MEASURING EPR ON THE BRUKER EMX SPECTROMETER

1.1 Temperature control systems

Dependent on the temperature range needed there are different options for measuring EPR samples:

I Measurements at room temperature
In this case no special preparations are needed. There is a set of collets available to accommodate different sample sizes. If you want to measure samples in aqueous solutions you have to use the special flat cells.

II Measurements at liquid nitrogen temperature (77 K)
This is the most convenient way to measure EPR spectra at a lower temperature. However, this method cannot be used for all samples. A liquid nitrogen finger dewar is inserted in the cavity. The dewar is filled with liquid nitrogen and the sample is inserted into the finger. This method works well for concentrated samples in combination with the less sensitive dual-mode cavity. The normal-mode cavity is too sensitive. The boiling of the liquid nitrogen causes spikes in the spectra. This effect is less with the dual-mode cavity.

III Measurements with the helium-flow system (4.2 – 200 K)
With this method you have the option to measure signals at different temperatures. This is important when looking at new samples that might show several signals at very different temperatures. To use a flow system, the helium cryostat has to be installed connected via the transfer line to a dewar with liquid helium.

IV Measurements with the nitrogen-flow system (100 – 300 K)
This method uses the same cryostat as used in method III, but now we use liquid nitrogen. Liquid nitrogen is much cheaper than liquid helium, so if you do not need temperature below 100 K use the nitrogen-flow system.

I Measurements at room temperature

1 **Turn on the nitrogen gas supply to the cavity.** The controls are placed on the wall left from the magnet. Set the flow to about 120 (black ball) on the flow regulator.

2 **Select the appropriate collet to accommodate the size of the sample**. Remove the protection collets that are placed on the top and bottom of the cavity to keep dust out. Replace the protection collets with collets that are wide enough to hold the sample. In the case of aqueous solutions special flat cells have to be used.

3 **Insert the sample tube and adjust the vertical position of the tube so that the sample area is in the center of the cavity.**

4 Continue with section 1.2.

II Measurements at liquid nitrogen temperature (77 K)

1 **Insert the nitrogen finger dewar.** Remove the protection collets that are placed on the top and bottom of the cavity to keep dust out. Replace the protection collets with the collets that are wide enough to hold the nitrogen finger dewar. Insert the nitrogen finger dewar and position it so that an EPR samples will be exactly positioned in the central part of the cavity. See also the figures on page 1-5. (You can use a dummy EPR sample to find the optimal position.) Tighten the collet nuts to keep the dewar in place.

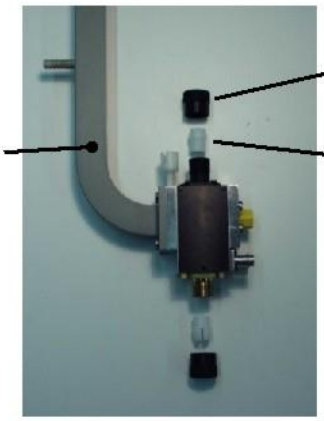
2 **Fill the finger dewar with liquid nitrogen.**

3 **Clean the sample tube to be inserted into the dewar.** Remove all the ice particles from the EPR tube. Continue to do this till the quartz feels smooth. This process will also remove any liquid nitrogen/oxygen in the sample. Liquid oxygen, or oxygen dissolved in liquid nitrogen, will interfere with the EPR measurement. *Take care not to thaw your sample.*

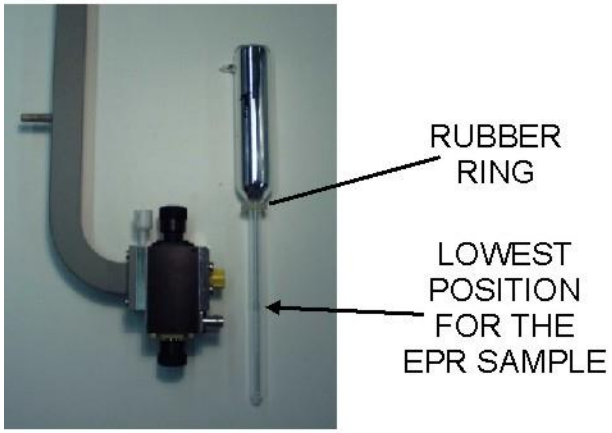
4 **Insert the sample tube.**

5 Continue with section 1.2

EPR CAVITY



EPR CAVITY WITH NITROGEN FINGER DEWAR



III Measurements with the helium-flow system (4.2 – 200 K)

In principle Dr. Evert Duin (844-6072) is responsible for preparing the EPR spectrometer for measurements at liquid-helium temperatures (and liquid-nitrogen temperatures). Except for the first day, the spectrometer will be fitted with the cryostat, and the transfer line will be connecting the helium transport dewar (60 or 100 l) to the cryostat. This is how you should find the spectrometer in the morning and should leave it behind in the evening. If this is not the case or you want to dismantle the system, please contact Dr. Duin.

The ***night before*** the measurement you should start pumping the cryostat. (Preferably the person who measured the day before you should have done this.)

A Preparing the cryostat

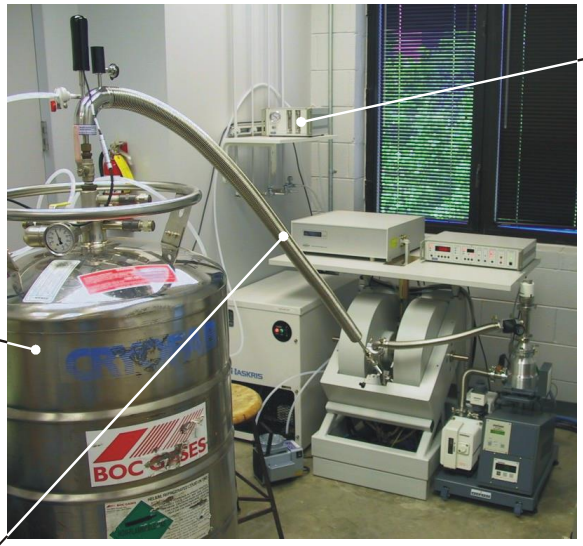
1 **Check whether the turbomolecular pump is connected to the cryostat.**

2 **Start the turbomolecular pump.** The power to the pump should be switched on already. (If not you should do this. The switch can be found at the back of the pump. The pump will additionally run a self-test for approximately 20 seconds.) Start the pumping process by pressing the Start/Stop key on the front panel of the display unit. The oil pump will immediately start running. After a certain vacuum is reached the turbomolecular pump will automatically start. ***Wait till a vacuum of 10^{-5} is reached before opening the cryostat to the pump.***

3 **Open the cryostat to the turbomolecular pump.** The turbomolecular pump is connected to the cryostat via a vacuum line. Both the turbomolecular pump and the cryostat have a knob that has to be opened. First turn the big black knob on top of the turbomolecular pump that opens the pump to the vacuum line connecting the pump to the cryostat. Turn the knob counter-clockwise till a green ring is shown. The width of the green band should be about 1/16’ (3 mm). This will pump the vacuum line space. Subsequently, turn the black knob on the cryostat at the entrance of the vacuum line. Slowly turn the knob counter-clockwise three or four turns.

4 Overnight a vacuum of about $2 \cdot 10^{-6}$ should be reached.

Overview of the EPR Components



Flow Controller/Meter

Helium Dewar

Transfer Line

Microwave Bridge

Temperature Controller

Cooling Unit

Turbomolecular Pump

Gas Flow Pump

EPR Magnet

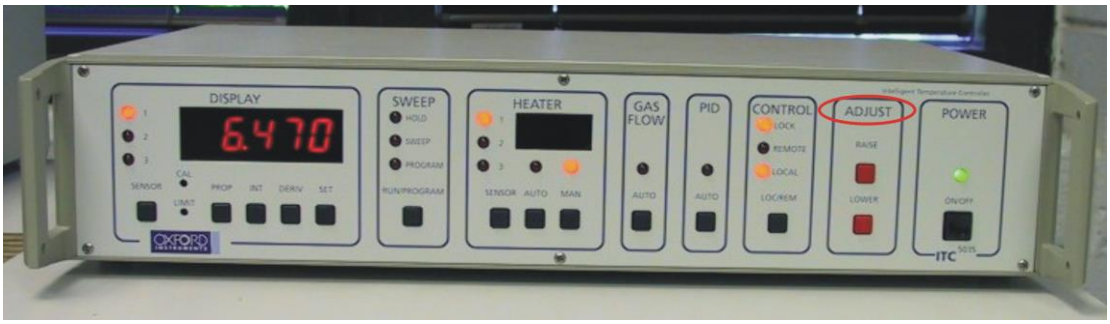
B Initial Cool-down

1 **Close the valve connecting the cryostat to the turbomolecular pump.** Turn black knob on the cryostat clockwise till closed.

2 **Switch off the turbomolecular pump.** Stop the pumping process by pressing the Start/Stop key on the front panel of the display unit on the vacuum pump. The oil pump and turbomolecular pump will automatically stop.

3 **Turn on the nitrogen gas supply to the cavity.** The controls are placed on the wall left from the magnet. Set the flow to about 120 (black ball) on the flow regulator.

Note on the liquid helium/temperature control units: The Temperature Controller unit with the digital display is placed on the shelve on top of the magnet. The Flow Controller/Meter is placed on the shelve on the wall left from the magnet. The Temperature Controller has several functions, which are activated by pressing the appropriate button. When a button is pressed and held down, the appropriate parameter is displayed on the digital readout. This parameter may be adjusted by simultaneously pressing the function button and either the red LOWER or RAISE button in the ***ADJUST*** *section of the controller. The digital display will change to indicate the target value for this parameter.* ***The longer you hold the buttons the faster the rate of change.*** *When the desired value is reached, release the buttons.*



4 **Turn on the Temperature Controller.** Push the ON/OFF button in the POWER section of the controller. This is the rightmost button on the front of the controller.

5 **Turn on the gas flow pump.** This is the small vacuum pump at the foot of the magnet connected to the transfer line by white plastic tubes. The switch is on the side of the pump.

6 **Open the needle valve from the transferline.**

- **Use the Temperature Controller:** Press the button in the GAS FLOW section of the Temperature Controller. The digital display should now read 0.0. Change the gas flow to 99.9 by simultaneously pressing the button in the GAS FLOW section and the RAISE button in the ADJUST section. Wait for a couple of minutes before checking whether this value is reached since it takes that long for the needle valve to completely open.
- **Manually**: When the temperature controller does not work you have to regulate the gas flow by hand. Turn the knob (the needle valve) on top of the transfer line (one the site closes to the liquid helium dewar) two whole turns.

7 **Wait for the temperature to fall to ~4.3 K.** This requires 30 min to achieve. When the gas flow pump is first activated, the needle of the pressure gauge on the Flow Controller/Meter will read approximately -800, and the flow indicators will read near zero. ***Both flow indicators will move slightly, however, which is your first check that the transfer line is not blocked.***

While you wait you can turn on the spectrometer (Section 1.2).

8 After the cryostat has reached 4.3 K, the plastic tube leading from the transfer line to the Flow Controller/Meter will begin to frost and the needle of the Flow Controller/Meter will fluctuate wildly (somewhere in between -400 and -200). Both flow indicators will be at their maximal position. At this point, reduce the helium flow to the minimum level required to maintain the temperature in the 6.0-8.0 K range.

- **Use the Temperature Controller:** Press the button in the GAS FLOW section of the Temperature Controller. The digital display should still read 99.9. Change this to 15.0 by simultaneously pressing the button in the GAS FLOW section and the LOWER button in the ADJUST section. Again wait a couple of minutes for the needle valve to reach this value and to see how the temperature changes. The value of 15.0 might be too high or too low, dependent on how much helium is left in the dewar. When the temperature is still too low or too high, adjust the needle valve settings accordingly in small increments.
- **Manually**: Close the knob (the needle valve) on top of the transfer line completely and open it again ¼ of a turn. Wait for a couple of minutes to see the effect of this procedure. Adjust the knob if needed to achieve a temperature reading in between 6 – 8 K.

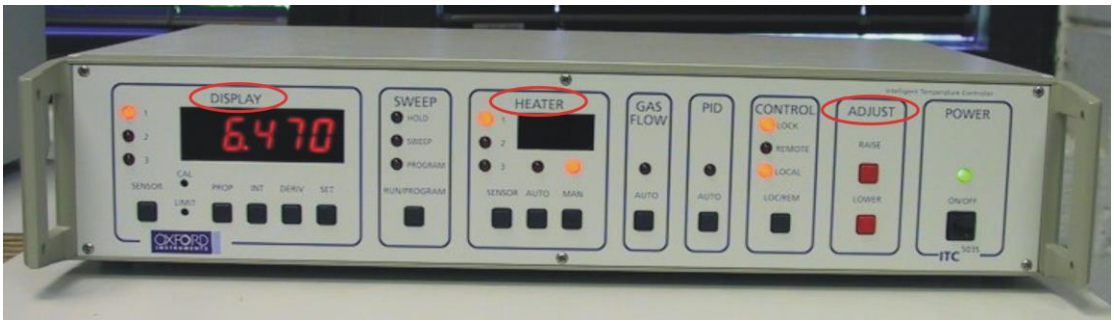
It is important that a constant temperature is maintained at around 8 K. Wild fluctuations around this value indicate that there is a partial blockage in the transfer line.

C Adjusting the temperature.

Every type of paramagnetic group has its optimum temperature for detection. With the helium flow system, temperatures between 4.5 K and 200 K can be easily obtained. (It is strongly advised not to use higher temperatures.)

1 **Adjust the needle valve settings to obtain temperatures between 4.5 and 8 K.** There is no point in using the heater when the helium flow is high. With the heater the temperature will not be very stable and the heater will be using too much current. This way too much helium will be used. **Liquid helium is very expensive.**

2 **For temperatures higher than 8 K the heater should be switched on.** Do not change the needle valve settings to obtain higher temperatures than 8 K. Closing the needle valve even further will result in higher temperatures but also in temperature gradients in the EPR sample. Since the temperature sensor is placed below the EPR sample the display will read a different (lower) temperature. In the **DISPLAY** section on the Temperature Controller press on SET. The display will now show the selected temperature (0.000). By simultaneously pressing the RAISE button in the **ADJUST** section you can change this setting to the desired value.



3 **Engage the heater**. In the **HEATER** section press on the MAN button (for MANUAL). Press simultaneously the RAISE button. Dependent on the desired temperature keep pressing the RAISE button till the heater display shows one or three red bars. Now wait till the temperature starts to rise. If the temperature display shows that this process is slowing down (only one red bar left) while the desired temperature is not obtained heat a little bit more. When the temperature is close to the desired value (< 2 K), press the AUTO button in the HEATER section. The Temperature Controller will now automatically keep the temperature at the desired value.

After you have set the temperature, do not use the AUTO button to obtain this temperature. The heater might use too much current. This might result in overheating of the heater and it will automatically be shut off. In that case you have to shut of the Temperature Controller and wait five minutes. There is a chance, however, that the heater will be permanently damaged. This will be the end of the cryostat and your EPR days are over too.

To use the AUTO option you have to enter these values for the PROP, INT and DERIV in the DISPLAY section:

Temperature	PROP	INT	DERIV
10 K	60	0.1	0.0
50 K	30	1.0	0.2
100 K	20	0.5	0.1

D Inserting the First Sample Tube

1 You are now ready to place the first sample in the spectrometer. Experienced users may desire to run cavity spectra ('blanks') for comparison and subtraction. This is a convenient time to do so.

2 **Clean the sample tube to be inserted into the cavity.** Remove all the ice particles from the EPR tube. Continue to do this till the quartz feels smooth. This process will also remove any condensed nitrogen/oxygen in the sample. Liquid oxygen, or oxygen dissolved in liquid nitrogen, will interfere with the EPR measurement. *Take care not to thaw your sample.*

3 **Turn off the gas flow pump.** The small vacuum pump at the foot of magnet connected to the transfer line by plastic tubes. The switch is on the side of the pump and can easily be operated by foot.

4 Observe the Flow Controller/Meter. The pressure gauge on the left side should rotate clockwise until it reads 0.0. **Wait until the pressure gauge read 0.0 and the left-most flow meter indicates a positive gas flow before proceeding.**

5 **Remove the plug from the sample entry port.**

6 **Take the sample from the dewar flask, wipe off any condensation with a tissue and insert into cavity slowly until it comes into contact with the insert. DO NOT PUSH DOWN ON THE TUBE WITH FORCE! This will damage the quartz insert. If this happens the temperature reading will be completely off and all your data will be useless!**

7 **Put the plug back on the sample entry port.**

8 **Turn on the gas flow pump.** The pressure and flow gauges and temperature readout should slowly return to their previous values. (This may require a few minutes. If the temperature does not return to the previous value, adjust the needle valve or heater settings.)

IV Measurements with the nitrogen-flow system (100 – 300 K)

In principle Dr. Evert Duin (844-6072) is responsible for preparing the EPR spectrometer for measurements at liquid-nitrogen temperatures. Except for the first day, the spectrometer will be fitted with the cryostat, and the transfer line will be connecting the nitrogen dewar (30 l) to the cryostat. This is how you should find the spectrometer in the morning and should leave it behind in the evening. If this is not the case or you want to dismantle the system, please contact Dr. Duin.

The ***night before*** the measurement you should start pumping the cryostat. (Preferably the person who measured the day before you should have done this.)

A Preparing the cryostat

1 **Check whether the turbomolecular pump is connected to the cryostat.**

2 **Start the turbomolecular pump.** The power to the pump should be switched on already. (If not you should do this. The switch can be found at the back of the pump. The pump will additionally run a self-test for approximately 20 seconds.) Start the pumping process by pressing the Start/Stop key on the front panel of the display unit. The oil pump will immediately start running. After a certain vacuum is reached the turbomolecular pump will automatically start. ***Wait till a vacuum of 10^{-5} is reached before opening the cryostat to the pump.***

3 **Open the cryostat to the turbomolecular pump.** The turbomolecular pump is connected to the cryostat via a vacuum line. Both the turbomolecular pump and the cryostat have a knob that has to be opened. First turn the big black knob on top of the turbomolecular pump that opens the pump to the vacuum line connecting the pump to the cryostat. Turn the knob counter-clockwise till a green ring is shown. The width of the green band should be about 1/16’ (3 mm). This will pump the vacuum line space. Subsequently, turn the black knob on the cryostat at the entrance of the vacuum line. Slowly turn the knob counter-clockwise three or four turns.

4 Overnight a vacuum of about $2 \cdot 10^{-6}$ should be reached.

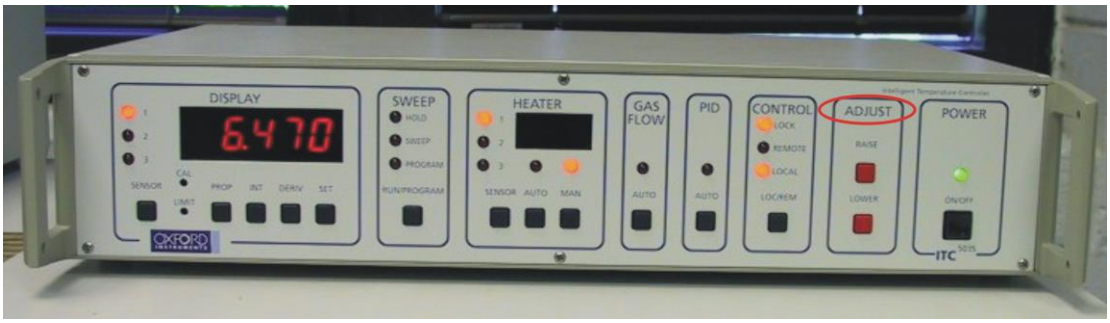
B Initial Cool-down

1 **Keep the cryostat connected to the turbomolecular pump.** When the cryostat is used in combination with liquid helium we do not pump the cryostat during the measurements. This is because liquid helium will freeze all gas particles that might enter the vacuum space of the cryostat. In other words the cryostat is cryo-pumped by the liquid helium itself. When using liquid nitrogen this process does not take place and therefore we have to keep pumping the cryostat during the measurements.

2 **Turn on the nitrogen gas supply to the cavity.** The controls are placed on the wall left from the magnet. Set the flow to about 120 (black ball) on the flow regulator.

An overview of the different components of the EPR spectrometer can be found on p. 1-7.

Note on the liquid helium/temperature control units: The Temperature Controller unit with the digital display is placed on the shelve on top of the magnet. The Flow Controller/Meter is placed on the shelve on the wall left from the magnet. The Temperature Controller has several functions, which are activated by pressing the appropriate button. When a button is pressed and held down, the appropriate parameter is displayed on the digital readout. This parameter may be adjusted by simultaneously pressing the function button and either the red LOWER or RAISE button in the ***ADJUST*** *section of the controller. The digital display will change to indicate the target value for this parameter.* ***The longer you hold the buttons the faster the rate of change.*** *When the desired value is reached, release the buttons.*



3 **Turn on the Temperature Controller.** Push the ON/OFF button in the POWER section of the controller. This is the rightmost button on the front of the controller.

4 **Turn on the gas flow pump.** This is the small vacuum pump at the foot of the magnet connected to the transfer line by white plastic tubes. The switch is on the side of the pump.

5 **Open the needle valve from the transferline.**

- **Use the Temperature Controller:** Press the button in the GAS FLOW section of the Temperature Controller. The digital display should now read 0.0. Change the gas flow to 99.9 by simultaneously pressing the button in the GAS FLOW section and the RAISE button in the ADJUST section. Wait for a couple of minutes before checking whether this value is reached since it takes that long for the needle valve to completely open.
- **Manually**: When the temperature controller does not work you have to regulate the gas flow by hand. Turn the knob (the needle valve) on top of the transfer line (one the site closes to the liquid helium dewar) two whole turns.

6 **Wait for the temperature to fall to ~66 K.** This requires 30 min to achieve. When the gas flow pump is first activated, the needle of the pressure gauge on the Flow Controller/Meter will read approximately -800, and the flow indicators will read near zero. ***Both flow indicators will move slightly, however, which is your first check that the transfer line is not blocked.***

While you wait you can turn on the spectrometer (Section 1.2).

7 After the cryostat has reached 66 K, reduce the nitrogen flow to the minimum level required to maintain the temperature in the 80-90 K range:

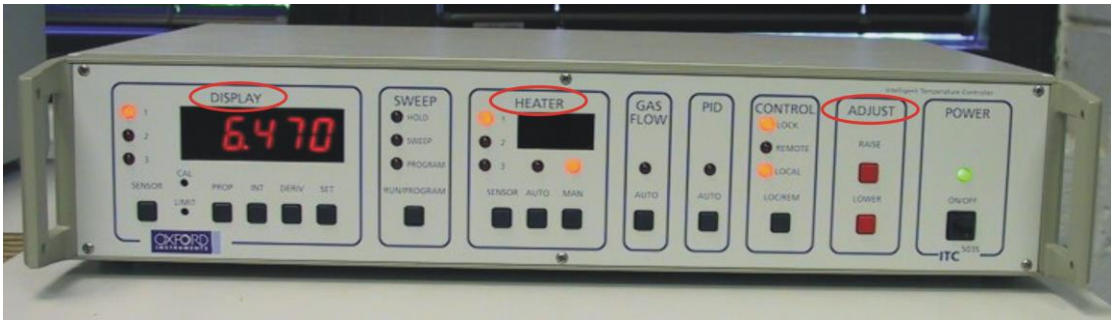
- **Use the Temperature Controller:** Press the button in the GAS FLOW section of the Temperature Controller. The digital display should still read 99.9. Change this to 15.0 (check) by simultaneously pressing the button in the GAS FLOW section and the LOWER button in the ADJUST section. Again wait a couple of minutes for the needle valve to reach this value and to see how the temperature changes. The value of 15.0 might be too high or too low, dependent on how much nitrogen is left in the dewar. When the temperature is still too low or too high, adjust the needle valve settings accordingly in small increments.
- **Manually**: Close the knob (the needle valve) on top of the transfer line completely and open it again ¼ of a turn. Wait for a couple of minutes to see the effect of this procedure. Adjust the knob if needed to achieve a temperature reading in between 80-90 K.

It is important that a constant temperature is maintained at around 80-90 K. Wild fluctuations around this value indicate that there is a partial blockage in the transfer line.

C Adjusting the temperature.

Every type of paramagnetic group has its optimum temperature for detection. With the nitrogen-flow system, temperatures between 100 K and 300 K can be easily obtained. (It is strongly advised not to use higher temperatures.)

1 **For temperatures of 100 K and higher the heater should be switched on.** After a stable temperature is reached in the 80-90 K region, the heater can be sued to obtain the desired temperature in the 100 – 300 K range. Do not change the needle valve settings to obtain higher temperatures than 100 K. Closing the needle valve further will result in higher temperatures but also in temperature gradients in the EPR sample. Since the temperature sensor is placed below the EPR sample the display will read a different (lower) temperature. In the **DISPLAY** section on the Temperature Controller press on SET. The display will now show the selected temperature (0.000). By simultaneously pressing the RAISE button in the **ADJUST** section you can change this setting to the desired value.



3 **Engage the heater**. In the HEATER section press on the MAN button (for MANUAL). Press simultaneously the RAISE button. Dependent on the desired temperature keep pressing the RAISE button till the heater display shows one or three red bars. Now wait till the temperature starts to rise. If the temperature display shows that this process is slowing down (only one red bar left) while the desired temperature is not obtained heat a little bit more. When the temperature is close to the desired value (< 2 K), press the AUTO button in the HEATER section. The Temperature Controller will now automatically keep the temperature at the desired value.

After you have set the temperature, do not use the AUTO button to obtain this temperature. The heater might use too much current. This might result in overheating of the heater and it will automatically be shut off. In that case you have to shut of the Temperature Controller and wait five minutes. There is a chance, however, that the heater will be permanently damaged. This will be the end of the cryostat and your EPR days are over too.

To use the AUTO option you have to enter these values for the PROP, INT and DERIV in the DISPLAY section:

Temperature	PROP	INT	DERIV
100 K	20	0.5	0.1

D Inserting the First Sample Tube

1 You are now ready to place the first sample in the spectrometer. Experienced users may desire to run cavity spectra ('blanks') for comparison and subtraction. This is a convenient time to do so.

2 **Clean the sample tube to be inserted into the cavity.** Remove all the ice particles from the EPR tube. Continue to do this till the quartz feels smooth. This process will also remove any condensed nitrogen/oxygen in the sample. Liquid oxygen, or oxygen dissolved in liquid nitrogen, will interfere with the EPR measurement. *Take care not to thaw your sample.*

3 **Turn off the gas flow pump.** The small vacuum pump at the foot of magnet connected to the transfer line by plastic tubes. The switch is on the side of the pump and can easily be operated by foot.

4 Observe the Flow Controller/Meter. The pressure gauge on the left side should rotate clockwise until it reads 0.0. **Wait until the pressure gauge read 0.0 and the left-most flow meter indicates a positive gas flow before proceeding.**

5 **Remove the plug from the sample entry port.**

6 **Take the sample from the dewar flask, wipe off any condensation with a tissue and insert into cavity slowly until it comes into contact with the insert. DO NOT PUSH DOWN ON THE TUBE WITH FORCE! This will damage the quartz insert. If this happens the temperature reading will be completely off and all your data will be useless!**

7 **Put the plug back on the sample entry port.**

8 **Turn on the gas flow pump.** The pressure and flow gauges and temperature readout should slowly return to their previous values. (This may require a few minutes. If the temperature does not return to the previous value, adjust the needle valve or heater settings.)

1.2 Turning the spectrometer on.

1 **Turn on the cooling water.** ON/OFF knob on the cooling unit on the ground left from the magnet.

2 **Turn on the power for the console.** The power switch for the console is located in the lower right corner of the back of the console. (Lower left corner when you approach the console from the front.)

3 **Turn on the heat exchanger and magnet power supply.** The power supply is located directly below the console. Simply pushing the POWER ON/OFF button on the front turns on the supply.

4 **Turn on the computer.**

User name: EPR
Password: _ _ _ _ _ _ _ _

The program BootPD will automatically start. Click on the minimized window in the task bar to show the contents. Wait till all three ethernet addresses for the different spectrometer components have been assigned. The window will now show a text similar to this:

```
bootp server ready
tftpd 'Listening' on port 69, protocol UDP
recvd pkt from IP addr 0.0.0.0
request from Ethernet address 00:00:AD:05:46:12
setarp 192.168.1.40 - 00:00:AD:05:46:12
recvd pkt from IP addr 0.0.0.0
request from Ethernet address 00:00:AD:02:85:12
setarp 192.168.1.50 - 00:00:AD:02:85:12
recvd pkt from IP addr 0.0.0.0
request from Ethernet address 00:00:AD:04:6A:12
setarp 192.168.1.30 - 00:00:AD:04:6A:12
```

Minimize the BootPD window.

5 **Start the WIN-EPR acquisition application.** By double-clicking the Bruker EMX icon  on the desktop, the program will start up and initialize all the modules of the EMX spectrometer. The program will start with the Microwave Bridge Control and the Signal Channel Options dialog boxes opened.

6 **Calibrate the cavity.** In the WINEPR ACQUISITION window, select File on the menu bar and additionally select New. A new window will pop up: Spectrum1. On the menu bar select Acquisition, and additionally select Calibrate Signal Channel. Again a new window will pop up: Spectrum1:2. The top of this new window displays the location and name of the current calibration file, normally this will read:

c:\program files\bruker emx\syscal\hs0315.cal

There are two cavities available: The standard cavity for normal (perpendicular, ⊥) mode measurements (file name: hs0315.cal), or the dual-mode cavity for both parallel (//) and perpendicular (⊥) mode measurements (file name: dm0301.cal). If the wrong calibration file is selected click on Change File to select the correct calibration file. This will open up a new window, Open or Create a SCT Calibration File, where the correct file can be selected.

When the correct calibration file is selected, click on Start Calibration. Wait till the calibration is finished (this can be followed in the Spectrum1:1 window) and then close the Spectrum1:2 window again.

7 The setting for AFC modulation leveler is different for the two cavities. The black dial and red switch of the AFC modulation leveler are found at the backside of the Microwave Bridge. For the standard cavity the value is 3/8 (See picture: black dial at 3, red switch at "x1/8"). For the dual-mode cavity the value is 4 (black dial at 4, red switch at "x1").

8 Activate and maximize the Microwave Bridge Control dialog box. Now you are ready to insert a sample.

1.3 Tuning the microwave cavity and bridge.

1 **Switch the microwave bridge to Tune mode in the Operating Mode display of the Microwave Bridge Control dialog box.** There are three states or modes for the microwave bridge, Stand By, Tune, and Operate. When you turn on the spectrometer, it is in Stand By mode.

2 **Set the microwave attenuator to 30 dB.** The microwave attenuation is set by clicking the arrows on either side of the Attenuation display in the Microwave Bridge Control dialog box. The right arrows are for adjusting the power in amounts of 1 dB, the left arrows are for adjusting the power in amounts of 10 dB.

3 **Find the "dip".** On the small display monitor on the left-hand side of the screen a pattern is shown. Adjust the Frequency slider bar to locate and center the "dip". Center the dip in the middle of the display. This pattern is a display of the microwave power reflected from the cavity and the reference arm power as a function of the microwave frequency. The dip corresponds to the microwave power absorbed by the cavity and thus is not reflected back to the detector diode. By centering the dip on the display monitor, the microwave source is set to oscillate at the same frequency as the cavity resonant frequency.

4 **Tune the signal (reference) phase.** While the dip is in the center of the display, adjust the Signal Phase slider, until the depth of the dip is maximized and the dip looks somewhat symmetric. The might be two positions for the Signal Phase slider where a symmetric negative dip is obtained. Always choose the position in the center (40-60%).

5 **Switch the microwave bridge to Operate mode in the Operating Mode display of the Microwave Bridge Control dialog box.**

6 **Fine-tune the microwave source frequency.** Adjust the Frequency slider until the needle of the AFC meter is centered.

7 **Adjust the bias level.** Change the microwave attenuation to 50 dB. Adjust the Bias slider until the Diode meter needle is centered. Keep an eye on the frequency. Sometimes the cavity will loose lock at 50 dB. (The meter may rush off either to the right or left.) The AFC will lock again at higher microwave power levels (More power means lower values in dB.)

8 **Critical coupling of the cavity.** Increase the microwave power by 10 dB (i.e. attenuator settings of 40 dB or 30 dB.) Click the ↑ or ↓ iris buttons for the iris screw motor until the diode current returns to the green area and the needle is centered. Repeat this procedure till you have reached an attenuator setting of 20 dB. *Make sure that also the AFC meter stays centered.*

Note: the ↑ or ↓ iris buttons work in opposite direction when using the dual-mode cavity!

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9 After adjusting the iris at 20 dB also adjust the Signal Phase slider until a local maximum is achieved in the diode current. Adjust the iris again when necessary to center the Diode meter needle.

10 Verify that you have achieved critical coupling by changing the microwave attenuation from 10 dB to 50 dB with virtually no change in the diode current. Repeat the coupling and bias level adjustment procedures (step 7 and 8) if necessary.

For power levels greater than 20 mW (lower than 10 dB), set the attenuator to 0 dB and once again adjust the diode current. The current may drift because the high microwave power starts to heat the sample. If this happens, wait a minute or two and readjust the coupling.

11 Finally, set the microwave power to the dB value you need for your measurement. (see also section 1.7).

12 **Leave the Microwave Bridge Control dialog box.** Click on the MW icon on the menu bar. Now you are in the WINEPR ACQUISITION [Spectrum] window and ready to measure.

The following table shows the values of the frequency, bias, and signal phase for different cavities and operating modes. Quartz will concentrate the field lines in the EPR sample. Therefore the setting will change dependent if you measure with or without a sample and with and without the quartz insert from the cryostat.

The dual mode cavity can measure in the normal (perpendicular, ⊥) mode or parallel (//) mode. When you tune this cavity you will find a separate dip for each measuring mode. Make sure you select the correct dip.

Settings for the dual-mode cavity with the nitrogen finger dewar

Mode	Frequency	Bias	Signal Phase	Q factor
(⊥) Dewar A + sample	9.62 GHz (65%)	60%	64%	±3000
(//) Dewar A + sample	9.33 GHz (29%)	60%	51%	±3000
(⊥) Dewar B + sample	9.62 GHz (65%)	60%	65%	±3000
(//) Dewar B + sample	9.33 GHz (30%)	67%	51%	±3000

Settings for the normal-mode cavity with the cryostat

empty	9.41 GHz (39%)	64%	47%	5000
+ sample	9.38 GHz (36%)	64%	45%	5000

Settings for the dual-mode cavity with the cryostat

Dual ⊥, empty	9.64 GHz (69%)	58%	67%	4700
Dual ⊥, + sample	9.63 GHz (66%)	58%	63%	3100
Dual //, empty	9.40 GHz (38%)	58%	58%	2200
Dual //, + sample	9.37 GHz (34%)	59%	54%	3500

1.4 Acquiring spectra

The most important step towards acquiring an EPR spectrum is to input appropriate parameters either by editing default parameters or to load from a previous EPR measurement.

1 **Open the Standard Parameter dialog box in order to check the parameters.** To edit the default parameters click on Parameter on the menu bar and select Experiment. There are several fields where you can make changes. The setting you need depend on the type of sample you run and the type of measurement.

Hall

CF (g=2)	Centers the center field on the g=2 value. Value in Gauss depends on the frequency.
Center Field	For initial broad scans, a Center Field around 3100 Gauss is recommended.
Sweep Width	For initial broad scans, a Sweep Width around 6000 G is recommended. This means that the scan will start 3000 Gauss below the Center Field value (100 Gauss) and stop 3000 Gauss above the Center Field value (6100 Gauss). **This scan will cover the complete area where signals might be detectable.** **When using different settings, make sure that the sum of Central Field and the half of Sweep Width is less than 6100 Gauss and their difference is more than zero.**
Static Field	You don't have to change this. This is used when you do a time scan at a certain field position.

Microwave Bridge

Frequency	There is no need to edit the microwave frequency parameter. It will be automatically read during the run.
Attenuator/Power	To optimize the microwave power level, the power is set or changed in the Microwave Bridge Control dialog box (see section 1.3). 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.
Step	This cannot be changed.

Temperature unit and Goniometer are not installed.

Signal Channel

Receiver Gain	For a new sample you can begin with the value of 8 x 10^{+5}. Then, after the first acquisition you can change it in accord with the experimental conditions. **NOTE that the software will scale the spectrum when your gain is too low. In principle you can measure that way but 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 a too high value of the receiver gain.**
Modulation Frequency	This is normally set to 100 kHz.
Modulation Amplitude	You can start with 6 Gauss. The larger this value the lower the value needed for the Receiver Gain, which means less noise. Excessive field modulation, however, broadens the EPR lines and does not contribute to a bigger signal. As a rule-of-thumb **this value has to be smaller than the line width of your signal**. With radical spectra you might have to change this to a lower value. With protein samples you normally have larger line widths, but watch out for hyperfine structure.
Modulation Phase	Leave this at 0.
Offset	You can change this with axial spectra and have the baseline start a little bit higher or lower on the screen.
Time Constant	Time constants filter out noise by slowing down the response time of the spectrometer. **Longer time constants need a slower scan rate (see below).**
Conversion Time	If the Time Constant is too large in comparison with the Conversion Time (the rate at which the field is scanned) the signals we want to detect will get distorted or will even be filtered out. The Bruker manual advises that the time needed to scan through a single scan should be ten times greater than the length of the Time Constant. This is true for very sharp signals. For signals normally detected in proteins the Time Constant can even be two times larger than the Conversion Time. A longer Conversion Time, however, also improves the signal to noise ratio in a different way: The signal channel

	incorporates an integrating ADC (Analog to Digital Converter) to transfer the analog EPR spectra to the digital data acquisition system. An important side effect of using the integration method for the conversion is that it integrates the noise out of the signal. The noise diminishes as the square root of the conversion time. This effect is independent of the time constant of the signal channel. With a sweep width off about **1000** Gauss a Conversion Time of **163.84** msec can be used. (Time Constant of **327.68** msec.)
Sweep Time	Set by the WinEPR software. The Sweep Time is the time actually needed for the complete scan. This is calculated as the amount of data points times the Conversion Time (1024 * 163.84 msec gives 168 sec (2 min 48 sec).
Harmonic	Leave at 1 for regular measurements.
Resolution in X	The Number of data points. If your sweep width is less than 2000 Gauss, 1024 data points will be sufficient. If your sweep width is more than 2000 Gauss the resolution might have to be increased to 2048 or 4096 data points. (Only when you want to enlarge certain parts of the spectrum later. However, a good advice is to remeasure interesting areas of a "broad" scan with a new set of settings.) If the spectrum has very narrow lines a higher resolution should be used. This can be the case when radical spectra of molecules in organic solvents are measured. This type of spectra or normally not found for proteins.
Number of X-Scans	If you are looking for very weak signals they might get lost in the noise due to the boiling of the liquid nitrogen. You can increase your signal to noise ratio by signal averaging. The resultant signal to noise is proportional to $\sqrt{N}$, where N is the number of scans.
Resolution in Y	This cannot be changed
Repetitive Mode	This sets the number of X-Scans

2 **Press OK when all the parameters are set.**

3 **Check whether the AFC and Diode meters are still in the middle.** If not go back to the Microwave Bridge Control dialog box by clicking on MW to center these meters again.

4 **Record a spectrum.** Click on RUN to start the scan. Dependent on the starting position of the scan, the spectrometer needs some time to change the current in the magnet. During this process the message "Waiting for Field to settle" will appear. Wait for this process to finish.

5 If necessary the scan can be stopped by pressing STOP.

6 **During the scan you can enter a comment.** On the menu bar click on Parameter and select Comment. The Comment dialog box will appear. In the Operator field you can enter your name. In the Comment field you can enter which type of sample you run and for example the temperature. Press OK when you are finished.

7 **The software will scale the spectrum when your gain is too low. In principle you can measure that way but the best resolution in the vertical position is obtained when the signal you are interested in fills most of the screen without extra scaling.** This means you have to scan a couple of times to get the optimal setting. This can be done at a much quicker scan rate to save time (Conversion Time 20.48 msec, Time Constant 40.96 msec for scans with a Sweep Width of 1000 Gauss).

8 **Saving the spectrum.** Click on the menu bar on File and select **Save As**. Save the spectrum in your own directory. (This is done because it will be easier to clean the hard disk after people have left.) Make a directory yourself or ask some of the people responsible for the EPR spectrometer. You can give the spectra any name you want, but for later purposes it is recommended that you start with two characters which identify you spectra as being yours and follow with four numbers: NN####. For example for Evert Duin: ED1234.

1.5 Changing samples

Now you are ready to change samples. **The sample tube should never be changed while the instrument (microwave bridge) is in OPERATE mode. Under no circumstance should the sample be moved during data acquisition. This will damage the detector diode. Replacing the detector diode will cost $ 2000.--.**

1 **Go back to the Microwave Bridge Control dialog box.** Click on MW. Decrease the power to 50 dB and change to the Tune mode. ***If you want to take a break you have to leave the EPR spectrometer in the Standby mode.***

When you are not using a gas-flow system you can simply exchange the samples and retune the bridge (section 1.3). When you are using a gas-flow system follow the next steps:

2 When you use relatively high heater settings it is better to turn the heater off while you change samples. Press the AUTO button in the HEATER section on the Temperature Controller to read the heater settings. Remember this value. Then press the MAN button and lower the current to zero (0.000) by simultaneously pressing the red LOWER button in the ADJUST section.

3 **Turn off the gas flow pump.** This is the small vacuum pump at the foot of the magnet connected to the transfer line by plastic tubes. The switch is on the side of the pump.

4 Observe the Flow Controller/Meter. The pressure gauge on the left side should rotate clockwise until it reads 0.0. **Wait until the pressure gauge reads 0.0 and the left-most flow meter indicates a positive gas flow before proceeding.**

5 **Remove the plug from the sample entry port.** Remove the current sample from the cavity by pulling straight up.

6 **Put the plug back on and start the gas flow pump again.**

7 **Clean the sample tube to be inserted into the cavity.** Remove all the ice particles from the EPR tube. Continue to do this till the quartz feels smooth. *Take care not to thaw your sample.*

8 **Turn off the gas flow pump.** The small vacuum pump at the foot of the magnet connected to the transfer line by plastic tubes. The switch on the side of the pump and can easily be operated by foot.

9 Observe the Flow Controller/Meter. The pressure gauge on the left side should rotate clockwise until it reads 0.0. **Wait until the pressure gauge reads 0.0 and the left-most flow meter indicates a positive gas flow before proceeding.**

10 **Remove the plug from the sample entry port.**

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11 **Take the sample from the dewar flask, wipe off any condensation with a tissue and insert into cavity slowly until it comes into contact with the insert. <u>DO NOT PUSH DOWN ON THE TUBE WITH FORCE!</u>**

12 **Put the plug back on the sample entry port.**

13 **Turn on the gas flow pump.**

14 **Reenter the setting for the heater**. Press MAN in the HEATER section and simultaneously press the RAISE button in the ADJUST section.

15 Wait till the desired temperature is reached (within a limit of < 2 K) and change the heater setting to automatic by pressing the AUTO button in the HEATER section

16 **Retune the spectrometer.** Go back to section 1.3 for the tune process.

If you are more experienced you can take out the old sample and put in a new sample at the same time (skip step 6 to 10). Make sure, however, that this process doesn't take a lot of time since there will be a higher chance that air gets into the sample compartment which will freeze and block the helium flow.

1.6 Shutting off the spectrometer

1 **Go to the Microwave Bridge Control dialog box.** Click on MW. Decrease the power to 60 dB and change to the Standby mode.

2 **Close the WIN-EPR Acquisition program.** The software will freeze when the console is turned off before the software is closed.

3 **Turn off the computer.**

4 **Turn off the heat exchanger and magnet power supply.**

5 **Turn off the power for the console.**

6 **Turn off the cooling water.** Close the two red valves on the wall. Open the red valve on top. This way, water in the cooling system keeps circulating.

7 **Turn off the nitrogen gas supply to the cavity.**

1.7 Shutting down the gas-flow system

1 **Turn the heater off**. Press the MAN button in the HEATER section of the Temperature Controller and lower the current to zero (0.000) by simultaneously pressing the red LOWER button in the ADJUST section.

2 **Turn off the gas flow pump.** This is the small vacuum pump at the foot of the magnet connected to the transfer line by plastic tubes. The switch is on the side of the pump.

3 Observe the Flow Controller/Meter. The pressure gauge on the left side should rotate clockwise until it reads 0.0. **Wait until the pressure gauge reads 0.0 and the left-most flow meter indicates a positive gas flow before proceeding.**

4 **Remove the plug from the sample entry port.** Remove current sample from cavity by pulling straight up.

5 **Put the plug back on and start the gas flow pump again.**

6 **Close the needle valve.** Press the button in the GAS FLOW section of the Temperature Controller and simultaneously the LOWER button in the ADJUST section. Change the setting to 0.0. Wait for a couple of minutes before checking whether this value is reached since it takes that long for the needle valve to completely close.

7 **Let the cryostat warm up for 30 min**. During this time the cryostat will warm up and the gas inside the sample compartment will expand. All this time the gas flow pump stays on. If you forget to put vacuum on the sample compartment the plug will pop off and water and air might get into the compartment.

8 **Turn off the gas flow pump.**

9 **Open the needle valve.** Press the button in the GAS FLOW section of the Temperature Controller and simultaneously the RAISE button in the ADJUST section. Change the setting to 20.0. Wait for a couple of minutes before checking whether this value is reached.

10 **Wait for the system to re-pressurize.** Watch the gauge on the Flow Meter/Controller. As the needle valve opens, the dial will rotate clockwise until it reads 0.0. At this point the flow meters should start to indicate gas flow. It is time to close the needle valve.

11 **Close the needle valve.** Press the button in the GAS FLOW section of the Temperature Controller and simultaneously the LOWER button in the ADJUST section. Change the setting to 0.0. Wait for a couple of minutes before checking whether this value is reached.

12 **If you have been using the heliun-flow system you have to start the turbomolecular pump to pump the cryostat overnight.** The power to the pump should be switched on already. (If not you should do this. The switch can be found at the back of the pump. The pump will additionally run a self-test for approximately 20 seconds.) Start the pumping process by pressing the Start/Stop key on the front panel of the display unit. The oil pump will immediately start running. After a certain vacuum is reached the turbomolecular pump will automatically start. ***Wait till a vacuum of 10^{-5} is reached before opening the cryostat to the pump.***

20 **Open the cryostat to the turbomolecular pump.** Turn the big black knob that is placed on the vacuum line connecting the pump to the cryostat. Turn the knob counter-clockwise till a green ring is shown. The width of the green band should be about 1/16' (3 mm). In addition, slowly turn the black knob on the cryostat at the entrance of the vacuum line counter-clockwise three or four turns.

21 Overnight a vacuum of about $2 \cdot 10^{-6}$ should be reached.