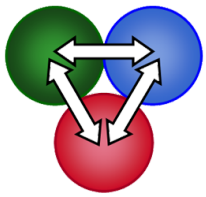

# *KinTek Corporation*

## Stopped-Flow
## Model AutoSF-120

## Instruction Manual

*Revision 1.4*

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# Table of Contents

# How to Use This Manual

The manual will offer instructions for the standard *KinTek AutoSF-120 Stopped-Flow*.

Section I describes the physical set-up of the equipment.
Section II provides basic instructions for preparing to conduct an experiment.
Section III provides detailed descriptions of individual software menu items.

We assume the user is familiar with basic Windows© procedures such as opening and saving files. A complete set of operating system instructions is included with the computer.

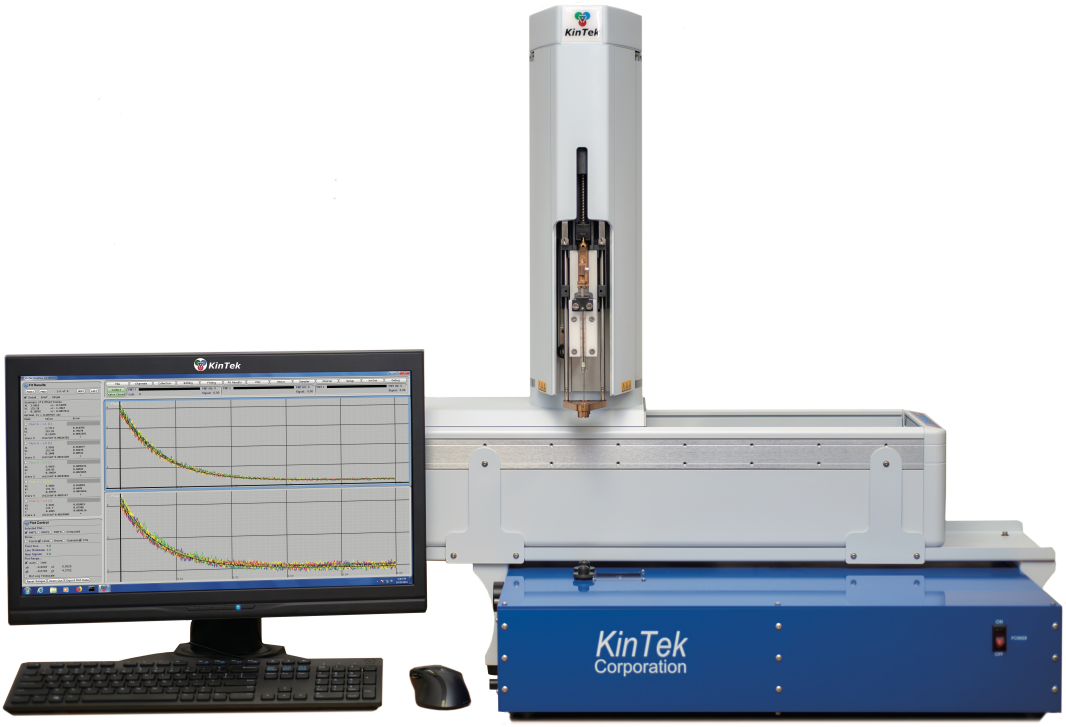



**Figure 1**. *Auto-SF 120 with optional robot autosampler*.

# I. Physical Assembly of the System

## *A. Electrical Connections*

Connections need to be made between the AutoSF-120 and computer. Each connection is unique as described separately below.

**Electrical Connections from the computer to the stop flow system**:

1. Setup Computer: The standard computer is a Dell computer running Windows applications. If unfamiliar with setting up a computer, see the instructions that are packaged with the computer.
2. Before attaching any cables insure power is off.
3. After computer connections are made (mouse, keyboard, and monitor), there are only three cables connecting the system and the computer.
    a. The first is an Ethernet cable that secondary network card on the computer. There are two connection ports labeled "*Ethernet*" on the back of the system, either port may be used.
    b. The other two identical connections are the 68-pin data cables and are marked "*Connector 1*" and "*Connector 2*". These cables have two distinct ends; the smaller silver connector is connected to the A/D board in the PC, while the larger blue connector is the inserted into the back of the AutoSF. Plug the cable into the A/D Board located towards the bottom of the computer in the back. The opposite ends are attached to the driver port marked "*Connector 1*" and "*Connector 2*", respectively.
    c. The final connection will be the power cables to the computer, monitor and stop flow system.

## *B. Temperature Control*

Temperature control is achieved by using a circulating water bath (not included). Inlet and outlet tubing connectors are accessed from the back of the instrument. Water circulates through the cube surrounding the observation cell, then flows through the syringe chamber and exits from the top of the syringe chamber. Connect the output of the water bath to the tubing connector entering the system marked "*IN*". Connect the input (return) to the water bath from the tubing connector marked "*OUT*". A solid-state temperature sensor is located in the syringe chamber, providing a continuous readout to the computer. A record of the temperature is included as part of each data file.

# C. Lamp Installation and Adjustment

1. Connect the starter unit (igniter) to the power supply according to instructions provided by the manufacturer (Hamamatsu Instruction Manual Page 13). It is best to place the light source on a shelf above the Stopped-Flow unit to limit the transmission of vibration from stopped-flow to the lamp. Alternatively, the light source can be placed on a granite block with vibration damping feet.
2. The lamp REQUIRES circulating water for operation. A pump and water bath are supplied for this purpose. The water in the supplied bath should be no cooler than room temperature to avoid condensation inside the light source housing. The water inlet and outlets are shown in *Figure 2*. Because mineral deposits from tap water can clog the capillary tubing in the lamp housing, ONLY use distilled water in the water bath. Change the water monthly, or whenever it appears turbid.

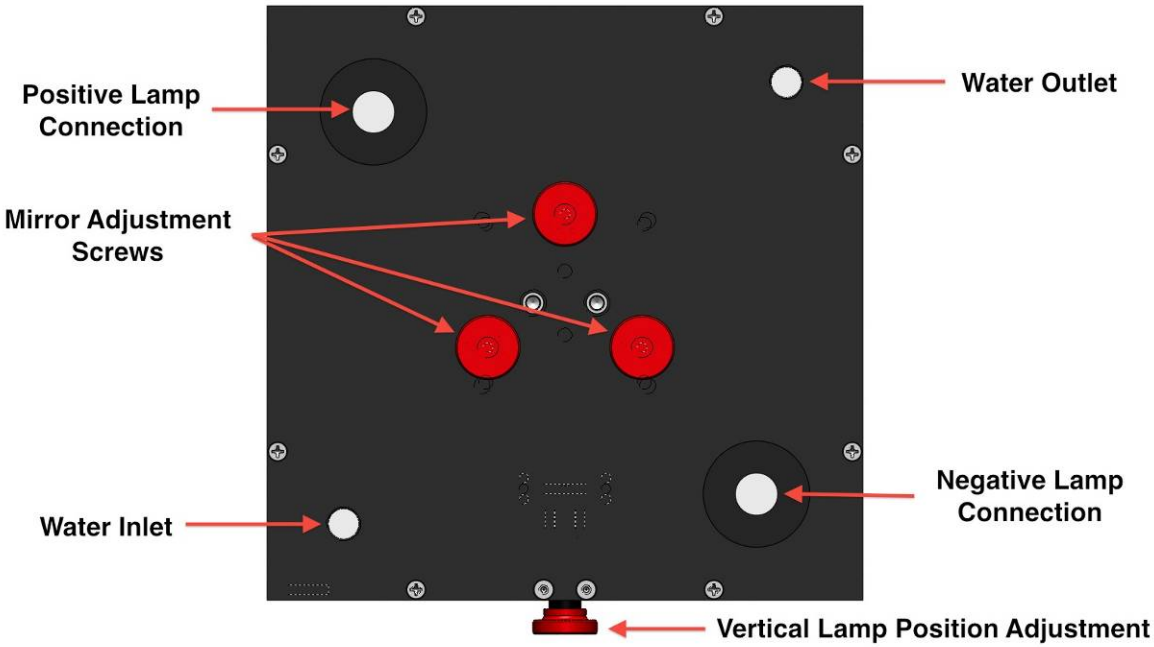



**Figure 2:** *Arc-Lamp Housing*

3. To install the arc lamp remove the eight flat head Phillips screws from the end of the lamp housing and pull the arc lamp housing out of the light source. The positive wire and cooling sleeve will be tied to prevent damage to the parabolic mirror during shipping. This must be freed by removing the twist tie. The arc lamp will be packaged separately. It is recommended to use gloves and safety glasses when installing the lamp. The gloves will prevent fingerprints on the mirror or lamp, and the safety glasses will protect your eyes in the unlikely event that the lamp breaks during installation. Remove the lamp from the packaging, and remove knurled nuts from each end of the lamp. The negative end (-) will be inserted into the fixed cooling tube. Place lamp through the tube, slide black wire over threaded stud, and then hand tighten the nut to secure the lamp. Do not over-tighten–it just needs to be snug. Slide the free-moving connector with the cooling tube over the positive end (+), slide the green wire over the stud, and then tighten the knurled nut. NOTE: there is a nipple protruding from the quartz bulb of the lamp. The lamp should be installed with the nipple facing away from the mirror. Inset the arc lamp housing back into the lamp housing and re-attached with the eight flat head Phillips head screws–do not over-tighten the screws.

4. The grating for the monochromator has been removed for transportation and will need to be installed. DO NOT touch the grating surface. If your light source is equipped with the auto wavelength drive you will need to remove the housing by removing the 3 Philips head screws that secure it. Next remove the four socket head cap screws that hold the monochromator lid in place. There are two screws located at the front bottom of the monochromator and two between the monochromator and lamp housing (*Figure 3*). You will use a 7/64” Allen wrench to loosen these screws (they do not need to be removed). Pull the lid upwards to remove it and set it aside. Looking from the front of the monochromator from the top, there will be two round braces. To the left of the left round brace will be a flap. Push the flap towards the left and install the grating into the rectangular slot. Hold on to the extension on the grating. The grating will seat into the slot with very little play in any direction. Return the lid and tighten screws.

5. Connect the igniter cables into the proper terminals. The white positive cable has a red end; this will be plugged into the red female receptacle on the arc lamp housing (*Figure 2*). The black negative cable has a black male plug that will be inserted into the black female receptacle on the arc lamp housing. You will then need to connect your tubing to the barbed fittings to cool the lamp. The water inlet is located in the lower left of the arc lamp housing. The water outlet is located in upper right corner of the arc lamp housing. Be certain water is flowing through the lamp housing. If water is not flowing through the lamp housing, damage WILL result. This should be done every time prior to igniting the lamp.

6. We recommend that the lamp power supply and the pump for the water cooling of the lamp be plugged into a power outlet strip with a switch, so that lamp can only be ignited when the water circulation is turn on.

7. Insert the optical fiber cable into the holder on the exit slit of the monochromator and insert the other end into the AutoSF (*Figure 4*). In each case, there is an o-ring to hold the optical fiber in place. Push the optical fiber end into the holder until it hits bottom.

8. Fine adjustment of the lamp requires use of the Stopped-Flow software to monitor the output signal using the photodiode channel. To do this make sure water is circulating through the arc-lamp housing, and then ignite the lamp. To ignite the lamp, turn on the power to the power supply and then press the light green button for about a second. You will hear a snap, and the button will light up. To check to see if the lamp has been ignited look through the viewing window located in the top of the light source. The power supply settings were made at the factory. For reference, we utilize is the 150 Watt L2274 Xenon or the L7047 Mercury-Xenon Lamp. The current (Amp) selection for this lamp is listed on the top of the power supply.

9. Let the lamp warm-up for approximately 15 minutes to allow it to stabilize. Next, boot up the computer and turn on the AutoSF. Load distilled water into the load cups and *Flush* the water through the system. See "Loading the Samples" section of manual. After flushing about 1.0 ml through the observation cell, click Collection then Scope Off to view the signal output of the detectors. Click Channels and then turn the display status off for all detectors other than PMT-3, and then *Autoset* the PMT-3 HV. This will provide a signal in the middle of the range on channel 3 so that you can monitor the intensity of the transmitted light while fine-tuning the lamp adjustment.

10. Adjust the lamp mirror to get the maximum intensity. Turn one of the three adjustment screws while watching the computer monitor to find the maximum intensity. Repeat the process for each of the three adjustment screws until the signal no longer increases. If the signal exceeds maximum (1V or 10V depending on A/D Scale) press Autoset to return the level to mid-scale. There are two additional lamp adjustments that can be made if acceptable intensity cannot be achieved. These typically do not need to be adjusted after the unit leaves the factory unless you install a lamp of a different type. On the bottom of the housing there is a knob that will adjust the vertical position of the lamp in relation to the mirror and removal of the 5/16-18 set screw from the side of the housing will allow access to adjust the horizontal position of the lamp with a 3/32" Allen wrench.

11. Note that there are slit width adjustments on each side of the monochromator. The bandpass of the light is approximately 6 nm per mm of slit width. The general recommendation is to use a wide slit width for fluorescence and a narrow slit for absorbance measurements, depending on the spectrum of the species that is absorbing light.

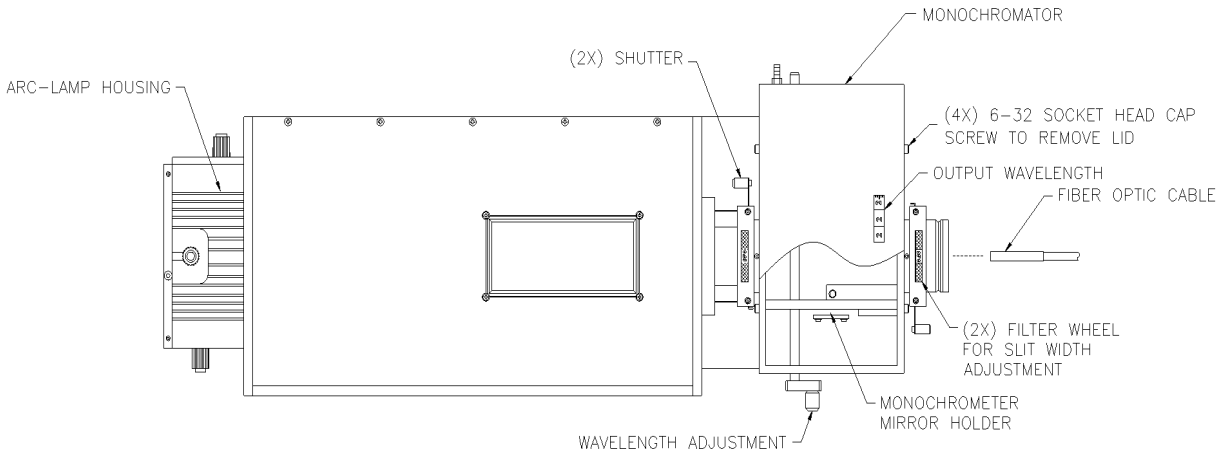



**Figure 3:** ***Light Source Schematic***

## *D. Filter Installation*

The AutoSF-120 uses sliding holders for installing filters in front of the photomultipliers used to detect fluorescence. Place the filter into the holder and then insert the included retaining ring to hold the filter in place. To remove, there is a groove inside of the retaining ring. Take the supplied flat head screwdriver in your tool kit, place it in the groove and pry the filter holder out. The sliding filter holders can accommodate either long pass or band pass filters that are 25.4 mm in diameter (1 inch). CAUTION: Never remove a filter slider if there is a voltage on the PMT. Make sure all detectors have no voltage before changing a filter. Voltage is supplied to the PMT only when the computer program is running and can be turned on and off under the Channel Setup menu.

The instrument is supplied with an inexpensive high-pass 320nm cutoff filter that is adequate for use with our fluorescence test assay or for protein fluorescence. We recommend purchasing band pass filters from **Semrock (www.semrock.com)** to match the emission wavelength of your fluorophores. Semrock filters provide a range of bandwidths and high transmittance to optimize signal throughput.

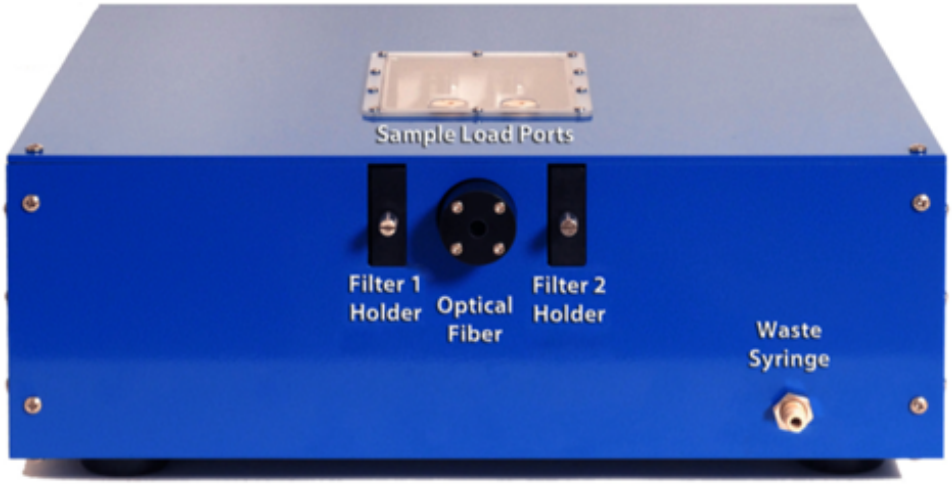



**Figure 4:** ***Optical fiber and Sliding Filter Holders***

# II. Operation of the Instrument

## *A. Summary of Instrument Setup Procedure*

In this section, we describe the sequential steps for setting up the instrument for data collection. Software command functions are described in detailed, encyclopedic fashion in *Section III: Description of Commands*.

With the exception of the order of turning on the equipment, the order of setting up the instrument for data collection is not mandatory. The recommended order is the easiest for getting ready to collect data. In some instances, the computer will automatically request the next step in the setup.

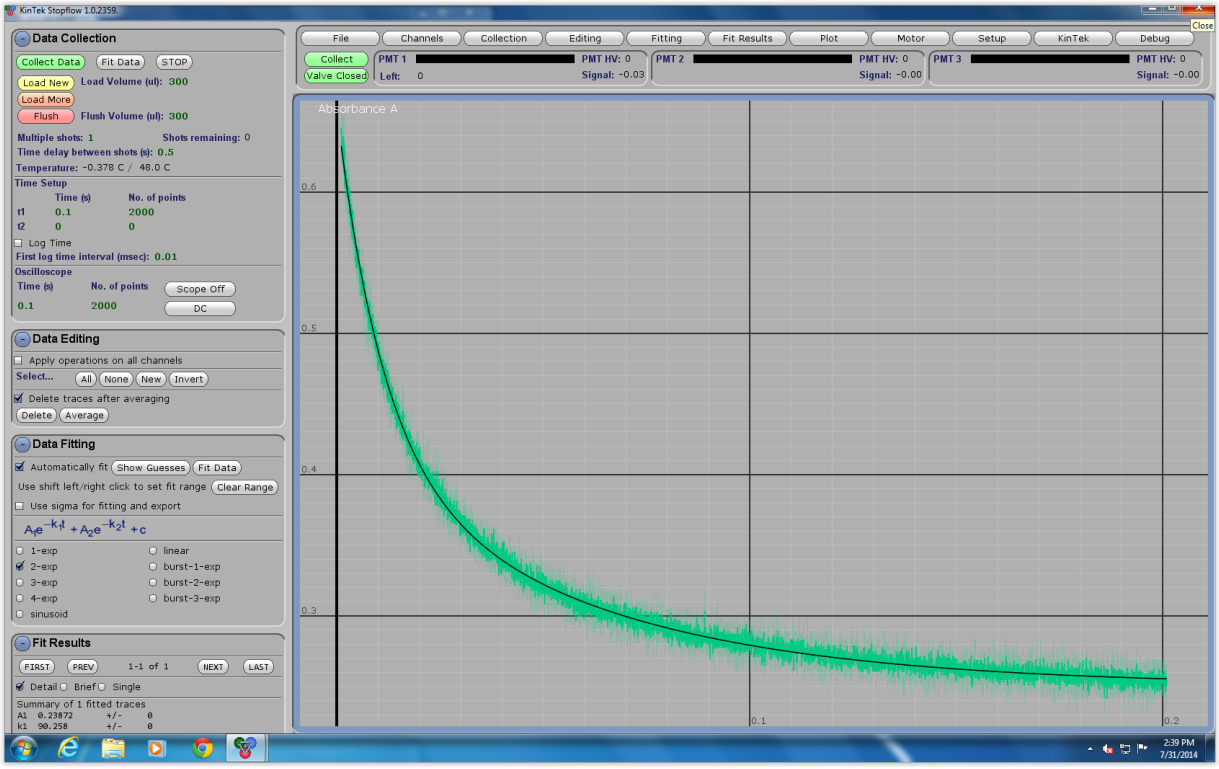



**Figure 4:** ***Stopped-Flow Main Screen***. This figure shows the main screen of the Stopped-Flow program. The window is divided into a Control Panel on the left and a Graphics Panel on the right. Across the top of the frame there is a row of buttons to jump to each control panel submenu and mini-panels to readout the status of each data of the three data collection channels. All functions can be accessed through the scrolling through or jumping to expandable menus in the control panel.

The program is written such that all of the commands are accessible at all times through the scrolling expandable menus on the control panel seen in the left side of Figure 4. Specific submenus can be accessed quickly using the buttons along the top of the screen. The use of most commands should be readily apparent.

**NOTE:** Any numeric value shown in dark green can be scrolled by clicking on the number and then dragging the value up and down with the mouse. Alternatively, after clicking and releasing, a text box will open to allow numeric values to be entered directly.

**NOTE:** Buttons that toggle between on and off states show the current status. For example, pressing 

toggles the oscilloscope mode to the on state and vice versa.

The general workflow is listed here, with detailed instructions to follow. Section III gives a more complete description of each command.

1. Ignite the lamp and then turn on the AutoSF-120 and computer.
2. Double click on the *KinTek Stopped Flow* icon to start the program
3. Set monochromator wavelength under the instrument Setup menu.
4. Flush Syringes and Load Samples
5. Adjust Detector Sensitivities
6. Read Dark Current and Reference (if in Absorbance Mode)
7. Set data collection time and channels to be used
8. Collect data
9. Save data
10. Analyze data
11. Shutdown the instrument

## *B. Turning on the Equipment*

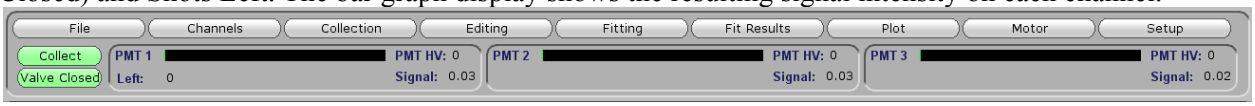
The KinTek Stopped-Flow system has three basic components, the light source, the main SF drive unit, and the computer. It is important that the computer be OFF when the lamp is ignited. The high frequency pulse from the lamp igniter may crash the computer program if it is on. Therefore, it is recommended to turn on the equipment in the following sequence.

The lamp should be cooled while in operation. We recommend using the supplied circulating water bath at ambient temperature. Do not use a refrigerated water bath set below room temperature since condensation on the mirror will tarnish the polished aluminum surface.

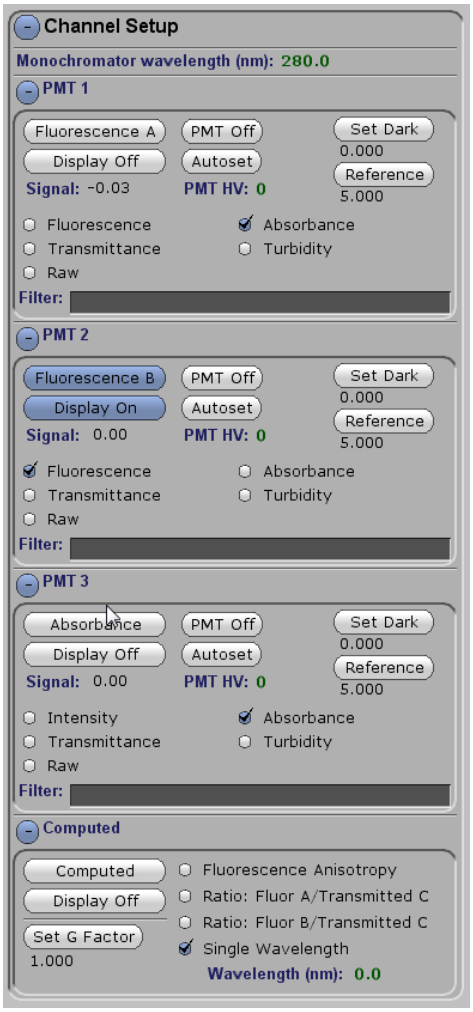
1. Turn on the arc lamp power supply and the pump to circulate water to cool the lamp. Press the light green button to ignite. Be certain lamp has been aligned (see *D. Lamp Installation & Adjustment*). Allow lamp at least fifteen minutes to warm up before beginning data collection.
2. Turn on the power to the stop flow system and allow 20 seconds before starting the computer program to allow time for the system to initialize.
3. Turn on the computer and run the KinTek Stopped Flow software. When the program begins, it causes the drive motor to find the "Home Position", which is used as a reference point to calculate the number of shots remaining and to easily be returned to this position.
4. If the message, "No Connection to Instrument" comes up, either the power was not turned on to the stop flow system or the connections have not been made properly.
   **Note:** You can run the software for data analysis only with the instrument turned off. The software will open once the **OK** button is clicked and data files can be loaded but instrument operation will not be possible until the issue is corrected and software restarted.
5. Confirm that the wavelength dial on the monochromator corresponds to the value listed under Channel Setup. If there is a discrepancy, enter the correct value under the Setup Instrument control panel.

## C. Setting the Wavelengths and Selecting Detectors

In the Stopped-Flow Software, the indicators along the top of the window display the current status of the following system settings: PMT HV, PMT Signal for each channel, Stop Valve state (Open/ Closed) and Shots Left. The bar graph display shows the resulting signal intensity on each channel.




To set the excitation wavelength on the monochromator enter the desired wavelength in the *Monochromator wavelength* field in the *Channel Setup* menu. This will cause the monochromator to dial to the desired wavelength. Check the dial readout on the monochromator to confirm.




Data can be collected either from one, two, or three channels simultaneously, and the user can choose to display one, two or all three channels as well as any computed data (such as Fluorescence Anisotropy). In the example at the left, the instrument is set to collect data on PMT-2 and to display the results in the graphics panel.

For each channel, the sensitivity of the detector is controlled by adjusting the PMT HV (photomultiplier high voltage) to get a signal in the middle of the range (0-10 volts by default, but the range can can be set to 0-1 volt for low signals). Pressing Autoset, initiates a process where the instrument increases the HV until a signal of 5 volts is obtained (or 0.5 for a 0-1 V scale). Alternatively, the value for the PMT HV can be entered directly.

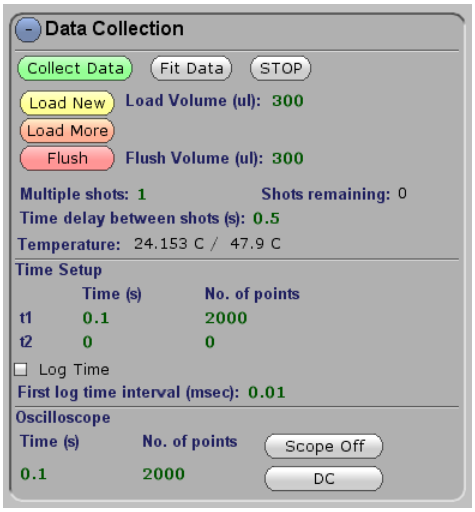
NOTE: These buttons display the current status and can toggle between the On and Off states. For example, see the "Display On" and "Display Off" buttons.

NOTE: Here and throughout the program, any number displayed in green text can be scrolled by simply clicking on the number with the mouse and dragging the value up or down with the mouse. Alternatively, clicking and releasing opens a text box to all entry of a number.

Also shown here are the buttons and measured valued for the dark current (Set Dark) and Reference value used for absorbance measurements.

For Absorbance measurements, set the monochromator wavelength and then turn on PMT3.
For Fluorescence measurements, the excitation wavelength is selected on the monochromator and a filter (25.4mm diameter) is placed in front of PMT 1 or PMT 2 to observe emission (*Figure 4*). Make sure the power is off to the PMT before removing the filter holder (click on the *PMT On* button to turn off the HV).

## D. Loading Samples




A single, automated valve is used to load the drive syringes by drawing solution from the pair of sample load cups on the top of the instrument. The standard sample load cups each hold about 1.5 mL of solution, but because they are conical, smaller volumes can be used without wasting material. We recommend a typical volume of 200 µL and a minimal volume of approximately 120 µL per sample.

Sample Load parameters are adjusted in the *Data Collection* menu section with two different volumes: *Load Volume (ul)* and *Flush Volume (ul)*.

This menu also allows control of the time for data collection, as described below, and the Oscilloscope mode, which is mainly used to lamp adjustment.

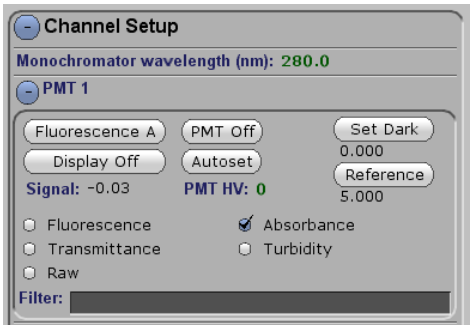
With your solutions in the load cups simply press Load New or Load More and the system will move the load/fire valve to the load position and draw in solution before returning the valve back to the fire position. If Load New is chosen the system draw in and discard 30 µL before loading the remaining solution. It will then fire the number of shots set in the *Bad shots to discard* setting (standard setting is 3 shots).

The *Bad shots to discard* setting is controlled in the *Instrument Setup* menu. This last setting is used to adjust the systems priming shots, which are used when you Load New samples in order to prime the system so that the next shot collected provides reliable data.

If Load More is used to load samples the same load volume is drawn into the system but no priming shots are fired.

## E. Adjusting Detector Sensitivity

The sensitivity of each detector must be adjusted to be within the appropriate range according to the intensity of the signal. Before adjusting the detector sensitivity flush fresh solution through the observation cell.




The PMT sensitivity is set by adjusting the photomultiplier high voltage power supply (PMT HV) which is done by computer control in the *Channel Setup* menu. You can enter a desired voltage and press return or if you click Autoset, the computer will increase the PMT HV until a half scale voltage (0.5 volt if working on 0-1.0 scale or 5.0 volts if working on a 0-10 volt scale) is achieved.

For fluorescence work it is important that a fluorescent sample be in the cell prior to adjusting the sensitivity. If a large fluorescence increase is expected, you may need to set the PMT high voltage manually, since using the *Autoset* feature sets the voltage at half scale and there is the possibility that the trace could go out of the scale.

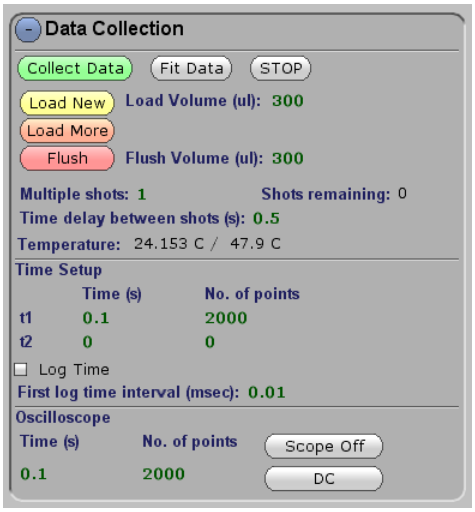
NOTE: The PMT High Voltage of PMT3 should be above 325 or you will see a wave in the PMT signal due to instability of the PMT. If your signal is too high (your HV is too low) you should narrow your monochromator slit width until the HV exceeds 325 V.

After the sensitivity of either detector is set, it is a good idea to measure the dark current (the signal in the absence of light). This is usually a small number and is subtracted from the readings. You can take a new dark current reading at any time by clicking on one of the Set Dark buttons under the *Channel Setup* menu. You will then be instructed to closed the slider on the monochromator and then re-open it after the reading is taken.

When working in absorbance mode you can take a new reference reading at any time by clicking on the Reference button under the *Channel Setup* menu. If a reference voltage is not recorded, the program will assume a value of half scale for calculation of a relative absorbance. The current reading for the reference voltage is displayed.

## *F. Setup for Data Collection*

Prior to collecting data, you must enter the data collection time under the *Data Collection* menu. In this menu you also have the option to collect over a log time scale.




You may collect data over either one or two time domains or on a log time scale. The log time domain option is particularly useful when there is uncertainty regarding how fast a reaction is occurring or if the reaction is biphasic and the two rates differ by greater than 10-fold.

Data are collected at a rate of 1 MHz (one million data points per second) and then data averaging is used to reduce the number of saved data points. The system defaults to save 2000 points for data collection per channel after averaging the raw data.

The time of data collection should be such that the reaction is monitored for 6-8 half lives.

If you desire one time domain, enter the time domain endpoint, e.g., 0.1 seconds, in the box immediately to the right of the *t1* label. If you desire two time domains, e.g., 0.1 seconds and 2 seconds, enter the first time domain endpoint and the number of data points you want collected, e.g., 2000, then in the t2 field enter the second time domain for which you wish to collect and the number of data points as with t1. When you enter and save two time domain endpoint values, a vertical line appears on the main screen dividing the field at the endpoint of the first time domain.

The software allows you to fire multiple sequential shots. Enter the number of shots you would like to fire with each data collection session in the *Multiple Shots* box. For example, multiple traces could be collected in succession and then averaged to improve the signal/noise ratio. Each time a shot is made, the number of shots remaining in the syringes is updated on the main screen at the *Shots Left* indicator.

In the *File* section there are fields for the contents of each syringe (A,B) as well as any experimental comments to be saved as part of the file. This is optional.

Finally under *Channel Setup*, select which detection channels you wish to use for the experiment: PMT-1, PMT-2, and/or PMT-3. In addition, the mode of data reduction must be selected for each

channel, i.e., Absorbance, Fluorescence, Transmittance, etc. Data can be collected from one, two, or three channels simultaneously. You may choose to view a single channel of data or any combination of channels as well as any calculated data (Anisotropy etc). Choosing to view a channel is independent from data collection on a given channel, you can collect data and not view that data if you prefer.

If you choose to collect in Absorbance mode, you should also set the reference value, if you have not already done so. To set a reference value, fill the cell with buffer, select Reference, and then take a reference reading at the desired wavelength. To set the dark current value press Set Dark. The computer will instruct you to close the input shutter on the light source, and then open it again to continue.

Additionally a calculated mode of data reduction can be selected in the *Channel Setup* menu. In this case, data will be collected on the channels selected and the ratio of either PMT-1 or PMT-2 will be compared with the transmitted signal. Or if your system is so equipped, Fluorescence Anisotropy can be selected here and your G Factor set. See Appendix F for more information on Anisotropy setup.

## G. Data Collection

Data Collection is initiated by clicking Collect Data in the upper section of the screen or in the *Data Collection* expandable menu.

## H. Saving and Recalling Data

Before manipulating any data, it is recommended that you save it first. Click on Save or Save As under the *File Menu*. Additionally, by pressing Export, data can be saved as a .txt file to allow you to import it into data fitting software or spreadsheets of KinTek Explorer software for fitting. Existing files can be recalled by clicking Open; in this case, previous files currently stored in memory will be overwritten. The Overlay button will overlay an existing file onto the currently loaded set of data. This can be used to combine multiple files.

As an alternative method of saving files, the program also has an auto filename function. By selecting the *Auto-Filename* check box in the *File Menu* section the software will save data (when Save is pressed) using the file location and name defined in the *Root name* field and add a numerical suffix which increments each time a file is saved (i.e. 0, 1, 2, 3 etc.).

## I. Data Editing and Fitting

Data from several traces can be averaged. There are several ways that traces can be selected for deletion or averaging. Individual traces are selected either by clicking on the curves directly or in the *Fit Results* menu by clicking the box next to a desired trace. The trace color changes to white when it is selected. Each trace can be de-selected in the same manner. There is also the option to select or deselect all traces in the *Data Editing* menu as well as the option to perform operations to all channels or only the selected channel. Upon clicking Average only the selected traces will be averaged, they will also be deleted if the "Delete traces after averaging" box is checked in the *Data Editing* menu. *NOTE: The original data will be lost in the process, so be sure to save the data before averaging if you want to recall it later.*

Data can be fit to various functions under the Date Fitting menu. One can also turn on the automatic fitting of each trace after it is collected.

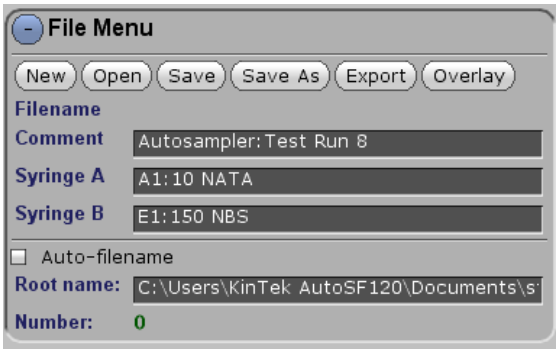
## *J. Instrument Shutdown*

Flush the syringes with buffer, and then water. Distilled water can be left in the system or for longer down times a 10% Ethanol solution can be used. The lamp, control unit and computer can be turned off in any order. *Leave the water circulating through the arc lamp housing for a minimum of 15 minutes after powering off the power supply to the lamp.*

# III. Description of Commands

In this section, detailed descriptions are given for individual menu commands.

## *A. File Menu*




New - Start a new file. Erases all current data and will prompt if that data is unsaved.

Open - Open and load a previously saved file.

Save - Save the current traces to the current filename. If a file has been saved previously with the same filename it will overwrite this file. If no filename has been specified you will be prompted to enter one.

Save As - Save the current traces to a new filename.

Export - Saves the currently open data to a .txt file, allowing import into KinTek Global Kinetic Explorer Pro, Excel or other software for data analysis.

Overlay - Overlay a previously saved data file on the currently open data for comparison.

**Filename** – Shows the current filename.

**Comment** – A text box the user can enter a description of the experiment.

**Syringe A** – A text box for the contents of syringe A

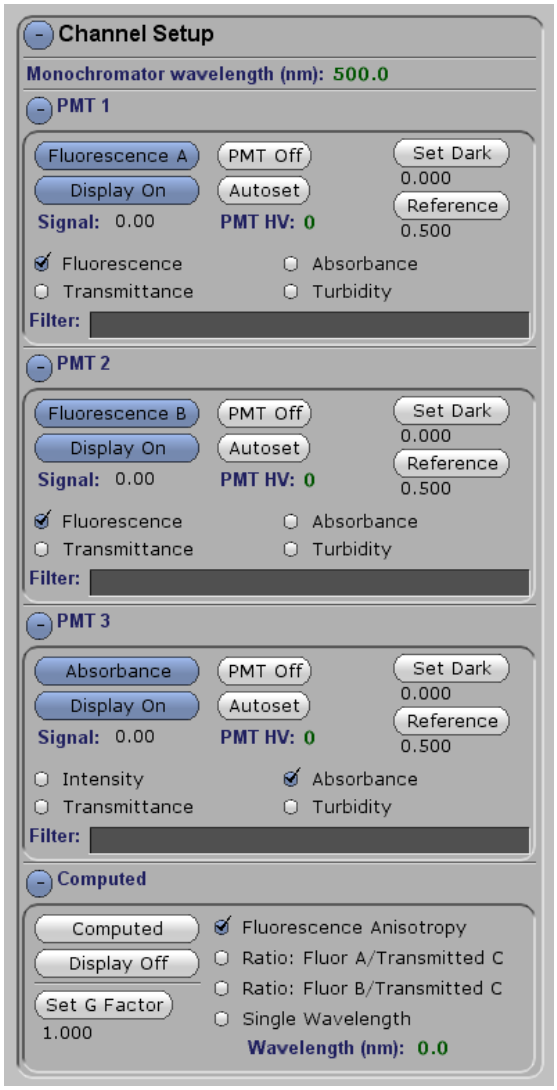
**Syringe B** – A text box for the contents of syringe B

**Auto Filename** – If selected, then an automatic filename is used which contains a count which increases with each save.

**Root Name** – The base filename for auto filename.

**Number** – The current file number count that is appended to the root filename with the Auto Filename function. The user can change this field, but it is incremented by 1 each time a file is saved.

## *B. Channel Setup*




**Monochromator wavelength (nm)** – Set the desired wavelength of the monochromator in this field. Auto wavelength drive will adjust the monochromator to the correct wavelength.

**PMT 1, PMT 2, Photodiode, Computed** – Select the + or – next to each label to open or close each section of the menu as desired. Menu does not have to be open to use that detector.

Fluorescence A Fluorescence B Absorbance Computed - These buttons select channels and toggle the channels on and off. When a button is filled in with blue that channel is on and collecting data, when the button is white it is not active. The labels of the buttons do not update with the type of reaction selected.

Display On Display Off - Display status button for each channel, status will update when selected as well as color. User may choose to display one, two, three or all four channels to be viewed and you do not have to view a channel to collect data on that channel.

PMT Off - Displays current state of photomultiplier power and allows the user to toggle its status.

Autoset - Autoset the PMT high voltage level. The software will adjust the PMT high voltage to achieve a signal level of half scale (0.5 volt if using the 0-1 volt scale or 5.0 volts if using the 0-10 volt scale).

Set Dark - Set the dark current level for each channel. When selected the software will prompt the user to close the light source shutter, and then after the reading has been made will again prompt the user to reopen. Selecting Set Dark will set the dark current level for all channels.

Reference - Used to set the reference level when needed for a given data type (eg. Absorbance) Selecting Reference will set the reference level for all channels.

**Signal** – Displays the current signal level of the detector.

**PMT HV** – Displays the high voltage level of the photomultiplier. User can click on the number and manually set the voltage if they do not want to use the Autoset feature or can be used to adjust the level if desired.

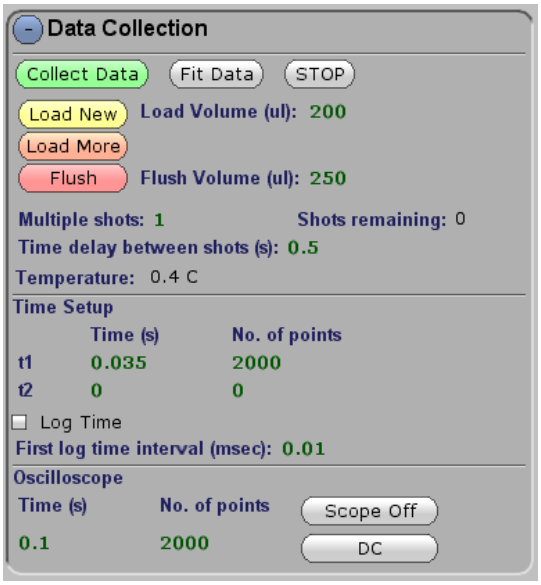
**Data Type** – Each detector has a list of types of data that can be collected. Fluorescence, Absorbance, Transmittance, or Turbidity.

**Filter** – Text field that allows the user to have a record of the filter used in each channel.

Set G Factor - Used to set the G Factor for fluorescence anisotropy experiments. Software will prompt user to set Glan Taylor prism to specific positions during this process (Optional accessory).

**Computed Data Types** – User can select from Fluorescence Anisotropy, Ratio: Fluor A/Transmitted C or Ratio: Fluor B/Transmitted C.

## *C. Data Collection*




Collect Data - Button used to fire a shot.

Fit Data - Fits currently collected data. If traces have not been averaged it will fit each trace individually.

STOP - Stops a multiple shot experiment.

Load New - Loads new sample (amount set by "Load Volume", first emptying the syringes of any remaining sample. After loading new sample the system will prime itself using the number of shots to discard set in the instrument setup section.

Load More - Loads additional sample (amount set by "Load Volume") without discarding any sample currently in the syringes.

Flush - Flushes the system with the following steps. 1) Discards any loaded sample remaining in the drive syringes. 2) Loads a small amount from the load cups and discards. 3) Loads the rest of the set flush volume and discards.

**Multiple shots** – Gives users the ability to let the system automatically collect sever shots with a single press of the Collect Data button.

**Shots remaining** – Displays the number of shots remaining. The software calculates the number of shots remaining based on the drive plate position, the syringe volume and the volume per shot.

**Time delay between shot (s)** – This is the amount of time the system waits between shots on a multiple shot experiment. The timer starts when data collection has completed.

**Temperature** – Displays the temperature as read by the temperature probe in the syringe tower.

**Time Setup** – Fields for data collection time(s) and number of points of data to be collected. Also allows user to select log time interval is desired.
**Oscilloscope** - Section allows system to be put into oscilloscope mode, which is useful for setting up the light source. User can set time period to be displayed and number of points over that time period.

Scope Off - Toggle oscilloscope on and off.

DC - Toggle between AC and DC signal.

## D. Data Editing


**Apply operations on all channels** – When selected any data editing will be applied to all channels.

All - Selects all traces of data.

None - Deselects all traces.

New - Selects all data that has been collected since the last averaging. If you take several traces of data that you fit (or open a previously saved data file) and then collect additional data, pressing New will select only the second set of newly collected data.

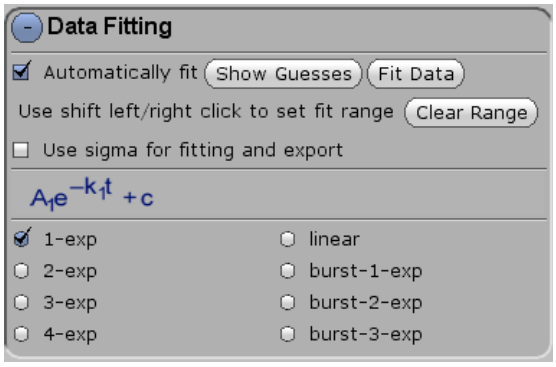
Invert - Inverts the selected traces. Any traces that are selected will be deselected and any traces not selected will be selected.

**Delete traces after averaging** – When selected all traces will be deleted when the Average button is pressed and replaced with the averaged data.

Delete - Deletes the selected traces.

Average - Average the selected traces.

## E. Data Fitting




**Automatically fit** – If selected the software will fit each trace as it is collected.

Show Guesses - Toggles whether the initial guesses for the fits for each trace is plotted as a white curve. It will also enable or disable the "Guesses" checkbox in the plot panel for each channel.

Fit Data - Fits currently collected data. If traces have not been averaged it will fit each trace individually.

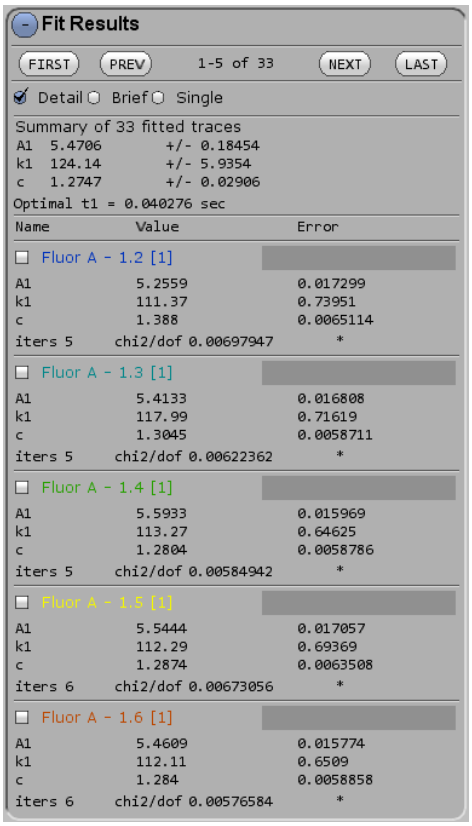
Clear Range - Clears any data fit range manually selected by the user.

**Use sigma for fitting and export** – When multiple traces are averaged, an error or sigma value is assigned to each averaged point, based on how much scatter there is in the values being averaged. These sigma values can be displayed via the 'Errors' checkbox in the Plot Control panel.

When selected, these sigma values are used to weight the fits, so that points with small sigma values are weighted more. Also, the sigma values will be saved in exported files. When unselected, sigma values are ignored.

**Equation Display and Selection** – The software displays the equation being used for fitting and allows 8 different equations to be used as listed.

## F. Fit Results




Displays trace and fit details for the currently selected channel. If there are fitted traces, a summary of the average of the fit coefficients is displayed near the top of the panel.

FIRST - Brings data set to the first page of data.

PREV - Changes to the previous (one page less) page of traces.

NEXT - Changes to the next page of traces.

LAST - Changes to the last page of traces.

**Detail** – Detail display state shows all fit information for each trace collected and the summary of the fit data. Displays 5 traces of data per page.

**Brief** – Displays only trace names but also includes the summary information for the fit data. Displays up to 20 traces per page.

**Single** – Displays one trace at a time and shows detailed data as well as the summary information for the data set.

## G. Plot Control


**Selected Plot** – This controls which plot channel is selected. The selected channel is displayed with a blue border around the plot. The plot controls in this panel apply to that selected channel only. Clicking on the plot panel will also select that channel.

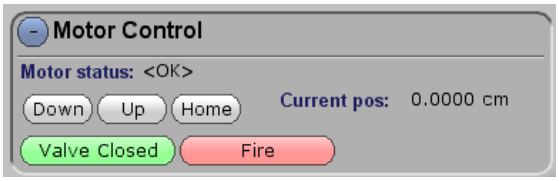
**Show** – Controls which elements of each trace are displayed. You can individually choose: Points at each data point, Lines between each data point, Errors showing sigma values as translucent bars, Guesses for fits as white curves, and Fits as black curves.

**Point Size/Line Thickness/Num Sigma Values:** Controls the size of the plotted trace elements.

**Plot Range** – Controls and displays the plot range. When Auto is selected, the range is automatically determined from the trace data, and the resulting range is displayed as read-only text. When User is selected, then the user can choose a specific range by editing the green values. The range can also be selected by left click and dragging the mouse to choose a selected range.

Reset Ranges - Resets all plot ranges to Auto.
Zoom Out - Zooms out the selected plot.

## *H. Motor Control*




**Motor status** – Displays current status of motor and drive.

Down - Moves the drive plate down when pressed. Hold for continuous movement.

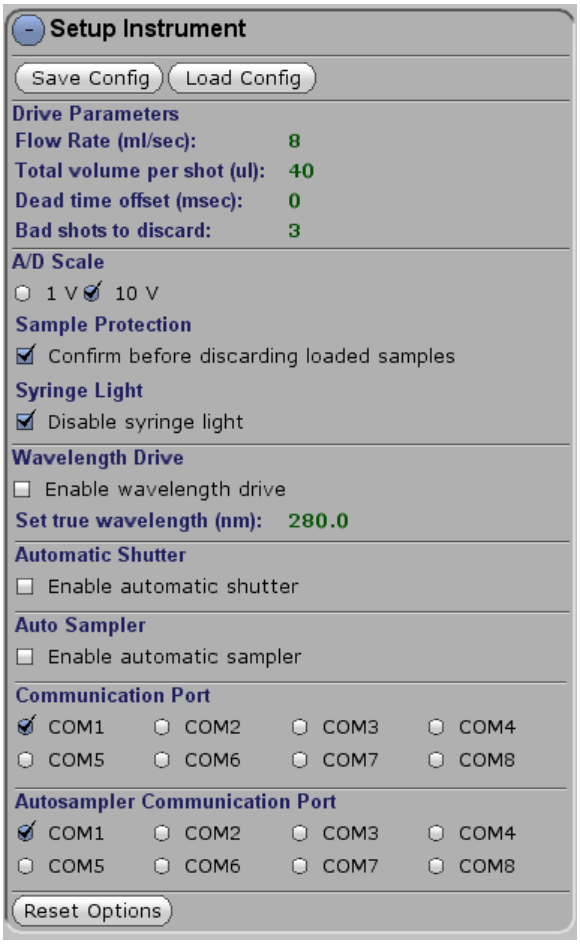
Up - Moves the drive plate up when pressed. Hold for continuous movement.

Home - Moves the drive plate to the "Home" position (upper most position) when pressed.

Valve Closed - Displays the current status of the stop valve and allows the user to toggle the valve open or closed.

Fire Load - Displays the status of the LOAD/FIRE valve.

## *I. Setup Instrument*




Save Config - Saves the current configuration to the instrument configuration file.

Load Config - Loads a saved instrument configuration file.

**Drive Parameters** – The instrument's "Flow Rate", "Total volume per shot", "Dead time offset" and "Bad shots to discard" can be set here and will be saved in the instrument configuration file.

**A/D Scale** – The user can select a 0-1 volt scale or a 0-10 volt scale. Default is 0-1 volt.

**Wavelength Drive** – If system is equipped with the optional wavelength drive this box should be checked. Enter the true (as read on the monochromator) in this field to calibrate the drive.

**Automatic Shutter** – Select if your system is equipped with the automatic shutter this box should be selected to activate its features.

**Communication Port** – Not used.

**Autosampler Communication Port** – This is the serial port used for communication between the PC and the Autosampler accessory. Typical setting is COM1

Reset Options - If pressed the software will reset to the default values.

# Appendix

## *A. Test Reactions*

Principle of the method:

Reaction of N-bromosuccinimide with N-Acetyl tryptophanamide leads to bleaching of tryptophan fluorescence as described by Peterman (1979) Analytical Biochemistry 93, 442-444. The reaction is done with NBS in excess, so the concentration of NBS determines the rate of the reaction. With a second order rate constant of 0.7 $\mu M^{-1}$ $s^{-1}$, a concentration of 200 µM NBS gives a reaction at a rate of 140 $s^{-1}$. The signal to noise ratio is governed by the concentration of N-acetyl tryptophanamide and we typically use about 10 µM giving a large signal that can then be used to evaluate flow artifacts.

Fluorescence is excited at 280 nM and observed at 340 nm with a band-pass filter or with a 320 nM high pass cutoff filter.

Stock solutions:

1 mM N-acetyl tryptophanamide is pH 7 phosphate buffer. It can be stored in the refrigerator for up to 6 months. Dilute 100 x to make working solution.

NBS must be made up fresh daily as it reacts slowly with water. It is slow to dissolve, so put approximately ~4 mg into 100 ml and stir for 10-15 minutes until dissolved. Use undiluted in the stopped-flow, or dilute to get slower reaction.

Calculations:

NBS (FW=178 g/mol )
178 g/mol x 0.1 L x 0.0002 M = 0.0036 g in 100 ml

N-acetyl tryptophanamide (FW = 245.3 g/mol)
245.3 g/mol x 0.1 L x 0.001 M = 0.0245 g in 100 ml

## *B. Maintenance*

The following procedure outlines the approved method for cleaning the AutoSF-120 syringe and observation cell assembly, and should be done as needed to maintain stable data for your instrument. In particular, if you observe erratic, irreproducible data it could be due to a plugged mixer, and can be remedied by following this cleaning procedure.

Materials needed:

- Deionized (DI) Water
- 2N NaOH solution
- 2N HCL solution
- Leur Lock syringes to load solutions
- Breaker for waste solutions

Warnings:

- Observe all regulations regarding the storage and disposal of hazardous materials.
- Always use appropriate safety precautions for handling of Acids and Bases to prevent injury.

Procedure:

1. Flush instrument with DI Water.
    a. Fill sample load cups with DI water.
    b. Flush the instrument using the KinTek SF program "Flush" routine.
    c. Repeat this process three times.
2. Flush system with 2N NaOH solution.
    a. Fill sample load cups with the 2N NaOH solution.
    b. Flush the instrument using the KinTek SF program "Flush" routine.
    c. Press "Load More" in the SF program to draw additional NaOH into drive syringes. Be sure that the load ports have sufficient remaining volume to avoid drawing air into the system.
    d. Allow solution to soak. The duration of this soak time is dependent on your reactants and the frequency this process is performed.
3. Flush with DI water (see step 1).
4. Flush system with 2N HCL solution by forcing it backwards from the waste syringe.
    a. Fill sample load cups with the 2N HCL solution.
    b. Flush the instrument using the KinTek SF program "Flush" routine.
    c. Press "Load More" in the SF program to draw additional HCL into drive syringes. Be sure that the load ports have sufficient remaining volume to avoid drawing air into the system.
    d. Allow solution to soak. The duration of this soak time is dependent on your reactants and the frequency this process is performed.
5. Flush with DI water (see step 1).

Duration of Soaks in steps 2.d. and 4.d.:

The duration required for the soaks of both NaOH and HCL solutions will depend on the frequency of cleaning and the reagents used in the instrument. For best results with the stopped flow it is important that the system be clean and flowing freely. Deposits in the Load/Fire valve, the fluid pathways, mixer or flow cell will impact the performance and longevity of the instrument. Some users run this cleaning between every sample, in this situation the duration of the soaks could be as short as 1-3 min depending on the likelihood of your specific reagents to deposit. If you choose to perform this cleaning daily we would suggest 5 min soaks and if you only perform it weekly then we would suggest between 5 and 10 min. If the instrument is in regular operation we do not suggest going longer than a week between cleanings.

## C. Electronic Adjustments

Several adjustments can be made electronically on the printed circuit board in the Control Unit. To access the circuit board, first remove the top from the control unit (attached by two screws on the top and three on the back). Refer to *Figure-4* to locate the potentiometers used for the following adjustments.

### 1. Temperature Calibration

The temperature probe can be calibrated by adjusting resistors in the upper left corner of the circuit board; Temp Offset and Temp Slope. Adjust the Temp Offset with thermometer and probe in ice to adjust values around zero. Insure probe has stabilized prior to adjusting. Adjust the slope to get the correct reading with the probe and thermometer immersed in a solution at a higher known temperature (~40 Degree C).

### 2. Output Zero Adjustment

If reading a dark current supplies a negative value, you will need to use the zero adjustment through the signal channels of the detectors. To adjust the photodiode zero value close the shutter. In the program under *Data Collection* menu click Scope Off. The other channels are adjusted the same way.

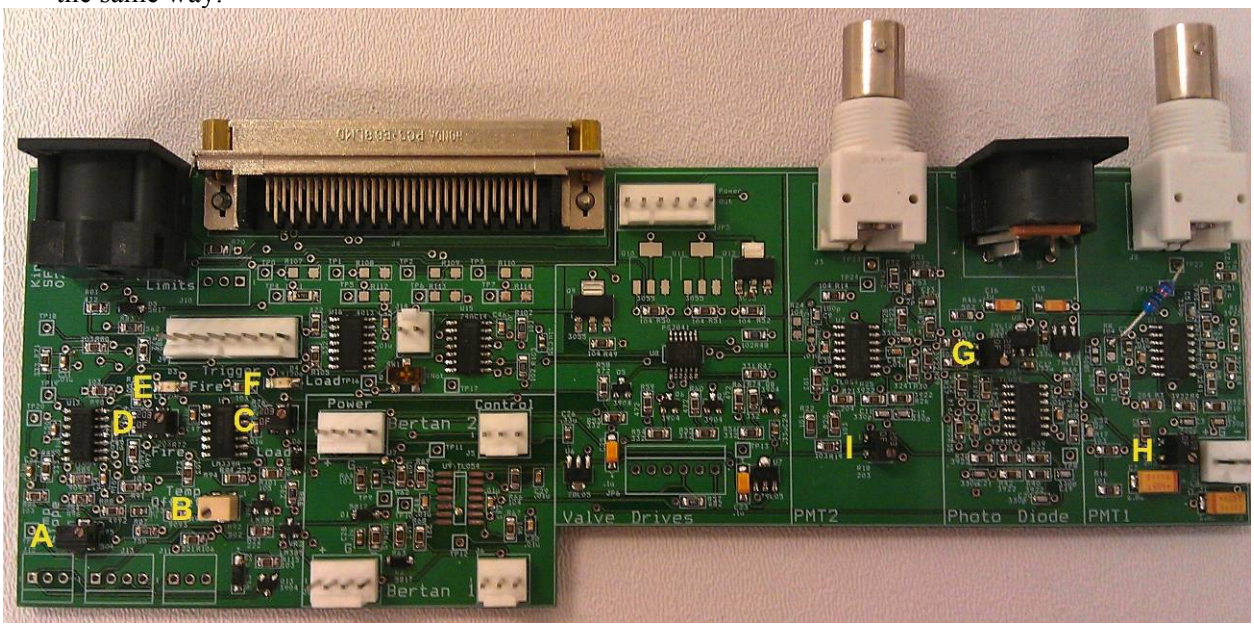



**Figure-4:** ***Printed Circuit Board.*** A. Temp Slope B. Temp Offset C. Load Sensor Adjustment D. Fire Sensor Adjustment E. Fire LED F. Load LED G. Photodiode Zero Adjustment H. PMT-1 Zero Adjustment I. PMT-2 Zero Adjustment

## *D. Parts and Accessories*

**Syringe Plungers:** Syringe plungers are available for order from the factory and are a custom part for KinTek.

KinTek Corporation
500 West Sycamore Road
Snow Shoe, PA 16874
Phone: 814-387-4678
FAX: 814-387- 4974

| Part Number | Description |
|---|---|
| 86-126 | 2.5 ml Plunger Assembly |

**Optical Filters:** Interference filters and all other types of filters can be obtained from several sources. The company that we recommend is listed below.

Semrock, Inc.
3625 Buffalo Road, Suite 6
Rochester, NY 14624
Phone: 585-594-7000
Fax: 585-594-7095
www.semrock.com

**Arc Lamps:** Our system is designed around a Hamamatsu Super Quiet Mercury/Xenon and Xenon Arc Lamps. We stock these items and part numbers are listed below.

27-225 150 Watt Super Quiet Mercury/Xenon Arc Lamp
27-226 150 Watt Super Quiet Xenon Arc Lamp

## *E. Anisotropy Setup and Experiments*

Fluorescence anisotropy experiments can be easily performed using instruments equipped with our fluorescence cell and the optional anisotropy filters. There are three filters in the anisotropy set, two side film polarizers and one Glan-Taylor polarizer.

The Glan-Taylor polarizer is held inside a holder that is attached directly to the fiber optic cable in place of the standard fiber optic cable holder. To change fiber optic cable holders, first pull holder from the rear of the cube block assembly, then loosen the setscrew and remove the light cable. Insert light cable into Glan-Taylor polarizer holder assembly, tighten the setscrew, align slot with pin, and then push on (*Figure-6*). The Glan-Taylor polarizer is in the vertical position when the position lever is pointing straight up (*Figure-11.a*). When its position lever is pointing to side, it is in the horizontal position.

The two side film polarizers are also set from the factory. The left PMT (PMT-1) is designated vertical, and the right PMT (PMT-2) is designated horizontal. The filter sliders in the Anisotropy set, including polarizers, should replace the normal filter sliders. Be sure that the PMTs are off when replacing the filter sliders to prevent damage to the PMTs. After loading your sample, set the HV for PMT-1 with the input Glan-Taylor polarizer in the vertical position. Then change the input polarizer to the vertical position and set the HV for PMT-2. Next select the *Computed* channel and check the box for Fluorescence Anisotropy and then measure the G-factor by pressing Set G Factor and following the software prompts. Namely, with the Glan-Taylor prism in the horizontal position (see *Figure-11.a*) click *OK* at which point the G-factor is measured and the program asks you to **return the Glan-Taylor polarizer to the vertical position**, which you can do by moving the position lever to the top of the fitting. Finally you must make sure that Computed is highlighted in blue indicating that it is turned on for collection (computation). Like all channels, you do not have to have the *Computed* display on to collect *Computed* data.

Now that the G-factors are measured, the program is in fluorescence anisotropy mode and the filters are in place you can perform anisotropy experiments. The anisotropy trace can be fit and manipulated just like a standard trace.

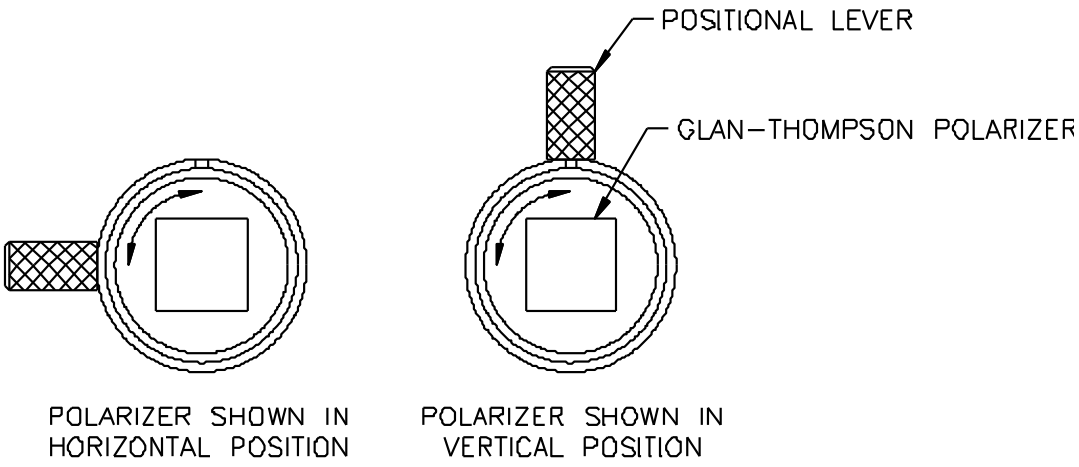



**Figure-11.a:** ***Polarizer Position.***

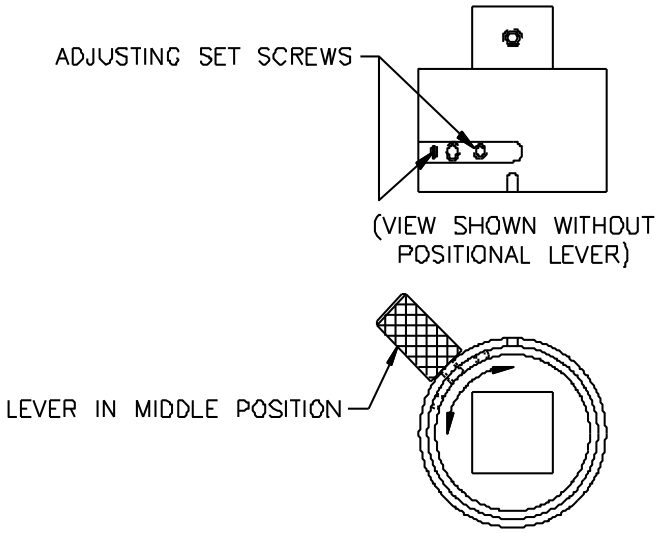



**Figure-11.b:** ***Polarizer Adjustment.***