Bruker EPR Procedure

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Sample Preparation

- **Sample Tubes:**
  a.) Standard EPR tube size = **4mm OD**, however, sizes do vary (2mm, 3mm, 5mm, 10mm) and can be accommodated by the instrument. (4mm and 5mm OD are the ONLY sizes for cryogenic experiments)

  b.) EPR tubes are made out of quartz whereas NMR tubes are made out of Pyrex, which contains some iron that may or may not, depending on your sample, contribute to the EPR signal.

  c.) LOW CONCENTRATION SAMPLES:
  Suprasil (synthetic) quartz tubes should be used over clear-fused (natural) quartz b/c Suprasil will not have contributing background signals due to e- holes (E' Signals) in the quartz (SiO2) matrix.

  The E' signal is very weak and should not be a factor if there is a strong signal from the sample.

- **Minimum:** **(a)** *Sample Volume:* ~ 20 µL
  (b) *Spin Concentration:* ~ 10^{-9} M (Minimum # of spins = 10^{11})

- **Aqueous Samples:** The water in aqueous samples absorbs the electric part of the microwaves and begins to heat (like your microwave at home), and therefore, the sample size needs to be limited, specifically the surface area of the sample size. Users have a few options, two of which are
  a.) Use special sample tubes that are available upon request
  b.) Use capillary tubes

Getting Started

1.) Turn on the following (if not already on):
- Water Chiller (allow 10-15 minutes for the chiller to come to temperature)
- Console
- Power Supply (located directly underneath console)

- N_2 gas flow (orange tubing connected to back of waveguide) set to ~ 1.0 SCFM

  ** This purges the waveguide of any oxygen that may cause erroneous signals in your spectra.

2.) Open the WinEPR Acquisition software 

located on the desktop

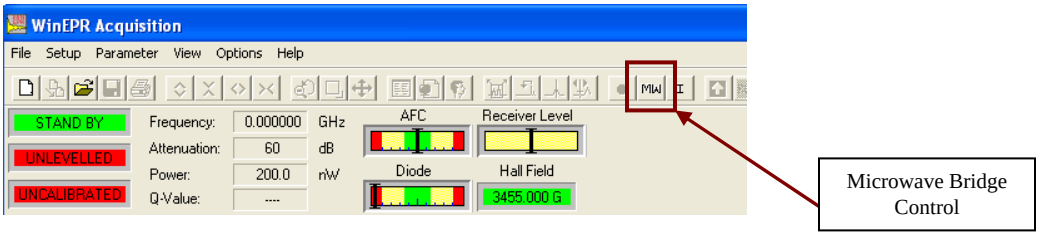
4.) Fill out the Recharge Log with your name, advisor, department, 13-digit recharge number, time started, and experiment being performed (i.e. Room T, VT, He, or ENDOR).

Tuning the Cavity with Sample Inserted

1.) Insert the correct size collet into the resonator and screw on the black nut; leave it loose. Be careful NOT TO STRIP OR CROSSTHREAD THE THREADS ON THE WHITE TEFLON PIECE. If there is ANY resistance STOP and retry.

2.) Wipe the outside of the tube with acetone or solvent of choice. Insert your sample to the appropriate depth (using the Depth Gauge), making sure to insert it straight down so you do not break the quartz dewar or your sample in the cavity, and tighten the black nut finger tight.

2.) Open the *Microwave Bridge Control* by clicking the "MW" icon. The system should be in ***Standby*** mode when opened.



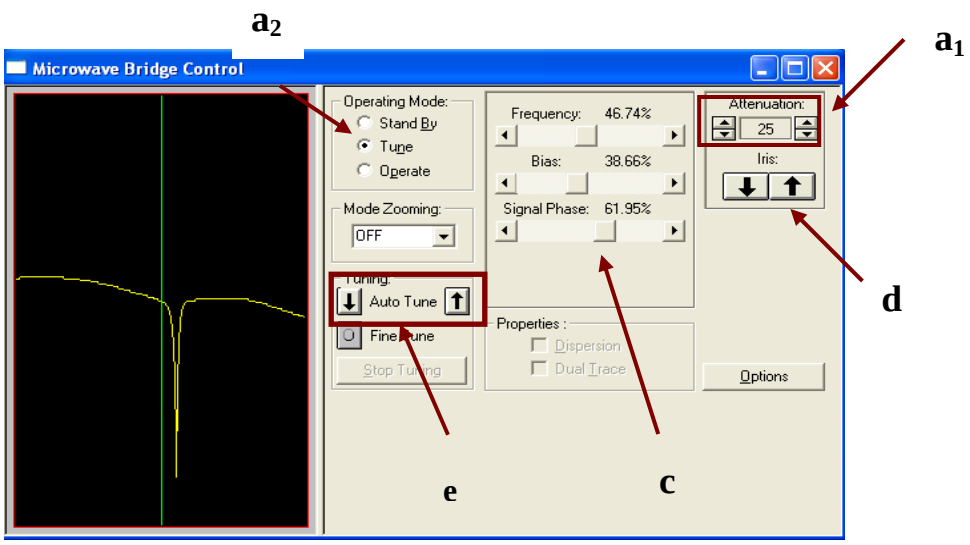
3.) a.) Click the ***Tune*** mode. Wait for the tuning dip to appear; it takes ~ 10 secs so please be patient. Set the ***Attenuation*** (i.e. power, however, they are inversely related, so a higher Attenuation = lower Power) to ***25 dB***. The arrow buttons 

on the left of the ***Attenuation*** value allow you to move in increments of ten and the arrow buttons on the right allow you to move in single digit increments.

If you see a line but no dip, you can find the dip by moving the ***Frequency*** cursor left or right; it should be around 9.44GHz (23%) if the dewar is installed in the cavity.

Using the ***Frequency*** cursor, get the dip near the center of the window.

(Refer to figure on next page)



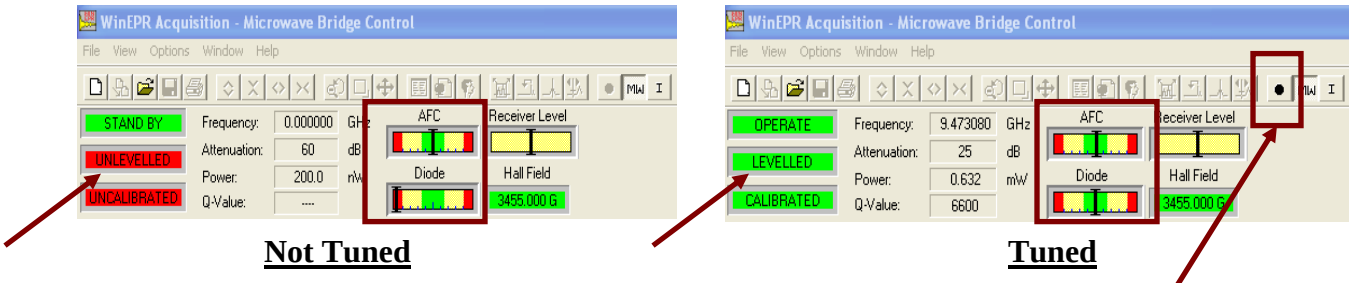
b.) Allow the system and your sample to thermally equilibrate for ~10 minutes.

Steps (c) and (d) are <u>OPTIONAL:</u>

c.) Move the ***Signal Phase*** until the dip is as symmetric as possible. While maintaining as much of the depth as possible.

d.) Move the ***Iris*** motor up or down until the tuning dip is as narrow and deep as possible. You may have to re-phase the dip afterwards.

e.) Click ***Auto Tune*** Up (↑) if tuning dip is to the right of the center line or Down (↓) if the tuning dip is to the left.
Wait for ***Auto Tune*** process to finish – Everything will be green and the *AFC* and *Diode* cursor will be in the center/green area.



If *AFC* and *Diode* are not exactly in the center, then you can hit the ***Fine Tune*** button.

<u>If the Auto Tune process cannot tune your sample</u>, then please follow the **Manual Tuning Procedure** below.

* It is sometimes helpful to use the Manual Tuning Procedure to tune your first sample and then use the Auto Tune feature to tune the rest of your samples.
* <u>If you have aqueous samples</u>, you will <u>always</u> have to manual tune.

Manual Tuning Procedure

1.) In the MW Bridge Control box go to ***Tune*** mode. Set the attenuation to **25 dB**. Locate and center the tuning dip using the ***Frequency*** slider control (it will be ~ 23% if the dewar is installed in the cavity and ~98% if there is no dewar).

2.) Adjust the ***Signal Phase*** slider to make dip as symmetric as possible. Adjust the frequency if necessary.

3.) Go to ***Operate*** mode (wait, it takes ~ 10 secs). Increase the attenuation to **50 dB**.

4.) Adjust the ***Bias*** slider so the diode current meter is centered.

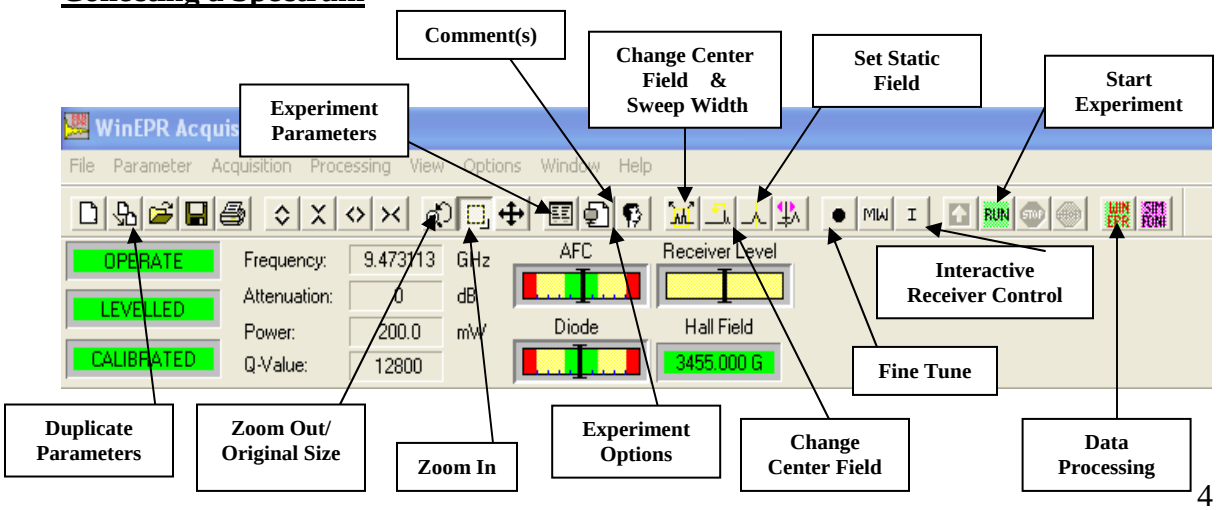
5.) Lower the attenuation down to **30 dB** and adjust the diode current back to the center by clicking and holding down the ***Iris up/down arrows***  . Center the ***AFC*** as necessary by using the ***Frequency*** slider. Repeat for **20 dB** and then **10 dB**, or the lowest desired (i.e. 2 dB for the Power Sweep). At 2 dB the diode takes a long time to stabilize and tends to drift.

6.) 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.

7.) 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.

<u>Once your Sample is Tuned</u> close the MW Bridge Control box.

Collecting a Spectrum



1.) Open the *Interactive Spectrometer Control* ("I" icon next to MW on toolbar) and make sure the *Calibrated* box is checked. Close the window.

* If you forget this step, then when you run your first experiment it will say "uncalibrated" in red letters at the top of your spectrum. Check the box and then click on "Set Parameters to Spectrum" and then click on the spectrum with the "P" that appears as the mouse cursor.

2.) (a) If you **do not know** where your signal will be – Use the DEFAULT PARAMETERS:

- *File => Open => My Computer =>Local Disk (C:) => EPR Data => Default Parameters*
- Open the file that best suites your sample
- AS SOON AS YOU OPEN IT save under a different name: *File => Save as…=> My Computer => Local Disk (C:) => EPR Data => Your Folder => New File name*
- Click **Run** (the green button on the top about 2/3 to the right)
- Optimize the parameters for your sample (refer to the "Optimizing Parameters" section below)

(b) If you **do know** where your signal will be*

- Open a New Spectrum Window
- Open the Experiment Parameters and fill in as applicable
- Click Run
- Optimize the parameters for your sample (refer to the "Optimizing Parameters" section below)

* To load parameters from a previously saved experiment:
Open the saved data set and then click the *Duplicate* button. This opens a new spectrum window but with all of the same parameters from the saved data.

(c) Once you have your parameters optimized for your sample, **TAKE A BACKGROUND** spectrum. The parameters for your background need to be identical to your scan parameters for your sample. In addition, an empty tube should be inserted into the cavity, preferably the same tube you will put your sample in.

Optimizing Parameters

1.) ***Center Field and Sweep Width*** – Center field should be set at the center of your EPR signal and the sweep width should include the signal and enough baseline. Once you can see your signal you can click on the "Change center field and sweep width" icon (refer to image on page 4) in order to set the appropriate values. Size the box to the desired size by clicking with the left mouse button and dragging at each corner. Once you have your desired size set it by clicking the right mouse button then RERUN your spectrum. The new values WILL NOT be saved until you rerun the experiment.

2.) ***Receiver Gain*** – Depends on the strength of the EPR signal from your sample

Strong Signal = Low R.G.

Weak Signal = High R.G.

3.) ***Power Level***

Perform a Power Sweep

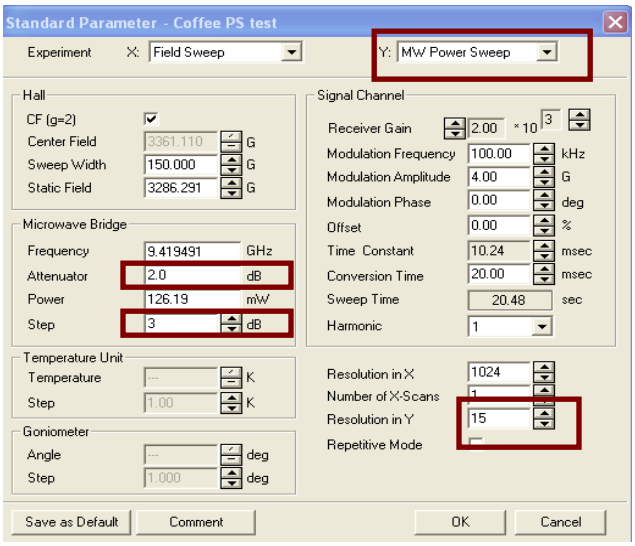
Once you've acquired a signal and have it centered with the correct receiver gain:

a.) Open the *MW Bridge Control*. Tune the cavity down to 2dB (Follow Manual Tuning Procedure). This is done b/c it is extremely difficult for the AutoTune function to tune to that high of power.

b.) Open the Parameters list. Select MW Power Sweep. Set the following (suggested values only):

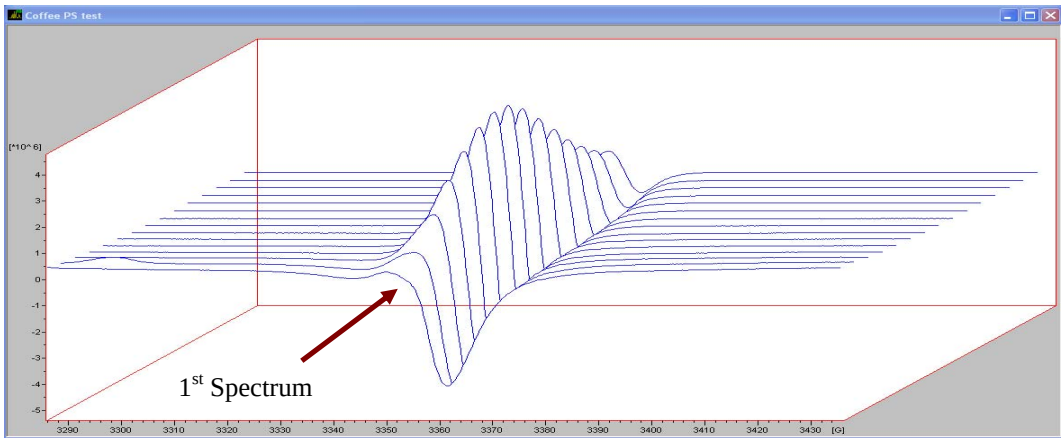
Attenuator = 4.0 dB Resolution in Y = 15 (number of spectra)

Step = 3



Click OK. Then click Run.

Once acquisition is finished, right-click on the spectrum and go to *Display Type => 2D Stack Plot.* Right click again and check *Whitewash* (this shows only positive values). The spectrum with the highest intensity is your optimal power.



If you would like a more precise power value, you can run the Power Sweep again with a smaller range and increment.

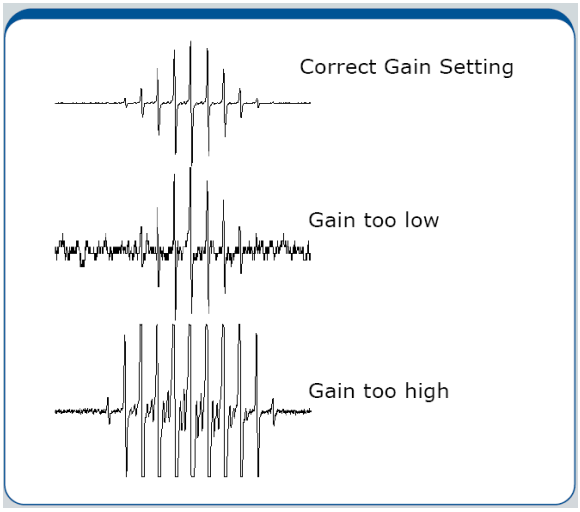
4.) ***Modulation Amplitude*** – Helps decrease low frequency noise (i.e. thermal fluctuations, electronic vibrations, drafts in the room, etc.)

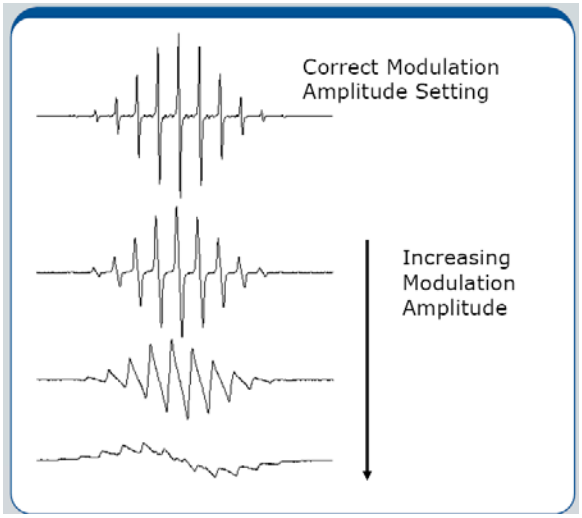
Refer to Appendix A on page 11 for more information

→ Compromise between sensitivity and resolution

→ If too large => Will distort/broaden signal
If too small => Disadvantage because S/N will not be as good

→ This value is sample dependent
* The more hyperfine splitting, the smaller the value should be



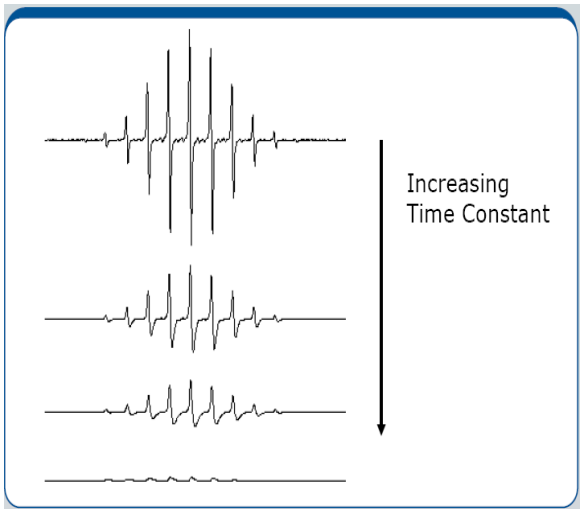


5.) ***Time Constant*** – filters out high frequency noise

→ Set to 1/10 the time it takes to sweep through the narrowest line of interest
**Want to increase for weak signals

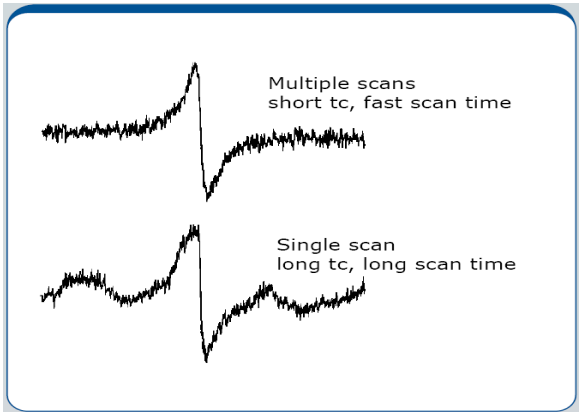
→ Too Low => Get a lot of noise

→ Too High => Filters out your EPR signal

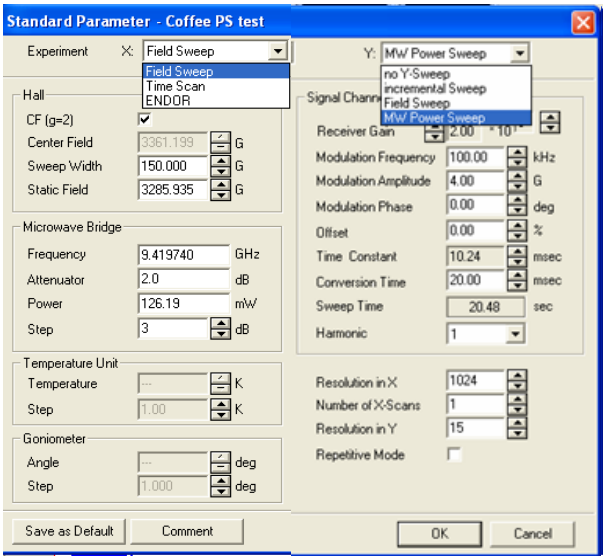


6.) ***Conversion Time*** – The amount of time the AC to DC converter spends at each point.
→ Rule of Thumb: Set = to the Time Constant

7.) ***Number of X-Scans (i.e. Signal Averaging):*** The baseline quality of your spectrum is improved by accumulating several short scans rather than taking one long scan.



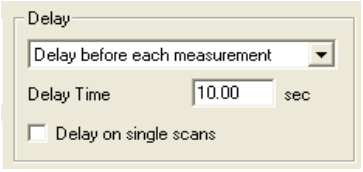
Performing 2D Experiments



1.) ***Incremental Sweep*** – Useful for:

(a) measuring a sample that changes with time and you want to observe the change over the entire field or

(b) putting together a 2D data set with several different samples (a time delay can be set between scans so there is time to change samples: Go to *Parameters=> Options => Delay*).



2.) ***Temperature Sweep**** – Useful for samples that change as a function of temperature (a time delay can be set).

***** Variable Temperature Training needs to be completed before attempting this experiment

3.) ***Time Scans*** – Useful for monitoring the kinetics of a sample that changes in intensity with time at one particular field value.

Please ask for help if you would like to run this experiment.

4.) ***Field Sweep*** – Allows you to run time scans at different field positions to create one 2D data set if used in conjunction with the time scan feature.

Saving Data

1.) As a WinEPR File

File => Save as… => My Computer => EPR Data => Your Folder name

** The software saves two files: one for the spectra and one for the parameters. Be sure that if you move one, you move the other by clicking on All Files from the drop down menu.

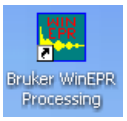
2.) As an ASCII file

File => Export… => Destination (notice the file type is ASCII format)

You can open this file in Excel and it is delimited with tabs; format as necessary.

Data Processing

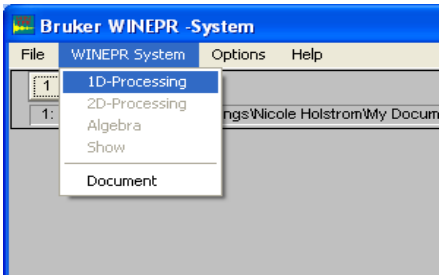
1.) Open the WinEPR Processing software by double clicking on the icon located on the desktop:



or by clicking  on the menu bar of the WinEPR Acquisition software.

2.) To open a spectrum, click *File => Load =>* and choose a file (more than one can be loaded at the same time by holding down the CTRL key and clicking on each file).

3.) Click once on the number that corresponds to the spectrum you want, then click on *WINEPR System => 1D Processing* (or *2D processing* if you performed a 2-D experiment)



To switch between loaded spectra, double click on the number and the corresponding spectrum will appear in the screen, but only after 1D processing has been selected.

4.) To create a 2D data set from 1D experiments:

a.) Load all spectra in the order you want them to be included in the 2D data set (you can load all of them at the same time by holding down the CTRL key and clicking on each file)

b.) Click *File => Create 2D File…* Select files and click OK.

c.) Click on the very last # at the top (this is your 2-D data set) then go to *WINEPR System => 2D-Processing*

5.) To get back to the original menu (i.e. you're in 2D Processing and want to do some 1D Processing on another spectrum) then go to *1D/2D Processing => WinEPR System* and repeat step (3)

When you are Finished

1.) Open the ***MW Bridge Control*** and put the system in ***Standby*** mode. Every time you change samples you must put the system in ***Standby***.

2.) Turn off the console, power supply, and N_2 purge gas. Turn the Chiller off **LAST**.

3.) Remove your sample and replace the plastic cover over the collet (this helps keep the cavity free of contamination.)

4.) Save any data and close the software; log off your account

5.) Finish filling out the rest of the recharge sheet.

Appendix A

Magnetic Field Modulation

Used to improve the sensitivity of the spectrometer by "encoding" the signal into a sine wave. The magnetic field is modulated (i.e. varied) at a certain frequency (normally 100 kHz) which will then cause the EPR absorption signal to have the same frequency. The phase-sensitive detector will only detect signals that are within +/- 1 kHz of this modulation frequency. This technique increases the S/N by filtering out low frequency noise.

Excessive modulation amplitude or frequency can lead to signal/line distortion. The setting of each depends on what is more important to the user: sensitivity or resolution.

If sensitivity is more important and some lineshape distortion can be tolerated, then the modulation amplitude should be increased until a maximum EPR signal amplitude is obtained with minimal signal distortion.

If resolution and true lineshape is more important, then the modulation amplitude should be set at ~ 0.2 x's LESS THAN the actual line width (only rule of thumb).

A compromise b/t sensitivity and resolution is to set the modulation amplitude to 4-5 x's LESS THAN the mod amp at max signal intensity (only rule of thumb).

Determining What Modulation Amplitude to Use (Range: 0.1 – 20G)

(1) Run an experiment, find the signal, set appropriate field sweep and gain
(2) Determine and set optimum power level
(3) Run a series of experiments where only the modulation amplitude is changed:

For Resolution – If by decreasing the mod amp decreases the line width, then continue decreasing the mod amp until there is no more line broadening – this will be the mod amp for maximum resolution.

For Sensitivity – Perform the resolution experiment above and then increase the mod amp until a maximum signal intensity is seen without compromising too much resolution (i.e. minimizing line distortion/broadening).