Introductory Training for the Bruker EMX EPR Spectrometer

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What is EPR ? A very brief introduction.

EPR is an acronym for Electron Paramagnetic Resonance. EPR, also referred to as Electron Spin Resonance (ESR) spectroscopy, measures the absorption of microwave radiation corresponding to the energy splitting of an unpaired electron when it is placed in a strong magnetic field. EPR / ESR is a spectroscopic technique that detects the presence of these unpaired electrons in a chemical system. This can yield meaningful structural and dynamic information, even from ongoing chemical or physical processes (i.e. kinetics, etc.) without influencing the process itself. This proves an ideal technique to complement other analytical methods in a wide range of application areas. These areas include Chemistry, Physics, Materials Research, Biology and Medicine, and Ionizing Radiation.

In the absence of an external magnetic field the two possible electron spin states (spin up and spin down) are degenerate. When an atom or molecule with an unpaired electron is placed in a magnetic field, the spin of the unpaired electron can become aligned either in the same direction (spin up) or in the opposite direction (spin down) of the applied field. These two possible electron alignments have different energies (i.e. are no longer degenerate) and are directly proportional to the applied magnetic field strength. This is called the Zeeman effect. There is a short introduction describing the physics behind the Zeeman effect at the HyperPhysics Page at Georgia State University.

Species that contain unpaired electrons:

1. Free radicals
2. Odd electron molecules
3. Transition-metal complexes
4. Lanthanide ions
5. Triplet-state molecules

The EPR instrument in a nutshell:

In a typical EPR instrument, a Klystron tube is used to generate monochromatic microwave radiation in the ~9.75 G Hz (known as the X-band) region. Recently, the Klystron tube was replaced with the solid state Gunn diode. The Gunn diode offers superior frequency stability and had a longer lifetime. Other regions of interest are the L-band (~1.1 GHz), the S-band (~3.0 G Hz), the Q-band (~34.0 G Hz), and the W-band (~94.0 G Hz). The microwave radiation travels down a waveguide (a type of rf pipe) to the sample, which is held in place in a microwave ‘cavity’ held between the poles of two magnets. Spectra are obtained by measuring the absorption of the microwave radiation while scanning the magnetic-field strength. EPR spectra are usually displayed in derivative form to improve the signal-to-noise ratio.

Before you start:

1. Quickly inspect the instrument to verify that there is no obvious damage to the spectrometer and that everything appears to be in working order.
2. Check to make sure that the cooling water is on and flowing. Inspect the delivery and return lines for signs of wear. If there is any question abut the condition of the lines please report this to a DCIF staff member.
3. Make sure that the cavity is in place and that the previous user has installed the 'blank' collets. It is important that the cavity have the 'blank collets' installed to prevent contaminants from entering the microwave cavity. If you have installed a different cavity, or the cryostat, make sure that the waveguide gaskets are properly installed. It is essential that the cavity be centered between the magnet poles. If it is not, carefully center the cell with the waveguide stabilizers.
4. Check to make sure you have the proper collets and pedestal for your sample cell.
5. The current cavity will only accept 4mm diameter EPR tubes. NMR tubes will not work. We recommend using Wilmad® #707-SQ-250mm tubes and Wilmad® can be reached at 1-800-220-5071.
6. If you plan on performing low temperature experiments, make sure that the N_2 delivery Dewar / thermocouple are in working order. If you will need cryogens, order them in advance. The DCIF does not supply cryogens for EPR experiments.
7. Verify that both the EMX console and the field power supply are turned on. Please remember that it takes ~1 to 2 hours for the field power supply to properly warm up! Without the proper warm up time, the cavity will not tune properly.
8. If any of the above cannot be satisfied, please contact a DCIF staff member.

Getting started:

Bruker supplies their family of EPR spectrometers with a proprietary application/ operation package called the WIN-EPR Acquisition software. This intuitive package is based on the common Windows® platform. Those familiar with Windows® operating system will find that the software allows the user easily perform sophisticated EPR experiments with a minimal learning curve.

1. **Open the Win-EPR Acquisition program**. The can typically be done by double clicking the 'acquisition shortcut' on the desktop. If the acquisition shortcut is not available, open the 'Acquisit' icon under the **Start → Programs → Winepr** menu option.
2. **Insert your sample**. Open the Microwave Bridge Control box by clicking the MW button on the program toolbar. Switch the bridge to '**Stand By**', then clean and carefully insert your sample into the microwave cavity. Center the

sample (horizontally) in the cavity (this is especially important while using solid samples), and make sure the pedestal is not protruding into the cavity.

3. Turn on the waveguide nitrogen purge. Only a very small flow is required. This will help to purge water, oxygen and any other undesirable species from the cavity.
4. **Tune the cavity**. This is probably the most important step in acquiring quality EPR spectra. In most instances, '**Auto-Tune**' will work fine, but for some instances the cavity must be tuned manually. To '**Auto-Tune**' follow these steps:
   a) Switch the microwave bridge radio button to '**Tune**'.
   b) Set the microwave attenuator to ~25 dB
   c) Center the cavity dip using the frequency slider bar. When centered, the analog AFC meter should be centered in the green.
   d) Adjust the phase slider bar. The cavity dip should be made to go 'negative'. The object here is to make the dip as sharp, narrow and negative as possible.
   e) Adjust the 'Bias' slider bar to center the analog Diode meter. This step isn't critical and the bias should be set to ~80%.
   f) Tune the Microwave Bridge by pressing either of the '**Auto-Tune**' arrows. The routine automatically adjusts the frequency, phase, bridge bias, and critically couples the cavity. If the auto-tune algorithm fails, repeat steps c through e then press the *other* '**Auto-Tune**' arrow.
   g) When completed, close the Microwave Bridge Control box.

5. If the '**Auto-Tune**' algorithm does not satisfactorily tune the cavity, you must manually tune the Microwave Bridge. This can be easily performed as follows:
   a) Open the Microwave Bridge Control dialog box
   b) Set the microwave bridge to '**Tune**' mode and set the attenuator to ~25 dB
   c) Observe the mode pattern for the Gunn diode microwave source on the display monitor. The mode patterns are fully described in Chapter 5 (Figure 5-2) of the EMX user manual.
   d) Tune the microwave source using the '**Frequency**' slide bar to optimize (center and maximize) the 'dip'. Experience has shown that the instrument prefers that the 'dip' be slightly to the left when tuning the cavity manually.
   e) Carefully insert your sample into the microwave cavity.
   f) Re-tune the cavity using the '**Frequency**' slide bar (repeat step d above).
   g) Optimize the signal using the '**Signal Phase**' slide bar. The key here is to look for symmetry.
   h) Click the '**Operate**' button and re-adjust the '**Frequency**' slide bar until the AFC meter is centered.
   i) Change the microwave attenuation to 50 dB and adjust the

‘**Bias**’ slide bar to center the Diode meter.

j) Critically couple the cavity. Decrease the attenuation by 10 dB and adjust the ‘**Iris**’ until the Diode meter is centered (200 μA). Repeat the procedure in -10 dB steps until you have reached an attenuator setting of 10 dB.
k) If the cavity still will not tune, you may have what is referred to as a ‘lossy’ sample. Ask a staff member for assistance if you are having difficulties.

6. **Prepare to Acquire Spectra**.
    a) Click the ‘**Experiment Parameter Button**’.
    b) Verify that the Experiment type is set to X: Field Sweep and Y: no Y-Sweep.
    c) Check the field sweep (‘**Hall’**) and ‘**Signal Channel’** parameters. Edit these values such that they correspond to the values given in Figure 3-14 of the EMX User Manual. It is recommended to start with a modulation amplitude of ~10 gauss for most transition-metal spectra and ~1 to 2 gauss for radical type species.
    d) Set the ‘**Microwave Bridge Power**’ to 2.00 mW and close the ‘**Experiment Parameter**’ dialog box. In order to determine the *optimal* microwave power, you will need to perform a few ‘survey scans’. The power required for individual experiments will be dependent upon such factors as concentration, solvent, and temperature. In order to determine the optimal power, several microwave power settings *must* be attempted. In the absence of saturation effects, the EPR signal will increase to the square root of the microwave power.
    e) The sweep width, receiver gain, center field, etc. can be easily adjusted using the 'interactive' buttons on the Win-EPR toolbar.
7. Once you are convinced that sample saturation is not occurring, click the ‘**Run**’ button to acquire a spectrum.

Important considerations:

1. The EPR cavity must be kept clean. Please wipe off all samples prior to inserting samples in the cavity. If you happen to break a sample in the cavity please notify a staff member immediately.
2. The N_2 transfer line along with the thermocouple assembly is extremely delicate and is easily damaged. Please exercise caution and care when using this accessory since others also depend on this piece of equipment. If it does become worn or damaged, inform a DCIF staff member immediately!
3. The cavity iris is *extremely* fragile. When tuning the cavity please do not run the iris adjustment all the way into the ‘in’ position. This will result in a broken iris (major $$) and considerable down time.

When you are finished:

1. **Open the** '**Microwave Bridge Control**' dialog box and switch the microwave bridge to '**Stand By**' and then close the '**Microwave Bridge Control**'. Note: **THIS IS VERY IMPORTANT.**
2. Carefully remove the sample from the microwave cavity.
3. Turn off the waveguide the nitrogen purge.
4. Insert an upper collet blank plug.
5. Exit the Win-EPR acquisition program.
6. Record your EPR usage in the logbook. Record any specialty equipment you may have used and report any irregularities to a staff member. If anything was damaged or has become suspect please report this directly to a staff member.

Common Problems:

No or Abnormal Cavity Dip

1. **Verify the waveguide gasket is properly installed**. The gasket in the waveguide will fit even though it has been rotated by 90º.
2. **Check that the cavity is neither undercoupled or overcoupled**. Look at the microwave frequency where you would normally expect the cavity to resonate and then adjust the iris screw for optimal coupling. Mode tuning patterns can be seen in Figure 5-2 of the EMX User's Manual. Poor coupling can be an issue when working with lossy samples such as aqueous solutions. Using a flat cell or capillary will help.
3. **Increase the microwave power**. If you are using insufficient microwave power, it can be difficult to see the cavity dip. Try increasing the attenuation value of ~20 dB.

Unable to Critically Couple the Cavity:

1. **Sample position**. If too much of a lossy sample is in the absorption region you will not be able to critically couple the cavity. Gently move the sample up and/or down until you are able to achieve critical coupling. Be careful not to position the pedestal in the absorption region, as the pedestal will absorb microwave radiation.
2. **Microwave reference phase**. The proper reference phase is crucial to achieve critical coupling. Follow the above procedure to re-tune the cavity properly.
3. **Condensation in the cryostat**. Water is a *huge* microwave absorber. If you are running a low temperature experiment, make sure that you have purged the cavity with dry nitrogen to minimize water losses. Water absorption can also be a problem on humid days.

No Signal When Everything Else Seems OK:

1. Check all of the cables. Check both the modulation and the preamplifier cables. Even if these look OK remove and re-seat them.
2. Check the sample position. Verify that the sample is well centered in the cavity. Also check to make sure that the pedestal is not up into the cavity region.
3. Check the magnetic field values. Verify that you are using the correct field values for your EPR signal.
4. Check that the magnet power supply is turned on. If the power *is* off, turn it back on by pressing the AC Power button (green), the Reset button (white), then the DC Power button (blue) in that order.

Optimizing Experimental Sensitivity Guideline:

Instrument sensitivity is critical in acquiring the best data possible. Following these simple guidelines will assist you in optimizing the signal to noise, obtaining the cleanest data possible, and minimize your instrument usage (and frustration) time.

Instrumental Factors:

Minimize microphonics:

The high Q of the microwave cavity makes the system susceptible to microphonic generated noise. Vibration of the sample within the cavity may modulate the mode patterns, frequency, and Q of the microwave cavity. Depending on the nature and frequency of the microphonics (the power spectrum of the noise), these vibrations may generate noise spikes or contribute to the white noise. Prevent microphonic noise pick-up by securing the waveguide with the waveguide stabilizers. Secure the sample in the cavity by tightening the collets on the cavity sample stack to hold the sample rigidly in place. Do not place objects on the microwave bridge or the magnet table that may vibrate or are free to move.

Maintain a controlled environment for the best spectrometer performance.

Air drafts past the spectrometer, especially the cavity, may induce temperature fluctuations or microphonics from sample vibration. Large fluctuations in the ambient temperature may degrade performance by reducing the frequency stability of the cavity. Very humid environments may cause water condensation when you are cooling your sample with a variable temperature system. Condensation inside the cavity may be reduced by maintaining a constant purging stream of dry nitrogen gas. Note that excessive gas flow rates may generate microphonic noise through sample vibration.

Minimize electrical interference.

Noise pick-up from electromagnetic interference (EMI noise) may be encountered in some environments. It may be possible to reduce EMI noise by shielding or perhaps by turning the noise source off if generated by equipment near the spectrometer.

Carefully follow the procedure in the spectrometer manual for positioning the sample inside the cavity.

This is particularly important for samples exhibiting a large dielectric loss. Improper sample positioning may perturb the microwave field mode patterns in the cavity, resulting in reduced sensitivity.

Periodically check the iris coupling screw for tightness of fit.

A worn iris screw thread will make the iris susceptible to microphonics which can modulate the cavity coupling.

Critically couple the cavity.

Best cavity performance is obtained with a critically coupled or matched cavity. Maximum transfer of power between the cavity and waveguide is realized in this condition.

Adjust the AFC modulation depth to minimize the noise level observed in the absorption EPR spectrum at full incident microwave power.

Adjustments of the AFC MOD LEVEL potentiometer, which is located on the rear of the microwave bridge, should be made while in the Operate mode. The sample to be investigated should be inserted in the cavity and tuned according to the spectrometer manual. This adjustment should be made for all experiments limited by signal to noise considerations. The optimum AFC modulation depth is a function of the loaded cavity Q. Consequently, slight variations in the optimum setting may be anticipated. If you are using a finger dewar with a boiling refrigerant such as liquid nitrogen, you should turn the AFC modulation level to maximum.

Insert a cryostat in the cavity.

Quartz has a dielectric constant of 3.8 but a low dielectric loss. Insertion of high purity quartz sleeves, such as the variable temperature dewar, actually concentrates the microwave magnetic field intensity at the sample. The increased field intensity produces an EPR signal that has a larger signal to noise ratio than is achieved in the absence of the dewar insert. Experiments limited by the signal to noise ratio may benefit by the use of the variable temperature quartz insert dewar, even if the experiment is run at room temperature.

Parameter Selection:

Optimize the receiver gain.

You need to have sufficient receiver gain in order to get a good signal to noise ratio. Clipped signals in a spectrum are indicative of an overload in the signal channel. Use the highest power possible with out saturation.

Optimize the time constant.

The time constant filters out noise; however, if an excessive time constant is chosen, the signal distorts and diminishes. A safe and conservative rule of thumb is to make sure that the time needed to scan through an EPR signal (i.e. one EPR line) is ten times greater than the length of the time constant.

Optimize the field modulation amplitude.

Excessive field modulation broadens the EPR lines and does not contribute to a bigger signal. An optimal field modulation amplitude for maximum sensitivity is approximately the width of the EPR signal. It is recommended to start with a modulation amplitude of ~10 gauss for most transition-metal species and ~1 to 2 gauss for radical type species.

Optimize the microwave power level.

The EPR signal intensity grows as the square root of the microwave power in the absence of saturation effects. When saturation sets in, the signals broaden and become weaker. Several microwave power levels should be tried to find the optimal microwave power.

Hints for Finding EPR Signals:

If you are having problems finding an EPR signal use the following steps to assist you in diagnosing the problem. If these guidelines don't work, contact a DCIF staff member immediately.

Make sure that the spectrometer is functioning properly.

If you followed the directions of the spectrometer manual, this should not be a problem. There are many common mistakes. Is the modulation cable connected properly to the cavity and console? Is the waveguide gasket installed properly? Is everything turned on?

Scan over the correct magnetic field range.

If you look at the wrong magnetic field values, you will miss your signals. This mistake occurs quite often when using a cryostat in the EPR cavity. Consult literature references to determine approximate g-values for the species in your sample. You can then choose the appropriate magnetic field for your sample. Most organic radicals will have a g-value of approximately 2. This corresponds to a field for resonance of approximately 3480 Gauss at a microwave frequency of 9.8 GHz. Metal ions can have large departures from $g = 2$ as well as large zero-field splittings, making it difficult to guess where the resonance might occur. Performing wide scans maximizes the probability of finding the EPR signal.

Use an appropriate microwave power level.

Using more microwave power does not necessarily result in a more intense EPR signal. Saturation can occur. Organic radicals in non-viscous solvents or many samples at cryogenic temperatures are susceptible to saturation effects. Try running the EPR experiment at several different power levels.

Use appropriate signal channel parameters.

The parameters should be optimized for the type of signal to be expected. **(See Parameter Selection.)** If you get a straight noiseless line for a spectrum, the signal channel may be overloaded. The needle of the Receiver Level meter will be pegged on either side of the meter. If this occurs, turn down the receiver gain, microwave power or the modulation amplitude.

Optimize the sensitivity.

You may have a very weak signal. Follow some of the suggestions in the "Optimizing Sensitivity" section above. You may also need to increase the number of averages.