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Chemical Instrumentation Facility

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Jasco J-715 CD Operating Instructions

02/07/2012 S.V.

Location: **1240 Hach Hall**
Contact: **Steve Veysey, 1234 Hach Hall**

Safety

All researchers working in 1240 Hach Hall must complete the EH&S course: *"Fire Safety and Extinguisher Training"*. When preparing samples in this room, please wear all appropriate personal protective equipment. Aprons, safety glasses, and rubber gloves are available in 1238A Hach Hall. If solvents are involved, consider preparing your sample in room 1238A.

Properly dispose of glass pipettes in the container provided. Waste solvents can be disposed of in the waste containers provided in 1238A. All of the computers in this lab have direct links from the desktop to MSDS sheets, the EH&S Laboratory Safety Manual and to the CIF Safety Manual.

Introduction

The Jasco J-710 CD is a research grade instrument used for measuring the circular dichroism of compounds in solution, including small molecules, peptides and proteins. The instrument was purchased in 1995 and has recently been upgraded to a J-715. The system will accept a number of sample devices, but the default is a Peltier controlled thermostated cell holder designed for standard 1cm x 1cm quartz cuvettes. Spacers are included to accommodate smaller cuvettes. The use of long path length liquid or gas cells requires removing the Peltier device and should only be done by Instrument Services staff.

Jasco J-715 CD Spectrometer

Overview

The J-715 is controlled by a Windows XP PC communicating via two serial ports, one to the instrument and one to the Peltier temperature control accessory. The computer is part of a local area network in 1240 Hach Hall and is safeguarded behind a hardware firewall from normal computer attacks. Printers, folders, files, et cetera, can safely be shared within the 1240 Hach Hall LAN. Because full access to the university network is available through the firewall, the computer is still vulnerable to viruses attached to any files downloaded from outside the lab. Do NOT browse the internet or download ANY files to the PC's in this lab. Do NOT attach a thumb drive.

The instrument uses a high intensity UV lamp to provide energy in the wavelength region from below 180 nm to above 700 nm. Mirrors, prisms, and slits are used to select frequency elements. A linear crystal oscillating at 50 kHz creates circularly polarized light which is directed through the sample compartment, and detected by a photomultiplier tube.

Although the PC is normally on at all times, the J-715 is normally OFF except when in use.

Startup

Nitrogen gas - Strong UV radiation produced by the source can create levels of ozone damaging to the optical elements unless steps are taken to remove oxygen from the instrument. These effects increase as the wavelength is decreased. Nitrogen gas is used to purge air from the instrument optics. We currently use nitrogen gas generated elsewhere in the building. At least 15 minutes prior to use, the nitrogen gas valve must be turned on; also check that the associated in-line regulator is adjusted to 20 psi. The nitrogen flow should then be adjusted using the needle valve above the vertically mounted flow meter.

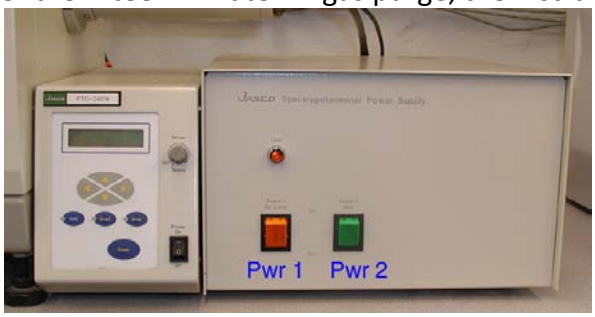
Turn on N2 gas; check pressure; open shut-off valve; adjust flow

Regardless of the spectral region being investigated, the instrument must be purged at a flow rate of 30 scfh (15 liters/min) for fifteen minutes before the instrument is turned on. After fifteen minutes the flow rate may be reduced (or increased) according to the following table:

Spectral Limit	Flow Rate (scfh)	Flow Rate (liters / minute)	Cylinder life
175 nm	60 scfh	30 l/m	4 hours
180 nm	30 scfh	15 l/m	8 hours
200 nm	15 scfh	7 l/m	15 hours

Water flow – *NOTE: Unless you plan to use the Peltier controller, it is NOT necessary to turn the water on.* We use the building "process chilled water" loop. There is both a supply valve and a return valve that need to be opened. The needle valve should be adjusted until the in-line flow-meter rotates slowly (one or two revolutions per second).

Spectrometer power – After the fifteen minute N2 gas purge, the instrument may be turned on.

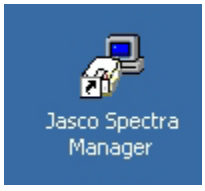


You MUST turn Pwr 1 on first, verify lamp ignition, then turn on Pwr 2

WARNING: You MUST turn Power 1 on first and verify that the UV lamp has ignited before turning on Power 2, or serious damage may be caused to the electronics.

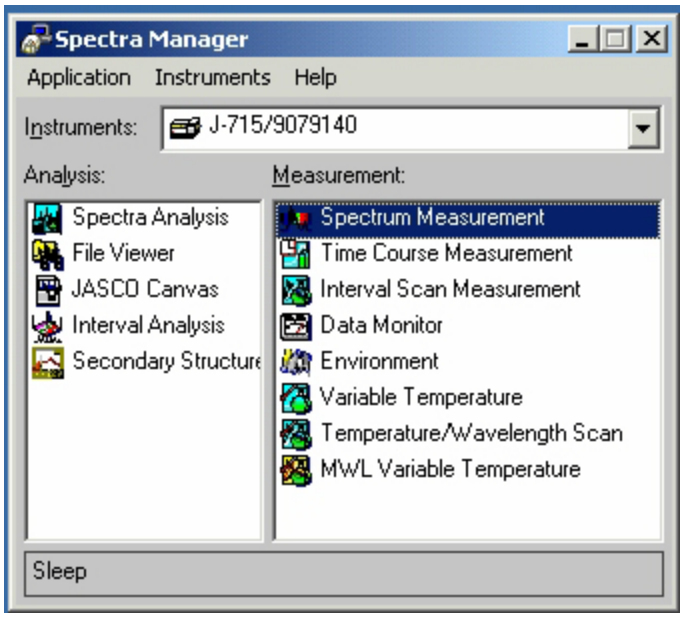
Computer access – Log in to the Jasco CD computer with your assigned username. This will usually be your ISU netID. Double-click the "Jasco Spectra Manager" icon to start the program.

Log in with your assigned username.



Start the Jasco program

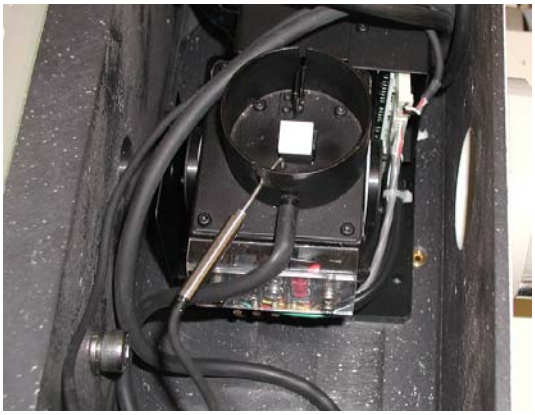
A suite of programs will be displayed, including five analysis programs, and eight measurement programs. Each of these will be discussed in later sections.



The software includes both analysis and measurement programs.

Loading Samples

Only the use of the Peltier temperature controlled sample accessory will be discussed. Begin by lifting the hinged beige sample compartment lid, and removing the round black cap from the sample holder. Place your sample cuvette in the holder. Cuvettes smaller than 1cm x 1cm require the use of spacers.



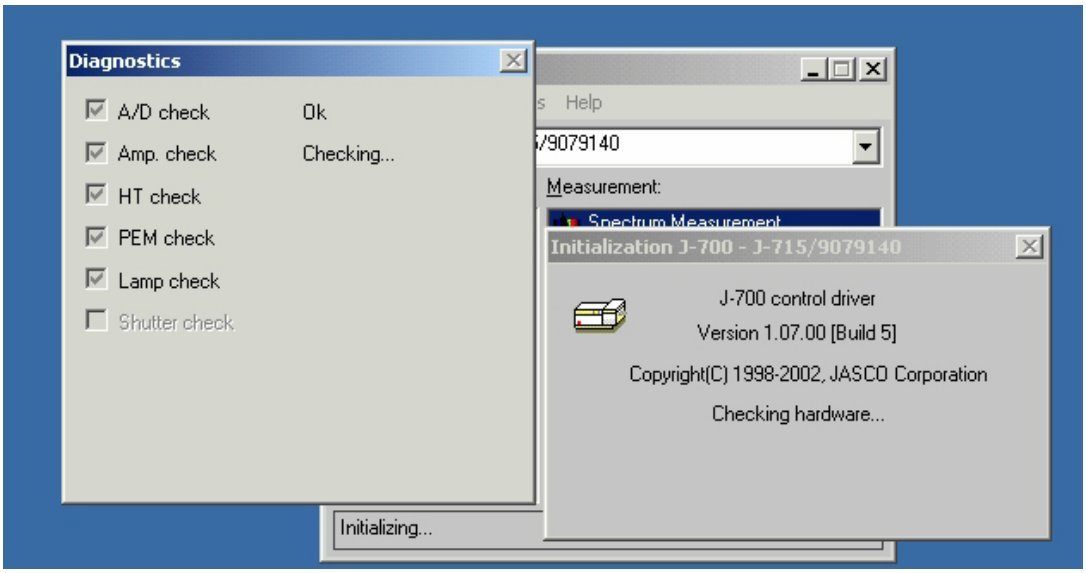
1. Lift the sample compartment lid; remove cap from sample holder; place cuvette in holder.

2. Replace the black sample holder cap; close the sample compartment lid.

Preparing for Data Acquisition

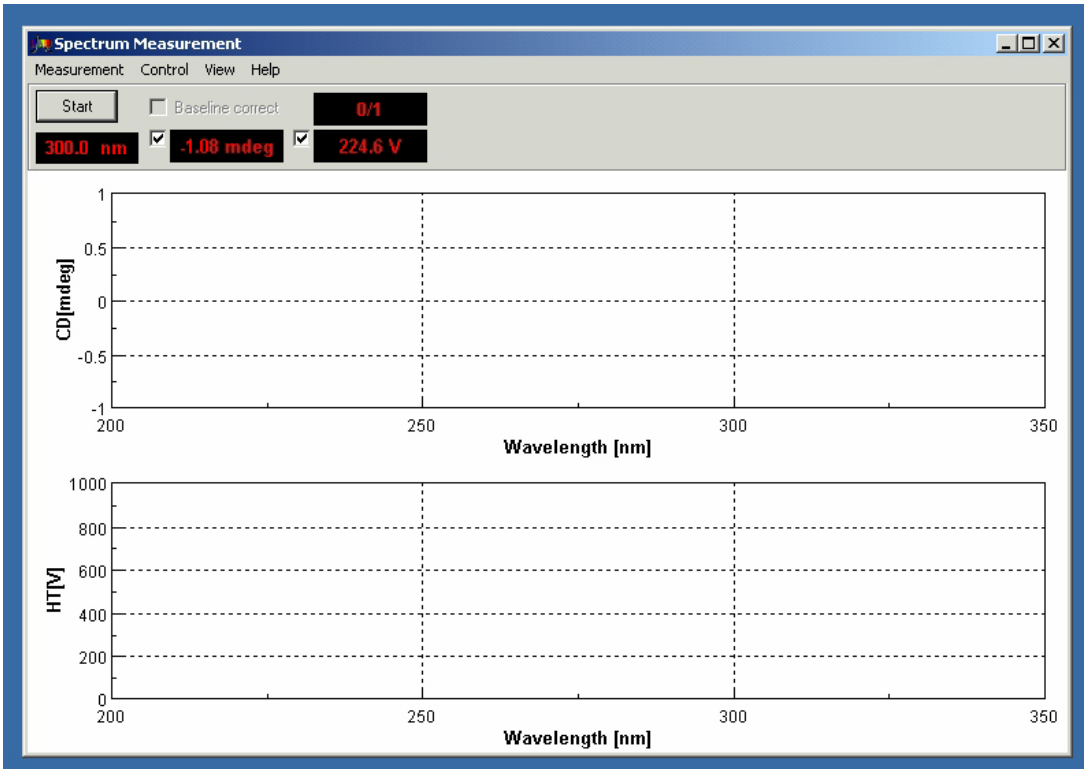
From the list of measurement programs presented in the Spectra Manager menu, double-click "**Spectrum Measurement**". This causes the software to perform a number of automatic diagnostic tests, and to download default measurement parameters to the spectrometer. The diagnostic tests will not be discussed in this document.

WARNING: If any of the diagnostic tests fail, do NOT attempt to "fix" the problem! Immediately cease operation of the instrument, return it to a safe mode by following the normal shutdown steps discussed later in this document, and notify Instrument Services staff.



Normal instrument start-up after selecting "Spectrum Measurement"

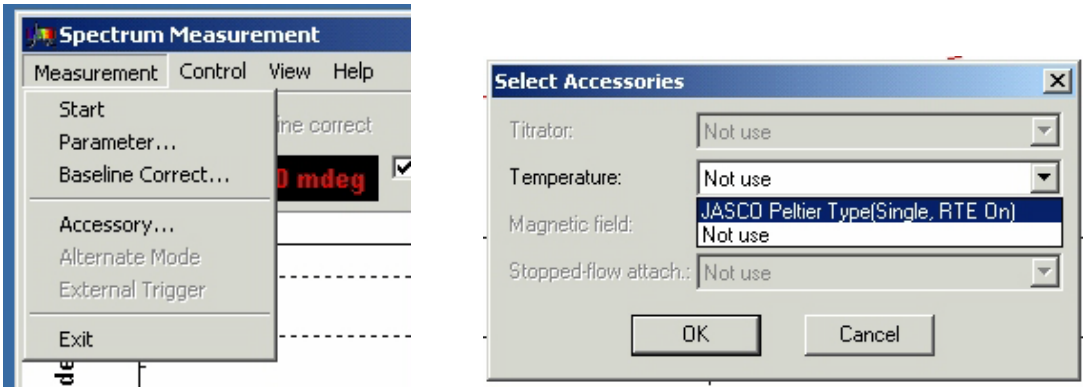
After completing the diagnostics, the software presents the "Spectrum Measurement" view.



"Spectrum Measurement" view

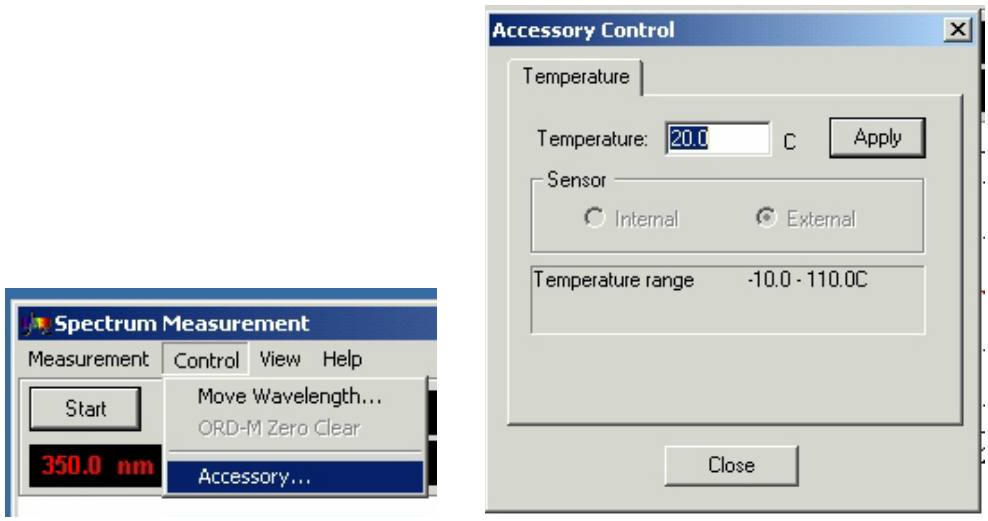
The current spectral position will be displayed (the default is typically 300 nm), as well as the CD signal (typically less than a few millidegrees, positive or negative, for an empty sample holder), and the PMT voltage (less than 250 V at 300 nm if the system is working properly).

From the "**Measurement**" pulldown menu, select "**Accessory…**" and enable the Peltier temperature controller.



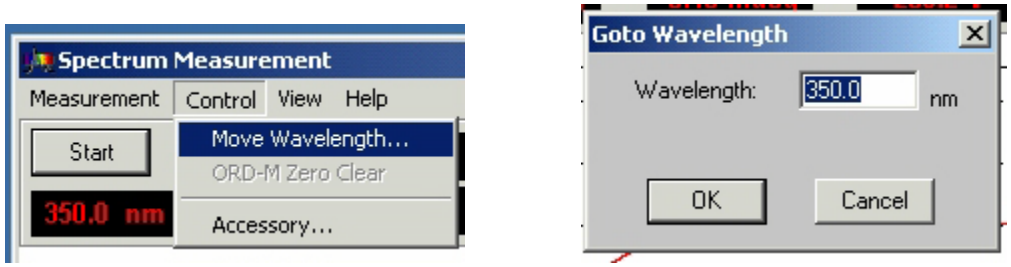
From "Measurement" pulldown, select "Accessory" and enable "JASCO Peltier".

From the "**Control**" pulldown, select "**Accessory…**"and select a temperature for the sample holder. Normally 20 degrees Celsius should be used as the default.



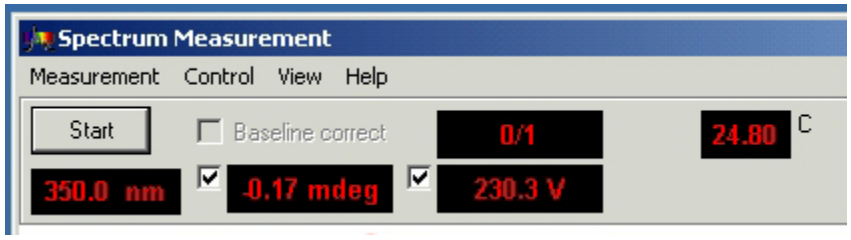
Set the default temperature of the sample holder to 20 degrees Celsius.

You may also use the "**Control**" pulldown to move to any specified wavelength.



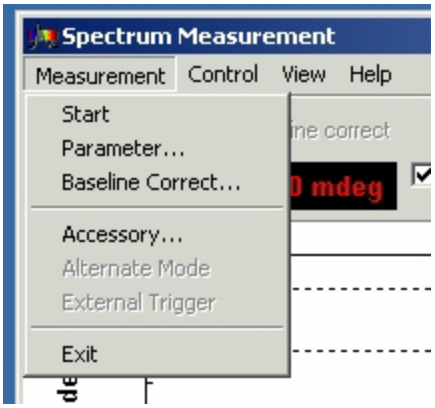
You can easily move the spectrometer observation to any wavelength.

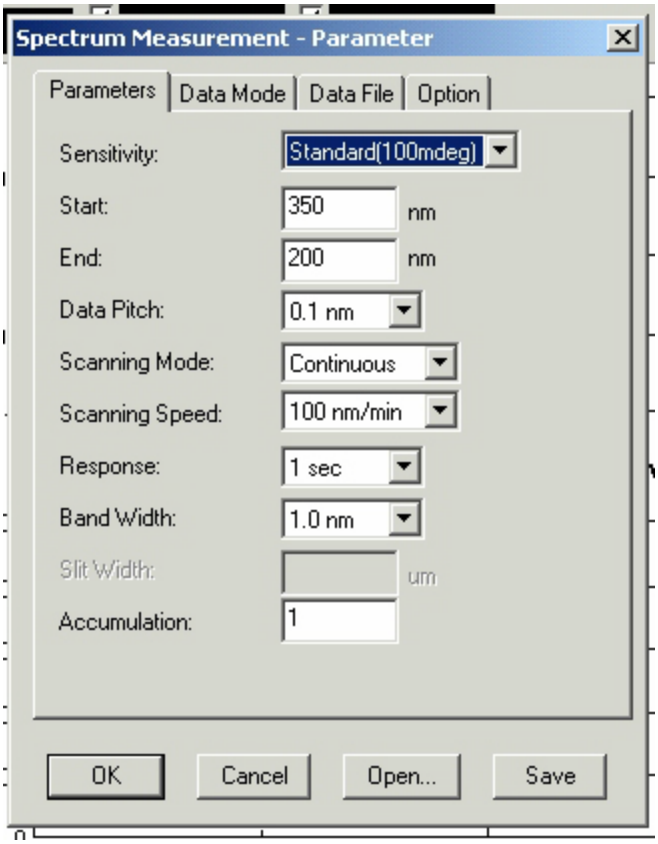
After enabling the Peltier, the Spectrum Measurement view will display the sample holder temperature in addition to the wavelength selected, CD signal, and PMT voltage.



Wavelength, CD signal, PMT voltage, and cell temperature displayed.

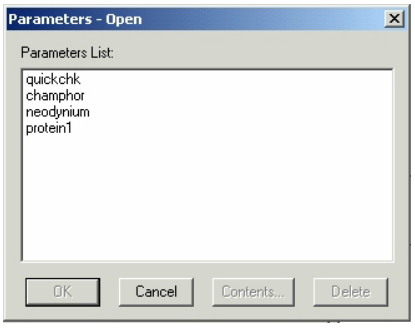
Returning to the "**Measurement**" pulldown, select "**Parameter…**"

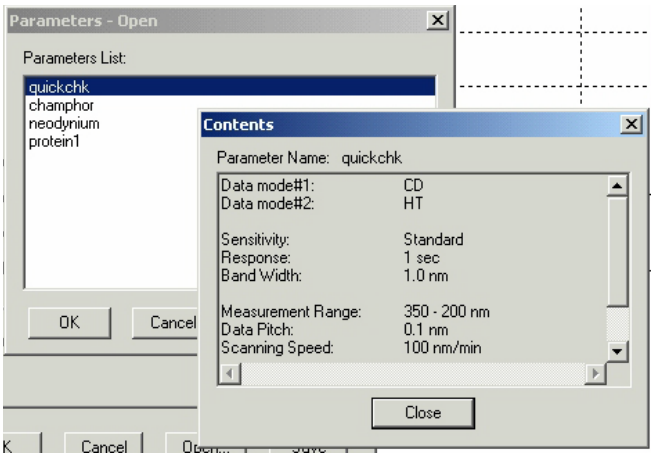




From "Measurement" pulldown, access the "Parameter" page.

All of the necessary instrument settings for your measurement are set on the parameter page. It is best to establish reasonable parameters for the measurements you will be performing, and save them as parameter sets. Open the proper parameter set, and then make any minor adjustments as necessary. The contents of each parameter set can be easily previewed.

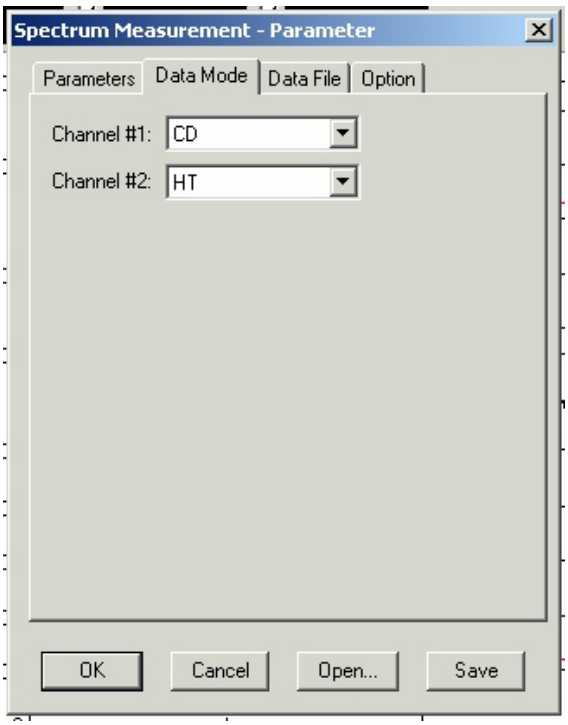


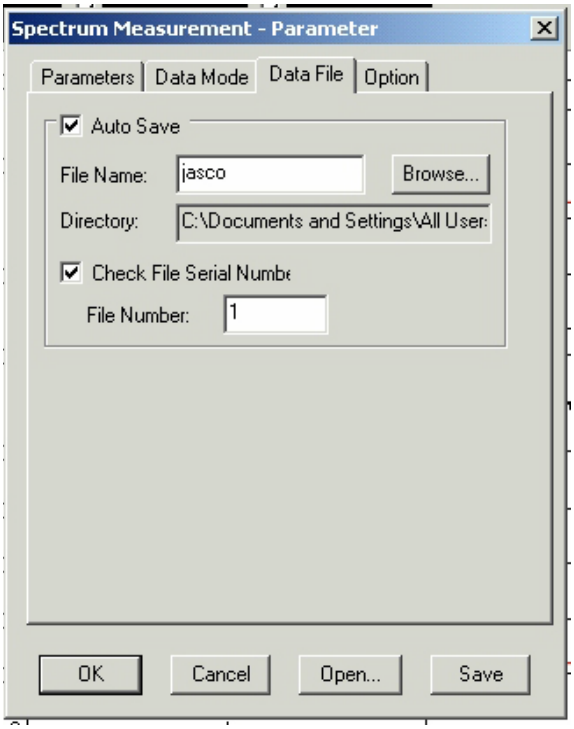


Choose a parameter set. The contents of parameter sets can be easily previewed.

The "**Parameter**" view contains four tabs. These are:
Parameters: Instrument settings such as scan range, scan speed, et cetera
Data Mode: Signals to be displayed on the two display channels

Data File: Location and naming protocol for acquired data files
Option: Not applicable





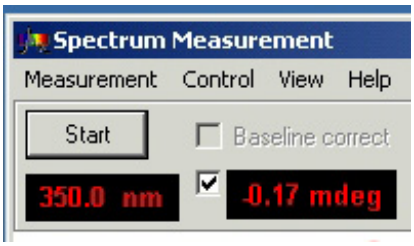
Data Mode tab *Data File tab*

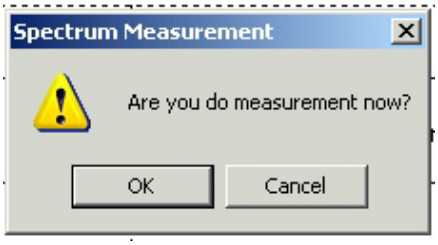
If significant modifications are made to the parameter set, consider saving it as a new parameter set.

Starting the Measurement

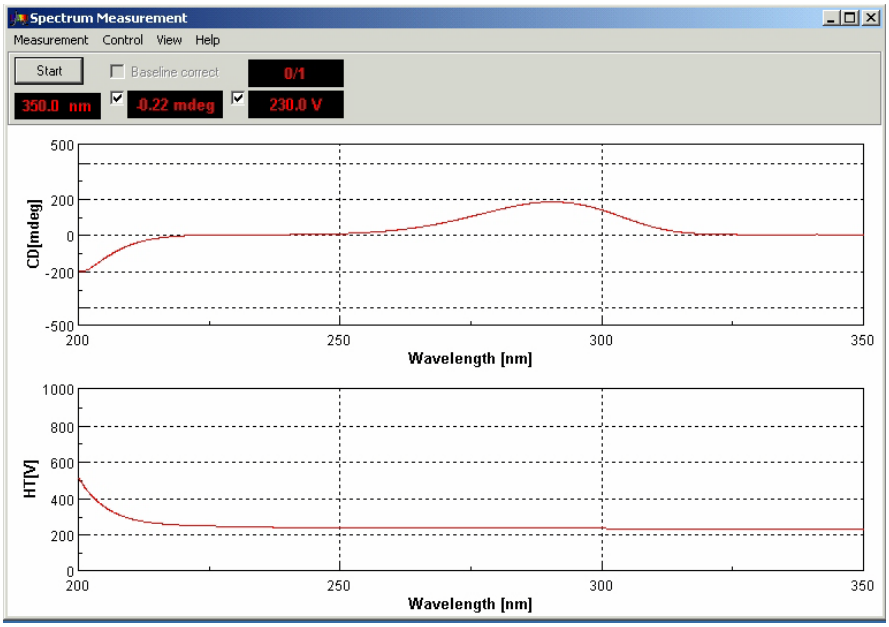
After enabling and setting the temperature controller, selecting a general parameter set, and modifying measurement parameters as necessary…

From **"Spectrum Measurement"** press **"Start".** You will be prompted to start the measurement.





The data acquisition will then be displayed in real time:

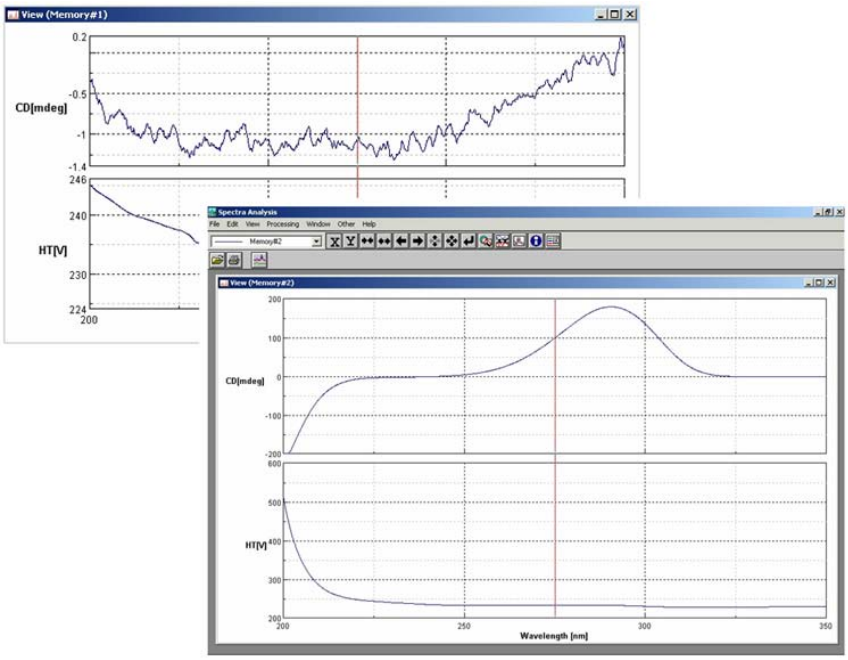


The data acquisition is displayed in real time in the "Spectrum Measurement" view.

When the measurement is completed, the software will automatically open and transfer the results to the "**Spectra Analysis**" program.

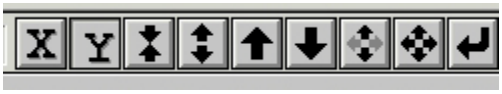
Analyzing Data

Each spectrum acquired may be stored in memory and viewed (i.e memory#1, memory#2… et cetera), or automatically saved on disk, depending upon the options selected on the *spectrum measurement -> parameter -> data file* tab. The most recently acquired spectrum automatically opens in "**Spectra Analysis**" at the top of the stack.

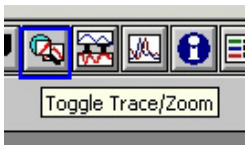


Spectra Analysis views of "memory 1" and "memory 2", background and camphor.

The display may be manipulated using the various elements shown in the ribbon bar.



Ordinate and abscissa may be scaled by selecting “X” or “Y” and the actions of the various buttons. Alternatively, the cursor “zoom” mode may be selected; the normal mouse operations of “left-click-hold-and-drag” can then be used to resize the display as required. In “trace” mode the cursor displays the wavelength position and CD value.



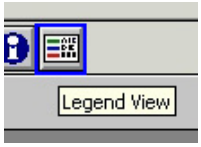
Toggle between “zoom” and “trace” functions of the mouse-controlled cursor.

Following are the functions of the other buttons:

Select the “active” data file from those in the view.

Choose between “overlay” or individual “normalize” when viewing multiple spectra.

View and modify the information stored with the spectrum.



Display a condensed summary of the spectra in the view.

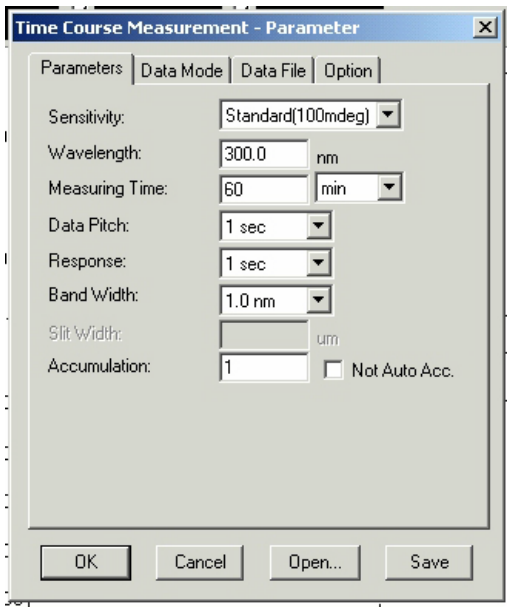
Spectra may be added to the view using the normal “file open” icon

The view may be sent to one of several printers in the lab.

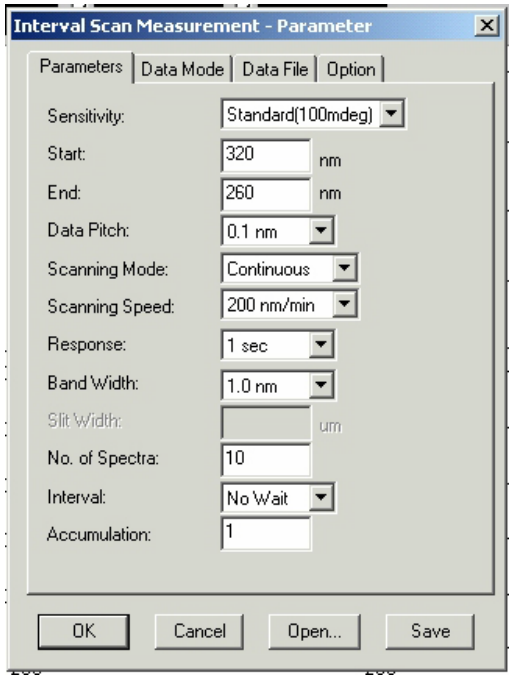
Advanced Measurement Programs

In addition to routine "**spectrum measurement**", the following "**Measurement**" programs can be selected from the **Spectra Manager** menu. Only one program may be opened at a time. As with "**spectrum measurement**", each program is configured from a unique parameter page with one or more tabs. Some require interaction with the Peltier temperature control parameter page.

Time Course Measurement – Monitor one selected wavelength versus time, i.e. kinetics.



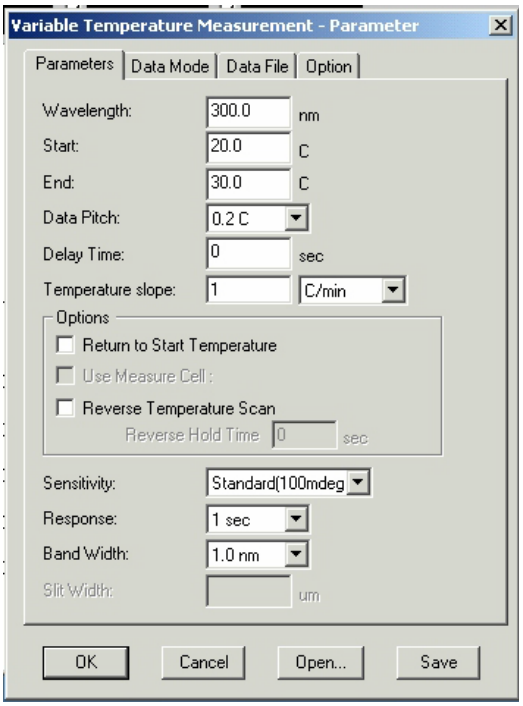
Interval Scan Measurement – Monitor a spectral region versus time by acquiring repetitive scans, i.e. kinetics.



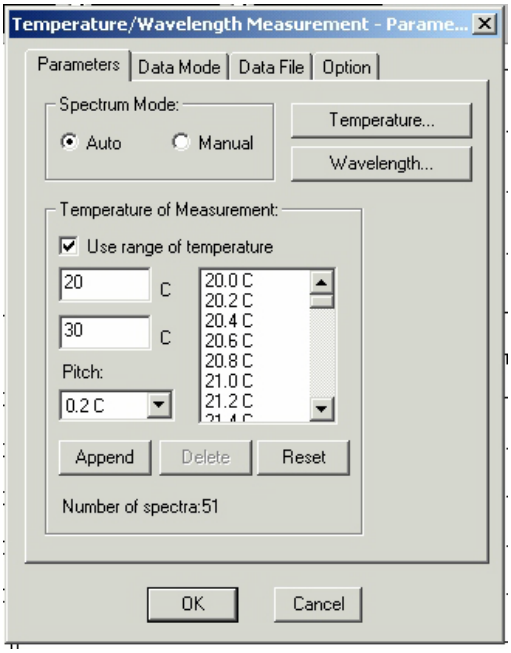
Data Monitor – Used for diagnostic purposes

Environment – Information not available

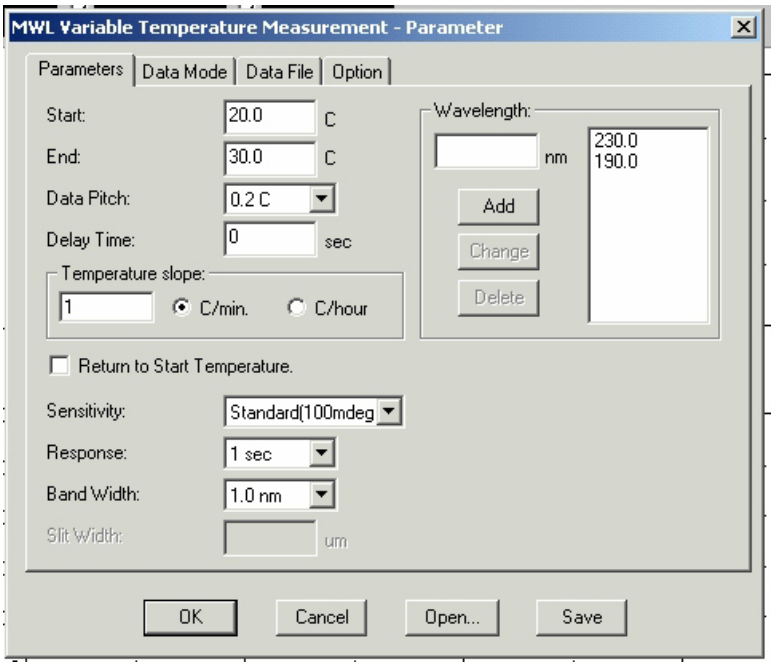
Variable Temperature – Monitor one wavelength versus temperature and time, i.e. kinetics.



Temperature/Wavelength Scan – Monitor one or more spectral ranges versus temperature and time. Opens the Variable Temperature parameter page and the Spectrum Measurement parameter page to allow multiple temperature ramps and spectral windows to be measured as part of one analysis.



MWL Variable Temperature – Monitor multiple wavelengths versus temperature and time.



Advanced Analysis Programs

In addition to routine "**spectra analysis**", the following "**Analysis**" programs can be selected from the **Spectra Manager** menu.

File Viewer – Special browser window to allow file search and retrieval based upon a variety of search parameters.

JASCO Canvas – No information

Interval Analysis – No information at this time

Secondary Structure – Use of this program for analysis of the secondary structure of proteins is NOT recommended by Jasco. There are much better third-party programs available for free via the internet for the analysis of secondary protein structure. Utilities are included to export Jasco data files to these programs.

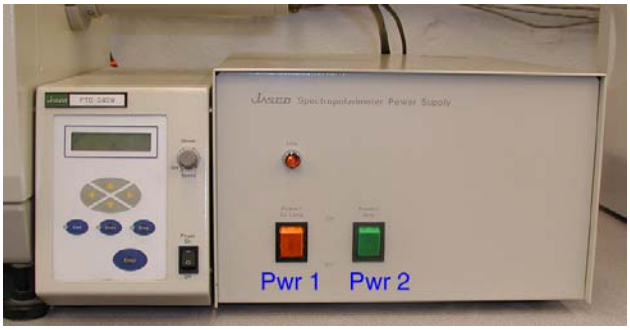
Shutdown and Record Keeping

1) Exit from whichever "Measurement" program is active, normally "Spectra Measurement".

2) Exit from whichever "Analysis" programs you are using.

3) Close "Spectra Manager".

4) Turn off "Power 2", THEN turn off "Power 1"



You MUST turn Pwr 2 off first, then turn Pwr 1 off.

WARNING: You MUST turn Power 2 off FIRST, or serious damage may be caused to the electronics due to a power surge when Power 1, (the UV lamp) is turned off.

5) Remove your sample.

6) If the Peltier temperature controller was used, turn off the cooling water supply and return valves.

7) Turn off the nitrogen gas by closing the valve.

8) Correctly record your instrument use in the logbook.

WARNING: Failure to correctly record your instrument use will result in suspension of usage privileges.

10) Log off of the computer. This computer records your instrument usage for billing purposes. Logging ON should be the first thing you do when you enter the lab to begin your session, and logging OFF should be the last thing you do before leaving the lab.