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AVIV MODEL 215

CIRCULAR DICHROISM

INSTRUCTION MANUAL



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Revision Date: 9/26/01

AVIV® INSTRUMENTS, INC.

215 SERIES CD SPECTROMETER MANUAL

AVIV MODEL 215

CIRCULAR DICHROISM

INSTRUCTION MANUAL



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ORIGINAL INSTRUMENT WARRANTY:

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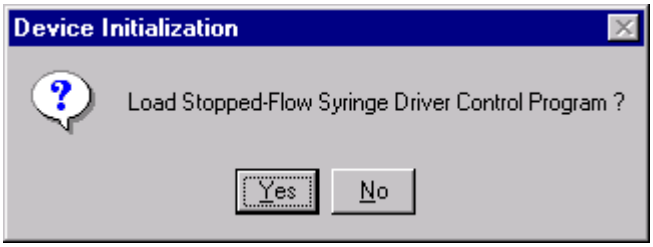
Chapter I: Software Details

This chapter provides a detailed explanation of each input to the CDS software. An explanation of how to run the instrument is given in the chapter entitled Quick Start.

I. A Starting the Software and Main Control Panel

When the instrument control software is started it first checks the individual pieces or hardware to ensure their functioning. Once this is completed the Main Control Panel is displayed. It is here that the current instrument readings are shown along with data from experiments in progress. If the instrument is equipped with the Stopped-Flow accessory then a message will appear that asks if the motor controller program should be loaded. Under most circumstances the appropriate answer is Yes. The only time No should be used is if it is undesirable to erase the current motor positions from memory.

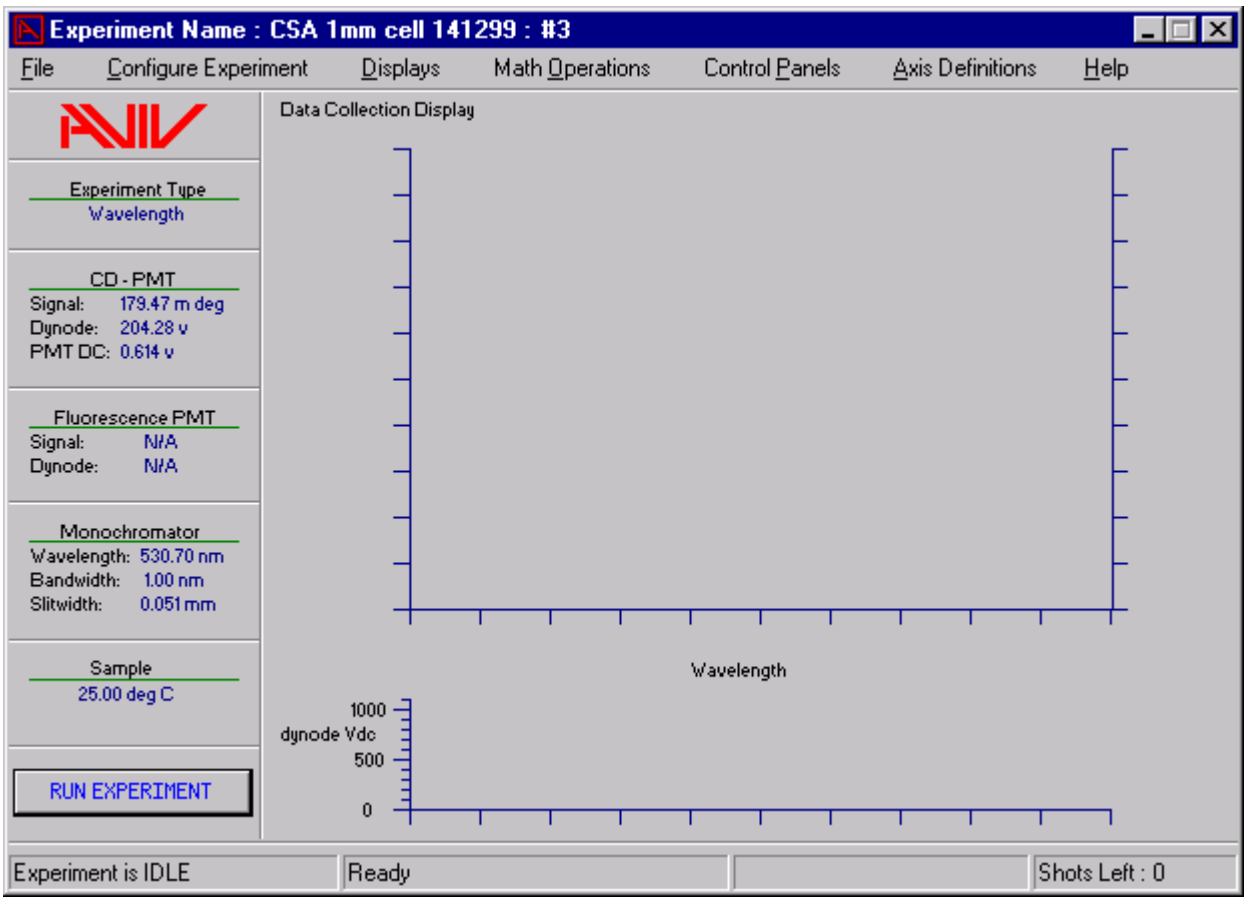
Figure I - 1: Start-Up Messages



Progress of the start-up procedure is shown in the bottom line of the Main Control Panel. If an initialization test fails then an error message will be displayed. These are explained in the Trouble Shooting chapter.

The **Main Control Panel** displays the data as it is collected and the current values of the different pieces of hardware. **Experiment Type** is the type of experiment that will be executed when the **Run Experiment** button is clicked on. **CD-PMT** is the in-line photomultiplier tube (PMT) used to measure the light transmitted through the sample. **Signal** is the current CD signal, **Dynode** is the high voltage applied to the dynodes of the PMT and PMT DC is the current through the PMT. One volt on the PMT-DC display corresponds to 500 nanoamps of actual current. Instruments equipped with a fluorescence accessory will show the current values for the **Fluorescence PMT**. **Signal** is the relative fluorescence signal (full scale is 0 to 5) and **Dynode** is the high voltage applied to the dynodes of the fluorescence PMT. Lines beneath **Monochromator** show the properties of the exciting light. **Wavelength** is the current selected wavelength. **Bandwidth** is the range of light wavelengths that are passing through the monochromator. These values are controlled in the Configure Experiment panel. **Slitwidth** is the mechanical opening between the slits. This

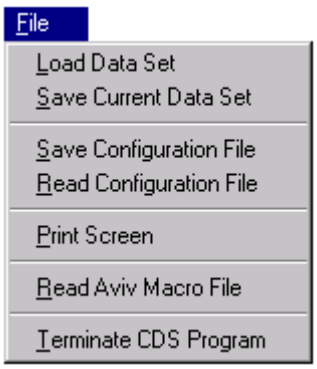
Figure I - 2: Main Control Panel



value is determined by the user selected values of the Wavelength and Bandwidth. Beneath **Sample** are the current readings for the temperature, pH and other items.

The **File** menu has options for reading and saving the different types of files. **Load Data Set** opens the Data Browser, where Read Data Set <- Disk can be used to load data into program memory. **Save Current Data Set** gives the user to save data currently displayed in the Data Collection Display. Saving of other data sets is done through the Data Browser. **Save Configuration File** allows the current experiment configurations to be saved to a file. This file includes the parameters for all of the experiments. **Read Configuration File** allows the configuration to be set using a configuration file already saved. The reading and writing of data files

Figure I - 3: File Menu

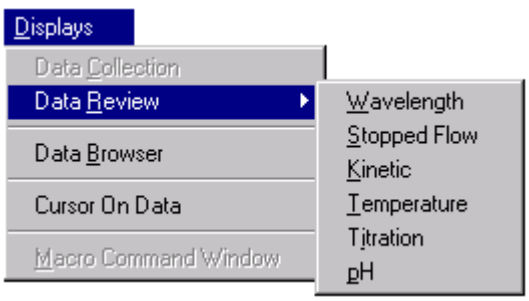


is done through the Data Browser, found under the Displays menu. Read Aviv Macro File opens the dialog box for reading macro file programs. This is explained in greater detail in the ReadMe.txt file that accompanies the macro file editor program (AMC.EXE). **Print Screen** launches a subprogram that will send the image of the current panel printer. The subprogram can be minimized and other panels brought forward for printing. **Read Aviv Macro File** loads a macro file into program memory so that it becomes available in the Experiment Type List. **Terminate CDS Program** exits the program. If data has not been saved then the user will be given a last chance to save data before it is lost.

The **Configure Experiment** menu option opens the Configure Experiment panel. This is where the experiment type and other parameters are selected. This explained later in Experiment Configuration section.

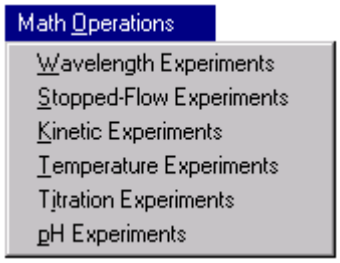
The **Display** menu allows the user to select from different displays of the data. **Data Review** selects the type of data to be graphically display in the Data Review panel. **Data Browser** allows the user to read and write data files, examine the raw numbers of a data set, and other file management options. These are explained in greater detail later in the Reviewing and Managing Data section. **Cursor On Data** turns on and off a cursor on the data point closest to the mouse arrow. The bottom, left, and right axis coordinates of the cursor are shown at the bottom of the Data Collection and Data Review panels. The Macro Command Window becomes available when a macro experiment has been loaded.

Figure I - 4: Display Menu



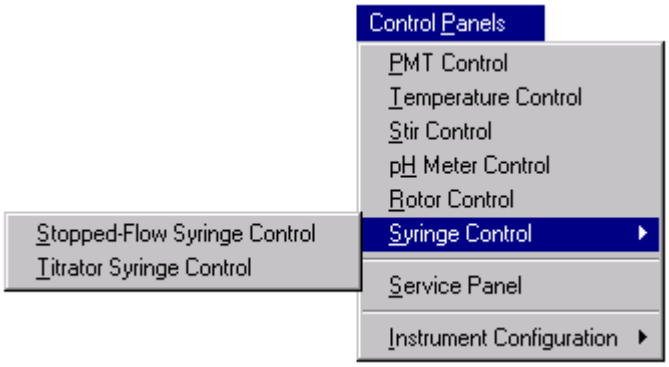
The **Math Operations** menu opens the Data Operations panel for performing math operations. The user must select the type of data that is to be used.

Figure I - 5: Math Operations Menu



The **Control Panels** menu allows the user to manipulate the individual pieces of hardware. These are explained in detail in the section entitled Controlling Hardware. The **PMT Control** allows the high voltages of the photomultipliers to be set. **Temperature Control** allows the user to set the temperature set point, equilibration time and other parameters associated with temperature control. **Stir Control** allows the user to turn stirring on and off and to set the speed. **pH Meter Control** reads back the current pH

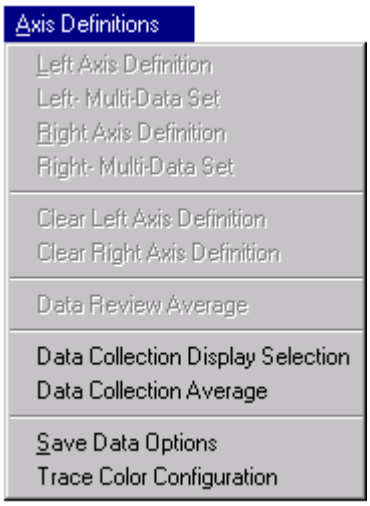
Figure I - 6: Control Panels Menu



values from the pH accessory. **Syringe Control** reads back the current volumes of the syringes, set the syringe sizes, and other syringe functions. **Service Panel** contains the current software version, a control for autoscaling the data and instrument utility functions. **Instrument Configuration** contains additional menu options for configuration of the hardware.

The **Axis Definitions** menu allows the user to control display of the data. The first set of options apply only to the Data Review panel.

Figure I - 7: Axis Definitions Menu



Data Collection Display Selection allows the user to turn off or on the display of the different types of data being collected. **Data Collection Average** allows the user to select and save data at the end of an experiment for averaging (see description for Data Review Average). **Save Data Options** selects the different types of data to be saved during future experiments. **Trace Color Configuration** allows the user to change the colors of the data being displayed.

The **Help** option in the Help menu brings up on-line help file. The **About** option displays information about the Aviv company and the software version history.

Figure I - 8: Help Menu

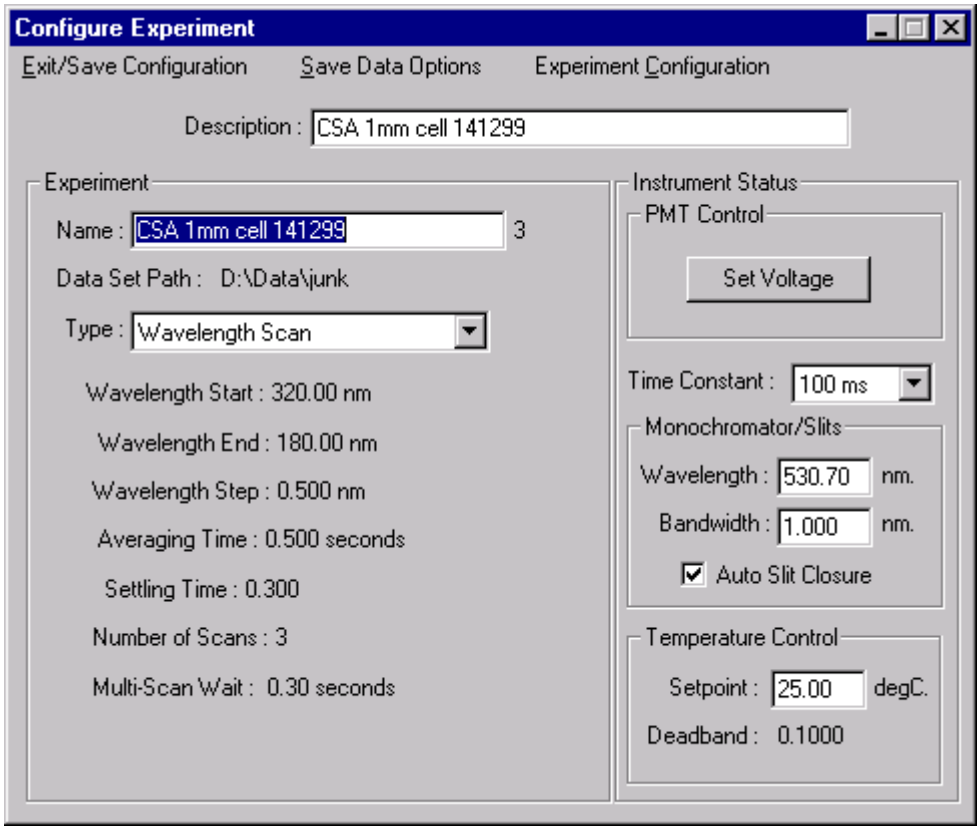


I. B Experiment Configuration

This is the location in which the current experiment parameters are displayed and

changed. The **Configure Experiment** panel

Figure I - 9: Configure Experiment Panel



allows the user to select the experiment type, name, and current instrument parameters. The **Description** is where the user can enter any descriptive information about the experiment. This can include the sample's contents, preparation, lot number, or what ever else is useful in identifying the experiment. Under the general heading of **Experiment** are the parameters that will be used for the next experiment. **Name** is used to name the experiment in the program's memory (as seen in the Data Browser) or on disk. This is followed by the **experiment number**. The number is incremented every time an experiment is started and then appended to the experiment Name. This ensures that every experiment has a unique name and yet doesn't have to be manually changed. Beneath the name is **Data Set Path**, the location on disk that the experiment will be saved. This can be changed in the Data Browser. **Type** is where the user changes the kind of experiment that will be ran next. Beneath Type are the current experiment parameters. These can be changed by going to the Experiment Configuration menu, discussed later.

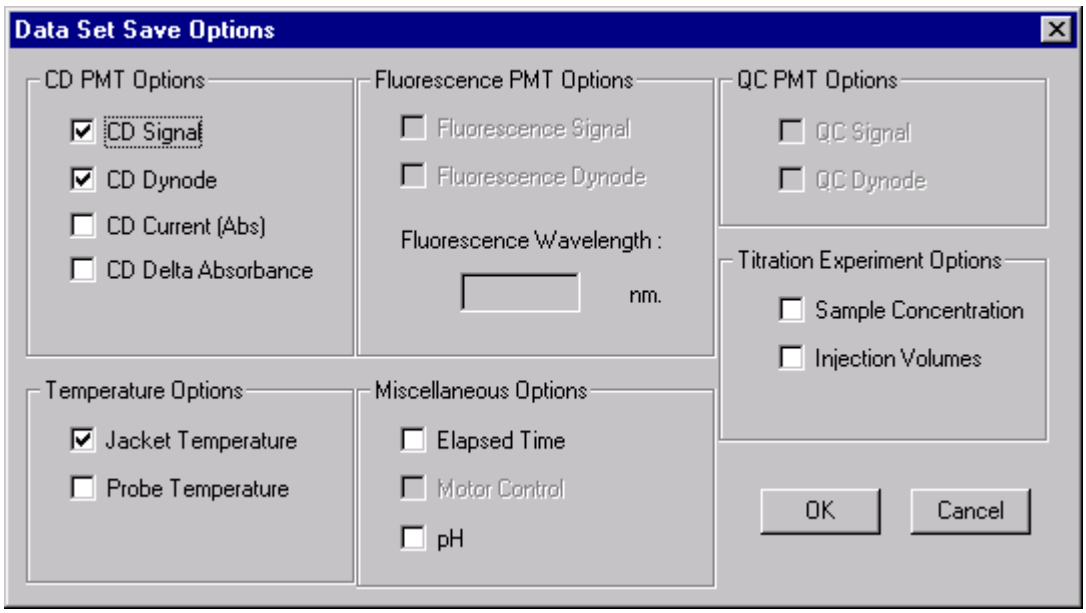
Under the general heading of **Instrument Status** are the current instrument parameters. First is **PMT Control** with the button **Set Voltage**. Clicking on this brings up the PMT

Control panel. If the instrument is in the Stopped-Flow experiment mode then **Time Constant** appears. This has a pop-up list of the available electronic time constants. If the experiment type is Stopped-Flow then the default will switch to 100 microseconds when the Experiment Configuration panel is closed. For other experiments types the default is 100 milliseconds. Under **Monochromator/Slits** are **Wavelength** and **Bandwidth**. If these values are changed then the instrument will immediately go to these values when the Return key is hit. When the instrument first starts it goes to the home wavelength, in the range of 530 nm. The Bandwidth determines the peak-half-width for the spectral dispersion of light. A larger Bandwidth allows more light to strike the sample at the expense of spectral resolution (good for highly absorbing samples). A smaller bandwidth passes less light with a narrower range of wavelengths (good for resolving sharp peaks). The bandwidth is controlled by adjusting the slits of the monochromator, as seen in the main control panel. If the **Auto Slit Closure** option is off then the slits are always left open. If this option is on then the slits will be closed when ever the instrument is idle between experiments, and during equilibration periods longer than 15 seconds. By closing the slits the sample will photobleach less and the life span of the instrument's optics will be extended. Under the heading of **Temperature Control** is the **Setpoint** for the temperature and the current **Deadband**. These are the same parameters as seen in the Temperature Control panel.

The menus in the Experiment Configuration panel are **Exit/Save Configuration**, Save Data Option and Experiment Configuration. The first closes the Experiment Configuration panel, making the current selections the values for the next experiment. If the panel is closed by using the X in the top right corner of the panel then any changes to the experiment configuration do not take place.

Clicking on the Save Data Options menu item brings up the **Data Set Save Options** panel. This is where the user selects which data is to be saved at the end of the experiment. Under the general heading of **CD PMT Options** are the optical signals. **CD Signal** is the measured Circular Dichroism signal. In all experiment modes except Kinetics and Stopped-Flow this signal is accompanied with the measured standard deviation for the CD signal (CD Error). **CD Dynode** is the high voltage applied to the photomultiplier tube used for CD measurements. The optimal range for this signal is 180 to over 500 volts. Beyond these limits the CD signal can be adversely affected. **CD Current (Abs)** is a signal proportionate to the current passing through the photomultiplier tube. A signal of 1 equals 50 nanoamps. In most experiment modes (expect Stopped-Flow) this value is constant and close to 1. **CD Delta Absorbance** is the raw signal proportionate to the CD signal. This signal is normalized by the CD Current signal and multiplied by 100 to give the CD Signal. Other optical signals may not be available if the software did not detect the necessary hardware at startup.

Figure I - 10: Save Data Options Menu



Under the heading of **Fluorescence PMT Options** the **Fluorescence Signal** is the current on the right-angle fluorescence photomultiplier tube. The current is proportionate to the amount of light illuminating the sensor. **Fluorescence Dynode** is the high voltage applied to the right-angle photomultiplier tube. Fluorescence Wavelength allows a different wavelength to be entered for fluorescence measurements (as opposed to CD or absorbance measurements). During Titration, pH Titration and Temperature experiments the wavelength will change, depending on the type of measurement being made.

Under the heading of **QC PMT Options** the **QC Signal** is the current on the quantum counter photomultiplier tube. This measures the total amount of light striking the sample, and can be used for correcting fluorescence and absorbance data. **QC Dynode** is the high voltage applied to this photomultiplier tube. Under **Miscellaneous Options** is **Elapsed Time**. This is the time since the start of the current experiment when the data was collected (in seconds). **Motor Control** is for Stopped-Flow experiments. **pH** allows the pH to be recorded in addition to other types of experiments when this accessory is used. For example, during a Titration experiment, the pH can be monitored while the ligand concentration changes.

Under the heading of **Temperature Options** are Jacket Temperature and Probe Temperature. The physical location of these sensors depends on the type of thermoelectric accessory being used, but in general the **Jacket Temperature** is the probe used for the thermal feedback loop, and is the temperature closest to the actual sample temperature. The **Probe Temperature** is usually associated with the external temperature probe accessory. This can be

inserted into the sample to allow measurements of the sample. For details pertaining to your particular thermoelectric accessory see Appendix A.

Miscellaneous Options has a collection of all the other data types. **Elapsed Time** is the time that has passed since the start of the experiment. **Motor Control** is a record of the transitions between different time domains in Stopped-Flow experiments. **pH** is a record of the pH (assuming the pH accessory is available).

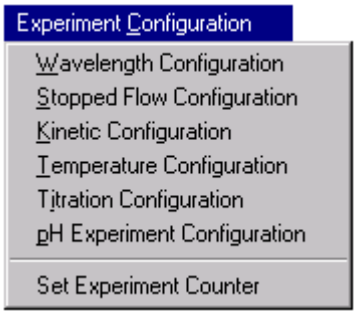
The **Titration Experiment Options** heading has **Sample Concentration**, which is the calculated sample concentration during a titration experiment. The data can be divided by the Sample Concentration to correct for dilution effects and normalize the signal. **Injection Volumes** is injection volume between Titration and pH Titration data points.

The **Experiment Configuration** menu gives access to the configuration panels for each of the experiment types. This is where the details of each experiment type can be modified. **Reset Experiment Counter** resets the number appended to the experiment Name.

I. B. 1 Experiment Configuration Panels

The Wavelength Configuration panel allows the user to enter the wavelength at which the scan will start at (**Wavelength Start**), the wavelength the experiment will end with (**Wavelength End**), the size of the wavelength step (**Sample Every**), and the

Figure I - 11: Experiment Configuration Menu



number of wavelength scans that will be made (**Number of Scans**). At each wavelength the signal will be averaged for the **Averaging Time**. If the averaging time is greater than 0.1 seconds then a standard deviation (error) will also be calculated for each data point. **Wait Time Between Scans** applies when more than one scan is made. This is an additional pause between wavelength scans, and is useful when long-term kinetic changes are being monitored.

The **Stopped-Flow Configuration** panel allows the user to define the shot size, timing, the number of data points, and the number of shots for stopped-flow experiments. The equipment for this is available as an accessory for the CD instrument. The experiment is divided into nine **Time Segments**. The duration (or **Time**) of each Time Segment is entered in the top row as milliseconds. During each Time Segment the volume in microliters to be delivered by each syringe is entered (**Syringe #1, #2, #3**). The ratio of the volume to time determines the flow rate for each individual syringe, and the sum of the flow rates is the total flow rate passing through the flow cell. Across the bottom is the number of **Data Points** to be

Figure I - 12: Wavelength Configuration Panel

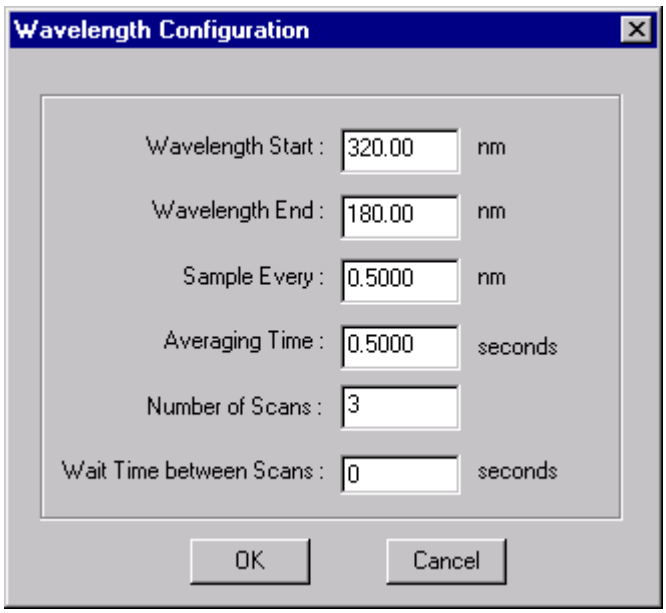
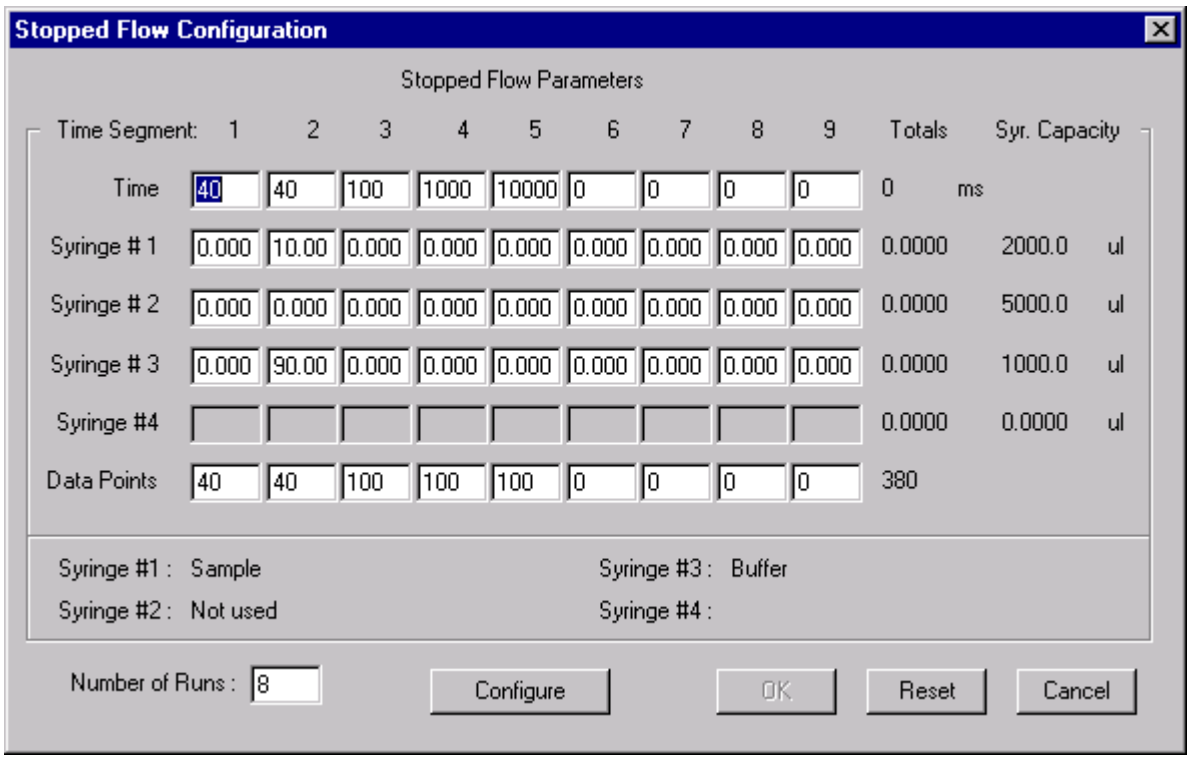


Figure I - 13: Stopped Flow Configuration Panel



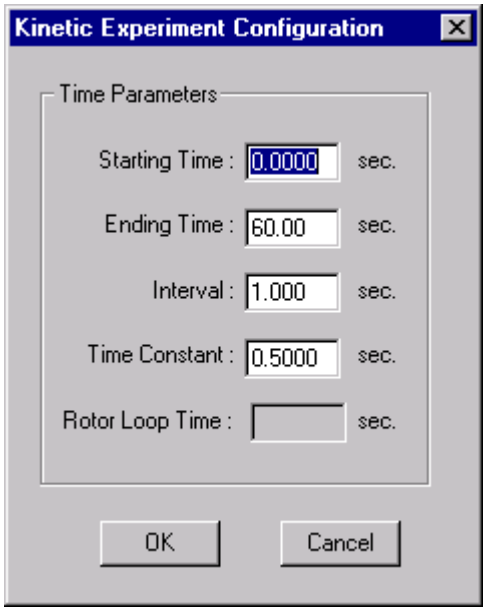
collected during each time segment. Likewise the ratio of the number of data points divided by the time gives the data collection rate. In the example shown the

first 20 milliseconds are spent collecting data at a rate of 0.1 milliseconds per data point with no flow (to measure the pre-trigger signal). In the second Time Segment 20 microliters is delivered syringe #1 (the 2 ml syringe) and 80 microliters from syringe #2 (the 5 ml syringe). Therefore the flow rates are 0.5 ml/second plus 4.0 ml/second for a total of 2.5 ml/second through the flow cell. The dilution ratio of the Stock Protein in syringe #1 is 1:4 with buffer, or 1:5 total dilution. The data collection rate continues at 10 data points every millisecond to measure the push profile. In the third Time Segment the high data collection rate continues for 100 milliseconds to measure the rapid kinetics. In the fourth and fifth Time Segments the number of data points is the same but the times are longer, so the data collection rates are 1 and 10 milliseconds per data point. The advantage of collecting data at slower rates is that more signal averaging can occur, and the signal-to-noise ratio will improve. By changing the data collection rates during a single shot the rapid kinetics at the start can be measured (albeit with more noise), then the slow kinetics can be measured more accurately, without creating data files with millions of data points.

The **Number of Runs** sets the number of shots to be made. If one to twenty shots are made then each individual shot is saved with all of the selected data sets. Each run will appear in the Data Browser as a separate data folder within the one experiment folder. If more than twenty runs are made then a running average is kept for all of the data sets plus the data sets from the last run.

The **Kinetic Experiment Configuration** allows the user to measure slow kinetics. The rate of kinetics that can be measured is determined by how fast the instrument can begin collecting data after the kinetics are initiated. The limiting factor is usually how

Figure I - 14: Kinetic Experiment Configuration Panel

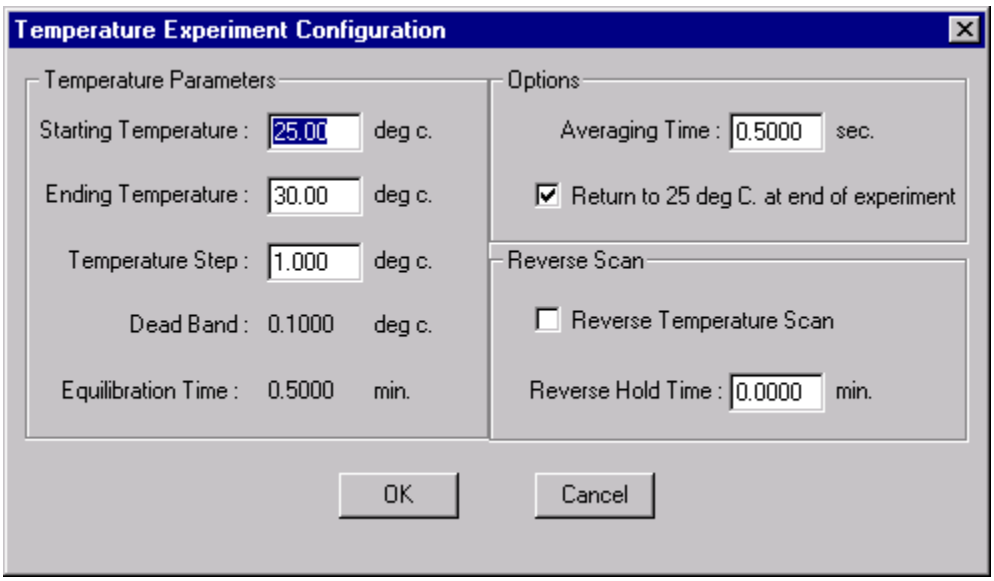


fast the sample can be placed in the cell holder and the sample compartment shut. A spring loaded syringe is available as an accessory so that solutions can be manually injected with the sample compartment closed. However, if the kinetics are faster than several seconds then the Stopped-Flow accessory must be used. The **Starting Time** is the time point at which data collection will begin after the experiment has been started. Normally data collection is started with the start of the experiment. **Ending Time** sets the duration of the experiment. **Interval** is the frequency at which data is collected. **Time Constant** sets the response time of the

signal. This is done with a software simulation of a RC circuit, and time constant of the signal. For example, if a response time of 1 second is used, then a step in the optical signal will result in a recorded signal with an exponential decay that at one second has changed 63% of the total. This can be used to optimize the response and noise for the experiment. The response time should be ten times faster than the fastest kinetics that will be measured. This way the response time of the instrument will not interfere with the measurement of the signal, yet there will be sufficient reduction of the noise. The **Rotor Loop Time** sets how frequently the five-position cell holder will be cycled to measure all of the selected cells.

Temperature experiments allow the thermal stability of a sample to be measured. The experiment is performed by the thermoelectric cell holder driving the temperature to a set point. When the is at the

Figure I - 15: Temperature Experiment Configuration Panel



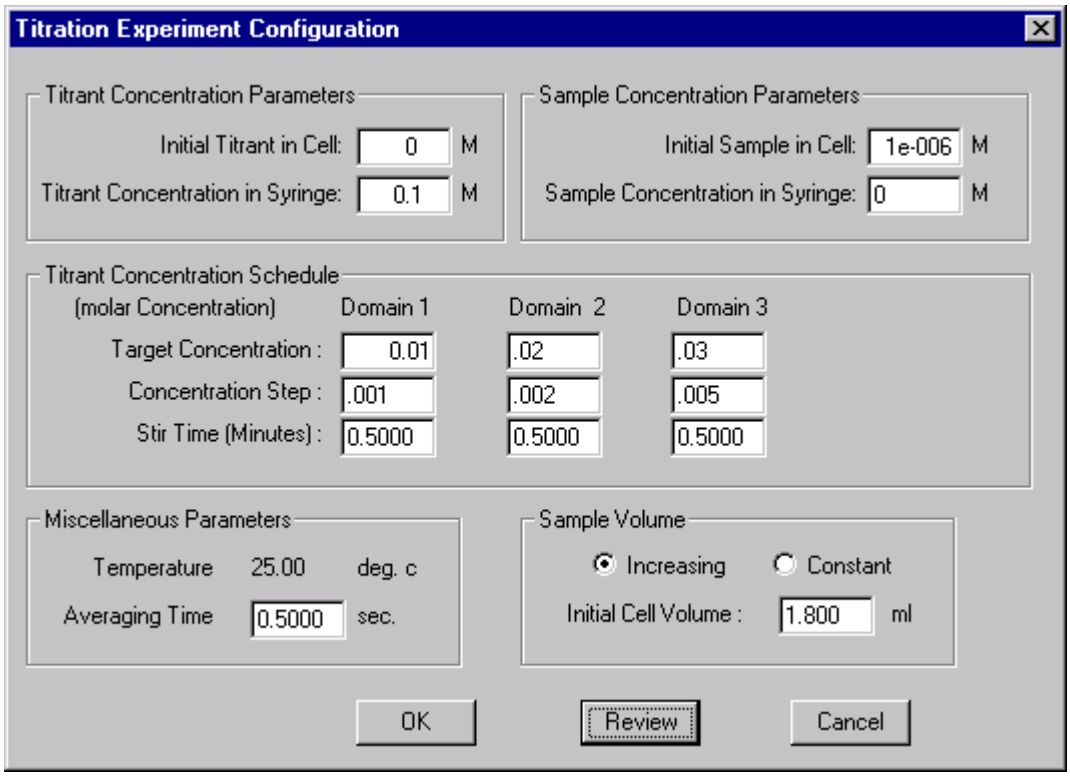
set point, plus or minus the Dead Band, the Equilibration Time starts counting down. If the temperature exceeds the Dead Band then the clock is stopped and restarted once the temperature re-enters the limits set by the Dead Band. When the Equilibration Time reaches zero then signal are collected for the Averaging Time. When this is complete the temperature set point is incremented to the next temperature and the cycle is repeated. Dead Band and Equilibration Time are set in the Control Panels: Temperature Control panel of the Main Control panel.

The **Starting Temperature** is the first temperature to be measured. Normally it is a low temperature (for a heating scan), but it can be set higher than the Ending Temperature for a cooling scan. **Ending Temperature** is the last temperature to be measured. **Temperature Step** is the size of the temperature increment between optical

measurements. **Averaging Time** is the time the optical signal will be averaged to create the data point. If the check box labeled **Return to 25 deg. C at end of experiment** is turned on then the temperature will automatically be set to 25 C when data collection is completed. Turning on the **Reverse Temperature Scan** check box will result in two temperature scans; the first with the Starting and Ending Temperatures defining the limits of the first scan, then the second scan with the Starting and Ending Temperatures exchanged. This type of experiment is good for testing the reversibility of the sample's thermal transition. **Reverse Hold Time** is the time held at the Ending Temperature before the reverse temperature scan is started.

Titration Experiments allow the user to control the concentration of a tattering in the cell. This is useful for measuring the effects of ligand binding, solute denaturation, dilution, or some other concentration dependent reaction. This experiment

Figure I - 16: Titration Experiment Configuration Panel



requires the syringe pump option. The software will keep track of two concentrations: the Titrant, whose concentration is generally ramped up; and the Sample, whose concentration may decrease due to dilution. The software asks the user for initial concentrations and volumes, as well as target concentrations, then the appropriate injection volumes are calculated. During the experiment the software keeps

track of the volumes in the syringes and will automatically refill or empty the syringes at the appropriate times. The order of events is measure the initial optical signals, make an injection, stir the sample, and make the next optical measurement. When this is done the next cycles of injection, stir, and measurement are performed until the experiment is completed.

Initial Titrant in the Cell is the concentration of Titrant (i.e., the ligand or denaturant) in the cell. Normally this is zero but it need not be so. **Titrant Concentration in Syringe** is the concentrated solution being injected. It represents the upper limit for the accessible concentration range. **Initial Sample in Cell** is the concentration of Sample (protein, macromolecule, or what ever) in the cuvette. **Sample Concentration in Syringe** is the concentration of the same material that is in the syringe. Frequently this is zero, so the software will calculate the dilution of the sample during the experiment. However, it is possible in some cases to put the same concentration of Sample in the syringe as in the cell. This will eliminate dilution effects of the Sample, making data easier to interpret. However, this can only be done safely if the Sample can withstand extended exposure to the Titrant and still exhibit reversible chemistry. For example, during solute denaturation experiments on proteins the volume of denaturant injected can exceed the volume of the original sample. This can result in an optical signal that changes more from the dilution of the sample than from any chemical transformations that may take place. If the Sample concentration is the same in the Syringe and Cell then there will be no dilution of the sample during the experiment.

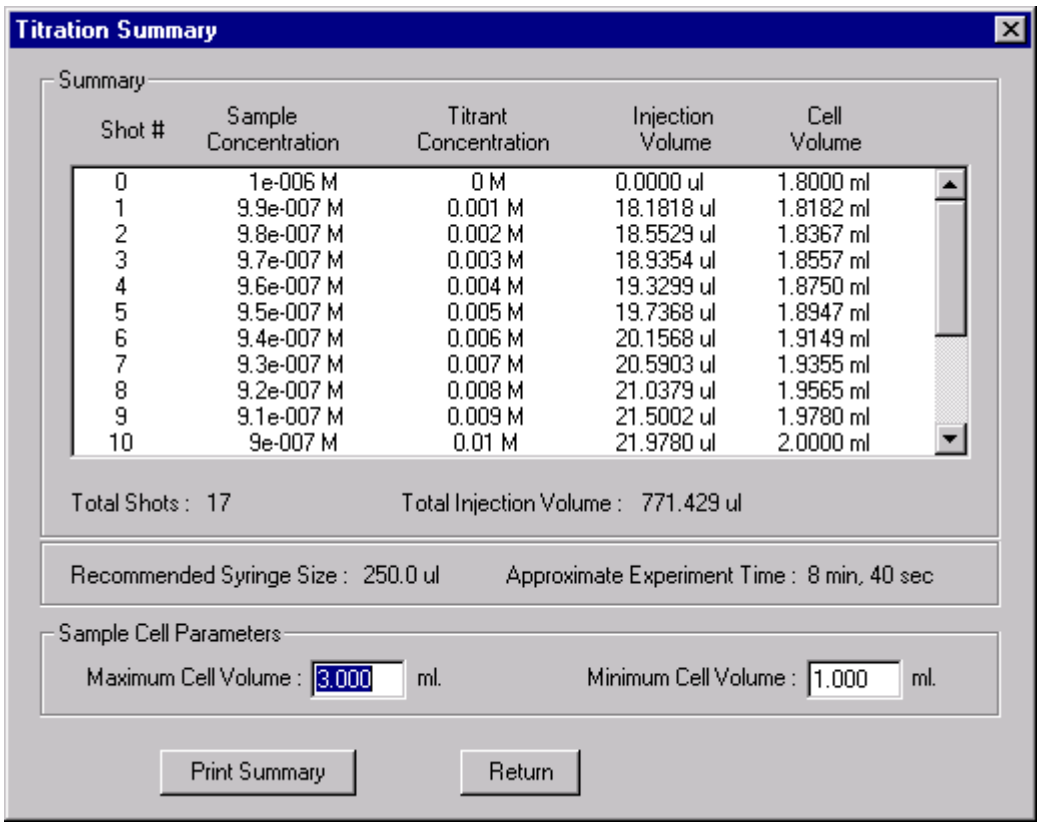
The intermediate concentrations of Titrant are determined by the **Titrant Concentration Schedule**. This is divided into three Domains, each with its own **Target Concentration**, **Concentration Step**, and **Stir Time**. The Initial Titrant in Cell is the starting concentration for the experiment. The first injection raises the Titrant concentration by the Concentration Step of Domain 1, then the software waits for the Stir Time of Domain 1 before making optical measurements. This repeats until the Target Concentration for Domain 1 is achieved. Then the parameters for Domain 2 are used. The experiment ends when the Target Concentration for Domain 3 is reached. The parameters for the three Domains can be customized for the sample's characteristics. For example, if a solute denaturation experiment is done that has a midpoint at four molar, then the initial baseline is of zero to two molar is moved through quickly. In the transition range of two to six molar the concentration steps are smaller to give better definition of the transition. The stirring time is increased to allow for the slower rate that the sample reaches chemical equilibrium. In the final baseline region of six to eight molar the concentration step is larger and the stirring time shorter to quickly end the experiment.

The temperature of the experiment is set in the Main Control panel, Control Panels: Temperature Control panel. The signal is measured for the **Averaging Time** and the standard deviation is calculated for each

individual data point. The **Sample Volume** can be allowed to increase (**Increasing** check box) or can be held **Constant**. If Increasing is selected then only injections will be made. If Constant is selected then before each injection the second syringe of the syringe pump will remove an aliquot, then the same volume of Titrant will be injected. This way the same volume is held during the whole experiment. The advantage of this approach is that the cuvette can never overflow, and the range of accessible Titrant concentrations greatly increases. Without this feature solute denaturation experiments must be broken up into several experiments covering different concentration ranges, then pasted together to obtain the entire concentration profile. When the **OK** or **Review** buttons are selected the software performs several checks of the validity of the experiment. Checks are made for overflowing the cuvette (Increasing volume mode), underflowing the cuvette (Constant volume mode), and negative injection volumes (concentrations wrong or out of sequence). OK accepts the experiment, closes the panel, and make Titration experiments the current experiment. **Return** closes the panel without accepting the changes.

If the Review button is hit the schedule of shots is shown with the calculated volumes and concentrations. Also shown are the Total

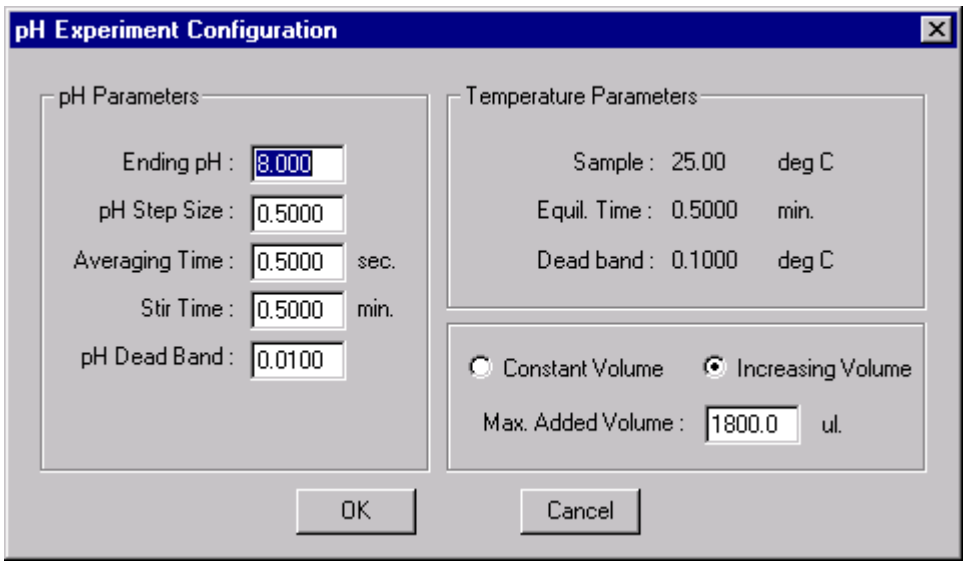
Figure I - 17: Concentration Summary Panel



Shots that will be made for the experiment, the Total Injection Volume that will be used, the Recommended Syringe Size, and the Approximate Experiment Time. The pump requires another 0.5 ml to fill the lines, so plan on making an excess of Titrant for the experiment. The Recommended Syringe Size is based on the smallest injection that will be made. The pump uses stepper motors to drive the plungers, which have 2000 steps for the full plunger distance. Therefore the limiting factor on the accuracy of delivery of an injection is how close is the requested volume is to an integer number of steps. Fractional steps are impossible, so anything less than 1/2 of a step is rounded up or down. To minimize this error the software wants to take a minimum of 100 steps per injection, so it recommends a syringe size that meets this requirement. If the larger injections are larger than the size of the syringe then the injection is broken up into several passes, so the syringe size does not limit the size of the injection. As describe before the software automatically performs overflow and underflow checks. To do this it must know the **Maximum Cell Volume** and the **Minimum Cell Volume**. **Print Summary** sends the volume and concentration information to the printer.

The pH Experiment Configuration panel allows the user to set up automated pH titration experiments. It requires both the syringe pump and pH meter accessories. This type of experiment differs from regular titration experiments in that the injection size is adjusted on the fly to give evenly spaced pH steps. The appropriate injection volume is determined by the buffering strength and volume of the sample, as well as the concentration of acid or base being injected. The pH electrode sits in the sample in the instrument, along with tubing from the pump. A feedback loop is created between the measured pH, the computer, the syringe

Figure I - 18: pH Experiment Configuration Panel



pump, and the sample. Instead of asking the user for the injection volumes the software has target pH values, and constantly adjusts the injection volumes of acid or base to achieve the target pH values. This makes experiment design much easier because the user does not have to be concerned about changes in the sample's buffering capacity, yet the experiment gives data at fairly constant pH increments.

The starting pH of the experiment is determined by the pH of the sample when it is placed in the instrument. The **Ending pH** is the pH at which the experiment will end. The **pH Step Size** sets the size of the pH steps at which optical measurements will be made. **Averaging Time** is the duration the signal will be measured to produce a data point and standard deviation. **Stir Time** is the time between when the final injection is made and the optical measurement is made. 0.5 minutes ensures a homogenous solution, but longer times can be entered to ensure complete chemical equilibrium of the sample. **pH Dead Band** is the allowable error in the pH measurement. If the software can not achieve the requested pH Dead Band, or of the pH has overshot the set point, then optimal measurements will be made despite the pH Dead Band. The Temperature Parameters are set in the Main Control panel, Control Panels: Temperature Control panel. The **Constant Volume** and **Increasing Volume** determine if the syringe pump should use two syringes (one to remove sample and the other to add acid or base), or only one syringe to make injections. The **Max. Added Volume** is the maximum volume of acid or base that can be added without overflowing the cell. If this value is reached then the software will stop the experiment.

I. C Reviewing and Managing Data

Data that has already been collected or loaded into the program's memory can be displayed by going to the Main Control panel and selecting the **Displays: Data Review** menu. This will show a list of experiment types (to select the X-axis). When a type is selected the Data Collection Display graph with the Dynode Voltage graph are replaced with the **Data Review Display**.

To select data for display use the **Axis Definitions** menu. The options for selecting data that were disabled in Data Collection are now available (and options specific to Data Collection are disabled). **Left Axis Definition** and **Right Axis Definition** menu options select single data sets for either axis. These open the **Data Browser** with the appropriate experiment type folder. To select a data set click on it to highlight it and then click on the **Select Data Set** button. If more than one data set is to be selected the **Left-Multi-Data Set** and **Right- Multi-Data Set** menu options are preferred. These give lists of all of the data that hasn't already been displayed. Several data sets can be selected at once for display. **Clear Left Axis Definitions** and **Clear Right Axis Definitions** menu options allow the user to select which data to remove from display. **Data Review Average** is described below. **Save Data Options** opens the Data Set Save Options panel, describe earlier. **Trace Color**

Figure I - 19: Data Review Display

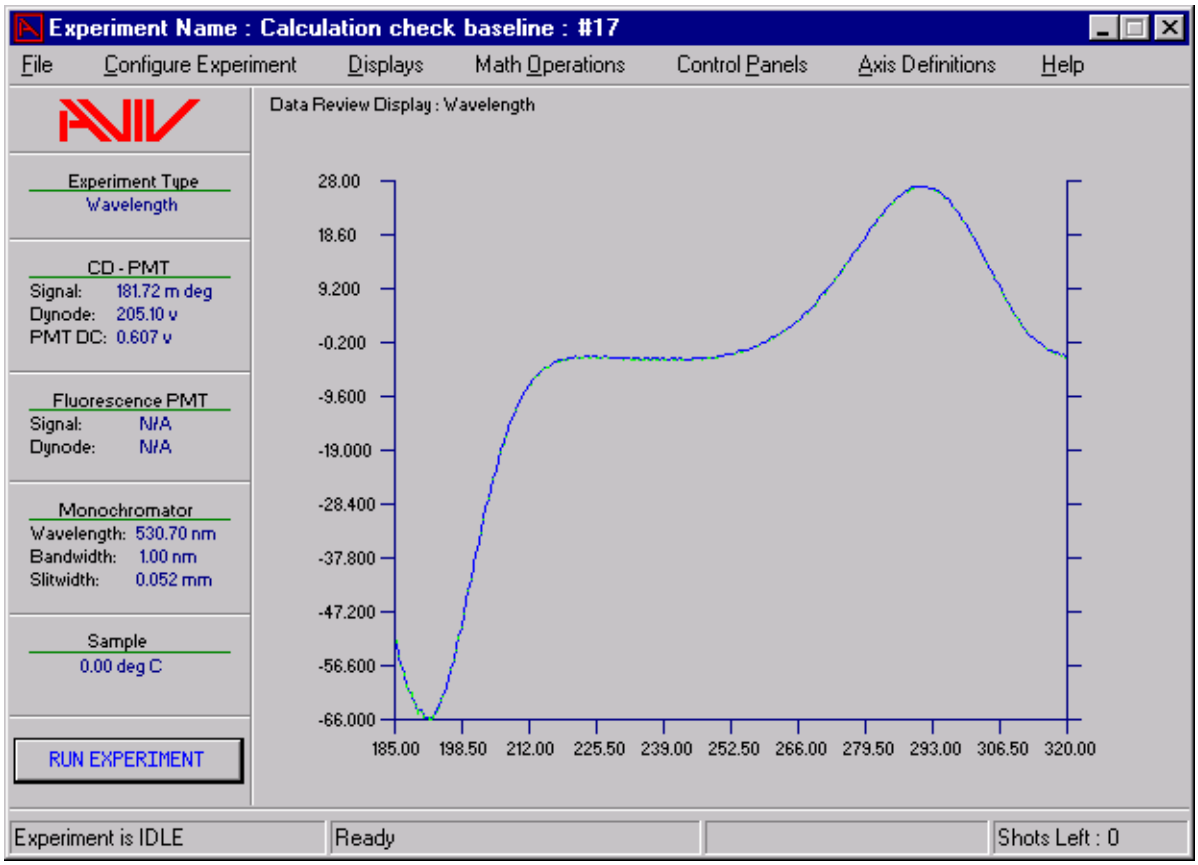
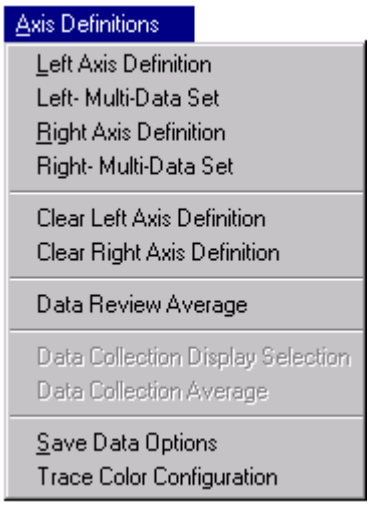


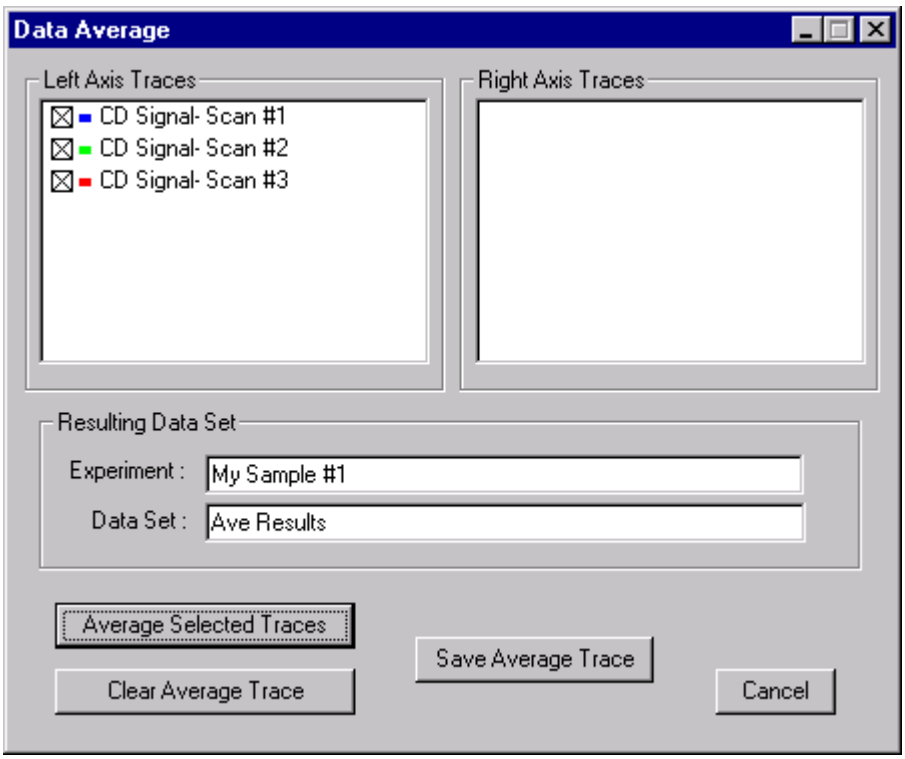
Figure I - 20: Axis Definitions Menu for Data Review



Configuration allows the user to define the colors of the displayed data.

The **Data Review Average** panel allows the user to select from the displayed data sets. Data from both the left and right axis can be averaged together. **Experiment** determines the experiment folder in which the new data set will be saved. In Data Collection Average the Experiment input will be the current experiment. In Data Review Average the Experiment input will not include the experiment counter (this must be supplied by the user). **Data Set** is the name of the new data set. When the panel is first opened the user should select the data to be averaged,

Figure I - 21: Data Review Average Panel

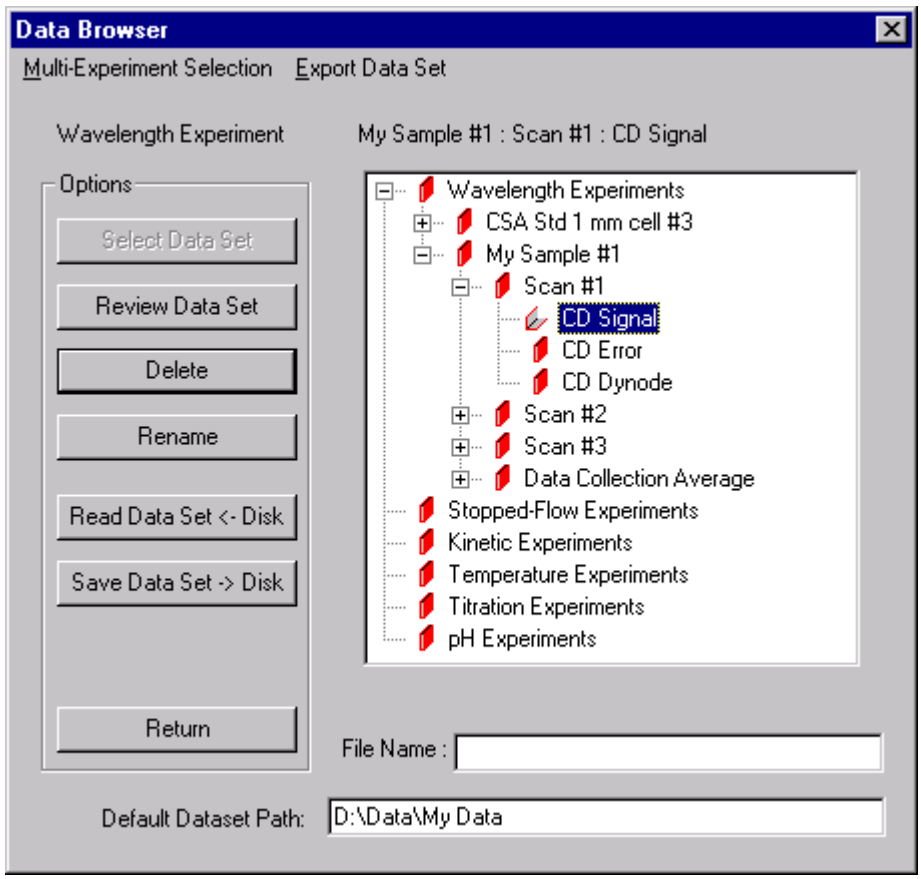


then push the **Average Selected Traces** button. This calculates the average, displays it in white, but does not save the data. Default inputs are placed into the Experiment and Data Set inputs. **Save Average Trace** saves the average to the Data Browser only (not to disk). **Clear Average Trace** removes the plotted average.

The data stored in the memory of the program is organized in the **Data Browser**. This is accessed through the Displays menu of the Main Control Panel. The Data Browser only shows data loaded into the program, not data on a hard drive or floppy. If the program is not exited properly then data shown in the Data Browser may be lost if it has not been written to a hard drive or floppy. Several opportunities are given to write data to disk, and safety checks have been placed on CDS.EXE to check for unsaved data, but no insurance can be made against power loss or inappropriate use of the software. Therefore the safest approach is to save the raw data immediately after it has been collected, and save the data again after calculations have been made. At the end of every experiment an opportunity is given to save the data to disk. Data can be saved to disk at any time with the Data Browser. Finally, when CDS is terminated it warns the user of any unsaved data and gives the opportunity to save.

The data is organized in the Data Browser by experiment type. The right half of the panel shows folders all of the experiment types. If an experiment type contains data then a + appears to the left of the type name. Clicking

Figure I - 22: Data Browser Panel



on this (or double clicking on the name) will open the folder. The data is further organized into sub-folders for each experiment. These can be opened in the same manner. In the cases of Stopped-Flow and Wavelength Experiments the experiment folders may contain another layer of sub-folders. These are for each Wavelength scan or Stopped-Flow shot. At the lowest level of sub-folders is the data. Data and sub-folders are selected by clicking on them to highlight the name. Along the left half of the Data Browser panel are buttons for various operations. The **Select Data Set** button becomes available when data is being selected for display in the Data Collection or Data Review panels. The contents of a data set can be viewed by selecting the data and clicking the **Data Review** button. Data and experiment sub-folders can be removed from memory by highlighting on an item and clicking on the **Delete** button. Data and experiment sub-folders can be renamed in the same fashion with the **Rename** button. Experiment files on disk are read into memory with the **Read Data Set <- Disk** button. **Save Data Set -> Disk** button writes the selected experiment folder to disk. The default name for the data file is the name of the experiment with .DAT appended. All data is saved in a text format, except for Stopped-Flow data which is binary due to it's large size. If other file formats are needed then use the Export Data Set button (explained below). If a file name different

from the experiment is desired the file name should be typed into **File Name** before selecting the Save button. Data is saved to the **Default Data Path**. If a different disk drive or directory is desired then the Default Data Path should be changed. If the new directory does not exist then the user will be given the opportunity to create a new directory on disk. The **Return** button closes the Data Browser panel.

The menu line of the Data Browser panel has several functions. If several experiments are to be manipulated in the same fashion then the **Multi-Experiment Selection** menu can be helpful. This allows the user to **Save**, **Export** to ASCII text, and **Delete** selected experiments of the same type. **Return** closes the Data Browser panel. The **Export Data Set** menu has options for saving to other data file formats. **Export to ASCII text** is the best if data must be exported to other programs. File name and data path selection is the same as described above. Entire experiments, individual shots or scans, or individual data sets can be saved to text files. If a single data set is selected then the file will contain several headers followed by X- and Y-axis columns of data. Text files use .TXT as the extension. **Export to Aviv Model 62** saves single experiments to the text format compatible with the Model 62 CD instrument.

Figure I - 23: Multi-Experiment Selection Menu

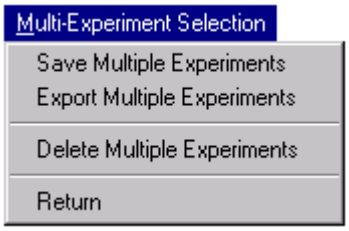


Figure I - 24: Export Data Menu

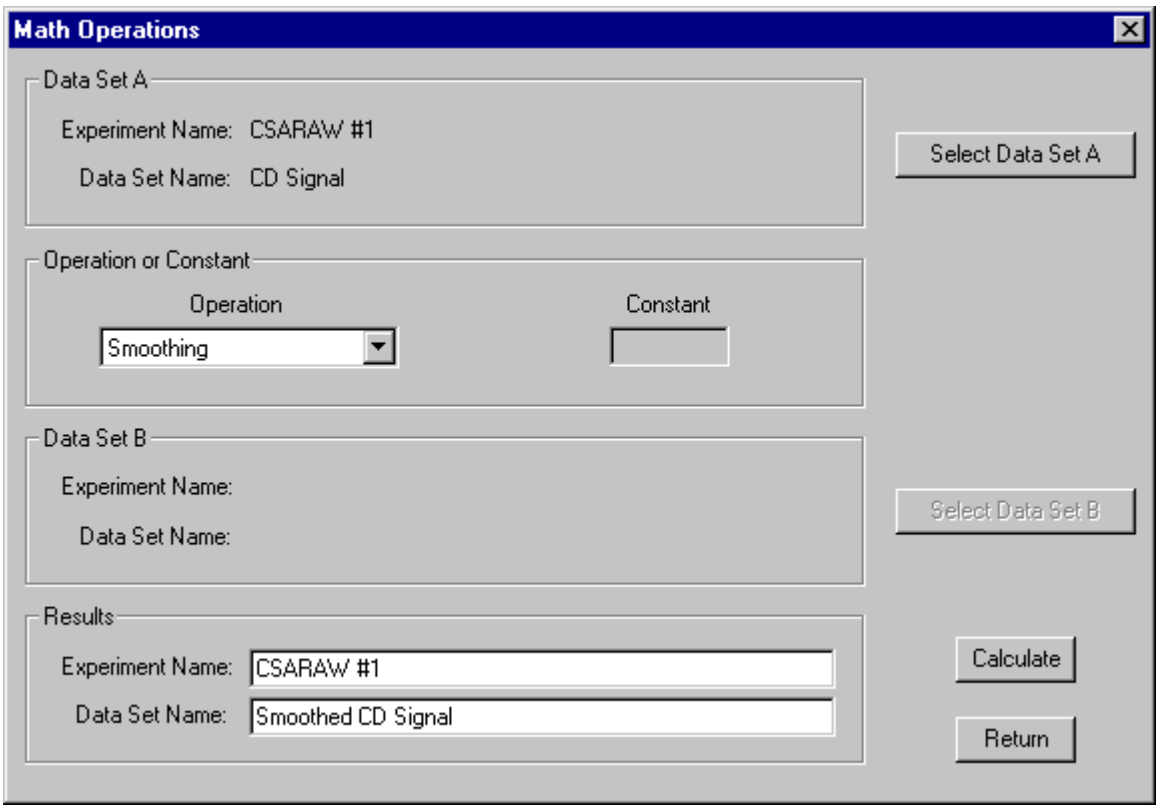


I. D Math Operations

Most of the math operations for data are available through the **Math Operations** menu of the Main Control panel. The exception is averaging multiple data sets, which is done through the Axis Definitions menu of the Data Collection and Data Review panels. The Math Operations menu gives a list of the experiment types that the user must select from.

To perform math operations the original data set must be selected. Select Data Set A opens the Data Browser, where the user click on a data set to highlight it, click on Select Data Set to make the selection. Next a math operation must be selected. All of the common math functions are available, both with a constant and between two data sets. If a constant is required it's value should be entered under Constant. If a second data set is needed then it is selected with the Select Data Set B button. The results of the math operation will automatically be placed in the same experiment folder that contains Data Set A. If a different location is desired then the input following Experiment Name should

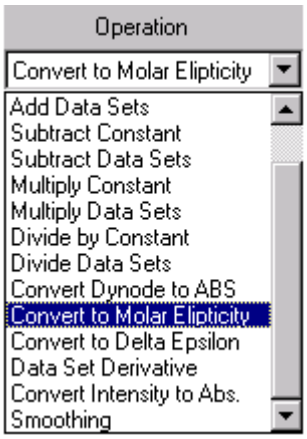
Figure I - 25: Data Set Operations Menu



be changed. The user must enter the name for the new data in Data Set Name. When these input have been made the Calculate button becomes available. Clicking on this performs the math operation and creates the new data set.

There are several specialized math operations that need more explanation. **Convert Dynode to ABS** estimates the sample's absorption based on the recorded dynode voltage. This works for all experiment types except for Stopped-Flow (see Convert Intensity to Abs. described below). The CD instrument can be used as a single beam spectrometer to record the light intensity. This requires two measurements; one of the sample and one of a reference. The reference

Figure I - 26: Math Operations Menu

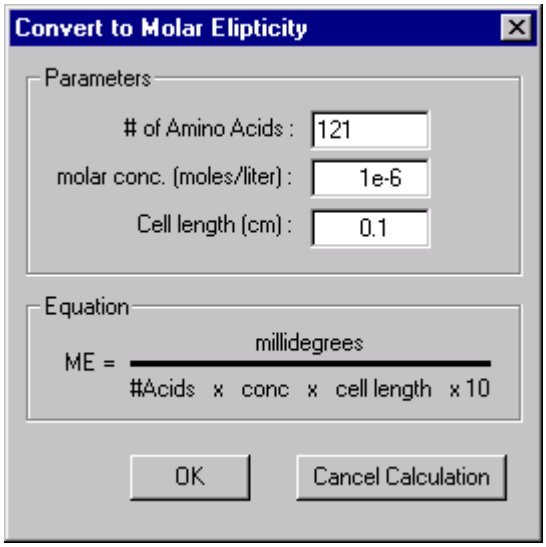


determines what absorbance will be calculated. If air is the reference then the absorbance value will include contributions from the sample, buffer, solvent and cuvette.

If the absorbance of only the sample is desired then a cuvette filled with buffer should be used as the reference. The sample should be selected as Data Set A and the reference as Data Set B.

The secondary structure analysis of peptide and protein CD spectra requires that the raw data recorded in millidegrees be normalized to molar ellipticity. Furthermore the

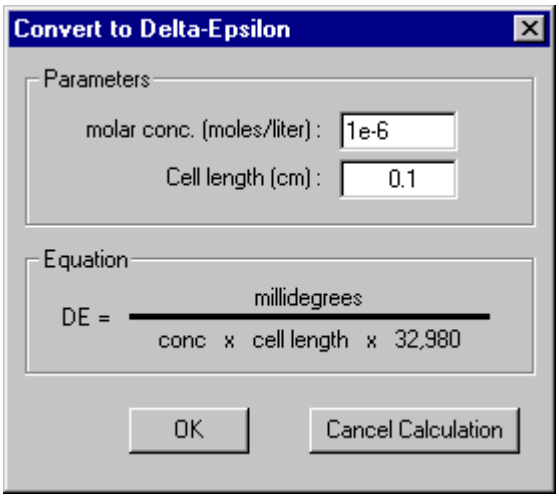
Figure I - 27: Convert to Molar Ellipticity Panel



normalization is based on the number of amino acids in the peptide or protein. This conversion allows for comparison of spectra between small peptides and large protein. To perform the calculation it is necessary to know the **number of amino acids** in the peptide or protein, the **molar concentration** of the peptide or protein, and the path length of the cuvette (**cell length**). The **Convert to Molar Ellipticity** math operation performs all of these functions.

Convert to Delta Epsilon calculated $\Delta\varepsilon$ (Delta Epsilon) based on the millidegrees signal. $\Delta\varepsilon$ is the difference in the extinction

Figure I - 28: Convert to Delta Epsilon Panel

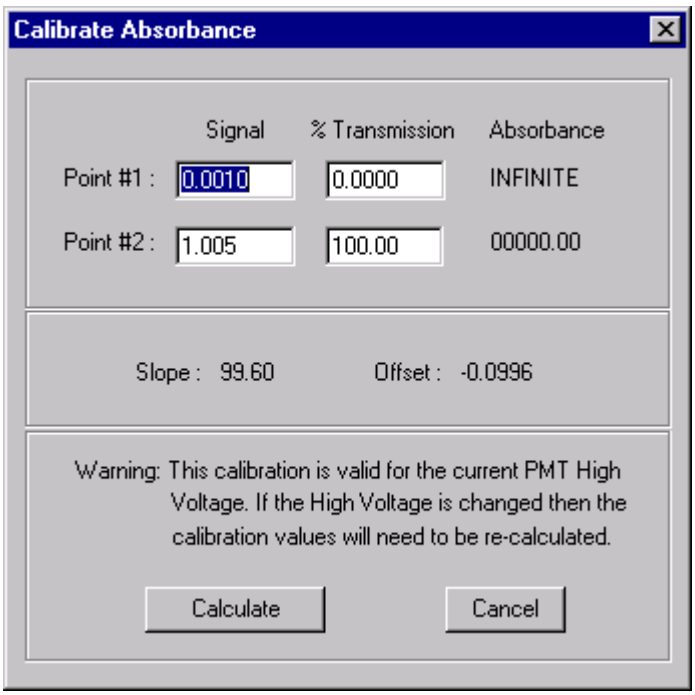


coefficients between the left and right circularly polarized light. This requires the **molar concentration** of the sample and the cuvette's optical path length (**Cell length**). It is used so that the CD signal can be directly compared to the absorbance signal.

Data Set Derivative takes the difference between adjacent data points to calculate the derivative.

The **Convert Intensity to Abs.** math operation converts the CD Current (sometimes called erroneously called the Absorbance signal) to absorbance. The DC current passing through the CD PMT is linearly proportionate to the transmission of the sample. If this signal is properly calibrated it can be converted to absorbance. To do this operation a two-point calibration is performed, where the **Signal** and percent

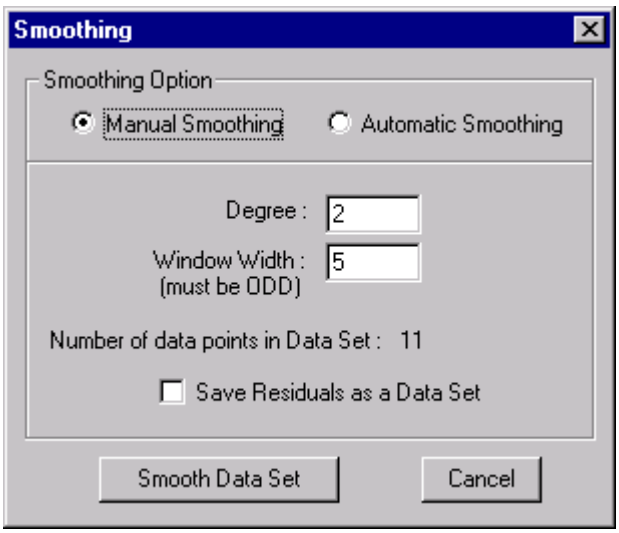
Figure I - 29: Calibrate Absorbance Panel



transmission (**% Transmission**) of two samples are entered. The most common points for this calibration are 0% and 100% transmission, however, any two signals with known absorbance values can be used. Ideally these points bracket the range of signals seen in the data. Based on these to points CDS.EXE will calculate the Absorbance values associated with the two points, give the Slope and Offset used to convert the Signal to % Transmission, then apply these values to first convert the data to % Transmission, then to Absorbance, which is saved using the data set name given by the user. For these calculations to be accurate the intensity of light from the source can not change once the signal levels for the two calibration points has been established. If the CD PMT high voltage level, the slit width, the wavelength, or any other factor affecting the intensity of the source is changed then the calibration will be wrong. For this reason CDS.EXE will automatically loose the calibration values when the CD PMT high voltage is changed.

Smoothing smooths the data using a sliding polynomial. The user may either type in the parameters for the **Manual Smoothing** or use **Automatic Smoothing**, which will find the optimal parameters based on the D_2/S_2 test. The D_2/S_2 test is a measure of the randomness of the residuals. Random residuals will have a D_2/S_2 value of 2. The **Degree** of polynomial and number of points in the window (**Window Width**) must be entered. The Window Width must be an odd number, so that there are an equal number of points in the window on either side of the data point being smoothed. **Save Residuals as a Data Set** takes the difference between the original data and the smoothed data, and

Figure I - 30: Smoothing Panel



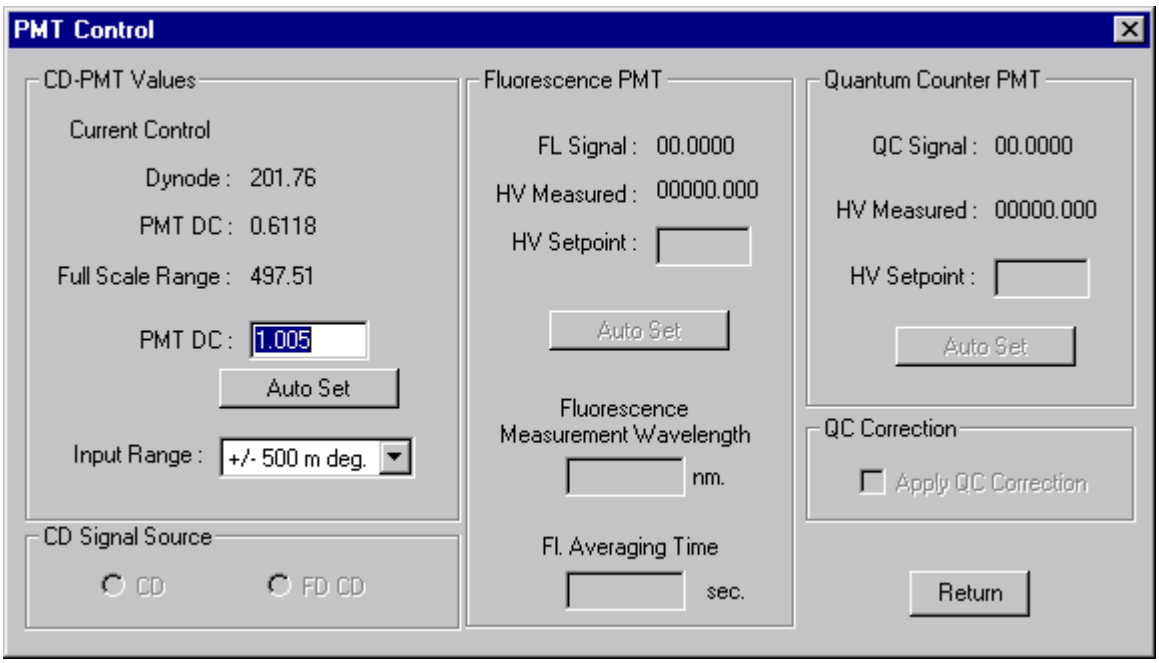
saves it with the name give to the smoothed data plus the word 'residuals'. Smoothing in general does not improve the quality of the data, as it does not remove noise and can introduce systematic errors. For these reasons all data presented by Aviv instruments is the raw data. Smoothing is left to the discretion of the user.

I. E Control Panels

I. E. 1 Hardware Control

The **PMT Control** panel is where the voltages, and hence the sensitivities of the photomultiplier tubes are control. There are two modes of control for PMTs. If the need is to measure light intensity then the high-voltage is fixed at a single point and the current passing through the PMT is treated as the signal. The current is linearly proportionate to the intensity of the light. This is the control mode used for fluorescence and quantum correction measurements. It is also used for stopped-flow CD measurements, where the rate of signal change is too rapid for adjusting the high voltage. All of the other CD experiment modes use the CD PMT is a constant current control mode. This mode of operation allows the CD instrument to operate over a larger range of light intensities. By holding the high voltage constant and measuring the PMT current the range of measurement covers roughly 3 magnitudes of light intensity. If the current is held constant by adjusting the high voltage then the range increase to 5 magnitudes. This is because the high voltage responds in an crude logarithmic fashion to the light intensity. This is not a problem for CD measurements because the CD calculation uses the ratio of the AC ('Delta Absorbance') to DC ('Absorbance') signals from the CD PMT. Both signals contain the same nonlinear gain of the PMT, so this factor drops out. This is not true for other types of measurements like fluorescence and quantum correction that.

Figure I - 31: PMT Control Panel



Another consideration of PMT signals is the effect of fluctuations in the intensity of the source of light. The CD signal is self referencing due to the above mentioned ratio. However the CD instrument is a single-beam spectrometer, so absorbance and fluorescence signals will be a convolution of the intensity of the light source, the sample's absorption and the quantum yield. To reduce instrument artifacts in these measurements can be corrected using the Quantum Counter PMT. This is a PMT located between the monochromator and sample. A beam splitter sends a small fraction of the light to the QC PMT, which measures the intensity of the source. This signal can then be used to correct the fluorescence and absorbance measurements.

The PMT Control panel is divided into three sections, one section for each of the photomultiplier. Under **CD-PMT Values** is the control mode in presently in use. **Current Control** means that the PMT current is held constant and the high voltage is changed. This is the mode used for all experiment modes except stopped-flow (described below). As light intensity goes down the PMT's high voltage is raised to increased it's sensitivity. The working range for this PMT is 170 to greater than 500. Values less than 170 result in nonlinear behavior of the PMT due to saturation. Values greater than 500 first result in a noisy signal, then the signal dropping to zero as stray light begins to dominate. **Dynode** and **PMT DC** are the real-time read back of those values. **Full Scale Range** is the range of CD signals that can be measured. This is determined by the PMT's current, where a current of 1 results in a full scale of ±500 millidegrees. Larger currents reduce the full scale range, and smaller currents increase the range. **PMT DC** is the set point for the

PMT's current. A value of 1 corresponds to 50 microamperes passing through the PMT. **Auto Set** has not effect in the Current Control mode. **Input Range** selects the full scale range that is to be used by the A/D converters. This need not be changed from +/- 500 millidegrees because the increase in sensitivity is not matched by improved electronic noise, which is the limiting factor.

High Voltage Control mode means that the CD PMT's high voltage is held constant. This mode is used only for Stopped-Flow experiments. This experiment mode has rapidly changing light levels that the high voltage servo can not keep up with, so the high voltage must be held constant. The PMT high voltage is set with the sample must be in the flow cell so that the current is the same as the PMT DC set point used for wavelength experiments. This requirement exists because this is the PMT DC level for which the instrument was calibrated. If the Stopped-Flow PMT DC is slightly different (less than $\pm$0.1) from the value used for wavelength experiments then the error is trivial, but larger differences should be accepted. The sample of the correct concentration should be in the cell when the PMT DC is set so that the changes in absorbance will not significantly affect the light level.

A description of how the CD signal is measured is appropriate at this point. The light first passes through a polarizer to create linearly polarized light. This light next passes through a modulator that converts the light linearly polarized into circularly polarized. The light passes through the sample where left and right circularly polarized light is selectively absorbed. The CD PMT then measures the intensity of light. Electronics separate the signal into AC and DC components. The DC component is the PMT DC, which is proportionate to DC current passing though the PMT. The amplitude of the AC component is proportionate to the CD signal. The computer measures both of the AC and DC signals and takes their ratio. The ratio removes changes in these signals due to changes in the light intensity. In other words, the CD signal is self referencing.

The CD instrument can be used as a single beam spectrometer to measure light absorbance. The method depends on whether the experiment mode is stopped-flow or one of the other experiment modes. In Stopped-Flow mode changes in the PMT DC are proportionate to changes in the Percent Transmission of the sample. This can be calibrated and converted to absorbance. In other experiment modes the high voltage changes with proportionately to the sample's absorbance. Two measurements of high voltage must be made, one of the sample and one of the reference. These two measurements can then be used to calculate the absorbance. The appropriate math functions are available through the Math Operations panel. The QC PMT can be used to improve the quality of absorbance measurements. See below for a description of this accessory.

Under the heading of **CD Signal Source** are buttons for **CD** and **FDCD**. Normally the instrument measures the CD signal. If the

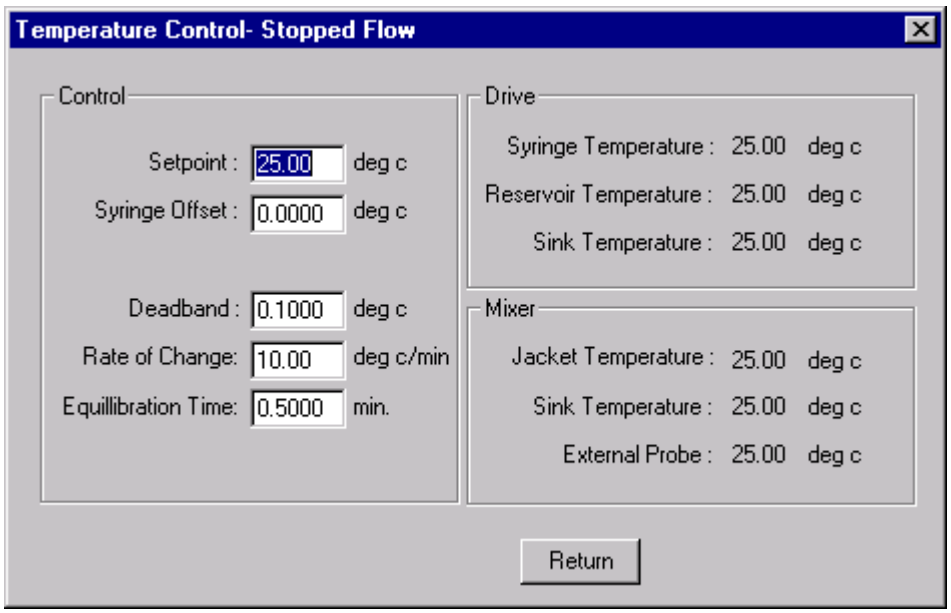
accessory is available then the CD signal can be measured through the fluorescence photomultiplier tube (Fluorescence Detected CD).

The **Fluorescence PMT** section of the PMT Control panel contains the controls for fluorescence measurements. These controls are only available if the software detects this accessory at startup. **FL Signal** and **HV Measured** are the current values for these parameters. The **HV Setpoint** allows the user to manually control the high voltage applied to the fluorescence PMT, which in turn controls the sensitivity of the device. Larger high voltages increase the sensitivity, but may saturate the PMT if the light level is too high. The working range is 170 to 1200 volts. The **Auto Set** button automatically raises the high voltage to achieve a signal level of 1. **Fluorescence Measurement Wavelength** is the wavelength that will be used for fluorescence measurements if both CD and fluorescence measurements are made during an experiment. The instrument will automatically toggle back and forth between the CD and fluorescence wavelengths to make these measurements over the course of the experiment. **Fl Averaging Time** is the time the fluorescence signal will be averaged to generate a data point.

The **Quantum Count PMT** is another accessory that is used to measure the light intensity output of the instrument. This signal can be used to correct absorbance and fluorescence signals. The CD instrument is fundamentally a single beam instrument, with no reference beam. The CD signal is self referencing, so it does not need this accessory (see above for explanation). The absorbance and fluorescence signals will change as a function of the light output of the instrument. To remove this unwanted influence these signals can be ratioed with the QC signal. **QC Signal**, **HV Measured**, **HV Setpoint**, and **Auto Set** all operate the same way as described above for the Fluorescence PMT. If the **QC Correction** button is turned off then the saved fluorescence and absorbance signals will be the raw signals. If this option is turned on then these signals will automatically be ratioed with the QC Signal and the corrected signals will be saved.

The **Temperature Control** panel control the temperature of the thermoelectric cell holder. There are several versions of this accessory, but they all fundamentally work the same. They use Peltier elements that act as heat pumps. When an electrical current is passed through a Peltier element one surface becomes hot and the other cold. The temperature of the cell holder is controlled by adjusting the current through the Peltier elements. Temperature sensors in the cell holder jacket measure the temperature, and the control box then uses this information to calculate the appropriate amount of heat to add or remove to the cell holder. The controller has built into it a dedicated microprocessor to do the decision making, and to communicate with the computer. When the controller is first turned on it initializes it self. As part of the initialization it determines the type of thermoelectric cell holder that it is controlling. This information is passed to the computer, that then selects the appropriate user interfaces and calibration constants. The user does not have to make

Figure I - 32: Temperature Control Panel



any software changes when cell holders are changed.

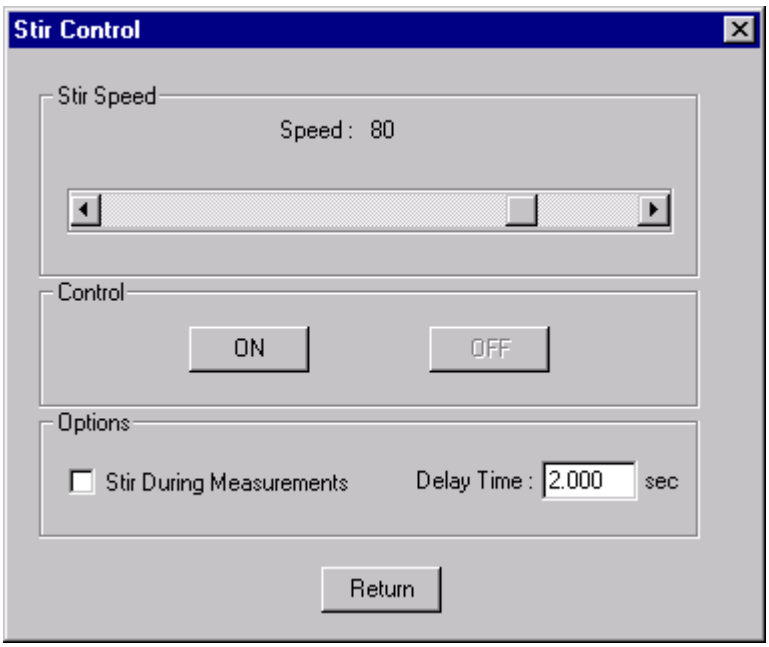
The left side of the Temperature Control panel contains the **Control** settings. **Setpoint** is the temperature set point. When this value is changed the temperature controller automatically drives the system to the new set point. If the cell holder is a Stopped-Flow accessory then a second control loop is used for the syringes and reservoirs. Normally this loop is held at the same temperature as the Mixer, however **Syringe Offset** can be used to set a different temperature. **Deadband** is the allowable error in the temperature before measurements are made. If the difference between the set point and the device's temperature is greater than Deadband then measurements will be delayed. **Rate of Change** is the rate at which a thermoelectric will approach the Setpoint. This is useful for controlling the amount of temperature overshoot when adjusting the temperature. The **Equilibration Time** is the time between when the measured temperature first arrives within the deadband, and when the optical measurements are started. This time allows thermal gradients to dissipate and the whole system reach the same temperature.

On the right side of the Temperature Control Panel are the measured temperatures. This display is different for the different types of thermoelectric cell holders. In all cases the top temperature is the temperature used for the feedback loop (usually **Jacket Temperature**). The **Sink Temperature** is the temperature of the water sink used to remove waste heat from the system. This temperature is normally a degree or two above the temperature of the incoming cooling water. During changes in the thermoelectric cell holder's temperature the Sink temperature may deviate, but will return once the cell holder has equilibrated. If there

is insufficient cooling (such as the water is not flowing) then the sink temperature will rise. If the Sink Temperature reaches approximately 38 C then power to the Peltier elements is turned off, and temperature control will be lost. When the sink temperature cools the power will automatically come back on. An **External Temperature** probe is available as an option. This probe sits in the sample compartment, and can be located where ever desired.

Most Aviv thermoelectric cell holders come equipped with a motor attached to a magnet.

Figure I - 33: Stir Control Panel

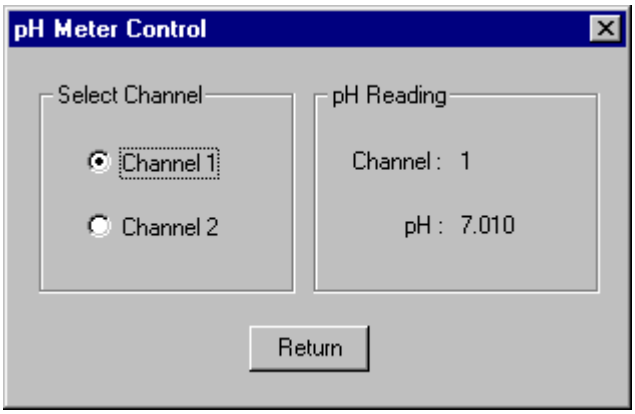


This is used to drive magnetic stir bars placed in samples. The speed of the motor is controlled by the temperature controller, which receives it's instructions from the computer. The Stir Control panel is the user interface to turn the stirring **ON** and **OFF**, as well as set the stirring **Speed**. The Speed units are arbitrary. During Titration experiments the maximum Speed is recommended. Under **Options** is **Stir During Measurements**. If this is turned on then the stirrer will continue to stir during optical measurements. Of this is turned off then stirring is stopped prior to optical measurements, then resumed when measurements are finished. When the stirring is stopped the software waits the **Delay Time** before starting optical measurements. The advantage of not stirring during measurements is that if there are bubbles or dust in the sample then not stirring give a chance for these to move out of the light beam, thus improving the measurement.

If the instrument is equipped with the pH Meter Accessory then this device can be communicated with through the **pH Meter Control** panel. The meter has two channels

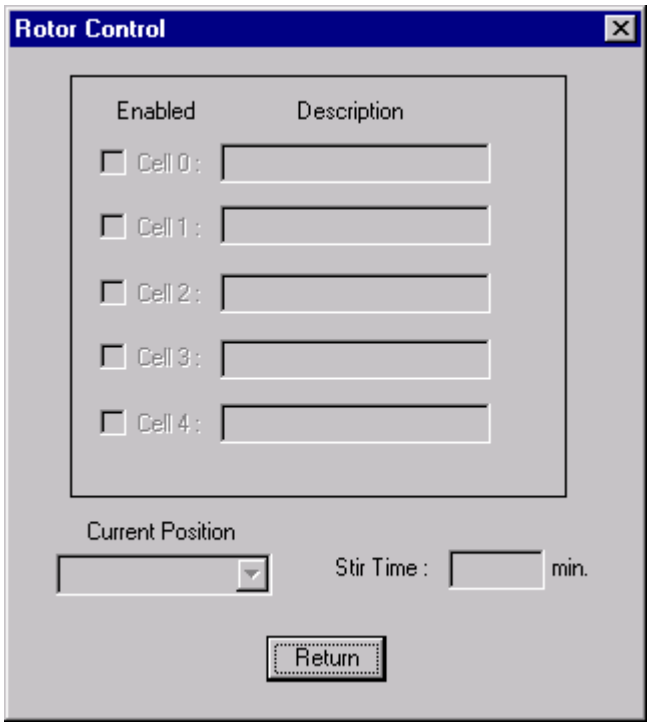
who's readings can be selected. Channel 1 is for experiments.

Figure I - 34: pH Control Panel



The **Rotor Control** panel controls the actions of the five position cell holder. To **Enable** measurements on select positions turn on the check boxes for the selected cells. Each

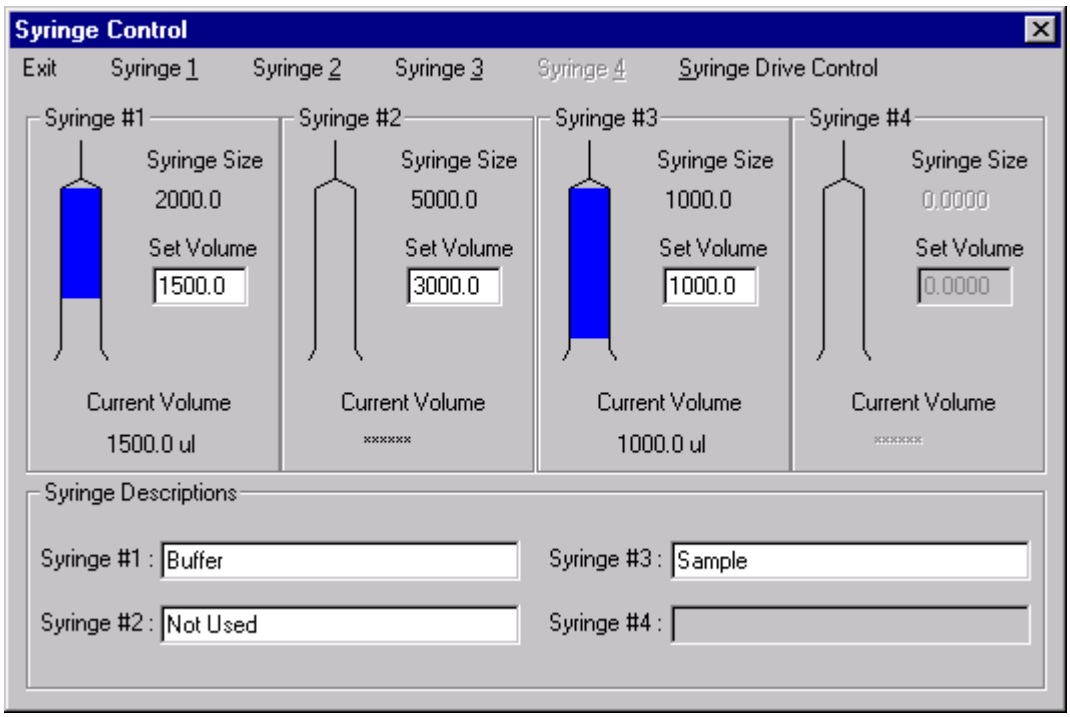
Figure I - 35: Rotor Control Panel



position can have its own **Description** that will be saved with the Data file. The cell selected for the **Current Position** is the one in the light path and being measured. Beneath this position is a magnet attached to a motor, used for driving magnetic stir bars. A **Stir Time** can be set to delay measurements after the sample has moved into the measuring position, to allow the sample to be stirring before optical measurements are made.

The Syringe Control line of the Control Panels menu pops out into options for the Stopped-Flow Syringe Control and Titrator Syringe Control. The **Syringe Control** panel

Figure I - 36: Stopped-Flow Syringe Control Panel



for the Stopped-Flow is divided into four sections, one for each syringe. Each section graphically shows the syringes volume, with the **Current Volume** below. The Current Volume is based on the actual read-back from the motor's optical encodes, so small variations may occur. The **Syringe Size** is the maximum volume for the syringe. **Set Volume** is the absolute volume that the plunger will move to when commanded. Below are the **Syringe Descriptions**, where the user can type in the contents of each syringe. This information is displayed in the Stopped-Flow experiment configuration panel and is saved to the data file.

In the Stopped-Flow Syringe Control panel the **Exit** menu item closes the panel. Menu items **Syringe 1** through **Syringe 4** contain identical menus, except they apply to different syringes. If the syringe size has been recently changed, then **Change Syringe**

Figure I - 37: Stopped-Flow Syringe Menu

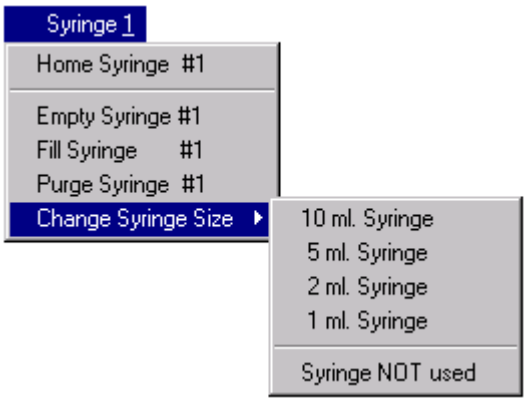
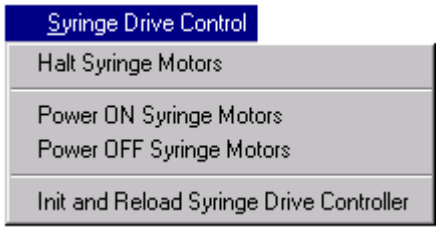


Figure I - 38: Syringe Drive Menu



Size must be updated. Before any motions are possible with the syringe must be homed. **Home Syringe** moves the plunger through the range of motions so the that hardware and software knows the limits. After this the other motion commands become operable. **Empty Syringe** moves the plunger to the zero volume position. **Fill Syringe** moves the plunger to the absolute volume that is entered in Set Volume. Therefore Fill Syringe can be used to partially empty the syringe. **Purge Syringe** is used for removing bubbles from the syringe. It will move the plunger to approximately 25% of the syringe then quickly empty. The command should be repeated until no bubbles are seen in tubing or reservoir. The Syringe Drive Control menu contains items not routinely used. **Halt Syringe Motors** will stop the motor movement immediately. However, a faster way to stop the motors is to use the Emergency Stop button on the front of the stopped-flow syringe power supply. **Power ON Syringe Motors** and **Power OFF Syringe Motors** is used to turn the power on and off to the motors. Normally this is done automatically. If it is necessary to work on the syringes then the motor power must be off so the plungers can be moved up and down by hand. As a safety precaution it is advised that the power switch to the stopped-flow motor power supply be turned off while the syringes are being worked on. When work is completed the switch power can be turned back, then the Power ON Syringe Motors command given to send power to the motors. It is recommended to Home the motors again after servicing them. **Init and Reload Syringe Drive Controller** will clear the program on the motor controller card and load the program fresh into it's memory. This should only be used if the motion control or position has become confused, and a fresh start is necessary. This will clear the syringe positions, so it will be necessary to repeat the Home function for each syringe.

Chapter II: Maintenance

II. A Introduction

Your Aviv 215 Circular Dichroism Spectrometer has been designed to be generally trouble free. However, certain simple tests should be periodically performed to insure that the instrument is operating properly. Wavelength calibration is tested using Holmium oxide. Energy measurements and noise tests can help identify lamp aging and other instrument problems. These tests should be performed at least once every 300 hours of instrument use.

This section assumes the user is familiar with the CD software. You should consult the software chapter if you have any questions

II. B Changing and Adjusting the Xenon Lamp

The lamp is located in the rear right side of the instrument. **Warnings**: SAFETY GLASSES & GLOVES must be worn. Please follow all of the manufacturer's safety warnings on the replacement lamp. Make sure the power has been off for at least 20 minutes. The lamp should always be cooled prior to handling.

Figure II - 1: Top Lamp Cover

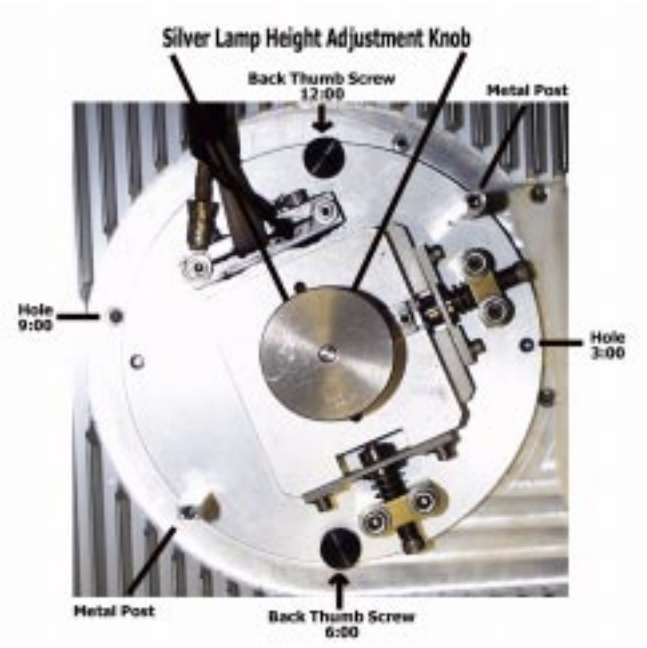


1) **Turn the instrument power off**.

2) Remove the silver nuts on the top of the white round lamp cover and lift it upwards and off.

3) Completely unscrew the back two thumb screws. They are located at the 12 o'clock and 6 o'clock positions.

Figure II - 2: Lamp Mechanism



4) Take the same two black thumb screws and simultaneously screw them into the two holes located at the 9 o'clock and 3 o'clock positions. Doing so will break the o ring seal and slightly raise the mechanism.

Figure II - 3: Raised Lamp



5) With the lamp mechanism now partially raised; grab the two metal posts. Slowly and gently pull the lamp holder up and out of the instrument.

6) Use captive screws to secure lamp holder in the upright position. Now remove the black thumb screws from the 9 o'clock and 3 o'clock holes.

7) Remove the two thumb nuts from the top and bottom of the lamp. Place the original plastic protective case around the lamp. Remove the lamp from the holder.

Figure II - 4: Protective Case



8 Place the new lamp (with protective cover) in holder. Adjust the two top back screws until the gold plate is in contact with the new lamp. Lamps vary in length so you will need to adjust the the top two back screws every time you insert a new lamp. If these adjustments are not made, the lamp may fail.

9 Remove plastic cover off lamp. Place and secure lamp mechanism back in instrument (reverse steps).

Adjusting the lamp

A) Ignite lamp and leave on for at least seven hours.

B) Start up the instrument and software.

C) The Main Control panel will appear. Monochromator settings will be displayed towards the bottom left of the screen. Double click on wavelength, enter 200 and hit return.

Figure II - 5: Top Back Screws



D) Single click on Control Panels (upper right), single click on Service Panel. The "Service" Panel will appear. Single click on Big Display (lower left).

E) The "Big" display will now appear as a large window that displays the dynode voltage and signal. This enables the user to monitor the Dynode Voltage (CD Dynode) while adjusting the xenon lamp position.

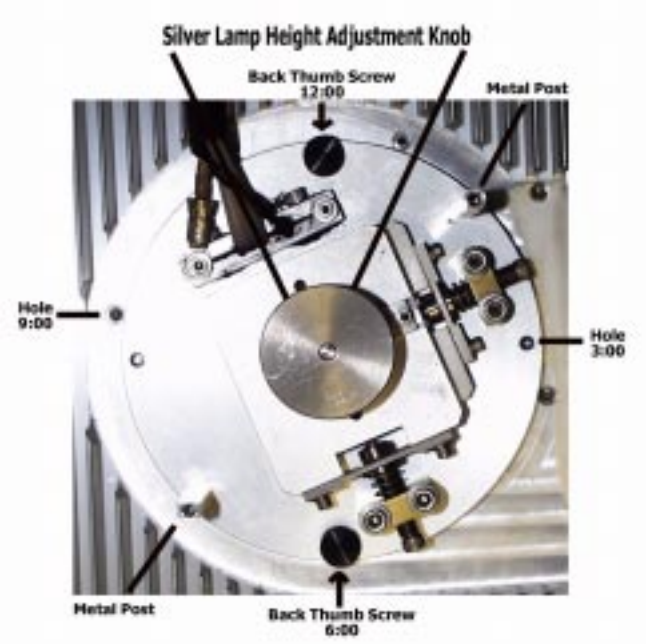
F) Turn the monitor towards the lamp house. Select a comfortable position by the lamp house to make adjustments. Make sure you can see the dynode voltage on the monitor.

G) The height position of the arc is the only difference between lamps.

H) The only adjustment needed is the silver lamp height adjustment located on the top of the lamphouse.

I) Slowly turn this large knob to obtain the lowest dynode voltage possible. The voltage should be below 250 volts.

Figure II - 6: Lamp Mechanism



Appendix I: Titration Accessory

I . A Introduction

The Aviv Automated Titration System is designed to automatically perform ligand and solute denaturation experiments. It is also required for the automated pH titration accessory. These types of experiments can be done manually, however the process can be laborious. For each measurement the user must at least open the sample compartment, remove the sample, make an injection, replace the sample and make the measurement. Besides being time consuming this process can introduce errors in the measurements due to varying cuvette positions in the sample holder, user error in the injection volume, possible fingerprints on the outside of the cuvette, dust that falls into the cuvette, et cetera. The Aviv automated titration system will add a precise volume to the cuvette without the need to remove it. Ligand titration and solute-induced denaturation experiments are discussed separately because of a number of differences in the methods and the intentions behind the two types of experiments. pH titrations are discussed in a separate section devoted to it. This part of the manual is principally devoted to the hardware. Detailed explanations of the software are explained in greater detail in the Software section.

I. A.1 Ligand Titrations

Ligand titrations are intended to add to the sample cuvette some substance while observing signal changes brought on by binding. The titrant added frequently is a small molecule (ligand) that binds to a larger molecule (macromolecule) already in the cuvette, however this arrangement can vary. Normally an optical signal sensitive to the physical states of the macromolecule is measured, and changes in this signal indicate when the ligand binds to the macromolecule. To obtain thermodynamic data, such as binding constants, the concentrations are kept low so that both free and bound species are present during the titration. This means that the ligand concentration in the cuvette is on the same order as the macromolecule, and the volume of ligand added is small in comparison to the total sample volume. The added volume will dilute the macromolecule, but corrections for this can be applied in the subsequent data analysis.

I. A.2 Solute Induced Denaturation Experiments

In the case of solute induced denaturation experiments the solution being added does not contain a ligand, but rather some denaturing solute that destabilizes the macromolecule's structure. Common denaturants are urea or guanidine hydrochloride (GuHCl). The concentration of denaturant required in the sample can be quite high, on the order of several molar. The maximum solubility of the denaturants is 10 M for urea and 8 M for GuHCl, so to achieve the appropriate concentration in the cuvette a large volume of denaturant must be added. This additional volume will seriously dilute

the macromolecule and possibly overflow the cuvette. To correct for the first problem a common technique is that of “the method of Job”. To avoid diluting the sample the same concentration of macromolecule in the denaturant is matched to the macromolecule concentration in the cuvette. **This approach requires that the conformation change be reversible**, because macromolecules in the denaturant must be capable of refolding in the new solvent condition. The problem of overflowing the cuvette is handled by the Aviv automated titration system. Normally the left syringe of the Hamilton pump is filled with ligand or concentrated denaturant. For ligand titrations only the left syringe is used to add titrant. However, for solute induced denaturation experiments the right syringe is filled with buffer and used to remove sample. The selection for these options are the “Increasing” and “Constant” buttons under “Syringe Volume” in the Titration Experiment configuration panel. The software automatically calculates the appropriate injection volume, and before every injection removes that volume from the cuvette using the right syringe. The concentrated denaturant is then added to bring the sample back to its original volume. The entire titration is performed in this manner, so the volume is held constant while the denaturant concentration is raised.

I . B Helpful Hints for Titration Experiments

Any time an injection is made into the cuvette there must be some stirring action to keep the sample homogenous. The Aviv thermoelectric cell holder will provide the necessary stirring, as well as tightly regulate the temperature. The stirring speed (set through the Stir Control panel) must be set to give adequate mixing, but not to create artifacts. The small “flea” stir bars are OK for ligand titrations, but solute denaturation experiments require more strenuous mixing. In these cases it can be difficult to push the dense, viscous denaturant up from the bottom of the cell. If the mixing is not adequate then the denaturant will collect at the bottom of the cell and an upper layer of nearly undisturbed sample will develop. In this case the experiment will be worthless because the concentration of denaturant in the light beam is unknown. For solute denaturation experiments we recommend that the bulky, cross shaped stir bars be used (figure VI-1).

Figure 1 - 1: Recommended Stir Bar



These generate a stronger mixing action and the large size helps by displacing sample. Care must be taken so that bubbles are not in the light path during optical measurements. This can be avoided by setting the speed low enough to avoid bubbles, or by turning off the option to “Stir During Measurement” in the Stir Control panel. A “Delay Time” must be set for several seconds so that after the stirring is stopped there is sufficient time for the bubbles to leave before measurements are made. With this approach the stirring can be set to the maximum without risk of interfering with measurements. The sample

volume can influence the mixing. Obviously the meniscus must sit above the light beam but too large sample volume is more difficult to mix. In our experience 1.8 ml of sample is best for the cross stir bar in the one by one centimeter cells.

The Hamilton pumps used by the Aviv automated titration system can deliver a wide range of volumes, but there are limits. The motion of the plungers is controlled by stepper motors, which move in discrete steps. For any given syringe size there are 2000 steps available, so the smallest increment that can be delivered is 1/2000 of the total syringe size. For example, with a 500 ul syringe the resolution is 0.25 ul. Smaller volumes are rounded up or down. To ensure that the round-off error does not adversely affect the injections we suggest that the smallest injection size be kept to greater than 1/20 of the syringe size (at least one hundred steps per injection). If smaller injection volumes are required then a smaller syringe size is recommended by the software. These can be purchased from Hamilton Syringe company. The software is designed to make the entire injection with a single stroke. If there is not enough solution in the syringe then the syringe will automatically be refilled. If the injection volume is larger than the syringe size then the injection will be made in two or more passes.

Diffusion of material from the tip of tubing is another concern. The injection tubing has been drawn out to make the opening smaller, but liquid in the end of the tube is still exposed to the sample. In the case of dense, viscous solutions (such as 8 M guanidine hydrochloride or 10 M urea) the tubing tip has been turned upward to prevent titrant from dribbling out. In this case the injection volumes are large and diffusion is insignificant. In the case of ligand titrations the injection volume may be very small (on the order of a microliter). In this case diffusion could prematurely release solutes into the sample. This problem can be reduced by making the titrant less concentrated, and making larger injections. Injections of 10 µl or larger work well.

The tubing that fits in the cuvette obviously must be cleaned between experiments. Frequently the tubing is wiped with a tissue to dry it. Many tissues are not lint free (including “lint free” KimWipes) and this can lead to problems in the measurements. Any lint present on the tubing will come off in the sample and pass through the light beam. The lint will scatter light and probably will deteriorate the measurements in other ways too. The best way to dry tubing is with lens paper (even though it absorbs liquids poorly) or to blow it dry with a stream of nitrogen gas.

I . C Installing Pump Hardware

Installing the titration accessory is fairly easy. The syringe pump should be placed on top of the instrument, next to the sample compartment.

1) Follow the Hamilton syringe pump manual for connecting the valve, syringes, and power cord. Consult chapter 2, pages 9-15.

2) The pump and computer should be turned off. It is good to confirm that the dials and switch on the back of the pump have not been moved during shipment. The correct positions are SWITCH in the UP position, BAUD is 3, and UNIT # is 0. The serial communication cable should be plugged into the "RS-232 IN" port of the pump and an available serial port of the computer (usually found on the serial extention box, plug # 5).

3) The software should be configured to communicate with the syringe pump. Click on Control Panel, Instrument Configuration, I / O Setup. Under Com Ports select the appropriate comport (usually com 7). In a typical configuration com 1&2 are found on the back of the computer. Com 3 and above are the ports on the serial expansion box.

4) The Hamilton tubing (serial number 240010 or 240000) should be attached to the left and right side ports of the valve head. The tubing for the center ports of the valve head have been modified to fit into the Aviv instrument. They already are attached a the titrator cuvette cap. The tubing with the curled tip should be attached to the left-center port, and the tubing with the straight tip goes in the right-center.

The Teflon tubing can behave like fiber optics and route stray light directly into the cuvette. To prevent this problem a light trap has been provided. The following instructions are for mounting the light trap to the instrument:

5) On the top edge of the CD sample compartment are two notches, with plastic plugs filling them. Remove the right plug.

6) The light trap is supplied with all necessary the parts connected in the correct positions. Remove the two round headed screws on the light trap (note that the longer screw has the wire loop attached to it) and detach the notched plastic plug.

7) Slide the notched plastic plug into the right notch of the sample compartment top, and insert the two round headed screws, with the wire loop positioned over the top of the notch.

8) Reassemble the light trap inside the sample compartment, beneath the notched plastic plug. Tighten the two round headed screws.

9) The tubing can be positioned now or left till later. To place the tubing in the light trap first remove the back thumb screw that holds the cover on the trap. Loosen, but do not remove the front thumb screw. Starting at about the middle of the tubing, place the tubing into the notches of the plastic plug and light trap. Once the tubing is around the 180 degree turn let the lid hang down. The edge of the lid will hold the tubing in place while the tubing is positioned around the last turn. Swing the lid up, make a last check that the tubing is in the right places, and tighten down the thumb screws. The wire loop is included to hold the tubing in place, and to provide strain relief. The tubing can be slipped into the center of the loop by pushing it into the groove between the loops, starting

at the bottom, and working to the top. It may be necessary to remove the tubing for cleaning. Simply reverse the process to remove the tubing.

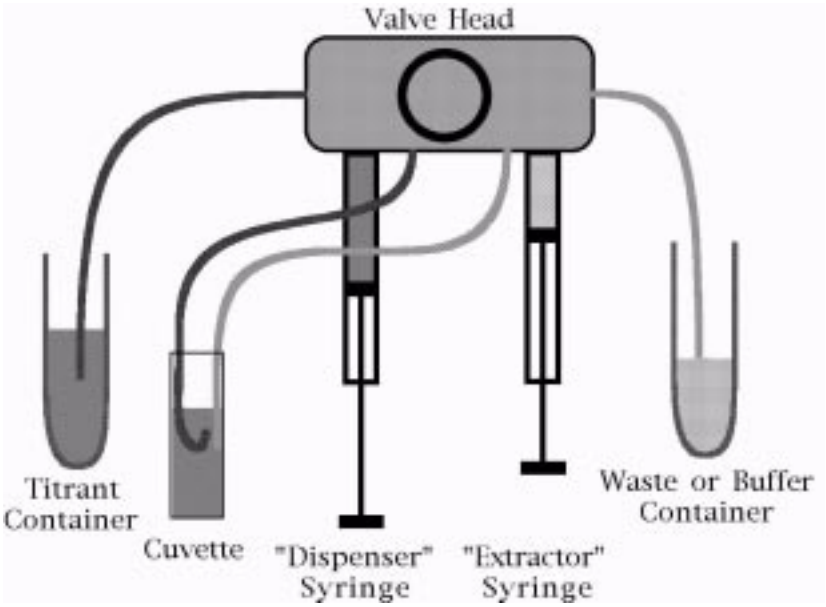
I . D Setting up a Titration Experiment

I. D.1 Loading the Pump and Cuvette

Gas solubilities decrease with increasing temperatures. If a solution is taken out of the refrigerator and immediately loaded into the syringe pump then bubbles will almost certainly appear later. The best approach is to degas solutions while they are allowed to warm up. Solutions can be degassed by placing them in a bell jar connected to a water aspirator for 15 minutes.

Titrant (ligand, denaturant, acid or base) is loaded into the left syringe, and is injected into the cuvette through the curled tubing. If two syringes are used then the right syringe is loaded with buffer or water. The contents of the right syringe are not injected into the sample, but are needed so that the plunger has something hard to work with. Air is compressible and it's volume can not be accurately controlled. It is suggested that the right syringe be filled first, so that if a mistake is made during loading then valuable titrant will be less likely to be involved. The syringes are filled and purged using the **Step/ Prime** button on the front of the Hamilton syringe pump. The Step/Prime button will purge the pump by moving solutions in from the two side ports, through the syringes, out the two center ports and through the cell cap

Figure 1 - 2: Syringe Pump



The cell cap can be positioned above the titrant and waste containers to recycle solutions. It is necessary to cycle the syringes several times to remove all air bubbles.

During experiments the flow of titrant continues in through the left side port to the left syringe and out the left center port to the syringe. If the right syringe is used then it's flow is reversed and sample moves from the cuvette, through the right syringe and out the right side port to the waste container.

Once the pump has been filled place the tubing into the light trap (if not already there) and the cell cap into the sample compartment. Put the stir bar in the cuvette and add sample. Put the cuvette cap on the cell. The hook is best positioned on the back wall of the cuvette. Make sure that the tubing is located in the corners of the cell. The goal is to position the tubing well into the sample but so that the light cleanly passes through the middle.

I. B.1 Cleanup

At the end of the experiment the cell should be removed and examined for complete mixing of the sample. The cell and stir bar can be cleaned as usual. The syringe pump can be cleaned by first pumping out the solutions, then rinsing with water, then pumping through a laboratory detergent (not SDS). The detergent should be rinsed out with large amounts of water. The syringes can run continuously by setting the Prime switch. When the lines are clean the syringes should be removed from the pump, and washed out separately. The system can then let to dry in the air. If there is a small amount of water left in the lines it can be blown out with nitrogen gas, or flushed out with sample and buffer at the beginning of the next experiment.

I . C Conclusions

Obviously this system is not trivial to learn. There are a number of details, and they are best learned by practice. Therefore we suggest that the users first perform experiments with water and a marker (CSA is good for CD measurements, L-tryptophan is good for fluorescence) only. Later, practice with inexpensive reagents with known transitions. After the user is familiar with the whole operation then the real experiments can be started.

Appendix I: Stopped-Flow

I . A Introduction

The stopped-flow accessory is designed for rapid mixing of reagents to measure kinetics on the millisecond and longer time scales. The principle components of the stopped-flow accessory are the Syringe Drive Tower, the thermostated Cell Holder, the Mixer / Optical Cell assembly, the Stopped-Flow Power Supply, and the Thermoelectric Power Supply. The Syringe Drive Tower is designed to deliver aliquots from multiple, independent syringes to the mixer in milliseconds. From the mixer a homogeneous solution flows to the optical cell, where measurements are made. These components are integrated through the software so the whole package behaves as a single instrument.

I. A. 1 Parts List

The stopped-flow accessory includes the following parts:

Syringe Drive Tower (1), including:
- 1 ml Syringe (1)
- 2mlSyringe(1normally,2ifdrive tower supports 4 syringes)
- 5 ml Syringe (1)
- ReservoirCaps(3normally,4ifdrive tower supports 4 syringes)
- Light Trap (1)

Thermoelectric Power Supply (1) (used by all temperature controlled cell holders), including:
- TemperatureControllerPower Cable (1)
- Serial Communication Cable (1)
- Power Cord (1)

Stopped-Flow Power Supply (1), including:
- Controller Card (1)
- Motor Power Cable (1)
- Motor Signal Cable (1)
- Ribbon Cable (1)
- Power Cord (1)

Cell Holder (1), including:
- Cell Adaptor for 1 cm cells (1)
- Alignment Target (1)
- Assembly Cover (1)

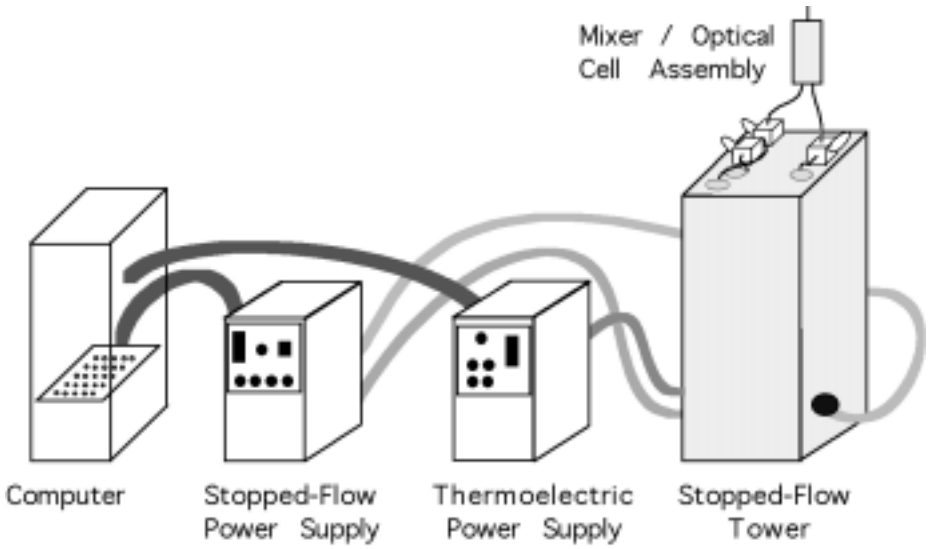
Mixer / Optical Cell Assembly (1), including:
- "T" Mixer (1)
- "Delta" Mixer (1)
- "Fersht" Mixer (1)
- Tubing (4)

"T" Connector (1)
25 ml Waste Syringe (1)
Miniature Torque Wrench (1)
Miniature Wrench (1)
Plastic Disposable Pipettes

I. A. 2 Overview

The principle components of the Stopped-Flow accessory are the Motor Controller card in the Computer that is connected to the Stopped-Flow Power Supply through the Ribbon Cable. The power supply has two cables that transmit power to the motors and read back the positions. The Thermoelectric Power Supply communicates with the computer through an RS-232 line. A cable sends power to the Peltier elements in the

Figure 1 - 1: Stopped-Flow Layout



Cell Holder Assembly and the Syringe Drive Tower. Temperatures measurements in these locations are sent back to the controller which then adjusts power to achieve the user specified temperatures. Inside the Syringe Drive Tower each syringe is connected to a separate motor allowing independent control of the fluid flow rates. At the top of the tower are valves connected to each syringe as well as reservoirs and the mixer. The syringes are filled and purged with the valves set to the reservoirs. When an experiment is run the valves are switched to the mixer. The fluid stream from the mixer passes through the optical cell where measurements are made and out into a waste syringe.

Data collection typically begins when the motors begin moving. The optical signals are processed by the instrument's electronics and sent to the computer where and Analog to Digital (A/D) board converts the voltages levels to numbers. This is done every 10 microseconds, so the signals are greatly over sampled. These numbers are then averaged by the software to produce the data points requested by the user.

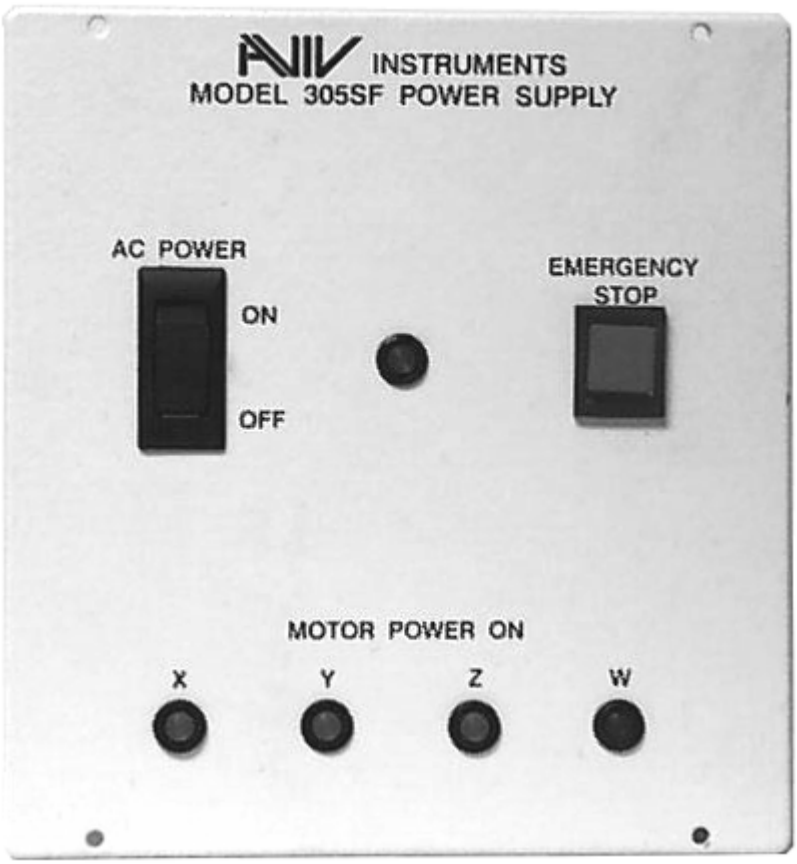
Routine use of the stopped-flow is through the same user interface software that controls the rest of the instrument. The software is explained in a separate chapter. Utility software for calibration and diagnostics is explained in the Cleaning and Maintenance section, below.

I . B Hardware Components

I. B. 1 Stopped-Flow Power Supply

The stopped-flow power supply receives signals from the controller card and relays that information to the motors. Encoders attached to the motors report back the positions to the power supply, which passes that information to the controller card. On the front panel the **AC Power** turns controls

Figure 1 - 2: Stopped-Flow Power Supply Front Panel



power to the box. Next to that is a power indicator light that comes on when there is AC power. This is feed to a DC power supply that separately switched on and off under control of the software. The DC power can also be shut off with the **Emergency Stop** button on the front panel and a safety circuitry built into the power supply. If the power supply detects that too much power is being delivered to the motors then the DC power is automatically turned off. When DC power is being delivered to the motors then the **Motor Power On** lights will come on.

I. B. 2 Stopped-Flow Drive Tower

Several items attach to the outside of the tower. It is attached to the instrument through a hydraulic jack, used to raise and lower the drive in and out of the sample compartment. The handle for the jack is located beneath the power switches. The handle can be pulled to swing it in and out of position. The tower has three plugs on it's sides: one for motor power, one for position feedback and one plug for temperature control. The top of the tower contains the reservoirs for loading reagents and valves to direct the flow of reagents. Syringes sit beneath the valves.

Inside of the syringe tower are DC servo motors, each connected to a single syringe. Power for the motors is supplied by the Stopped-Flow Power Supply. Position control is achieved through optical encoders on the motor shaft. Signals from the encoders are sent to the motor controller card, which adjusts power to the motors to maintain the correct position and speed during movement. With this setup a feedback loop is created to ensure accurate performance. The optical encoders produce 4,000 steps per revolution, and approximately 80,000 counts for the full distance of plunger travel. Therefore control on the microliter scale is possible. The positions are measured and corrected every 0.5 milliseconds enabling motor control on the millisecond time scale.

Each motor has it's own feedback loop and programmed motion profile. This arrangement gives independent control over each syringe so that any combination of flow ratios or total flow is possible within the limits of the motor's performance. A motor can cover slightly over one magnitude of speed range, so variations in fluid rates and hence dilution ratios can be achieved. Larger variations can be achieved by changing the syringe sizes. The available sizes are one milliliter, two milliliter and five milliliter. Changing the syringes is described below, in the maintenance section.

The syringes are housed in a thermostated block. The block contains Peltier elements with water cooled heat sinks to remove waste heat. There are three temperature probes: one next to the syringes (used for feedback control), one next to the reservoirs and one attached to a heat sink. The Thermoelectric Power Supply recognizes which thermoelectric device is attached at startup and uses the appropriate tuning constants. Power is delivered to the Peltiers to drive the syringes and Cell Holder to the temperature entered in the user software.

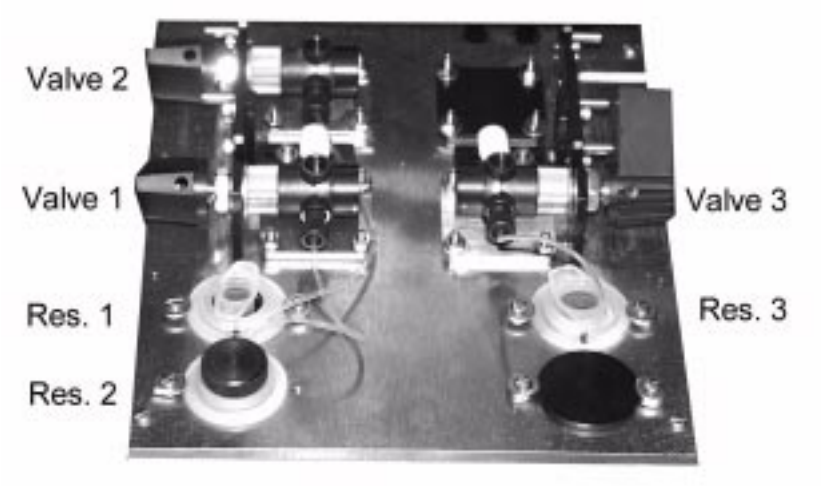
On the top of the syringe drive are the reservoirs and valves. The reservoirs can hold up to 11 milliliters. Samples and buffers can be loaded into the reservoirs directly or two and five milliliter disposable slip-luer syringes can be slid into the reservoirs so that a seal is made in the base. Using disposable syringes makes cleaning easier and reduces the possibility of cross-contamination. A luer-lock syringe can be made to fit by cutting off the locking sleeve, leaving only the nipple. Caps are supplied to cover the reservoirs when they are not used.

The valves direct the flow of liquids. The syringes sit directly beneath the valves and are always connected. The valves switch between the reservoirs (handles in the back positions, flow through the front ports) and the mixer (handles in the front positions, flow through the top ports. When filling and purging the valves should be switched to the reservoir (back) positions. When the mixer and optical cell assembly are being flushed or experimental shots are made the handles should be switched to the mixer (front) positions.

I. B. 3 Mixer and Optical Cell

The flows from the valves are sent to the mixer and optical cell assembly. It is here that

Figure 1 - 3: Stopped-Flow Valves and Reservoirs



the solutions are rapidly mixed and sent through the optical cell. A selection of mixers are available: "T", "Fersht" and "Delta". The selection is available so that the mixing and dead time can optimized to the sample. The dead time is the time between when the fluid flow has stopped and good quality kinetics data can be measured. The "theoretical dead time" is the volume of sample between the mixing and observation points, divided by the flow rate. Obviously the smaller the volume the better (shorter) the dead time. The shortest dead time is supplied by the "T" mixer, but this also does the least mixing. Highly concentrated samples may not be completely mixed resulting is bad kinetic data. The "Fersht" mixer (named after Dr. Alan Fersht) does an improved job of mixing, but still does not handle the highest concentrations. The "Delta" mixer does the best job of mixing (it will handle saturated guanidine and urea solutions) but has the largest volume and hence the longest dead time. Below is a table showing the performance that can be expected.

Figure 1 - 4: Stopped-Flow Mixer Capabilities

Component	Volume (µl)	Total Volume (µl)	Dead Time (ms)*	Maximum [Urea] (M)
"T" Mixer	1	14	4.6	1
"Fersht" Mixer	3	16	5.3	5
"Delta" Mixer	16	29	9.7	Saturated
Optical Cell	13	-	-	-
* Theoretical Dead Times at 3 ml/sec. total flow rate.				

If the theoretical dead time were the only influence then the shortest dead time would be achieved by running at the highest flow rate. But the actual measured dead time is the time the solution travels between the mixing and observation points, starting when the motors stop to when the signal is stable enough for measurements. Factors that affect the actual dead time are the deceleration of the motors, depressurizing of the system

(which keeps the flow moving after the stop), sample properties that affect the mixing (viscosity and density), and the electronic response times (controlled through the software). Deceleration of the motors and depressurizing take longer with faster flow rates, so very high flow rates actually have longer dead times. The compromise between these affects and the theoretical dead time results in an optimal flow rate of 2.5 - 3.0 ml/ sec.

After the mixer the solution flows through the optical cell, through a check valve and out into the waste syringe. The optical path length of cell is 1 mm, the width is 2.2 mm. The beam height is ~3 mm so the volume optically sampled is ~6 µl. The check valve the isolates the sample from old fluids. The cap screw that sits on the check valve has a standard 1/4 inch diameter, 28 threads to the inch that can accept other chromatography fittings if desired. A diagram that shows the various parts is located below, in the section on Cleaning and Maintenance.

I . C Installing the Stopped-Flow in the Model 202 CD Spectrometer

The stopped-flow accessory can be moved into and out of the sample compartment for rapid kinetic measurements or steady-state types of experiments. To install the stopped-flow:

I. C. 1 Remove the existing cell holder

1) **Quit instrument control software** if it is running.

2) **Turn off the Thermoelectric Power Supply.**

3) **Unplug the water lines** to the front panel of the cell holder by pushing the steel buttons and pulling off. They are self sealing and will only release a few drips of water.

5) **Unplug the nitrogen line** from the front panel in the same fashion as the water lines.

6) **Disconnect the power cables** to the front panel of the cell holder. These have a locking ring near the base that must be unscrewed before the cable can be pulled off.

7) **Unscrew the four screws** on the corners of the front panel.

8) **Pull the front panel and cell holder away** from the instrument. It rests on rollers in the sample compartment. Be prepared to support the unit when it comes off the last set of rollers.

9) **Remove the bottom plate** from the sample compartment. Unscrew the six thumb screws and save them with the cover.

I. C. 2 Raise the Stopped-Flow Drive

1) **Remove the two bolts** anchoring the stopped-flow drive to the instrument's frame. These are located on the side walls of the drive near the bottom. The right-angle brackets may need to be loosed so that the drive does not bind between them.

2) **Position the tubing and cables** on the top of the stopped-flow drive away from the edges. As the drive is raised these can become pinched between the drive and edges of the sample compartment.

3) **Turn the hydraulic crank** clock-wise to raise the stopped-flow drive. Raise the drive so that the top is roughly 3 cm above

the bottom of the sample compartment.

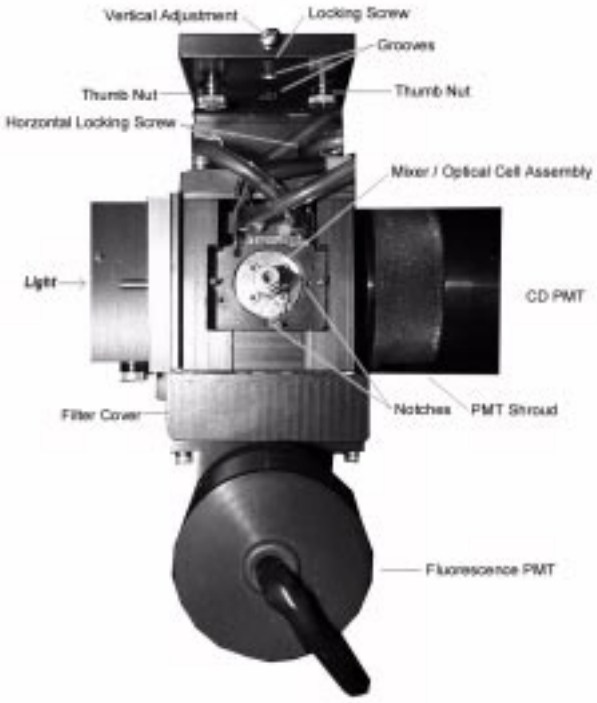
4) **Connect the power cable** between the top of the Stopped-Flow Tower and the Cell Holder.

5) **Replace the power cable** between the Thermoelectric Power Supply and Stopped-Flow Drive. Remove the cable used for the original cell holder and install the cable specific for the stopped-flow.

6) **Connect the cables** between the Stopped-Flow Power Supply and the Syringe Drive Tower.

7) **Slide over the Syringe Drive Tower the Light Trap**. The Light Trap rests on the bottom of the sample compartment and fits around the Tower to prevent light from entering the sample compartment. It rests on the bottom of the sample compartment. Tubing and cables should not pass beneath it.

Figure 1 - 5: Top View of Stopped-Flow Cell Holder



I. C. 3 Mount the Cell Holder to the Sample Compartment

1) **Remove from the back plate of the sample compartment two Thumb Nuts**. These are on two screws protruding from the center of the back plate.

2) **Slide the Cell Holder into place**. Position the Cell Holder in front of the Sample Compartment then slide it back into place. It may be necessary to fold the nitrogen tubing (clear tubing) out of the way. If the space is not wide enough to accept the cell holder then screw the PMT Shroud on the right into it's mount. The notches on the back wall of the cell holder go over the screws and two pins in the sample compartment's back wall. The screw passing through the top of the cell holder's back mounting plate should rest on the top pin.

3) **Attach the two Thumb Nuts** to the screws snugly to hold the cell holder in place.

4) **Connect the electrical, water and nitrogen lines**. The electrical connector is for the temperature control and is of the same fashion as other electrical connectors. The nitrogen tubing (black) plugs into the nitrogen line entering through the bottom of the sample compartment. The electrical and water lines connect to the lines coming in through the top of the Stopped-Flow Drive.

5) **Connect the cable to the Fluorescence PMT.** This cable emerges from the left side of the sample compartment. Store the terminator formerly attached to the cable in a safe location.

I. C. 4 Check the Optical Alignment

1) **Start the lamp** in the usual manner: purge the instrument with nitrogen; shutdown the computer and turn off the CPU and Instrument power switch; turn on the Lamp Power and Cooling System switch; fire the lamp. Leave the other electronics off.

2) **The light beam should be visible**. Insert the Alignment Target so that the flat surface faces the light. The Target has a pin in it's side that should drop into the Side Notch of the Cell Holder.

3) **The light should be centered** around the hole in the target. If not then the cell holder's position will have to be adjusted.

4) **Vertically adjust the cell holder** by slightly loosening the two Thumb Nuts that hold the cell holder to the back wall. Loosen the Locking Screw that holds the Vertical Adjustment screw in place. Turn the Vertical Adjustment screw to raise and lower the cell holder. When in place tighten down the Locking Screw and tighten the two Thumb Nuts.

5) **Horizontally adjust the cell holder** by slightly loosening the two Horizontal Locking Screws. Move the Cell Holder forward or back by hand until the light is centered around the target's hole. Tighten the two Horizontal Locking Screws.

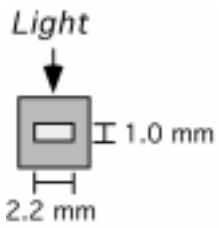
6) **Screw out the shroud covering the PMT** (on the right) so it makes contact with the cell holder. This blocks out light and reduces the amount of nitrogen needed to purge with.

I. C. 5 Mount the Mixer / Optical Cell in Cell Holder

If the Mixer / Optical Cell Assembly is already mounted in the cell holder then this operation can be skipped.

1) **Determine the orientation of the optical cell** in the assembly. The optical cell cross section is 1 by 2.2 mm. The 2.2 mm surface should be facing the beam so that the optical path length is 1 mm. Hold the cell up so that light can be seen through the window. The 1 mm path length will have a clear view while in the other orientation the walls of the cuvette will faintly seen. The view can be enhanced if air (not water) is in the optical cell. The side showing the walls should be towards the front of the instrument so that the instrument's light will pass entirely through the sample. Note the orientation of the cell relative to the pin on the side of the assembly.

Figure 1 - 6: Optical Cell



2) **Slide the Mixer / Optical Cell Assembly into the Cell Holder**. The assembly has an orientation pin that drops into a notch in the top of the cell holder. There are two notches for the two possible orientations of the assembly. Be sure that the pin is in the notch so the light passes through the 1 mm path length of the cell, with the 2.2 mm surface facing the light.

3) **Attach the assembly cover**. Slide the black cover over the mixer / optical cell

assembly, aligning the notches with the black tubing. Use a 5/64" hex wrench to fasten the screws.

4) **Attach the waste syringe**. This is a 25 ml disposable syringe that screws onto the luer lock adaptor in the top of the mixer / optical cell assembly.

I. C. 6 Attach the Mixer's Tubing to the Valves

1) **Fasten the green tubing** from the mixer / cell assembly into the tops of the valves. They should screw down thumb tight. If the mixer is a single stage mixer then the two fluid paths are symmetric and can be connected to any valve. If two or more stages of mixing are used then the fluid paths are not symmetric and the appropriate tubing should be connected to the right valve. If the valves are not at the right height then the stopped-flow drive can be adjusted with the hydraulic lift.

I. C. 7 Finishing Touches

1) **Turn on** Thermoelectric Power and Stopped-Flow Supplies. These will not be activated until after the control software has been started.

2) **Attach the front plate** to the sample compartment. This plate is similar to the front plate of the thermoelectric cell holder, only without the electrical, water, or mechanical connections.

3) **Store the bottom plate** of the sample compartment it's with screws.

4) **Start the user interface software**. It will automatically recognize that the stopped-flow is in place. Answer Yes to the initialization question about downloading stopped-flow software. The temperature calibration constants will be automatically selected for the stopped-flow.

5) It will be necessary to spend extra time and water purging air from the stopped-flow as the connections have been exposed.

I . D Running Stopped - Flow Experiments

I. D. 1 Optimizing Conditions

For optimal performance it is recommended that the one ml syringe be used to inject the smaller volume and the two milliliter syringe be used for the larger injection. For example, the sample is to be diluted in buffer at a ratio of 1:10. In this case the sample should be put in the one milliliter syringe and the buffer placed in the two milliliter syringe. If larger dilution ratios are needed, or a large number of repeat shots are required, then the five milliliter syringe should be used instead of the two milliliter syringe. Likewise the optical mixer should be selected. The appropriate mixer should first ensure complete mixing, then be selected for the shortest dead time. Changing the syringes and mixer are explained in the Cleaning and Maintenance section.

The dilution ratio has to be calculated to deliver the right concentrations to the flow cell given the initial concentrations of reagents. The total flow rate should be in the range of 2.5 to 3.0 ml/sec. total flow. The dilution ratio and flow rate are determined by the volumes and times entered in the stopped-

flow experiment configuration panel (described in the software section of this manual). The final concentration has to be in the range so it is concentrated high enough that a signal can be seen yet the concentration still allow light to pass through. The optimal absorbance is in the range of 0.8 OD at the wavelength being measured.

I. D. 2 Loading Solutions

Before the sample and buffers can be loaded the stopped-flow should first be purged of air bubbles. Bubbles have several undesirable effects: they cause the light to be scattered resulting in ruined measurements; bubbles are highly compressible, resulting in delayed fluid flow at the start of the push and delayed stoppage at the end of the push; and the excess compressibility cause mismatched flow rates resulting in the dilution ratio being wrong. To make purging easier and reduce the likelihood of bubbles all solutions should be degassed. This can be done by putting the solutions in a bell jar connected to a water aspirator for 15 minutes. Degassing solutions will help bubbles in the stopped-flow to dissolve back into solution. Degassing also reduces the likelihood that cavitation of the solutions in the mixer results in bubbles. If the stopped-flow has not been used in the same day it probably is best to purge using degassed water first, then replace the water with the buffers and samples once all bubbles have been removed.

To load buffers and samples one of two different strategies can be used. If the solution is abundant and inexpensive (buffers, for example) then it can be used to rinse out the water. Go to the Control Panels: Syringe Control: Stopped-Flow Syringe Control panel. Change the Set Volume value to 100 µl (press Enter for the new volume to take effect). Switch the valve to the fill position (handle back). Go to the menu for the syringe to be filled and choose the option to Fill Syringe. Switch the valve to the mixer position (handle forward) and in the syringe menu choose Empty Syringe. Repeat this process several times until no bubbles are seen rising in the waste syringe. Afterwards switch the valve to the fill position (handle forward) and in the syringe menu select Purge Syringe. This will partially fill the syringe then quickly empty it. Repeat this process until no bubbles are seen rising in the reservoir or in the tubing connecting the reservoir to the valve. Afterwards change the Set Volume to the syringe's maximum value and use Fill Syringe to load the solution. Switch the valve to the mixer position.

If the solution to be loaded scarce then it can not be used to rinse the lines. It will be necessary to dilute the solution with the water remaining in the syringe and tubing. In principle the water could be removed before the sample is solution is loaded, but removing the water requires removing the syringes to dry them and blowing the tubing with gas to remove the water, steps that are troublesome to perform. Afterwards the sample must be used to remove bubbles, a process that requires more handling. In practice it easier to first purge with water, then slightly dilute the sample and determine the dilution if the concentration is important. All of the water should be removed from the reservoir before

the sample is loaded. Next use Purge Syringe to flush the bubbles from the lines and mix the solution. Use Fill Syringe to load the solution in the syringe. It is not necessary to completely fill the syringe. If the solution volume is less than the syringe volume then Set Volume can be used to entirely load the solution without drawing bubbles into the syringe. It is now necessary to fill the tubing between the valve and mixer. Switch the valve to the mixer (handle forward). Reduce the Set Volume value by 100 µl (press Enter) and choose Fill Syringe. This will push 100 µl into the tubing.

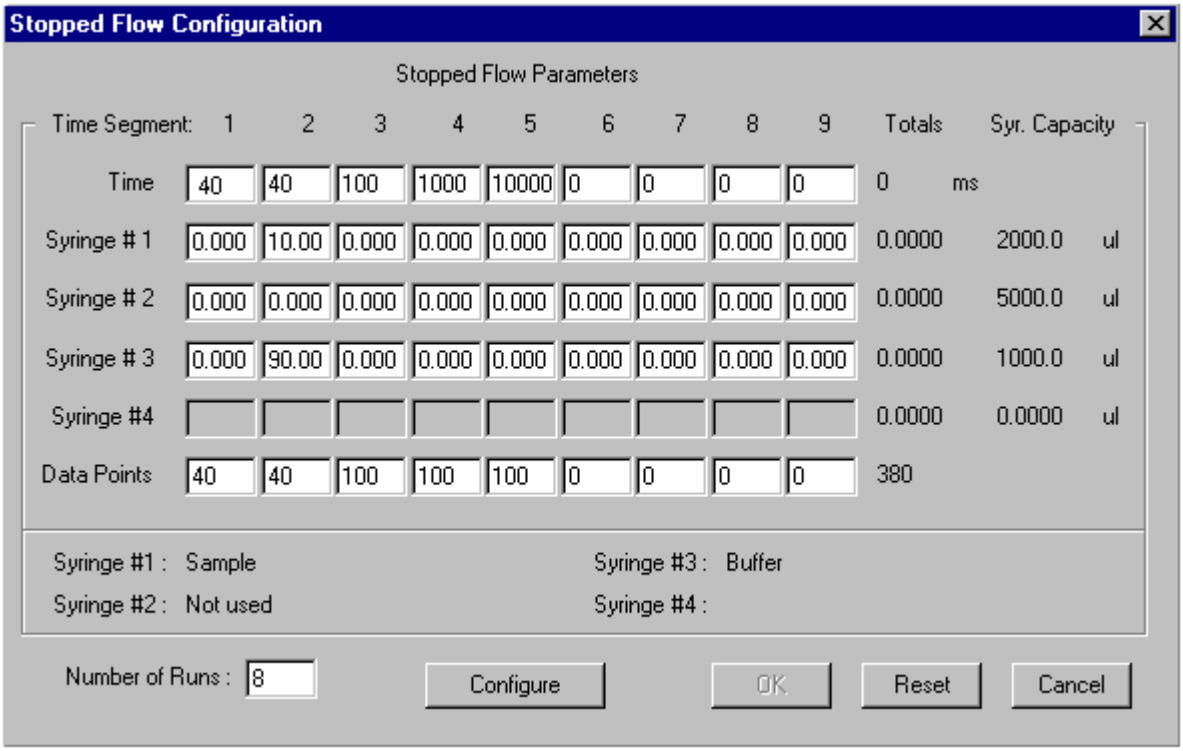
After the solutions are loaded their descriptions should be entered in the Stopped-Flow experiment configuration panel. This information becomes part of the data files.

I. D. 3 Making Measurements

After the solutions have been loaded it is necessary to set the experiment parameters in the software. This is also explained in the software section of this manual. Go to the Configure Experiment panel and under Experiment Configuration choose Stopped-Flow Configuration. This panel has the experiment divided into nine time segments, each with it's own time, volume for each syringe to deliver, and number of data points to collect. The flow rates and is determined by the ratio of the volumes to the times, the dilution ratio by the ratio of the volumes and the data collection rates by the ratio of the number of data points to the times. Sometimes it is desirable to measure the signals before the syringes are moved (pre-push). This can be done by entering zero for the volumes and typically 40 milliseconds for Time Segment 1. The second time segment can be used for the push. Typical values for this are for a 30 millisecond push with total volume of 90 microliters. This gives a flow rate of 3.0 milliliters per second. The total volume is split between the syringes to achieve the desired dilution ratio. For a single push the subsequent time segments will have zero for their volumes.

The number of data points specified for each time segment determines the data collection rate and total number of data points. The limits are 10,000 data points per time segment and no faster than 10 microseconds per data point. During the pre-push and push a collection rate of 1 data point per millisecond is adequate. In the time domain following the push the same collection rate should be maintained to capture fast kinetic signals, for example 100 data points for the next 100 milliseconds. Subsequent time domains should be made longer to collect the slower kinetics. The data collection rate can similarly be slowed down; for example, 100 data points for 1000 milliseconds (1 data point per 10 milliseconds) then 100 data points for 10,000 milliseconds (1 data point per 100 milliseconds). The hardware always collects data at the highest possible rate then the software averages the signals to produce a data point. As a result the slower data collection rates have superior noise levels due to the increased averaging. This approach allows fast signals to be collected during and immediately after the push while having excellent noise levels later to measure small signal changes.

Figure 1 - 7: Stopped-Flow Configuration Panel



The solution descriptions are shown in the Stopped-Flow Configuration panel (as entered by the user in the Stopped-Flow Syringe Control panel). The Number of Runs sets the number of shots that will be made as part of a single experiment. Initially this should be set to one until good data is being collected. After the instrument is delivering quality data the Number of Runs can be increased to automatically collect a series of shots. Before the stopped-flow experiment parameters can be activated the Configure button must be pushed. This performs a series of checks to make sure the experiment parameters are valid. Checks are made of the minimum and maximum flow rates, the number and rate of data points collected, and other items. If one of the checks fail then the user should make the appropriate changes. Some checks will prevent the experiment from being done while other checks will allow experiment although the instrument is operating beyond it's calibrated range. If both the user and software are satisfied then the OK button becomes available. Pushing OK accepts the experiment parameters, closes the panel and makes Stopped-Flow experiments the current experiment.

The Configure Experiment parameters also need to be set. The Time Constant normally defaults to 100 milliseconds for experiments other than stopped-flow. When stopped-flow experiments are configured (by the Exit / Save Configuration menu item) the default switches to 100 microseconds so that fast kinetics can be measured. If only slow kinetics are to be measured then the Time

Constant can be made slower to filter out more noise. The Time Constant should be more than three times faster than the fastest kinetics to be measured otherwise the instrument's response time will distort the signal. The Time Constant switches to an experiment's default when ever the experiment type is changed, so customizing the Time Constant requires returning to the Configure Experiment panel after the experiment type has been set.

Use Save Data Options to select the types of data to be saved. In stopped-flow experiment mode the Dynode high voltage is static so recording it is of little use. In CD instruments the CD Current (Abs) signal will change linearly with the sample's percent transmission, so it can be saved to follow absorbance changes. An equation exists in the Math Operations menu to convert this signal to absorbance. The X-axis for stopped-flow experiments is time, so it is not necessary to record the Elapsed Time. Turn on the Fluorescence and QC Signals if these are desired. Leave the panel using OK for the selections to take effect.

While in the Configure Experiment panel the wavelength and bandwidth should be set for the stopped-flow experiment. If the temperature is changed then the user should wait until after the drive and mixer have equilibrated to the new temperature. Detailed temperature information is available through the main panel's Control Panel: Temperature Control menu. The Description, Name and Experiment Counter can be updated as well. In fluorometers the dark correction based on a single dark measurement at the start of the experiment is possible, but correction based on dark measurements for each data point is not possible.

At this point in time the optical cell does not contain the correct concentration of diluted sample, so it is not practical to set the PMT high voltages yet. Leave the Configure Experiment panel with Exit / Save Configuration. Make a final check to make sure the valves are in the correct positions. Leave the covers off the sample compartment and Press Run Experiment to make a single shot. Watch the waste syringe to make that no bubbles emerge from the Mixer / Optical Cell assembly. Repeat making shots until no bubbles are seen. Replace the covers on the sample compartment.

By this time the line and mixer are purged and approximately the correct concentration of sample is in the optical cell. Go to the Control Panels: PMT Control menu. In fluorometers the fluorescence and quantum correction measurements are made with the PMT high voltages held constant. This remains true for stopped-flow experiments. Set the high voltage for these detectors as described in the software chapter.

CD measurements are different matter though. In experiment modes besides stopped-flow the CD PMT dynode high voltage is automatically adjusted to maintain a constant current passing through the PMT. In stopped-flow experiment mode the signals change too fast for the high voltage servo, so in this experiment mode only the dynode high voltage is locked and the current is allowed to

change. This requires setting the dynode high voltage manually to the correct value (close to 1.0). Deviations from this value can result in calibration errors. Pressing Autoset under CD-PMT Values will automatically ramp the dynode high voltage until a current value close to 1 is achieved. If this does not happen then pressing Autoset a second time usually finishes the job. Manually setting the Dynode can be used for fine adjustments if necessary. The Input Range need not be changed. Fluorescence measurements can be made simultaneously with the CD measurement if the fluorescence accessory is available. If the CD instrument is equipped with the Fluorescence Detected CD option (FD CD) then the user will most likely want to the CD Signal Source to be CD. The Fluorescence Measurement Excitation Wavelength should made to match the current wavelength. Fl Averaging Time does not matter because the CD and fluorescence signals are measured simultaneously. Press Auto Set at Excitation WL to set the fluorescence PMT's dynode high voltage. If the fluorescence signal makes large changes the HV Setpoint can be manually changed to make better use of the signal's full scale (0 - 5). If the quantum counter (QC) accessory is present then it's high voltage should be set as well. Press Auto Set to turn it on. The QC signal can be used to automatically correct the "absorbance" and fluorescence signals by choosing the Apply QC Correction check box. Press Return to go back to the main panel.

At this point it is desirable to make a few more single stopped-flow shots to ensure the right sample concentration in the optical cell. Changes in the concentration can be followed by the optical signals reported in the main panel. If the values migrates from the original values then the high voltages should adjusted in the PMT Control panel. Once these parameters have stabilized then the instrument is ready to collect data. If a single shot does not provide data with sufficient signal-to-noise ratio then it will be necessary to average multiple shots together. Data from different experiments can be averaged together by displaying the data in Data Review and using the Axis Definitions: Data Review Average (see software portion of this manual for a description). A single experiment can also be programmed to make multiple shots by changing the stopped-flow experiment configuration. The multiple shots can then be averaged using the Axis Definitions: Data Collection Average menu.

Saving data is done in the same fashion as other experiment types, except the default file format is binary. Binary files are smaller and move more quickly than text files, a desirable feature for data rich stopped-flow experiments. If all of the data points possible were collected in a stopped-flow experiment and all of the data types were turned on then the result is over 1 million numbers. If then multiple shots are made in a single experiment then the data file becomes even larger. Stopped-flow experiment can be manually saved to text in the Data Browser by using the Export Data Set: Export to ASCII Text option. The file format is the same as used for other experiment types. If a single data set is selected then only it and the time are saved to the same text file format. Probably the best approach is to save all of the raw data to a binary file as soon as the

experiment is done, then later save the averaged and other calculated data to text files for exporting to other programs.

I . E Cleaning and Maintenance

Good cleaning of the instrument is important for it to continue collecting quality data. Cleaning should be done in the same manner as for regular cuvettes. First the solutions should be pushed back into their reservoirs by switching the valves to fill positions and choosing Empty Syringe in the Syringe Control panel. Remove the solutions and replace them with distilled water. Use the Purge option to rinse out the syringes. Replace the water with fresh water and fill the syringes. Switch the valves to the mixer positions and empty the syringes through the mixer / optical cell assembly. Empty the waste syringe and repeat this process several more times. This cleaning is sufficient for salt solutions, but sticky substances require further cleaning. Detergents can be used, although SDS should be avoided (it forms insoluble precipitate when mixed with potassium ions). Commercially available detergents or Tween are good choices. Afterwards the system should be rinsed with water to remove the bulk of the detergent, ethanol to remove detergent on the surfaces, then more water. If the stopped-flow is to be used in the next week then it can be stored with water in the lines. If it is to be stored for longer periods then it should be filled with a 10% ethanol solution.

It may be necessary to clean the system more severely than is possible by rinsing through solutions. For example, proteins may stick to the optical cell, causing poor optical baselines or high light absorption. Fluid lines may clog if samples contain particulates. In these cases it is necessary to disassembly the mixer / optical cell assembly and individually clean the parts. To do this:

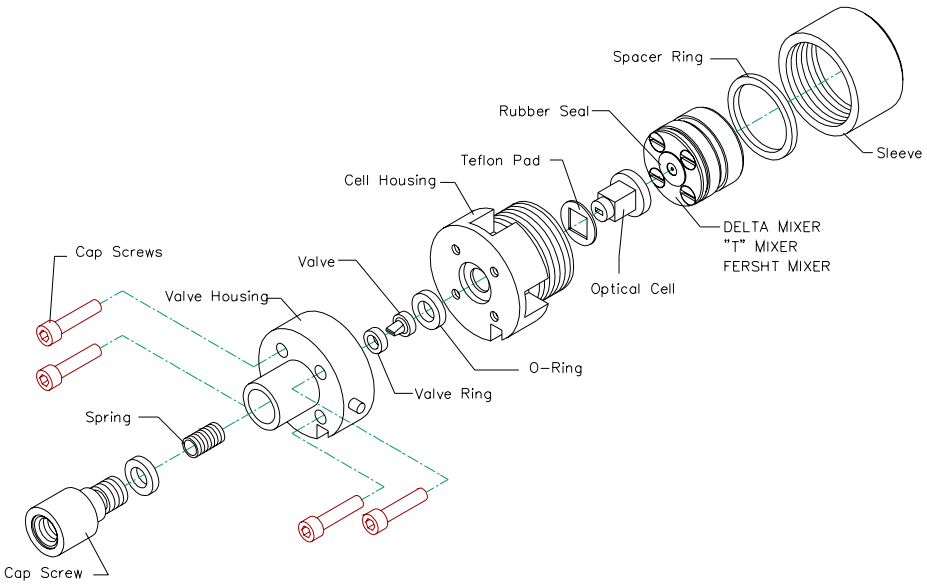
I. E. 1 Remove the Mixer / Optical Cell Assembly

1) **Unscrew the fluid lines** from the tops of the valves
2) **Unscrew the waste syringe** from the top of the assembly
3) **Remove the cap** covering the assembly by loosening the two screws (use a 5/64" hex wrench) and lift off the cap.
4) **Lift the mixer / optical cell assembly** out of it’s holder.

I. E. 2 Disassemble the Assembly

1) **Remove the mixer** by unscrewing the Sleeve that threads onto the bottom of the assembly. Slide the mixer out. If the mixer is tight then grip the base of the tubing and rock the mixer while pulling out.
2) **Remove the Cap Screw** from the top of the assembly
3) From this point forward there is a risk of the optical cell unexpectedly separating from the assembly. Either place your hand or some soft material underneath for the cell to fall onto. Use a 3/32" hex wrench to **remove the four bolts** from the top of the assembly. The Valve Housing and Cell Housing are now separate.
4) The Spring, Valve and Valve Ring are now loose. **Remove the optical cell** by

Figure 1 - 8: Mixer and Optical Cell Assembly



pushing on the it's top in the center of the O-Ring.

I. E. 3 Disassemble the Mixer (optional)

1) **Remove the four screws** in the mixer's top. The order and orientation of the layers are important. As the unit is taken apart note the details.

2) **Remove the tubing** from the base with the miniature wrench supplied with the stopped-flow.

I. E. 4 Clean the Parts

1) Use standard laboratory cleaning procedures for cleaning the parts. A bath (not probe) sonicator can be used to speed the process. The optical cell is made of quartz and can be etched by pH's greater than 10. It is immune to low pH's so cleaning it with acids is acceptable.

I. E. 5 Reassemble the Assembly

1) **Reassemble the mixer** in the reverse order it was taken apart. Leave the tubing off until later. Tighten down the screws hand hard.

2) **Insert the optical cell** into the Cell Housing. Be sure the Teflon Pad is in place over the Optical Cell. Keep the assembly in it's side to keep the cell from falling out.

3) **Place the Rubber Seal** in the center of the top of the mixer. Tilt the Cell Housing and slide the mixer into housing. **Screw on the Sleeve** to hold the Mixer and Optical Cell in place. The sleeve should be snug but not screwed on hard.

4) **Place the O-Ring** over the top of the Optical Cell in the Cell Housing.
5) **Put the Valve Ring over the Valve and insert both into the bottom of the Valve Housing**. Hold the Cell Housing on its side and put the Valve Housing in position so that the groove in the sides of the two parts is aligned. **Screw the four bolts in** to fasten the two housings together.
6) **Place the spring into the top of the valve housing**. **Screw the Cap Screw** down all of the way.
7) **Screw the tubing on loosely** to the base of the mixer by hand. Use the miniature wrench to attache the tubing. Use the miniature torque wrench to tighten down the fittings. The torque wrench has two arms: a shorter, thinner one labeled "5 ozf-in" and a longer, thicker are labeled "10 ozf-in". When one arm is pushed so that it barely touches the other arm then that amount of torque is being applied. The fittings should be tightened with a minimum of 5 ounce-inches, but no more than 10 once-inches. Over tightening will damage the fittings resulting in leaks.
8) **Mount the assembly** using the procedures given earlier in "Installing the Stopped-Flow".

I . F Changing Mixers

The mixers may be changed to optimize the performance for the sample's characteristics. The necessary instructions are in section entitled Cleaning and Maintenance. The subsections describing the valve and optical cell can be skipped.

I . G Changing Syringes

Normally it is not necessary to remove the syringes: they can be cleaned in place. Occasionally the syringes may be removed to inspect their conditions or exchange for a different size.

I. G. 1 To Remove a Syringe

1) **Quit the software** if it is running.
2) **Turn off power** to the stopped-flow motor power supply.
3) **Remove the front, middle plate** of the stopped-flow tower. It is attached with two thumb screws. It may be necessary to remove the tubing from the tops of the valves and use the hydraulic jack to lower the stopped-flow drive to a more accessible position.
4) Syringes one and three are accessible from the front. Syringes two and four sit behind one and three. To service syringes two and four it is easiest to detach the plunger from syringes one and three by unscrewing the caps and pushing their plungers up into the syringes to make room.
5) **Detach the plunger from the drive**. Unscrew the cap that attaches the plunger and slide it from around the plunger. Push the plunger up into the syringe.
6) **Push the drive down to nearly the bottom**. It will require some weight to force the drive to move downward. If the drive is too stiff to push then turn the lead screw by hand to move the drive down. Try not to push it all of the way down.
7) **Loosen the two thumb screws** next to the syringe. Swing the locking cams away from the syringe.

8) **Unscrew the syringe**. When it is free it will drop down.

I. G. 2 To Install a Syringe

1) Follow steps 1 - 4 under "To Remove a Syringe" if they have not already been done.
2) **Screw the syringe up** into the stopped-flow. The locking cams may move into the wrong position and impede progress. The syringe should be screwed in until it is hand tight.
3) **Swing the locking cams into position** beside the syringe and tighten the thumb nuts. The locking cams prevent the syringe from backing out.
4) **Raise the drive** so to within 5 cm of the plunger. If the drive can not be raised then the lead screw can be turned by hand to raise the drive.
5) **Pull the plunger down** into the top of the drive and slide the cap around the plunger. Screw down the cap firmly to fasten the plunger to the drive.
6) **Replace the middle cover** on the stopped-flow tower. **Turn on power** to stopped-flow motor power supply.
7) If the syringe is the same one as earlier removed then the unit is ready to use. If not then it will necessary to recalibrate the syringe drive. Calibration instructions are given below.

I . H Calibration of Syringe Drive

The motion of the plungers, and hence the flow rates and dilution ratios, is based on a feedback loop. This loop measures the motor speeds and adjusts power to the motors to achieve the correct speeds. This loop relies on several tuning parameters that convert the motion information to the appropriate power levels. If the mechanical loading of the system changes then it will be necessary to recalibrate the stopped-flow. Examples of when calibration should be done are if the syringe size has been changed, the unit has been used extensively and wear has changed the performance, or the mixers have been changed.

Calibration is done with the syringe utility program SCT.EXE (Syringe Control Terminal). The name may be slightly different if the version number has been added to the filename. When SCT is started it asks "Do you want to reload existing calibration data?" Answer "Yes". The program performs several functions related to the stopped-flow drive, one of which is calibration. **Syringe Motor Control** turns the power **On** and **Off** in the stopped-flow power supply. The **Syringe Control Panel** is the same as in the user programs. Under **Options** the item **Syringe Status** shows the absolute positions of the motors and the limits switches, and **Service Panel** gives the software version number.

Calibrate Axis contains the options necessary to calibrate the stopped-flow. **Calibrate Syringe #1**, **#2** and **#3** starts calibration of those individual syringes while **Stop Current Calibration** aborts the process. **Constant Volume Calibration** starts a process where two syringes are moved simultaneously to maintain the same flow rate through out the calibration. The advantage of this approach is that the

hydraulic back pressure is the same as during actual experiments yielding more accurate calibration values. A second (reference) syringe is used to deliver the additional volume needed to maintain the selected flow rate. This approach depends on the calibration of the reference syringe. **Display Calibration Results** shows the values obtained for each syringe shot while **Display Calculated Results** shows the calibration values obtained for each syringe speed. **Write FV Data File** saves the calibration values to file.

I. H. 1 Before Calibration

To calibrate the stopped-flow must first be assembled in the configuration that it will be used in, then it must be filled and purged with freshly degassed water. This is done in the same manner as in the user software, with the exception that the motor driver software must be manually loaded.

1) Go to the **Syringe Control** panel and under the **Syringe Drive Control** menu select **Init and Reload Syringe Drive Controller**. The stopped-flow power supply will turn on.

2) If the syringe size(s) have been changed then use **Change Syringe Size** for the individual syringes. Next **Home**, **Purge** and **Fill** the same as always. When all of the bubbles have been removed leave the Syringe Control panel.

I. H. 2 Single Syringe Calibration

1) **Select the syringe in the Calibrate Axis menu**.

2) The user is prompted to **move the valves at the appropriate times**. The program starts by moving the syringe at the slowest speed (5000 Counts Per Second) and measures the error in the speed. Ideally the graph will show a straight line, but in reality acceleration and deceleration errors will appear. The feedback values are adjusted to minimize the error during the steady-state portion of the movement. Once the appropriate feedback value is found the speed is raised and the optimization process is repeated.

3) The user is prompted to **move the valves to refill the syringes** at the appropriate times.

4) The speed is increased until either it reaches 80,000 CPS, the user stops the calibration, or the steady-state speed can not be maintained. When one of these conditions is reached the **user is asked if the calibration values should be saved**. Answer Yes. The calibration values and the range of speeds covered is saved to the calibration file. This information is automatically read every time the user software is started.

I. H. 3 Constant Volume Calibration

1) **Select the Reference syringe**. This syringe should be calibrated prior to using the Constant Volume Calibration, either by calibrating it individually, or by using another calibrated syringe as the reference.

2) **The Flow Rate must also be selected** to be close to experiment conditions. For stopped-flow drives without electric valves the optimal flow rate is 2.5 - 3.0 ml/ sec.

3 - 4) **The Constant Volume calibration proceeds the in the same fashion** as

calibrating a single syringe. The volume of each syringe is tracked and the user will be prompted to move the appropriate valves when refilling is necessary.

I. H. 4 After Calibration

When the calibration is completed a record of the results can be saved to other file formats beside that used by the user programs. Both Display Calibration Results and Display Calculated Results allows the data to be saved and exported. These are convenient so that a record of the calibration is kept.

I . I Removing the Stopped-Flow from the Model 202 CD Spectrometer

Before removing the stopped flow the system should be thoroughly cleaned. The system should be left containing distilled water and 10% ethanol.

I. I. 1 Remove the Mixer / Flow Cell Assembly

1) **Quit the software**. **Turn off the power** to the Stopped-Flow Power Supply and Thermoelectric Power Supply.
2) **Remove the sample compartment covers** from the front and top.
3) **Unscrew the fluid lines** from the tops of the valves
4) **Empty and remove the Waste Syringe** from the top of the assembly.
5) **Remove the Assembly Cover** over the mixer / flow cell assembly. Use a 5/64 inch hex wrench to loosen the screws.
6) **Lift out the Mixer / Flow Cell Assembly** from the cell holder.

I. I. 2 Remove the Cell Holder

1) **Disconnect electrical, water and nitrogen lines.** The water and nitrogen lines separate by pushing on the metal tab. They are self-sealing. The electrical lines disconnect by unscrewing the knurled ring then pulling the ends apart.
2) **Screw back the PMT shroud.** Screw it back far enough to give a few millimeter clearance.
3) **Remove the two thumb nuts** attaching the Cell Holder to the sample compartment's back wall. Be prepared to support the cell holder with one hand.
4) **Remove the Cell Holder** by sliding it forward and out of the sample compartment. The tubing and cables will have to be moved to clear the optics.
5) **Reattach the thumb nuts** to the back wall and place the cell holder in a safe location.
6) **Attach to the fluorescence cable** in the sample compartment the terminator.

I. I. 3 Lower the Stopped-Flow Drive

1) **Empty the reservoirs** if not already done.
2) **Place the caps on the reservoirs**.
3) **Remove the two bolts** anchoring the stopped-flow drive to the frame. These are located on the side walls of the drive near the bottom. The bolts on the right-angle brackets may have to be loosened so the drive does not bind between them.

4) **Turn the hydraulic crank** counter clock-wise to lower the drive. Lower it until the electrical connections at the base of the stopped-flow tower nearly touch the table.

5) **Remove the light trap** from the sample compartment. This is the square metal plate that sits loose on the bottom of the compartment. Store it in a safe location.

6) **Position the tubing and connectors** so that they are out of the sample compartment.

7) **Install the bottom plate** in the sample compartment and fasten down with the six thumb screws.

I. I. 4 Install the new cell holder

1) The sample compartment sits on the rollers attached to the sides of the sample compartment. **Position the end of the cell holder on the first roller** and push it into the sample compartment.

2) **Fasten the cell holder into place** with the four spring screws on the front cover of the cell holder.

3) **Detach the two water lines** from the side of the Stopped-Flow Tower. Attach these to the connectors on the front of the cell holder.

4) Hanging beneath the sample compartment is an unused nitrogen line. Attach this to the front of the cell holder.

5) If the new cell holder has a fluorescence PMT then remove and store the terminator and attached the cable in the sample compartment to the PMT.

5) The power cable for each type of cell holder is different. **Attach the appropriate power cable to the front of the cell holder**. Detach from the back of the Thermoelectric Power Supply the power cable that runs to the Stopped-Flow Tower. Attach to the Thermoelectric Power Supply the power cable from the cell holder.

6) **Turn on power** to the Thermoelectric Power Supply. Wait at least 15 seconds before starting the software.

7) **Run the user software**. The software will automatically configure itself for the new hardware.

Appendix I: CD Macro Language

I. A Introduction

The goal behind AMC.EXE, and the versions of instrument software that supports the macro language, is to create a set of commands that control the basic functions of the instruments. By doing this it is possible to control any aspect of the instrument and have complete flexibility in doing so. The disadvantage of such an approach is that convenient all-in-one commands are not supported. This sort of command places constraints on what can be done, for better or worse. We have chosen to use fundamental commands as it offers the greatest power to the greatest number of people. This approach makes it necessary to write a command for each function. For complex experiments the scientist may reduce the number of commands entered by loading a configuration file.

The editor has each of the commands available in buttons or menus. Selecting one of these opens a panel with the inputs appropriate for that command. This approach avoids having to memorize each of the commands or the command syntax.

The editor is suitable for all of the Aviv instruments that support the macro language. These are the Model 202 CD, Models ATF-105 and ATFF-212 fluorometers, and Model 14 spectrometer. Each of these have different sets of commands, so the editor must select which set to use at start up. If the editor is being run on a computer connected to an instrument then the selection is made automatically. If the editor is being run on a computer not connected to an instrument then a dialog box will allow the user to select the appropriate instrument.

I. A. 1 To install Macro Language:

1. Copy all of the specified files into the C:\AVIV\CDS directory.

2. If you have a previous macro language version, you may copy over it. There is no reason to save older, previous versions.

3. You will need to create a SHORTCUT in order for the icon to appear on the desktop.

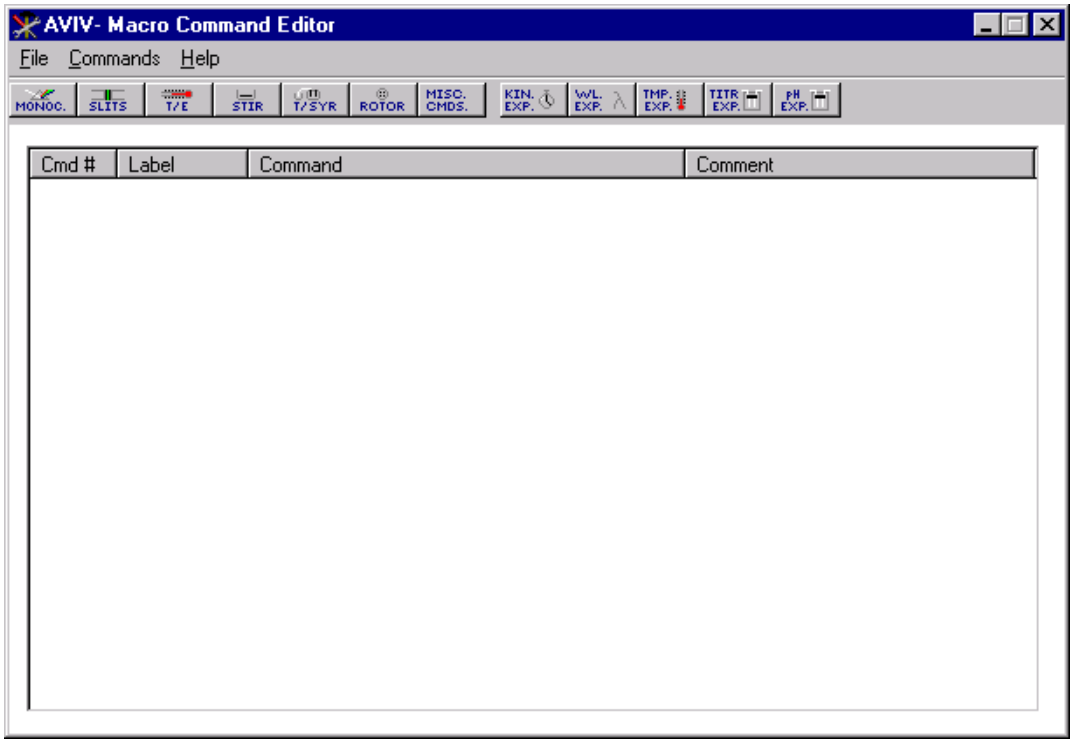
4. When installation is complete start the program and go to "Commands" and select "Edit Command Header". The message should display the current software version and "Instrument Type: CD Spectrophotometer".

I. B Getting Started

I. B. 1 Editor Panel

The main portion of the AMC.EXE interface is the editor panel. The first column is the command line number (Cmd #). The next column is the Label. This is used for controlling the flow of the program when using the loop command. The following column is the Command, and the final

Figure I - 1: Editor Panel



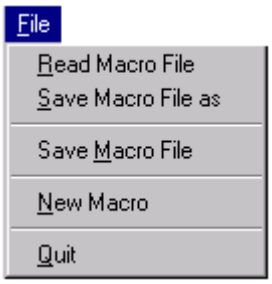
column is an optional Comment. Commands are added by either using buttons with lists of commands relevant to a given device or experiment, or by using menus to select commands. When a command is chosen a panel appears with the appropriate inputs. When this panel is closed the command appears in the editor. Normally commands are appended to the end of the list, but if a command is highlighted then the new command will be inserted after the selected line.

I. B. 2 Buttons

The buttons that appear beneath the menu line are the easiest way to insert commands. The left buttons show different devices of the instrument (for more information on device configuration, please refer to the hardware section of the 202 Manual). Buttons on the right show parameters associated with different experiment types. Clicking on a button shows the commands associated with that device. Selecting a command opens a panel showing the inputs for the command. Every command has a Comment line which will display any helpful text. The parameters for the command should be filled in before hitting the OK button. Some commands (such as Wavelength) have either Absolute or Relative buttons. In this case selecting Absolute will send the device to that absolute value. If Relative is selected then the device will move by the select amount away from the current value (what ever that may be). For example, if a fixed temperature for a titration experiment is desired then the Absolute button should be used. If on the

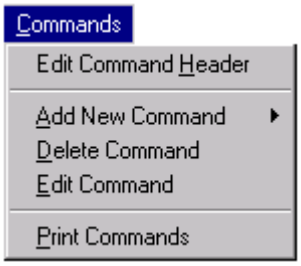
other hand the goal is to increment the temperature in a loop then the Relative button is needed

I. B. 3 File Menu



The file menu allows the user to Read Macro Files into the editor. In the **Save Macro File As** Panel the user **must specify the file extension (.AMC).** When typing in the file name simply add .amc; for example "wavelength test.amc". New Macro enables the user to create new macros. Quit exits the program.

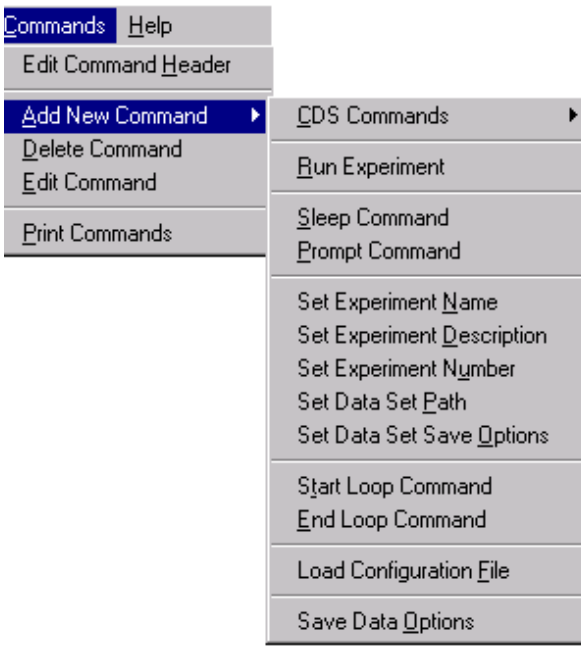
I. B. 4 Commands Menu



Edit Command Header brings you into the Aviv Macro Language Description Box. The software version number, instrument type (CD) and number of commands in your current experiment will be displayed. We suggest entering a description of the experiment and date. This will help keep multiple projects well organized. The information will be displayed in the header (line 0) of the editor's main control panel and keeps multiple projects well organized. You may modify this information at any time by double clicking on the command header.

To delete a command first highlight the command (line of text) you wish to eliminate and then click on Command and select Delete Command. To Edit Commands: highlight the command (line) you wish to change and then click on Command and select Edit Command; or simply double click on the command you wish to edit. The appropriate box will appear. Print Commands will print all the macro commands in the format displayed on the screen.

When clicking on Add New Commands the add new commands window will appear. All of the commands available through buttons



are displayed, plus a few additional options. Additional options not available through buttons are described below:

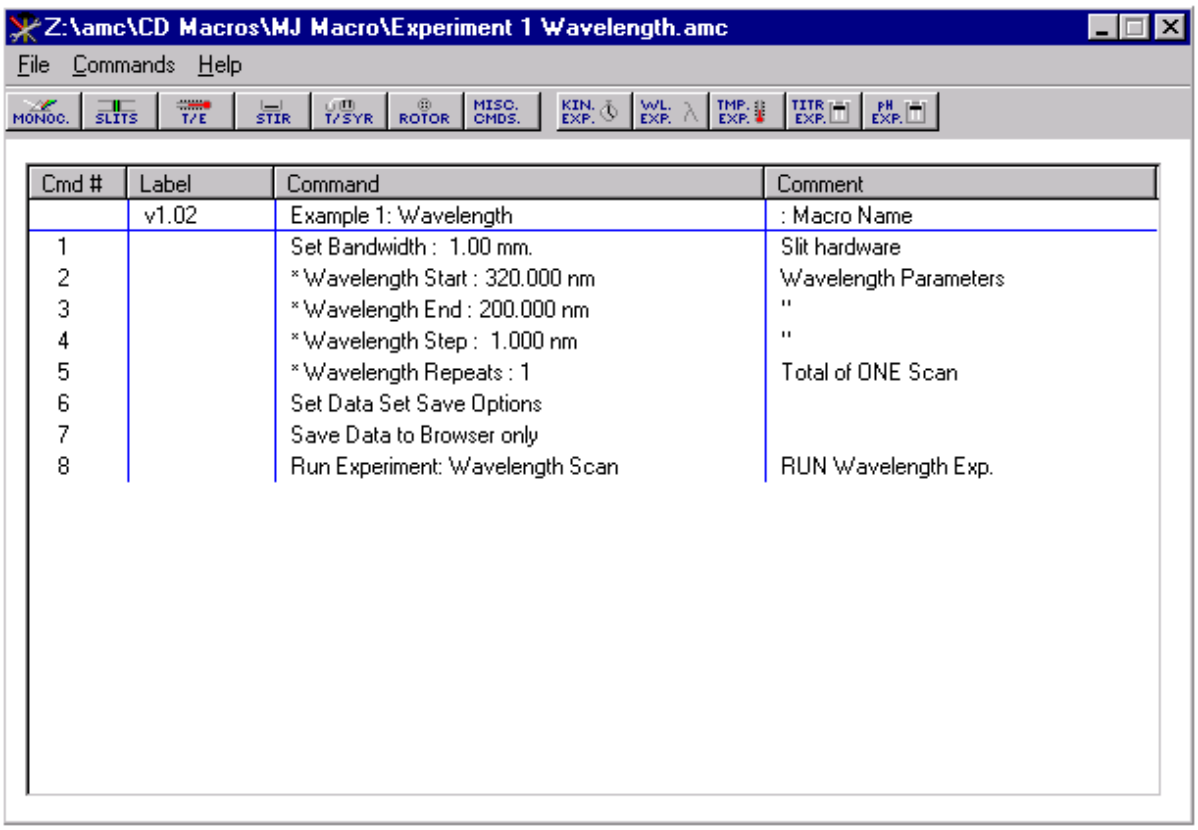
Run Experiment must be selected in order to run an experiment. Sleep command enables the user to pause the instrument for a specified amount of time. Prompt command alerts the user and pauses the program until

the user presses "enter". Set Data Set Save Options gives the user a choice of how the data should be saved, including the option of prompting the user when it is time to save the data. Save Data Options enables you to save to the data browser or hard drive. In Set Data Set Path you may enter the desired path to the hard drive.

The loop command enables you to repeat a sequence of commands within the experiment. Clicking on Start Loop will bring you into the Start Loop Command Box. Be sure to enter the label name. The label name will appear in the label column and serves as a reference point for creating loops. End Loop is the ending point of the loop, and will also refer to a label in the same fashion. The macro will cycle between the start loop label and the end loop label for the number of specified times.

CDS commands displays the same hardware and experiment configuration options available through buttons. Miscellaneous Commands allow the user to set averaging time and fluorescense averaging time.

Figure I - 2: Example 1: Wavelength Experiment



I. C Writing a Macro

The easiest way to write a program is to simply think about the steps that are taken when manually running the instrument. For example: after turning on the instrument and inserting the sample; the temperature, wavelength, and bandwidth are set. Next the experiment parameters are set, the experiment name given and perhaps the experiment counter reset. Finally the experiment is started. At completion the data is saved. Perhaps the conditions are then adjusted and the experiment rerun. Each of these functions are possible through the command language. Once a macro program has been written it should be saved to a file. This file is then read into the interface program. The name given in the header appears in the list of available experiments.

Let's write a macro! Go to Commands, edit command header and enter the necessary information (Example 1 Wavelength Experiment). Select all the hardware you'll be using. In this wavelength experiment you may want to go to Commands, CDS Commands, band width or simply hit the slits button. Enter desired slit width;1 for this experiment (Command 1). Now that the hardware selections are made you may enter your experiment parameters. You may hit the WL. Exp. button or go to Commands, Add New Command, CDS commands and select Wavelength Scan Experiment Configuration. We're going to scan 320nm to 200nm with a 1nm step size (Command 2-4). We want to make only ONE scan; so we're going to enter the number of wavelength repeats as ONE (Command 5). Next, you will want to save your data: Go to Commands, Add New Command, Set Data Set Save Options: the Data Set Save Options Box appears (same as CD Software). We decided to save our CD Signal and CD Dynode Voltage (command 6). Go to Save Data Options and select where you would like to save your data. We decided to save to the data browser only (command 7). Select Run Experiment from the CDS menu (command 8). Remember to save your experiment as .amc.

I. C. 1 Helpful Hints:

Always remember to:

Select Data Set Save Options.

Save file as .amc.

Select RUN Experiment.

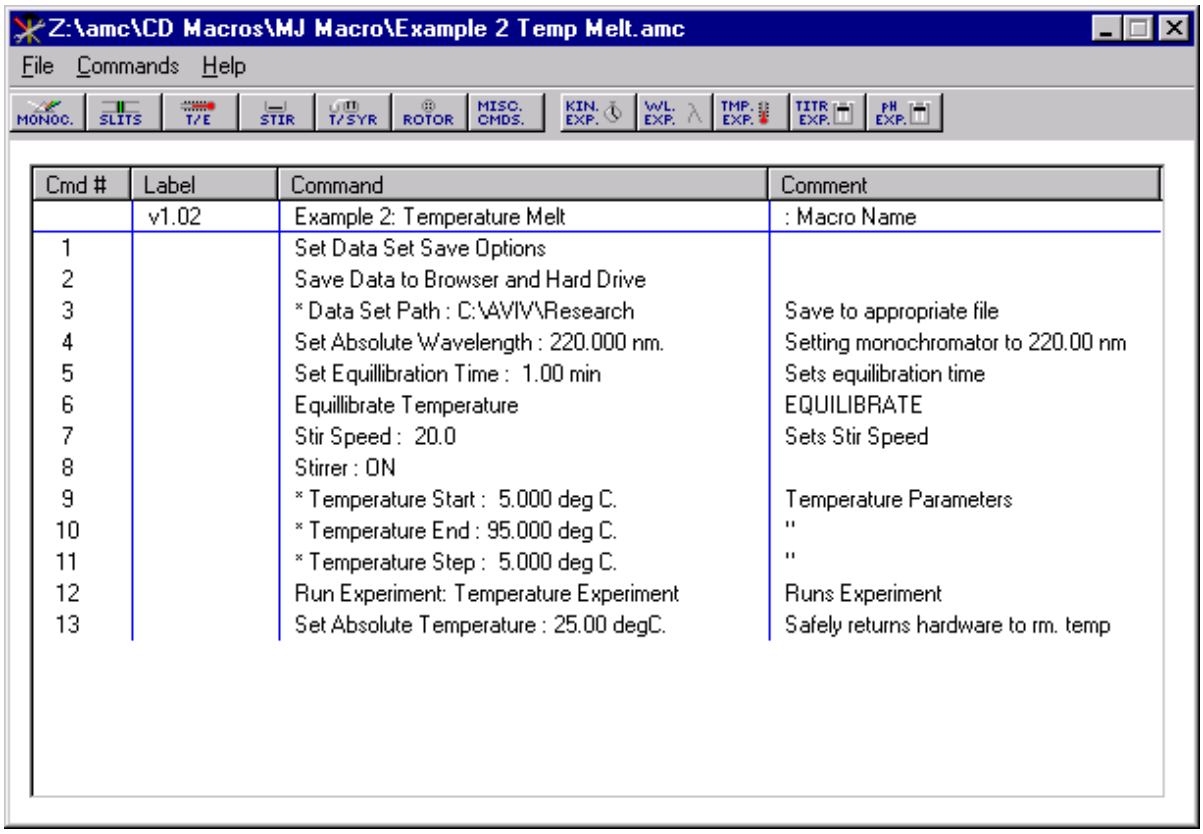
When selecting RUN Experiment, make sure you select the right type of experiment (ex: for wavelength experiment; RUN wavelength experiment).

I. C. 2 Loading Files

READING A MACRO INTO THE EDITOR: Start the Macro Command Editor the same as usual. Under the File menu, select Read Macro File. Select desired macro language program. If you did not save the macro file as .amc it will not appear as an option. Click O.K. when Read Macro File Box appears.

RUNNING A MACRO in the CD SOFTWARE: Start the 202 Software the same as always. In the 202 Main Control Panels click on File and select Read Aviv Macro File. Select your macro file. If you did not save the macro file as .amc it will not appear as an option. Click O.K. when the Read Macro File Box appears. The file will load and the CD Software's Main Control Panel will be displayed. Click on Configure Experiment and the Experiment Configuration Panel will appear. Click on the arrow which displays Type of experiment; a list of available experiments will appear. Your macro file will be the last item on the list. After you click on your macro language experiment name hit Exit/Save Configuration. Click on RUN in the CD Software Main Control Panel to start the programmed experiment.

Figure I - 3: Example 2: Temperature Melt Experiment



Cmd #	Label	Command	Comment
	v1.02	Example 2: Temperature Melt	: Macro Name
1		Set Data Set Save Options	
2		Save Data to Browser and Hard Drive	
3		* Data Set Path : C:\AVIV\Research	Save to appropriate file
4		Set Absolute Wavelength : 220.000 nm.	Setting monochromator to 220.00 nm
5		Set Equillibration Time : 1.00 min	Sets equilibration time
6		Equillibrate Temperature	EQUILIBRATE
7		Stir Speed : 20.0	Sets Stir Speed
8		Stirrer : ON	
9		* Temperature Start : 5.000 deg C.	Temperature Parameters
10		* Temperature End : 95.000 deg C.	''
11		* Temperature Step : 5.000 deg C.	''
12		Run Experiment: Temperature Experiment	Runs Experiment
13		Set Absolute Temperature : 25.00 degC.	Safely returns hardware to rm. temp

I. D Example Experiments

I. D. 1 Example 2

Temperature Melt.AMC. This instrument is located in a high volume lab with multiple users. Whenever writing a macro the scientist elects to enter data save options first. In command 1 the researcher decides what type of data he would like saved (CD Signal, Jacket Temperature, etc.). The data is saved to the data browser and hard drive (#2). Because the data was saved to the hard drive, the scientist enters a data set path (#3). In command 4 the researcher sets the monochromator to an absolute wavelength of 220nm. In step 5 the researcher sets the equilibration time. Command 6 is very important because it equilibrates the temperature. If you only enter an equilibration time (#5), equilibration WILL NOT occur. You must enter the Equilibrate Temperature command (#6). Line 7 sets the stir speed. Command 8 turns stirring on. If the stir time (#7) is entered without turning stirring on (#8) stirring WILL NOT occur. In this particular experiment the researcher chose to have continuous stirring. If you do not want continuous stirring, you must enter the command to turn the Stirrer Off. In steps 9-11 temperature parameters are entered. Line 12 is very important because it runs the experiment. If you do not enter Run Experiment, the experiment will not run. Command 13 sets the temperature back to room temperature (25 C) to ensure proper cooling of instrument prior to shut down.

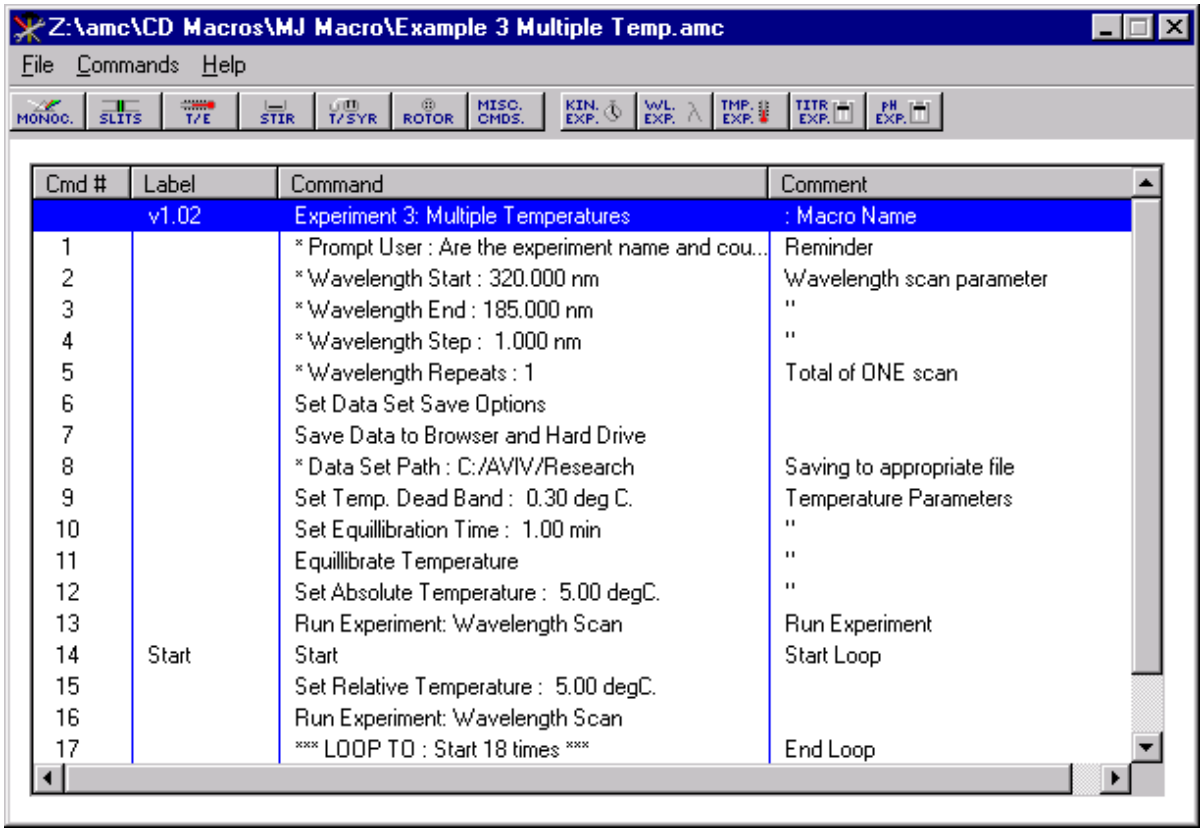
I. D. 2 Example 3

Multiple Temperatures.AMC. Command #1 prompts the user by beeping and having a dialog box appear displaying the message of your choice. This particular scientist chose to use this feature to remind his associate to set the experiment name and counter. The desired wavelength parameters were set by clicking on WL EXP (commands 2-5). Please note there will be a total of ONE scan (#5). In line 6 the researcher indicates what type of data is to be saved (CD Signal). Command 7 saves data to browser and hard drive. Because the scientist elected to save data to the hard drive he enters a data path (line 8). In steps 9-12 temperature parameters are entered. The scientists use of Command 13 is very important because it tells the computer which type of experiment to run. If you chose the wrong experiment type, your experiment will not run properly. If you fail to enter Run Experiment, your experiment will not run. Command 14 starts the loop and command 17 ends the loop. The user has a choice of how many times to let the loop run. This researcher chose to run the loop 18 times. Therefore in this particular macro the temperature will span 5-105 C in 5 C steps; over the 320 nm to 185 nm wavelength range. In line 18 the researcher sets the temperature back to room temperature (25 C) to ensure proper cooling of instrument prior to shut down.

I . E Conclusion

This appendix was written for our basic users performing general experiments. If you are interested in writing more complex macro

Figure I - 4: Example 3: Multiple Temperature Experiments



language programs, please refer to "Advanced Macro Language Appendix".

If you would like additional information about Aviv Macro Language Software or any other Aviv Instruments, Inc. product; please visit our website at www.avivinst.com.

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