

Recording EPR Spectra using SHQE Resonator

This protocol gives step-by-step instructions for recording an EPR spectrum using the high sensitivity Bruker SHQE cavity (**assuming the SHQE is already in place and you have been trained on the instrument**).

- Reading this protocol **does not replace** the **training** provided by Dr.Erbse. (*Annette Erbse, Office C316, ext. 2-0528, erbse@colorado.edu*)
- Before you are allowed to use the instrument by yourself, **one-on-one training** with Dr.Erbse **and** an okay directly from Dr.Erbse is **required**.
- After training you will receive access to the Google Calendar. All users should use the Google calendar to reserve instrument time and should log into the logbook at the beginning of each use. When you sign up using the on-line booking schedule **please check which resonator was in use**. If a different resonator was or will be used before the time you want to use the SHQE Contact Dr.Erbse (2-0528, erbse@colorado.edu). She will help you to change the resonator. Keep in mind that you must notify her at least a day in advance to allow time for the resonator change to be fit into her schedule. The earlier she knows the more likely she can make time!
- **Do not attempt to change the resonators without Dr.Erbse** or with the help of anyone else but her. You can **damage the instrument** severely and **you will lose the privilege** to use the instrument, regardless of damage.

Good Practice and Required Procedure:

Always take a spectra with a clean, solvent filled capillary/tube (or an empty tube if your sample is solid) **before you start and after you have measured the last sample for the day**. This is to check for possible contamination of the SHQE. Please save the spectra so that we have an up-to-date record in case some contamination should happen.

Important:

- **The SHQE resonator can only be cleaned by Bruker in case of heavy contamination. This might mean sending it to Germany. It will take several weeks and will be very expensive. Please be extremely careful to avoid contaminations.**
- **If you notice/suspect any contamination, inform Dr.Erbse immediately. The faster we can start clean up measures, the higher the chance of success without sending the resonator in.**

- *You are encouraged to read the Bruker manuals for a more details and in depth explanations. They are kept next to the instrument. Please do not remove them!*

Reserve Instrument Time

1. Make sure your PI has provided a speedtype for the EPR time.
2. We are charging \$5/hour. The money is used for upkeep and minor repairs.
3. After training you will receive access to the Google Calendar. This will give you access to the instrument signup. It would be best if you could plan your experiments well in advance. But please try to reserve time at least 2 days in advance. Please make a note to the admin during signup stating the resonator you want to use.
4. In order to allow everybody to have a chance to get instrument time we have the following rules:
 - Up to **Friday the week before** you want to use the EPR, you can reserve up to two days in a block.
 - In the week that you were planning to use it, if nobody else has reserved time, you can reserve additional days.
 - Users that were part of the SGI Grant that paid for the instrument can reserve 3 days in a block up to **Friday the week before** they want to use the EPR and more days in the week if nobody else has signed up.
 - If your experimental plans change, please adjust your reservation accordingly.
 - When you reserve the EPR send Dr.Erbse an email about your reservation **at the same time**.

Power up the EPR

1. Sign the logbook.
2. Switch the water on by opening both the inlet and the outlet valve.

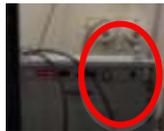
3. Turn on the heat exchanger.



4. Switch on the console (green button).



5. Switch on the magnet.



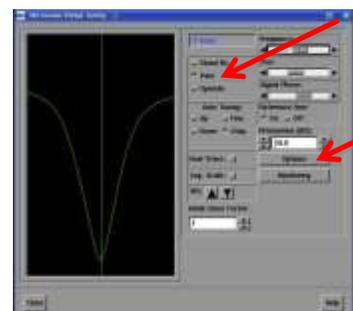
6. Watch the green LED on the bridge. It should go from off to blinking green to steady green. If it does not something is wrong. **Stop and inform Dr.Erbse.**



7. Open Xepr program (one  click)
8. Go to acquisition menu → connect to spectrometer → Local host → OK
9. Load the calibration data file: Go to acquisition menu → spectrometer configuration → signal channel → calibration data set → SHQE → apply → save → close

10. Note: If you don't have the right calibration file loaded you will damage the modulation coils of the resonator resulting in over \$5000 in repair costs!

11. Open tuning panel by clicking  → option → AFC option:
AFC AC gain%: 30
DC gain: 1/10
Time constant: medium high
Modulation%: 50



12. Close AFC option window
13. **Set the Iris limit. This is important! If the limit is not set properly you can break the Iris screw!!!**
14. Open tuning panel again. Go to options → *Iris run down*,
15. A window will open and ask if the Iris is disconnected. **Stop!!!**
16. Physically disconnect the iris from the iris motor.
17. **Only after the IRIS is disconnected**, click yes. The iris motor will start to rotate. Make sure it does not get caught on something.
18. Turn down the iris screw by hand all the way down and one turn back up.
19. Reconnect the iris motor.
20. In the tune window, click tune, wait for the Frequency, Bias and Phase slider to be activated, set the attenuation to 25 dB.
21. If you need publication quality data, warm the instrument up for at least 15 min before you start tuning and recording spectra. If you just want to work out conditions and parameters you can start right away. Keep an eye on the diode current and frequency lock since they might drift more if the EPR is not warmed up yet.

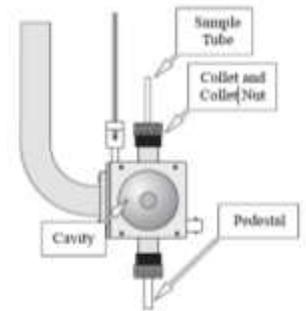
Inserting Your Sample

1. Make sure you are in Tune Mode and that the attenuation is set to 40 dB or higher.
2. **Never** move a sample in or out of the resonator without first switching into **Tune Mode** and setting the attenuation to 40dB or higher.
3. Fill your EPR tube with your sample. Make sure you add enough sample to fill the active volume of the resonator. Avoid air–solvent transitions in the active volume.
4. Be extremely careful to avoid contaminating the outside of your tube.
5. Wipe the tube several times with a fresh Kim wipe.

Important:

The SHQE cavity can only be cleaned by Bruker in case of heavy contamination. This might mean sending it to Germany. It will take several weeks and will be very expensive. Please be extremely careful to avoid contamination.

6. Make sure you have the right collet size mounted for your tube. If it is not tightened the tube should sit in it slightly loose.
7. Insert the tube gently into the cavity. Make sure the active volume of the cavity is filled with your sample.
8. Stabilize the lower end of the tube with the pedestal.
9. Tighten the collet around the top end.

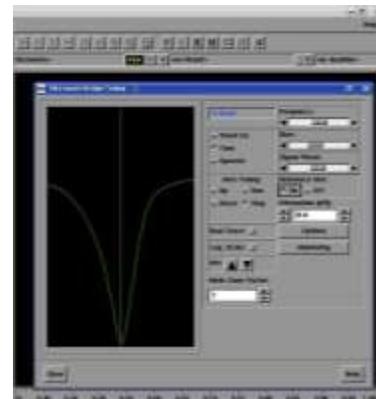


Be gentle; Never use force. If you break the tube, while it is in the cavity, you will contaminate it!!!!

Accidents do happen. If you break the tube in the resonator notify Dr.Erbse **immediately**. The faster we can start clean up measures the less dramatic it will be.

Tuning (Manually)

1. Select “tune mode”.
2. If you just did the Iris run down, press the **Iris-up** button until you see a tune picture.
3. You will work with the Iris buttons and the frequency, bias and signal phase sliders during tuning.
4. Note on working with the sliders: Dragging the slider – changes in big steps.
Clicking in the white to the left and right of the slider – medium steps
Clicking the black arrows – smallest steps
5. Adjust the attenuation to 25 dB.
6. Look for the dip and center it using the frequency slider.
7. Use the iris button (up or down, after an iris run down you will probably have to go up) to have the signal as deep as possible.



Note:

**You can break the iris screw by going over the lower Iris limit.
Be sure it is set correctly! Listen to the motor. Stop pushing the iris-down button, if it sounds strange or stops moving!!!**

8. Use the signal phase slider to get a symmetric signal/dip (approximately).
9. Click the reference arm off and on a couple of times. The dip should only move up and down. If it moves to the side the reference phase is off. If it moves to the side re-center the dip with the frequency slider. Click the reference arm back on. The dip will move to the side again. Re-center it with the Signal Phase Slider and try switching the arm off and on again.
10. Set the attenuation to 30 and 20 dB. Watch the dip; it should only get smaller/bigger not change form. If it does you are still off with the phase.
11. Return to attenuation of 25 dB.
12. There are two ways to continue manual tuning from this point on.
 - A. Tuning with the reference arm off**
 - B. Tuning with the reference arm on**

A. Reference Arm Off

- a. With the reference arm off, select the “operate mode”.
- b. The diode current should be close to 0. If it is not use the iris buttons (up or down as needed) to set the diode current close to 0 (anything under 10 is ok).
- c. Use the frequency slider to center the lock offset at around 0. At this point it is best to use the black arrows to move the frequency slider.
- d. Decrease the attenuation by step of 1 and adjust the diode current too close to 0 (anything under 10 is ok) and re-center the lock each time it is necessary, using the iris buttons for the diode current and frequency slider for lock.
- e. If you lose the lock, increase the attenuation a little bit, if the lock does not jump back, go back to the “tune mode” and redo what you did to get the signal centered and deep.
- f. Continue to decrease the attenuation by step of 1 until you reach 10 dB (or lower if you want higher power for your experiment) adjusting the diode

current and the lock each time it is necessary, using the iris button and frequency slider.

- g. Go back to 25 dB and switch the reference arm on.
- h. Use the Bias slider to center the diode current at 200.
- i. Go to attenuation of 20 dB or 25 dB and optimize the phase. Use the phase slider clicking the left or right arrow and watch the diode current. You want to be in the maximum of the diode current if it goes down as you click in one try the other direction. If it goes up keep going in that direction. Once you have reached the maximum, the current will plateau for a few clicks and then will decrease again. If you see it go down go a few clicks back with the phase. Re-center diode current at 200 with bias.
- j. Decrease and increase the attenuation in steps of one, a few steps each way.
- k. If you tuned well, you should only have small shifts in diode current. If you have strong shifts (more than 20 for low ϵ samples or more than 50 for high ϵ samples) repeat tuning.
- l. You can try the auto tune after the first manual tuning. If you are close to well-tuned it should work.

B. Reference Arm On

- a. With the reference arm on, select the “operate mode”
- b. Use the bias slider to set the diode current to 200 and frequency slider to center the lock offset at 0.
- c. Decrease the attenuation by step of 1 and re-center the diode current and the lock each time it is necessary, using the iris buttons (up or down as needed) for the diode current and frequency slider for lock. At this point it is best to use the black arrows to move the frequency slider.
- d. If you lose the lock, increase the attenuation a little bit, if the lock does not jump back, go back to the “tune mode” and redo what you did to get the signal centered and deep.

- e. Once an attenuation of 10 dB is reached optimize the phase. Use phase slider to go up or down and watch Diode current. You want to be in the maximum. If Diode current goes over 300 during this use the Bias slider to set it back to 200. Once you have reached the maximum, the current will decrease again. Go a few clicks with the phase back. Re-center diode current at 200 with bias.
- f. Continue to decrease the attenuation by step of 1 until you reach 3 dB (or lower if you want higher power for your experiment) and re-center the diode current and the lock each time it is necessary, using the iris button and frequency slider.
- g. If you tuned well you should only have small shifts in Diode current while going back with the power to the level you need. If you have strong shifts (more than 20 for low ϵ samples or more than 50 for high ϵ samples) repeat tuning.
- h. You can try the auto tune after the first manual tuning. If you are close to well-tuned it should work.

Note:

If you want to remove or change samples, put the attenuation at 40 dB or higher and go to tune mode (**Never move sample or cavity while in Operate at high power. (Remember high power means low dB!)**)

You have to tune each time you move or replace a sample. You should not have to do much if you tuned well. The automatic tune should be able to tune for you after the initial tune up. If it gets stuck retune manually.

Automatic Tuning

1. **Make sure the Iris limit is set correctly. If it is not, the automatic tuning will break the Iris screw!**
2. Select “tune mode”
3. If you just did the Iris run down, press Iris-up button until you see tune picture.
4. Look for the dip and center it using the frequency slider

5. Use the iris button (up or down, after a iris run down you will probably have to go up) to have the signal as deep as possible

Note:

You can break the iris screw by going over the lower Iris limit. Be sure it is set correctly! Listen to the motor. Stop pushing the iris-down button, if it sounds strange or stops moving!!!

6. Use the signal phase slider to get a symmetric signal (approximately).
7. Click auto tune up or down.
8. Watch what the instrument is doing. With time you can recognize if things are going completely wrong and you can stop it.
9. Observe what is happening with the Iris screw. If the motor sounds strange at any point stop the process.
10. If everything works the EPR should stop in “operate mode” with the diode current and lock off set centered. If they are slightly off click fine tune, if they are very off start tuning again. Manually might work better.

Collecting an EPR spectrum

1. Create a new experiment by clicking. 
2. The Build Experiment window will open. Choose the right settings for your experiments (field or time, 1D or 2D). If you are doing a simple 1D experiment at room temperature scanning the field, the default settings are right and you can click create without changing anything. 
3. Click on the Parameter  icon.
4. The parameter window will open. If you have previous spectra you can load one of them and simply load the parameters from the spectra. By using this icon:  If not you have to adjust the parameter by hand. Parameters will depend on the nature of your sample.
5. For Nitroxide radicals the settings in the figures below are good starting points.

Note:

The maximal Modulation Amplitude at 100 kHz is 15 G, at 30 kHz it is 10 G.

- Once you have set the parameters, click the  gearbox to transfer parameter to the instrument. The wheels should go from not touching to touching and the signal output window will be active.
- You can use the set up scan to adjust your parameters if your receiver gain is ≤ 70 .
- Click setup scan, activate the set up scan by clicking set up scan in the new window. You should see the center field signal in the window (if not shift center field in steps of 10 G until you see it). Optimize the receiver gain so that the signal is a little smaller in amplitude than half of the screen. Switch the set up scan off, close the window.
- Adjust receiver gain so that signal fills half of the window.

Note:

Do not use set up scan with a receiver gain >70 dB. The set up scan will not work right with high gains and will confuse the software.

- Adjust the offset to set signal in the middle.
- If you have set the parameters, close the parameter window, check that the bias and lock offset meters are still centered, if not fine tune again.
- Start the scan.



13. Stop the scan at the end of the first scan, if you have a good signal or no signal at all. Do a few scans if you have a weak signal before you stop.
14. If you have no signal at all increase the Sweep Width. You might be off with the center field and start the scan again.
15. Adjust your parameters according to the intensity of your signal.
16. Start scanning again and average as many scans as you would like to achieve a good signal to noise ratio.
17. If you have to average for a long time keep an eye on the bias and lock offset. Make sure they are not drifting too much. You can ask the program to fine tune before each scan. If you are working with high power or with the N2-Temperature controller the automatic fine tuning might not work well and the scanning might be stopped
18. If the data acquisition is ended save your spectrum. If you have done any manipulations or have a second spectrum loaded as secondary make sure that you really save the primary (which is your newly acquired spectrum). If you want to open your spectrum in any other application (EXCEL, WinSIM ...) you have to export it as ASCII file as well.
19. To remove the sample from the resonator, change to "tune mode" and set attenuation to 40 dB.

Note:

Never move a sample in or out of the resonator without first switching into "tune mode" and setting the attenuation to 40dB.

20. For a new sample start from Tuning the EPR.

Note:

It is important to avoid line shape distortions due to over modulating and/or power saturation.

You should always do some **exploratory scans** with **varying modulation amplitudes** to determine what amplitude works best for you. Higher modulation

amplitudes will produce a better signal to noise but might also start flattening your spectra. A rule of thumb is your modulation amplitude should not be higher than a 1/4 of the width of your narrowest line to avoid spectra distortion. But you have to decide what is acceptable for you.

You should always do some **exploratory scans** with **varying power** to be sure that you are not saturating your sample. Saturation effect can lead to line broadening and to loss of amplitude.

Turning the EPR Off

1. Turn the instrument off in the reverse order of turning it on:
2. Using Xepc and the tune panel set the instrument into standby mode.
3. Go to acquisition menu → disconnect from spectrometer. The instrument and parameter control panels should vanish.
4. Close the program.

Important:

Click no when you are asked if you want to save changes. **Never click yes.**

Yes would save changes for everyone!

5. Switch off the magnet power supply.
6. Switch of the console.
7. Switch off the heat exchanger.
8. Switch off the water.
9. Make sure the door is locked when you leave.
10. **Better safe than sorry – If you are the last to use the EPR on the day double check that everything is switched off on your way out of the building.**