

Operating the CD Spectrometer

Turning on the instrument

- **Confirm that more than ¼ tank of liquid nitrogen remains.** The middle of the red pin is the most precise place to make the reading. If the tank has been sitting idle for a while, the pin may not reflect the actual amount, so be sure to tap the gauge – this will sometimes cause the pin to drop to its true value.
- **Open the liquid nitrogen tank valves.** Fully open (turn left / counterclockwise) the **PRESSURE BUILDING** valve, then open the gas use valve (near the pressure regulator). The pressure should be set to **30 psi**. Check that the O₂ indicator (cylindrical tube with silica and sand) is brownish-black.
- **Open the valves of the oxygen scrubber.** The scrubber is found behind the instrument. Open the two valves in order of nitrogen gas flow (first the left, then the right) with a 90° turn for each.
- **Wait 20 minutes** to flush and cool the system.
- **Turn lamp power on** using the black switch at the front/center of the instrument. After about ten seconds an orange “Lamp Ready” light will come on, and then press the red button to activate the lamp. Immediately record the lamp hour (shown on the digital display near the button) in the log book, including other pertinent info.
- **Wait 30 minutes** to allow the lamp to warm up, providing a stable baseline.
- **Turn the CPU on** using the black switch at the front/center of the instrument. During the startup sequence, you will be prompted to type “YES” – do so. Don't be surprised if you unexpectedly find your productivity tripled.
- **Turn the Mac on** using the power key (triangle/arrow in the top-right corner of the keyboard). If the computer is frozen, press the ⌘+control+power keys at the same time to reboot.

Data acquisition

After turning on the Mac, navigate to the Datafiles folder and create a new folder (⌘+N) for your data, labeled with your name. Open the Star 3.0 Stationary software using the desktop icon and allow it to connect to the instrument, which takes a minute or two. The program will prompt you to change the directory where data are saved; choose “Yes” and navigate to the directory you previously created for your data, and click the “Select Current Folder” button at the top of the window. After opening, if the right side of the program's user interface says “Temperature signals and controls are OFF-LINE”, the software and instrument did not connect properly and you should quit the program and open it again.

Before starting the experiment, input a filename for your data (8 characters maximum or else the filename may become distorted upon transfer to a PC). To record detailed information on your sample, use the “Notes” section near the top of the screen: this text will be saved inside the data file. Choose “wavelength” or “temperature” mode from the drop-down menu on the left, then input experimental setup parameters. **Wavelength:** Start @250 nm, end @190 nm is recommended. **Bandwidth:** Describes the spacing of your datapoints. 0.5-1 nm is recommended. **Temperature:** Choose a single temperature for a wavelength scan or choose the range for a thermal denaturation; the instrument can go from 4-100°C. **Averaging time:** This determines how long the instrument collects data at a single wavelength or temperature point. 5 second is typical, and this value can be increased to improve the quality of the resulting data. **Scans:** The instrument can perform data in duplicate, triplicate, etc. as desired. A potential drawback arises if the scan is interrupted by excessive dynode voltage, in which case some data can be lost (see below). Before setting up multiple scans, be sure that the entire wavelength range can be examined without freezing up due to the dynode voltage issue. **Other parameters** not mentioned here don't need to be changed from default settings.

Dynode voltage error: This is a problem that occurs in the 190-210 nm range, due to properties of the solution components (typically the buffer or aggregating protein). It happens when the dynode voltage crosses a threshold marked by a red line. This causes the instrument to freeze in the middle of its wavelength scan. If a single scan was being performed, the user can click “stop” to interrupt the scan, and then save the run to salvage the collected data.

Saving your data: You must actively save your data after each run. It's not necessary to change the file name between runs, but you must click “save” after each experiment that has been completed. The program will ask if you want to save the info from the “Wavelength” window that pops up – “No Save” is OK. Similarly, when you exit the program, it's not necessary to save the experimental parameters (although this can facilitate setup of future runs).

Turning off the instrument

- **Transfer your data** from your data folder on the Mac using a floppy disk. Drag the disk icon from the desktop into the trash to eject the disc. Use the PC attached to the ITC instrument to transfer the data from the disk to a flash drive.
- **Record the lamp hour, and turn off the lamp** using the power switch at the front/center of the instrument.
- **Wait 10 minutes** to allow the lamp to cool down.
- **Shut the valves in the reverse order** (right side then left side of scrubber, then gas use, then pressure build).
- **Check the area** to make sure it's clean, and return the keys, syringe, or cuvette as discussed with CD manager.

Contact Ian Kleckner with any issues:
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