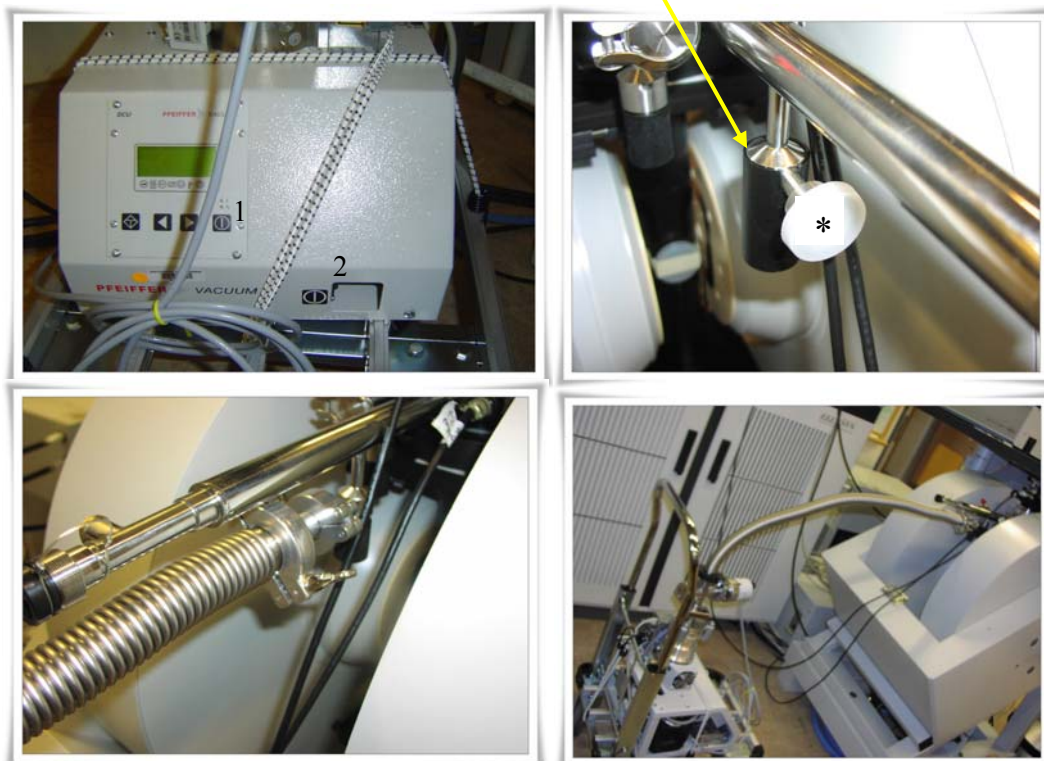


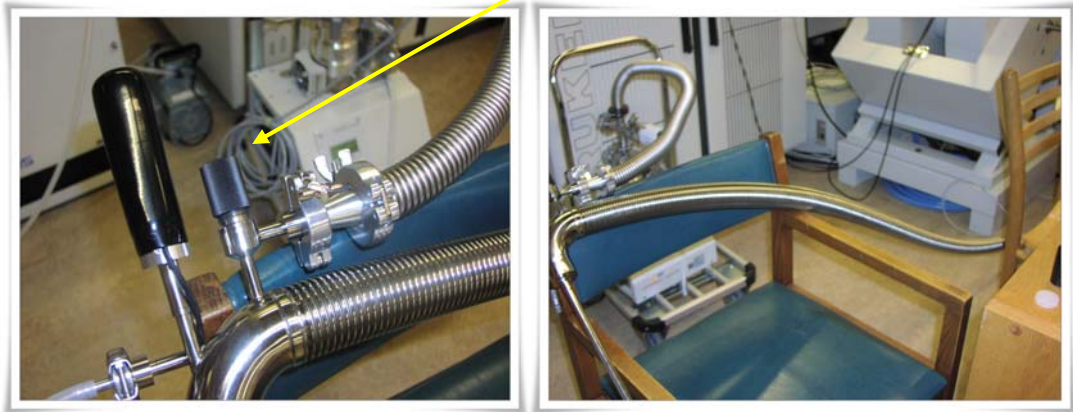
DEER pulse EPR procedure (revised September 2008)

- Put sample into flame-sealed capillary, spin down to the bottom, make sure the height of sample >6mm (12 μ l should be fine for CV1518Q capillaries, 25 μ l for CV2024Q), place the sample in -80 freezer.
- Check if we have low-pressure liquid nitrogen tank.
- The day before you are going to use it, pull vacuum for the cryostat overnight.
 - 1) Connect the vacuum pump line to the machine (shown in *).
 - 2) Turn on the main power (#2).
 - 3) If the window says "OK" turn on the second power (#1) and it will show one pump sign on the left (✓).
 - 4) Tighten the screw (black knob) in the back of the pump.
 - 5) Use "<" or ">" to set the menu at 340 which indicates the pressure "340: Pressure".
 - 6) Wait and then it will show two pump signs in the right (✓).
 - 7) Then take out the cover and loosen the connection in the machine to allow the pumping.



- In the morning (before you start), finish pulling vacuum for the cryostat and pull vacuum for the transfer tube for 3-4 hours until you get the pressure down to $\sim 10^{-6}$.
 - 1) Check the pressure of cryostat. If the pressure is $\sim 10^{-6}$, then it will be enough.
 - 2) Tighten the knob and put the black cover back.
 - 3) Turn off power (#1) – green light blinks.

- 4) Wait until two signs (✓) disappear and then start to loosen the screw in the back to release the pressure. It will go up to $> 1e3$ and then you can tighten.
- 5) Disconnect the vacuum pump line from the cryostat and put the white cover back (*).
- 6) You don't need to turn off the power (#2).
- 7) Connect the vacuum pump line to the transfer tube (tip end with a protective cover, leave the cover on) with black o-ring in between.
- 8) Turn on pump (#1) now, (#2 is already on).
- 9) Make sure the screw in the back of the pump is tightened.
- 10) Wait until it shows two pump signs (✓) on the right.
- 11) If it shows them, take out the black cover and loosen (2-3 turns) and pump 2-3 hrs until the pressure goes down to 10^{-6} .



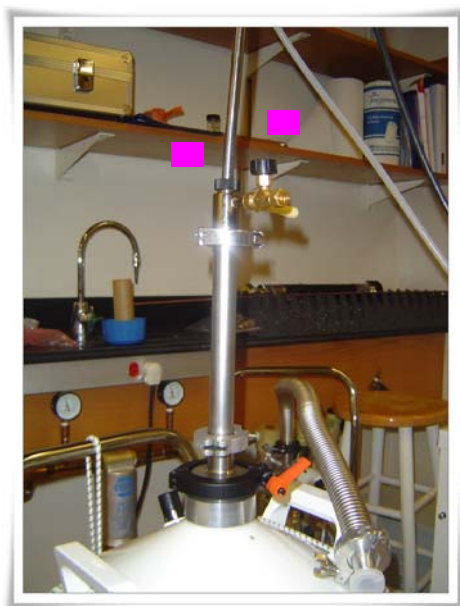
- If the pressure is below 10^{-6} , you can stop pulling the vacuum.
 - 1) Close knob and put the cover back on transfer tube. (yellow arrow above)
 - 2) Turn off switch #1.
 - 3) Wait until the two signs disappear, and release the pressure by loosening the screw in the back until the pressure goes to $> 1e3$ and then tighten the screw.
 - 4) Disconnect the line (undo clamp).
 - 5) Turn off switch #2 (main power).
- Here, you need to open and close the valve on the transfer tube manually, not automatically. From the completely closed valve position (all the way clockwise), one turn 360° counter-clockwise is 100% open. For the duration of the experiment, about $\frac{1}{4}$ open is correct (you want the heater level to be half-way) but for cooling the instrument to 80K you can open the valve to $\frac{1}{2}$ open and later close it to $\frac{1}{4}$ open.
- Turn on temperature controller (main power). *Note: As of Sept. 2008, temperature control is manual (by turning valve) not automatic. The motorized needle valve is not on the transfer line, contrary to shown in photos.*



- You need to fill the nitrogen (use low pressure nitrogen) dewar up to the top (it's 21 inch, 26L) (usually, if everything is going well you can do two experiments using one full dewar).
- Filling nitrogen dewar:
 - 1) Make sure rubber hose is attached to liquid valve on nitrogen tank.
 - 2) Put rubber hose a few inches below dewar opening – hold with 2 gloved hands.
 - 3) Open liquid valve on low pressure nitrogen tank slowly and just a little.
 - 4) After temperature inside dewar stabilizes, open valve on nitrogen tank more.
 - 5) When dewar is full (liquid comes out) close valve slowly.
 - 6) Don't move rubber hose until it thaws!
- Turn on two water pumps



- Put the connector on to the liquid N₂ dewar, with green O-ring between dewar and connector, tighten the connection (black clamp tight) and open the vent (knob “on”/ “off”).
- Take out the protective cover for the transfer tube and insert the transfer tube (leg part) into liq. Nitrogen dewar very slowly to prevent the splash of liquid N₂. Tighten the screw and vent (“off” direction) after the leg is all the way down to the bottom.
- Insert the tip end of transfer tube into a bottle of EtOH to check the flow of Nitrogen gas (you will see bubbles) → wipe the end using dry towel and tighten two black closures, screw and vent (shown in magenta).



- Insert the tip end of transfer tube into the cryostat until the nuts almost touched , keep loose for present (shown below as yellow arrow)
- Connect white TEFLON tube on the transfer tube with the same tube from the vacuum pump (shown in blue) and turn the pump on.
- check the pressure on gauge, the pressure will drop down to -850 immediately and wait until it goes up to -800 (it will go > -800) (another sign is that the two probes in flow meter will become stable, it may take ~ 5 min). Then tighten the connection between the transfer tube and cryostat.
- In ITC temp. controller, change sensor in display to “3” and heater to “3” for internal temp. measurement
- Set ITC temp at 80K (sometimes 50K, if you need and using helium): set and adjust → lower to set the temp. and push 1) heater “auto” 2) change PID, “I” from 2.5 to 1.0 (for nitrogen use).

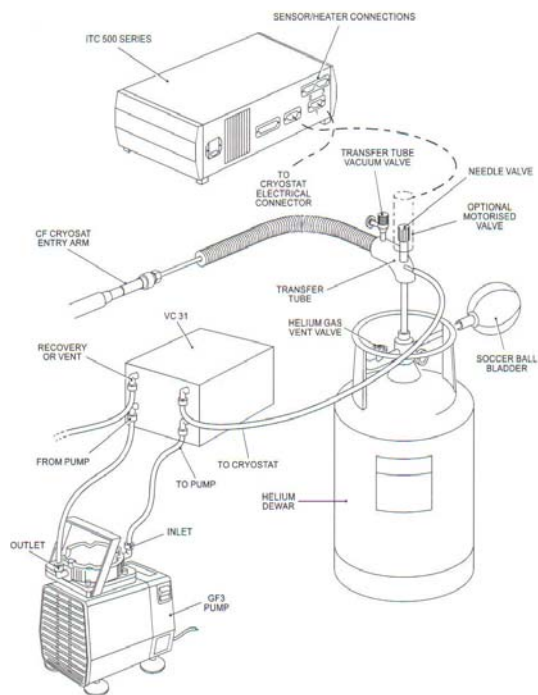


Figure 2 Cryogen, exhaust gas and electrical connections (GFS transfer tubes)





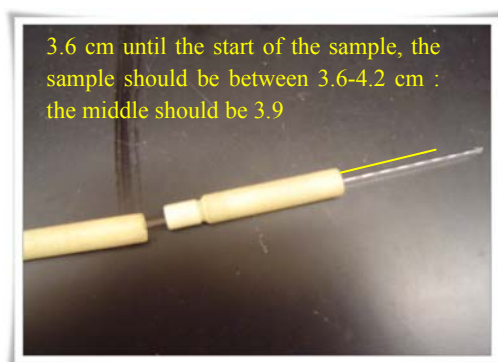
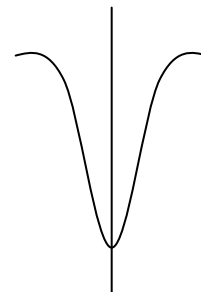
- Make sure water pumps are on – “Haskris” by machine and the other one by filing cabinet/ white board == “water pump temperature should be below 72F (21C) for both of them. If they’re above that temp, the machine goes back to standby!!” : check water level regularly!!
- Turn on the 1) machine, 2) bridge, 3) amplifier(TWT) power
- Wait until temp. reaches to 80K : it should go down to 80K within 30 min, if not it means there is a problem...! Check the vacuum and check if any part is blocked!! And wait a little bit more after the temp reaches at 80K.
- Check one more time the connection between the transfer tube and the cryostat if it’s tightened before you start!! Also, check periodically – it may loosen on the first day.



“Computer : click “Xepr” icon on the right bottom of the desktop”

1) Tune

- Click EPR icon at the bottom of the screen.
- Menu (Acquisition) → (Connect to spectrometer) → type “e580” → OK
- [Open tuning panel : 4th in the first group] → Opens “Microwave Bridge Tuning” window
- click Tune
- Make sure the bias is all the way to left
- Attenuation 60dB to 20dB
- Lower Iris by rotating disk to make a sharp dip (the deepest dip possible)
- Use the frequency bar to move the dip to the middle (see right)
- Now the temp is ~ 80K (look at page 17)
- Turn off the blue vacuum pump wait until the pressure increases and goes to zero...
- Put the capillary into the holder, inserting it into the side with the threads and measure its position.
- Take out the stopper in the sample holder to prevent the capillary from breaking inside the machine.



- Freeze the sample in liquid nitrogen (or isopropanol/ dry ice then wipe capillary with kimwipe) quickly
- Quickly, take out the black stopper from the machine
- Put the sample holder into the cryostat and tighten and wait 10-20 sec: In the tuning window, you will see the dip shifted after sample insertion, which suggested that the sample is located in the detection window.
- And then put the stopper back into the sample holder
- Turn on blue vacuum pump wait until the temp goes down to 80K
- Move frequency bar to make the dip in the center again.
- Lift up Iris to overcouple, you will see the dip become shallow, decrease attenuation down

to 15 or 10, keep Iris all the way up, and move frequency bar to make the center of the dip align with the screen center.

- Change to Operate, Attenuation back to 60 dB → Close
- Make sure pressure is at -800 to -700 before beginning

- PULSE

- [Open FT bridge panel : 8th in the first group] → go to standard configurations → select “pulse” mode
- [Exp : new experiment] → Go to Pulse → Advanced → Experiment Name; type pulse (pulse2, pulse3... etc.) → Create
- (File) → (Load) → 7_19_05 → pulse 1 (You can select something else, previous parameters for the same mutant, if it is FSW file) → Load → Close
- (File) → (Dataset table) : it will show the window in which file 1 and 2 are loaded
- [Get parameters from dataset : 1st in the second group] → select the file you need → Load
- click [Parameters to hardware : 4th icon in down left corner]
- [Open Specjet panel : 7th in the first group] : it will show “SpecJet Display” window
- [Open FT EPR parameters : 5th in the first group “23”] → Patterns → select acquisition trigger → set position at 0 and set the length at 20 → in +x, make sure there are two pulse (16ns and 32 ns pulse) (first a pi/2 and a pi) at 0 and 400 ns, respectively.
- [Open FT bridge panel : 8th in the first group] → Opens “FT bridge” window → Receiver Unit → (Depending on the signal intensity... you might need to adjust gain) Video gain to 48dB → Video bandwidth to 20 MHz → MW attenuation to 60dB
- click “start” on both FT EPR parameter window and specjet window, change # pts to 512, to make sure the defense pulse will be observed.
- Turn on TWT from standby to operate
- Slowly decrease attenuation to see the echo (FT bridge window), like to 20 dB.
- There are two lines on the window of “specjet”, One is green (real part) and the other one is yellow (imaginary part): you need to focus on the green one; here you can check the signal intensity for the first time.
- [Open parameter panel : 5th in the first group “23”] → Opens “FT EPR parameters” window → Pattern → select acquisition trigger → set position at 1200 → start in both → you will see a shift in echo signal position in specjet display
- In order to get the biggest positive and symmetric echo signal [green] [Open FT bridge panel : 8th in the first group] → Opens “FT bridge” window → Receiver Unit → 1) change the *phase* 2) adjust *MW attenuation* and [Open parameter panel : 5th in the first group “23”] → Opens “FT EPR parameters” → Field 3) change the center field

FT bridge window	signal phase	
	video gain	66
	video bandwidth	20
	MW attenuation	

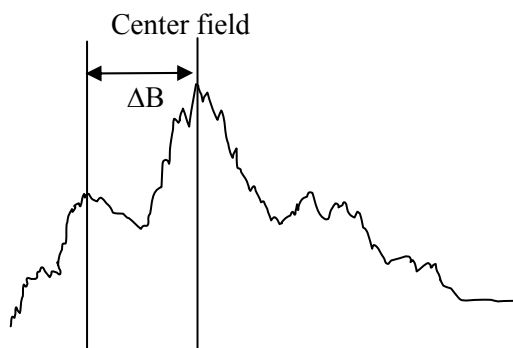
- If it's the best, [Open parameter panel : 5th in the first group "23"] → Opens "FT EPR parameters" → Acquisition → click "start transient" → and then start (▷) in the main window (left bottom corner)



- And then you will see this signal

Acquisition trigger	start at	
= echotop-echowidth/2	echo top	
	echo width	
	Acquisition trigger starts at	

- Find the echo width (dx) and echo top point.
- Calculate (echo top – bandwidth/2)
- [Open parameter panel : 5th in the first group "23"] → Opens "FT EPR Parameters" window → Go to "Patterns" → Select "Acquisition trigger"
- Set position at 1200+(echo top – bandwidth/2) and set the length at dx value. Set sweep width to 150G, field tab in FT EPR Parameters.
- Acquisition and click "run from the tables" → and then run (▷) in the main window (left bottom corner)



- You will see the field swept echo-detected EPR spectrum shown above

- Record the center field, low field value and calculate the difference ΔB



- Write down (1) to (4)
- Calculate the Eldor frequency : this value will be used in the final 4P-DEER window as a final ELDOR frequency
- Save this file : mutant name....._FSW (field scan wave)

3) Set to DEER

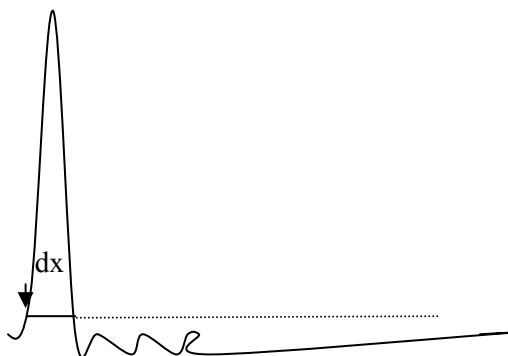
- [Exp : new experiment] → DEER → Experiment Name ; type DEER (DEER2, DEER3... etc.) → Create
- (File) → (Load) → 7_19_05 deer file (or you can load your previous deer file) → Load
- (File) → (Dataset table) : it will show the window in which file 3 is loaded
- [Get parameters from dataset : 1st in the second group] → select the deer file → Load
- [Parameters to hardware : 4th icon in down left corner]
- [Open parameter panel : 5th in the first group "23"] → open "DEER" window
- [Open Specjet panel : 7th in the first group] : it will show "SpecJet Display" window
- [Open parameter panel : 5th in the first group "23"] → in "DEER" window, "setup" tab → d0=380, d2=1200, pi(ELDOR)=0, Field = local Maximum field that you get before (low field), frequency=current frequency, attenuation=30dB, and phase cycling to be 8step,
- [Open FT bridge panel : 8th in the first group] → Opens "FT bridge" window → Receiver Unit → Video gain to 60dB → Video bandwidth to 20 MHz → MW attenuation to 20dB, start in DEER window
- [Open FT bridge panel : 8th in the first group] → Opens "FT bridge" window → Receiver Unit → In order to get the biggest positive green signal shown in specjet display window
1) change the *phase* 2) adjust *MW attenuation*

Set to DEER	Phase	
	MW attenuation	

- [start (▷) in the main window (left bottom corner)

You will see this signal and then check the starting point (arrow) and dx and write them down

- GO to 4p DEER window → in d_0 (usually 380), add (echo top – bandwidth/2) and then input this value in d_0 .
- Go to “4p DEER” tab, change “field position” to low field maximum, set dx to be 8, set shots/point=1024, set pi(ELDOR)=32, set Attenuation = 0, set $d_2=1200$ ns (or greater value depending on anticipated distance), change gate to “dx” value
- Set phase cycling to 2 step, give a number of scans (like 1000), type in current ELDOR frequency with the value just calculated.
- Start (▷) in the main window (left bottom corner)



4P-DEER window	pi/2 (ns)	16
pulse sequence	gate (ns) : final dx	
	d_0 (ns) : 380+(top – width/2)	
	d_1 (ns)	200
	d_2 (ns)	1200 (or longer)
	pi (eldor) (ns)	32
	d_3 (ns)	100
	phase cycling	2 step
general parameters	shots/point	1024
	number of scans	1000
	SRT (us)	1999.6
	field position (G)	low field
abscissa1 :eldor	number of points	Depends on d_x and d_2
	d_x (ns)	8
	Eldor frequency (GHz)	Calculated eldor freq.
	attenuation (dB)	0

Nutation experiment:

- after tune, overcouple, set to operation, change FT bridge to pulse mode, TWT to standby
 - new experiment, select advanced, click create
 - open and load a nutation experiment file from data set table or
1. click “parameter to hardware”
 2. open parameter, in “pattern” tab, setting up a stationary two pulse echo with a tau of 400 ns and an acquisition trigger here.

Edit						Start
	1	2	3	4	5	Stop
Position [ns]	2000	2400	0	0	0	
Length [ns]	16	32	0	0	0	
Pos. Disp. [ns]	0	0	0	0	0	
Length Inc. [ns]	0	0	0	0	0	

Enter the following parameter values:

+x Position(1) 2000 ns

+x Length(1) 16 ns

+x Position(2) 2400 ns

+x Length (2) 32 ns

Acquisition Trigger Position 2000 ns

Acquisition Trigger Length 4 ns

Shot Rep. Time 1999.2 μ s

Shots per Loop 10

1. In FP-bridge, the “receiver unit” tab, video gain to 48, MW attenuation 60dB
2. Open “specjet”
3. Change the field (FT EPR parameters, Field Tab) to the global max. that you obtained in field swept experiment
4. Turn on TWT, adjust signal phase and MW to maximize the echo - positive (they should be

pretty close that those values in FSW measurements)

5. In the pulse pattern of “acquisition”, Increase the Acquisition Trigger Position until the top of the echo is at the left edge of the SpecJet display. Stop – parameters and specjet
6. In the pulse pattern of “ELDOR”, Set the ELDOR Length to 0 ns, Set the Length Inc. to 4 ns
7. Set the Pos. Disp. of the Acquisition Trigger and the +x pulses to 4ns (twice)
8. Set Shots Per Point to 1024.
9. In “Acquisition” tab, Set the X-Axis Quantity to Time. Set the X-Axis Size to 32, Y-Axis to 1

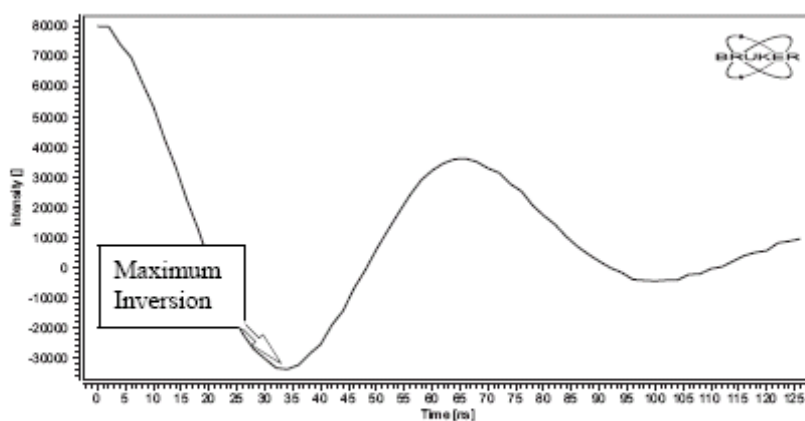


Figure 4-5 Nutation data to determine the maximum inversion.

10. FT EPR Parameters – Microwave Tab – set current ELDOR frequency = frequency displayed, ELDOR Attenuation = 2
11. Click “run”, and find the max inversion

Sample change and turn off the system:

1. After the measurement was completed or stopped, save the spectrum
2. Click stop in both “EPR parameter” (or DEER) window and “specjet” window, and close both windows
3. Turn off TWT – press stdby
4. Change from “pulse” to “CW” in the “FT-bridge” window, close
5. Open “tuning” window, change from “operate” to “tune”
6. Turn off pump (the small, blue one)
7. Wait until the pressure goes up to 1 atm, (you will see the jump of probe)
8. While waiting, rotate the iris down and see the dip – should reach minimum.
9. Take out sample rod (the dip on the screen should disappear) and put the black stopper in and tighten
10. If you finished your measurement and want to shut off the system, go to step 16

11. Take out your old sample from the rod and clean the frost on it.
12. Wait until the rod’s temperature become equilibrium with room temperature, put new sample in and make sure the center of your sample is 3.9 cm away from the tip of rod.
13. Freeze your new sample with liquid N₂ or isopropanol/ dry ice.
14. Take out the stopper and quickly and carefully put the sample rod in (without its stopper), watch the computer screen to see the appearance of dip to make sure your sample is in. Tighten the rod and reinsert stopper into sample rod. Turn on pump.
15. To continue your measurement, see previous pages

16. To shut down the system, Tuning window – click stdby, Acquisition – disconnect (wait until screen clears), turn off the power of TWT, bridge and machine
17. Disconnect the TEFLON tubes between transfer tube and pump (after pressure goes down).
18. Carefully take out the transfer tube from liquid N₂ dewar, wait until it reaches room temperature, clean any water on it and put the cover on the tip end and put it onto the shelf carefully
19. Take away the connector and put it on shelf too
20. Put the stopper on the dewar
21. Turn off the power for the water cycling system
22. Turn off temperature controller
23. Turn off turbo pump if need (see the instruction before).

Data Analysis, Basic Operation

1. On a Cafisolab PC open Secure FX. Connect to xep1. Open xepfiles. In your folder, save .DSC and .DTA files to PC's hard drive.
2. Open MATLAB.
 - a. In current directory, choose C:\Program Files\DeerAnalysis200#. (# Depending on latest version.)
 - b. Type DeerAnalysis in MATLAB prompt.
 - c. In DeerAnalysis 200# window (applies for the remaining steps), load file(s) .DTA.
 - d. Click off imaginary, set the zero time to peak of echo decay.
 - e. Click “!” (auto calc.) in background.
 - f. In background model, homogeneous – 3 dimensions for soluble/ membrane proteins or 2 dimensions for membrane proteins.
 - g. Click Tikhonov reg. and click Compute L curve. Click fit.
 - h. You can move the blue cursor by typing value in start (nm). But must set at the original value to re-fit.
3. See <http://www.esr.ethz.ch>, Software for downloads and Users Manual.