

Things to know before operating an EPR Spectrometer

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I. (Very) Basic theory of EPR.

Unpaired electrons (and the nuclei of certain atoms such as ^1H and ^{13}C) behave like spinning bar magnets. If you place many ($\sim 10^{15}$) unpaired electrons in a magnetic field, then a little bit more than 50% will line up with the magnetic field and the rest will line up against the magnetic field. It is possible to induce transitions between these two states using electromagnetic radiation. The precise frequency of this process is given in equation 1

$$h\nu = g\beta B \quad \text{eq. 1}$$

where h equals Planck's constant, ν equals the frequency of the electromagnetic radiation, β equals the Bohr magneton and B equals magnitude of the magnetic field. You can think of g (also known as the g value) like chemical shift in NMR, because its value can reveal important properties of your molecule.

EPR spectroscopy differs from other types of spectroscopies (such as NMR) in two major ways. First, in EPR spectroscopy the frequency of the electromagnetic radiation is constant, and the magnitude of the magnetic field is varied to satisfy eq. 1. One important consequence of this difference is that the x-axis of an EPR spectrum is in units of magnetic field, usually gauss, although the SI unit is milli Tesla (mT) where $1 \text{ mT} = 10 \text{ gauss}$, rather than frequency. Second, EPR spectra are recorded and displayed as the first derivative of the absorbance. Figure 1 shows the EPR spectrum of the benzosemiquinone radical anion.

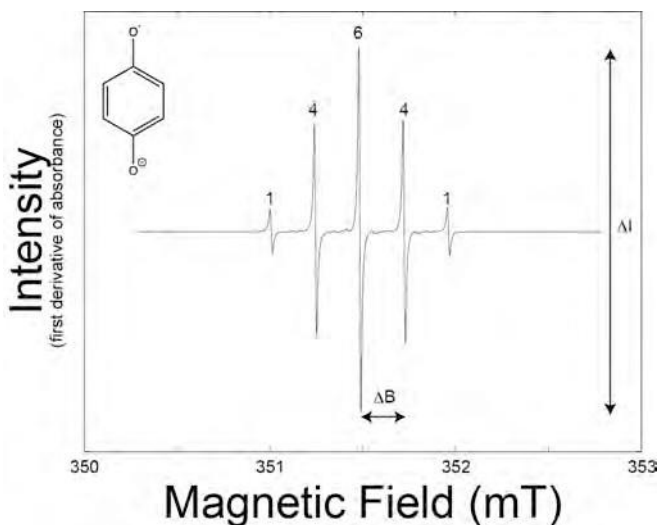


Figure 1. X-band EPR spectrum of benzosemiquinone radical anion. The intensity ratio of the five-line pattern is 1:4:6:4:1 with splitting ΔB and intensity ΔI .

Figure 1 introduces many of the most important parameters that can be measured in an EPR spectrum. The g value was discussed above. The coupling of the unpaired electron to the four $I = \frac{1}{2}$ protons splits the EPR peak into five lines. The effect is called “hyperfine coupling” and is analogous to scalar coupling in NMR spectroscopy. The relative intensities of the peaks (1:4:6:4:1) and the value of the splitting (ΔB) can be diagnostic for a molecule. Also, the intensity of the EPR signal (or double integral of the EPR spectrum) is related to the number of spins in a manner analogous to the integral in NMR spectroscopy.

This guide will focus on introducing the basic aspects of recording an EPR spectrum. After studying this guide, you should be prepared for one-on-one training with Justin. This training should prepare you to record EPR spectra.

II. A tour of the EPR lab.

Figure 2 shows the layout of the EPR lab at KU. Familiarize yourself with the major pieces of equipment in the lab so that you can follow the rest of this document.



Figure 2. A picture of the KU EPR lab.

The **magnet** is an electromagnet. The **cavity** (or resonator) holds the sample. The **bridge** contains the microwave source and the detector. The **console** houses various electronics used to acquire and process the signals to generate a spectrum. The **power supply** provides current to the electromagnet. The **Haskris** provides cooling water for the magnet, as well as the power supply and the bridge. Cryostat is used for low temperature EPR.

III. The Cryostat.

You will only need to use the cryostat if you plan on doing EPR at low temperatures. If you plan to use the cryostat, please see Justin for more information.

IV. Turning on the EPR spectrometer.

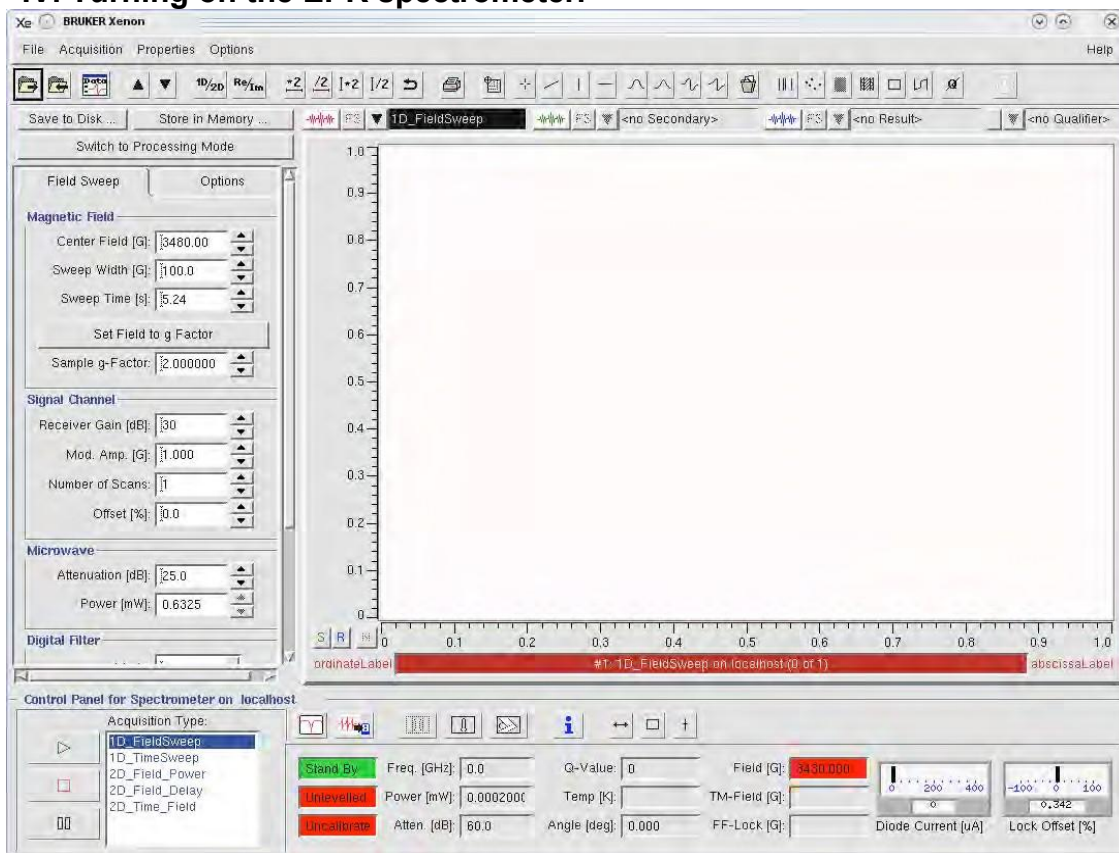



Figure 3. Bruker Xenon Software.

Because the EPR spectrometer is not used daily, you may find the system turned off. It is not difficult to turn on the system. First, turn on the power for the system by flipping the switch on the breaker boxes on the east wall of the room. Second turn on the Haskris to start cooling the water. Wait a minute or two for the Haskris to reach 65 °F. Third, turn on the power for the system. This switch is on the back of the power supply. Next, turn on the console by

pressing the flashing blue button in the middle near the bottom of the front panel. Fifth, on the back left-hand side of the bridge there is a power switch. Flip it and the status indicator will start flashing on the bridge. After a few moments, the bridge will initialize, and the status indicator will remain lit on both the console and bridge. Next, log onto the computer. Click the Xenon icon on the desktop to open the software. Figure 3 shows the layout of the Xenon software. Click the  button to open the Microwave Bridge Tuning window. Switch to tune mode and allow the spectrometer to warm up for ~1 hour. Finally, turn on the power to the electromagnet by flipping the switch on the front of the power supply. The EPR spectrometer should now be operational and ready to record a spectrum.

V. Loading a sample (when the cryostat is not in use).

Note: See the document “Using the cryostat for low temperature EPR” for instructions on how to load a sample if the cryostat is in use.

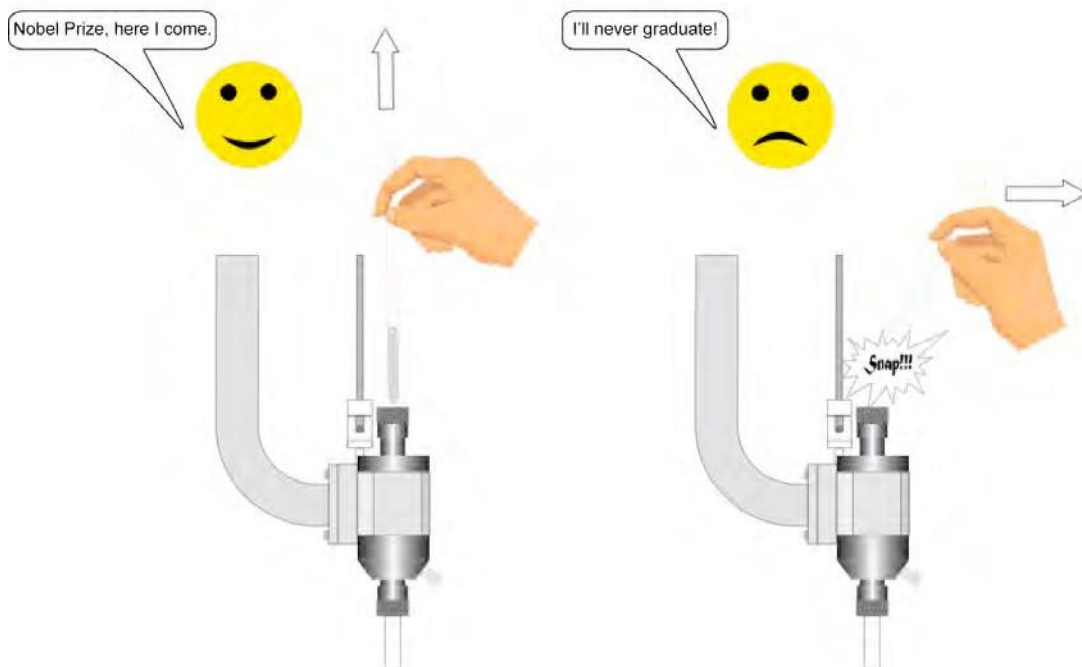



Figure 4. The correct and incorrect way to load an EPR sample

We never want to break an EPR tube inside the cavity. The sample will spill on the inside of the cavity and show up in every spectrum we record in the future. Hence, it is very important that everyone uses the appropriate amount of caution when loading and removing a sample from the cavity. See figure 4 for the proper and improper way to remove a sample from the cavity.

First, you want to make sure the spectrometer is not in “Operate Mode”. The mode is displayed in the bridge status lights in the spectrometer monitoring panel

below the spectrum window. To change, click the  button. Figure 5 shows the microwave bridge tuning dialog box. Switch mode to “Tune” and set the microwave attenuation to 30 dB. Make sure the Calibration Data Set is correct for the cavity (ask Justin if you are unsure).

Next, to avoid contaminating the cavity wipe the EPR tube with a Chem wipe. Loosen the nut at the top of the cavity and gently insert the EPR tube into the cavity. Tighten the nut to hold the sample in place.

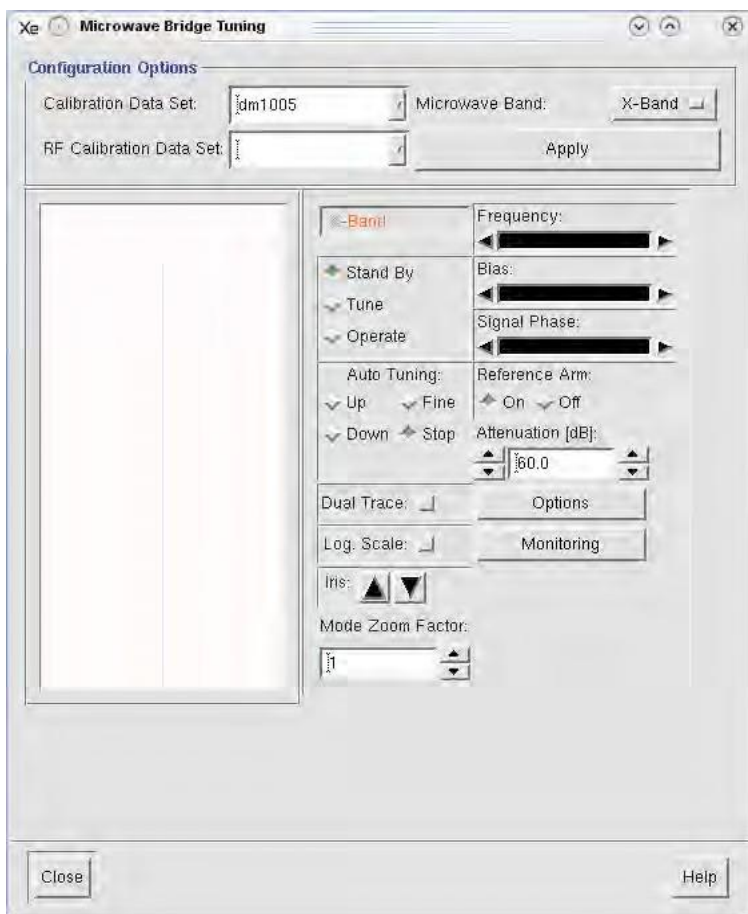


Figure 5. Microwave bridge tuning dialog box.




VI. Tuning the EPR spectrometer.

The first step to acquire an EPR spectrum is to tune the spectrometer. You may be asking yourself – “What are we really doing when we tune the spectrometer?” We are doing two things. First, we are setting the frequency of the microwaves to match the resonance frequency of our cavity and sample. This step turns on the Automatic Frequency Controller (AFC), a feedback loop that measures and adjusts the microwave frequency to make sure the cavity and sample stay in resonance during the entire experiment. Second, we are “matching” or “critically coupling” the bridge with our cavity and sample. The idea is that we want 100%

of the microwaves going into the cavity absorbed by the cavity and sample. To match the spectrometer a small screw, called the iris, is adjusted. Use the procedure in Appendix 1 for tuning.

When the spectrometer is properly tuned there will be three green lights on the left-hand side of the Spectrometer Control Panel. The bottom two will alert you that the spectrometer is “Levelled” and “Calibrated”. The Diode Current will read 200 μA ($\pm 20 \mu\text{A}$) and the Lock Offset will read 0% ($\pm 10\%$). Note that there will be some fluctuations in the diode current and lock offset. As long as the “Levelled” and “Calibrated” buttons stay green during the entire experiment your data should be fine.

VII. Spectral Acquisition.

The Xenon software has many options for data acquisition. This section explores some basic options. Click on the 1D_FieldSweep experiment in the Acquisition Type window. This loads the simplest possible EPR experiment, a scan of the magnetic field. Click the  button to load sample information. Click either the Organic Radicals or Transition Metals button near the bottom of the Field Sweep Panel. Alternatively, you can load parameters from a file. Use the file menu to navigate to the file and click the  button to load the parameters. Click the  button to start the acquisition.

After you have acquired your spectrum, you will have to save the data. You can either “Store in Memory” (create a temporary copy that is lost when you exit Xenon) or “Save to Disk” (write a permanent copy on the hard drive). Obviously, anything you think will be needed later should be saved to the disk! Click the Save to Disk button to open the Save dialog box. Choose a good filename and enter a descriptive title in the title box. Navigate to `/home/data/your-username` and click the Save button to save the data. Note that you can create sub-directories in your data directory and organize your files any way that seems appropriate to you.

VIII. Optimizing Data Acquisition.

Often, you can use the generic parameter sets for Organic Radicals or Transition Metals and get a reasonable spectrum. Alternatively, if you or other members of your group have optimized acquisition for your types of samples, you can load in an old data set and use these parameters. Otherwise, see Justin about setting up initial parameters. You will want to run an exploratory scan to make sure you see signal and then optimize your acquisition parameters to collect the highest quality spectrum possible. This section discusses several possible parameters and offers some basic guidance to help optimize data acquisition. The most important parameters to optimize are the microwave power and (if you have very narrow lines) modulation amplitude.




A. Microwave Power.

Technically, microwave power is measured in watts. When we speak of power, we often speak of power attenuation, which is measured in decibels (dB). The larger the attenuation, the less power. Hence, 0 dB attenuation is full power and 60 dB is minimum power.

Naively, we may think that more power (less attenuation) means more signal. This statement is not true because of relaxation. Think of an extreme case in which the microwave power is so high that the rate of energy absorption by the system is greater than the relaxation rate. In this case, the difference in the populations of the energy levels decreases. Subsequently the signal intensity decreases! This process is called saturation. Samples that relax slowly (have a large T_1 and/or T_2) will saturate at lower powers (higher attenuation) than samples that relax fast (i.e. have small T_1 and/or T_2).

Overall, it is useful to record a spectrum at several different attenuations to make sure there is no saturation. In fact, if you are doing quantitative EPR or plan to simulate your data, then saturation will complicate your analysis. Justin will demonstrate an easy way to check for saturation in the one-on-one training.

B. Sweep Width.

Click the  button to open the sweep tool. Drag the center marker to the middle of your EPR spectrum and drag the one end to the high or low boundary of your spectrum (either end of the tool works). The  button will remove the tool when you are finished. Click the  button to start the acquisition again.

C. Receiver Gain.

Examine your spectrum for evidence of clipping. You can tell if your spectrum is clipped because the top of the peak will plateau. Turn down the receiver gain if your spectrum is clipped.

D. The Signal Channel.

EPR spectrometers use a technique called phase-sensitive detection to reduce noise and baseline artifacts in EPR spectra. There are three relevant parameters for phase sensitive detection: time constant (and conversion time), modulation amplitude and modulation frequency.

1. *Time constant (and conversion time).*

In general, the larger the time constant, the less the noise. Unfortunately, we cannot increase the time constant to our hearts content: the time constant is limited by the scan rate. A general rule is that the time needed to scan through the narrowest feature should be ten times greater than the time constant.

Bruker Xenon software also uses a related parameter called “conversion time”. It is recommended that the time constant is set to a value that is approximately equal to the conversion time.

2. Modulation amplitude.

WARNING: If the modulation amplitude is too high the coils in the cavity could be damaged, which would require us to send the cavity to Bruker for a costly repair. The Xenon software has safeties to protect the cavity, however as a **RULE IN THE EPR LAB EVERYONE SHOULD AVOID EXCEEDING 4 GAUSS FOR THE MODULATION AMPLITUDE**. Help keep hourly costs low by treating the cavity with care.

With this warning in mind, we recommend setting the modulation amplitude to the maximum value (4 G) for most transition metals. There are situations where it is necessary to increase beyond 4 G for transition metals. For organic radicals, 4 G is a reasonable starting point, however, it is good practice to record a spectrum at lower modulation amplitude, as well. The reason is that if the modulation amplitude is larger than the EPR linewidth, the spectrum becomes distorted.

3. Modulation frequency.

For transition metals and most organic radicals, you should set the modulation frequency to the maximum (100 kHz). For organic radicals with very small super hyperfine couplings (~50 milligauss), there is signal broadening at the maximum modulation frequency, hence this parameter should be reduced.

VIII. Shutting down the EPR spectrometer.

After you have saved your data you will need to remove your sample and turn off the spectrometer. Be sure to exit from the software in an orderly manner to ensure that the instrument parameters are set to safe values for shut down. **DO NOT** just exit the software and log out!

- Switch the bridge to standby mode and close the Microwave Bridge Tuning dialog box. Now it is safe to exit Xenon and log out of the computer.
- Turn off the electromagnet using the button on the front of the power supply.
- Turn off the console by pushing the blue button in the center and turn off the bridge (back, left-hand side). Turn off the power supply.
- Turn off the Haskris and flip the breakers.

Before you leave, make one last check of the room, and make sure everything is off.

Appendix 1. Spectrometer Tuning.

You may need to go through the entire procedure several times to get the cavity and sample tuned.

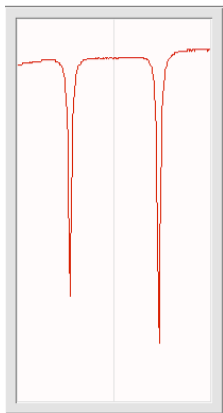


Figure A1. Excerpt of Microwave Bridge Tuning dialog box showing two dips for dual mode cavity.

First, open the microwave bridge tuning dialog box and switch to tune mode. Set the attenuation to 20 dB. Set the bias to ~50%. You should see something that looks like an upside-down NMR spectrum in the Microwave Bridge Tuning dialog box (Fig. A1). Most likely, the cavity in our spectrometer will be the dual-mode resonator, meaning that it can be used for both perpendicular and parallel mode EPR. If you have no idea what the difference between these modes is, then you should probably be using perpendicular mode. Because there are two modes, you will see two dips in the tune window. Hence, it is critical that you choose the correct dip when tuning the spectrometer. Use the frequency tab to center the appropriate dip and adjust the phase tab so that the dip is symmetric.

Next, switch the bridge to operate mode and increase attenuation to 50 dB. Adjust the bias tab to set the diode current to 200 μA (± 20 μA). At this point, the lock offset should be 0% ($\pm 10\%$). If not, you probably have a problem with the frequency (and/or phase). Make a very small adjustment of the frequency using the fine arrows. Give it a moment to equilibrate. If you can't get lock offset to 0%, then go back to tune mode, correct and switch to operate mode.

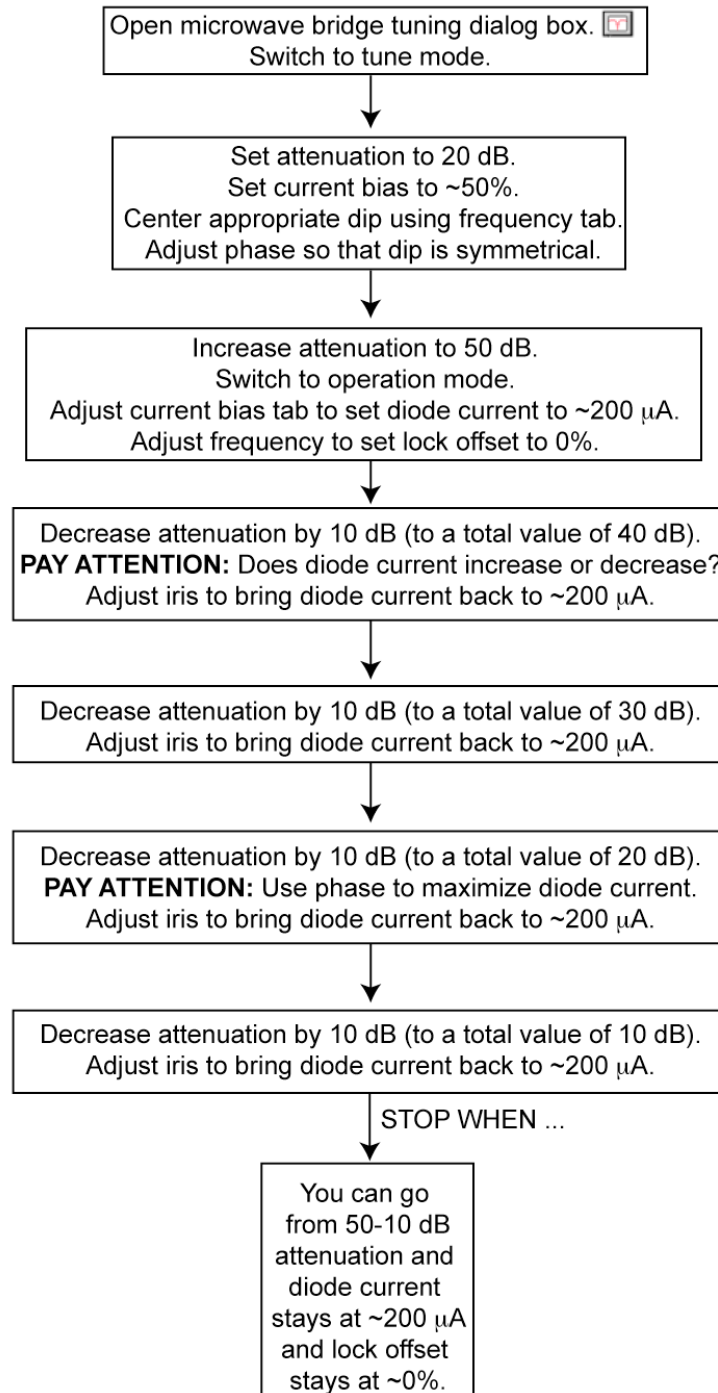
Decrease the attenuation by 10 dB (to 40 dB). Pay close attention to the diode current. Does it increase or decrease? Use the iris to bring the diode current back to ~200 μA . Usually, decreasing the iris will decrease the diode current and increasing the iris will increase the diode current. Don't go crazy with the iris. Over-tightening the iris could strip the threads of the screw and potentially damage the cavity! If you have problems find Justin.

Decrease the attenuation by another 10 dB (to 30 dB). Use the iris to bring the diode current to ~200 μA . Decrease the attenuation by another 10 dB (to 20 dB). At this point, make a fine adjustment to the phase. The goal is to maximize the diode current. Then use the iris to bring the diode current back to ~200 μA . Decrease the attenuation by another 10 dB (to 10 dB). Use the iris to bring the diode current to ~200 μA . The spectrometer is properly tuned when you can change the attenuation for 50 to 10 dB and the diode current is at 200 μA (± 20 μA) and the lock offset is at 0% ($\pm 10\%$). This procedure is summarized in a flow chart on the next page.

Before you are finished, set the attenuation to 33 dB and put the spectrometer in tune mode. This instrument will display the Q value, which is an important

parameter. Flip back to the operate mode and set the attenuation to the desired level. You may need to touch up the tuning at this stage.

A flow chart for tuning an EPR spectrometer



Caution: diode current will become more responsive as attenuation decreases.