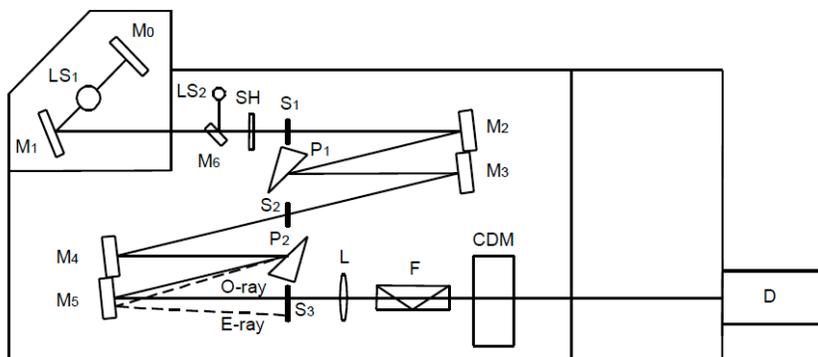


Operating the Circular Dichroism Spectropolarimeter Noah Osman

In the Barrera lab, we use the circular dichroism spectropolarimeter (more often called the “CD”) to obtain structural information about proteins—usually, membrane proteins. Resources for a thorough overview of methods and theory for CD/OCD studies of proteins are listed at the end of this document. This guide details best practices for operating the CD and gives some solutions for troubleshooting common issues.



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|--|---------------------------|
| M ₀ ~ M ₆ : Mirrors | O-ray : Ordinary ray |
| LS ₁ : Xenon lamp (for sample measurement) | E-ray : Extraordinary ray |
| LS ₂ : Mercury lamp (for the instrument inspection) | L : Lens |
| SH : Shutter | F : Filter |
| S ₁ ~ S ₃ : Slits | CDM : Modulator |
| P ₁ : First prism (horizontal optical axis) | D : PMT |
| P ₂ : Second prism (vertical optical axis) | |

Figure 1: Diagram of optical system of Jasco spectrometer

Starting Up the CD

Before beginning any experiments with the CD, be sure to record your name, current level of N₂, and “start time” in the user log. Usually, the form is located near the CD on the table. The first step in operating the CD should always be to flush the instrument with N₂. Never turn on the CD or the lamp without first purging the instrument with N₂ for at least 10 minutes; failure to do so can ruin the optics. To turn on the gas, locate the flow regulator valve on the N₂ tank (this is *not* the larger knob on top of the tank), and turn it counterclockwise.

After flushing the CD for at least 10 minutes, you are clear to turn on the instrument. Making sure that the computer and monitor are on, flip the green switch on the front of the instrument (you will hear a “beep” after doing so). Then, click the “Spectra Manager” icon on the desktop. Doing so will open the Spectra Manager program, within which you will find the ‘spectrum measurement’ option. You will hear some whirring, a click, and other mechanical sounds—this is the machine undergoing its initialization process, and this happens each time you start the CD. Be sure that you have a folder setup to export your data to.

Setting the Parameters

In general, the parameters for measurements are the same. However, there may be slight differences from experiment to experiment, so ensure that the parameters mentioned here are appropriate for your experiment. Parameter settings can be found, fittingly, under 'Parameter Settings'. Select the 'General' tab.

CD Parameters

Start wavelength ('Start')	260 nm
End wavelength ('End')	185 nm
Data pitch	1.0 nm
Sensitivity	Standard
D.I.T.	4 sec
Accumulations	25
Start mode	Immediately
Scanning mode	Continuous
Scanning speed	50 nm/min

Turning the CD Off

Once you have successfully saved/exported your data, you can begin the process for turning off the CD. To successfully exit the spectrum analysis program, select file>exit. To exit the Spectra Measurement program, select measure>exit. Finally, to exit Spectra Manager, select program>exit. You should then see the desktop displayed, indicating you have successfully exited the program. Log off of the computer. It is not necessary to turn off the computer; just turn off the monitor.

After making sure that there is no sample in the sample compartment, turn off the power switch on the CD. Then, shut off the nitrogen. Never turn off the nitrogen before shutting off the machine first, for the reasons described in the first section. Lastly, be sure to log the N₂ level and your "end" time.

Troubleshooting

If you are having trouble with the CD, this section may provide some solutions to your issue. This section is adapted from the Jasco J-1100-1500 manual.

Cannot turn on CD

Check that the power cable is plugged into the wall outlet. In the event that the fuse burns off, it needs to be replaced.

Lamp does not come on

Make sure that the light source is set to turn on at startup. If the anode holder is incorrectly installed, install it correctly. If a sparking sound is heard when you try to turn on the lamp, replace the lamp.

No increase of HT voltage

Check that the shutter is open, and that the photometric mode is set correctly (it should be set to “CD”, not “test signal”). Check that the HT voltage mode is correctly set to “auto”, not “manual” or “off”. Make sure that the lid is completely on top of the sample chamber.

HT voltage increases; will not decrease

Remove the sample if there is one in the sample chamber. Make sure that the sample bandwidth is not too small; increase if necessary. Make sure that the cable is connected correctly to the detector unit on the rear panel of the amplifier unit. Make sure that the wavelength is not set to a value at which the detector is not sensitive. Check the N₂ flow rate—is it set highly enough if the wavelength is set below 180 nm? If not, increase flow rate.

High noise level

The spectral bandwidth setting may be too small, in which case you may need to increase the spectral bandwidth. If the sample absorbs light strongly, try reducing the concentration of your sample or shortening the light path of the cell. If noise is detected in the HT voltage, you need to replace the lamp.

Baseline curves strongly

Make sure that the curvature of the baseline is within ± 10 mdeg; if it is not, perform a baseline correction.

Refer to the CD manual for further troubleshooting guidance and contact a Jasco field technician if necessary.

References

Jasco. (2013). Model J-1100/1500CD Spectrometer Hardware/Function Manual. Tokyo, Japan.

Further Reading

Bulheller, B. M., Rodger, A., & Hirst, J. D. (2007). *Circular and linear dichroism of proteins. Physical Chemistry Chemical Physics, 9(17), 2020.* doi:10.1039/b615870f

Bürck, J., Wadhvani, P., Fanghänel, S., & Ulrich, A. S. (2016). *Oriented Circular Dichroism: A Method to Characterize Membrane-Active Peptides in Oriented Lipid Bilayers. Accounts of Chemical Research, 49(2), 184–192.*

Greenfield, N. J. (2007). *Using circular dichroism spectra to estimate protein secondary structure. Nature Protocols, 1(6), 2876–2890.* doi:10.1038/nprot.2006.202