

Raman Training Notebook

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September 30, 2019 (rev. 2)

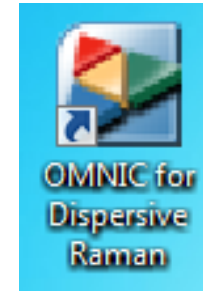
Before you begin...

- Receive a user name and temporary password for Faces scheduling
- Identify your ENGR username and Password from Systems
 - If you don't have an ENGR account, send me the following:
 - Full name
 - Principal Investigator (PI)
 - SID
 - email
- Coordinate a time with the lab manager for training
- Schedule a 1 hour block on Faces for your training

Raman Operation

- I. Initiate Software
- II. Selecting Sample Holder
- III. Sample Holder Alignment
- IV. Collection Parameters
- V. Collect Background
- VI. Collect Sample
- VII. Collect Sample Holder
- VIII. Saving Data
- IX. Background Subtraction
- X. Manual Baseline Correction
- XI. Cleanup

I. Initiate Software – 1/3

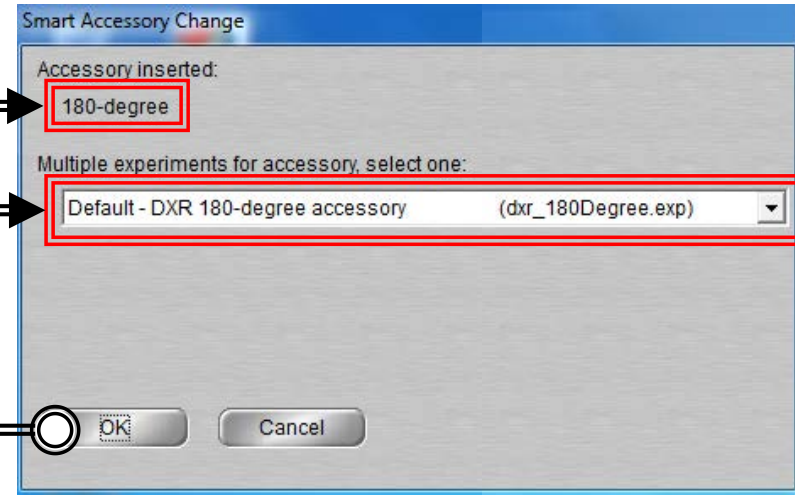


1. Double left-click on the OMNIC for Dispersive Raman

2. A dialogue may appear indicating installed accessory and confirm that it is correct:
180-degree

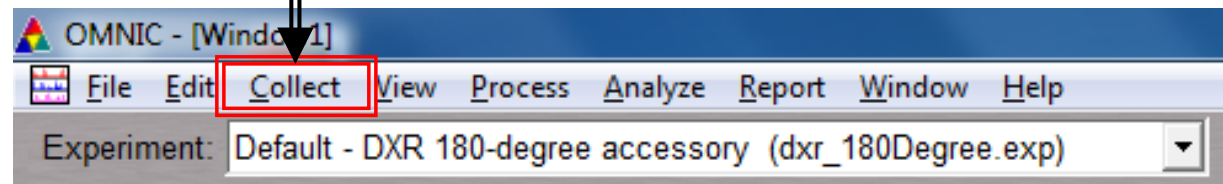
3. Select the
“Default – DXR 180-degree accessory”

4. Click **OK**



5. A dialogue showing ***“Resetting Step Motors”*** may appear

7. Select ***Collect -> Experiment Setup***
at the top window



I. Initiate Software – 2/3

8. Select the **Advanced** tab

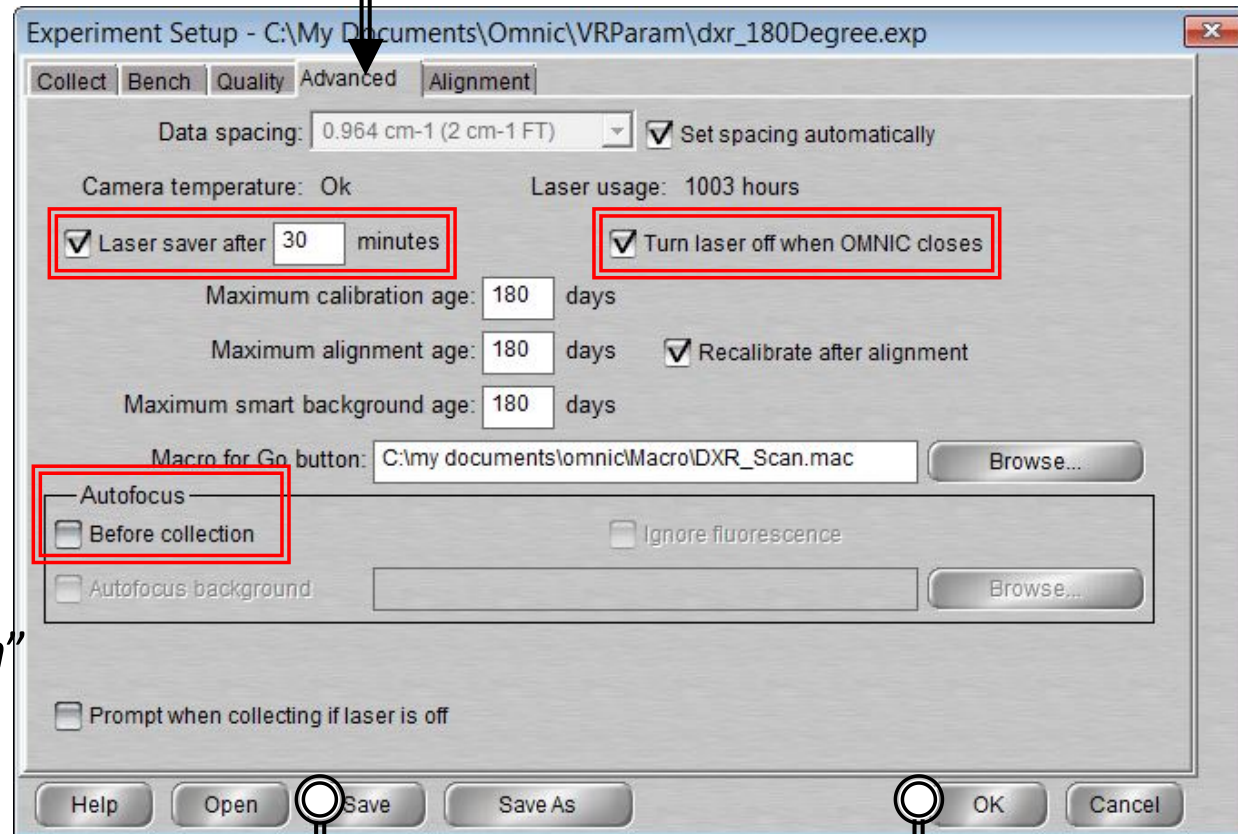
9. Confirm “**Laser saver**” is checked and set to “**30 minutes**”

10. Confirm “**Turn off laser**” is checked

11. Confirm Autofocus option “**Before Collection**” is unchecked

12. Click **Save**

13. Click **OK**

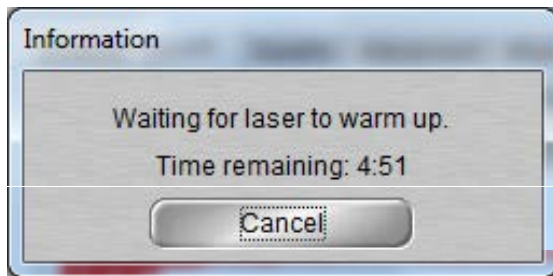


I. Initiate Software – 3/3

14. Select the **Bench** tab

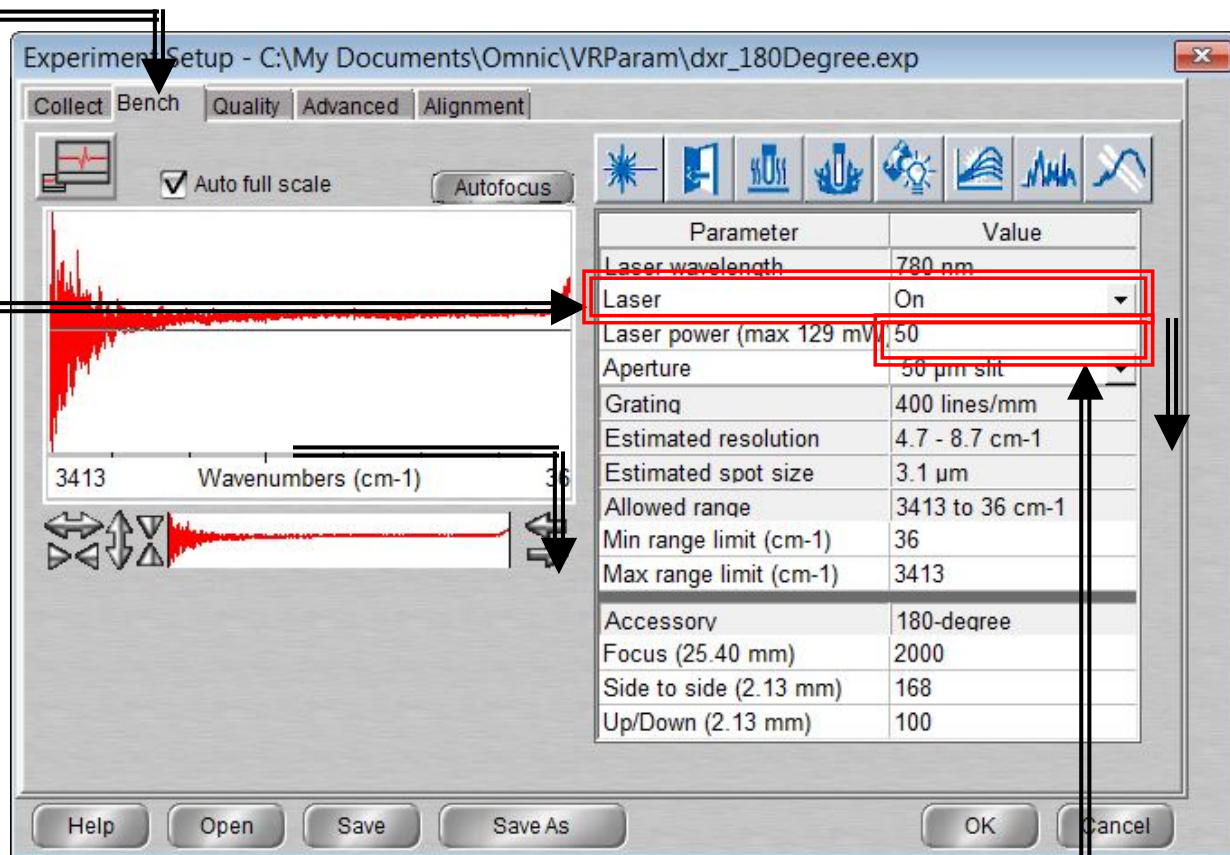
15. Turn on **Laser** by clicking and selecting “On”

16. A dialogue box will



17. Set Laser power to “50” as a suitable level by clicking and entering value

18. The laser will only be emitted when the enclosure is closed



II. Sample Holder – 1/2

1. Depending on your sample, the sample holder and preparation will vary...
2. Several sample holders are available for use located in the storage container
3. **CLEAN UP AFTER EACH USE AND WIPE DOWN!**

NMR Tube Holder



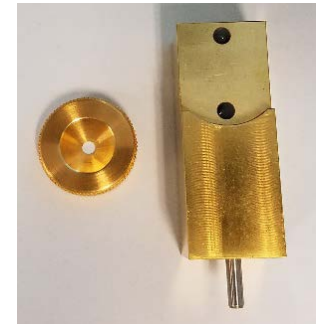
Large quantities of liquid and powder

Capillary Tube Holder



Small quantities of liquid and powder

Thin Film Holder



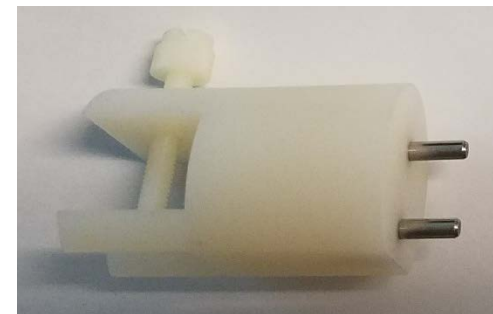
Thin film or thin planar samples

Flat Bulk Sample Holder



Flat bulk samples too large for the Thin Film holder

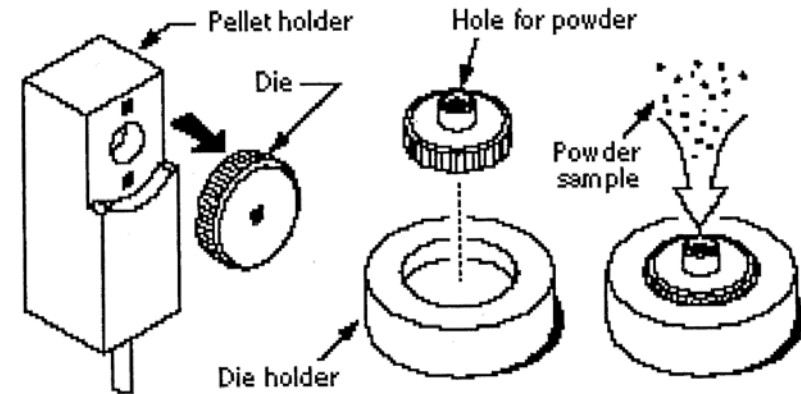
Bulk Sample Holder



Irregular shaped bulk samples

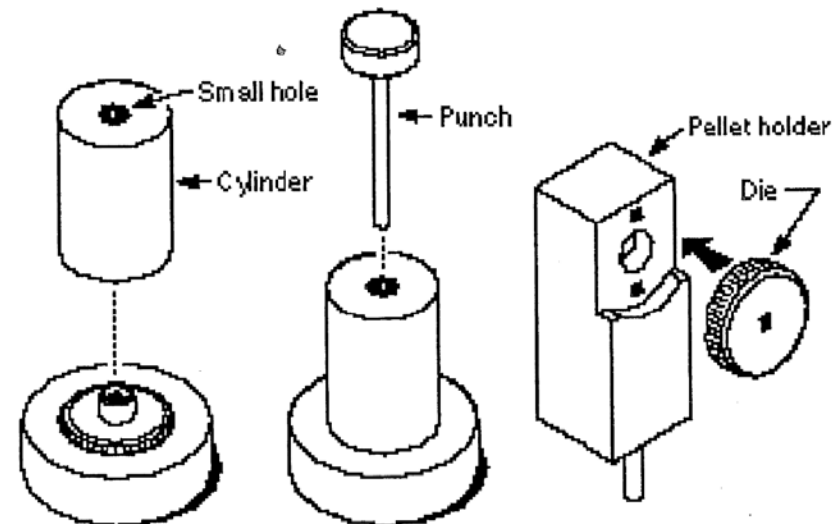
II. Sample Holder : Pellet – 2/2

1. Remove the **Die** from **Pellet Holder**
2. Place **Die** in **Die Holder**
3. Fill hole in **Die** with the powdered sample



4. Place **Metal Cylinder** over **Die** with the small hole facing up
5. Insert **Punch** into hole at top of **Cylinder** and apply force with hand to push **Punch** down as far as it will go

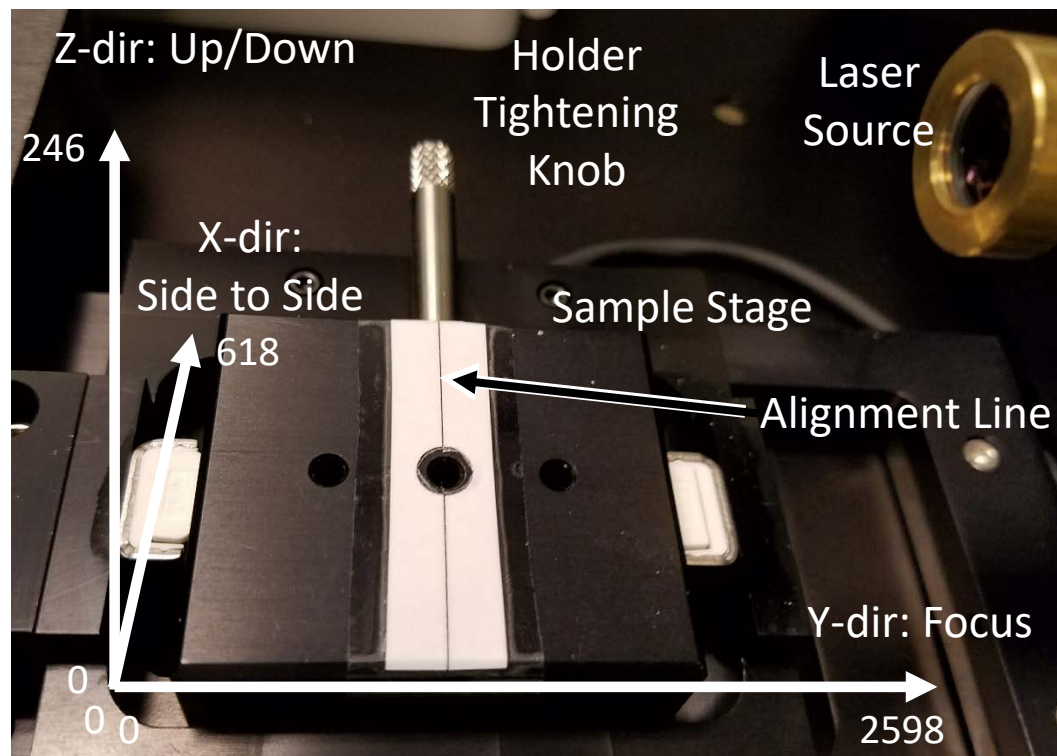
6. Remove **Cylinder** from **Punch Die**
7. Mount **Die** on **Pellet Holder** so stem of die is inserted into hole in the **Pellet Holder**



III. Sample Holder Alignment – 1/3

1. Depending on your Sample Holder, the appropriate **Focus** position will be different
2. Double-Click and enter the following **preliminary** settings for your Sample Holder

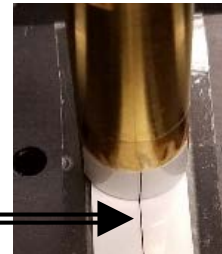
Accessory	180-degree	
Focus (18.81 mm)	1481	
Side to side (2.46 mm)	194	
Up/Down (2.46 mm)	130	



	NMR Tube	Capillary Tube	Thin Film	Flat Bulk	Bulk	Pellet Holder
Focus	2542	2432	2356	2397	1570	2475
Side to Side	168	168	168	168	168	168
Up/Down	150	160	180	151	108	180

III. Sample Holder Alignment – 2/3

3. Position the NMR and Capillary Tube sample holders using the alignment line as a guide and tighten the holder knob

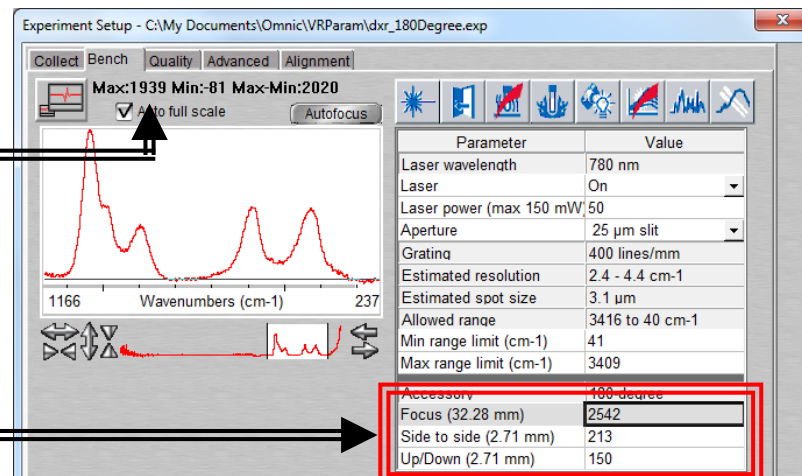
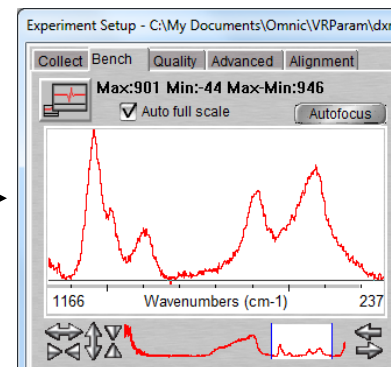
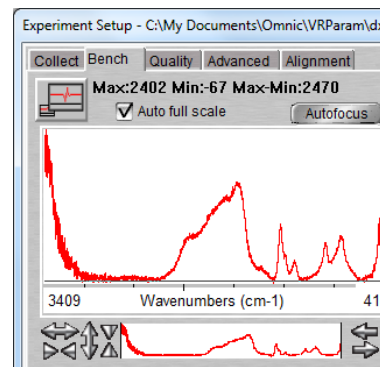


4. Insert the 50 wt% H₂SO₄ reference samples



5. Focus on any relevant peak(s) by moving the cursors

6. Achieve the max signal by optimizing the settings



Sensitivity of the settings:

± 1 Focus = ± 10 µm

± 1 Side to Side = ± 20 µm

± 1 Up/Down = ± 20 µm

III. Sample Holder Alignment – 3/3

7. Place your sample into position for the Thin Film, Bulk Flat, and Bulk sample holder

8. Position the Thin Film and Bulk Flat sample holder using the alignment line as a guide and tighten the holder knob

9. The Bulk sample holder does not require any additional alignment

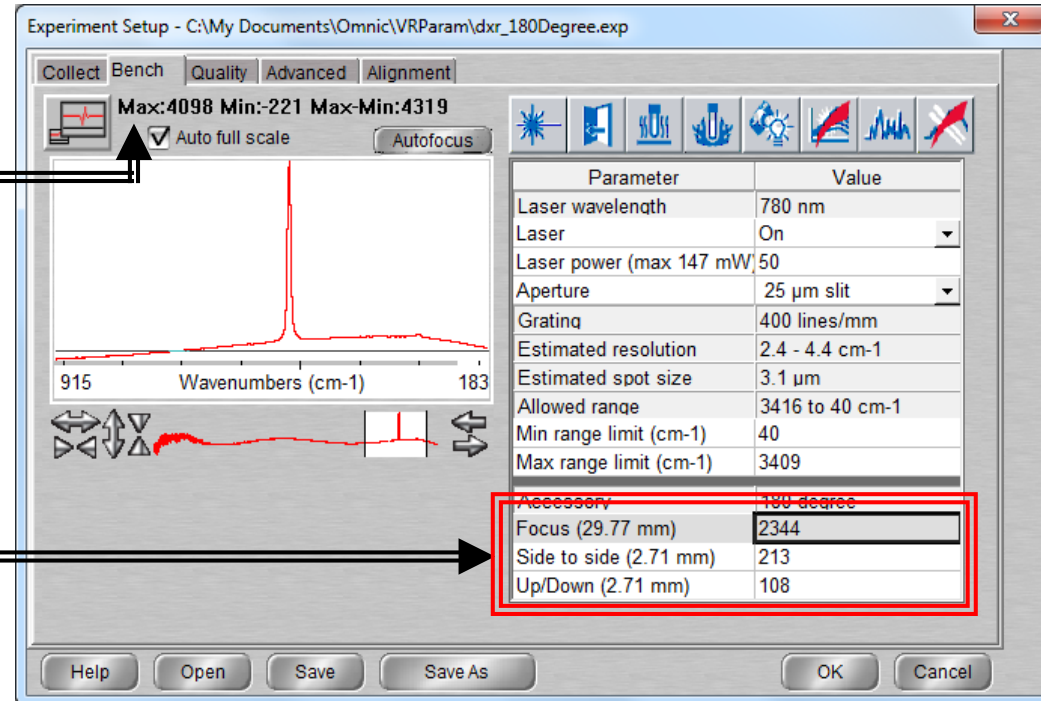
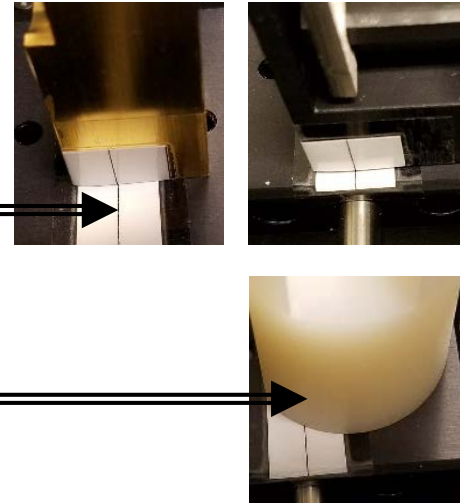
10. Achieve the max signal by optimizing the settings

Sensitivity of the settings:

± 1 Focus = $\pm 10 \mu\text{m}$

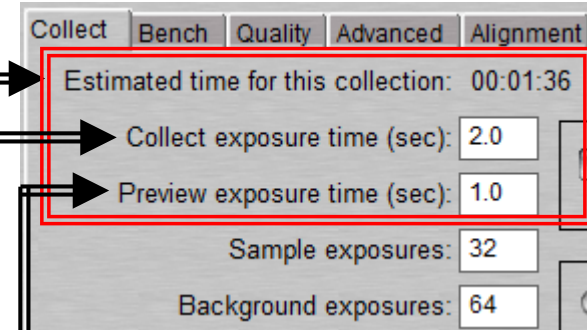
± 1 Side to Side = $\pm 20 \mu\text{m}$

± 1 Up/Down = $\pm 20 \mu\text{m}$



IV. Collection Parameters – 1/6

1. Select the **Collect** tab



2. Estimated time for collection is shown here

- Remember to check this value after changing collection parameters!

3. Determine the **Collect exposure time (sec)** – recommend starting at 2 sec

- Increase value to improve signal-to-noise ratio until desired result is achieved
- **Note:** If CCD overflow occurs, increase # of exposures instead

4. Determine the **Preview exposure time (sec)** – recommend 1 sec

- This value also determines the exposure time for the live display on Bench tab

IV. Collection Parameters – 2/6

5. Determine # of **Sample exposures** – recommend starting at 32

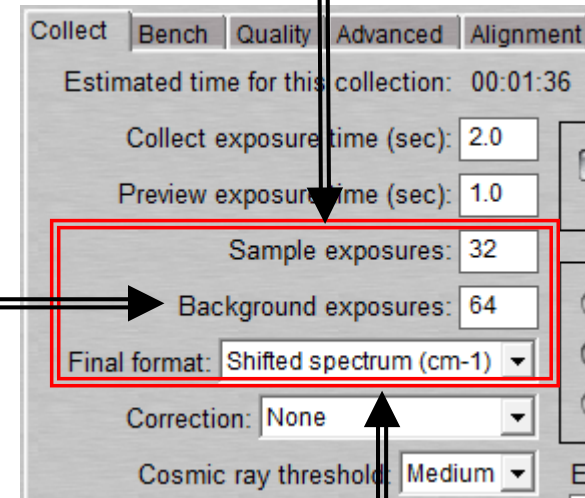
- Increase value to improve signal-to-noise ratio
- **Note:** For weak signals, set longer **Collect exposure times** instead of increasing # of **Sample exposures**

6. Determine # of **Background exposures** – recommend greater value than # of **Sample exposures**

- Increase value to avoid having background add noise to the spectrum

7. Select the **Final Format** – recommend Shifted spectrum (cm⁻¹)

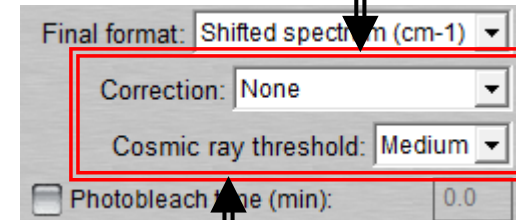
- Raman spectrum (nm): nanometer vs Raman intensity
- Raman spectrum (cm⁻¹): wavenumber vs Raman intensity
- Shifted spectrum (cm⁻¹): shifted wavenumber vs Raman intensity
- Photoluminescence (nm): nanometer vs emission



IV. Collection Parameters – 3/6

8. Select desired **Correction** – recommend None

- Raman Efficiency – corrects for intensity differences related to frequency
- Fluorescence – corrects the effect on baseline curvature due to fluorescence
 - Use Polynomial – specify a polynomial of order 1 to 6 for the operation
 - Default is 5th order polynomial
 - Use Reference File – specify a reference spectrum that contains fluorescence artifacts you want removed



9. Select desired **Cosmic ray threshold** – recommend Medium

- None – will not reject cosmic ray spikes
- Low – rejects random cosmic ray spikes with low intensity and higher intensity
- Medium – rejects random cosmic ray spikes of least moderate intensity
- High – rejects only random cosmic ray spikes of high intensity

IV. Collection Parameters – 4/6

10. Select desired **Photobleach time (min)**

- Photobleach may be occurring if baseline offset decreases on successive exposures
 - Estimate the appropriate photobleach time by observing how long it takes the baseline of the spectrum to reach a steady state in the Bench tab

Photobleach time (min): 0.0
 Preview data collection:
 Auto exposure Desired S/N: 100
Maximum collect time (min): 2

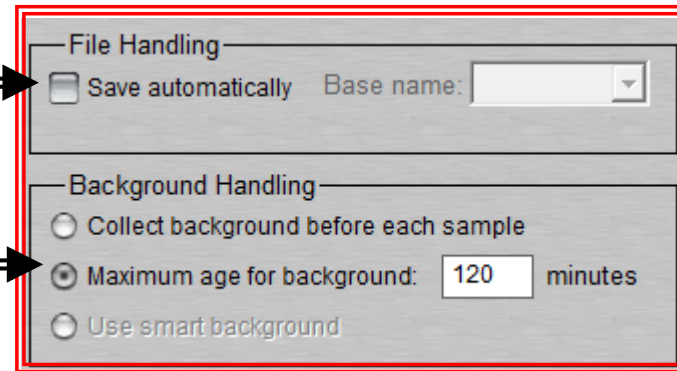
11. Confirm **Preview data collection** – recommend Medium

- None – will not reject cosmic ray spikes
- Low – rejects random cosmic ray spikes with low intensity and higher intensity
- Medium – rejects random cosmic ray spikes of least moderate intensity
- High – rejects only random cosmic ray spikes of high intensity

12. Confirm **Auto exposure** is not checked

IV. Collection Parameters – 5/6

13. Confirm **Save automatically** is not checked



14. Determine **Background Handling**

- Collect background before each sample
- **Maximum age for background** – default choice and recommend 120 minutes
- Use smart background

15. Select the **Bench** tab



16. Determine desired **Aperture**

- 50 μm slit – higher signal – recommended default
- 25 μm slit – higher resolution

Parameter	Value
Laser wavelength	780 nm
Laser	On
Laser power (max 129 mW)	50
Aperture	50 μm slit
Grating	400 lines/mm
Estimated resolution	4.7 - 8.7 cm^{-1}
Estimated spot size	3.1 μm
Allowed range	3413 to 36 cm^{-1}
Min range limit (cm^{-1})	36
Max range limit (cm^{-1})	3413

17. Adjust the desired scan range

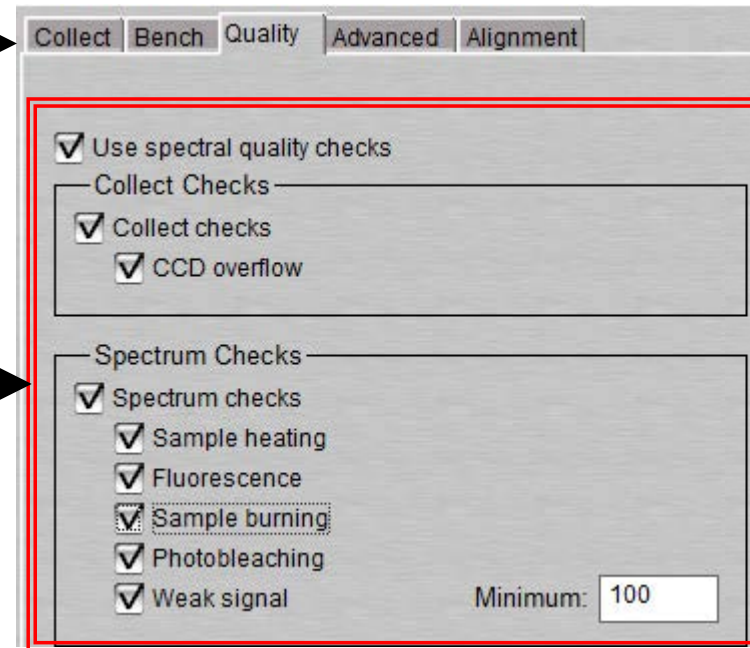
IV. Collection Parameters – 6/6

18. Select the **Quality** tab

19. Confirm all checks are selected

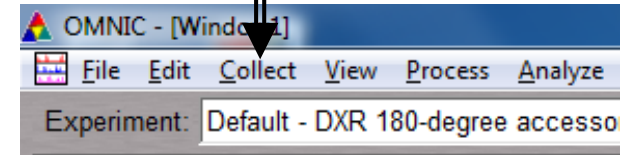
20. Perform the following if checks are found:

- **CCD overflow** – reduce exposure time or decrease aperture size
- **Sample heating** – reduce laser power, exposure time, and # of exposures
- **Fluorescence** – apply correction
- **Sample burning** – reduce laser power
- **Photobleaching** – include **Photobleaching time** in **Collect** tab
- **Weak signal** – check focus is correct, increase exposure time, increase aperture size, and increase laser power



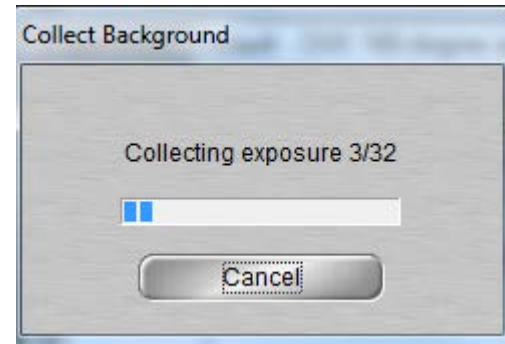
V. Collect Background – 1/1

1. Select **Collect -> Collect Background**



Note: Background measures the response of each pixel in the CCD with camera shutter closed, and does not take into account the sample holder background signal

2. A dialogue box will appear indicating the background exposure progress



VI. Collect Sample – 1/1

1. Select **Collect -> Collect Sample**
2. Enter **title** for collected spectrum, click **OK**
3. A live display of the collection will appear
4. The following shows the collect status indicator during your collection

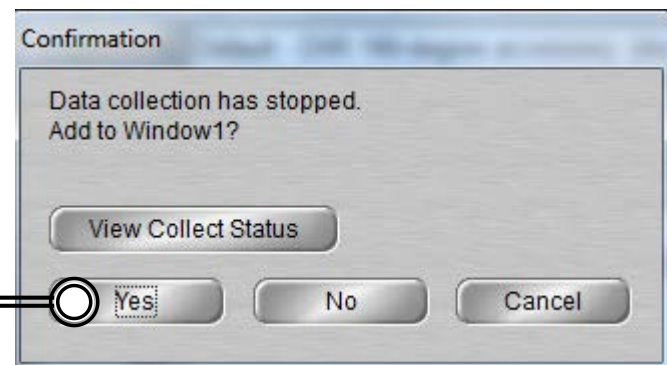
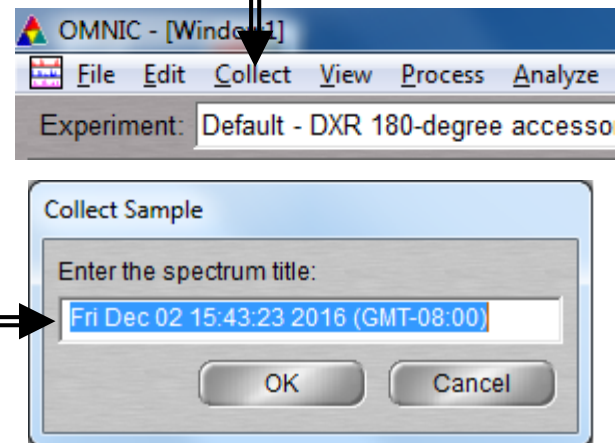
 ○ The spectrum has passed all quality checks

 ○ The spectrum has failed a spectral quality check but not serious




 ○ There is a problem with quality of spectrum, correct problem before collecting the spectrum again

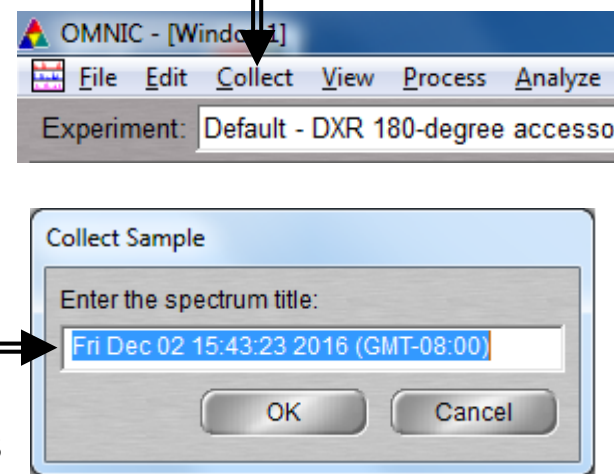
5. The current background will be automatically subtracted from sample data

6. Choose to add the collected spectrum in window specified, click **Yes**


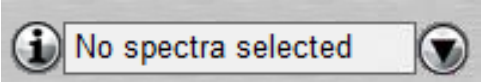


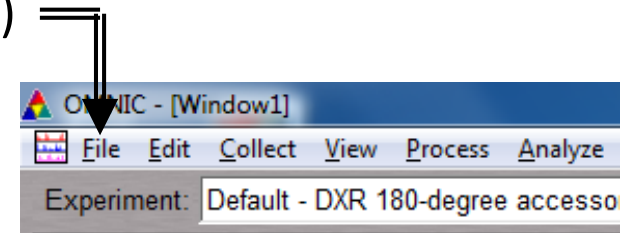
VII. Collect Sample Holder – 1/1

1. If your sample is transparent or are using a secondary sample holder like a glass NMR or capillary tube, you will need to collect background spectrum from primary sample holder
2. Remove sample from the sample holder and insert an empty glass NMR or capillary tube or glass slide if applicable
3. Select **Collect -> Collect Sample**
4. Enter **title** for collected spectrum, click **OK**
5. A live display of the collection will appear
6. The following shows the collect status indicator during and after your collection
 -  The spectrum has passed all quality checks
 -  The spectrum has failed a spectral quality check but not serious
 -  There is a problem with quality of spectrum, correct problem before collecting the spectrum again
7. The current background will be automatically subtracted from sample data
8. Choose to add the collected spectrum in window specified



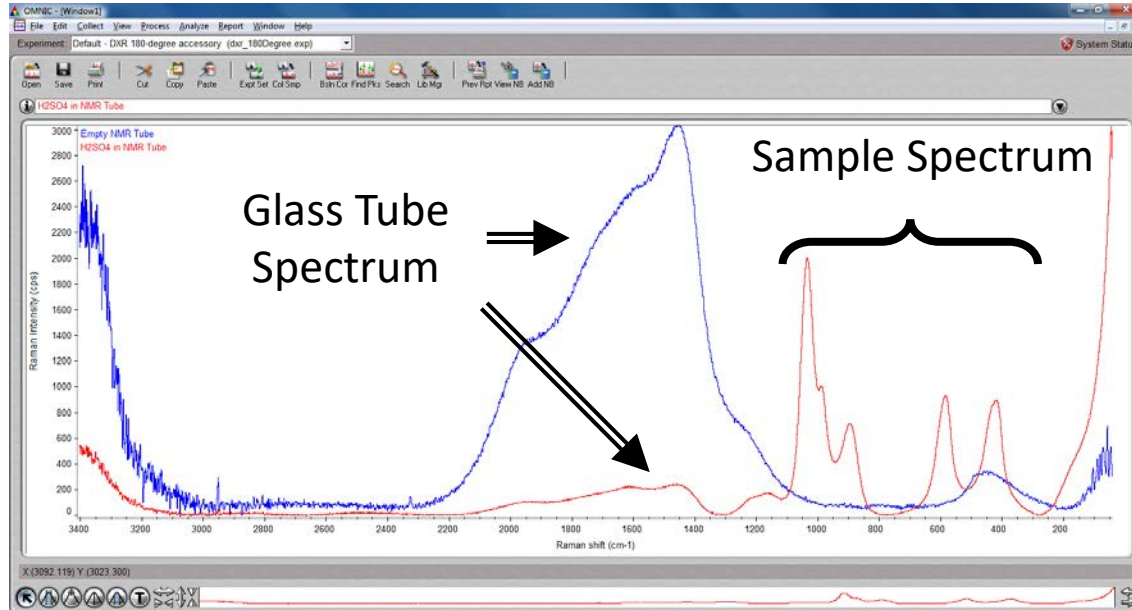
VIII. Saving Data – 1/1

1. Specific spectra can be selected using the  selection tool at the bottom of window and clicking on it or selecting  from dropdown
2. Multiple spectra can be selected/deselected by holding down the **Ctrl** key and clicking spectra
3. Click **File -> Save** to save spectrum (e.g. default is SPA) using the current filename
4. Click **File -> Save As** to save a spectrum into another file type (e.g. CSV or TIFF)
5. Click **File -> Save Group** to save more than one spectrum as a group in one file having file extension .SPG to open later
6. Click **File -> Save Current Background** to a named file if desired for later referencing or processing (optional)



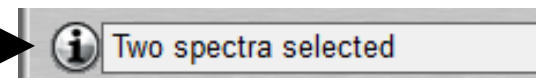
IX. Background Subtraction – 1/3

1. Perform a background subtraction to remove effects of a sample holder

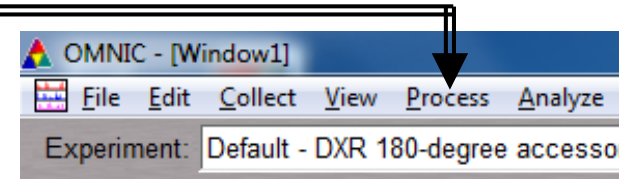


2. Select the sample spectrum (A) first, then hold **Ctrl** key and select the reference spectrum (B)

3. **“Two spectra selected”** appears at top

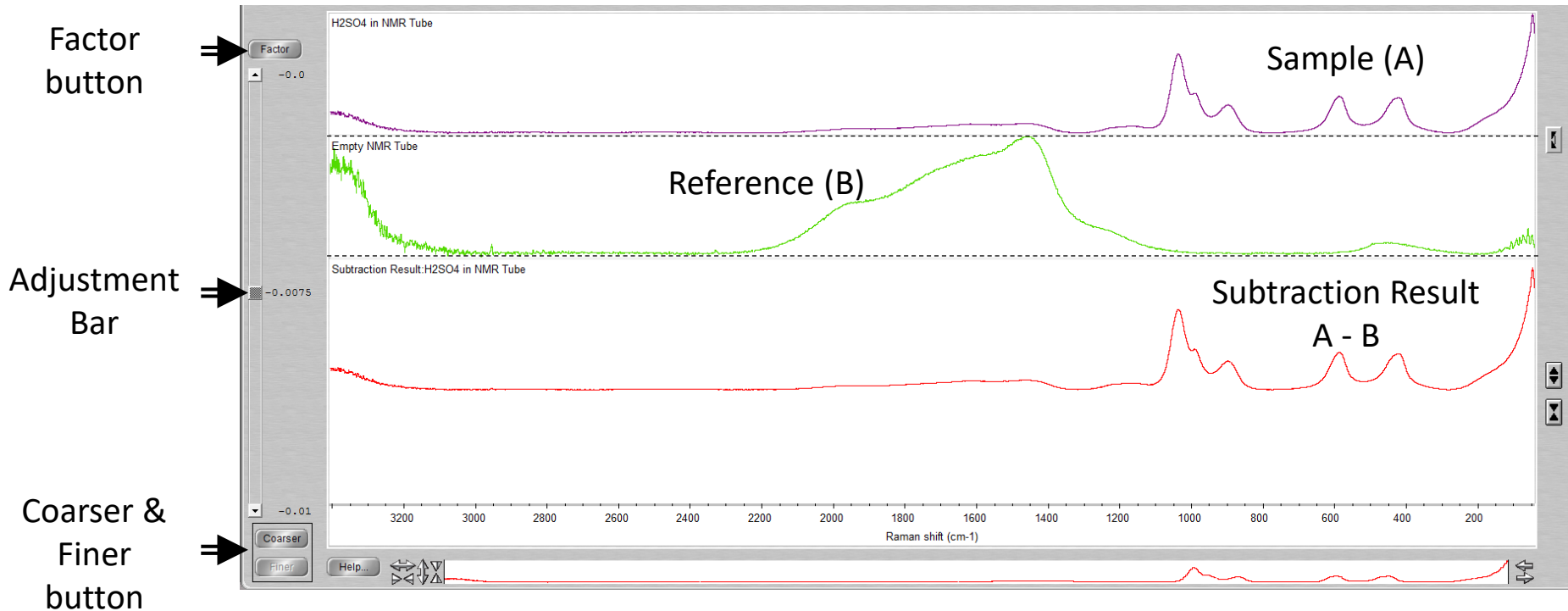


4. Click **Process -> Subtract**



IX. Background Subtraction – 2/3

5. The subtract window appears with the sample spectrum (A) in top pane and reference spectrum (B) below it



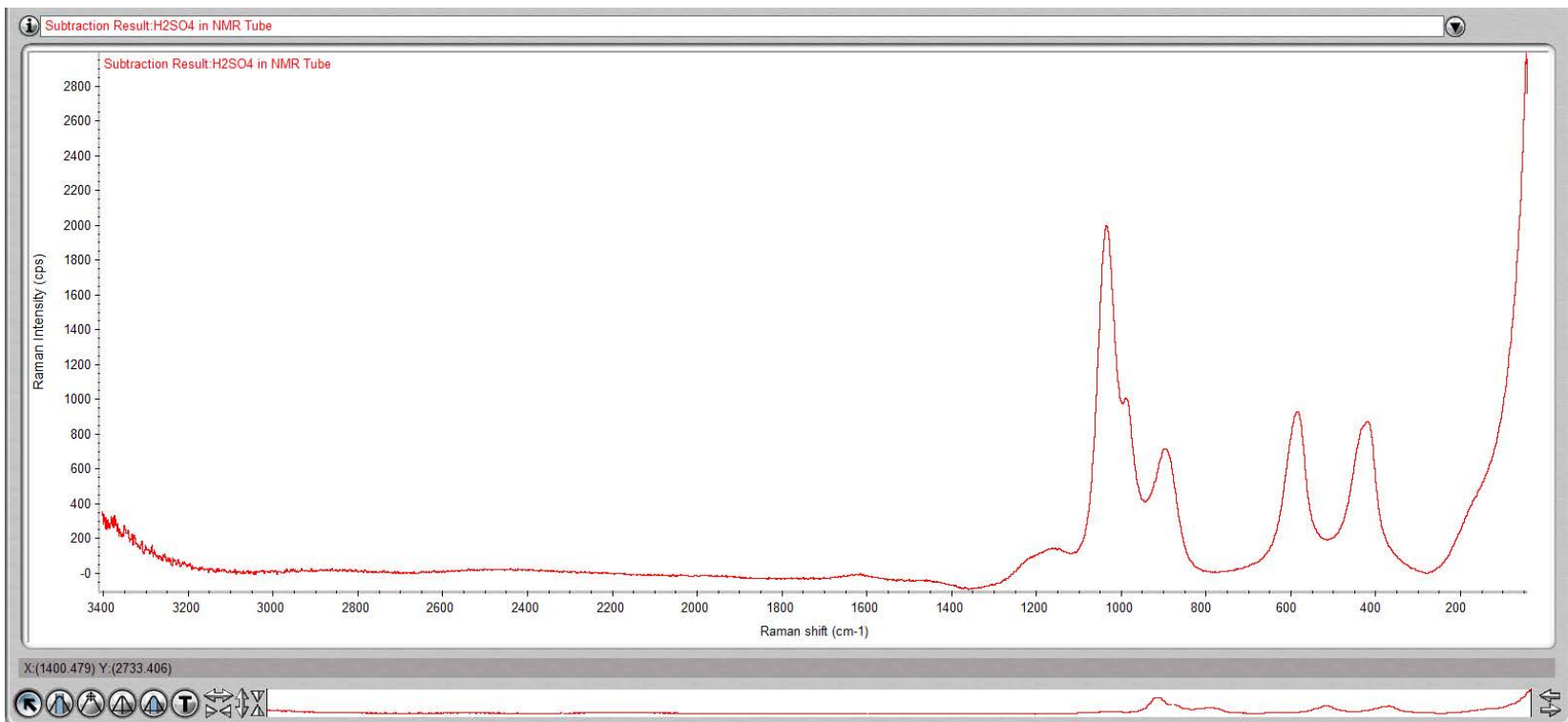
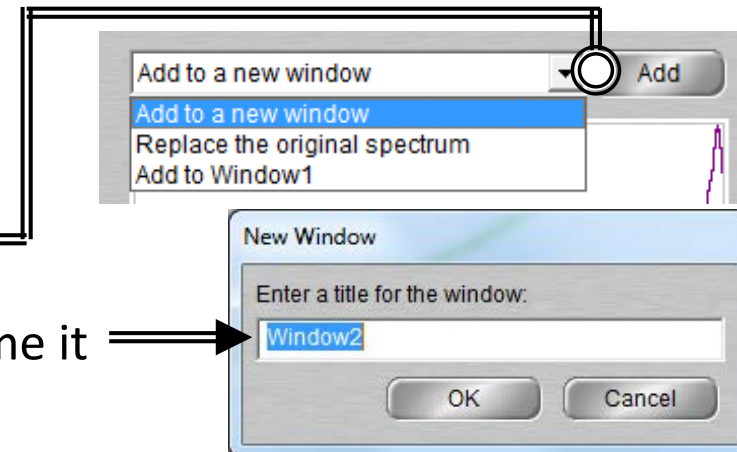
6. Click and move **Adjustment Bar** to achieve desired subtraction
7. Click **Coarser** or **Finer** to increase or decrease the sensitivity of adjustments
8. Click **Factor** button to enter in a specific factor value for subtraction

IX. Background Subtraction – 3/3

9. Click on top dropdown to determine where the new subtracted spectra will appear

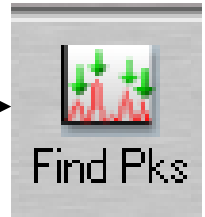
10. Click **Add** to add to desired window

11. If new window is selected, you will need to name it



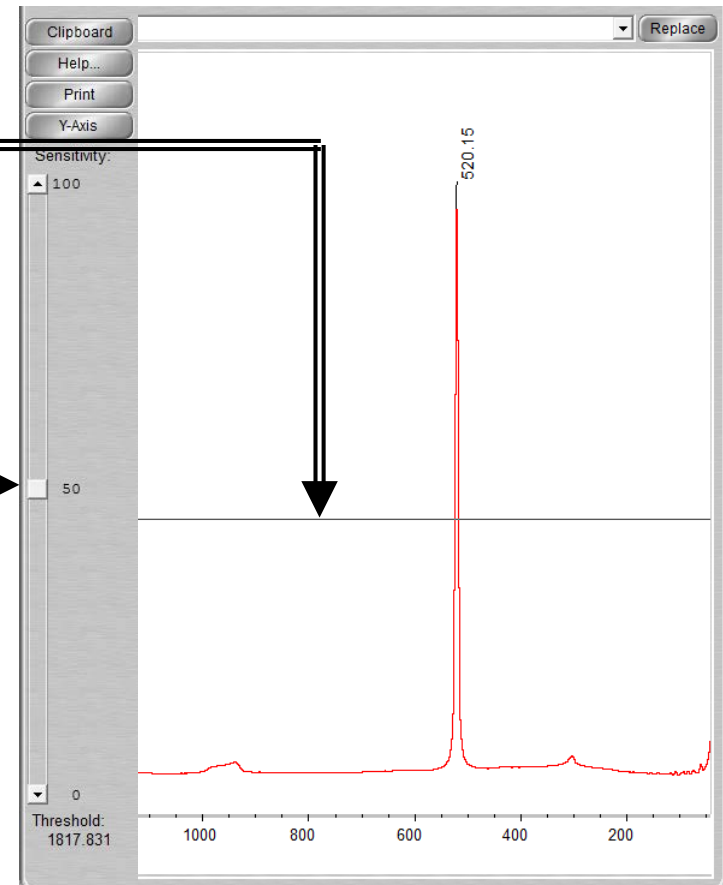
X. Peak Identification – 1/1

1. Click on “*Find Pks*” button at the top



2. Click the spectrum window to adjust the *Threshold* position on where peaks are to be considered

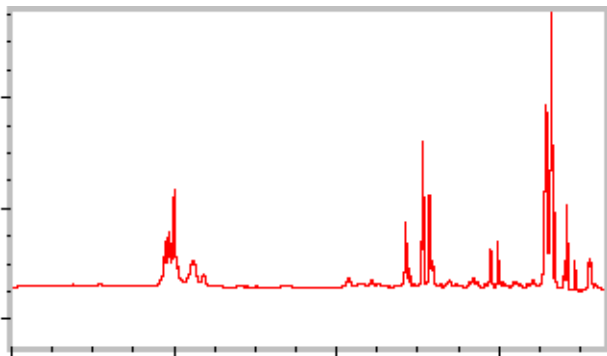
3. Adjust the *Sensitivity* button to separate peaks from noise



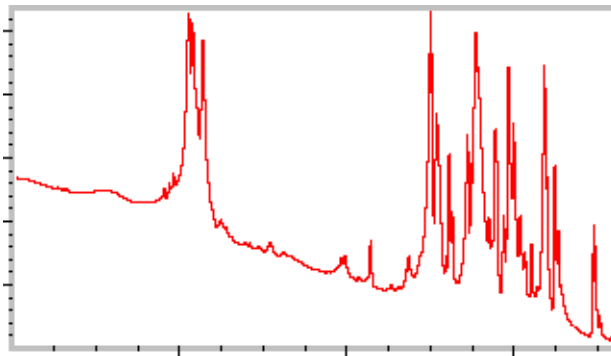
XI. Manual Baseline Correction – 1/3

1. If your spectra has a shifted, tilted, or curving baseline, you can choose to correct it manually using the software

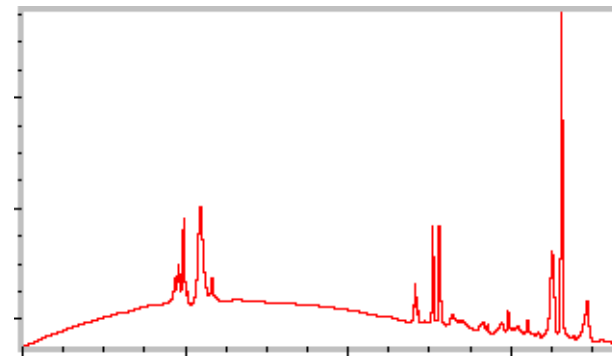
Shifted (above zero)



Tilted

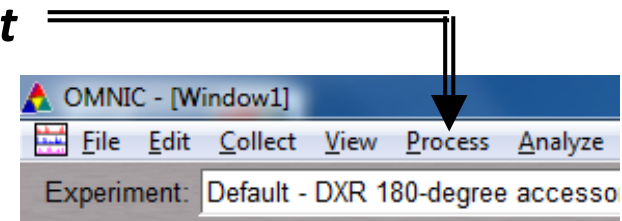


Curved



2. Select the spectrum you wish to correct

3. To correct a baseline, click **Process -> Baseline Correct**



XI. Manual Baseline Correction – 2/3

4. Select an algorithm from the drop-down list box near upper-left corner of window
 - **Linear**: For tilted or elevated baselines
 - **Spline**: For curved baselines
 - **Polynomial**: Suitable for all, with max order of 6

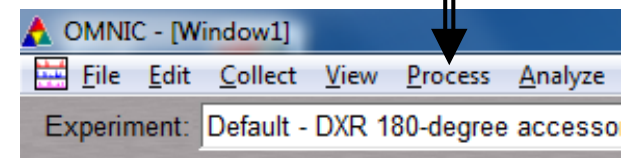
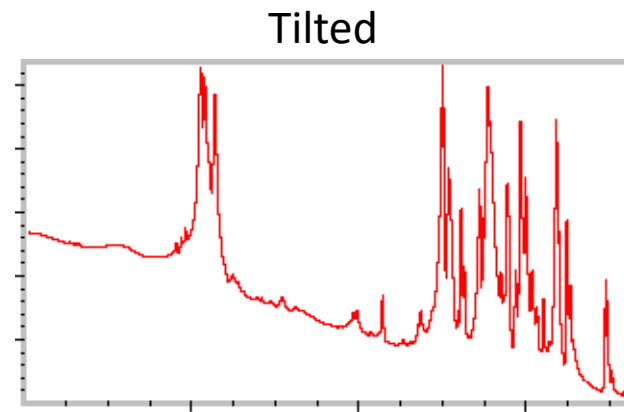
5. Select **Auto Y** to have baseline points coincide with points on spectrum

6. Click as few as necessary to straighten pronounced curves or slopes in upper pane

7. Add corrected spectrum to new spectral window

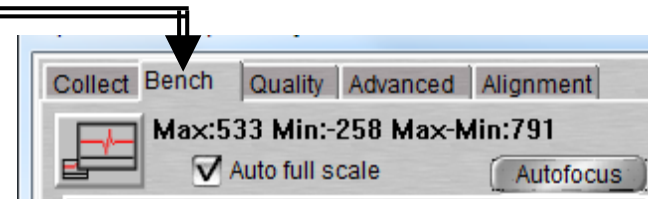
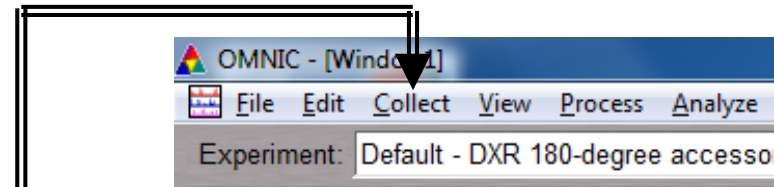
XI. Manual Baseline Correction – 3/3

8. You may choose to let the software automatically correct a tilted baseline
9. Select the spectrum you wish to correct
10. To correct a baseline, click ***Process -> Automatic Baseline Correct***
11. Click ***Edit -> Options***
12. Set the ***Polynomial Order and Number of Iterations*** in the ***Process*** options



X. Cleanup – 1/1

1. Remove the sample and holder from the stage
2. Clean up the sample holder and return back to cabinet
3. Select **Collect -> Experiment Setup** and click **Bench** tab
4. Click on Laser and turn to “**Off**”
5. Reset the position of stage to:
Focus = 2000
Side to side = 168
Up/Down = 100
6. Click on **File -> Exit** to shut down the software
7. Log off of your ENGR account



Parameter	Value
Laser wavelength	780 nm
Laser	Off
Laser power (max 147 mW)	5
Aperture	25 µm slit
Grating	400 lines/mm
Estimated resolution	2.4 - 4.4 cm-1
Estimated spot size	3.1 µm
Allowed range	3416 to 40 cm-1
Min range limit (cm-1)	40
Max range limit (cm-1)	3409
Accessory	180-degree
Focus (18.81 mm)	1481
Side to side (2.46 mm)	194
Up/Down (2.46 mm)	130

