Purpose of this Instrument: This instrument is for measuring differences in the absorption of left-handed polarized light versus right-handed polarized light arise due to structural asymmetry.

Location: 381 Chemistry Research laboratory building

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The Shared Research Facilities are operated for the benefit of all researchers. If you encounter any problems with this piece of equipment, please contact the staff member listed above immediately. There is never a penalty for asking questions. If the equipment is not behaving exactly the way it should, contact a staff member.
INSTRUMENT START UP

1. Reserve the equipment in CORES.
2. Sign into the logbook at the instrument.
3. Turn on the nitrogen generator from the back.

![Figure 2. Nitrogen generator back panel.](image)

4. Turn on the gas flow from the front the N2 generator.

![Figure 3. Nitrogen generator front touch screen panel.](image)

5. Purge the instrument with N2 gas for 10 min, before lighting on the lamp.

**WARNING:** Lighting the Xe lamp in air will generate ozone gas, which is harmful to human body. Feed nitrogen gas before lighting the light source.
6. Check the gas flow meter for correct gas flow rate.

![Figure 4. Nitrogen gas flowmeter.](image)

**Table 1.** The nitrogen gas flow rate required for experiment.

<table>
<thead>
<tr>
<th>Wavelength of measurement</th>
<th>Nitrogen gas flow rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measurement to 200 nm</td>
<td>3 to 5 λ/min</td>
</tr>
<tr>
<td>Measurement to 185 nm</td>
<td>5 to 15 λ/min</td>
</tr>
<tr>
<td>Measurement to 180 nm</td>
<td>15 to 20 λ/min</td>
</tr>
<tr>
<td>Measurement to less than 180 nm</td>
<td>More than 20 λ/min</td>
</tr>
</tbody>
</table>

7. The computer and monitor are on. (Users login as WVUSRF, password: wvusrf)

8. Turn on the instrument from the front. Then peltier temperature controller, then water bath.
9. If the user will be measuring spectra at very low temperatures, change the water bath temperature to 14 degree. The normal temperature setting for the water bath should be 18 degree.

10. Start the software. Double click the “spectra manager”
11. For a simple far-UV or near-UV measurement over a given wavelength range, double click the spectrum measurement. The instrument will go through self-checking. If all OK, then it will show the measurement page. And the meantime, the lamp is turned on and the shutter is open.

12. The lamp should be allowed to warm up for 10 min. When the lamp has reached 400+ hours, allow the lamp 15 - 20 min of warm up time to ensure reproducibility.

**NOTE:** The xenon 150 w lamp life time is about 1000 hours. It is important for users to record the start and end time. Go to CONTROL tab, then choose light source, then record the lamp hours in logbook before and after experiment.

13. Using Peltier temperature controller

If user needs use the temperature control, activate the peltier controller. Go to MEASUREMENT, choose ACCESSORY. Select “Jasco peltier type (six cells)”. Then OK.
Figure 8. Select pettler temperature controller

To change the temperature, go to CONTROL, choose ACCESSORY, then change the temperature, then “Apply” and close it.

Figure 9. Adjust temperature controller temperature.
Prepare sample and load sample

1. Please consult literature for sample solution preparation.


**Note:** Suggest users to consult with the literature for appropriate parameters and to have basic expectation for the output.

   a. Buffer consideration:
      1. Buffer for CD must not contain any materials that are optically active and should be as transparent as possible.
      2. The total absorbance of the sample, including the buffer and cell, should be below 1 for high quality data.
      3. Samples for CD analysis should be particle free. 0.2 μM filter is suggested to filter your sample.

   b. Prepare proteins and peptides
      1. Determine the concentration of your sample by UV absorption or amino acid analysis.
      2. The protein should be dialyzed or desalted into the CD buffer immediately before the spectrum is obtained and filtered through 0.1-0.2 μM filter to reduce light scattering.

2. Place sample in sample compartment. Close the lid.

3. Go to **MEASUREMENT** tab, then **PARAMETERS**. Use the following as guideline for peptide/protein solutions.

   Sensitivity: standard (100 mdeg)
   Start: 260 nm
   End: 190 nm
   Data pitch: 0.5 nm
   Scanning mode: Continuous
   Scanning speed: 50 nm/min
   Response: 1 sec
   Bandwidth: 1 nm
Accumulation: 5

4. Go to **DATA MODE** tab. 2 channels are default. Channel 1 and 2 should always
   monitor CD signal and HT. (The voltage increases proportional to the absorption of
   the sample. If the voltage is too high (>700), the user should dilute the sample/buffer
   concentration. Always test a blank buffer first before preparing sample. Be sure to
   check absorbance over the testing wavelength range)

5. Go to **DATA FILE** tab.
   
   1) Create your own folder under BNRF user file folder.
   2) You can select autosave.

6. Click **OK**.

7. Click **Start** tab.

8. When baseline scan in complete a CD spectrum will appear in the spectral analysis
   program.

9. In Spectra manager select Baseline Correction, and select the Blank Baseline File to be
   used. Leave a check mark next to Baseline correction, click OK (this will correct all
   your subsequent scans for the baseline file selected).

10. Save your file also as ASCII file for further data analysis.

11. Replace the blank with your solution. If the baseline file was saved via autosave,
    please change the file name is data file tab of parameters menu. Otherwise, your new
    sample file will overwrite Baseline file.

12. Data analysis. Read Nature protocols.2006, 1(6), 2876 for data analysis software
    suggestions.

    [http://www.cryst.bbk.ac.uk/cdweb/html/home.html](http://www.cryst.bbk.ac.uk/cdweb/html/home.html) is an online data analysis server.
    We suggest you go to this website and set up your academic account for your data
    analysis.
TURNING OFF THE SYSTEM

WARNING: Please follow the procedure to turn off the instrument.

1. Turn off Xenon lamp by going to “Control” then “light source”. Uncheck the box next to the lamp and select apply. **Note the lamp hours in logbook. Make sure the lamp indicator has switched off.**
2. If in use, turn off the water circulator and the temperature control device.
3. Exit spectra analysis program.
4. Exit spectrum manager program.
5. Turn off the CD.
6. Wait for 10 minutes then **SHUT OFF the N2** gas tank.

References:

- Jasco 810 spectropolarimeter operation. Laser spectroscopy facility, department of chemistry, UCI.
EMERGENCY SHUT-DOWN PROCEDURES

If, at any time, the user needs to contact someone for help, call or locate the following staff of the Shared Research Facilities (SRF):

**Primary Staff Contact:**

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If no one is available and the instrument is not acting as expected, the user should do the following:

- Turn OFF the Xe lamp
- Turn OFF the water circulator and temperature control.
- Turn OFF the software control
- Turn OFF CD power

Then, if possible, the user should stay by the instrument while trying to contact a Shared Research Facilities staff member. If it becomes necessary to leave the instrument then the user should leave a large, legible note on the CD SPECTROPOLARIMETER stating:

- The problem (describe what happened and steps taken)
- When it occurred (date and time)
- User name and phone number

If it becomes necessary to leave the instrument then the user should leave a large, legible note at the CD SPECTROPOLARIMETER stating the instrument is DOWN.

If a dangerous situation is evident (smoke, fire, sparks, etc.), or if the sound of shattering glass is heard emitting from the lamp housing, ONLY if it is safe to do so, the user should turn off system, lamp controller, and vacuum pump or unplug the instrument and leave the cleanroom immediately. The user should notify all other cleanroom persons within the cleanroom to evacuate. The user should then contact proper emergency personnel. The contact numbers can be found posted outside of the cleanroom or on the cover of the instrument log book.