Operating the OSU Chemistry Jasco J-815 Circular Dichroism Spectrometer
Brandon Sullivan and Prof. Thomas Magliery – Updated 18 Oct. 2010

Training:
Before you use the Jasco CD Spectrometer for the first time, you MUST use it once with Dr. Bakhtina (mbakhtin@chem.osu.edu), even if you previously were a user of the Aviv CD. *Failure to follow this simple request will result in your losing the privilege to use the CD without supervision for one year.*

Signing up:
Once your account has been activated for use on the Jasco CD, you can reserve time using the Chemistry department's Calcium calendar system. Acquisition of wavelength spectra will be much faster (~2 min) than it was on the Aviv (~20 min), so please adjust your reservations accordingly. Try to sign up for 4 hour blocks of time or less unless you need to do a thermal melt or titration. Note that 15 min. are required for nitrogen purge and 30 min. for lamp warm-up if the machine is not already on.

Preparing your sample:
As a general rule, the absorbance of the sample should be about 1 at the wavelength of interest in a cuvette with the path length that will be used for CD spectral acquisition. For typical far-UV protein spectra, that means A_{220} should be about 1, typically using a 1 mm rectangular quartz cuvette (which you must provide, e.g., Hellma 110-QS). A ballpark starting concentration would be 0.5 mg/mL protein, or maybe half that for α-helical proteins and twice that for β-sheet proteins. If there is insufficient CD signal (< 5-10 mdeg), you should increase the concentration of the sample. However, since CD measures the difference between absorbance of orthogonally polarized light, using arbitrarily high concentrations will prevent enough light from passing through the sample for meaningful measurements. If the HT (dynode) voltage is > 600 V, the total absorbance of your sample is too high. It is possible to use even shorter path length (0.5-0.1 mm) cuvettes to solve this problem. Note that if your buffer/solvent absorbs in the region of interest, it will add to the total absorbance (HT voltage) without improving signal. Avoid buffers, detergents or other molecules that contain aromatic groups, thiol reducing agents (βME, DTT), imidazole, metal ions, and chloride salts. The best buffer for CD is usually low-concentration (5-50 mM) phosphate or acetate with no salt (or less than 300 mM, anyway). Note that you need to use a sealable cuvette for thermal melts.

Log book:
Sign the log book when you arrive. Write your name, advisor, phone number (cell is best), time on, time off, and HT voltage of the empty chamber after warm-up. Failure to accurately log your usage of the instrument will result in your losing the privilege of using the instrument with supervision, or at all, for a period of 3 months to indefinitely.

Turning the Instrument on:
1. Behind the computer monitor one will find the on/off valve for nitrogen gas and the pressure regulator. In Figure 1, the nitrogen is off. To turn the nitrogen on, aim the blue handle towards the wall as seen in Figure 2. One will hear a slight hiss. Using the black knob located on top of the flow control, slowly adjust the flow of nitrogen such that the CD reads 25 ft^3/hr (Fig. 4). The measurement should be read at the top of the silver cylinder. Allow the nitrogen to purge the CD for **15 minutes**.
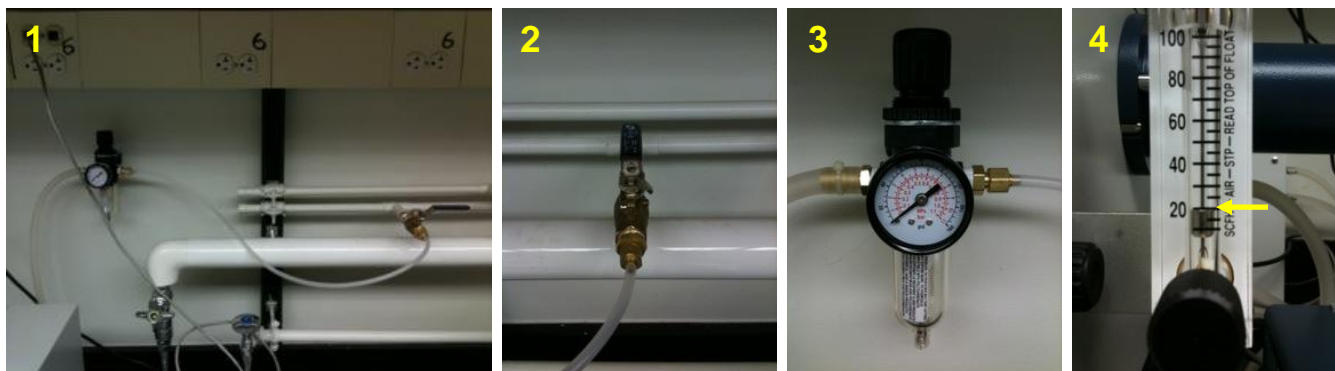

2. Turn the CD on (Fig. 5), followed by the Peltier device (Fig. 6) and the water circulator (Fig. 7). Allow the lamp to warm for **30 minutes** before taking data. The circulator must be on for the Peltier temperature controller to function properly. Attempting to cool the Peltier device to near or below room temperature without the circulator on can damage it permanently.
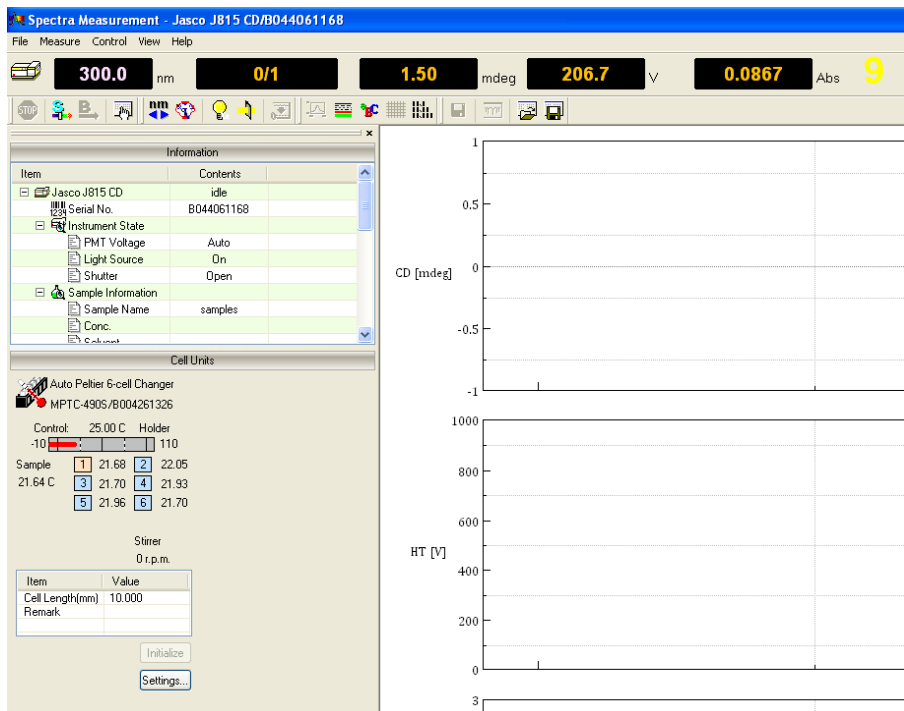
Starting the Spectra Manager 2 Software:

1. Select the Spectra Manager 2 icon from the desktop by double clicking. The following window will open (Fig. 8). The left pane is subdivided into two main features. The top half (Jasco J815 CD) are the software programs needed to run and collect data. The lower half (Analysis) is the software required for viewing and analyzing recorded data. The Analysis suite may be downloaded on lab computers.
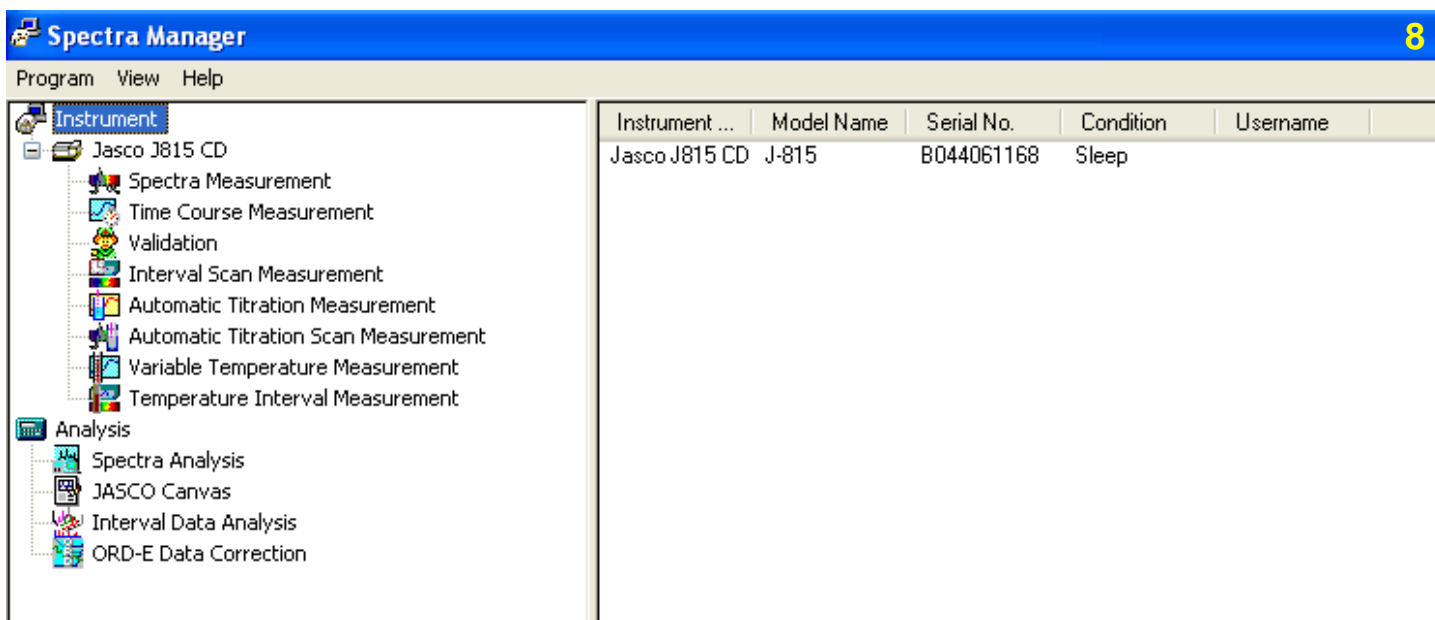
Collecting a Spectrum

1. Double click on the **Spectra Measurement** icon seen in Figure 8. This will prompt the software to "initialize instrument." Next the system will purge for several minutes with nitrogen gas. Do not skip this automated step. If double clicking the Spectra Measurement icon does not initialize the instrument, restart the computer and reopen the software. *For best results, the **Spectra Manager 2 Software** suite should be closed when the instruments are turned on.* When the CD is ready to begin measurements, a new window will open as seen in Figure 9.

By default, the software will open and set the monochrometer to 300.0 nm. **Please record the HT Voltage value in the log book for record keeping purposes**. *This recording should take place in the absence of a cuvette. In the current screen capture we see 206.7 V at 300 nm. If the value is significantly different than those recorded by previous users, make sure the cell is clear of samples/cuvettes/probes, the lid is closed and the nitrogen is on. If this is not the problem, contact Besik Kankia, Brandon Sullivan or Prof. Magliery. The HT Voltage will read near zero if the sample door if open.*

If the Peltier device is turned on, the software will automatically open in the left pane. In Figure 9, one sees this as "Cell Units." Note: If the Peltier device were not on when the **Spectra Manager 2** was initiated, the control panel would not appear.

2. Entering the Parameters for a Wavelength Scan. To open the control panel, click the icon called "Parameters," which is represented by a white hand hovering over a keyboard (Figure 10-Blue circle). This act will open a new window entitled "Parameter Settings."
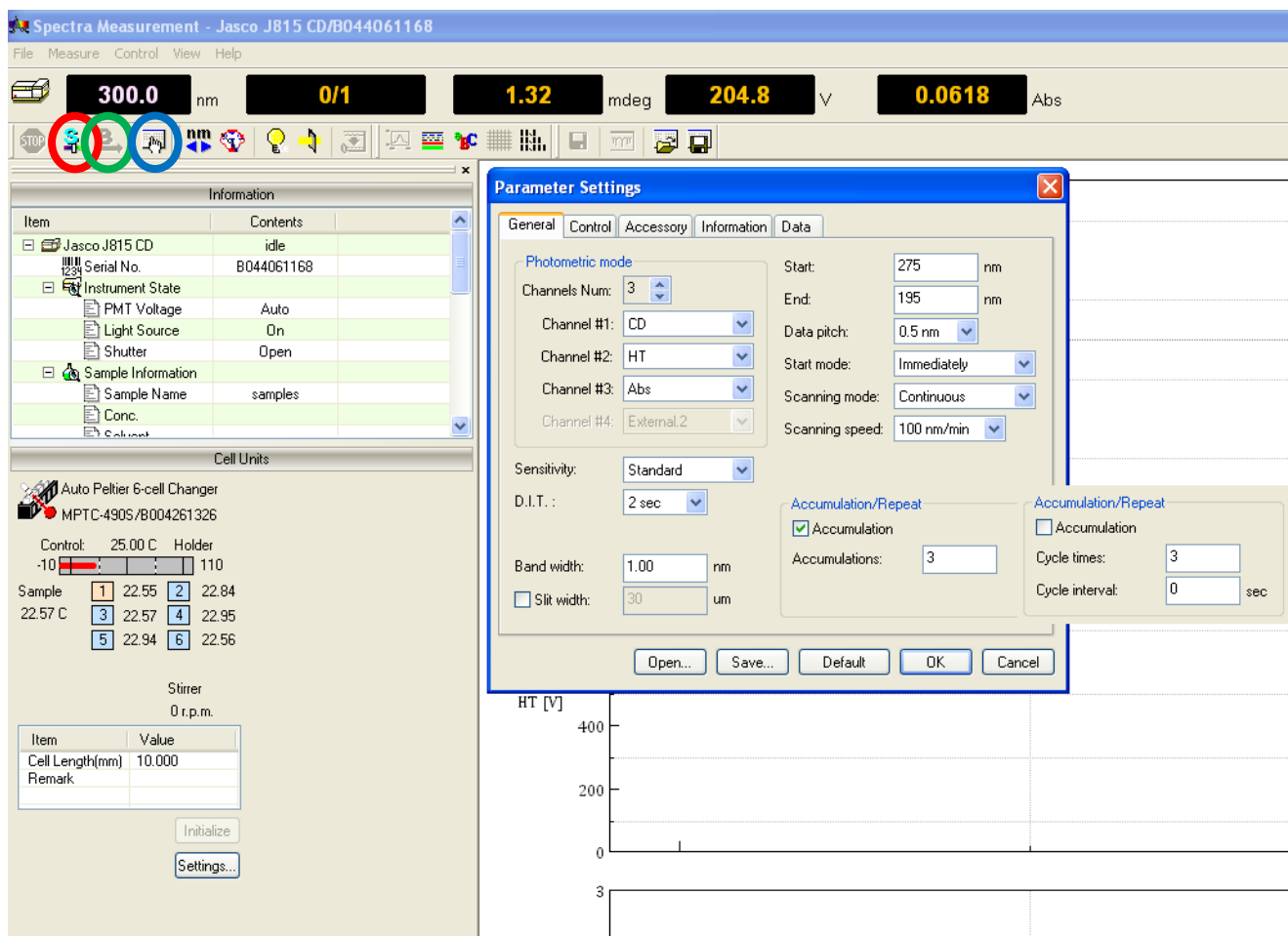
The following will walk one through all of the parameters in the "General" tab.

- **Photometric mode:** Select the number of channels one wishes to view during the collection period. It is generally advised to monitor HT voltage with CD signal. The HT voltage is analogous to the dynode voltage plotted by the AVIV Circular Dicrhoism. For the Jasco, any data collected with HT voltage 600-800 V is suspect and > 800 V is not valid. It may also be useful to monitor the absorbance of one's sample in the third channel.
- **Sensitivity:** For most samples the sensitivity can remain at Standard. If one's sample has very small differential absorbance of the circularly polarized light, one may wish to experiment with the high sensitivity setting.
- **D.I.T.:** Data Integration Time is the amount of time the detector will read the sample before taking the average. Cleaner data can be obtained with longer integration times, but for most samples 2 seconds is sufficient. Longer integration times will average over more nm of spectral width, smoothing out finer features of the spectrum.
- **Band width:** Try 1 nm first for most samples, but 2 nm is fine if the signal is too low (especially for beta proteins).
- **Start and End:** These parameters determine the start and end points for the spectrum to be collected. For proteins, the far-UV range for estimating secondary structure is 190-250 nm and the near-UV range, which reports on the environment of aromatic amino acids, is 250-350 nm.
- **Data pitch:** The data pitch determines the number of data points taken during the scan. This is more important for "step mode" which will be discussed with "Scanning mode."
- **Start mode:** Should be left on Immediate
- **Scanning mode:** The two most common modes on the Jasco are STEP and CONTINUOUS. The STEP mode will simply start at the beginning wavelength, 275 nm, and collect the signal for 2 seconds (D.I.T.) and move to 274.5 nm (Data pitch). The process is repeated every 0.5 nm until 195 nm. This is how the AVIV CD collects data. The CONTINUOUS mode collects data as a sliding window while still averaging for each point in the Data pitch. This method is dramatically faster and no less accurate. The rate at which CONTINUOUS mode collects data is determined in the "Scanning speed" drop box. 100 nm/min is appropriate for most applications. Use the CONTINUOUS mode unless you absolutely need discrete wavelength data.
- **Accumulation/Repeat:** The user has two options for collecting multiple wavelength scans on one sample. Under ACCUMULATION mode one specifies the number of scans (accumulation) and the software automatically averages the scans and exports the data as one curve. In REPEAT mode one enters the number of scans and each curve is exported independently. REPEAT mode makes it easier to see the reproducibility of the spectra.

Note that with 100 nm/min scanning speed, 2 sec data integration time, and data pitch 0.5 nm, the effect is that a value is reported at each 0.5 nm wavelength (e.g., 250.0 nm, 249.5 nm, etc.) that is a moving average of 2 sec, or 3.33 nm, of data. This has the effect of smoothing out fine features (although in general CD spectra do not have fine features) and underestimating the ellipticities at minima and maxima. Reducing the scanning speed and integration times will reduce this effect, but reducing the scanning speed will make the experiment take longer and reducing the integration time will increase the random error in each point.

The following will walk one through the parameters in the "Control" tab. (Figure 12)

- The Jasco gives one the opportunity to manually or automatically subtract background. On the AVIV there was only the option of manual subtraction. Under CORRECTION, if one selects NONE, the Jasco will report spectra in a manner similar to the AVIV. In this scenario, one would scan their buffer as a sample, their sample, and manual subtract he blank using the **Spectra Manager Analysis** or **Excel**. If one selects BASELINE, at the start of the run, the software will prompt one to insert the blank and collect the background spectra. This spectrum will automatically be subtracted from the following samples. It is recommended to use NONE and manually subtract the reference sampled (e.g., buffer) after acquisition.

The following will walk one through the parameters in the "Accessories" tab. (Figure 13)

- The Accessories tab is where one controls external devices like the Peltier device. The temperature one wishes to collect the spectra at can be adjusted in this window as well as the method for temperature monitoring. In general it is OK to monitor the temperature by selecting HOLDER in each drop down menu. If one is concerned with thermal conductivity and the exact temperature of the sample, external probes (the thin clear wire) may be placed within the cuvettes (Figure 11).

The following will walk one through the parameters in the "Information" tab. (Figure 14)

- The Information tab allows one to annotate the file with useful information. When exported to the Analysis software these inputs will remain as headers within the file. This is very useful especially when looking back at data that may have been collected several months or even years ago.

The following will walk one through the parameters in the "Data" tab. (Figure 15)

- This is the location one enters the directory in which the data will be saved to. **Save your data on your network (U:) or group (Z:) drive.** Data stored on the local hard drive will periodically be deleted without notice.

12 Parameter Settings — General | Control | Accessory | Information | Data
Correction: ⦿ None ○ Baseline
☐ Shutter is opened and closed automatically
Fluorescence units: Detector [0] Volt
[Open...] [Save...] [Default] [OK] [Cancel]

13 Parameter Settings — General | Control | Accessory | Information | Data
☑ Control   Temperature: [20.00] C
Control sensor [Holder ▾]
Monitor sensor [Holder ▾]
Start conditions
⦿ Start measurement immediately
○ Keep within +/- [0.10] C of the target temperature, for [5] seconds.
○ Across the target temperature [3] times.
☐ Timeout: [30] sec.
[Open...] [Save...] [Default] [OK] [Cancel]

14 Parameter Settings — General | Control | Accessory | Information | Data
Sample name: [samples]
Operator: [ ]
Division: [ ]
Comment: [ ]
Concentration: [ ] [mol/L ▾]
Solvent: [ ]
☐ Display the [Comment] dialog box before measurement
[Open...] [Save...] [Default] [OK] [Cancel]

15 Parameter Settings — General | Control | Accessory | Information | Data
Save data
☑ Auto save
Save in: [C:\Documents and Settings\Thomas Magliery Ph.] [Browse...]
Format: [Date-No. ▾]
String: [ ]
Send to analysis
☑ Send data to Spectra Analysis
Print
☐ Print measured data
Template: [ ] [Browse...]
[Open...] [Save...] [Default] [OK] [Cancel]

If one wishes to save these parameters to run future scans under identical conditions you may select the SAVE feature located at the bottom of the Parameter Settings window. Likewise, this is the location to OPEN previously saved methods. Once all the parameters are set, press OK to save and exit Parameter Settings.

3. Running a Wavelength Scan.
- For NONE Correction – Press the cyan "S" in the toolbar (Figure 10-Red circle). The user will be prompted asking which cells to read (Figure 16). Selecting MEASURE, will being the run. At the conclusion of the run, **Spectra Analysis** will automatically open. This is the recommended method of operation.
- For BASELINE Correction – Press the green "B" in the toolbar (Figure 10-Green circle.) The user will be prompted asking which cell contains the blank. You may select a specific cell, or all cells. Press MEASURE to collect the BLANK spectrum. Note that this data is not saved as a file or displayed in **Spectra Analysis**. It is saved to memory and auto-subtracted from each sample. If you need to analyze of collect the background spectrum select NONE correction in the Control tab of Parameters Settings. Once the background sample is collected, press the cyan "S" in the toolbar (Figure 10-Red circle). Select the cells one wishes to read and press MEASURE. **Spectra Analysis** will automatically open and display data at the conclusion of the run.

Collecting a Thermal Melt

There are two methods for collecting thermal denaturation data. The first method is analogous to the AVIV, where one monitors 1 or 2 wavelength with increasing temperatures. The second allows one to take an entire spectral scan at each temperature.

1. To Collect 1 or 2 Wavelengths with Increasing Temperature
- Open **Spectra Manager 2**
- Double Click Variable Temperature Measurement
- Open Parameter Setting by selecting the white hand hovering over the keyboard in the toolbar. Note that this icon is the same used in **Spectra Measurement**.
- Temperature Tab: Enter the starting Temperature and select whether or not to perform a reverse melt. Sampling(C) refers to how often one wishes to obtain data. The Target(C) temperature is the final temperature of the melt and the Ramp Rate(C/m) indicates how fast the Peltier device will increase the temperature. The final tab, Wait(sec) indicates how long the CD will allow the sample to equilibrate at each temperature before taking data. This tab also allows one to select temperature monitoring within the cell or holder.

16 Parameter Settings — Temperature | Start/End | General | Control | Information | Data
Start temperature: [25] C ☐ Reverse Hold Time: [0] sec

	Sampling(C)	Target(C)	Ramp Rate(C/m)	Wait(sec)
1	1.0	95	1.0	6.0
2				

Control sensor [Holder ▾]
Monitor sensor ⦿ Holder ○ Cell
[Open...] [Save...] [Default] [OK] [Cancel]

  - o A standard protein melt is shown in Figure 16. Sampling every 1 C° from 25 °C to 95 °C at 1 C°/min with 6 sec wait time means that the sample will be heated 1 C° in 1 min and then data will be acquired after 6 sec of equilibration.
  - o If you know the protein melts around, say, 60 °C, it would save time to only collect data every 5 °C between, say 25-40 °C, then every 1 C° from 40-60 °C, then every 5 C° from 60-95 °C.
  - o Depending on the rate of equilibration for your specific protein, you might wait from 0 sec to 60 sec. Note that this expands the time of the experiment by Wait (sec) times the number of points.

17 Parameter Settings — Temperature | Start/End | General | Control | Information | Data
Start condition
⦿ Keep within +/- [0.2] C of the target temperature for [3] seconds.
○ Equilibrate across the target temperature [3] times.
End condition
○ Return to start temperature
○ Keep end temperature
⦿ Move to specified temperature [25.0] C
[Open...] [Save...] [Default] [OK] [Cancel]

- Start/End Tab (Figure 17): This tab allows one to control the settings similar to the "Dead band" in the AVIV software. *We recommend keeping the sample within 0.2-0.3 C° of the target temperature for 2-3 seconds.* Once these criteria are met, the CD will equilibrate the sample (Wait) at the temperature. One
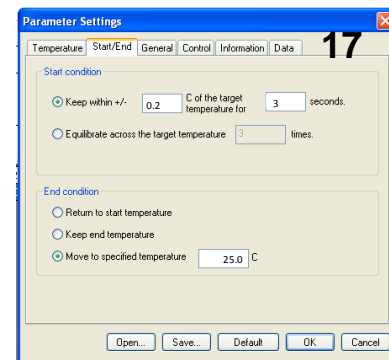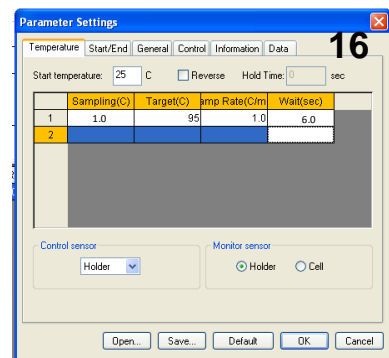
can also dictate what the Peltier device will do after the data is collected. It is recommended to return the block to room temperature (25 ºC).

- General Tab (Figure 18): This tab displays the same material we discussed in **Spectra Measurement** Figure 10. Additionally, this is where the user selects 1 or 2 wavelength to monitor with increasing temperature.
- Control/Information/Data: Are Identical to the tabs from **Spectra Measurement**.

Once the parameters are finalized, collect the data in the same manner as a wavelength scan.

2. To Collect the Spectra with Increasing Temperature

- Open **Spectra Manager 2**
- Double Click Temperature Interval Measurement
- Open Parameter Setting by selecting the white hand hovering over the keyboard in the toolbar. Note that this icon is the same used in **Spectra Measurement**.
- Because we are essentially taking a wavelength scan, the Parameter settings tabs, General, Control, Information and Data are identical to those described in the **Spectra Measurement** software.
- Temperature Tab (Figure 19): Here one enters the start and ending temperatures as well at how often to take wavelength scans. The screen capture in Figure 19 shows a standard protein melt profile. Here we melt the protein from 25 to 95 °C in 1 °C steps. The temperature is raised 1 °C in 1 min and data are collected after 6 sec once the deadband requirement (0.2 Cº for 3 sec) is met.
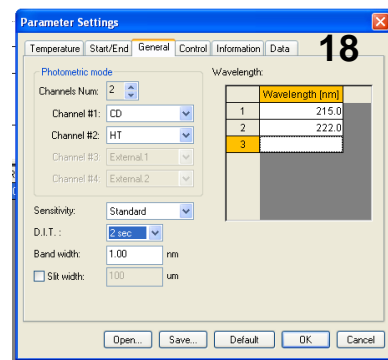
Once the parameters are finalized, collect the data in the same manner as a wavelength scan.
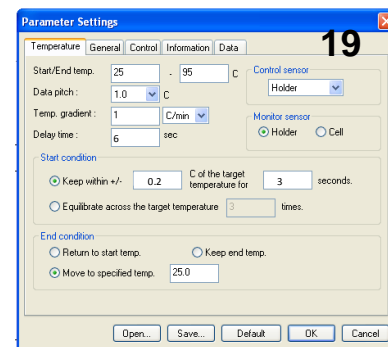
Collecting a Sample with Time

The Jasco CD can be used to monitor ellipticity with time. These experiments are useful for measuring kinetic processes.

Titration

Please contact Dr. Kankia or Prof. Magliery if you wish to use the titrator.

Turning the Instrument off:

1. Close out of the **Spectra Manager 2** software. Leave the PC on at all times.
2. Turn off the Water Circulator, the Peltier Device and the Jasco CD.
3. Wait 5 minutes, then turn off the nitrogen gas using the blue valve located behind the computer monitor.
4. Sign out of the log book, and note any issues.

Please note that the lamp is quite expensive and only good for 1-2 months of solid usage. This and the nitrogen are the two main things that result in the per hour charge for the CD. Please do not warm up the lamp more than necessary (~30 min). Please do not leave the CD on for the next user unless you are certain they are about to use it (i.e., they have confirmed this with you the day of the appointment, and they will use it within about an hour). And please make sure that the nitrogen is turned off after the instrument is turned off.

Contact information:
Dr. Besik Kankia, 0044A McPherson, bkankia@chem.osu.edu, 8-8799
Brandon Sullivan, 3039/3040 Evans Lab (Magliery Lab), bsulliva@chem.osu.edu, 7-8882
Prof. Magliery, 1043 Evans Lab, magliery@chem.osu.edu, 7-8425, cell 614-546-8585 (emergencies only)