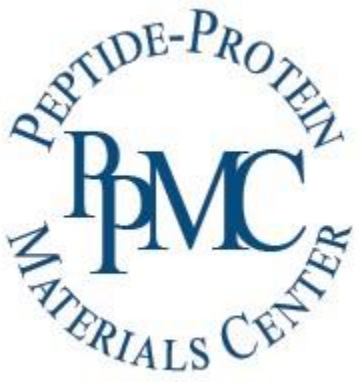
Jasco J-1500 Circular Dichroism Spectrophotometer Standard Operating Procedures



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About

The Jasco J-1500 Circular Dichroism Spectrophotometer is an instrument designed for analytical spectroscopy for analyzing chirality in molecules through their optical activity. Circular dichroism (CD) measures the difference in absorption of left- and right-handed circularly polarized light. This technique is often used to determine the secondary structure of peptides and proteins. A temperature control system is coupled with a six-cell holder to run thermal melts which can be analyzed for thermodynamic and kinetic data sets for conformational and folding studies of peptides and proteins. The wavelength range for the instrument is extensive, which can obtain measurements in both the vacuum-ultraviolet and near infrared spectral regions using the standard photomultiplier tube detector (163 – 950 nm).

Point of Contact

Primary Contact: Nolan Petrich – Peptide Synthesis and Instrumentation Specialist, PPMC

Email: npetrich@udel.edu

Instrument Location

Ammon Pinizzotto Biopharmaceutical Innovation Center

590 Avenue 1743, 2nd Floor, Room 244

Newark, DE 19713

Note: Swipe access to the building, floor, and lab are required. Contact Joe Chubbs (jrchubbs@udel.edu) to coordinate swipe access.

Preparation Checklist

Before You Start:

1. Fill out the log sheet.
2. Open flow meter to begin Nitrogen purge (40 SCFH).
3. Turn on the spectropolarimeter and heat exchanger.
4. Turn on the computer, open Spectra Manager, and turn on the light source.
5. ***Allow the system to purge for at least 30 minutes.***
6. Adjust Nitrogen flow (15 SCFH).
7. Check 300 nm wavelength voltage.
8. Prepare sample(s) and run experiment(s).

When You Are Done:

1. Remove cuvette(s) from the instrument.
2. Export data.
3. Close the software.
4. Power off the heat exchanger and spectropolarimeter.
5. Purge the instrument for 10 minutes with Nitrogen then close the float valve.
6. Remove sample(s) from cuvettes and clean cuvette(s).

A. Setting Up

1. Begin by filling out the logbook located in proximity to the spectropolarimeter. The following information should be included:
    a. Name
    b. Date
    c. Advisor/Group
    d. Time
    e. Detector's Voltage at 300 nm. Save this for after steps 13-15.
2. ***Prior to turning on the CD, open the flow meter on the left side of the instrument by turning the dial counterclockwise. Ensure that the flow rate is set to 40 SCFH (standard cubic feet per hour).***
    a. The flow meter is read using the top of the spinning metal cylinder.
    b. If the metal cylinder is not moving up despite turning the dial, contact the individual who oversees the instrument.
3. Double check that pressure is supplied to the regulator located on the wall to the right of the instrument. The gas is supplied by house nitrogen.
4. Turn on the spectropolarimeter via the on-off switch located on the left side of the instrument.
5. Turn on the heat exchanger by switching on the black switch located on the top, back, left-hand side of the exchanger.
    a. ***Ensure that blue fluid is circulating through the heat exchanger by observing the spinning blue wheel connected to tubing leading from the heat exchanger to the spectropolarimeter.***
    b. If the wheel is not spinning, contact the individual who oversees the instrument.
6. Power on the computer.
    a. The computer is typically left on and logged in.
    b. If signed out:
        i. Username: User
        ii. There is no password.
7. Open Spectra Manager software on the computer's main desktop.
    a. The icon appears as a computer and spectrometer.
8. To turn on the instrument's light source, double click on any of the programs on the left-hand side of the software. In general, these programs can be found under Instrument > CD Spectrometer.
9. Allow the lamp to purge for the set five minutes after opening the experiment program.
10. Verify that the lamp is on by checking the Xe lamp icon at the top of the window. It should be yellow.
    a. If the lamp did not manually turn on, click the Xe lamp icon to turn on the Xenon lamp.

11. **Very Important Step: Leave the lamp on and Nitrogen flowing at 40 SCFH for a minimum of <u>30 minutes</u> prior to the start of experiments.**
    a. This allows time for the lamp to warm up and purge with nitrogen while ignited, as well as have the entire system (optics, chromators, etc.) purged with Nitrogen.
12. After at least 30 minutes of purging at 40 SCFH, set the flow rate to 15 SCFH.
13. Move the wavelength to 300 nm by selecting Control at the top of the window > Move Wavelength. In the pop-up, type in 300. Hit OK.
14. Open the shutter by clicking the door icon in the tool bar at the top of the screen.
15. Record the initial voltage reading in the logbook.
16. Close the shutter by clicking the door icon in the tool bar at the top of the screen.
    a. To be certain the shutter is closed, ensure that the voltage reading goes back to zero.
17. Once these steps are completed, proceed to **section B** to prepare samples for measurement.

B. Sample Preparation

1. Begin by weighing out lyophilized peptide or protein in a microcentrifuge tube using a microbalance.
    a. It helps to use a static ionizer to ensure an accurate measurement.
2. Based on the molecular weight of the peptide or protein, dissolve in water or buffer solution to make a 0.1 mM solution.
    a. 0.1 mM is the recommended starting point for standard CD measurements.
    b. Note: Concentration may need to be adjusted based on the optical activity of your sample.
3. Prepare a blank sample in a different microcentrifuge tube.
    a. A blank sample refers to a cuvette filled with the solvent that was selected to dissolve the peptide or protein for the measurement.
4. Repeat these steps for up to six samples to be processed.
    a. Note that at least 300 µL of sample are required for the 0.1 cm path length. It is recommended to make 500 µL of sample.
    b. Before running experiments, the samples should be analyzed with UV-Vis to determine the actual concentration of peptide.
5. Once sample(s) are prepared, proceed to **section C** to load the sample(s) into the instrument.

C. Loading Samples

1. Once sample(s) have been prepared, at least 300 µL should be pipetted into the cuvette for a 0.1 cm pathlength cuvette.
    a. It is recommended that 500 µL of sample be used.
    b. Make sure that the cuvette is clean.
2. After adding the sample to the cuvette, place the cap on the cuvette.
3. Use a KimWipe to clean the outside of the cuvette while holding the sides of the cuvette (the sides that are not a part of the pathlength).
4. Ensure that the shutter of the instrument is closed.
5. Carefully open the door to the cell holder and place the cuvette in the desired slot.
6. Repeat this procedure for all samples.
7. Once this has been completed, experiments can be performed. The most common experiments are detailed in **section D** (fixed temperature CD spectrum) and **section I** (variable temperature scan with wavelength monitoring). **Sections E**, **F**, **G**, and **H** also briefly detail other experiments that can be run with the software.

D. Performing Spectra Measurements

To measure a sample(s) CD spectrum at a fixed temperature, select the Spectrum Measurement program. This measurement is typically used to measure blank cells. This ensures that appropriate background subtraction can be calculated based on the selected solvent, as well as check the conditions of the cuvette.

1. Double click the Spectrum Measurement program after opening the main spectra manager program from the desktop.
2. Prior to acquiring a spectrum measurement, it is first necessary to acquire a blank measurement. The settings for blank samples are similar to those for regular spectra measurements.
    a. The baseline should be run under the same conditions (solvent, gain, time constant, spectral band width, scanning speed, etc.) as the sample.
3. To set the measurement conditions, click Measure > Parameters.
    a. Set the desired parameters. The following tabs have different parameters below. For an initial scan, recommended parameters are provided.
        i. General:
            1. **Channels Num:** Number of channels.
                a. Recommended: 2
            2. **Channel 1:** The data recorded for channel 1.
                a. Recommended: CD
            3. **Channel 2:** The data recorded for channel 2.
                a. Recommended: HT
            4. **CD Scale:**
                a. Recommended: 200 mdeg/1.0 dOD
                b. If you have a small CD signal, set to 20 mdeg/1.0 dOD
            5. **FL Scale:**
                a. Leave at 200 mdeg/1.0 dOD
            6. **D.I.T.:** The digital integration time, which is the length of time the detector collects photons before transferring the signal to the A/D converter for processing.
                a. Recommended: 4 sec
            7. **Bandwidth:** A measure of the precision with which a monochromator selects light of a chosen wavelength. Increasing the bandwidth will allow more light to fall on the sample and hence on the photomultiplier, but will decrease the ability to resolve spectral bands.
                a. Recommended: 1.00 nm
            8. **Start:** Starting wavelength.
                a. Recommended: 250 nm
            9. **End:** Ending wavelength.
                a. Recommended: 185 nm
            10. **Data Pitch:** The number of data points taken during the scan.

a. Recommended: 1.0 nm (one data point every nm)

11. **Start Mode:**
    a. Recommended: Immediately
12. **Scanning Mode:**
    a. Recommended: Continuous (measurement time does not depend on step resolution)
13. **Scanning Speed:** How quickly the monochromator moves to obtain data points.
    a. Recommended: 100 nm/min
14. **Accumulation Check Box:** Determines whether accumulations are taken for spectral measurement(s) or not.
    a. Recommended: Checked
15. **No. of Accumulations:** The number of spectral scans that are automatically obtained and averaged together.
    a. Recommended: 3

ii. Cell Unit:

1. **Control Check Box:** Determines whether the temperature is controlled during the spectral measurement or not.
    a. Recommended: Checked
2. **Temperature:** The set temperature during the spectral measurement.
    a. Recommended: 25.00 °C
3. **Start Conditions:** The conditions that must be met before the experiment begins.
    a. Recommended: Keep within +/- 0.10 C of the target temperature for 10 seconds.

iii. Control:

1. **Correction:** Determines whether a baseline measurement is performed or not.
    a. Recommended: None
    b. It is recommended that blanks be run, and that correction is performed in post-processing.
2. *Shutter is opened and closed automatically* should be checked.
3. *Shutter is closed between measurements* should be checked.

iv. Information:

1. **Operator:** The name of the user running the instrument.
2. **Division:** The Advisor/Group Name of the user running the instrument.
3. **Sample Information:** Allows users to provide Sample name, Comment, Conc., Conc. unit, and Solvent for each of the six cells.
    a. If using the R script detailed in **section L**, be sure to use proper naming conventions.

v. Data:
    1. **Auto save Checkbox:** Determines whether data is automatically saved or not.
        a. Recommended: Checked
    2. **Save in:** The data path for where data should be saved.
        a. Recommended: Please only save data in your designated group folder.
        b. C:\Users\User\Desktop\CD User Groups
    3. **Format:** The naming format for saved files.
        a. Recommended: Sample-Comment-No.
        b. If using the R script detailed in **section L**, be sure to use proper naming conventions.
    4. **Send data:**
        a. Check *To Spectra Analysis*

b. Once the desired parameters have been set, save them by selecting OK.

4. After setting the required parameters, ensure that the shutter is open and go to Measure > Sample Measurement. The instrument will prompt you to select which cell is holding your cuvette. Deselect all other cells that do not have cuvettes.
    a. After hitting OK, measurements will be taken.
5. If done running experiments after completion of the measurement(s), proceed to **section J** for shutdown.

E. Performing Time Course Measurements

To monitor the structure of a sample at a specified wavelength and fixed temperature over time, select the Time Course Measurement program.

1. Double click the Time Course Measurement program after opening the main spectra manager program from the desktop.
    a. Note that prior to acquiring a time course measurement, it is first necessary to acquire a blank measurement. The settings for blank samples are similar to those for regular spectra measurements in the Spectra Measurement program (**section D**).
    b. The baseline should be run under the same conditions (solvent, gain, time constant, spectral band width, scanning speed, etc.) as the sample.
2. To set the Time Course Measurement conditions, click Measure > Parameters.
    a. Set the desired parameters. The following tabs have different parameters below. For an initial scan, recommended parameters are provided.
        i. General:
            1. **Channels Num:** Number of channels.
                a. Recommended: 2
            2. **Channel 1:** The data recorded for channel 1.
                a. Recommended: CD
            3. **Channel 2:** The data recorded for channel 2.
                a. Recommended: HT
            4. **CD Scale:**
                a. Recommended: 200 mdeg/1.0 dOD
                b. If you have a small CD signal, set to 20 mdeg/1.0 dOD
            5. **FL Scale:**
                a. Leave at 200 mdeg/1.0 dOD
            6. **D.I.T.:** The digital integration time, which is the length of time the detector collects photons before transferring the signal to the A/D converter for processing.
                a. Recommended: 2 sec
            7. **Bandwidth:** A measure of the precision with which a monochromator selects light of a chosen wavelength. Increasing the bandwidth will allow more light to fall on the sample and hence on the photomultiplier, but will decrease the ability to resolve spectral bands.
                a. Recommended: 1.00 nm
            8. **Wavelength:** The wavelength that is monitored during the experiment.
                a. Recommended: Depends on the applications. If monitoring α-helix structure, 222 nm. If monitoring β-sheet structure, 218 nm. For collagen-like peptides, 225 or 200 nm.

9. **Measuring Time:** The time length of the experiment.
    a. Recommended: Depends on the length of time the structure would like to be monitored.
10. **Data Pitch:** The number of data points taken during the scan.
    a. Recommended: Depends on how many data points desired. 1.0 sec is a good starting point (one data point every second).
11. **Start Mode:**
    a. Recommended: Immediately
12. **Accumulation Check Box:** Determines whether accumulations are taken for spectral measurement(s) or not.
    a. Recommended: Checked
13. **No. of Accumulations:** The number of spectral scans that are automatically obtained and averaged together.
    a. Recommended: 3

ii. Cell Unit:
1. **Control Check Box:** Determines whether the temperature is controlled during the spectral measurement or not.
    a. Recommended: Checked
2. **Temperature:** The set temperature during the spectral measurement.
    a. Recommended: 25.00 °C
3. **Start Conditions:** The conditions that must be met before the experiment begins.
    a. Recommended: Keep within +/- 0.10 C of the target temperature for 10 seconds.

iii. Control:
1. **Correction:** Determines whether a baseline measurement is performed or not.
    a. Recommended: None
    b. It is recommended that blanks be run, and that correction is performed in post-processing.
2. *Shutter is opened and closed automatically* should be checked.

iv. Information:
1. **Sample Name:** The name of the sample being run.
2. **Operator:** The name of the user running the instrument.
3. **Division:** The Advisor/Group Name of the user running the instrument.
4. **Comment:** Any comments about the sample.
5. **Concentration:** The concentration of peptide/protein in the sample.
6. **Solvent:** The solvent used for dissolving the sample.

v. Data:

1. **Auto save Checkbox:** Determines whether data is automatically saved or not.
    a. Recommended: Checked
2. **Save in:** The data path for where data should be saved.
    a. Recommended: Please only save data in your designated group folder.
    b. C:\Users\User\Desktop\CD User Groups
3. **Format:** The naming format for saved files.
    a. Recommended: Sample-Comment-No.
4. **Send data:**
    a. Check *To Spectra Analysis*

b. Once the desired parameters have been set, save them by selecting OK.

3. After setting the required parameters, ensure that the shutter is open and go to Measure > Sample Measurement. The instrument will prompt you to select which cell is holding your cuvette. Deselect all other cells that do not have cuvettes.
    a. After hitting OK, measurements will be taken.
4. If done running experiments after completion of the measurement(s), proceed to **section J** for shutdown.

F. Performing Variable Temperature Measurements

To monitor the structure of a sample at a specified wavelength over a temperature interval, select the Variable Temperature Measurement program.

1. Double click the Variable Temperature Measurement program after opening the main spectra manager program from the desktop.
    a. Note that prior to acquiring a variable temperature measurement, it is first necessary to acquire a blank measurement. The settings for blank samples are similar to those for regular spectra measurements in the Spectra Measurement program (**section D**).
    b. The baseline should be run under the same conditions (solvent, gain, time constant, spectral band width, scanning speed, etc.) as the sample.
2. To set the Variable Temperature Measurement conditions, click Measure > Parameters.
    a. Set the desired parameters. The following tabs have different parameters below. For an initial scan, recommended parameters are provided.
        i. Temperature:
            1. **Start Temperature:** The starting temperature for the interval of temperatures to be scanned.
            2. **Reverse Check Box:** Determines whether the reverse interval will be performed after the temperature ramp or not.
            3. **Input Table:** For each cell, users can select the Interval(C) that data points are taken, the Target(C) temperature the interval will run to, the Gradient(C/min) for how fast the ramp is, and the Wait(sec) for how long the cell holder will wait for temperature equilibration before taking another measurement, for each cell.
            4. It is recommended that users select the *Halt temperature ramping during measurement* checkbox.
        ii. Start/End:
            1. **Start condition:** The conditions that must be met before the experiment begins.
                a. Recommended: Keep within +/- <u>0.10</u> C of the target temperature for <u>10</u> seconds.
            2. **End condition:** The temperature conditions desired after the completion of the experiment.
        iii. General:
            1. **Channels Num:** Number of channels.
                a. Recommended: 2
            2. **Channel 1:** The data recorded for channel 1.
                a. Recommended: CD
            3. **Channel 2:** The data recorded for channel 2.
                a. Recommended: HT

4. **CD Scale:**
    a. Recommended: 200 mdeg/1.0 dOD
    b. If you have a small CD signal, set to 20 mdeg/1.0 dOD
5. **FL Scale:**
    a. Leave at 200 mdeg/1.0 dOD
6. **D.I.T.:** The digital integration time, which is the length of time the detector collects photons before transferring the signal to the A/D converter for processing.
    a. Recommended: 4 sec
7. **Bandwidth:** A measure of the precision with which a monochromator selects light of a chosen wavelength. Increasing the bandwidth will allow more light to fall on the sample and hence on the photomultiplier, but will decrease the ability to resolve spectral bands.
    a. Recommended: 1.00 nm
8. **Wavelength:** The wavelengths that will be monitored during the course of the experiment.

iv. Control:
1. **Correction:** Determines whether a baseline measurement is performed or not.
    a. Recommended: None
    b. It is recommended that blanks be run, and that correction is performed in post-processing.
2. *Shutter is opened and closed automatically* should be checked.

v. Information:
1. **Operator:** The name of the user running the instrument.
2. **Division:** The Advisor/Group Name of the user running the instrument.
3. **Sample Information:** Allows users to provide Sample name, Comment, Conc., Conc. unit, and Solvent for each of the six cells.

vi. Data:
1. **Auto save Checkbox:** Determines whether data is automatically saved or not.
    a. Recommended: Checked
2. **Save in:** The data path for where data should be saved.
    a. Recommended: Please only save data in your designated group folder.
    b. C:\Users\User\Desktop\CD User Groups
3. **Format:** The naming format for saved files.
    a. Recommended: Sample-Comment-No.
4. **Send data:**
    a. Check *To Spectra Analysis*

    b. Once the desired parameters have been set, save them by selecting OK.
3. After setting the required parameters, ensure that the shutter is open and go to Measure > Sample Measurement. The instrument will prompt you to select which cell is holding your cuvette. Deselect all other cells that do not have cuvettes.
    a. After hitting OK, measurements will be taken.
4. If done running experiments after completion of the measurement(s), proceed to **section J** for shutdown.

G. Performing Temperature Interval Scan Measurements

To measure a sample(s) CD spectrum at fixed temperature intervals, select the Temperature Interval Scan Measurement program.

1. Double click the Temperature Interval Scan Measurement program after opening the main spectra manager program from the desktop.
    a. Note that prior to acquiring a temperature interval scan measurement, it is first necessary to acquire a blank measurement. The settings for blank samples are similar to those for regular spectra measurements in the Spectra Measurement program (**section D**).
    b. The baseline should be run under the same conditions (solvent, gain, time constant, spectral band width, scanning speed, etc.) as the sample.
2. To set the measurement conditions, click Measure > Parameters.
    a. Set the desired parameters. The following tabs have different parameters below. For an initial scan, recommended parameters are provided.
        i. Temperature:
            1. **Start/end temp:** Provides the starting and ending temperatures of the data analysis.
                a. Recommended: 10 – 90 °C
            2. **Data interval:** The interval with which CD spectra will be measured during the temperature ramp.
                a. Recommended: 10.0 °C (a CD spectra will be measured every 10.0 °C)
            3. **Temp. gradient:** The rate at which the temperature is changed throughout the temperature interval.
                a. Recommended: 1 °C/min
            4. **Wait time:** The amount of time the instrument will wait at each temperature interval before measuring the CD spectra.
                a. Recommended: 600 sec
            5. **Start condition:** The conditions that must be met before the experiment begins.
                a. Recommended: Keep within +/- 0.10 C of the target temperature for 10 seconds.

6. **End condition:** The temperature conditions desired after the completion of the experiment.
    a. Recommended: Select Change to specified temp. 25.0 °C
7. **Halt temperature ramping during measurements:**
    a. Recommended: Checked
8. **Reverse:**
    a. If desired, it is recommended to have a wait time of 600 seconds before running the reverse temperature interval.

ii. General:

1. **Channels Num:** Number of channels.
    a. Recommended: 2
2. **Channel 1:** The data recorded for channel 1.
    a. Recommended: CD
3. **Channel 2:** The data recorded for channel 2.
    a. Recommended: HT
4. **CD Scale:**
    a. Recommended: 200 mdeg/1.0 dOD
    b. If you have a small CD signal, set to 20 mdeg/1.0 dOD
5. **FL Scale:**
    a. Leave at 200 mdeg/1.0 dOD
6. **D.I.T.:** The digital integration time, which is the length of time the detector collects photons before transferring the signal to the A/D converter for processing.
    a. Recommended: 4 sec
7. **Bandwidth:** A measure of the precision with which a monochromator selects light of a chosen wavelength. Increasing the bandwidth will allow more light to fall on the sample and hence on the photomultiplier, but will decrease the ability to resolve spectral bands.
    a. Recommended: 1.00 nm
8. **Start:** Starting wavelength.
    a. Recommended: 250 nm
9. **End:** Ending wavelength.
    a. Recommended: 185 nm
10. **Data Pitch:** The number of data points taken during the scan.
    a. Recommended: 1.0 nm (one data point every nm)
11. **Start Mode:**
    a. Recommended: Immediately
12. **Scanning Mode:**
    a. Recommended: Continuous (measurement time does not depend on step resolution)

13. **Scanning Speed:** How quickly the monochromator moves to obtain data points.
    a. Recommended: 100 nm/min
14. **No. of Accumulations:** The number of spectral scans that are automatically obtained and averaged together.
    a. Recommended: 3

iii. Control:
1. **Correction:** Determines whether a baseline measurement is performed or not.
    a. Recommended: None
    b. It is recommended that blanks be run, and that correction is performed in post-processing.
2. *Shutter is opened and closed automatically* should be checked.
3. *Shutter is closed between measurements* should be checked.

iv. Information:
1. **Operator:** The name of the user running the instrument.
2. **Division:** The Advisor/Group Name of the user running the instrument.
3. **Sample Information:** Allows users to provide Sample name, Comment, Conc., Conc. unit, and Solvent for each of the six cells.
    a. If using the R script detailed in **section L**, be sure to use proper naming conventions.

v. Data:
1. **Auto save Checkbox:** Determines whether data is automatically saved or not.
    a. Recommended: Checked
2. **Save in:** The data path for where data should be saved.
    a. Recommended: Please only save data in your designated group folder.
    b. C:\Users\User\Desktop\CD User Groups
3. **Format:** The naming format for saved files.
    a. Recommended: Sample-No.
    b. If using the R script detailed in **section L**, be sure to use proper naming conventions.
4. **Send data:**
    a. Check *To Interval Data Analysis*

b. Once the desired parameters have been set, save them by selecting OK.

3. After setting the required parameters, ensure that the shutter is open and go to Measure > Sample Measurement. The instrument will prompt you to select which cell is holding your cuvette. Deselect all other cells that do not have cuvettes.
    a. After hitting OK, measurements will be taken.

4. If done running experiments after completion of the measurement(s), proceed to **section J** for shutdown.

H. Performing Interval Scan Measurements

To measure a sample(s) CD spectrum at fixed temperature intervals, select the Temperature Interval Scan Measurement program.

1. Double click the Interval Scan Measurement program after opening the main spectra manager program from the desktop.
    a. Note that prior to acquiring a temperature interval scan measurement, it is first necessary to acquire a blank measurement. The settings for blank samples are similar to those for regular spectra measurements in the Spectra Measurement program (**section D**).
    b. The baseline should be run under the same conditions (solvent, gain, time constant, spectral band width, scanning speed, etc.) as the sample.
2. To set the measurement conditions, click Measure > Parameters.
    a. Set the desired parameters. The following tabs have different parameters below. For an initial scan, recommended parameters are provided.
        i. Interval:
            1. **Delay time:** The amount of time delayed before taking measurements.
                a. Recommended: 0 min
            2. **Interval time:** The amount of time between every scan.
                a. Recommended: 5 min
            3. **Interval time x no. of scans:**
                a. Recommended: 100
            4. **Time Unit:** Users have their choice between seconds and minutes.
        ii. General:
            1. **Channels Num:** Number of channels.
                a. Recommended: 2
            2. **Channel 1:** The data recorded for channel 1.
                a. Recommended: CD
            3. **Channel 2:** The data recorded for channel 2.
                a. Recommended: HT
            4. **CD Scale:**
                a. Recommended: 200 mdeg/1.0 dOD
                b. If you have a small CD signal, set to 20 mdeg/1.0 dOD
            5. **FL Scale:**
                a. Leave at 200 mdeg/1.0 dOD

6. **D.I.T.:** The digital integration time, which is the length of time the detector collects photons before transferring the signal to the A/D converter for processing.
    a. Recommended: 4 sec
7. **Bandwidth:** A measure of the precision with which a monochromator selects light of a chosen wavelength. Increasing the bandwidth will allow more light to fall on the sample and hence on the photomultiplier, but will decrease the ability to resolve spectral bands.
    a. Recommended: 1.00 nm
8. **Start:** Starting wavelength.
    a. Recommended: 250 nm
9. **End:** Ending wavelength.
    a. Recommended: 185 nm
10. **Data Pitch:** The number of data points taken during the scan.
    a. Recommended: 1.0 nm (one data point every nm)
11. **Start Mode:**
    a. Recommended: Immediately
12. **Scanning Mode:**
    a. Recommended: Continuous (measurement time does not depend on step resolution)
13. **Scanning Speed:** How quickly the monochromator moves to obtain data points.
    a. Recommended: 100 nm/min

iii. Cell Unit:

1. **Cell Changer - Control Check Box:** Determines whether the temperature is controlled during the spectral measurement or not.
    a. Recommended: Checked
    b. Below – Select the cells being used.
2. **Temperature Control:** The set temperature during the spectral measurement.
3. **Start Conditions:** The conditions that must be met before the experiment begins.
    a. Recommended: Keep within +/- 0.10 C of the target temperature for 10 seconds.

iv. Control:

1. **Correction:** Determines whether a baseline measurement is performed or not.
    a. Recommended: None
    b. It is recommended that blanks be run, and that correction is performed in post-processing.
2. *Shutter is opened and closed automatically* should be checked.

3. *Shutter is closed between measurements* should be checked.

v. Information:
    1. **Operator:** The name of the user running the instrument.
    2. **Division:** The Advisor/Group Name of the user running the instrument.
    3. **Sample Information:** Allows users to provide Sample name, Comment, Conc., Conc. unit, and Solvent for each of the six cells.

vi. Data:
    1. **Auto save Checkbox:** Determines whether data is automatically saved or not.
        a. Recommended: Checked
    2. **Save in:** The data path for where data should be saved.
        a. Recommended: Please only save data in your designated group folder.
        b. C:\Users\User\Desktop\CD User Groups
    3. **Format:** The naming format for saved files.
        a. Recommended: Sample-Comment-No.
    4. **Send data:**
        a. Check *To Interval Data Analysis*

b. Once the desired parameters have been set, save them by selecting OK.

3. After setting the required parameters, ensure that the shutter is open and go to Measure > Sample Measurement. The instrument will prompt you to select which cell is holding your cuvette. Deselect all other cells that do not have cuvettes.
    a. After hitting OK, measurements will be taken.
4. If done running experiments after completion of the measurement(s), proceed to **section J** for shutdown.

I. Performing Temperature/Wavelength Scan Measurements

To measure a sample(s) CD spectrum at fixed temperature intervals, as well as monitor a specific wavelength to obtain a melting curve (CD as a function of temperature) select the Temperature/Wavelength Scan Measurement program.

1. Double click the Temperature/Wavelength Scan Measurement program after opening the main spectra manager program from the desktop.
    a. Note that prior to acquiring a temperature interval scan measurement, it is first necessary to acquire a blank measurement. The settings for blank samples are similar to those for regular spectra measurements in the Spectra Measurement program (**section D**).
    b. The baseline should be run under the same conditions (solvent, gain, time constant, spectral band width, scanning speed, etc.) as the sample.
2. To set the measurement conditions, click Measure > Parameters.
    a. Set the desired parameters. The following tabs have different parameters below. For an initial scan, recommended parameters are provided.
        i. Tm Setup:
            1. **Start/end temp:** Provides the starting and ending temperatures of the data analysis.
                a. Recommended: 10 – 90 °C
            2. **Data interval:** The interval with which a measurement will be performed for a specified wavelength during the temperature ramp.
                a. Recommended: 1.0 °C (the CD at the specified wavelength will be measured every 1.0 °C)
            3. **Temp. gradient:** The rate at which the temperature is changed throughout the temperature interval.
                a. Recommended: 1 °C/min
            4. **Wait time:** The amount of time the instrument will wait at each temperature interval before measuring the CD at the specified wavelengths.
                a. Recommended: 90 sec
            5. **Start condition:** The conditions that must be met before the experiment begins.
                a. Recommended: Keep within +/- 0.10 C of the target temperature for 10 seconds.
            6. **End condition:** The temperature conditions desired after the completion of the experiment.
                a. Recommended: Select Change to specified temp. 25.0 °C
            7. **Halt temperature ramping during measurements:**
                a. Recommended: Checked

8. **Reverse:**
    a. If desired, it is recommended to have a wait time of 600 seconds before running the reverse temperature interval.

ii. Tm Param:
1. **Channels Num:** Number of channels.
    a. Recommended: 2
2. **Channel 1:** The data recorded for channel 1.
    a. Recommended: CD
3. **Channel 2:** The data recorded for channel 2.
    a. Recommended: HT
4. **CD Scale:**
    a. Recommended: 200 mdeg/1.0 dOD
    b. If you have a small CD signal, set to 20 mdeg/1.0 dOD
5. **FL Scale:**
    a. Leave at 200 mdeg/1.0 dOD
6. **D.I.T.:** The digital integration time, which is the length of time the detector collects photons before transferring the signal to the A/D converter for processing.
    a. Recommended: 4 sec
7. **Bandwidth:** A measure of the precision with which a monochromator selects light of a chosen wavelength. Increasing the bandwidth will allow more light to fall on the sample and hence on the photomultiplier, but will decrease the ability to resolve spectral bands.
    a. Recommended: 1.00 nm
8. **Wavelength:** The wavelength that will be monitored within the data interval for the creation of a melting curve.
    a. Recommended: Depends on the applications. If monitoring α-helix structure, 222 nm. If monitoring β-sheet structure, 218 nm. For collagen-like peptides, 225 or 200 nm.

iii. Spec Temp:
1. **Temperature table for spectra scans:** The specified temperatures where the software will measure a full CD spectrum.
    a. Recommended, 10.0 C, 20.0 C, 30.0 C, 40.0 C, 50.0 C, 60.0 C, 70.0 C, 80.0 C, 90.0 C

iv. CD Param:
1. **Channels Num:** Number of channels.
    a. Recommended: 2
2. **Channel 1:** The data recorded for channel 1.
    a. Recommended: CD

3. **Channel 2:** The data recorded for channel 2.
    a. Recommended: HT
4. **CD Scale:**
    a. Recommended: 200 mdeg/1.0 dOD
    b. If you have a small CD signal, set to 20 mdeg/1.0 dOD
5. **FL Scale:**
    a. Leave at 200 mdeg/1.0 dOD
6. **D.I.T.:** The digital integration time, which is the length of time the detector collects photons before transferring the signal to the A/D converter for processing.
    a. Recommended: 4 sec
7. **Bandwidth:** The full width of the Gaussian distribution at half the peak maximum (smaller spectral bandwidth, more peak resolution, less light throughput).
    a. Recommended: 1.00 nm
8. **Start:** Starting wavelength.
    a. Recommended: 250 nm
9. **End:** Ending wavelength.
    a. Recommended: 185 nm
10. **Data Pitch:** The number of data points taken during the scan.
    a. Recommended: 1.0 nm (one data point every nm)
11. **Start Mode:**
    a. Recommended: Immediately
12. **Scanning Mode:**
    a. Recommended: Continuous (measurement time does not depend on step resolution)
13. **Scanning Speed:** How quickly the monochromator moves to obtain data points.
    a. Recommended: 100 nm/min
14. **No. of Accumulations:** The number of spectral scans that are automatically obtained and averaged together.
    a. Recommended: 3

v. Control:
1. **Correction:** Determines whether a baseline measurement is performed or not.
    a. Recommended: None
    b. It is recommended that blanks be run, and that correction is performed in post-processing.
2. *Shutter is opened and closed automatically* should be checked.

vi. Information:
1. **Operator:** The name of the user running the instrument.
2. **Division:** The Advisor/Group Name of the user running the instrument.

3. **Sample Information:** Allows users to provide Sample name, Comment, Conc., Conc. unit, and Solvent for each of the six cells.
    a. If using the R script detailed in **section L**, be sure to use proper naming conventions.

vii. Data:

1. **Auto save Checkbox:** Determines whether data is automatically saved or not.
    a. Recommended: Checked
2. **Save in:** The data path for where data should be saved.
    a. Recommended: Please only save data in your designated group folder.
    b. C:\Users\User\Desktop\CD User Groups
3. **Format:** The naming format for saved files.
    a. Recommended: Sample-No.
    b. If using the R script detailed in **section L**, be sure to use proper naming conventions.
4. **Send data:**
    a. Check *To Interval Data Analysis*

b. Once the desired parameters have been set, save them by selecting OK.

3. After setting the required parameters, ensure that the shutter is open and go to Measure > Sample Measurement. The instrument will prompt you to select which cell is holding your cuvette. Deselect all other cells that do not have cuvettes.
    a. After hitting OK, measurements will be taken.
4. If done running experiments after completion of the measurement(s), proceed to **section J** for shutdown.

J. Shutdown

The following shutdown routine should be performed after every time slot.

1. Ensure that the shutter is closed, and lamp is powered off (click the lamp icon).
2. Remove cuvette(s) from the spectropolarimeter.
3. Properly export data. For instructions, see **section K**.
4. Close the software.
5. Power off the heat exchanger and spectropolarimeter via their power switches.
6. Once the instrument is shut off, wait ten minutes for the Nitrogen to purge the system. Then, close the Nitrogen float valve by turning the knob clockwise.
7. Remove samples from the cuvette(s) and rinse them five times with Millipore water.
8. Clean the cuvette(s) with a cleaning solution. This is done by adding two drops of the solution to the cuvette and filling the rest of the cuvette with Millipore water.
    a. ***It is highly recommended that users make a 5 M HCl solution and use this instead of the Starna cleaning solution.***
    b. For more information about cleaning cuvettes, see Starna's website:
        i. https://www.starna.com/cells/care-and-cleaning-of-cells
9. Let the cuvette(s) sit for five minutes (or ideally one hour if using the HCl solution).
10. After letting sit for five minutes, rinse the cuvette(s) five more times with Millipore water.
11. Finally, rinse the cuvette(s) twice with acetone before putting them away.

K. Exporting Data for Analysis

Regardless of the type of experiments performed, the data can be exported as a CSV file that can be opened in Microsoft Excel or used with a provided R script (see **section L**) for automated data analysis. Follow these instructions for exporting the data.

1. If the data is not already opened in Spectra Analysis or Interval Data Analysis, select File > Open and select the data that you would like to export.
2. Select a file window that you would like to export and select File > Export. Next, choose your group folder or other location you would like the CSV file saved.
3. If you would like to use the R script, when saving the data file, ensure that the naming convention is identical to the originally saved file. This can be done by selecting the file name in the folder the raw data is in. This will populate the File name. Select .CSV as the Save as type and hit Save.
4. Repeat these steps for all data that needs to be exported.
    a. Note that for multiple cells monitoring a wavelength over a temperature range, each individual cell's data set must be exported, despite it being in one single data analysis window.

L. Data Analysis

There are a variety of ways that the CD data can be analyzed. It is recommended that a blank sample be used to background subtract from the sample measurements. Then, it is common for CD measurements to be reported as the mean residue ellipticity (MRE), as defined by **Equation 1**.

$$MRE = \frac{CD}{\ell \cdot \mathrm{c} \cdot \mathrm{n}} \qquad \textbf{Equation 1}$$

Where *CD* is the ellipticity, ℓ is the path length, *c* is the peptide concentration, and *n* is the number of amino acid residues in the peptide.

To simplify this data processing, there are two different resources provided by the PPMC. Under the instrumentation page for the Jasco J-1500 Circular Dichroism Spectrophotometer, under supplementary material, are two different options for data processing. The first is a Microsoft Excel document that can be used for analyzing your data. This file contains different sheets within the document where users can input their sample conditions, blank data, as well as raw data for temperature and wavelength scans (see **section G** or **section I**). From there, preliminary MRE plots are provided in Excel, as well as two different sheets that can be used to export the data to Origin or some other plotting software. This sheet is set up for Temperature/Wavelength Scan Measurements with blank data from Spectra Measurement. The second option is the recommended option, which is a script in R that will automatically process the data and provide plots of both CD spectra and melting curves. More information about using this R script is provided on the PPMC website. For this code to work, it is important that the proper naming convention be used for files. The convention detailed in **section G** and **section I** is correct. It is also provided in the material detailing the script on the PPMC website.

M. Troubleshooting

The following section provides information on common errors that may occur when using the instrument, as well as ways to rectify these issues.

Symptom	Check	Corrective Action
Power cannot be turned ON.	Is the power cable plugged into the outlet?	Correctly plug in the cable.
	Does the fuse burn off?	Replace the fuse.
The light source does not come on.	Is the flow rate of coolant supplied to the 450 W light source sufficiently high?	Increase the coolant flow rate.
	Anode holder is correctly installed.	Install correctly.
	Is the cathode fixing screw tightened?	Tighten the cathode fixing screw.
	Can a sparking sound be heard?	When a sparking sound can be heard, replace the lamp.
HT voltage does not increase from zero.	Is the shutter open?	Open the shutter.
	Is the photometric mode set correctly?	Set the measurement mode to “CD” (not “Test signal”).
	Is the HT voltage mode set correctly?	Set it to “Auto” (not “Manual” or “Off”).
	Is the sample chamber lid completely closed?	Completely close the lid.
The noise level is high.	Is the spectral bandwidth setting too small?	Increase the spectral bandwidth.
	Does the sample have high light absorption?	Reduce the sample concentration or shorten the light path of the cell.
	Is noise detected in the HT voltage?	Replace the Xe lamp.
	Is the HT voltage at below 250 nm too high?	Adjust the M_0 and M_1 mirrors.
	Is there any noise source that generates electromagnetic waves nearby?	Remove the noise source from the vicinity of the instrument.
	Is there any source of mechanical vibration nearby?	Remove the source of vibration.
	Does the line voltage vary abruptly?	Use stabilized line voltage.

A CD value is displayed even though the sample is not optically active.	Is the sample fluorescent?	Decrease sample absorbance to 2 or less by adjusting the concentration of the sample.
	Is the sample a film or liquid crystal?	A spurious CD signal from the sample is probable.
	Does the cell accidentally contain any optically active residues?	Prepare a new sample and clean the cuvette.
Repeatability of CD values is low.	Has the instrument warmed up sufficiently?	Before performing CD measurement, warm up the instrument for approximately one hour after turning on the light source.
	Has the sample deteriorated due to irradiation by the light from the light source?	Use the shutter function or narrow the spectrum bandwidth.
	Is the scanning speed too high?	Slightly lower the scanning speed.
	Is the noise level too high?	Increase D.I.T. or refer to the “The noise level is high.” symptom.
No communication with the computer.	Is the USB cable connected properly?	Reconnect the cable correctly. Also try shutting everything down and restarting.

N. Recommended Consumables

Users must provide their own supplies for using this instrument. The following products are recommended from Fisher scientific. These can be purchased at a discount through UD exchange or through the chemistry store. The Fisher catalog numbers are also provided below.

Microcentrifuge Tubes: 14-666-310
Kimwipes: 06-666
Starna Cuvette Cleaner: NC9716202
Water: Water from the Millipore system in the laboratory is acceptable and recommended to use. If you would like to purchase water, the recommended catalog number is W64.

For those that need to purchase pathlength cuvettes, they can be purchased from Starna Cells, Inc. The 1 mm (0.1 cm) cuvette is what is typically used on this instrument (21-Q-1).

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If you use this instrument, then the following acknowledgement statement must be included in related publications:

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