University of Ottawa, Faculty of Science: NMR Facility
Standard Operating Procedure: EPR-001

I. General information:

Procedure Name	**Acquiring EPR spectra on the EMX Plus EPR spectrometer**
SOP No.	EPR-001
Audience	All users of the EPR spectrometer
Version	Version 2
Author	Volodymyr Semeniuchenko, NMR lab technician
Date	July 12, 2024

II. Applicable to instrument:

Manufacturer	Bruker	**Magnetic Field Strength**	1.7 T
Instrument	EMX Plus	**^{1}H Frequency**	not applicable
Location	STEM0028	**Serial Number**	EMP2111
Type	Solids and liquids		

III. Emergency contacts information:

Primary Emergency Contact	Patrick Szell, NMR Lab Manager 613-562-5800 ext 6077 pszell@uottawa.ca	**Secondary Emergency Contact**	Volodymyr Semeniuchenko, NMR Lab Technician 613-562-5800 ext 6078 vsemeniu@uottawa.ca

IV. Standard Operating Procedure Information:

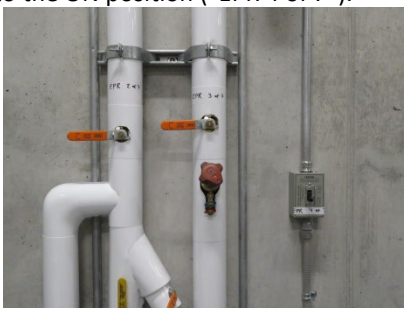
Purpose	The purpose of this standard operating procedure (SOP) is to provide users with the procedure on how to operate the EMX Plus EPR spectrometer located in STEM0028.
Scope	None.
Responsibilities	All users are responsible for their safety and the safety of others in the laboratory as set out by the office of risk management.
Hazards	**Cryogens**: The NMR lab contains liquid nitrogen (77 K, -196°C) and liquid helium dewars (4.2 K, -269°C) which can cause severe burns. Always wear safety glasses or a face shield, insulated gloves, and protective clothing when working with cryogens. These dewars release gas periodically as part of an overpressure protection. **Compressed gasses**: The NMR lab contains cylinders of compressed helium and is equipped with compressed air lines. Use caution when handling compressed gasses. **Asphyxiates**: The NMR spectrometers contain large quantities of cryogens. In the event of a magnet quench, large volumes of helium and nitrogen will be released from the NMR spectrometer, displacing the oxygen in the room. Immediately evacuate the room in the event of a magnet quench. **Chemicals**: The NMR lab may contains hazardous chemicals, and lab bench surfaces may be contaminated. Use gloves when handling chemicals or touching the lab bench. **Magnetic field**: The NMR lab operates high magnetic field instruments (Avance III 400: 9.4 T, Avance IIIHD 500: 11.7 T). Users with pacemakers and metallic implants should not enter the laboratory. Remove any metallic and/or magnetic objects (credit cards, keys, etc…) from your pockets before entering the 5-gauss line (denoted by the yellow chain perimeter). **RF field**: The NMR spectrometers uses high radiofrequency fields as part of the NMR experiment.
Location of documentation.	There is currently no central location for the documentation in STEM0028.
Version control	This SOP is subject to version control. In the event that a new version is released, users will be notified by email.

Standard Procedure

1. Switch on the EPR spectrometer:

1.1 On the wall to the left of the EPR spectrometer: Switch the mains supply ON by lifting the handle upwards all the way to the ON position. The mains supply box is marked “EPR 1 of 7”.

1.2 Located on the wall behind the EPR spectrometer: Start the chilled water flow by turning both valves to be parallel with the pipe for the chilled water supply (marked “EPR 2 of 7”) and chilled water return (“EPR 3 of 7”). Turn on the water flow meter be flipping the switch to the ON position (“EPR 4 of 7”).



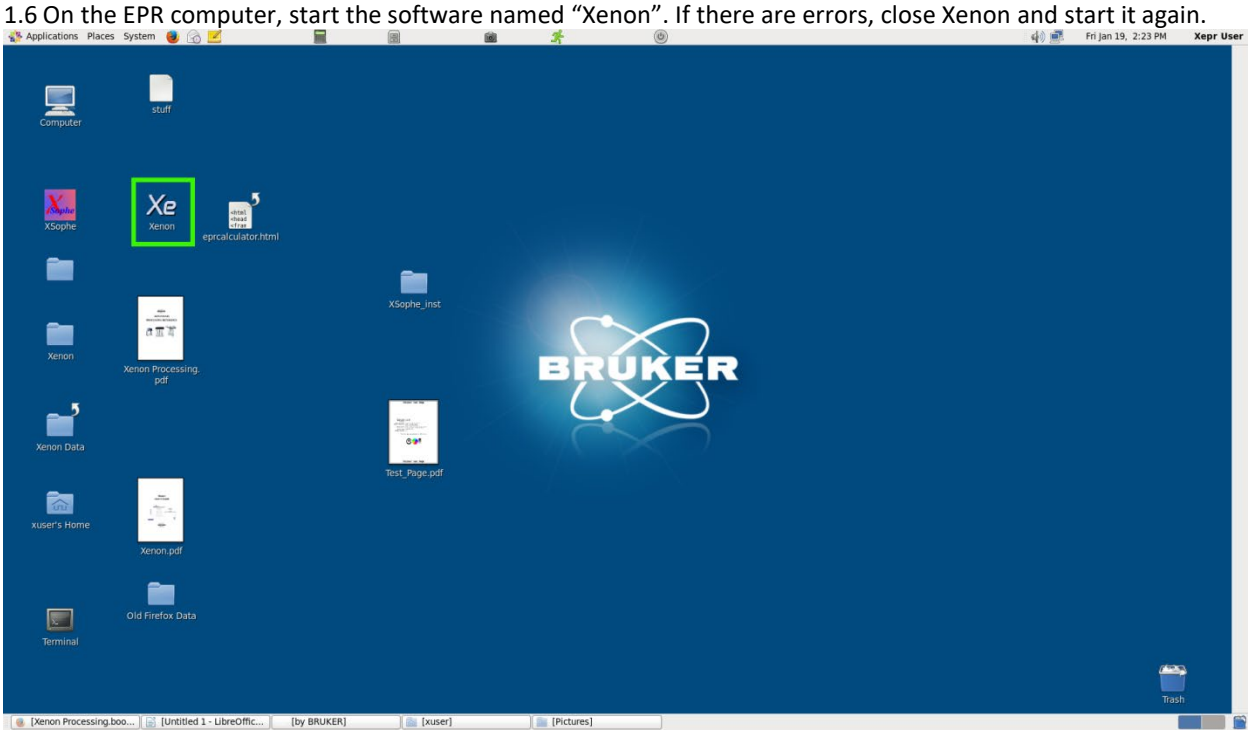
1.3 Turn on the magnet chiller (marked “EPR 5 of 7”) by flipping the switch to the ON (I) position and wait a few seconds until it has booted and shows the temperature. The temperature will initially read 69 °F, and will drop to 65 °F gradually.

1.4 If the temperature on the chiller is below 70 °F, turn on the magnet power supply by flipping the switch to the ON position (I), marked “EPR 6 of 7”.

1.5 Switch on the EPR console by pressing the round button marked “EPR 7 of 7”. The LED light should turn on shortly.

Wait until the bridge is booted, blue LED should shine constantly, without blinking (takes 30 sec approx).

1.6 On the EPR computer, start the software named “Xenon”. If there are errors, close Xenon and start it again.



2. Insert the sample, wipe it before insertion to avoid cavity contamination.

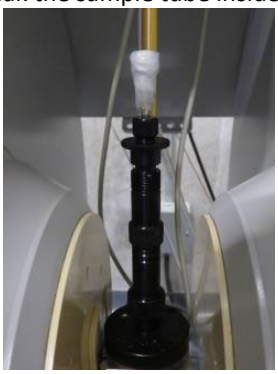
2.1 If liquid helium cryostat is installed, but it is at room temperature:

2.1.1.1 This procedure is applicable to 4 mm EPR tubes: If you have a sample in a capillary, place the sample in the capillary inside a 4 mm EPR tube. On the top of the EPR cryostat (green box in the below picture), unscrew the top

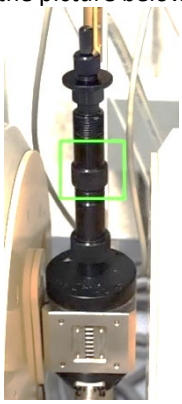
connector for the sample. Disassemble the removed parts into the plug (save for later), plastic screw, metal compression ring and o-ring. The pictures below show the top connector and cavity plug. The cavity plug is shown on the picture below on the right.

2.1.1.2 Insert the sample tube through the screw, compression ring and o-ring.

2.1.1.3 Insert the sample to the cryostat all the way down, tighten the screw. On insertion do not apply extensive force in order to not break the sample tube inside or damage the cryostat quartzware.



2.1.2.1 This procedure is for 3 mm EPR tubes: Be cautious to not break an expensive cryostat quartzware. Unscrew a part of cryostat, marked by the green box on the picture below.



2.1.2.2 Once opened, you will see a top of quartzware:

2.1.2.3 Insert a sample and let it stay loose inside. The height of sample cannot be adjusted.

2.2 If the liquid helium cryostat is cooled, refer to SOP of EPR sample cooling.

2.3 If the liquid helium cryostat is not installed (this procedure was taken from the Xenon documentation):

Take care if you are wearing an analog (mechanical) watch. The magnetic field in the air gap of the magnet is sufficiently strong to magnetize your watch! Therefore, to avoid damage to your watch, remove your watch before putting your hands in the magnet air gap.

4. **Remove the sample.** If there already is a sample in the resonator, remove it. Loosen the top collet nut (You do not need to remove the collet nut.) and carefully remove the sample from the resonator. Pulling the sample tube out as straight as possible prevents you from breaking the sample tube thereby destroying your valuable samples. (See Figure 3-12 and Figure 3-13 for details.) The ER 4119HS resonator is shown in the figures; however, the procedure of removing and inserting a sample is the same for other resonators.

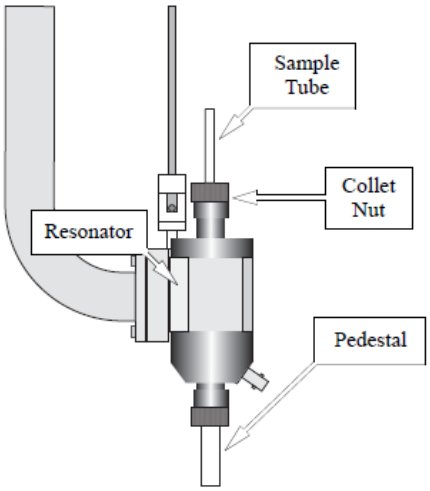


Figure 3-12 A Bruker ER 4119HS resonator.

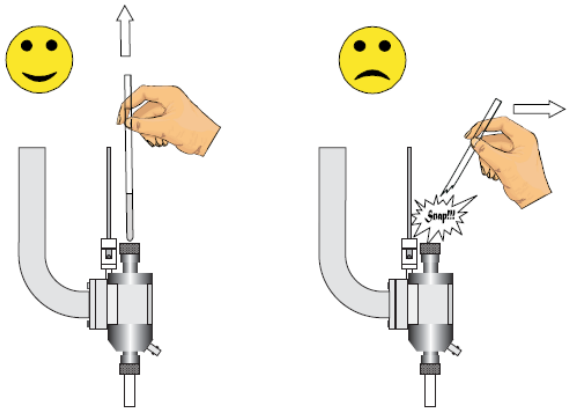


Figure 3-13 The right and wrong technique for removing a sample.

Be careful not to break your sample inside the EPR cavity.

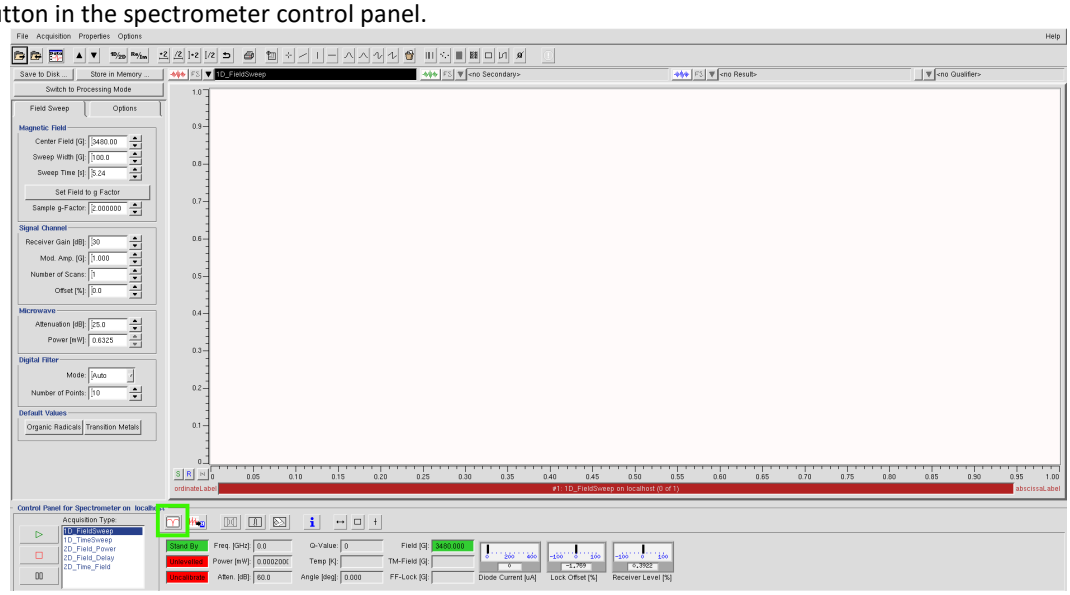
If this is your first time operating the spectrometer, we recommend that you use the strong pitch sample supplied with your instrument. Our instructions in this chapter are based on using this sample.

5. **Clean the sample tube to be inserted into the resonator.** It is vital to avoid contaminating the microwave resonator with paramagnetic contaminants that produce spurious EPR signals or distorted base lines. Wiping the outside of the sample tube with tissue paper is usually adequate.

6. **Insert the sample tube carefully into the resonator.** (See Figure 3-12 and Figure 3-13.) Make sure you have the appropriate collet size for your sample tube size. The tube should be slightly loose before you tighten the collet nut. The bottom of your sample should rest in the indentation on the pedestal. This ensures that your sample is centered horizontally. The height can be adjusted by loosening the bottom collet nut and moving the pedestal up and down. Tighten the top collet nut to firmly hold the sample tube in place and the bottom collet to firmly hold the pedestal.

3 Tune the cavity with sample.

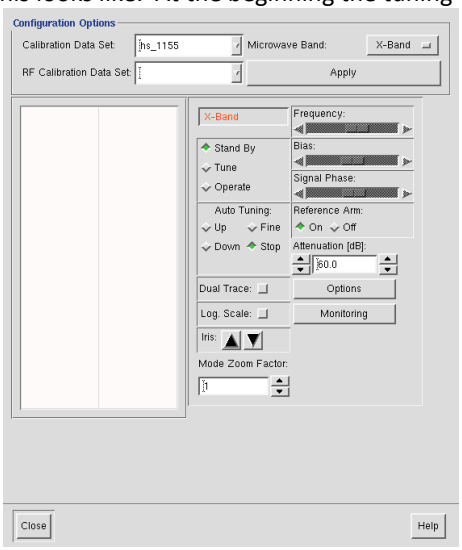
On the computer, in Xenon, open the *Microwave Bridge Tuning* dialog box (green box in the figure below). Click the Tuning button in the spectrometer control panel.



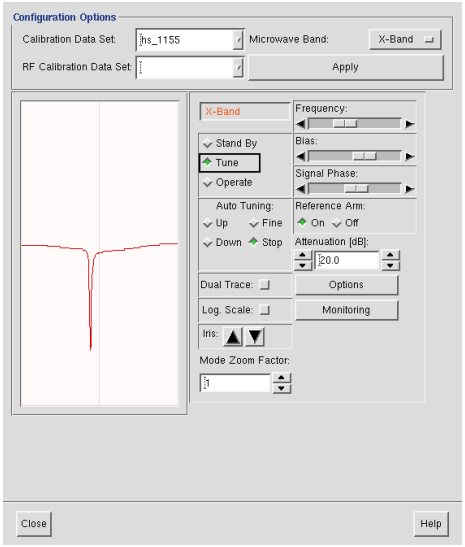
3.1 Follow the following procedure taken from the Xenon documentation (section 9.1 there). The parameters adjusted by sliders are changed by dragging a button of a slider, or by pressing the buttons with arrows ◄► on the left or right side of the slider. Pressing and holding the button will result in continuous action, as if multiple clicks are applied. The same pertains to Iris adjustment.

	EPR Manual Tuning Instructions	
	Open tuning dialog 	
Coarse Match	Click "Tune", **Reference Arm** off, **Attenuator** = 25 dB Adjust **frequency** bar to center the tuning dip Adjust **zoom factor** to maximize amplitude of the dip Adjust Iris to maximize the depth of the dip	Note: Reference arm off makes finding the resonator dip easier. There may be hysteresis. Click reference arm off and on twice. Make sure Bias is set to 0 or is inactive.
Intermediate Match	Attenuator = 30 dB, then click "**operate**" Change **frequency** bar to center **LOCK** offset Adjust **Iris** to minimize **Diode Current** (2-10 µA)	Note: Hardware bug might change frequency too much (below -50% or above 50%). If so, click "tune" again, make sure the peak is centered, and click "operate" again.
Intermediate Match	Decrease **Attenuator** by 1 dB, minimize **Diode Current** Repeat this step until **Attenuator** = 10 dB	
Adjust Bias	**Attenuator** = 60 dB, **Reference Arm** = ON adjust **Bias** slider until **Diode Current** = 200 µA	Note: there may be hysteresis. Click reference arm off and on twice.
Tune signal phase (coarse)	**Attenuator** = 33 dB, click "**Tune**" adjust **Signal phase** slider for good symmetric dip Center the dip by **frequency** slider if necessary	Note: after finished, the dip should not move when toggling reference arm on and off.
Fine Tune	Click "**operate**" Adjust **frequency** to center **Lock Offset**	Note: Hardware bug might change frequency too much (below -50% or above 50%). If so, click "tune" again, make sure the peak is centered, and click "operate" again.
Fine Match	Decrease **Attenuator** by 1 dB Adjust **Iris** for **Diode Current** = 200 µA repeat this step until **Attenuator** = 10 dB	Note: if necessary, adjust frequency to center lock offset, as you change microwave attenuation.
Tune signal phase (Fine)	Adjust **Signal phase** slider to maximize **Diode current**	Note: You should not have to adjust it very much.
Check Tuning	**Diode Current** should change little when **Attenuator** change from 10 to 50 dB. If not, repeat from **ADJUST BIAS** step	
	Click **Apply**	

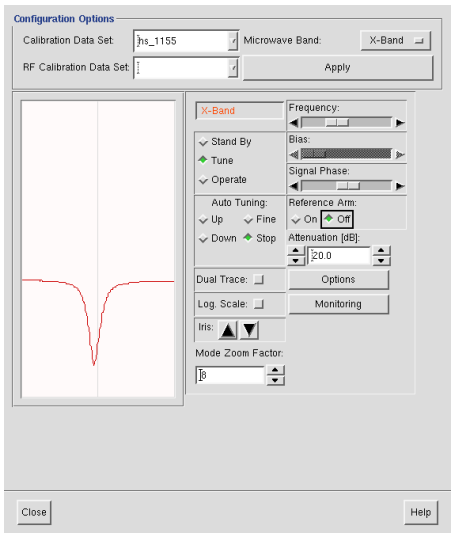
3.2 The following tuning procedure was taken from Bruker EPR tutorials (https://www.bruker.com/en/news-and-events/webinars/2021/Bruker-CW-EPR-User-Training-Course.html). It might fail for problematic samples. It is based on the procedure mentioned above, but is significantly simplified, and uses autotuning capabilities of Xenon software. Here is a step-by-step guide on what this looks like. At the beginning the tuning window looks like this:



3.2.1 Click “Tune”. Set the attenuator to 20 dB. If the peak is too intense, set it to 25 dB. The tuning window will look like this:

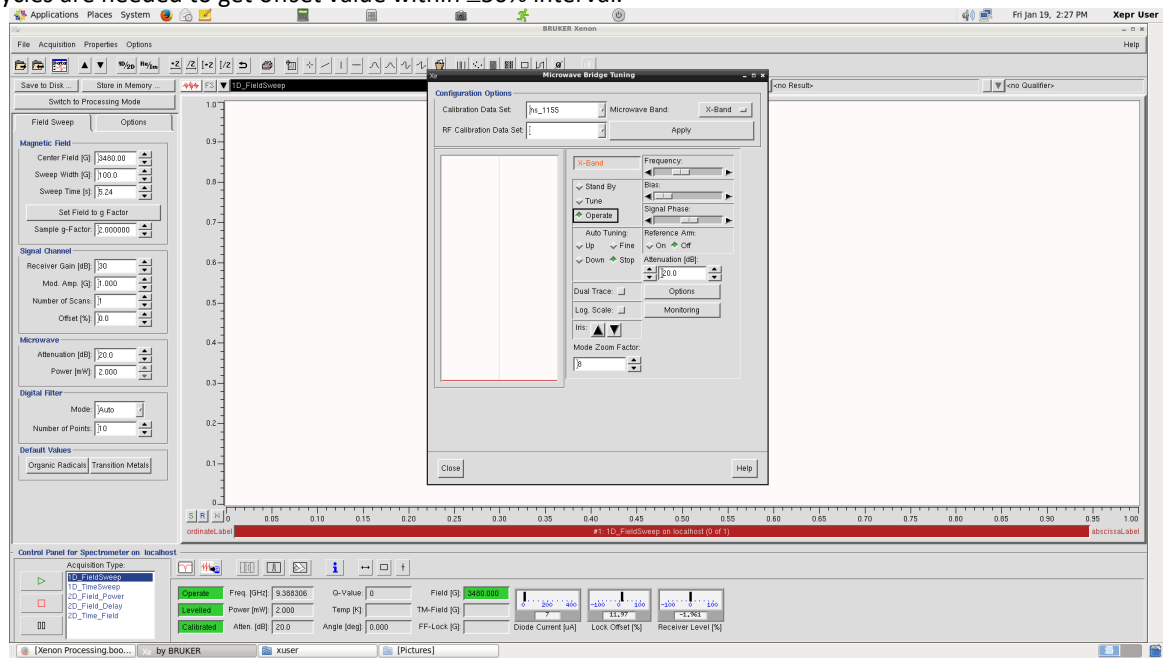


3.2.2 Switch off the reference arm, make sure the bias slider is either inactive or in the most left position, i.e. bias is not applied. Adjust the frequency slider to center the peak. Apply maximal zoom to center it better. Then adjust the Iris to make the peak as deep as possible; sometimes the iris should be more opened, sometimes more closed, this is hard to predict. At this stage do not try to adjust the phase of the peak. The tuning window will look like this:



If the peak is too shallow, it is a symptom of lossy sample (sample absorbing microwave radiation).

3.2.3. Click “operate”, **no need to change the attenuation**. Observe the value in “lock offset”. The hardware has a bug that sometimes after clicking “operate” frequency changes too much, and lock offset shows a value lower than -50% of higher than +50%. If so, click “tune” again, make sure the peak is centered, and click “operate” again. Sometimes a few cycles are needed to get offset value within ±50% interval.



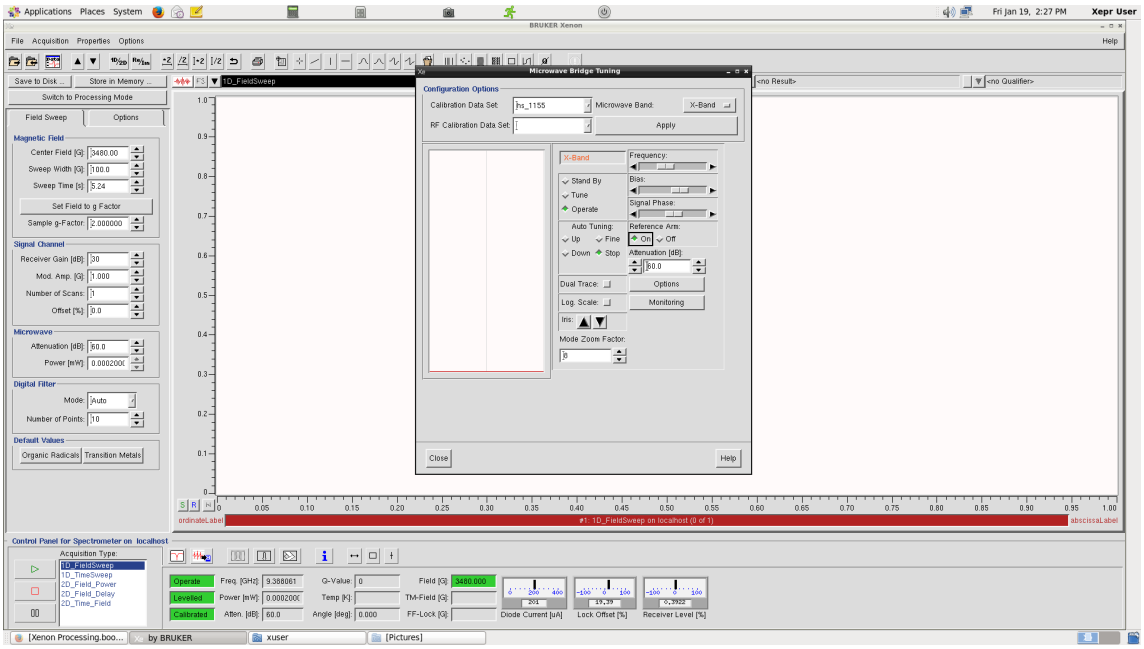
3.2.4 Adjust the frequency to get lock offset higher than -5% or lower than +5%. Adjust slowly, as it is very easy to overtune it.

3.2.5 Adjust the iris to minimize the diode current. Typically it is 6-7 mA. Also make sure that reference arm is off and bias slider is inactive or moved fully left.

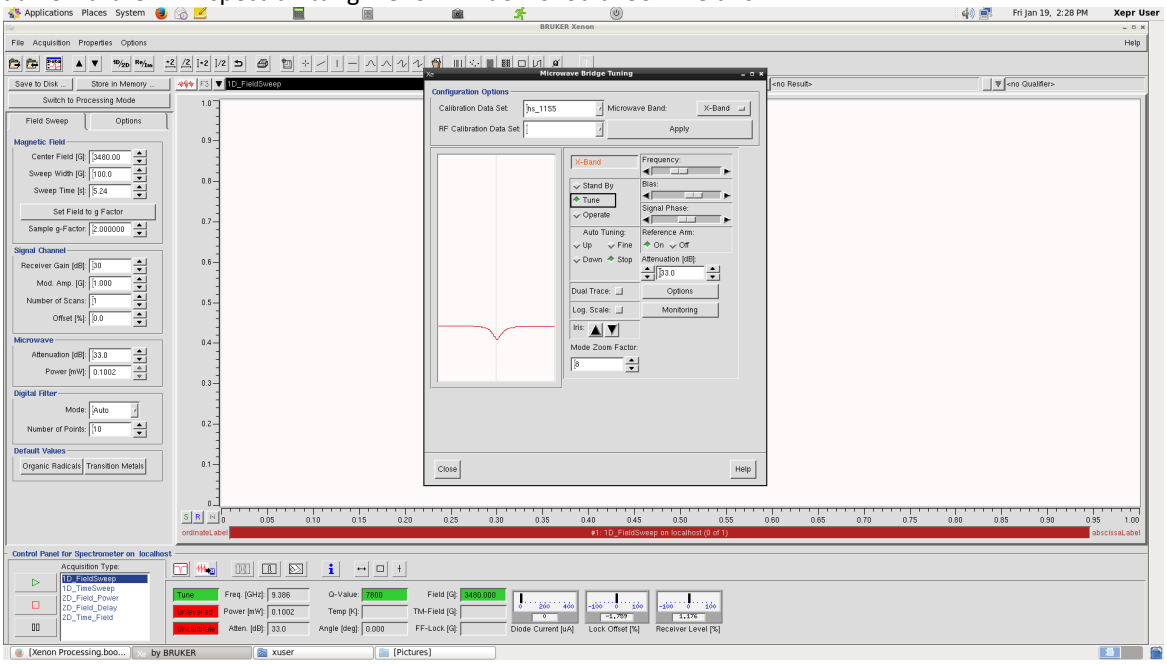
A high value of the diode current, which cannot be adjusted by iris, is a symptom of lossy sample, a value above 100 mA shows that your sample is definitely lossy. Iris can be fully closed, in that case diode current falls to 6-7 mA, but on minimal iris opening, for lossy samples, the diode current will jump to >100 mA value.

Set attenuation to 15 dB, adjust Iris to minimize Diode Current. Then set attenuation to 10 dB, adjust Iris to minimize Diode Current. If needed, adjust frequency to get lock offset higher than -5% or lower than +5%.

3.2.6 Set the attenuation to 60 dB, switch on reference arm. Because of hysteresis (or hardware bug) it might be not switched on after the 1st attempt. So switch it off, and immediately again on. After the 2nd switch on it must be active. Adjust the bias to make the diode current 200 mA. **Do not adjust the frequency.**

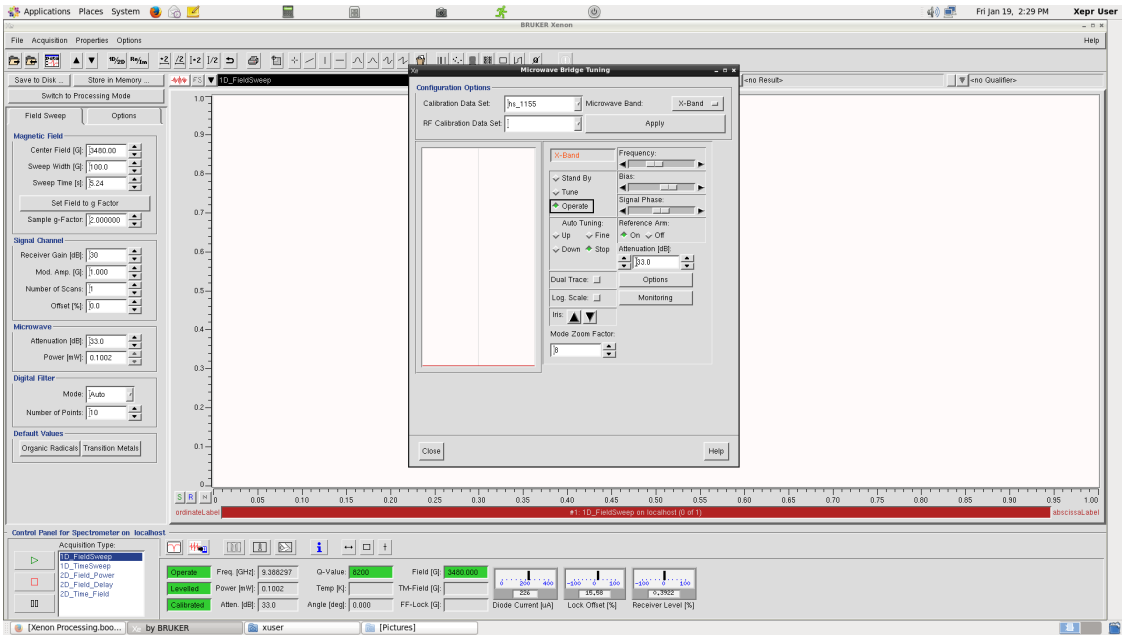


3.2.7. Set the attenuation to 33 dB. Click tune and observe the peak (zoom factor remains 8). Make sure Q-Value is measured, it might be needed later for quantitative EPR. Adjust the frequency to center the peak. Adjust the Signal Phase to make the peak symmetric. At first, click right or left button of Signal Phase slider, and wait a few seconds until the peak shape is stable. Only then adjust the phase by clicking these buttons multiple times. Right phase is essential for further EPR spectra fitting. Xenon window should look like this:



3.2.8. Click “operate”, **no need to change the attenuation**. Observe the value in “lock offset”. The hardware has a bug that sometimes after clicking “operate” frequency changes too much, and lock offset shows a value lower than -50% of higher than +50%. If so, click “tune” again, make sure the peak is centered, and click “operate” again. Sometimes a

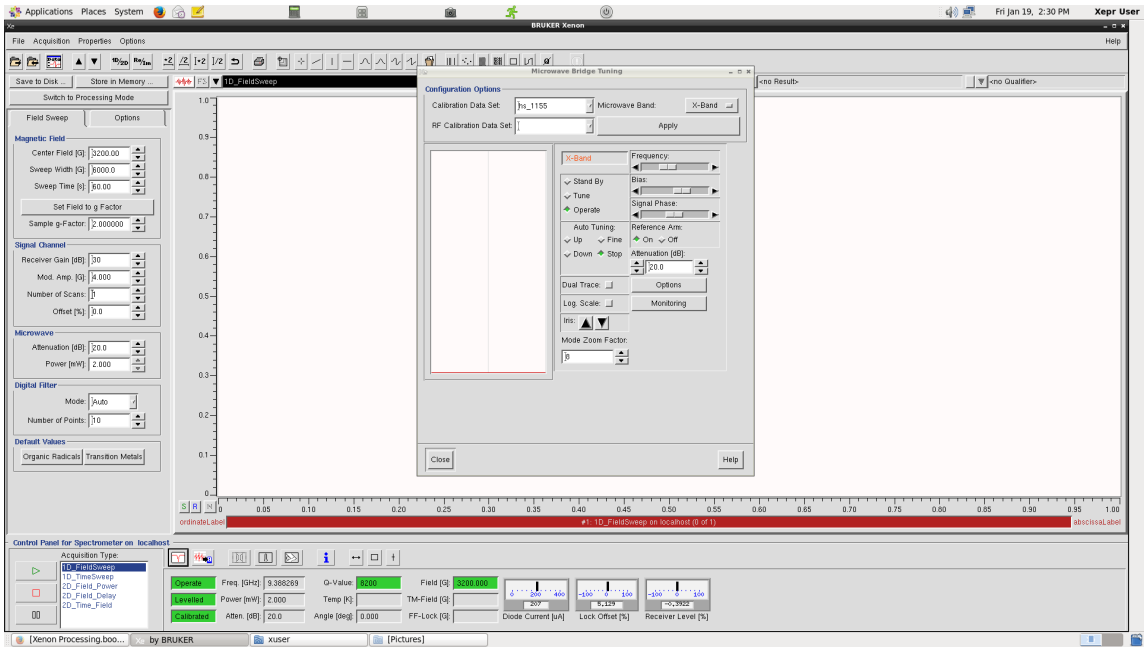
few cycles are needed to get offset value within ±50% interval. In almost all cases the diode current will be not 200 mA. If it is 400 mA, make the attenuation higher, but never less than 55 dB. Xenon window will look like this:



3.2.9. Manually adjust the frequency to make lock offset higher than -5% or lower than +5%. Adjust slowly, as it is very easy to overtune it. **Do not use autotuning**, as it does poor job resp. frequency adjustment.

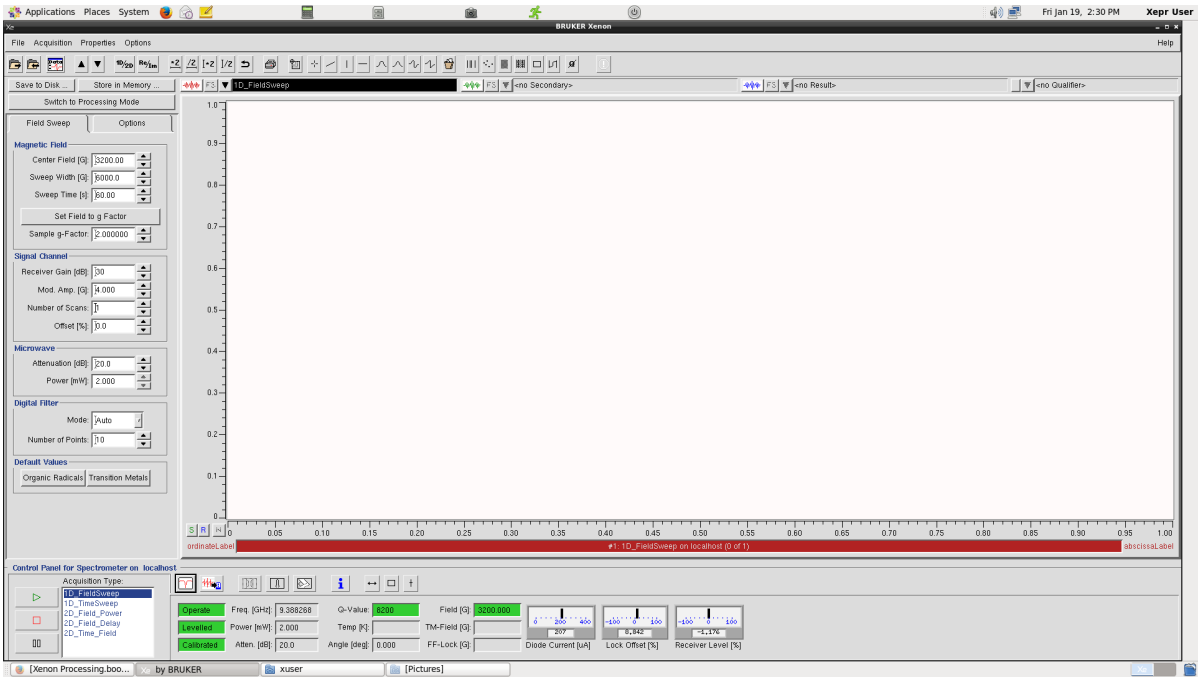
3.2.10 Click Autotuning:Fine, this will adjust Iris to make Diode current 200 mA.

3.2.11 Set desired attenuation (20 dB is a reasonable default value), click Autotuning:Fine again to adjust iris. Xenon window will look like this:



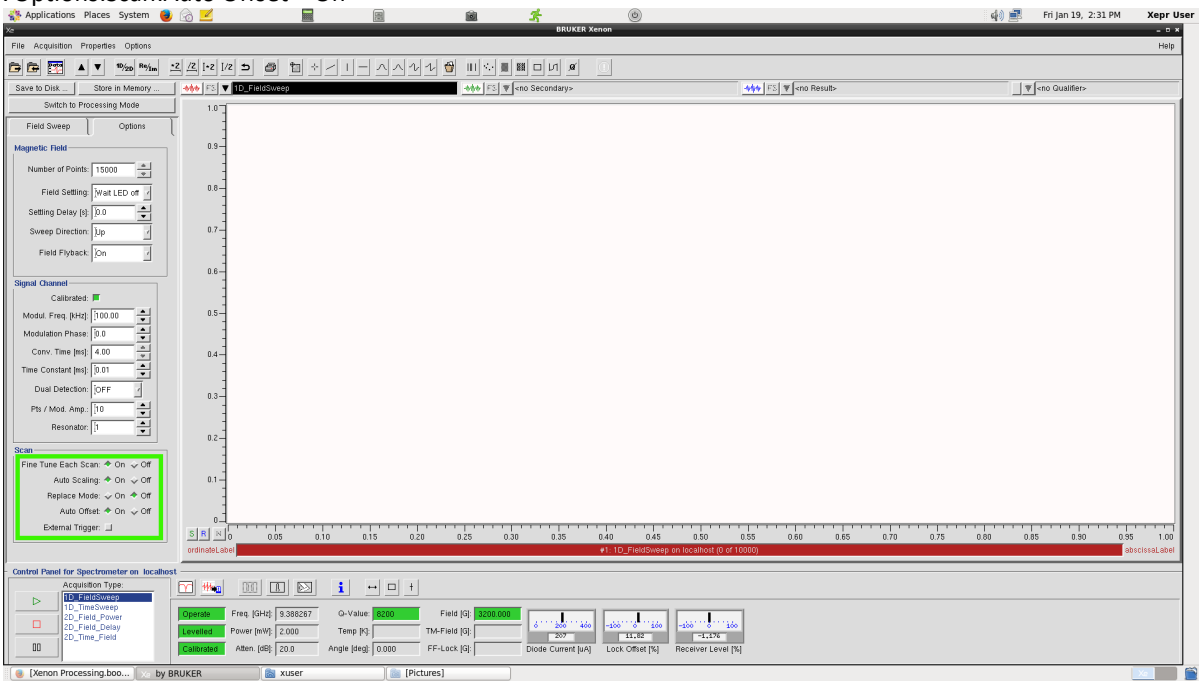
3.2.12. Click apply and close tuning window.

3.2.13. For a few seconds observe Diode current and Lock offset if they do not drift too much. Constant increase of diode current with constant shift of frequency are the symptoms of lossy sample, whose temperature slowly increases due to microwaves absorption. Xenon window should look like this:

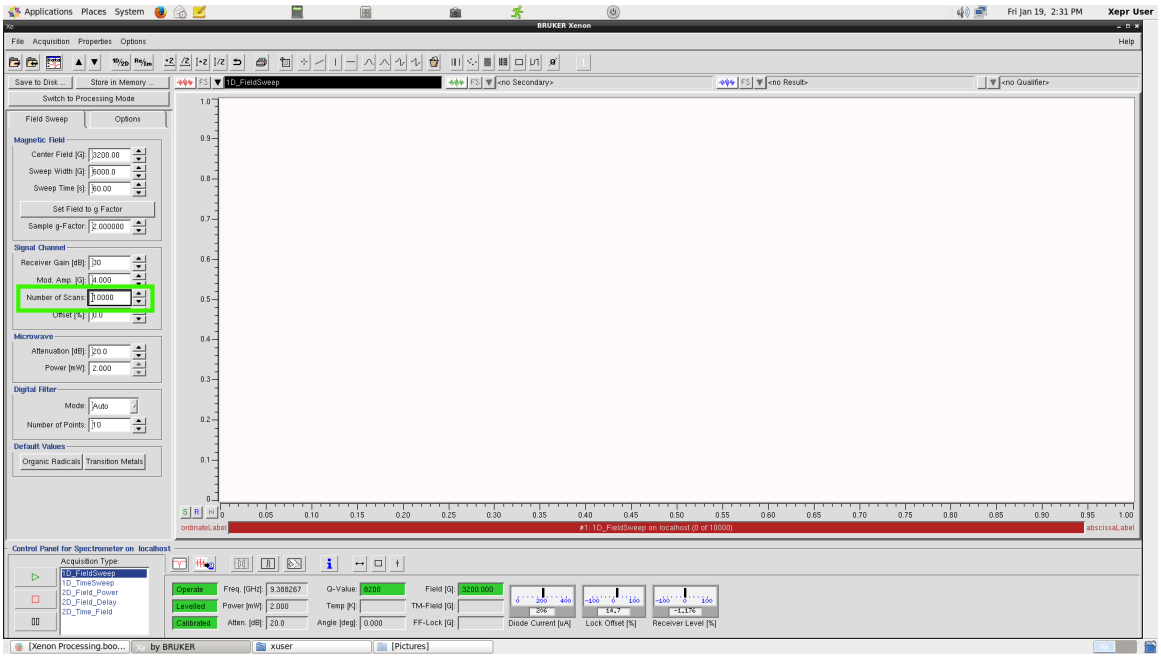


4 Load the default parameters for organic radical or transition metal compound. The default parameters are good for most cases, but sometimes other parameters must be adjusted. Refer to Xenon documentation and Bruker EPR webinars to learn what is the impact of them (Modulation amplitude and microwave power are the most important of all).

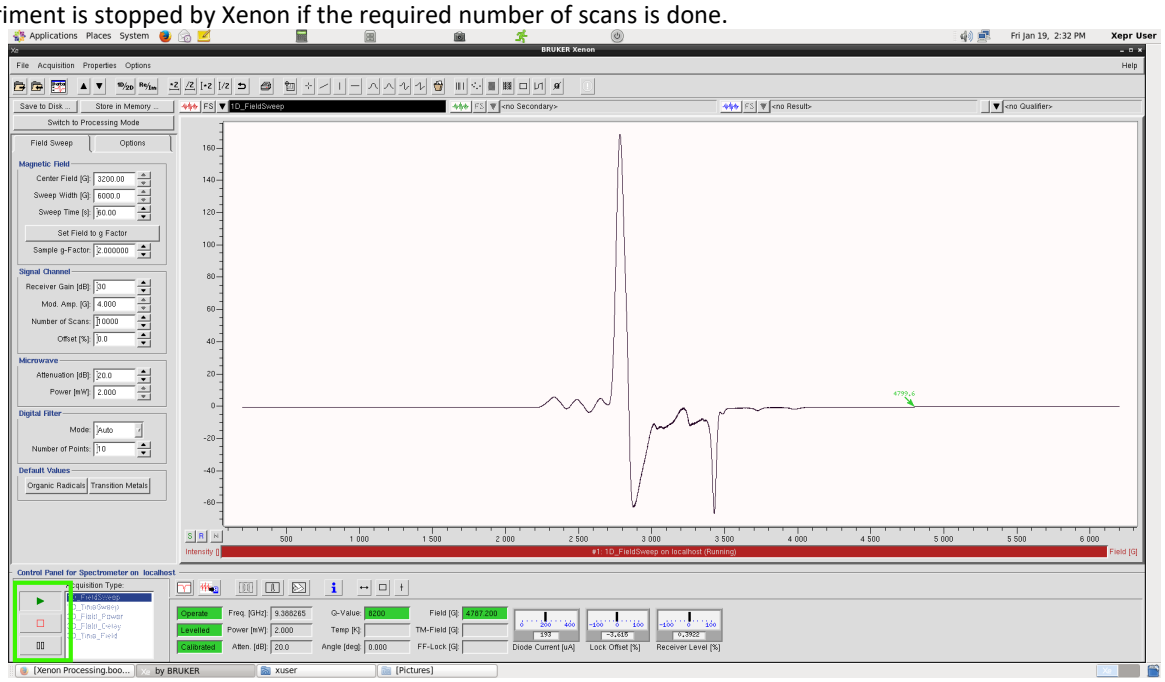
5 See the green box on the figure below. Click Options:Scan:Fine Tune Each Scan - On
Click Options:Scan:Autoscaling - On
Click Options:Scan:Auto Offset – On



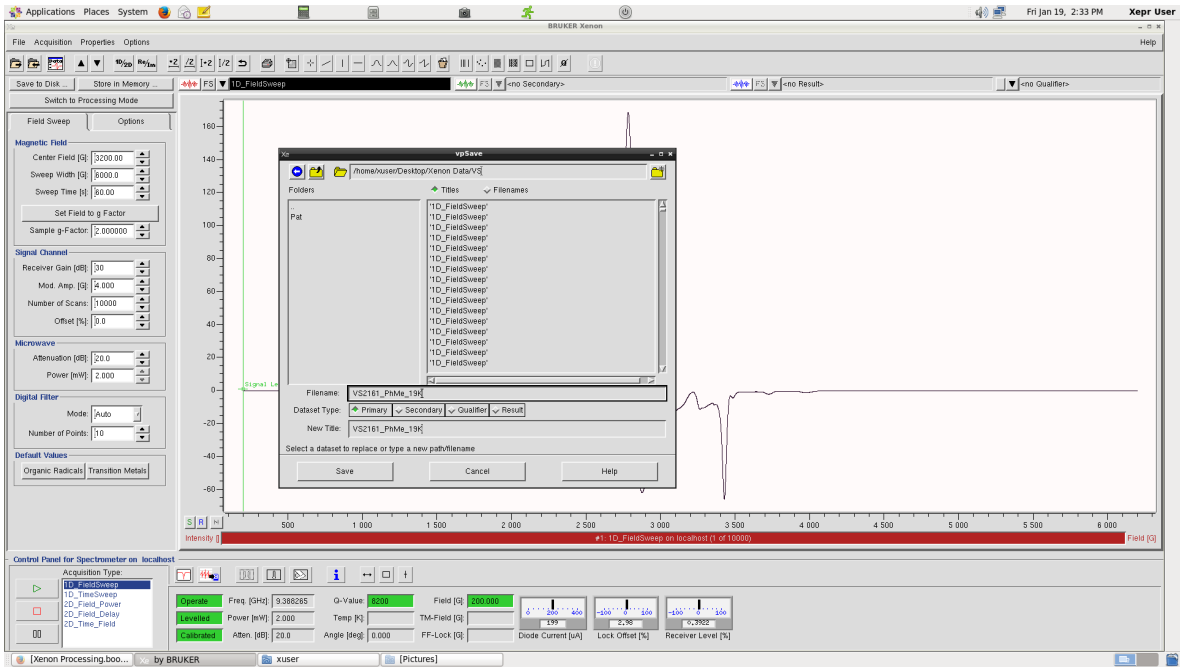
6 Return to Field Sweep and set desired number of scans. It is suggested to set more than you expect, as the measurement can always be halted retaining the collected data.



7 Press the Play button to start the measurement. The stop button is used to finish the current scan and stop the experiment, retaining the data in memory (without saving on disk). Pause button is used to pause field sweeping, fixing magnetic filed at constant strength. Press the stop button when you are happy with signal/noise ratio. The experiment is stopped by Xenon if the required number of scans is done.



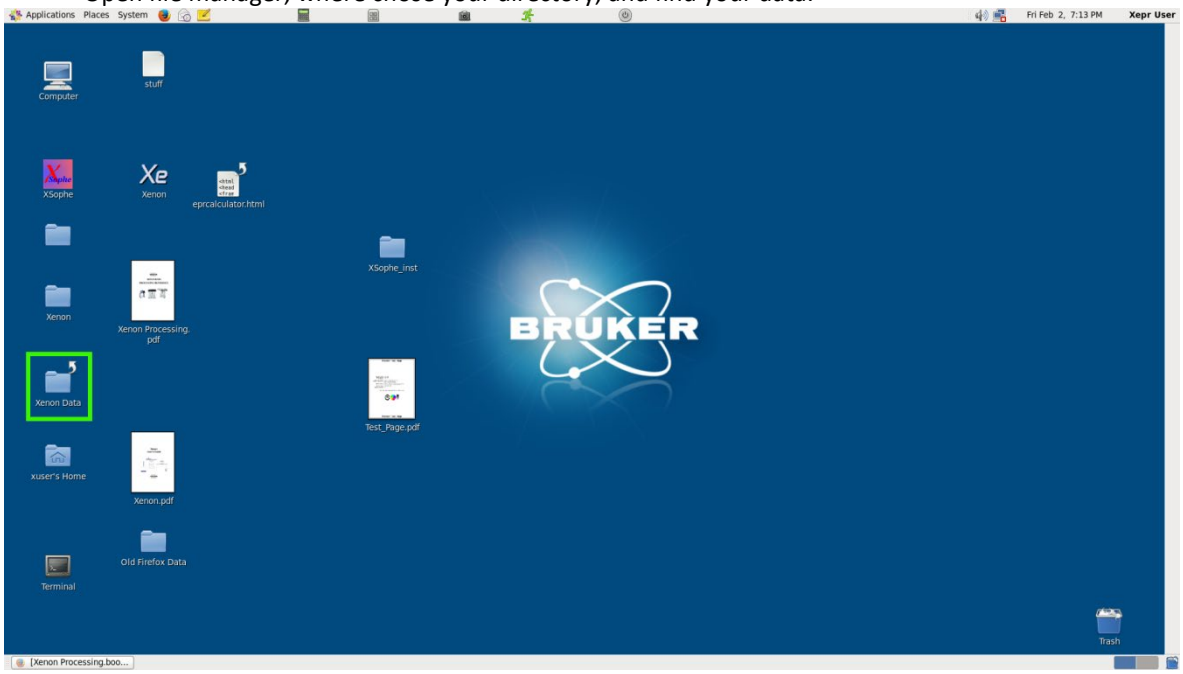
8 To save data, click “Save to Disk” button. Save the spectrum to your directory (created during EPR training). Filename and Title can be different, but to avoid confusion it is better to keep them the same. Make sure you save Primary dataset. Data are saved in Bruker BES3T format. If you want to open data in other software, make sure BES3T is supported. Otherwise, export data to ASCII (File:Save ACSII) or use a script to convert BES3T to ASCII (https://github.com/mortenalbring/BES3Tconvert). **The sample temperature is not saved with the EPR spectrum and we suggest that you add it to your file name manually.**

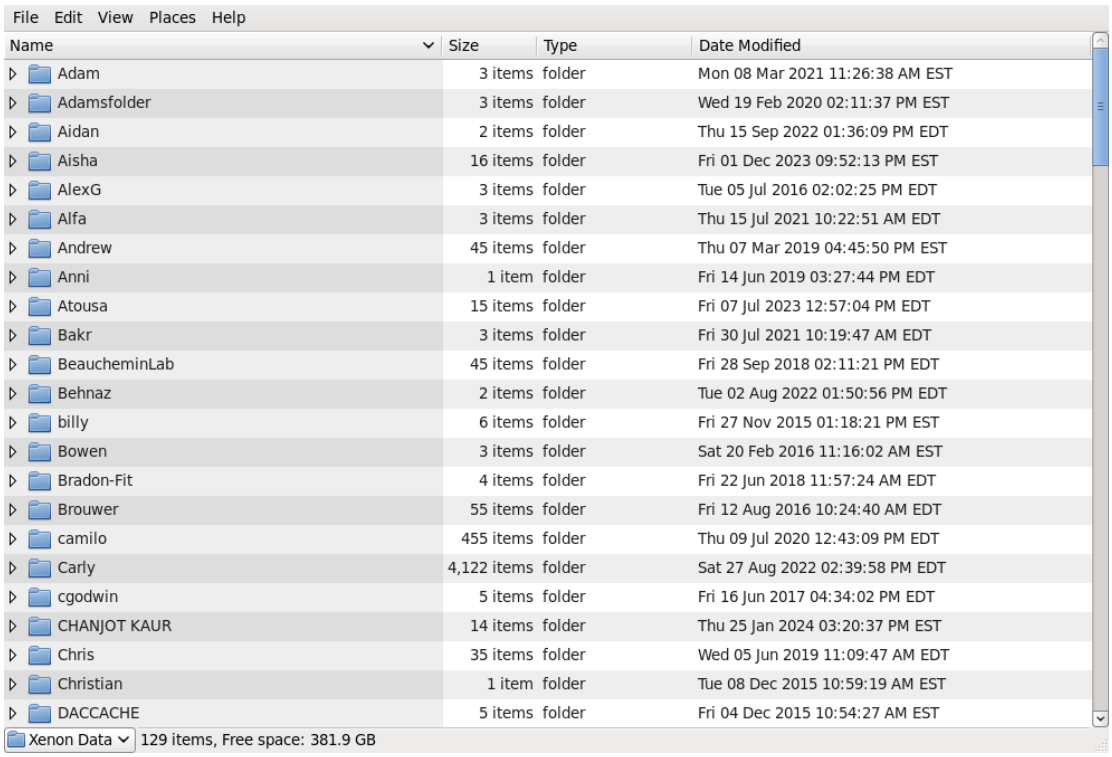


9 To remove the sample, refer to sample insertion procedure and do everything in reverse way. Insert a new sample if needed. If your work has finished, reassemble the cryostat (if needed), insert the plug to protect the empty cavity from dust.

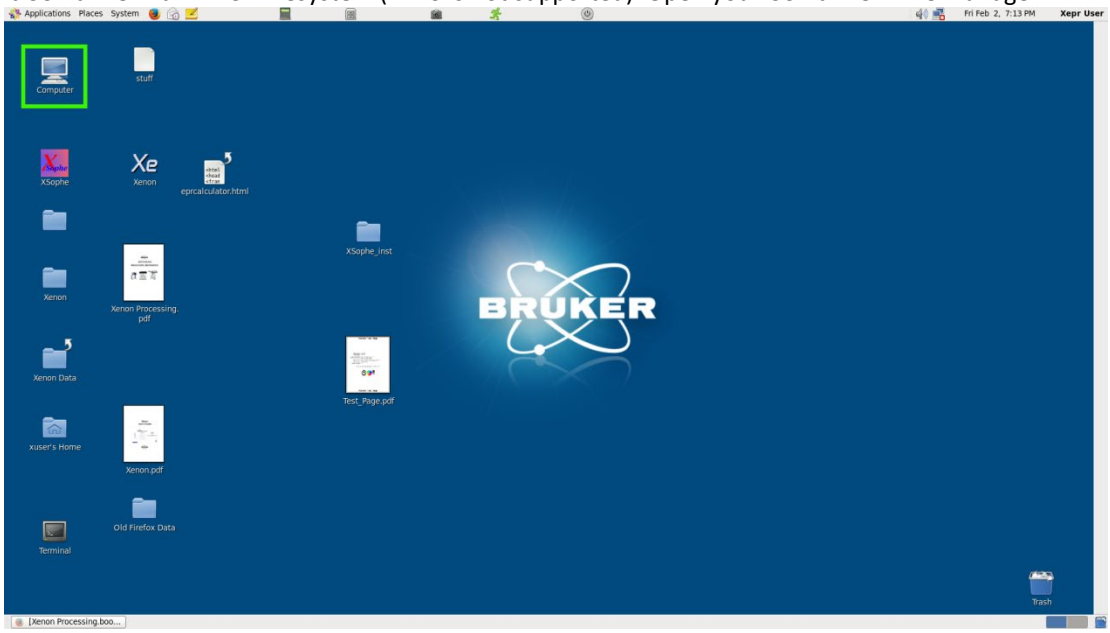
10 If all spectra are measured, close Xenon (answer that no data should be saved when it asks), then switch off the console, the magnet power supply, the chiller, the water flow meter, the water flow, and the mains power switch. To do this, follow the procedure of switching ON the system by reversing it, noting that: the “O” position on switches is off, the mains power is off when the lever is pulled downwards to down position, the EMX plus console is off when the LED light is off, and the chilled water is off when the handle is perpendicular to the pipe.

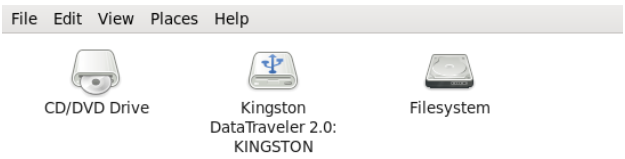
11.1 Open file manager, where chose your directory, and find your data.





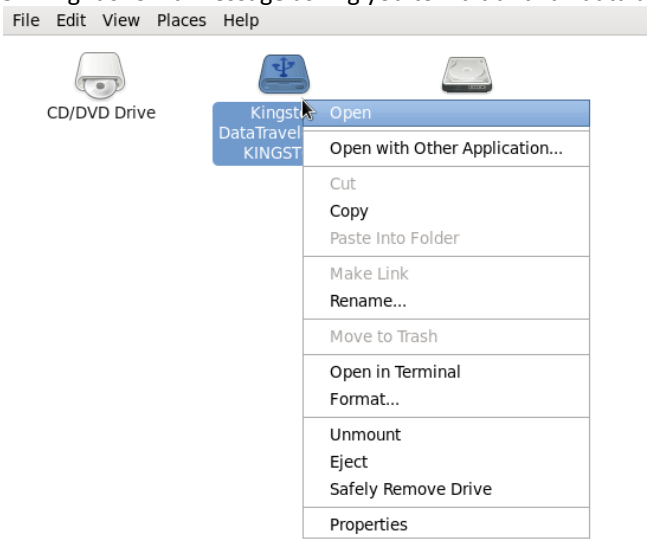
11.2 Insert USB drive with FAT32 filesystem (NTFS is not supported). Open your USB drive in file manager.





11.3 Drag the files containing your spectra to USB drive.

11.4 Dismount USB drive (right mouse button click on USB drive in disk manager, click “safely remove drive”) and take it with you. Operation system might show a message asking you to wait until all data are copied.



11.5

Additional Comments

1. Xenon has a SpinFit instrument to simulate and fit isotropic spectra (Switch to Processing Mode:SpinFit), refer to documentation to use it. Isotropic, anisotropic, slow motion regime, and all other spectra can be simulated and fitted by Easyspin software (https://easyspin.org/). Easyspin works in Matlab, reads Bruker BES3T files, and is available free of charge. Youtube Easyspin Academy channel is a good resource to get started with it.
2. BES3T format presumes that two files are created: the DAT one contains EPR spectrum in binary format, DSC one contains all parameters of measured spectrum in text format. The most important for simulation and fitting are: MWFQ (bridge resonance frequency, in GHz, this is needed to calculate g-factor of a given peak), XPTS (number of points), AVGS (number of scans), CenterField (centre of field, in Gauss), SweepWidth (sweep width, in Gauss). ModAmp (modulation amplitude, in Gauss), ModFreq (modulation frequence, in kHz), Power (microwave bridge power, in mW). For publication it is a good practice to mention ConvTime (conversion time), although it does not affect spectrum fitting, it affects S/N ratio.
3. Lossy samples could be measured at room temperature either in 3 mm tube (if microwave absorption is medium, like for samples in chlorinated solvents, or THF) or in 1 mm quartz capillary (if microwave absorption is high, like for samples in water, alcohols, acetone, DMSO, etc). Cooling will help to stabilize the temperature, but might cause solvent freezing or become too viscous, so it is practical only if the goal is to freeze the sample with liquid nitrogen or liquid helium, and register anisotropic EPR spectra.
4. There are more parameters to change in Xenon. Refer to documentation (available on desktop of EPR computer and on NMR FTP server) and to Bruker tutorials (https://www.bruker.com/en/news-and-events/webinars/2021/Bruker-CW-EPR-User-Training-Course.html) to learn more.
5. Do not use glass NMR tubes or glass capillaries for EPR. Glass contains traces of Fe(III) compounds, which make it EPR active.

Emergency Procedure

1. In the event of a magnet quench in the NMR lab, large volumes of helium and nitrogen will be released from the NMR spectrometer, displacing the oxygen in the room. Immediately evacuate the room, call protection (5411), and the NMR lab.

EPR Training Protocol

1. Ensure trainee has completed a new user registration form and perform in-lab training.
2. Have the trainee document, in short form and in their own words, their training in the specific fields on the form.
3. Provide the trainee with the SOP documentation or a link to the SOP documentation.