EPR Operating Procedures – Spectra Acquisition

Creating New Experiment:

1. Turn EPR machine ON according to "EPR Operating Procedures – Machine".
2. Log on to user computer, open **XEPR** from desktop.
3. Click **Acquisition** from top toolbar, select **Connect to Spectrometer** from dropdown menu, popup window should say **Server Name: localhost**, click **OK** and wait.
4. Create a New Experiment by clicking **New Experiment** from bottom toolbar, name the experiment by the following convention: Initials_ Date(YYYYMMDD)
   - Ex: MH_20170619
5. Ensure you are on the **C.W.** tab, with Abscissa 1 displaying **Field**, Abscissa 2 displaying **None**, and Ordinate as **Signal Channel**, click **Create**. This creates a spectrum with field strength as the X-axis and signal intensity as the Y-axis. If another Abscissa is preferred, select it here.

Acquiring Calibration Spectra:

6. Click the button on the bottom toolbar labeled **Open tuning panel**.
7. Select **Tune** from the popup window. Wait for green line to stabilize.
8. Ensure Attenuation [dB] is set to **30.** Press Enter. You should see a v-shaped dip in the tuning channel. If not, move the **Frequency** slider until it comes into view. The frequency shown on the microwave bridge should be around 9.8 GHz with no cryostat, and 9.3-9.7 GHz with one.
9. Take a labeled standard sample from the EPR rack, either DPPH or Strong Pitch, and insert it into the resonator to an appropriate height (~8 cm into the collet). Tighten the collet. The v-shaped dip should slightly shift position if inserted correctly.
10. Adjust the dip so that the trough is right at the center line using the **Frequency** slider. Clicking on the grey bar makes a macro adjustment, clicking on the black arrow makes a micro adjustment.
11. Adjust the dip so that both wings left and right of the trough is leveled using the **Signal Phase** slider. Readjust frequency if necessary.
12. Adjust the dip to be as deep as possible using the **Bias** slider.
13. Once the dip is appropriately tuned to the center line, switch from tune to **Operate**. The tuning channel should now be blank.
14. Click **Fine** under the **Auto Tuning** panel.
15. Notice the bottom two sliding panels of **Diode Current** and **Lock Offset**. The fine tuning should adjust both values to approximately the middle of the slider, 200uA for Diode Current and 0% for Lock Offset. A slight drift to the left or right is expected. The fine tuning should stop eventually and the **Auto Tuning** panel will be selected to **Stop**.
16. Set the **Attenuation** to 20. Repeat steps 14-16. Then set it to 10, repeat steps 14-16. Then 0, and repeat steps 14-16. Now the resonator and the standard sample is tuned all the way down.[1]
17. Close the tuning panel. Click the **Open parameter panel** button next to it.
18. Under **Signal Channel**, select the **Detection** panel and set the parameters to the following:

Calibrated:	Selected	Receiver Gain:	40
Modulation Frequency:	100.00	Time Constant:	10.24
Modulation Amplitude:	1.000	Conversion Time:	20.48
Modulation Phase:	0.0	Sweep Time:	20.97
Harmonic:	1	Offset:	0.0

19. Under **Absc. 1: Field**, set the Field Position to **Left.**
20. Under **Microwave**, set the **Attenuation** to 25, select the **Acq Fine Tuning** to **Each Slice Scan.**
21. Close the parameter panel.
22. Click the **Run/Abort Experiment** button on the bottom left corner. Click away any popup message if applicable.
23. The resulting spectra should show one clear absorption peak around 3480 G.
24. Click on the small red spectra button near the top toolbar, click **Save on Disk**. Navigate to the /xeprFiles/Data directory. Select or make a new folder with your name by naming the folder in the tool bar next to /xeprFiles/Data/_____, and then hit the new folder button next to the navigation bar. Name both the **Filename** and **New Title** as Standardname_Calib_YYYYMMDD. Ensure **Dataset Type** is set to **Primary**, select **Save**.
      - Example: StrongPitch_Calib_20170619

Acquiring Sample Spectra:

25. Open the **Tuning Panel**. Select **Tune**. Set the **Attenuation** to 30. Hit Enter.
26. Remove the calibration sample from the machine, replace it in the EPR rack. Insert your own sample to the appropriate depth into the machine and tighten the collet.
27. Tune the machine to your own sample by following steps 10-17. Decrease the attenuation by 5 if no dip is visible at 30 attenuation even after adjusting the frequency slider.
28. Close the tuning panel. Click the **Open parameter panel** button next to it.
29. Adjust parameters in the **Signal Channel: Detection** panel by the following guidelines:
      - Calibrated: Always selected.
      - Modulation Frequency: Keep at 100.
      - Modulation Amplitude: Adjust at most to 8 to increase peak resolution, too much and you may risk over broadening the signal. 2 is recommended to start. Spectrum must exist independently from modulation amplitude.
      - Modulation Phase: Change from 0 to 360 in increments of 30 to find optimal resolution.
      - Harmonic: 1
      - Receiver Gain: Adjust between 20 – 70 to adjust peak resolution and height. 40's a good place to start.
      - Time Constant: Higher value = longer scan = cleaner signal. Start with 10.24.
      - Conversion Time: Double time constant.
      - Sweep Time: Coupled to Conversion Time value, set automatically.
      - Offset: 0
30. Adjust parameters in the **Field** panel by the following guidelines:
      - Field Position: Will be set automatically
      - Center Field: Set to 3480 if g value is unknown
      - Sweep Width: Set to 300 for unknown organic radical, 6000 for unknown transition metal complex.
      - Sample g Factor: Enter it if known
      - Set Field position to start on the LEFT
      - Number of points: More points = better resolution. Start with 1024.

- Set Field to Sample g-Factor if known.
31. Microwave:
    - Attenuation: Higher attenuation = lower microwave power. 10 is recommended to start.
    - Acq Fine Tuning: Each slice scan, this means fine tuning will occur before each scan.
32. Scan:
    - Auto Scaling: On
    - Replace Mode: Off
    - Auto Offset: Off
    - Number of Scans: More scans mean less noise and clearer resolution for signal. 1 – 20 recommended.
33. Close the parameter panel.
34. Click the **Run/Abort Experiment** button on the bottom right corner. Wait for auto fine tuning to occur. Click away any popup message if applicable, then wait for spectrum to be acquired.
35. Adjust parameters as necessary to optimize spectrum until satisfied. Repeat step 34 to run experiment.
36. Click on the small red spectra button near the top toolbar, click **Save on Disk**. Navigate to your own folder with your name under the /xeprFiles/Data directory, select that folder. Name both the **Filename** and **New Title** by your convention. Ensure **Dataset Type** is set to **Primary**, select **Save**.
37. Repeat steps 26-36 for different samples.

Disconnecting Spectrometer:

38. Open the **Tuning Panel** and put the spectrometer in **Stand By**. The tuning window should now be blank again.
39. Remove your sample from the resonator.
40. Go to **Acquisition** and select **Disconnect From Spectrometer**.
41. **Optional:** Turn EPR machine OFF according to "EPR Operating Procedures – Machine".

Baseline Correction:

42. Have the spectrum you want to export pulled up as the primary spectrum. It should be displayed as red and its name would be in the primary window, next to the little red spectrum icon. Click on the little black box next to the red spectra icon to see a list of your spectra. Baseline correction deletes the uncorrected spectra after your correction, so be sure to make a copy if you want previous versions.
43. Click **Baseline Correction** on the left-hand toolbar. Select either **Polynomial** or **Spline**.
44. For Polynomial, select the applicable correction. **1ˢᵗ order** is a good starting point. You'll see a yellow line appear on your spectra.
45. Click **Subtract Line** and then **Ok**. Save the new spectra by a name of your convention.
46. The corrected spectra will be saved in both the primary spectra slot and the yellow result spectra slot. You can click on the black box next to it and select "no result" to get rid of it.

Integration:

47. Use this function if you want to quantitatively compare amongst samples. The resulting integration is the area under the curve and can be taken as a measure of intensity. For the comparison to be meaningful, ALL parameters must be identical amongst samples.
48. Have the spectrum you want to integrate pulled up as the primary spectrum.
49. Click **Integration** on the left-hand toolbar. Select **Double Integration**.
50. Click **Show Report**. Mark down the area in your notes. Close the tab.
51. Click **Ok**. The double integration curve is now saved under the yellow result spectra slot.
52. Pull up the next spectrum you want to integrate to the primary spectrum screen. Repeat above steps for all spectra.

Exporting Data:

1. Have the spectrum you want to export pulled up as the primary spectrum. It should be displayed as red and its name would be in the primary window, next to the little red spectrum icon. Click on the little black box next to the red spectra icon to see a list of your spectra.
2. Go to **File**, click **Export ASCII** and navigate to your folder. Save it by your own convention.
   Note: It is recommended to copy all three files, DTA, DSC, and ASC, to your own USB. Different programs use different formats.

[1] – Troubleshooting Tuning:

A few things may happen in the tuning process that prevent the machine from automatically tuning to your sample:

1. The machine cannot find the signal in the resonator. Solve this by manually tuning the frequency and signal phase sliders to create the deepest trough possible. Adjust the iris arrows to assist. Lower attenuation in increments of 5 starting from 40 and keep trying to fine tune it until it finds the signal.
2. Fine tuning may reach a point where the machine displays: **Lower/Upper Iris Limit**. Close the popup and use the arrows underneath the iris to correct the iris position until adequate. If the Iris has visibly spun out of its guide, follow the steps to replace it:
   i. Put the machine in **Stand By** mode from the tuning panel.
   ii. Click the button labeled **Options** in the tuning panel.
   iii. Physically go to the resonator, visually confirm that the iris has spun out of its white guide, and if so, gently move the iris arm (bronze colored) out of the way and turn the guide **counter clockwise** until it is all the way to the bottom.
   iv. Click **Iris Run Down** from the Iris control panel. Make sure **Autotune Iris Preadjustment** is off and **Iris Motor Present** is selected. Wait.
   v. Physically replace the Iris arm back to its guide, gently turn the guide to make this possible if necessary. The Iris arm can slide laterally and move horizontally.
   vi. Close the options panel and resume tuning.
3. If above steps don't resolve the tuning issue, you may try one of the following options:
   a. Adjusting sample concentration/volume. Adjust and attempt until success.

      b. If you think your solvent may be of too high dielectric constant, you may request to use the **flat cell resonator**[2]. Contact the facility manager for this.

   4. Ask your facility manager for the blue binder EPR operations manual. Consult sections 8 and 9.

[2] – Flat Cell Resonator Instructions:

The Flat Cell Resonator is designed to allow a minimal surface area of your sample to be exposed to the magnetic field so that the signal acquired may be of higher resolution. It makes obtaining spectra from samples with high dielectric solvents possible. Aqueous solutions are included in that list.

1. The Flat Cell is only compatible with the 4102ST resonator. The resonator should be gold plated. Ask the facility manager to help you switch resonators if necessary.
2. Obtain the Flat Cell Resonator from the facility manager.
3. Ensure that the glass stopper is inserted into the end which aligns it through the collet direction, as in the tip of the glass stopper points to the unperforated, thinner part of the white collet.
4. Put the machine in **Stand By** mode and physically remove the collet currently in the resonator by unscrewing the black collar and pulling the white collet out. Do not replace the collar yet.
5. Fill the flat cell resonator using a pipette. Draw your sample throughout the entire area of the rectangle by gently pulling out the glass stopper, very slightly to release pressure.
6. Wipe clean the outside of the flat cell resonator. Insert it into the resonator gently.
7. Align the resonator so that the edge, rather than the face, of the flat cell is aligned with the magnetic field. This means if you are to stand in front of the resonator, you will face the large surface, not the edge.
8. Replace the collar and begin tightening. Before completely tightening, pull the flat cell resonator up so that the entirety of the smoked glass part is above the collar and collet. Tighten fully.
9. Put the machine into **Tune** mode and follow tuning procedures.
10. When the spectrum has been acquired to satisfaction, put the machine into **Tune** mode, remove the collar, and remove the flat cell with the collet. Drain the sample by removing the glass stopper and draw out any remaining sample using a paper towel. Use water and methanol to clean the interiors and blow dry with a can of compressed air. Let air dry if necessary.
11. Return the Flat Cell to the facility manager when done.

Notes from Matt:

This guide serves as a procedure manual so that you may operate the machine and obtain spectra smoothly. Think of it more as an answer to how than why. For those answers, please refer to two wonderful books on EPR, both of which may be found in the McGill Library system:

Electron Paramagnetic Resonance, Second Edition by John A. Weil and James R. Bolton

Biomolecular EPR Spectroscopy by Wilfred Raymond Hagen