

## I. Getting Started

### A. Safety

#### **\*\*\*Safety Issues\*\*\***

- ⚠ If you, or people working with you, have magnetic metal implants, please consult your doctor for possible effects of magnetic field;
- ⚠ For those who have pacemakers, please stay away from NMR magnets;
- ⚠ Please remove all personal belongings from your pocket (anything ferromagnetic or vulnerable to magnetic field):
  - Your wallet, bank cards, credit cards, and any cards with magnetic stripes;
  - Electronics: cell phone, ipad, etc.;
  - Analog watches;
  - Keys and other magnetic items.

## B. FBS



To Schedule time and to use the instrument computer a FBS account is required. After training you will receive an invite for FBS or if you already have a FBS account the 400MHz DNP calendar will be added to it.



The instrument time is billed through FBS. Your FBS time = your recharged time. Recharge is calculated at an hourly rate. For current recharge rates go to the MRL website, [http://www.mrl.ucsb.edu/sites/default/files/mrl\\_docs/rechargerate.pdf](http://www.mrl.ucsb.edu/sites/default/files/mrl_docs/rechargerate.pdf)

- ❖ To begin an instrument session, you must log onto FBS first and either click on **Start Timer** if a reservation has already been made or select **Walk-Up**

To do so use the FBS designated computer in the lab or any internet connected device, and navigate to <http://ucsb.fbs.io>

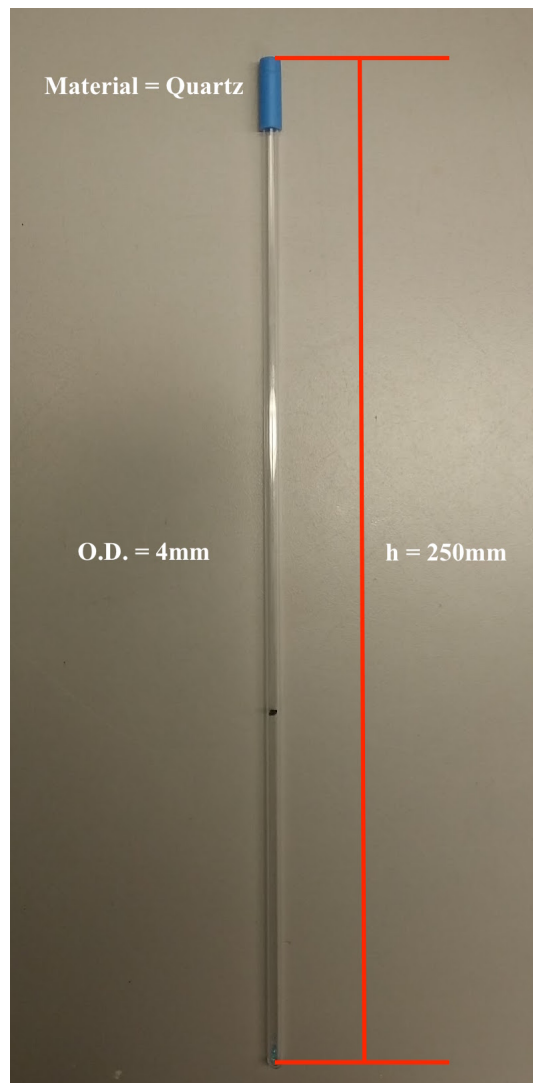
- ❖ Now you can log onto your account on the instrument computer. Remember to log off your computer account when finished.
- ❖ Once you are finished you must log into your FBS account and click **Stop Timer**. This will stop your FBS time. If you do not do this you could incur extra charges.
- ❖ The paper log sheet by the instrument is used as back up for the FBS system. Remember to mark you time, recharge number, and any notes or problems you would like to convey, feel free to use as many lines on the page as needed to be clear.
- ⚠ No shows will be charged **75%** of the scheduled time. If you cannot use your reserved time please cancel it.
- ⚠ FBS records the billable time as the longer time between the scheduled time and the time used. So if your scheduled time is longer than the actual time on the instrument then the scheduled time will be charged.

**C. Log-sheet**

1. Please fill out the log-sheet next to the computer. You will complete the columns up to “Start Time”. The last few columns will be completed once your experiments are finished.

**D. Sample Preparation**

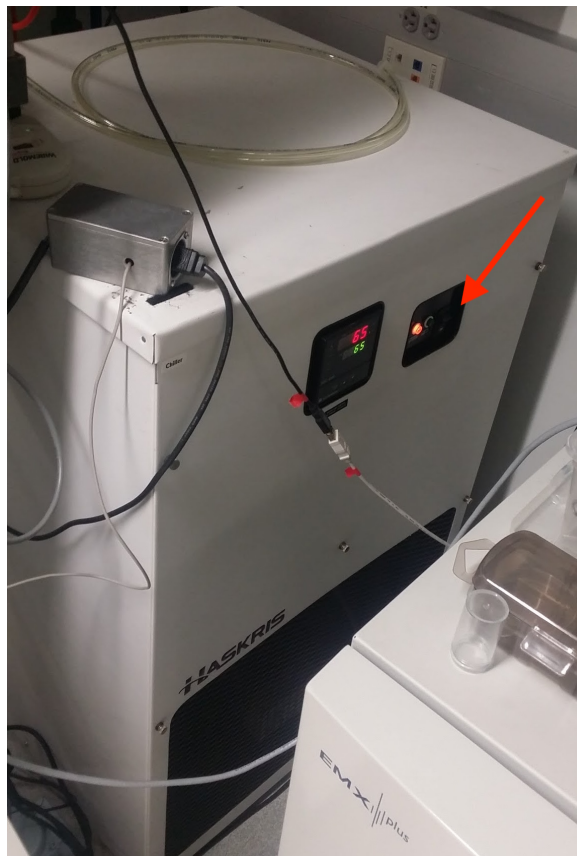
1. The standard EPR tube is made out of QUARTZ and has an outer diameter of 4mm. (However, the machine can accommodate 2mm, 3mm, 4mm, 5mm, and 10mm outer diameters). The standard length is 250mm.



2. **EPR tubes can purchased from Wilmad Glass, Norell, etc. EPR tubes can also be obtained from the Spectroscopy Lab on a recharge basis.**
3. **Minimum Sample Volume Recommendation ~ 20  $\mu$ L**
4. **Minimum Spin Conc. ~  $10^{-9}$  M (Minimum # of spins ~  $10^{11}$ )**
5. **Capillary Tubes (or flat-cell)**
  - a) If your sample(s) is aqueous, highly polar, and/or conducting you may have trouble tuning the cavity and therefore will need to use capillary tubes. This reduces the overall volume of sample so that the sample does not significantly interfere with the microwaves in the cavity. For example, the water in aqueous samples absorbs the electronic component of the microwaves and begins to heat etc.

#### **E. Turn-on Instrument**

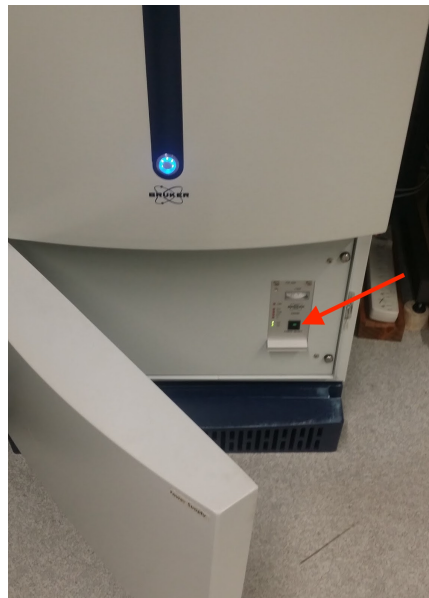
1. **Turn on the chiller (this is always turned on first) (wait 5 minutes)**



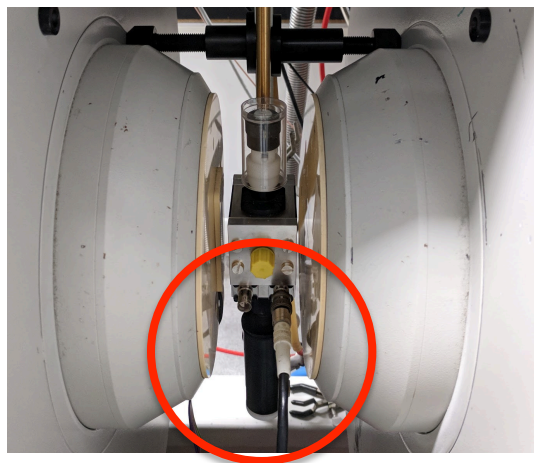
**2. Turn on the Console**



**3. Turn on the Power Supply**



**4. Check that the black quartz dewar is installed below the cavity.**



5. Check that the N<sub>2</sub> purge is connected in the back of the waveguide. This purges the waveguide of any oxygen that may cause erroneous signals in your spectra.

**F. Log-on EPR Computer**

1. Please log-on the computer with your username and password.

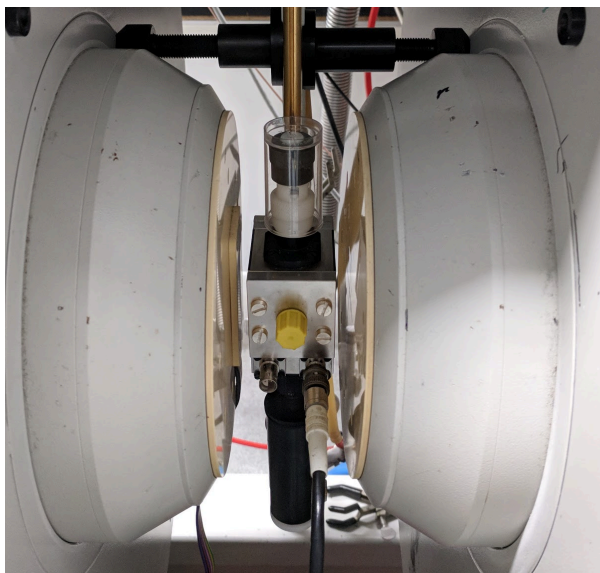
**G. Start Xenon Software**

1. Open the Xenon software.



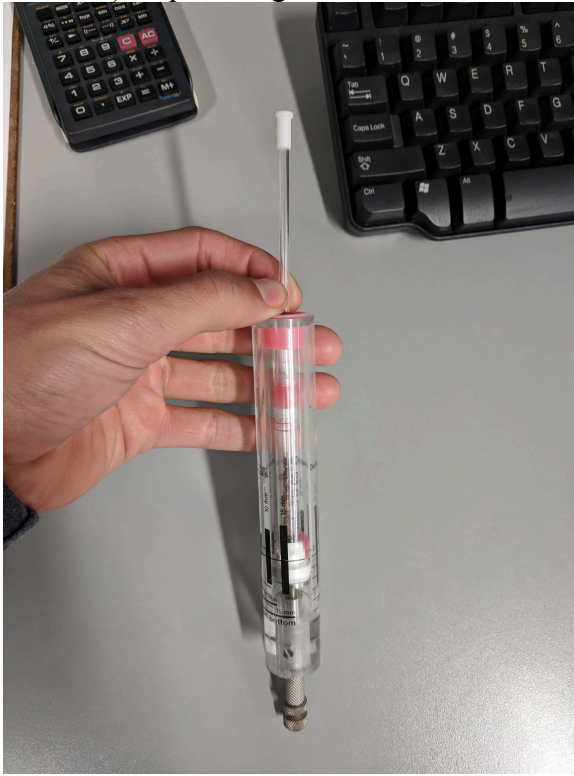
**H. Load Sample**

1. Remove plastic cover

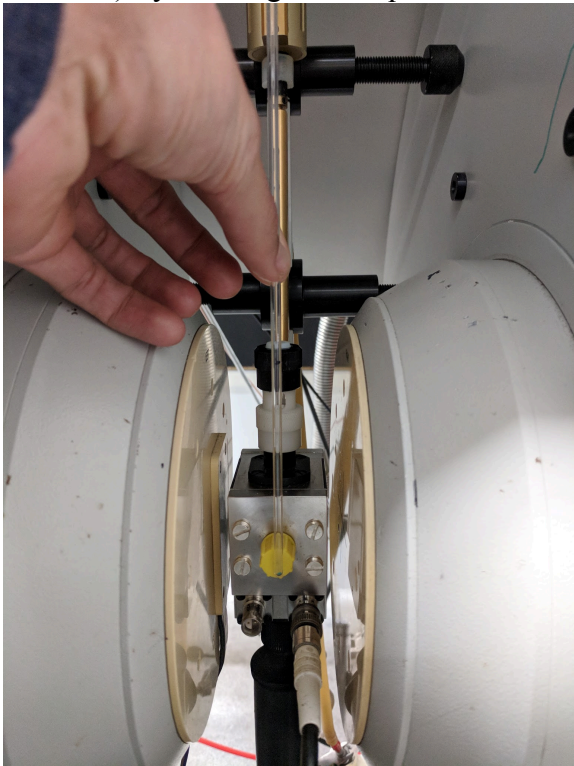


2. Insert sample to the correct depth using either...

a) Depth Gauge



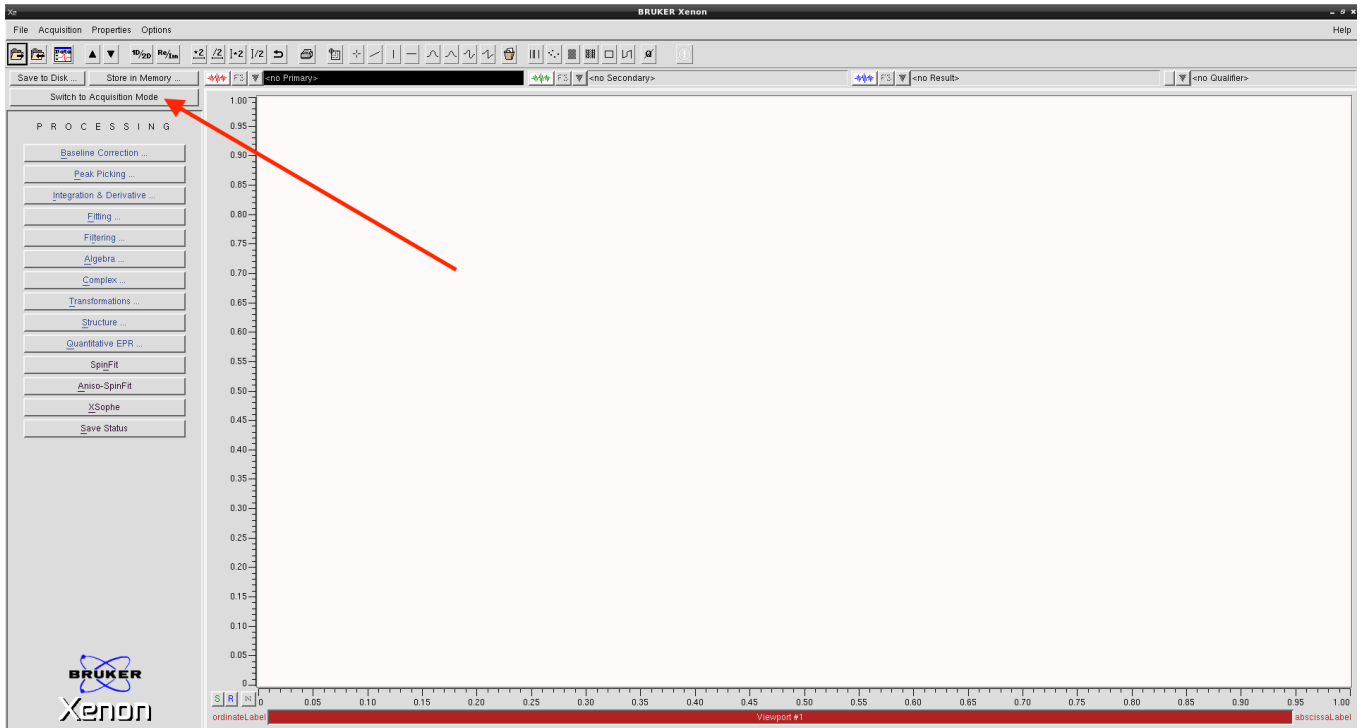
b) Eye-balling Technique



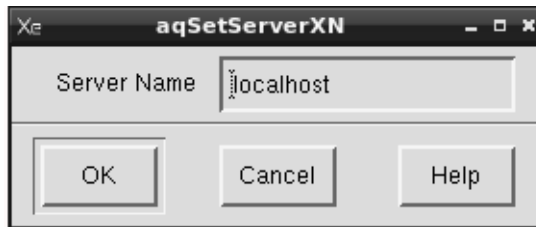
## II. Acquisition

### A. Acquisition Mode

1. Click “Acquisition Mode” in the upper left-hand corner

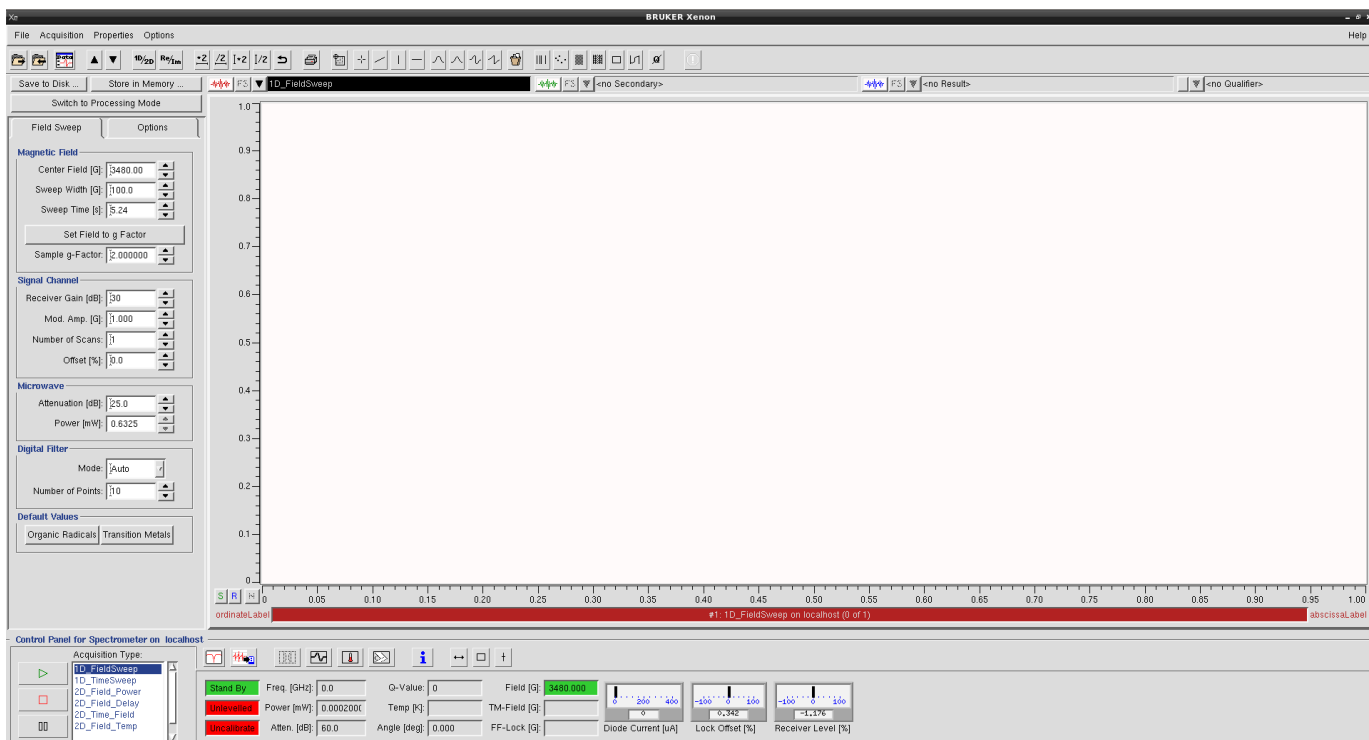


2. A “localhost” window will appear – click “OK” and wait.



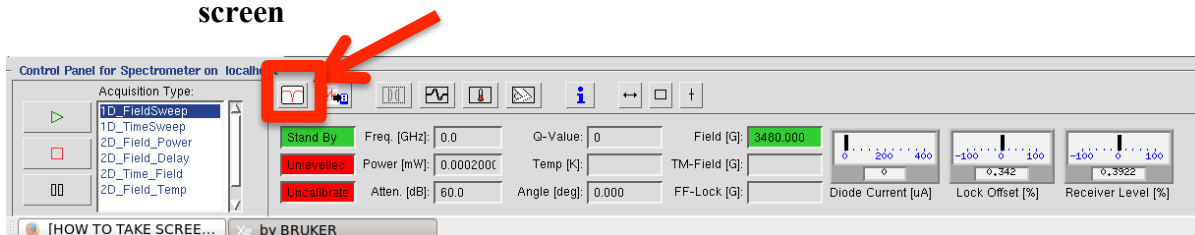
3. The software should now be in “Acquisition Mode” and look as follows:



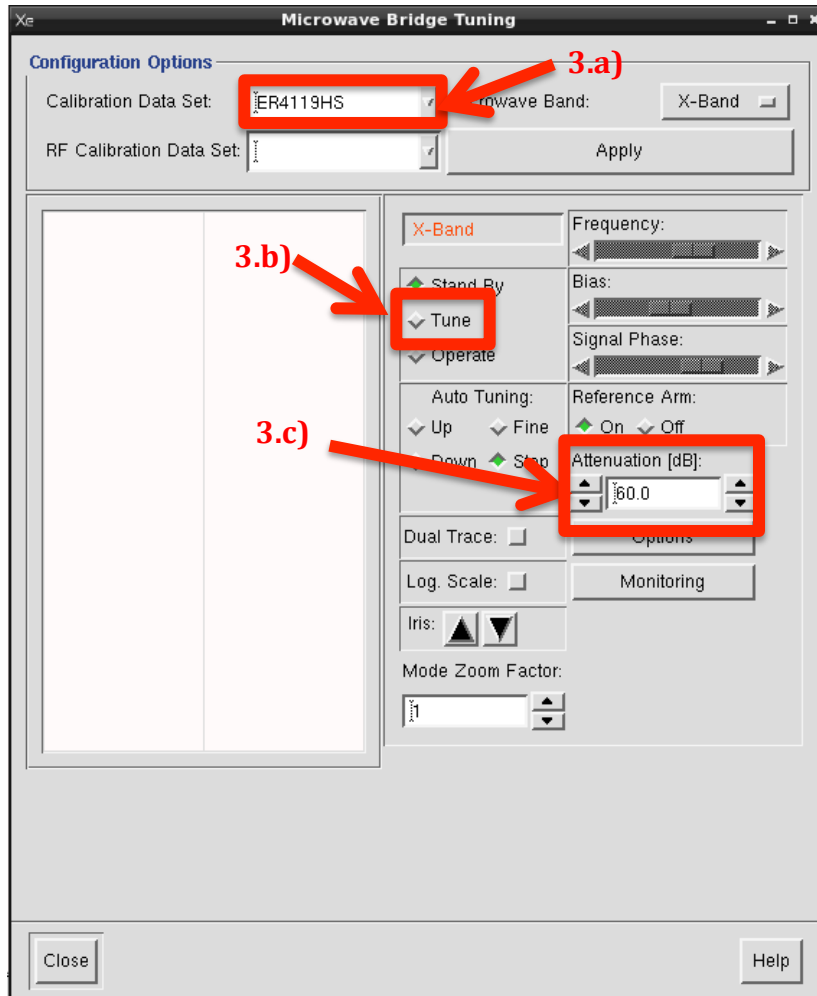


## B. Tune the Cavity

1. Press the "Microwave Tuning" button at the bottom of the screen



2. The tuning window will appear

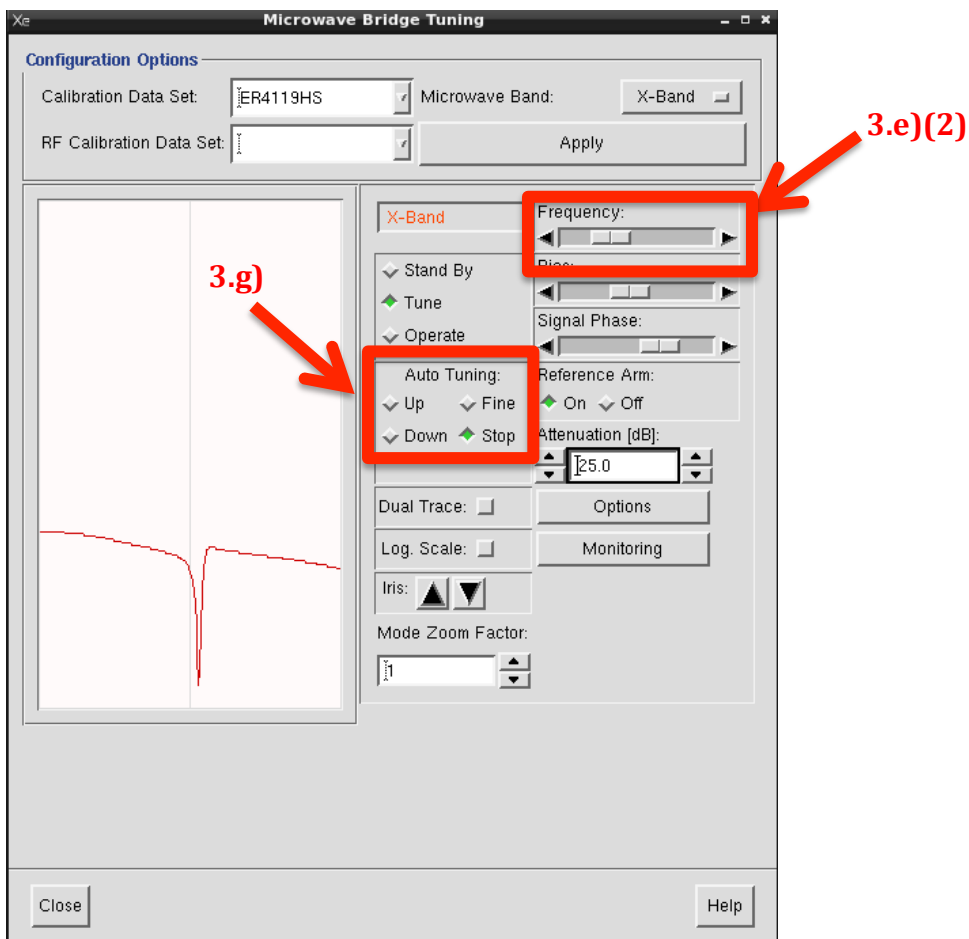


### 3. Auto-tune the cavity

- a) Select the correct calibration file for your current cavity. If the installed cavity has a black rectangle – choose ER4119HS. If the installed cavity has a yellow circle – choose ER4116DM.
- b) Select the radio button next to “Tune” to enter tuning mode.
- c) Change your microwave attenuation to 33dB and the Q-value will then be visible at the bottom of the software
- d) The arrows on the left of the attenuation change it by 10 and the arrows on the right of the attenuation change it by 1.
- e) Microwave forward power is related to attenuation by the following equation:

$$\text{Attenuation (dB)} = 10 * \log \left( \frac{P_{max}}{P_{desired}} \right)$$

f) Wait for you tuning dip to appear in the window



(1) Your dip should be located automatically

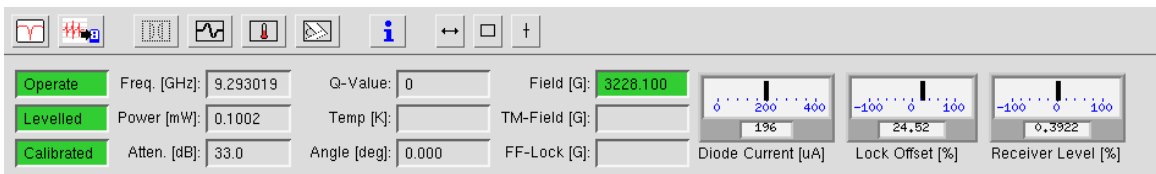
(2) If you do not see a tuning dip, move the “frequency” sliding bar around until you see a tuning dip. It should be located around ~9.3 GHz if you are using the single-mode cavity (black rectangle) and have the quartz dewar installed.

(3) \*\*There will be two dips if you are using the dual-mode cavity (cavity with the yellow circle ER4116DM). The lower frequency dip of the two is the special “parallel mode” EPR.

(4) If you don't see a tuning dip at any frequency, please see next section for instruction on how to use capillary tubes.  $Q$ -values  $< 1000$  will most likely not allow you to auto-tune.

g) Press the Auto-tune “Up” button or Auto-tune “Down” button if the dip is to the right of the center-line or to the left of the center line, respectively.

h) The Auto-tune process is complete when the “Operate”, “Levelled”, and “Calibrated” boxes are all green.



i) Once complete, you will have a tuned resonant cavity that will work from 10dB to 50dB of attenuation.

j) Close the tuning window

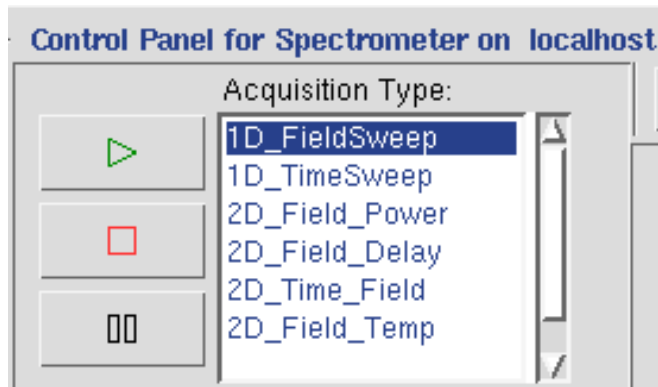
#### 4. Optional: Capillary Tubes

a) If you are using aqueous solvents or solvents with high polarity, conductivity or salt concentration, a capillary tube may help you reach a higher Q-value and successfully tune the cavity.

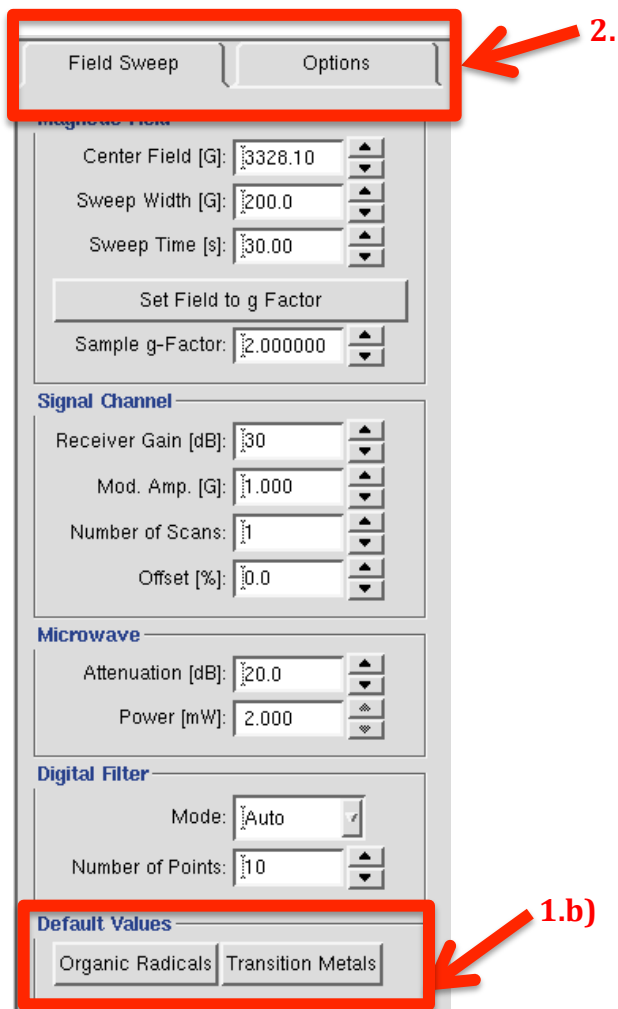
### C. Create Experiment

#### 1. Choose a default starting experiment

a) Choose “1D\_FieldSweep” from the control panel section



b) Choose “Organic Radicals” or “Transition Metal” as your starting point

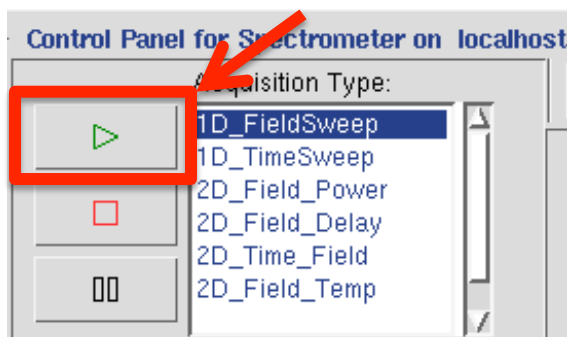


**2. Edit parameters under the “Field Sweep” tab and “Options” tab as needed**

- a) The lowest receiver gain is 0dB and the highest is 74dB
- b) See *Section E* for information on optimizing parameters

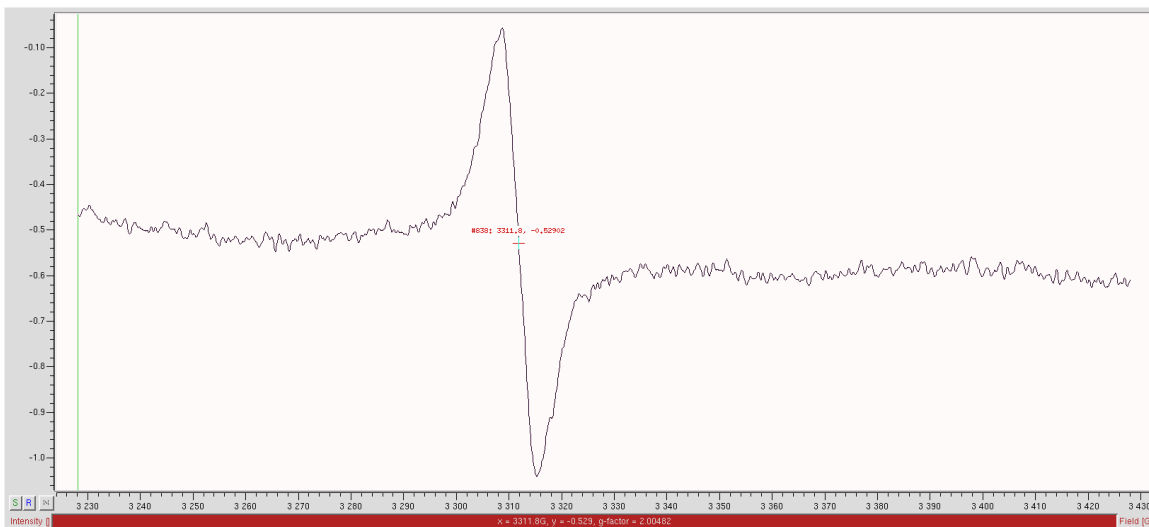
**D. Run**

- 1. Press the PLAY  button to begin your acquisition**



## 2. Check your spectrum

- a) After a moment, the software will auto-scale your spectrum for you.
- b) Click and hold on the signal and move your mouse around to see the magnetic field and g-factor at the bottom in red.



- c) Use right-click and hold to expand and contract the spectrum. Use left-click and hold to choose a zoom region. You can also use the \*2 and /2 buttons at the top of the software to expand and contract the spectrum. Please see **Part III. Processing** section for more information on manipulating the spectrum.

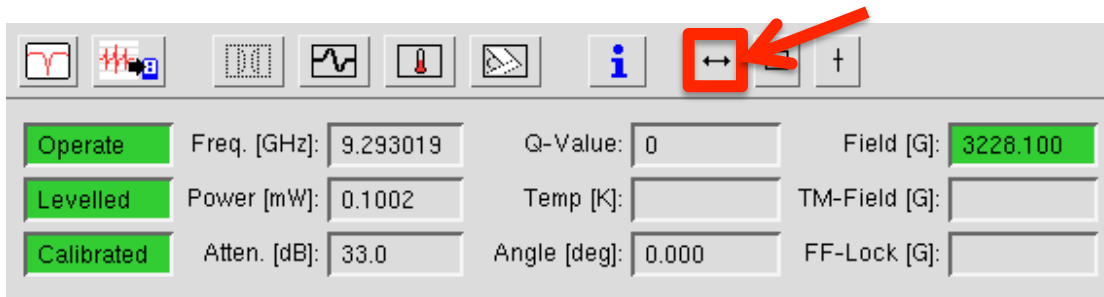


## E. Optimize parameters

1. Further edit your experiment parameters under the “Field Sweep” tab and “Options” tab as needed and re-run if necessary

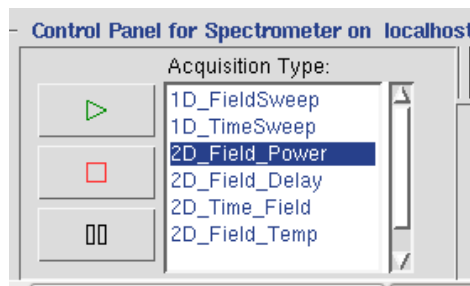
### 2. Center Field and Sweep Width

- Use the “Sweep Tool” to automatically find the optimum center field and sweep width
- Left click and hold over the center point to move the center. Right click and hold on one of the ends of the width line to move the width wider or narrower.
- The new center and new width are automatically updated.

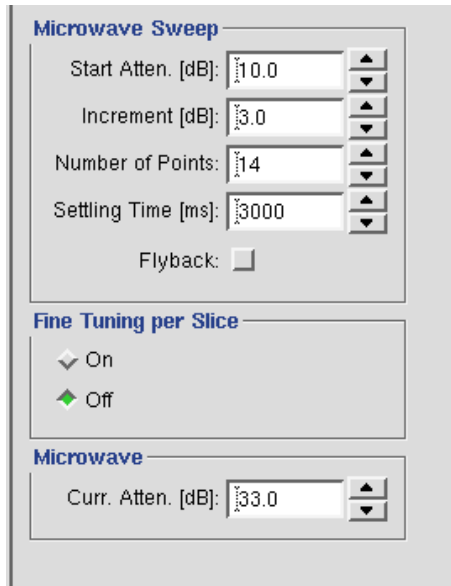


### 3. Microwave Power

- A MW attenuation of 0 dB will be your highest power of 200 mW.
- Optional: run a power sweep to determine MW saturation.
  - Choose “2D\_Field\_Sweep” from the control panel in the bottom left corner. You will then see a task bar associated with the power sweep on the left side of the window.



- Fill the corresponding parameters out to your liking...

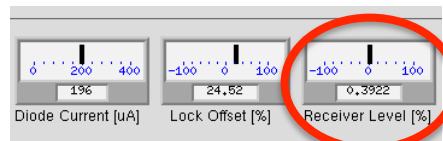


(3) Click 

(4) Scroll through your power swept spectra and choose a MW power that gives you a spectrum which is just below the maximum.

#### 4. Adjust the receiver gain accordingly

- a) The lowest receiver gain is 0dB and the highest is 74dB
- b) If the receiver gain meter moves more than  $\frac{1}{4}$  of its total width, you should decrease your receiver gain.

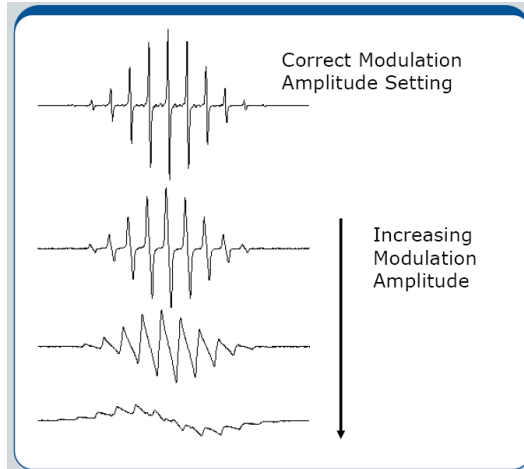


#### 5. Modulation Amplitude

- a) The correct modulation amplitude will help decrease *low* frequency noise (i.e. thermal fluctuations, electronic vibrations, drafts in the room, etc.)
- b) It is a compromise between sensitivity and resolution
- c) The default modulation amplitude will always work well because it has been set and tested during the creation of the calibration file for the current probe.

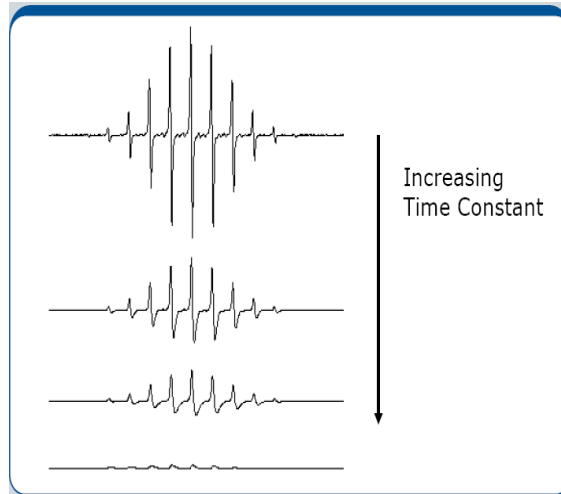


- d) The optimum modulation amplitude is somewhat sample dependent. The more hyperfine splitting, the smaller the value should be.
- e) If the modulation amplitude is too small → S/N will be poor
- f) If the modulation amplitude is too large → Signal will distort/broaden



## 6. Time Constant

- a) The time constant filters our **high** frequency noise (slows down the response time of the spectrometer)
- b) If time constant is too low → signal will be more noisy
- c) If the time constant is too high → signal will be filtered out
- d) Ideal to increase time constant for weak signals. If a long time constant is used, make sure to use a slower scan rate.
- e) For very narrow lines increase the time constant by TWO and increase the number of data points (resolution in X)

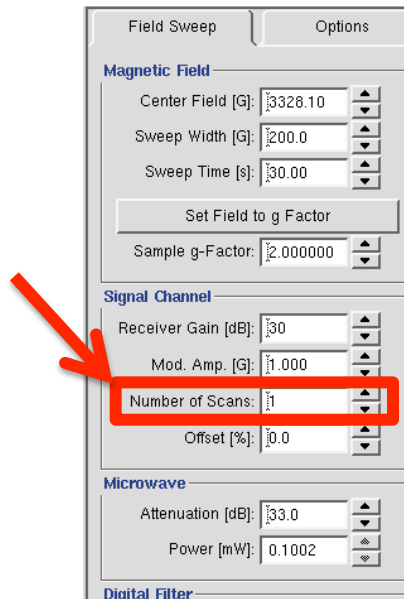


**7. Conversion Time**

a) The conversion time is the amount of time the AC to DC converter spends at each point. Rule of thumb → set conversion time = time constant

**8. Number of Scans**

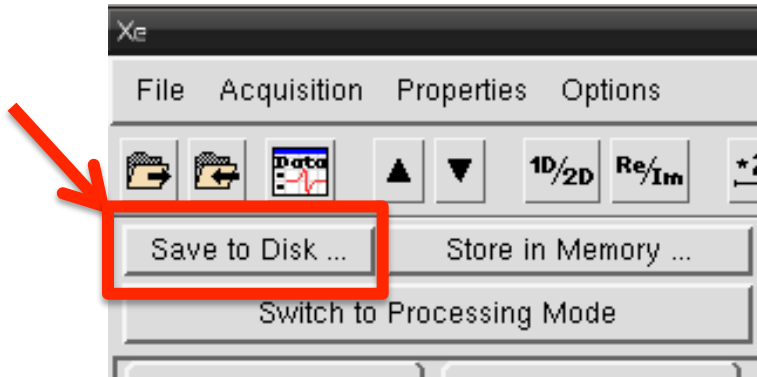
a) The number of scans can be changed under the “Field Sweep” tab



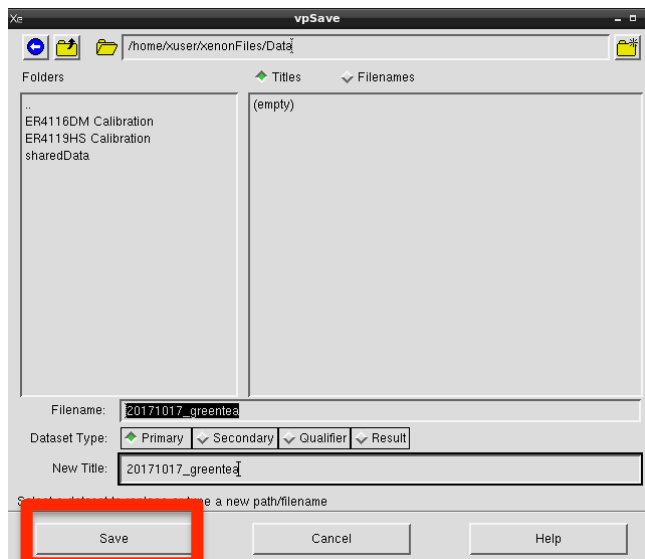
b) Signal to noise ratio will increase with increased number of scans.  $\frac{S}{N} \propto (N.S.)^{\frac{1}{2}}$

## F. Saving the data

1. **ON THE DESKTOP...Navigate to your EPR folder /home/<username>/xenonfiles/data/ and then create a new folder for each sample**
2. **Click “Save to Disk”**



3. **Navigate to your newly created folder**
4. **Once you have created a new folder for the corresponding sample, then fill out the “Filename” for that same sample**
  - a) Fill out the “Filename” box
  - b) Erase the text in “New Title” box
  - c) Highlight the text in the “Filename” box
  - d) Then hover your mouse over the empty “New Title” box and click the center mouse scroll button to paste it “Filename” in the “New Title” box.

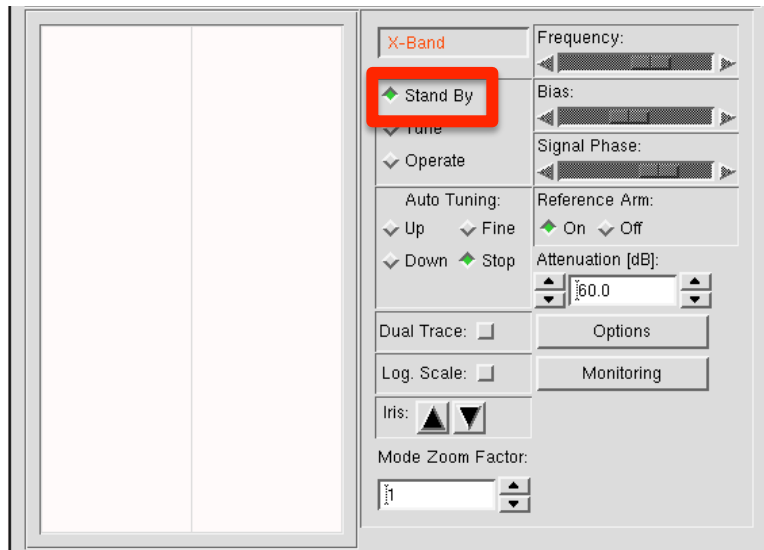


5. Then click “Save”

**G. Run background/empty cavity**

1. Switch back to “Acquisition Mode”

2. Before changing samples...open the microwave tuning window and put the system in “Standby” mode



3. Take your sample out and re-tune the cavity following the steps in Section B

4. Repeat Section D to run a background spectrum.

- a) If you see any background signal, please notify Shamon.
- b) If you just see noise, you are good to go!

## **H. Exporting Data**

### **1. Export Data as ACSII file**

a) File → EXPORT ASCII... → Choose desired file name and location → Click “Save”

### **2. Export Data as ESP file**

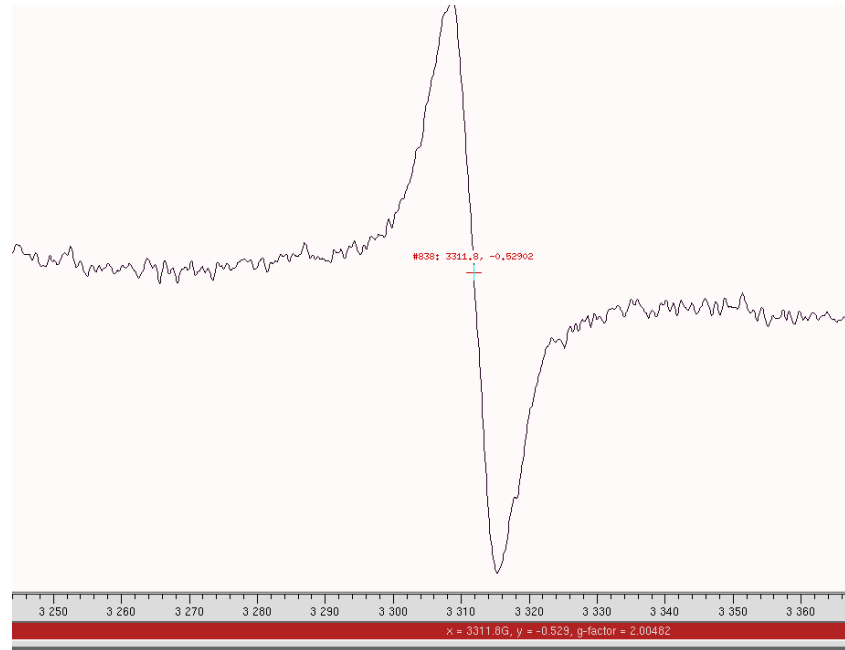
a) File → EXPORT ESP... → Choose desired file name and location → Click “Save”

### III. Processing

#### A. Zoom and Navigation Tools

##### 1. Mouse features

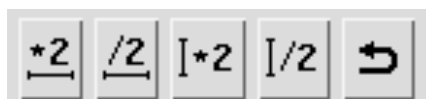
- a) Left-click and hold on the EPR signal line to see exact x,y coordinates and g-factor (this is displayed at the bottom of the spectrum)



- b) Left-click, hold, and drag across the screen to create a zoom window
- c) Right-click, hold, and move the mouse up/down or left/right to expand/contract the spectrum window in the vertical or horizontal directions, respectively.
- d) Click and hold the middle scroll button on the mouse to move the entire spectrum in any direction you move your mouse

##### 2. Tool Bar Features

- a) There are vertical and horizontal zoom buttons at the top of the software that look as follows:



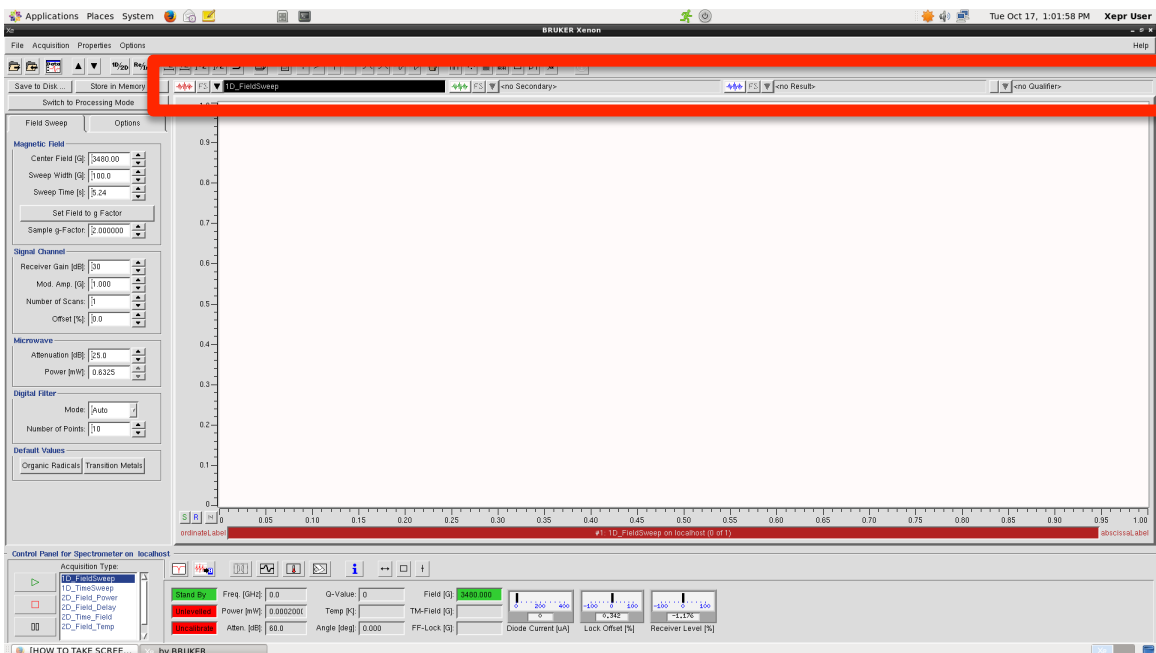
b) The \*2 and /2 buttons will expand or contract your spectrum by a multiple of TWO, respectively. The horizontal and vertical lines indicate in what direction you will expand and contract.



c) The button steps backwards to your previous action.

## B. Understanding DATASETS

1. There are FOUR available datasets on the Xenon software.

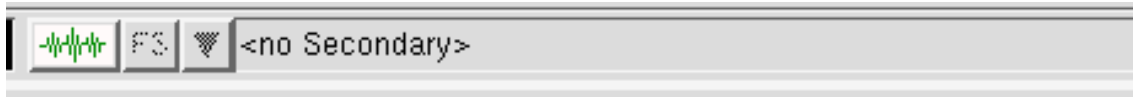


### PRIMARY DATASET



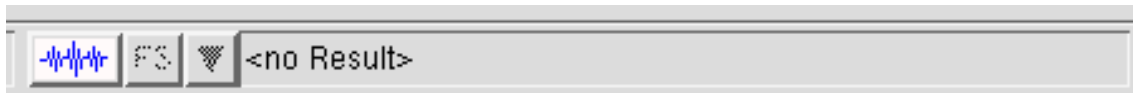
\*\*This is the Primary data set where your loaded spectrum will go automatically. The drop down arrow will let you quickly choose from files to load into the primary dataset that have been stored in “Memory”.

## SECONDARY DATASET



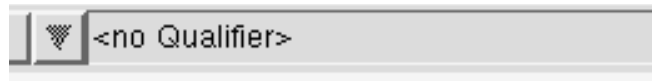
\*\*This is the Secondary dataset. This is not used frequently but can be useful when overlaying two spectra on top of each other. The drop down arrow will let you quickly choose from files to load into the secondary dataset that have been stored in “Memory”.

## RESULT DATASET



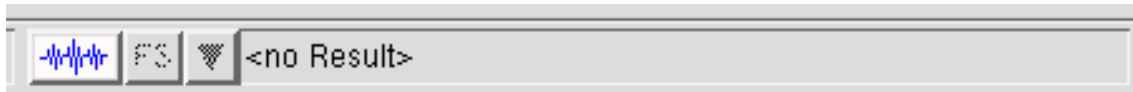
\*\*This is the Result dataset. This dataset will be automatically filled when you have completed a processing step that generates a result such as a double integral, or peak picking values.


## QUALIFIER DATASET



\*\*This is the Qualifier dataset. This dataset will be automatically filled when you are choosing a “qualifier” such as integral regions, baseline region, etc.

## 2. Removing Datasets from view

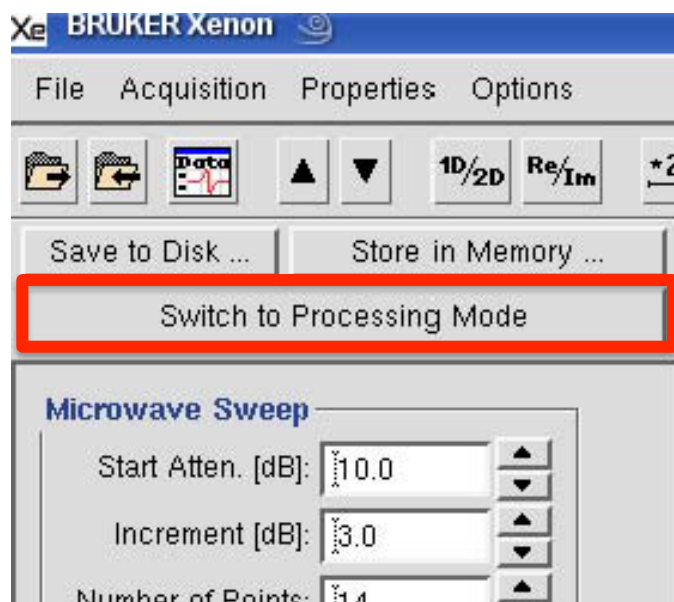


\*\*To clear any of the datasets you do not want to see over-layed on your spectrum, you simply click the black arrow  and choose “<no Result>” or “<no Qualifier>”, etc.



## C. Processing Mode

1. Click “Switch to Processing Mode” near the left Task Bar



2. Numerous processing options are displayed on the Task Bar

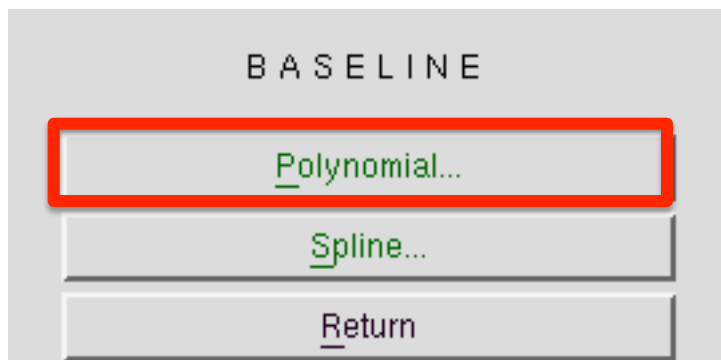


## D. Baseline Correction

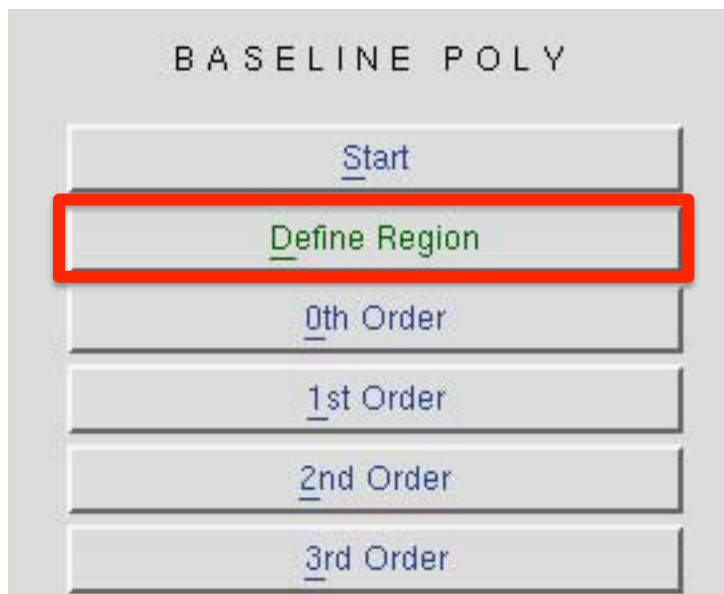
1. Load a spectrum (automatically loaded in your “Primary” Dataset)
2. Click “Baseline Correction ...” from the Processing Task Bar



3. Choose “Polynomial” from the Task Bar

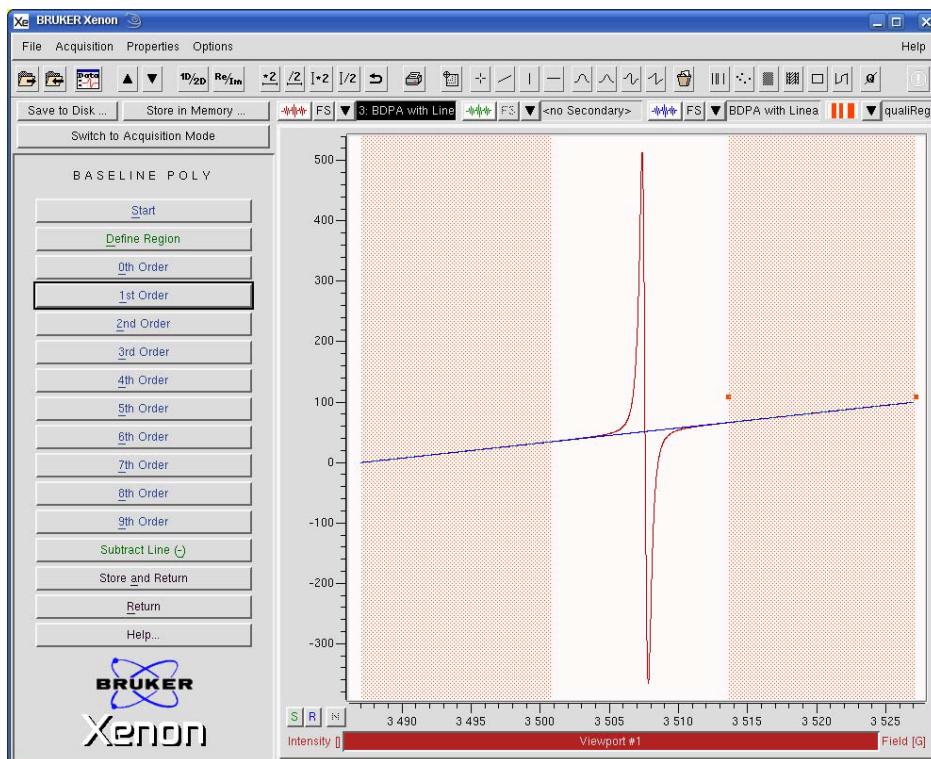


4. Define the baseline region by clicking “Define Region”



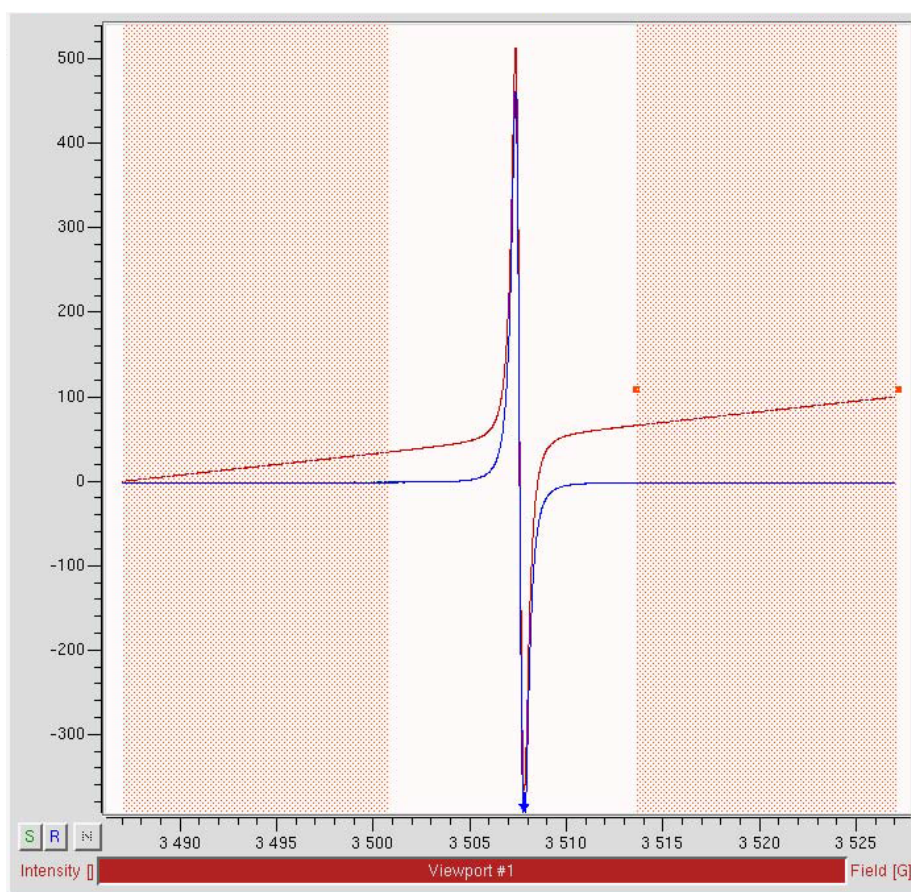
- a) Click “Define Region”
- b) Click and drag the cursor to define the region(s) as the baseline
- c) If you need to modify the region DO NOT CLICK on the screen again. Go to the Task Bar and choose “Change Region”

## 5. Fit a polynomial



- a) Choose one of the Nth Order buttons. In most cases the 1<sup>st</sup> Order button works well.
- b) The fitted polynomial then appears in the “Result” dataset and is the blue trace in the viewport
- c) If your first choice is not satisfactory, you can try again by choosing a another order polynomial
- d) The “Define Region” button switches to “Modify Region” after you define a region and you can click and drag to modify if necessary

## 6. Subtract the baseline

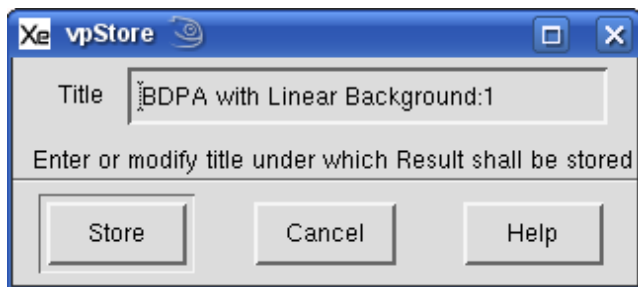


- a) Click “Subtract Line” in Task Bar
- b) The baseline corrected spectrum then appears in the “Result” dataset

## 7. Store the baseline-corrected dataset in memory if desired

- a) Click “Store and Return” from the left Task Bar.

b) Then put in a title and click “Store”



c) This stores the new baseline-corrected data in the “Primary” dataset

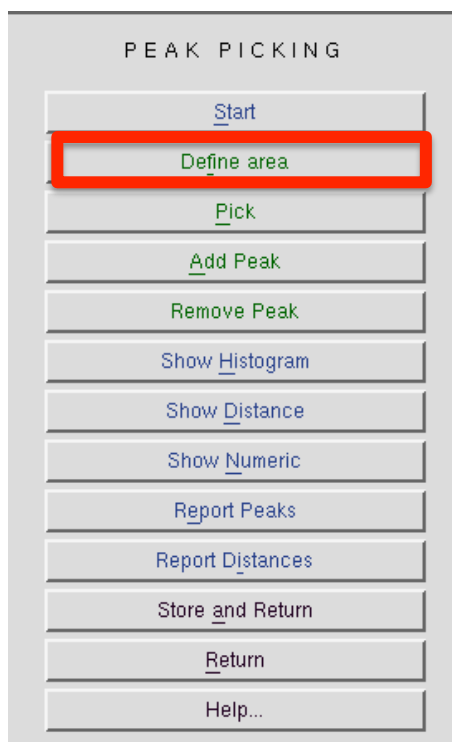
d) If you click “Return” from the left Task Bar, then the baseline corrected data remains in the “Results” dataset and the original spectrum in the “Primary” dataset

## E. Peak picking

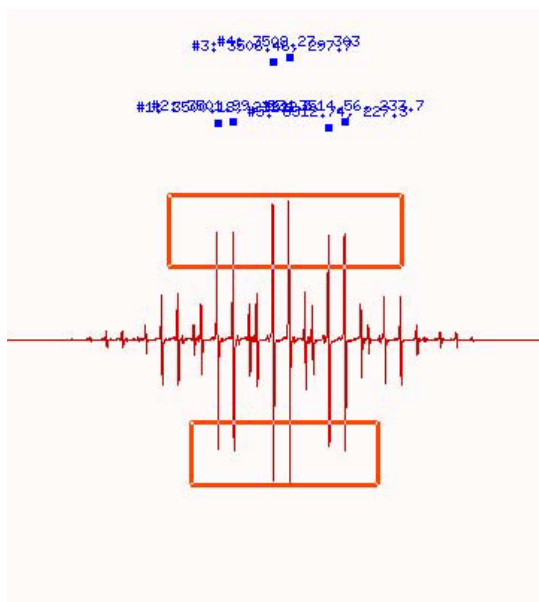
1. Load a spectrum
2. Click “Peak Picking...” from the left Task Bar



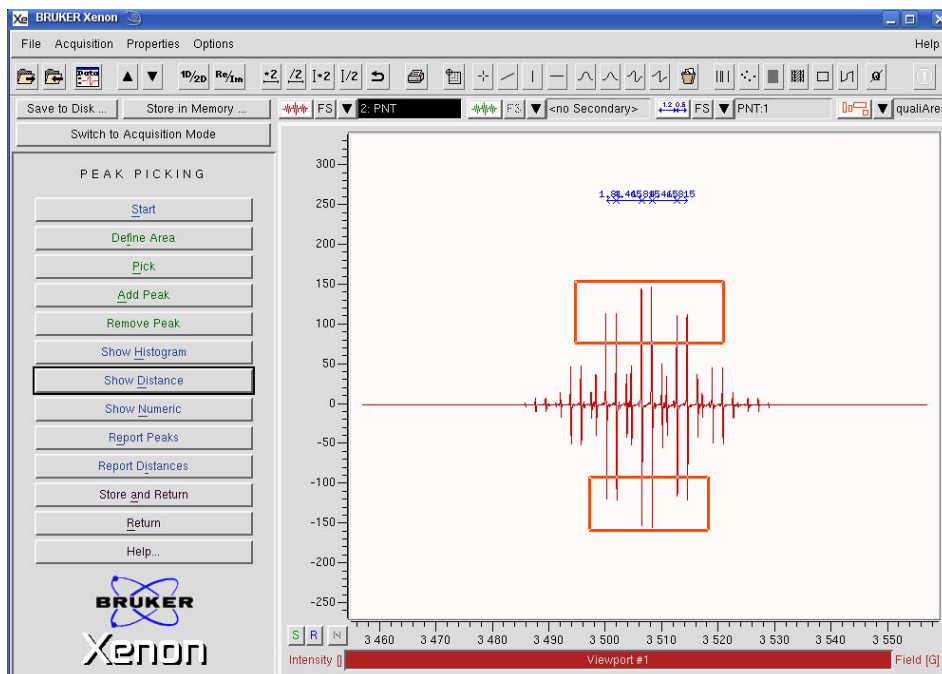
3. Click “Define Area” from the left Task Bar



4. Click and drag on spectrum to choose peaks (make sure to choose "Derivative" as the type)



5. Add Peak or Remove Peak
  - a) This allows you to manually click to add (or remove) peaks anywhere you'd like.
6. Click "Show Distance"



a) This shows the distance between peaks in the selected area.

7. **View the “Peaks” report**

8. **View the “Distances” report**

9. **Store the peak picking results in memory if desired**

a) Click “Store and Return” from the left Task Bar.

b) Then put in a title and click “Store”

## F. Integration

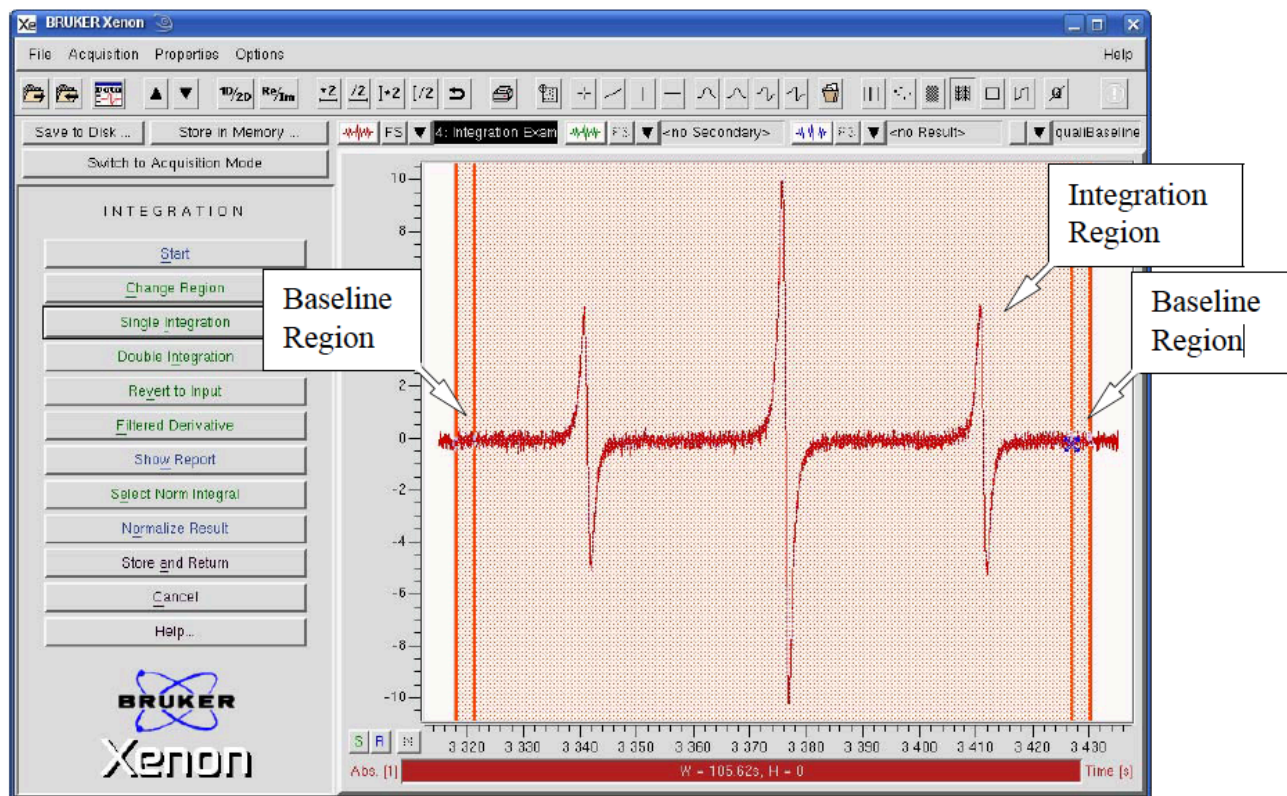
1. **Load the spectrum**

2. **NOTE: Offsets and a bad baseline can significantly skew integration results**

3. **Click “Integration and Derivative...” from the left Task Bar**



**4. Define the baseline and integration region by clicking and dragging on the spectrum**

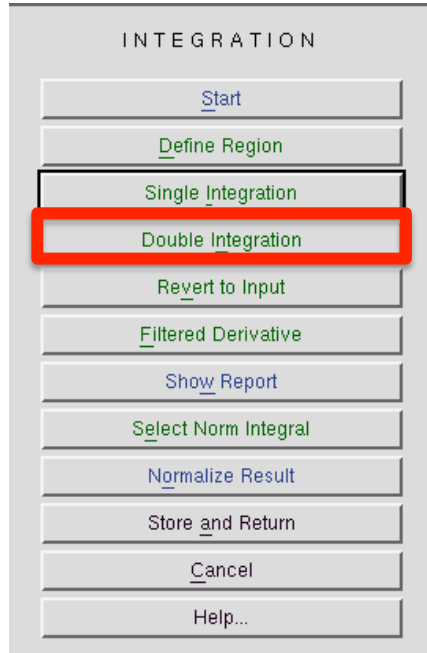


a) Repeat this to define and integrate multiple peaks

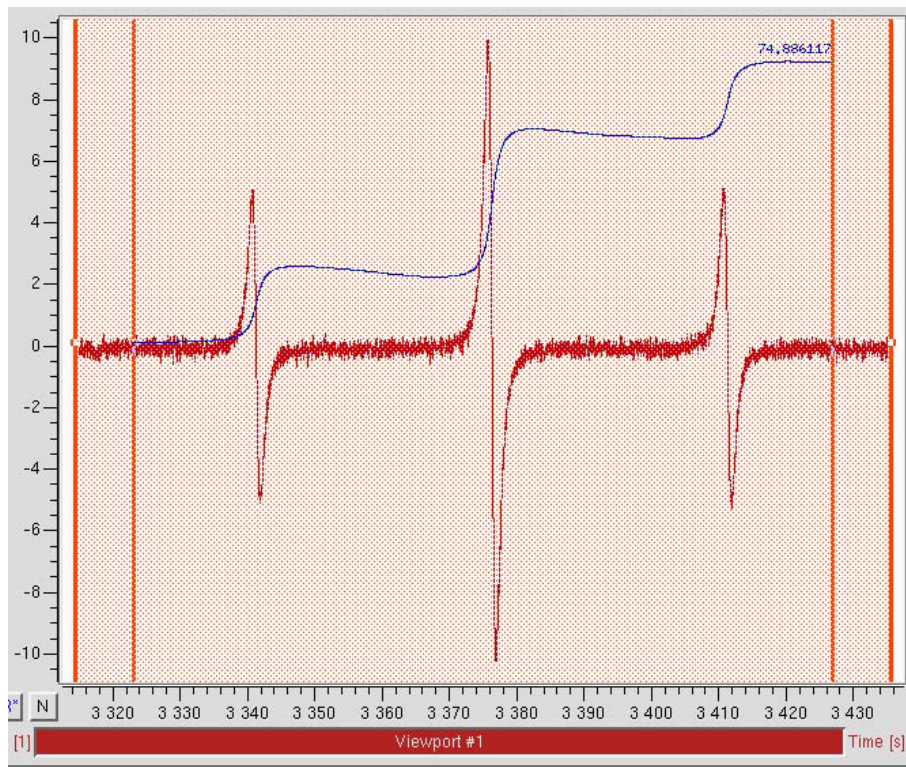


b) Click “Change Region” to modify the baseline and integration regions until you are satisfied.

**5. Choose “Double Integration” from the Task Bar to begin integration**



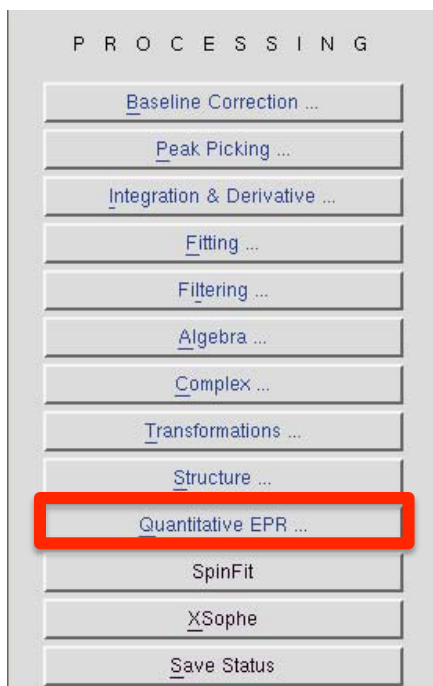
**6. Double integral results are displayed in the “Result” dataset**



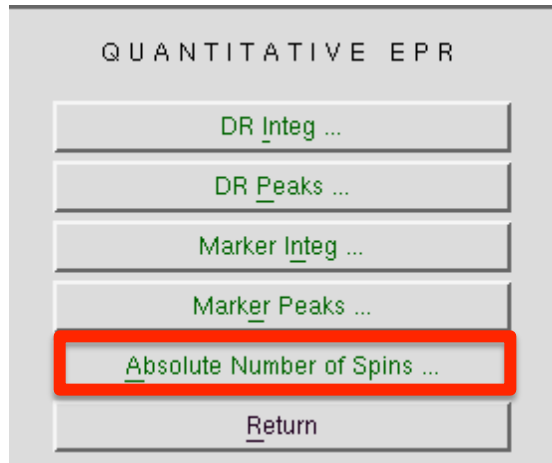
7. **Normalize integrals to one peak**
  - a) Click “Select Norm Integral” from the task bar and then click “Normalize Result”
  - b) Click the up or down arrow to select the integral you want to normalize, then click “Normalize” from the task bar
8. **Inspect and save integral values**
  - a) Click “Show Report” to see a list of all integrals
9. **Store the integration result(s) by clicking “Store and Return”**

## G. Quantitative EPR

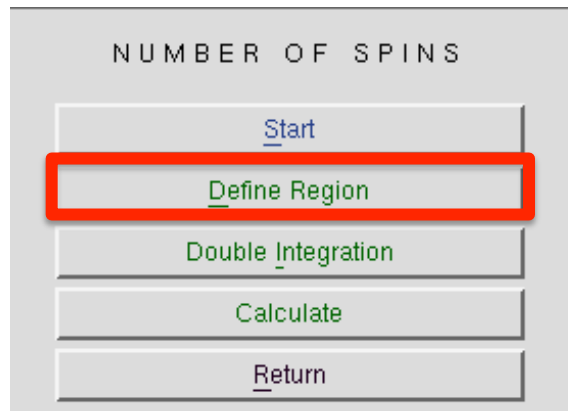
1. **NOTE: YOU MUST KNOW YOUR Q-value (see section II.B.3.b) and have the microwave power set sufficiently low such that the EPR signal is not saturated. Finally the sample must be centered in the resonator.**
2. **Load the spectrum**
3. **Click “Quantitative EPR...” from the left Task Bar**



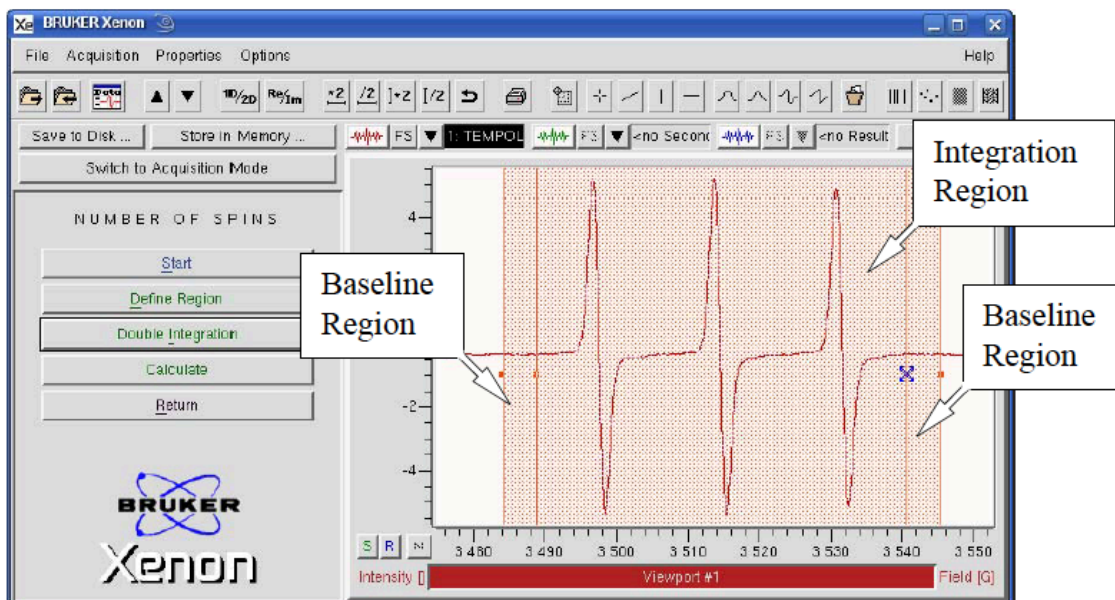
4. **Click “Absolute Number of Spins...” or “Spin Count”**



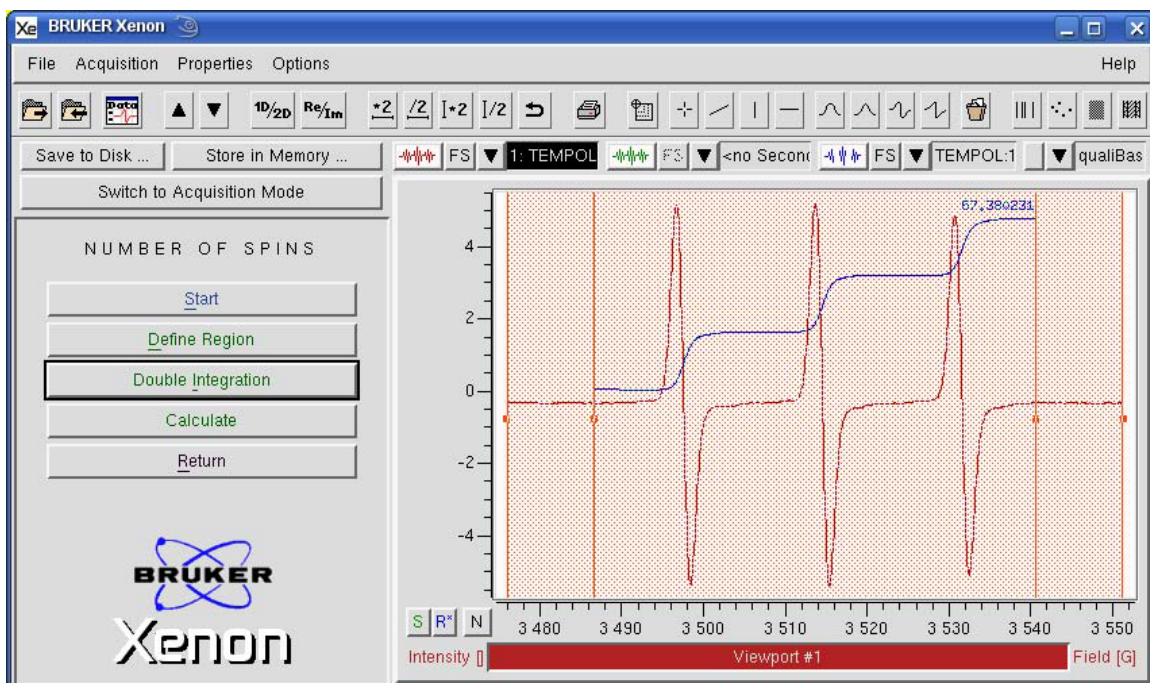
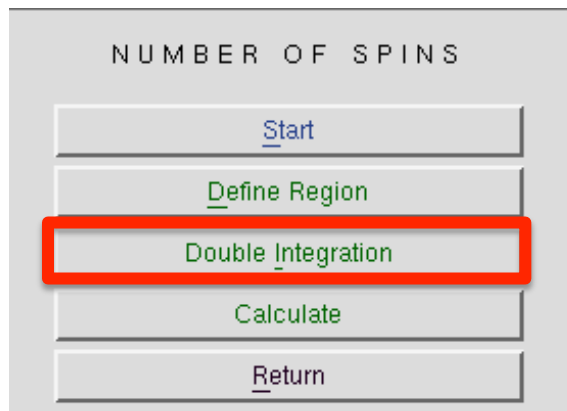
5. Click “Define Region”



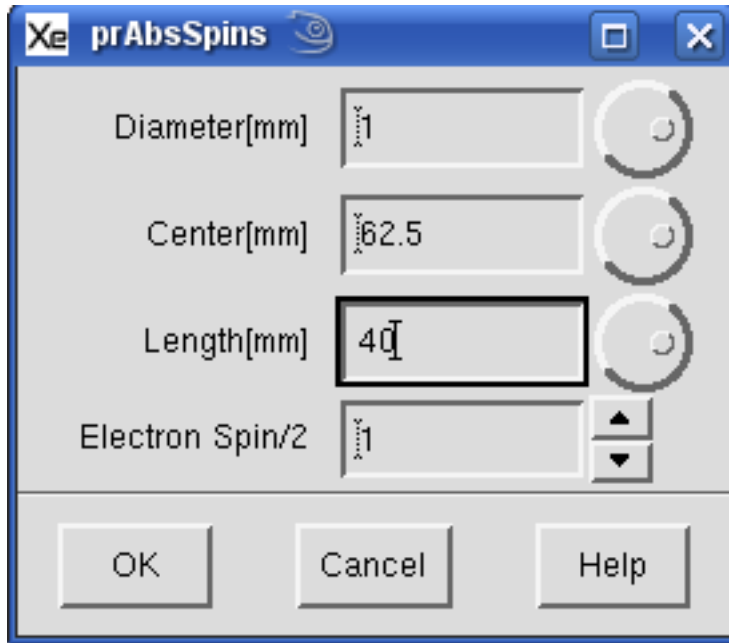
a) Click and drag on the spectrum to define the baseline and integration regions



6. Click “Double Integration”



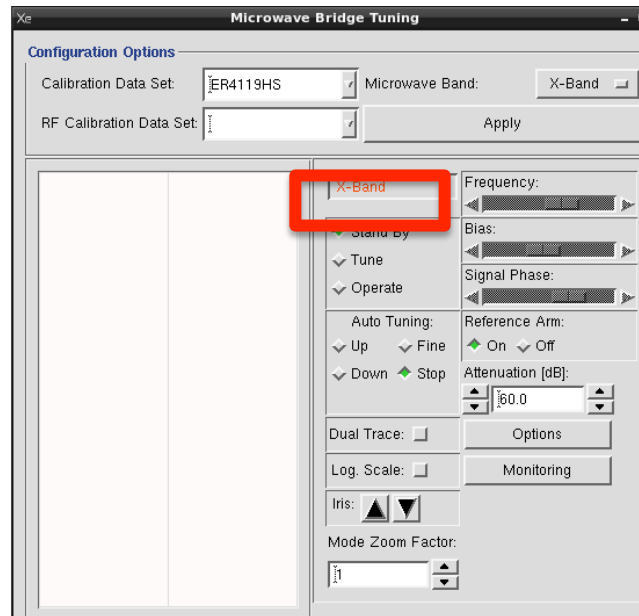
7. Click “Calculate”



- a) A new window appears and you fill out the corresponding information
  - b) “Diameter[mm]” of the sample is essentially the inner diameter of the tube
  - c) “Center[mm]” is the distance from the center of the sample to the top collet. This is 62.5 mm for the single mode cavity (black rectangle).
  - d) “Length[mm]” is the length of your sample inside your tube (not your tube length).
  - e) “Electron Spin/2” is the spin of the paramagnetic source i.e. a value of “1” corresponds to a spin  $\frac{1}{2}$  paramagnetic source.
8. **Click “OK” to view the results**
  9. **Save the values**

## IV. Finishing Up

### A. Change to “Standby” mode in tuning window



### B. Remove sample if present

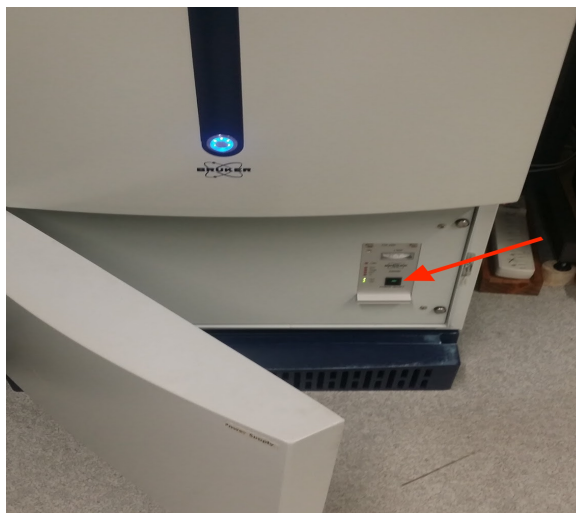
### C. Close the tuning window

### D. Close the Xenon software

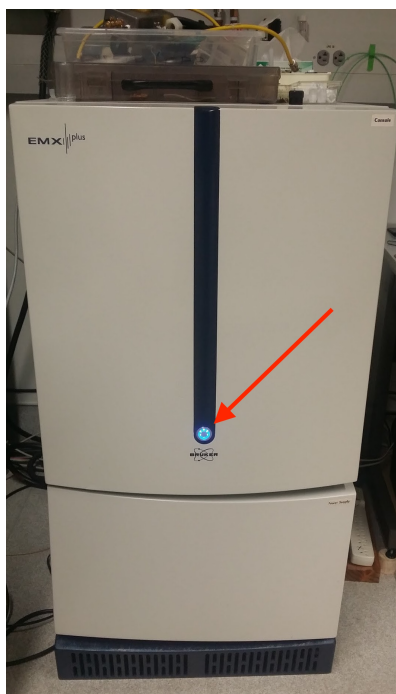
### E. Choose whether to save all datasets or not

### F. Log-off the computer

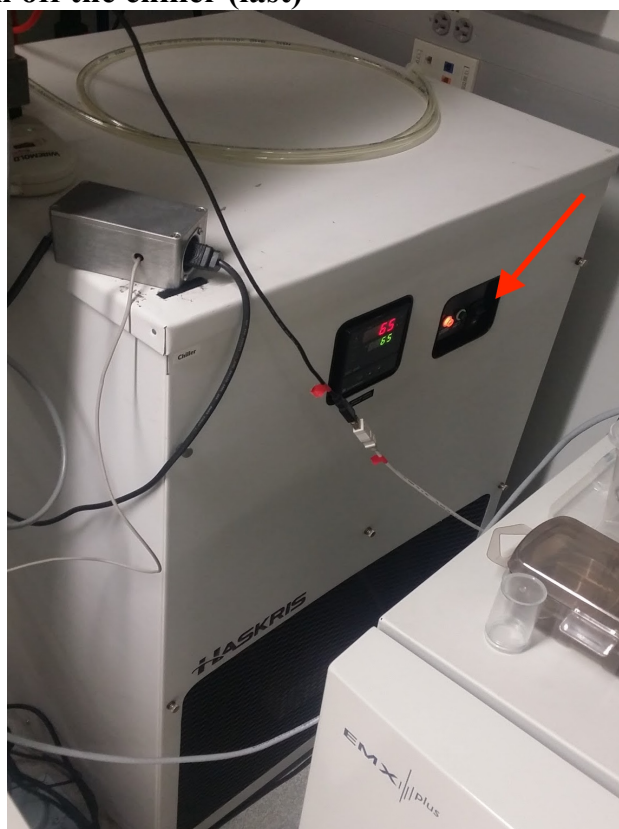
### G. Turn off the power supply



**H. Turn off the console**



**I. Turn off the chiller (last)**



**J. Fill out the log-sheet**

**K. Stop your FBS timer** 



## V. Appendix

### A. Manual Tuning

1. Adjust the *Signal Phase* slider to make dip as symmetric as possible. Adjust the frequency if necessary.
2. Go to *Operate* mode (wait, it takes ~ 10 secs). Increase the attenuation to 50 dB.
3. Adjust the *Bias* slider so the diode current meter is centered.
4. Lower the attenuation down to 30 dB and adjust the diode current back to the center by clicking and holding down the Iris up/down arrows . Center the AFC as necessary by using the Frequency slider. Repeat for 20 dB and then 10 dB, or the lowest desired (i.e. 2 dB for the Power Sweep). At 2 dB the diode takes a long time to stabilize and tends to drift.
5. Adjust the attenuation back up to 50 dB in 10 dB steps while watching the diode current meter. If the meter moves, then proceed to Step 7. If it does not move, then the sample is tuned and the experiment is ready to be run, proceed to collecting a spectrum.
6. Adjust the attenuation back down to 10 dB. Next, adjust the *Signal Phase slider* until a local maximum on the diode current meter is reached (i.e. until the meter is as far to the right as possible). Using the *Iris up/down arrows*, adjust the diode meter back to the center. Repeat Step (6). The diode meter should stay centered and not move.
7. Close microwave tuning window
8. C

### B. B

1. A
2. B
3. C

### C. C

1. A
2. B
3. C
- 4.