camera to 15 degrees
3. Once camera reaches 15 degrees, close software shut off spectrometer

Analysis Setting

- The first setting to consider is the laser wavelength (λ). In general it is usually best to use the smallest λ as possible. Both the signal and spot size are optimized with smaller λ. If a sample is prone to fluorescence, then a longer λ can help reduce the fluorescence.

- In general, the grating will be the main settings that will be used to control spectral resolution, such that the more groves on the grating the better the resolution but also restricting how much of the spectrum can be viewed at one time along with an increase in spectral noise. The exception to this rule is with the 75/echelle grating this is a specialized grating that offers the highest spectral resolution of the instrument.
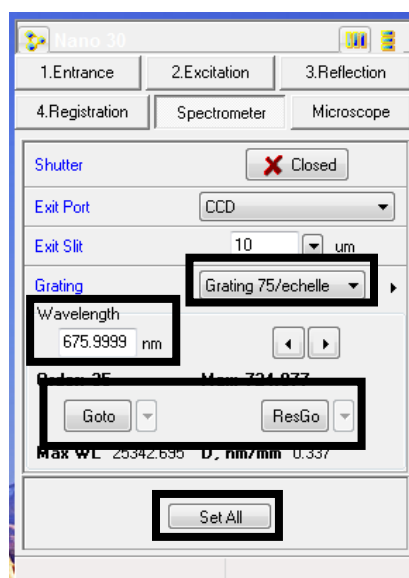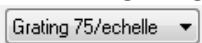

Figure 1: Spectrometer setting menu

- Select the desired grating from the drop-down menu and click "Set All" (Figure 1).

- Select the wavelength that you would like your spectrum to be centered this is done by entering a value under "Wavelength" and clicking "Goto" or "ResGo" (Figure 1).

  a. "Goto" will change the position of the spectrometer from where it is currently to where you set it

  b. "ResGo" will reset the spectrometer to the 0 position before moving to the set position, this is the most accurate way to set it

Keep in mind that the raman shift that we are measuring is greater than the excitation wavelength of the laser, for example if you set it to 632.8 when using the 632.8 laser you will have centered the spectrum at 0 cm-1 in wavenumbers.

- **Once the desired wavelength and grating have picked the instrument must be calibrated, this must happen every time the laser or grating are changed.** (See Calibration section for more detailed)

- Picking a ND (natural density) filter setting; the setting is on a relative scale from 0 to 3, 0 is the strongest laser power and 3 is the weakest (Figure 2). Remember dark samples and organic samples can be burned by the laser, and the longer the ⋀ the higher the absorption of energy.
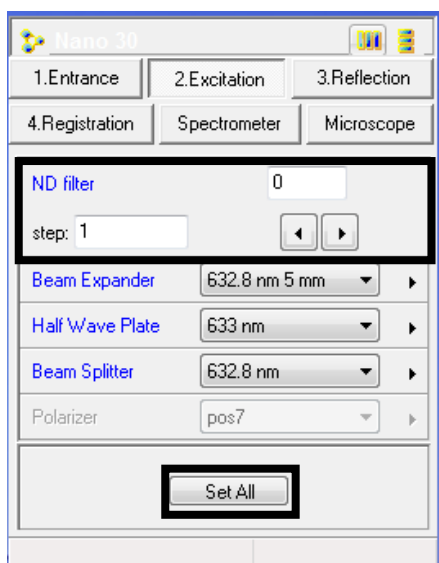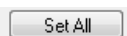

Figure 2: Excitation settings menu

Here we can set the step size of each increment of change on the ND filter and then click the arrows to increase or decrease the filter. Or we can just type in a value between 0 and 3 and click "Set All".

Generally speaking the higher the laser power the better the signal to noise to demonstrate (Figures 3-5); here are 3 spectra with the same acquisition parameters except for the ND filter notice the difference in noise and overall counts.
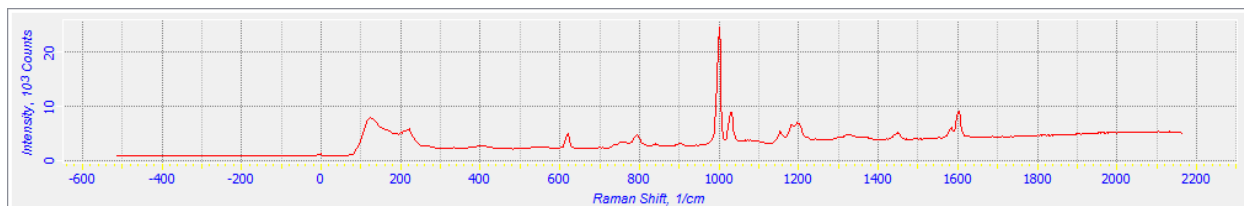

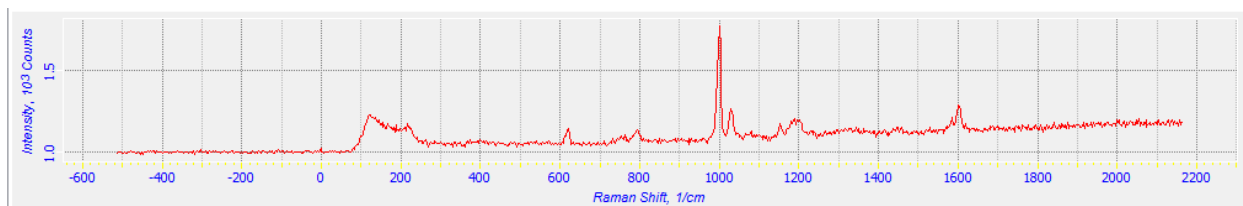Figure 3: ND filter of 0 which is 100% laser power
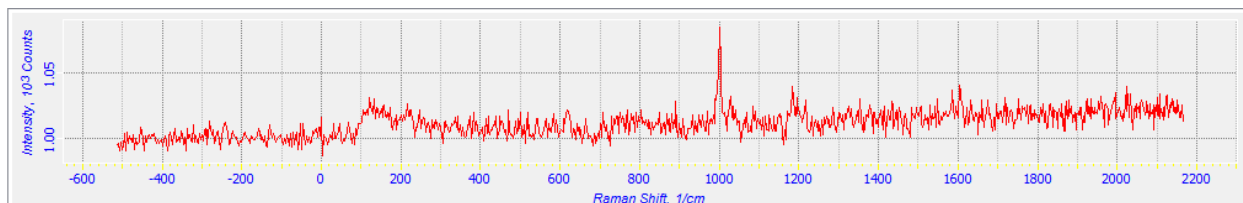
Figure 4: ND filter of 1

Figure 5: ND filter of 2

- Next we can also control signal strength and peak width by adjusting the pin hole under the Registration tab (Figure 6).
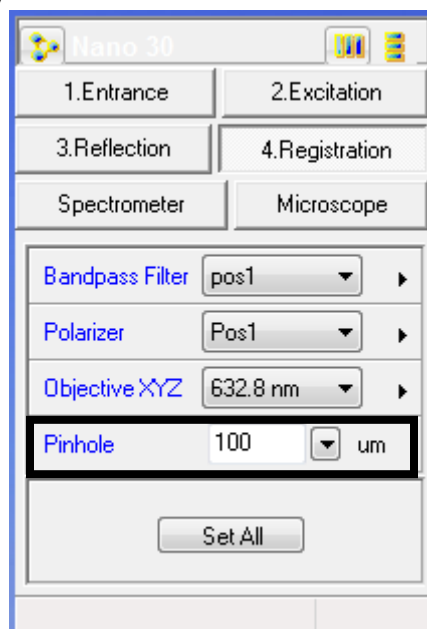

Figure 6: Registration setting menu

Keeping all acquisition parameters the same other than the registration pin hole we can notice a large difference in our spectrum (Figures 7-10).
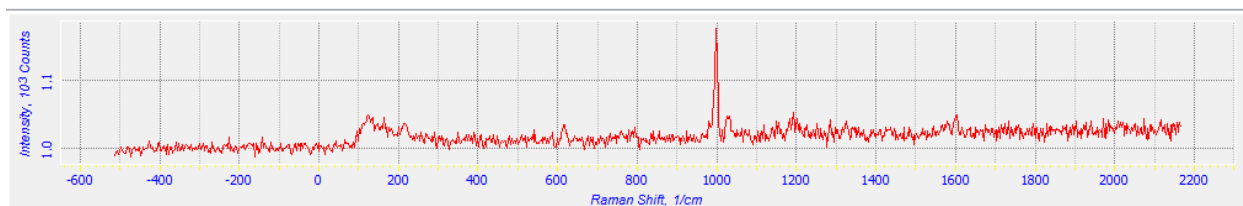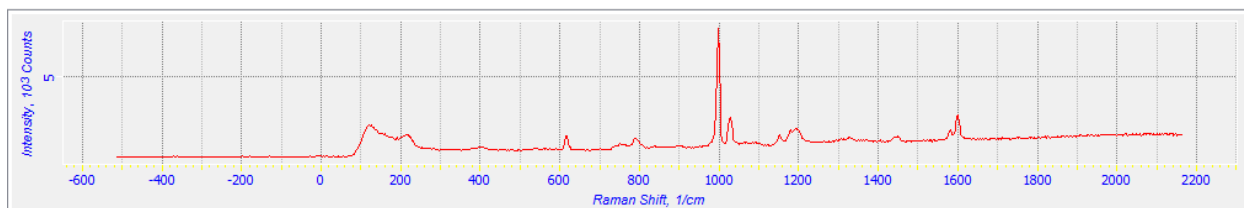

Figure 7: Pin hole of 10um
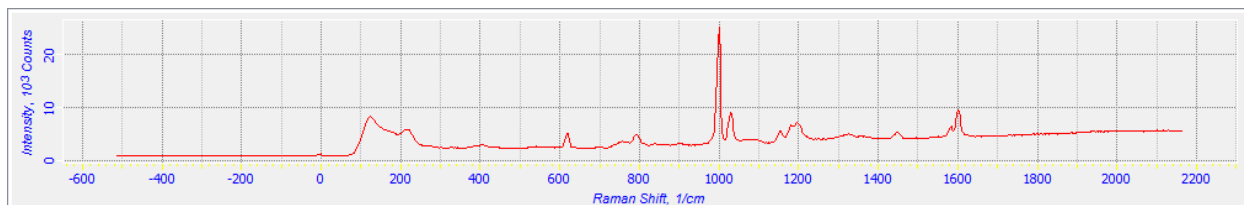
Figure 8: Pin hole of 50um
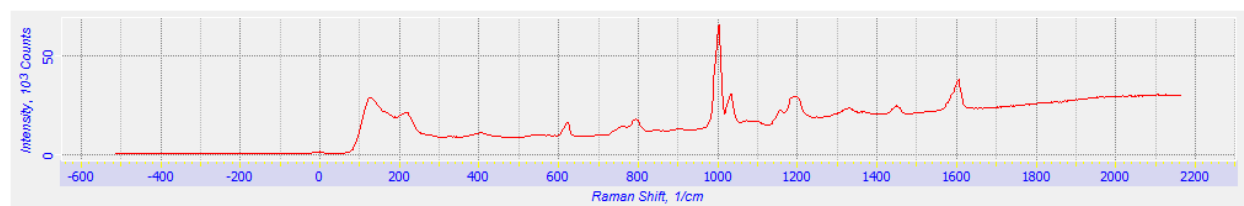

Figure 9: Pin hole of 100um

Figure 10: Pin hole of 250um

Notice how 100um provided the highest counts, low noise, low fluorescence and maintained narrow peaks. It is important to experiment with this parameter as it will vary from sample to sample.

- Another parameter that has a big effect on our spectrum is exposure time. In most cases the longer the exposure time the better the signal to noise ratio is and the higher the counts, but this can cause an increase in fluorescence depending on the sample
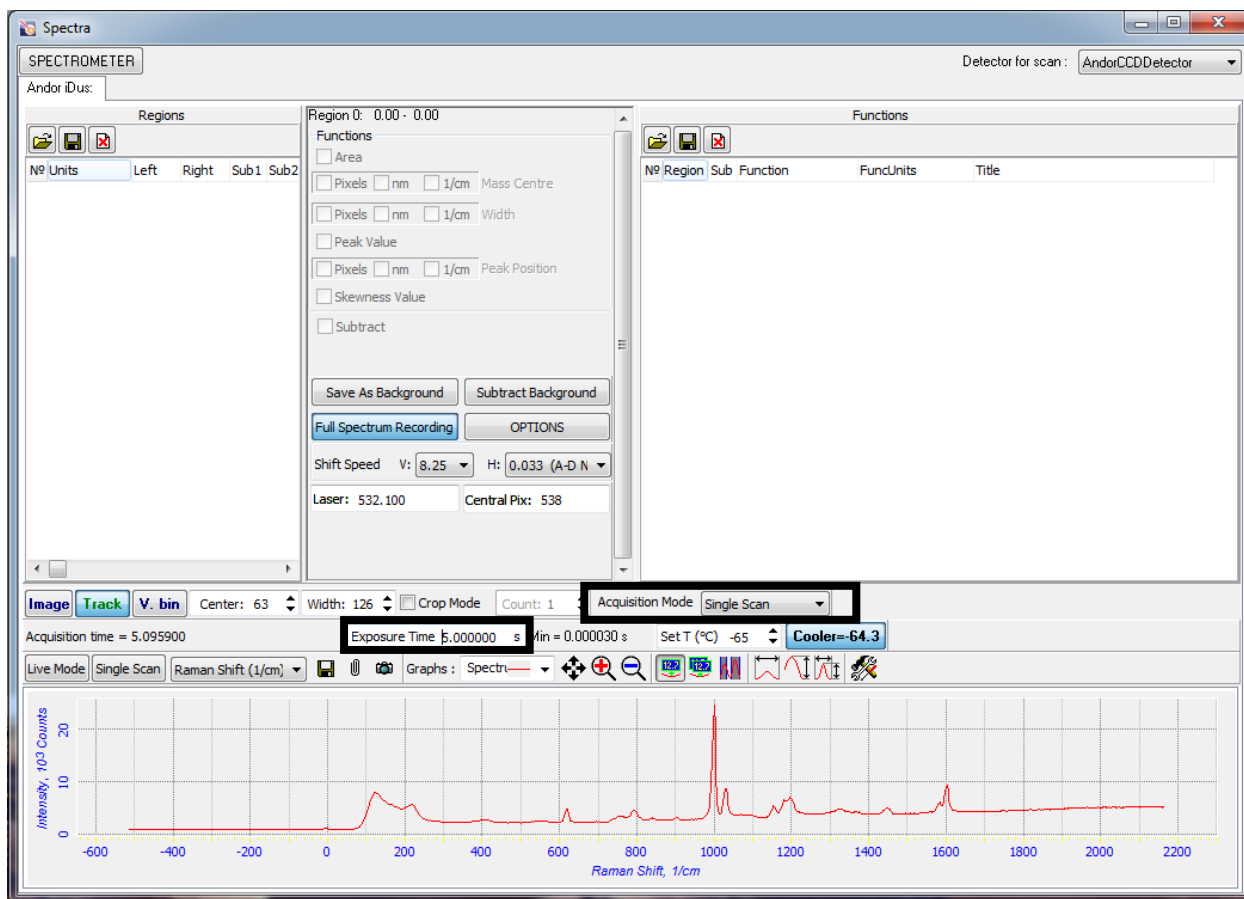
Figure 11: Exposure time

Exposure time can be change by entering in a value in the box and pressing enter (Figure 11).

Take note of the three spectra below all parameters are held constant other than exposure time (Figures 12-14).
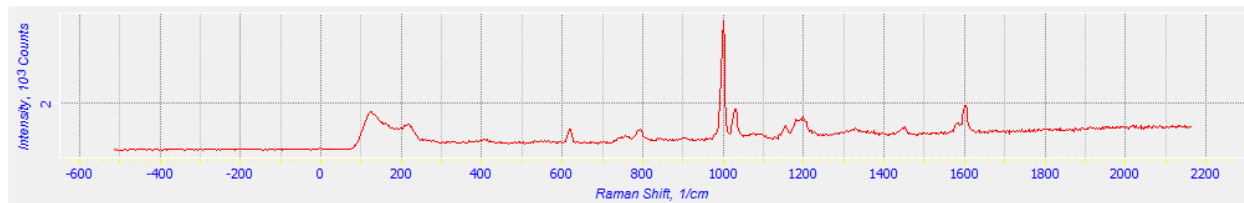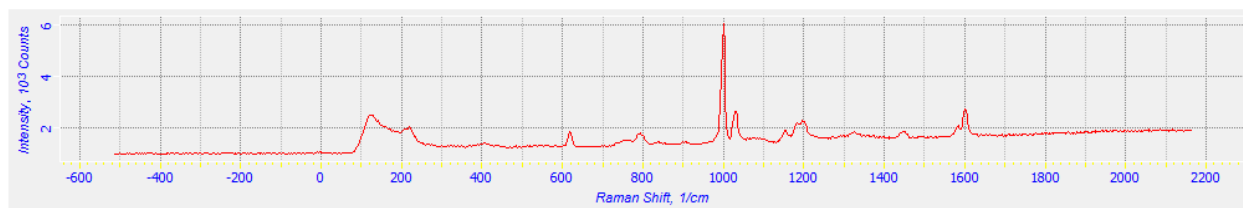

Figure 12: Exposure time of 0.5s
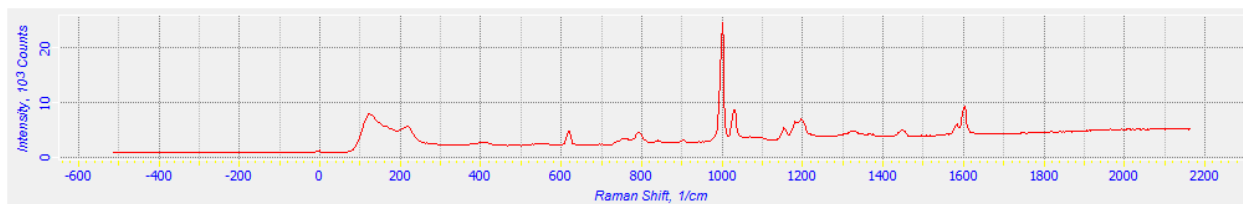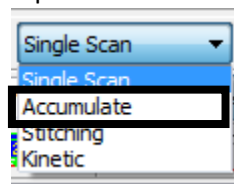
Figure 13: Exposure time of 1.0s

Figure 14: Exposure time of 5.0s

- There also is an option for acquiring more than 1 spectrum and integrating them together this

can be found under accumulate (Figure 11)

Enter in how many accumulations are needed (Figure 15).

As well as exposure time for each accumulation, keep in mind the minimum cycle time (Figure 15).
 is also dictated by the minimum exposure time, meaning the exposure time can't be higher than the cycle time.
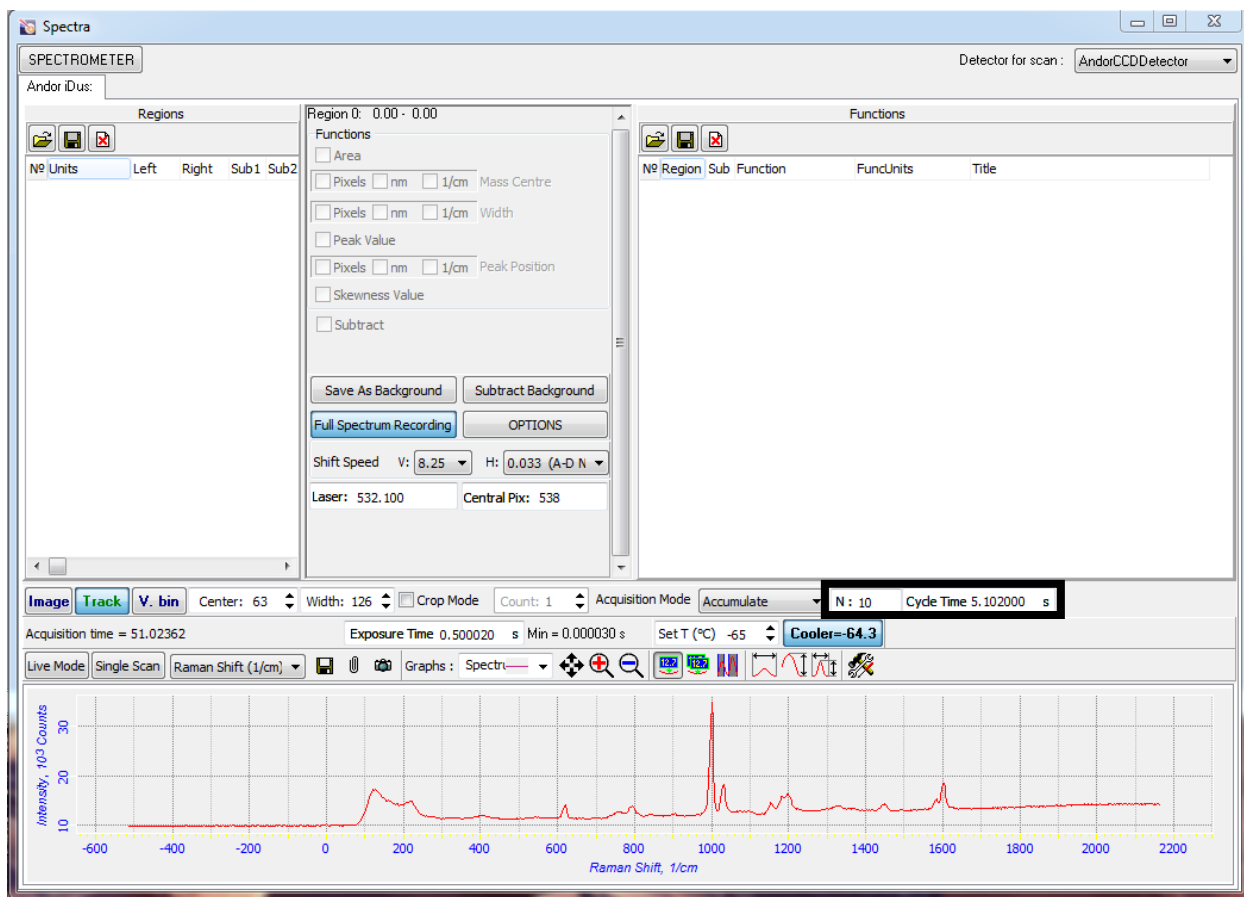
Figure 15: Accumulation settings

Here is a comparison of two spectra one was collected with a exposure time of 0.179s and 10 accumulations (Figure 16) the other was collected with an exposure of 2 s and 1 accumulation (Figure 17).
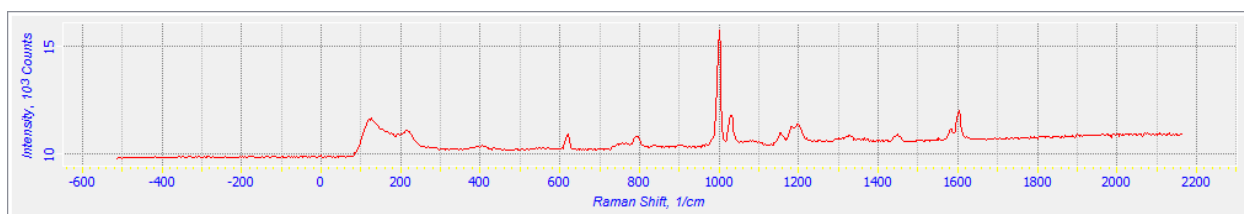

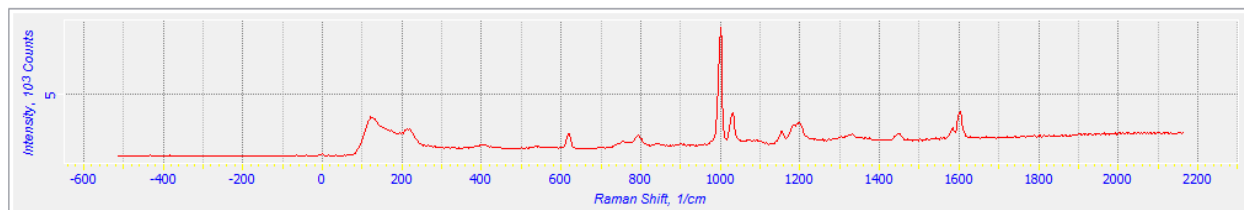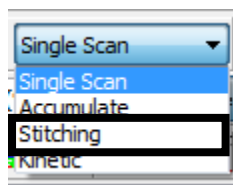Figure 16: Exposure of 0.179s and 10 accumulations

Figure 17: Exposure of 2s and 1 accumulation

Collection of multiple spectra over a shorter exposure time can help elevate fluorescence.

- The next acquisition mode available is stitching

Stitching allows collection across multiple spectral windows and stiches them together. Now the full spectrum of the polystyrene can be viewed at once. The past examples have used the 600 grove grating which has an approximate spectral window of 2600 cm-1. By using stitching any size window can be picked in the example below 3400 cm-1 was selected using the limit boxes (Figure 18).
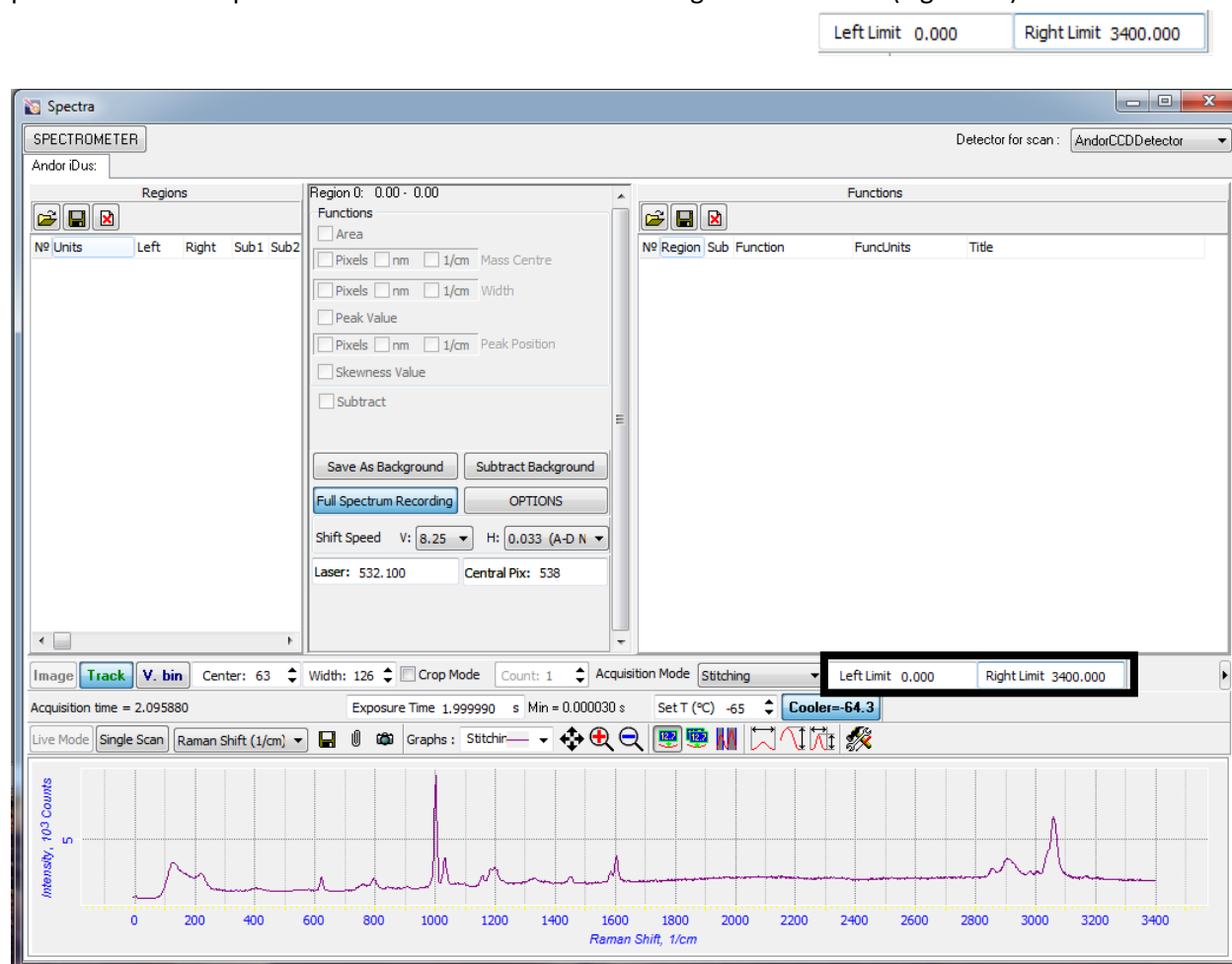

Figure 18: Stitching use to collect a 3400 cm-1 spectrum with 600 grove grating

Calibration Procedure

(For details on loading sample or spectrometer settings review other sections)

1. Load polystyrene sample
2. Focus laser on the surface of sample
3. Set mirror selection to 100%
4. ND filter to 0
5. Registration pin hole to 100um
6. Select the desired laser and grating
7. Acquisition time set to 1s
8. Collect a single spectrum
9. Center the 1001.4cm-1 peak in the center of the spectral window (the 1001.4 cm-1 peak is always has the highest intensity out of all the peaks in polystyrene, so even if it isn't at 1001.4 it can be identified)
10. Recollect the spectrum
11. Zoom in on the 1001.4cm-1 peak using the magnifying glass icon (Figure 1).
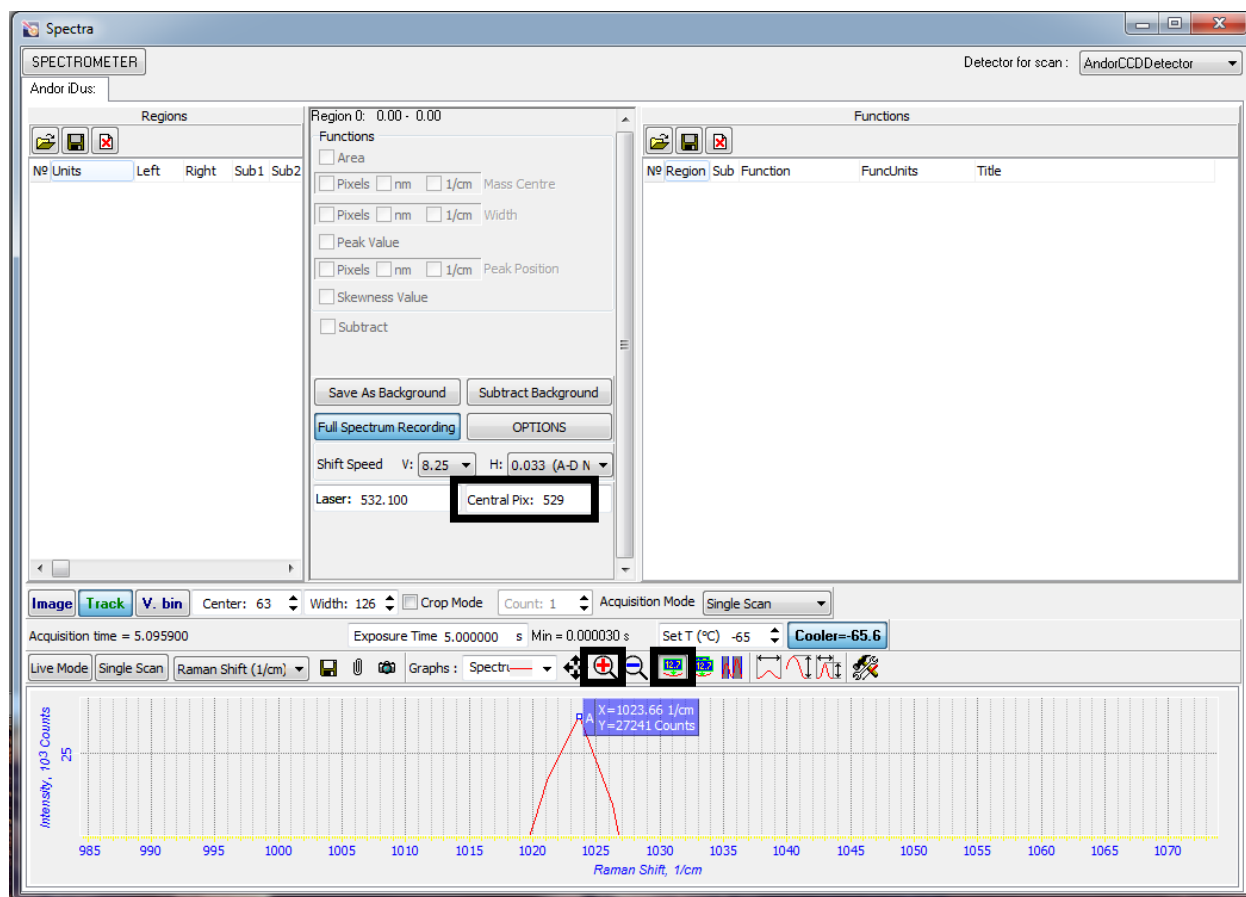12. Measure the peak using the peak position icon (Figure 1).

Figure 1: Uncalibrated 1001.4 cm-1 polystyrene peak, measured at 1023.66 cm-1

13. To calibrate the peak the "central pix" (Figure 1) value needs to be adjusted, the represents a physical pixel on the CCD of the camera that means it must be an integer. By changing the "central pix" the calibration peak can be shifted towards 1001.4 cm-1 (Figure 2).
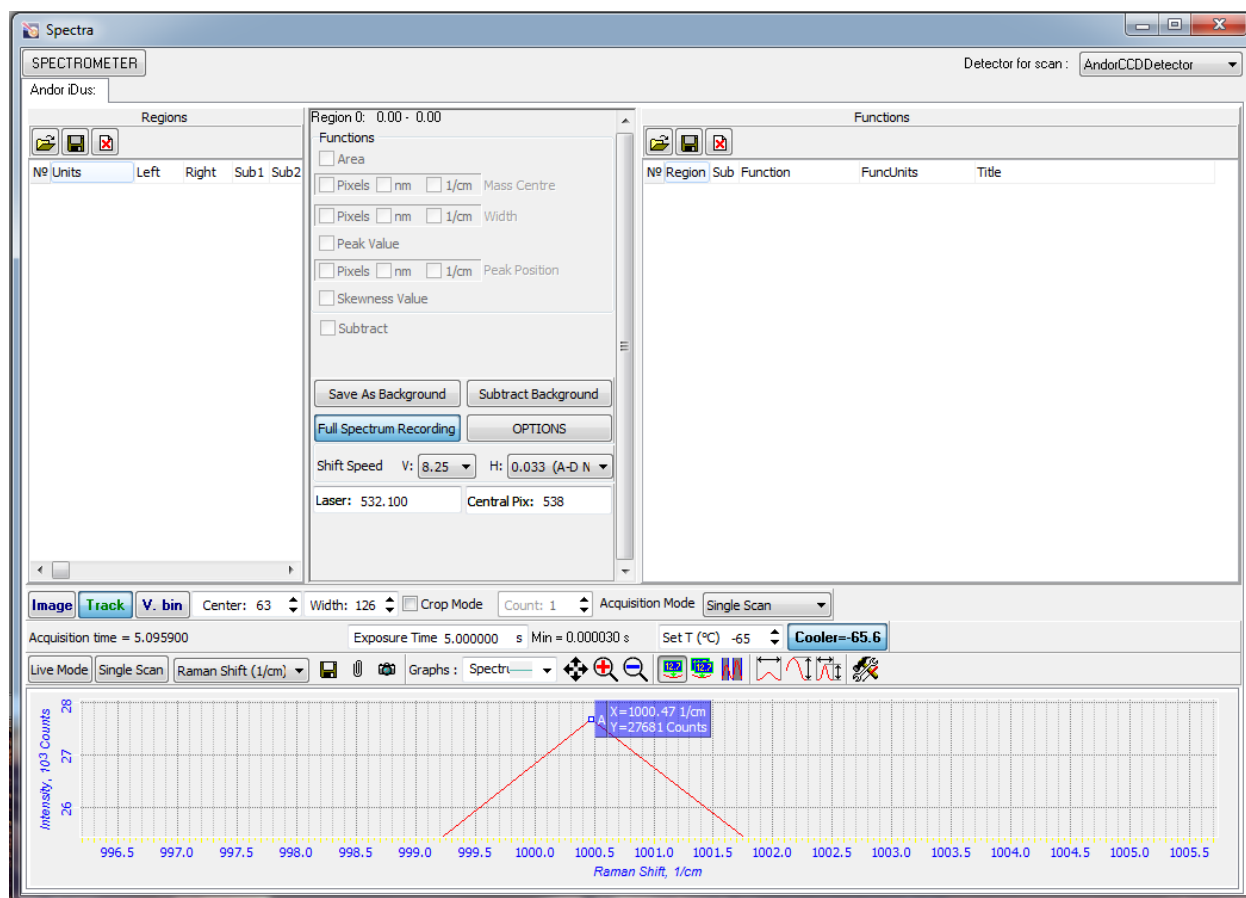

Figure 2: This is the 1001.4 cm-1 peak of polystyrene calibrated, even though it is not the exact value this is as close as this grating and laser combination can achieve. This also speaks to the spectral resolution of this laser and grating combination.

14. Once the "central pix" is adjusted so that the 1001.4 cm-1 peak is a close to its value as it can, record the peak position, grating, laser and central pix value in the calibration log.