POWER UP PROCEDURE

1) **TURN THE NITROGEN ON 15 MINUTES BEFORE YOU TURN THE INSTRUMENT ON.**

2) **BE SURE YOU KNOW WHICH NITROGEN SOURCE IS IN USE – THE LN2 TANK (IN 835) OR THE N2 GAS (NEXT TO GC-MS)**

NOTE! YOU WILL CAUSE EXTENSIVE DAMAGE TO THIS SYSTEM IF YOU RUN WITHOUT N2 –BE SURE IT DOES NOT RUN OUT WHILE IN USE

3) SET THE N2 FLOW TO 60 SCFH IF YOU ARE SCANNING BETWEEN 210-170 NM **OR** SET IT TO 40 SCFH IF YOU ARE ONLY SCANNING ABOVE 210 NM.

4) THE SYSTEM CONSUMES A LOT OF N2, BE READY TO RUN WHEN TO WHEN YOU TURN THE INSTRUMENT ON AND TURN THE N2 OFF AFTER YOU TURN THE INSTRUMENT OFF.

KEEP AN EYE ON THE NITROGEN PRESSURE REMAINING IN THE TANK, ESPECIALLY IF YOU ARE USING THE N2 GAS CYLINDER NEXT TO THE GC-MS.

5) YOU MUST HAVE A TRICKLE OF WATER FLOWING WHENEVER YOU RUN THE TEMPERATURE CONTROLLER, ONLY USE A TRICKLE, OR YOU WILL OVER PRESSURE THE CELL.

1. **<u>BE SURE THE NITROGEN IS ON AND FLOWING AT LEAST 40 SCFH.</u>**

2. Turn on the temperature controller.

3. Turn on the instrument.

4. Start the Spectra Manager 2 software.

5. Double-click on Spectra Measurement to open the spectrum acquisition software.
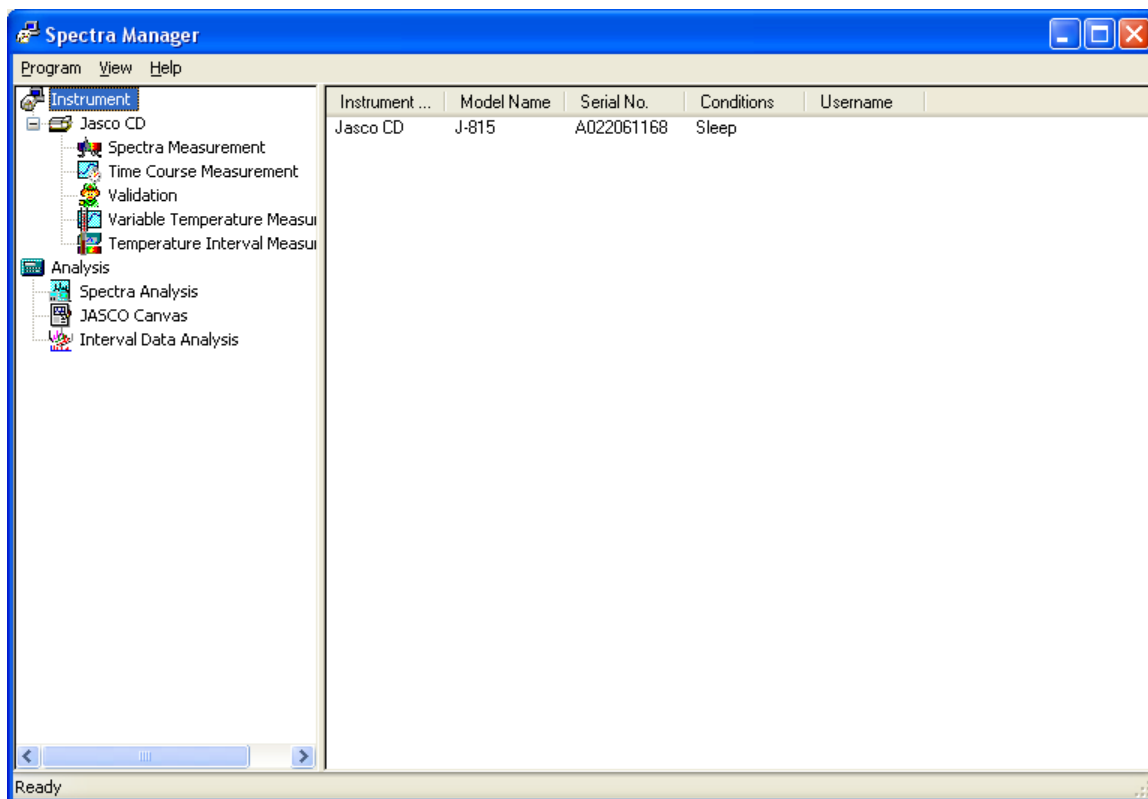
6. The software will initialize the instrument. Once the software is running, the lamp will turn on. Wait at least 15 min. for the lamp to stabilize (up to 1 hour wait for very sensitive samples).

7. Note the HT voltage (see screenshot below) in the logbook before you begin your acquisitions. If the voltage has made a large increase from the previous entry, notify Sara **immediately**.
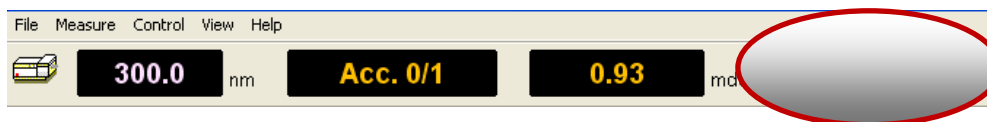
8. Click on [icon] to change the acquisition parameters.
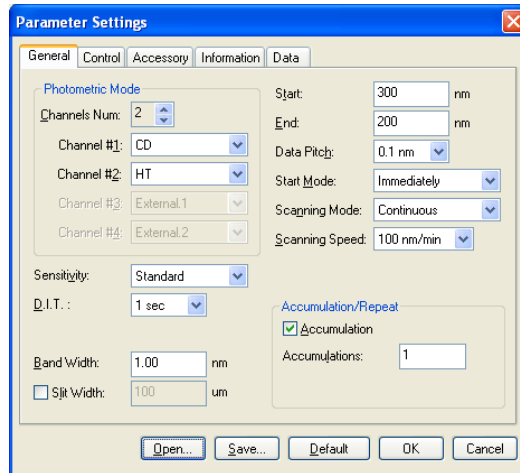
9. On the right side of the General tab, change the start and end wavelengths as you need. Start at the higher wavelength and end at the lower one.

10. The data pitch is the same as resolution.

11. Scan speed needs to be set so that it meets this rule:

    (DIT) x (scan speed) < 200

    (DIT is the integration time per data point.)

12. Accumulation is the number of spectra you want to average together to make one dataset. Can be set to 1 for a single spectrum acquisition.

13. Click on the Control tab. Click on the button next to baseline to acquire a background signal before sample acquisition.

14. Click on the Accessory tab.
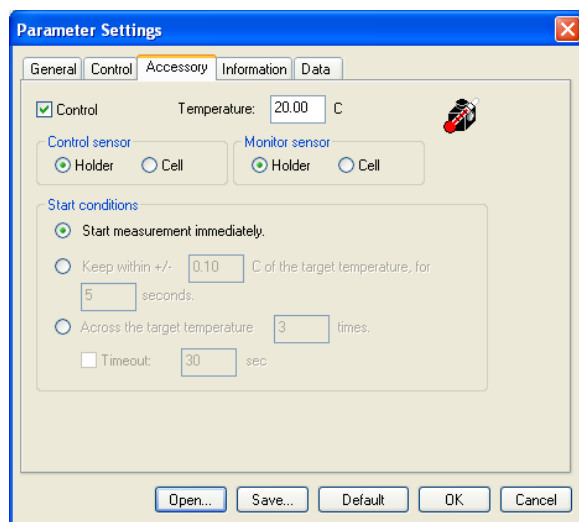
15. Change the temperature of the cell as needed for your sample.

16. If you want the temperature to stabilize before acquiring you can change the "start conditions" at the bottom of this tab.

17. To make any comments on your dataset, click on the Information tab and add any comments (concentration, buffer, etc).

18. Click on the Data tab and change your autosave folder and name.

19. Click on OK.

20. Click on to acquire a background.

21. Put your solvent-filled cuvette in the spectrometer and click Measure.

22. Remove your solvent cuvette and replace with the sample cuvette. Click on to acquire your spectrum.

23. Your spectrum will open in the Spectrum Analysis software when it is done acquiring. You can save and do processing there.

Data Analysis

1. In Spectra Manager 2, double click on "Spectra Analysis" under the analysis tab.

2. Open your file with either File->Open or the folder icon.

3. Use the ![icon] button to zoom in. Click and drag a box around the region you want to zoom in.   Righ-click to zoom out to full scale. Use the

    ![arrows] buttons to move left/right in your spectrum.

4. To peak pick, click on the ![icon] .

    a. Click on "Scale" and select "auto" for both axes, then click OK.

    b. Click on the checkbox next to "lower limit".

    c. Click on the black box on the upper x-axis and drag the cursor down below the peaks you wish to have automatically selected. Click "apply".

    d. If the software is picking too many peaks, you can change the noise level and click "apply". Larger number means higher noise threshold.

    e. Click OK.

5. To run a secondary structure analysis, click on ![icon] to run CDPro.

    a. You need to know your cell path length and an accurate concentration of your protein.

    b. You should pick a reference that includes the wavelengths over which you acquired data, and may need to try several different calculation methods to find one that works. Further information on this program and the calculations can be found by searching the internet for "CSU and CDPro".