KinTek Corporation

Stopped-Flow
Model SF-300X

Instruction Manual
Rev 7.0.2

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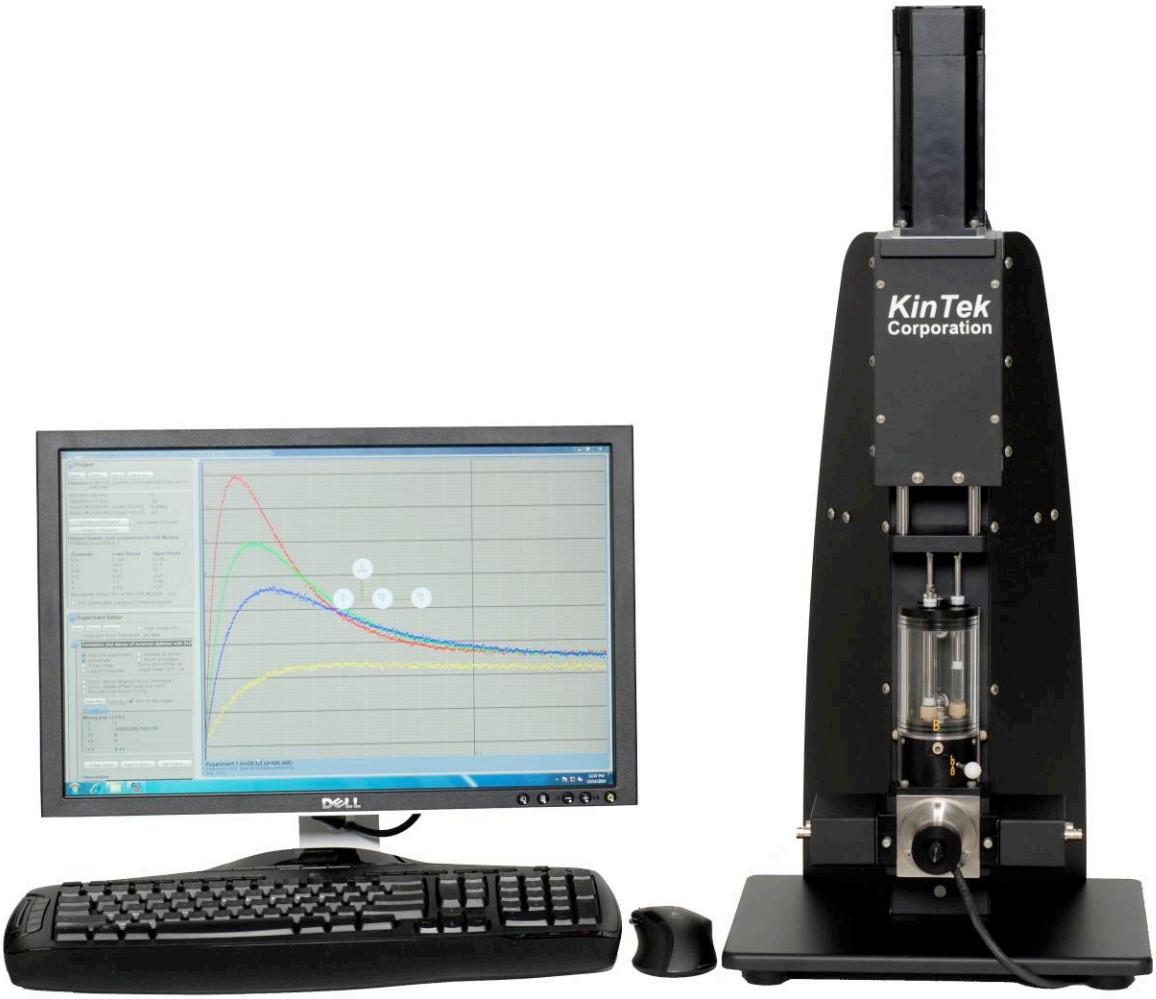
Table of Contents

How to Use This Manual

Section I describes the physical set-up of the equipment. Section II provides basic instructions for preparing to conduct an experiment using the KinTek Stopped-Flow system. Detailed descriptions of individual software menu items can be found in Section III. The manual will offer instructions for the Stopped-Flows manufactured by KinTek, the standard SF-300X. 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.



I. Physical Assembly of the System

A. Electrical Connections

Connections need to be made between the Motor Control Box and computer, and the Motor Control Box and the Stopped-Flow Drive Stand. With one exception (noted below), the electrical connections cannot be made incorrectly. Each connection is described separately below.

Electrical Connections from the Computer to the Motor Control Box:

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. (If facility provides the computer use instructions supplied by manufacturer).
2. Before attaching any cables insure power is off.
3. After computer connections are made (mouse, keyboard, and monitor), there are only two that need to be connected between the driver and the computer. The first cable is a 9-pin serial cable. It has a male and female end. The male end is attached to the driver. The connector is labeled "*Serial Cable Connection*" on the back of the driver. The female end is attached to the computer. There is only one 9-pin port in the back of the computer; it is marked with "*lOlOl*" markings below the port. The second connection is the 68-pin data cable. This cable has two distinct ends, the smaller silver connector is the PC end and the larger blue connector is the drive end. Plug the cable into the A/D Board located towards the bottom of the computer in the back. The opposite end is attached to the driver port marked "*A/D Board Connection*". The final connection will be the power cables to the computer and monitor.

Connections from the Motor Control Box to Stopped-Flow Drive Stand:

1. Plug the valve position/temperature sensor cable (7-pin connector) into the back of the Motor Control Box. This connection is marked *"Temp, Load/Fire*". The cable is coiled, twist tied, and tucked under the rear brace of the stand. The valve position sensors and temperature probe are already connected in the back of the Stopped-Flow Stand (*Figure-3)*. If the incorrect position is shown on the computer readout, refer to Section C-3 of the Appendix.
2. Plug the Photodiode Cable into the back of the Motor Control Box. This connection is marked "*Diode Signal*". The photodiode is attached to the front of the Steel Cube with three Socket Head Cap Screws (6-32 x 3/8 inch) using a 7/64" Allen Driver *(Figure-2)*.
3. Connect the Photomultiplier (PMT) to the Steel Cube with four Socket Head Cap Screws (6-32 x 3/8 inch) using a 7/64" Allen Driver *(Figure-2)*.

   NOTE: Due to the nature of its operation the PMT is highly sensitive to light. Any time a PMT is removed from the SF-300X the lights in the room should be dimmed and any ambient light should be limited. Covers are provided for each PMT to protect it when not in use.

4. If a second PMT was ordered, please repeat previous installation step. If only one PMT is ordered it can attach to either side of the cube. Insure black cover is installed on the opposite side. A 320 nm Long-pass filter for protein Fluorescence is provided with the unit. (See Filter Installation Instructions)
5. There are two cables to connect for each PMT. These cables are not identical and you cannot connect the wrong cable to the wrong connector on either the Motor Control Box or the PMT. The small connectors on flexible grey cable are for the Photomultiplier High Voltage. The BNC cables are for the Photomultiplier Signal, they are black in color with silver BCN connectors. Connect the high voltage cable from the PMT to the high voltage output on the Motor Control Box. Connect the signal cable from the PMT to the PMT signal input on the Motor Control Box.

Repeat if second PMT is supplied. If the system only has a single PMT, a 50-ohm BNC terminator must be put in place on the Motor Control Box where the 2nd PMT signal cable would attach.

6. The Stop-Valve should be connected to the bottom of the stainless steel cube (*Figure-1*). If the line has already been connected to the cube you must connect the line to the Stop-Valve itself. Please insure plastic ferrule is between the Stop-Valve and 2-Inch Line. If any of the Minstac connections are disconnected, always insure the ferrule is installed between tube and surface. The end of the Stop-Valve Cable has a black female 4-pin connector. This will be attached to the male connection marked "*Stop-Valve*" on the back of the control unit.
7. The servomotor is connected to the Motor Control Box with two cables, Motor Power and Motor Feedback. The Motor Power cable is a heavy black cable with 7-pin spin on connectors. Either end of the cable can be connected to the drive stand or the Motor Control Box. The Motor Feedback cable is a tan 15-pin D-Sub cable. The male end of this cable is connected to the drive stand and the female end is connected to the Motor Control Box connection marked *"Motor Feedback"*.

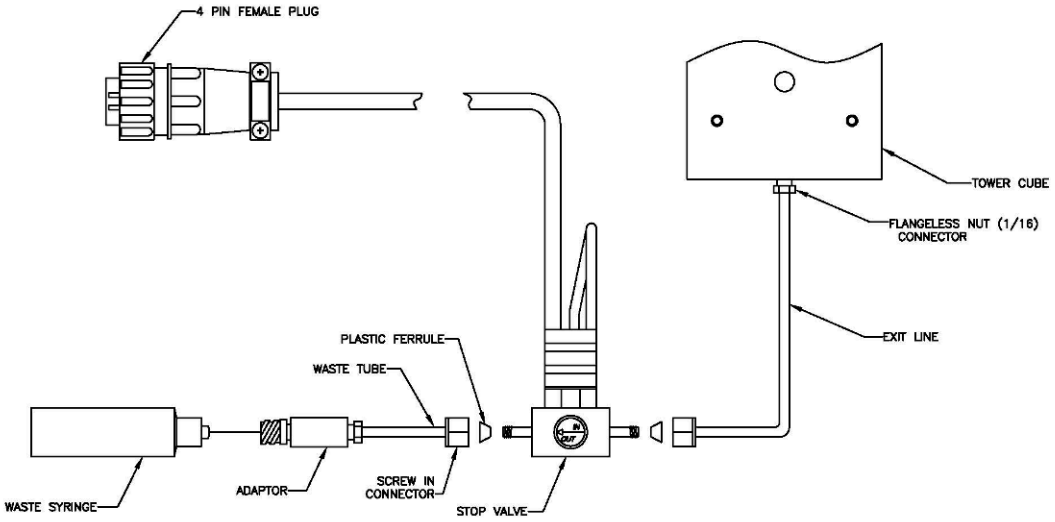


Figure-1: ***Drawing of Stop-Valve and connections.***

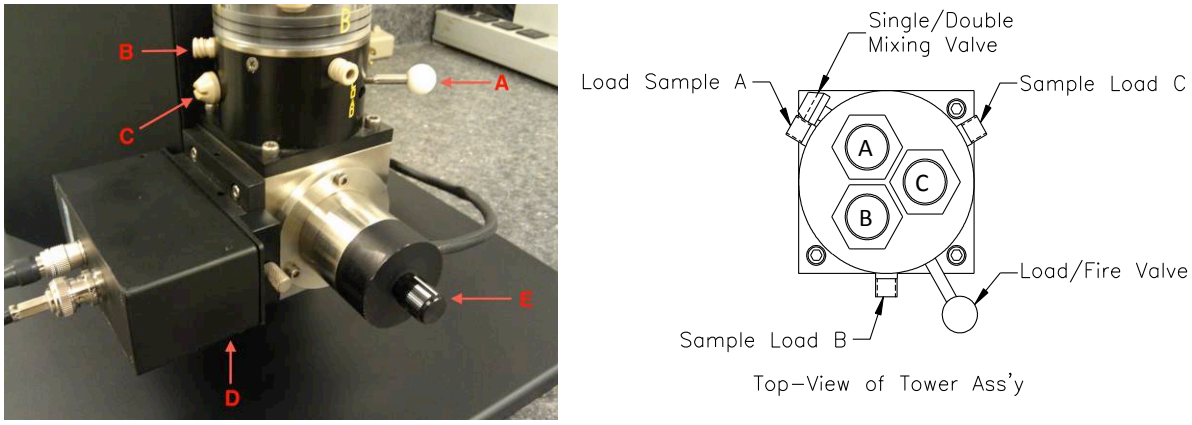


Figure-2: ***The Tower Assembly***. A. Load/Fire Valve for syringes
B. Sample loading fitting C. Double Mixing Valve D. Photomultiplier E. Photodiode

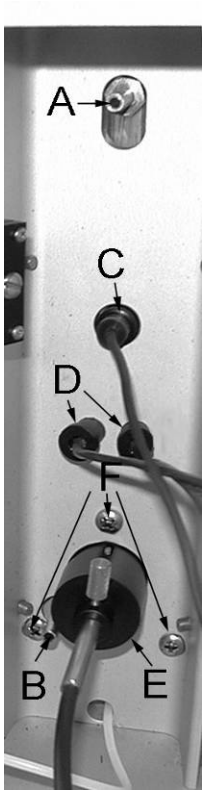


Figure-3: ***The connections to the Stopped-Flow drive stand.*** A. Water Out.
B. Water In. C. Temperature probe insert. D. Optical Load/Fire sensors for valve position. E. Fiber optic cable adapter with fiber optic cable inserted. F. Three attaching screws for Tower Assembly.

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 (*Figure-3)*. Water circulates through the stainless steel 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 stainless steel block (Water In). Connect the input (return) to the water bath from the tubing connector located at the top of the syringe chamber. A solid-state temperature sensor is located in the syringe chamber, providing a continuous readout to the computer (*Figure-3*). The temperature readout can be calibrated as described in Section C-1 of the Appendix.

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). Because vibration from the surrounding sources can transmit noise to the arc lamp if they are placed on the same table, it is best to place the light source on a shelf above the Stopped-Flow unit. 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-4*. Although tap water can be used, mineral deposits from tap water can clog the capillary tubing in the lamp housing after several years of use. For this reason, it is preferable to use distilled water in the supplied water bath and to change the water regularly.

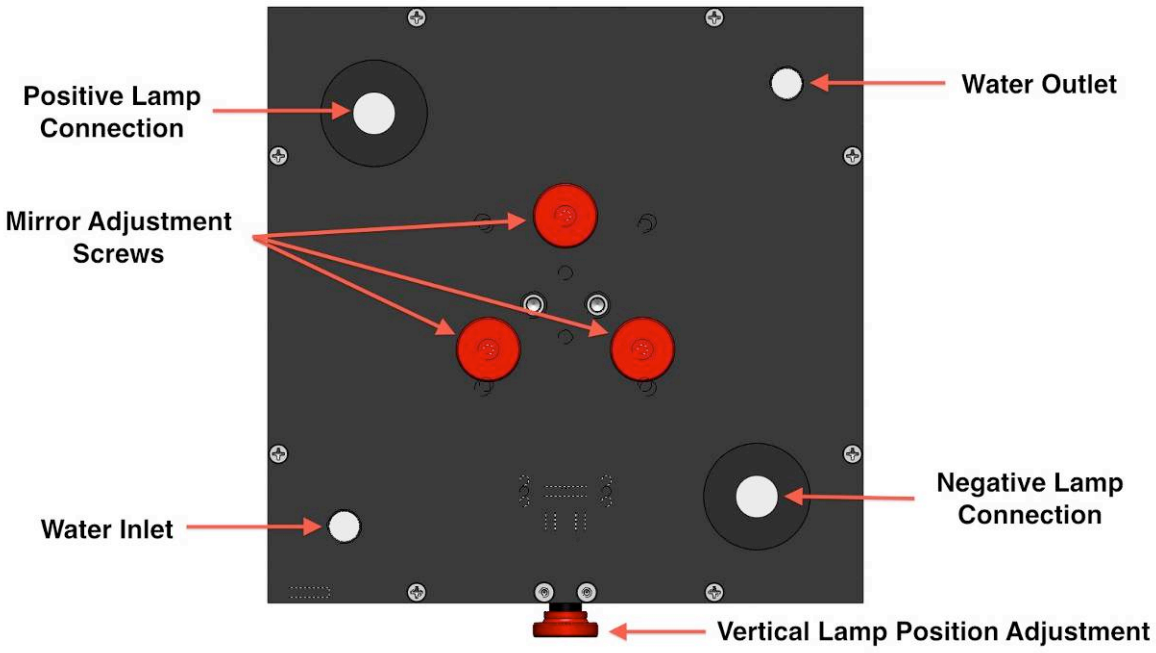


Figure 4: Arc-Lamp Housing

2. 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 the eyes in the unlikely event that the lamp cracks during installation. Remove the lamp from the packaging. Remove thumbscrews 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 tighten thumbscrew to hold. Slide the free-moving cooling tube over the positive end (+), slide the green wire over the stud, and then tighten the thumbscrew. There is an exhaust nipple protruding from the lamp. The lamp should be installed with the nipple facing away from the mirror. Screw the arc lamp housing back into the light source.
3. The grating mirror for the monochromator should be removed for transportation and will need to be installed. 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 at the rear bottom (*Figure-5*). You will use a 7/64" Allen wrench to loosen these screws. Pull the lid towards the ceiling. 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 mirror into the rectangular slot. Hold on to the extension on the mirror. The mirror will seat into the slot with very little play in any direction. Return the lid and tighten screws.
4. 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. The black negative cable has a black male plug; this 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.
5. Insert the optical fiber cable into the holder on the exit slit of the monochromator. There is a black plastic adapter that fits onto the other end of the fiber optic cable (*Figure-6*). The adapter should be attached. The last connection is to press the black adapter onto the lens holder in the back of the instrument. There is a small steel pin to orient in the lens holder and a slot in the adapter for positioning.
6. Final 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. This is done by turning on the power to the power supply and then pressing the light green button to ignite. 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 references the lamp we utilize is the L2274 Xenon or the L7047 Mercury-Xenon. The current selection for this lamp is listed on the top of the power supply. It is standard to let the lamp warm-up for at least 15 minutes. Next, you will boot up the computer and turn on the driver. Load distilled water into syringe "A" and "C". Do not fill syringe "B", this syringe is only used for double mixing experiments. See "Loading the Samples" section of manual. Place the "LOAD/FIRE" selector knob to the "FIRE" position. Go into the KinTek Software. Across the top menu, click 

and then 

to drive water from the syringes into the observation cell. After pushing about 1.0 ml through the observation cell, click 

then 

to view the signal output of the photodiode. Click 

and then turn the display status off for all detectors other than the photodiode channel. Increase the output

signal by turning the knob of this detector clockwise; if the signal is too high then turn counterclockwise. Each click increases/decreases the signal by a factor of two. To start, the output signal should be around 5.0 volts. To adjust the lamp intensity, turn each of the three screws while watching the computer monitor to get the maximum intensity from each position. Work your way around several times until the signal no longer increases. If the signal exceeds 10.0 volts, turn the output knob one click counterclockwise to decrease the signal. For checking the maximum intensity, turn the photodiode knob all the way counterclockwise, then turn clockwise until trace is around 5 volts (+/- 1.5 volt). This should happen after five to six clicks. 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. 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.

Figure-5: ***Light Source***

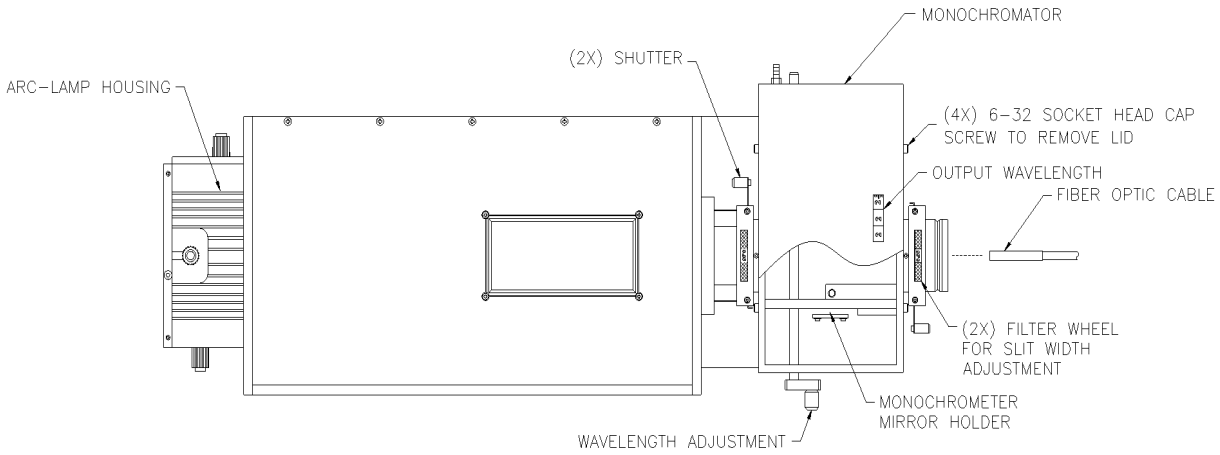
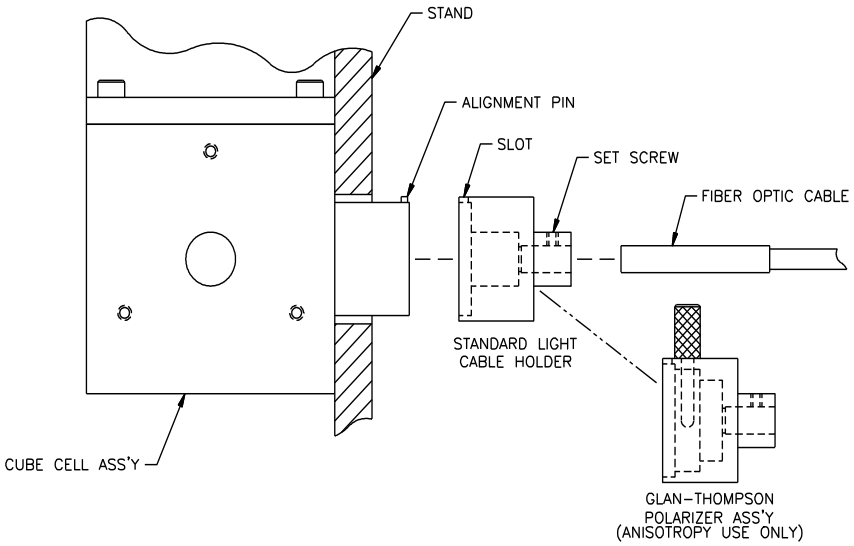


Figure-6: ***Connection of fiber optic cable to instruments equipped with the fluorescence flow cell.***



D. Filter Installation

The SF-300X uses sliding filter holders for to installing fluorescence channel filters. To install a long-pass filter, place the filter into the holder and then insert the included retaining ring to keep the filter from falling over. 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 25.4mm in diameter.

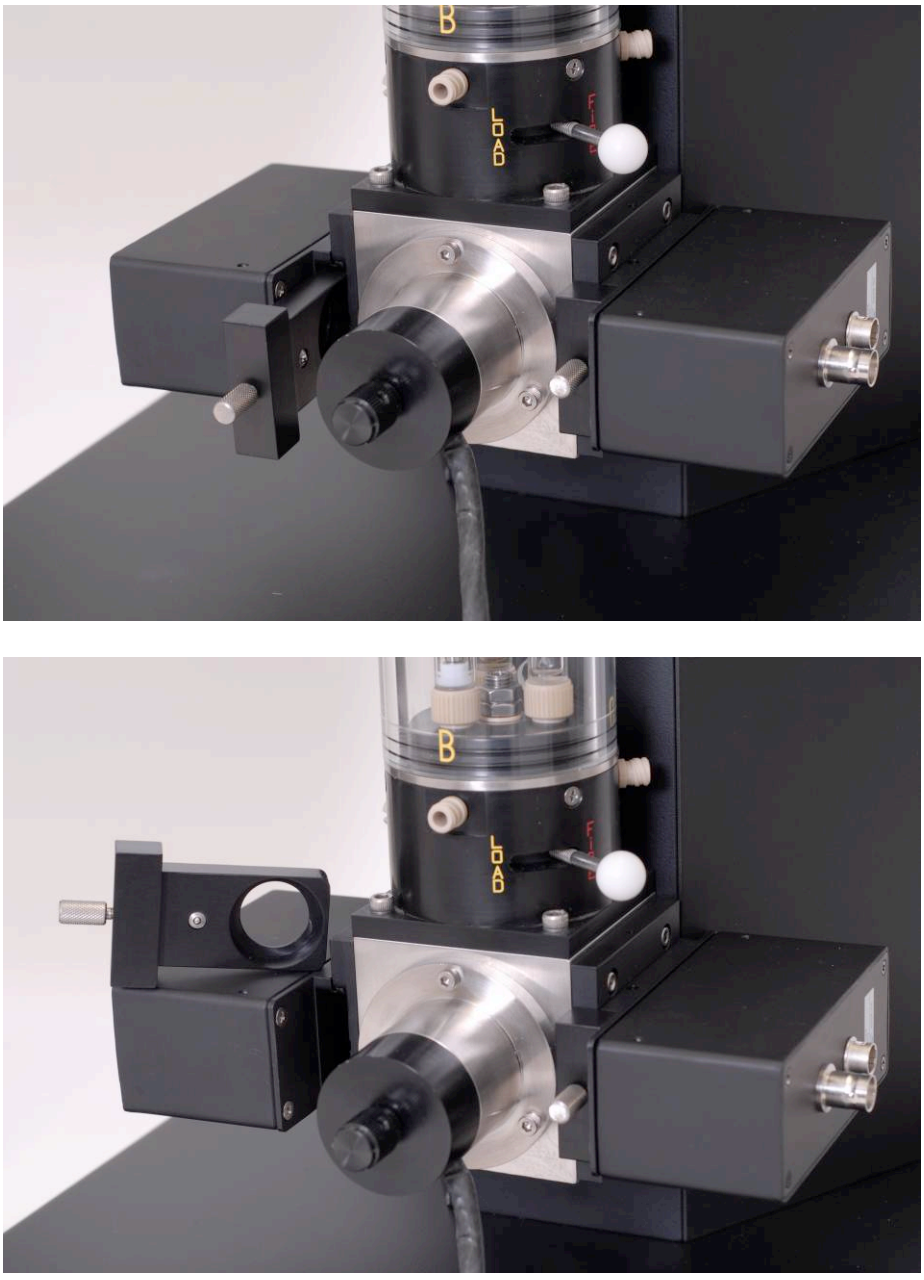


Figure-7: ***Sliding Filter Holders***

II. Operation of the Instrument

A. Summary of Instrument Setup Procedure

The recommended sequence for conducting a stopped-flow experiment is described below. 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 if it has not already been done.

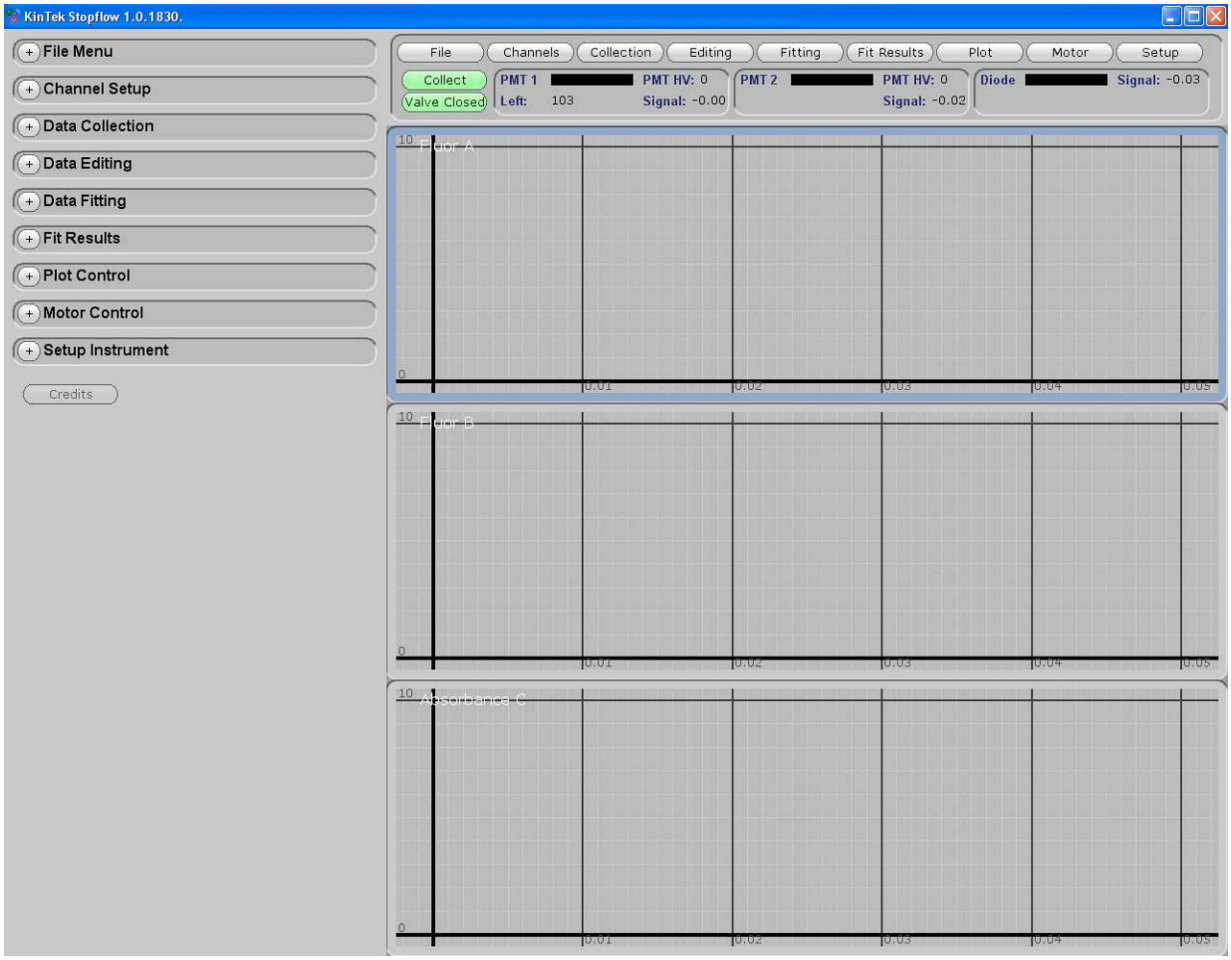


Figure-9: ***Stopped-Flow Main Screen***. This figure shows the main screen of the Stopped-Flow program. The buttons on the right display data collection channels (PMT-1, PMT-2 or DIODE). Manually opening and closing of the Stop-Valve and data collection can be accessed from this screen. All functions can be accessed through the scrolling expandable menus along the left side of the screen.

The program is written such that all of the commands are accessible at all times through the scrolling expandable menus, and specific sections can quickly be accessed using the radio buttons along the top of the screen. The use of the commands should be readily apparent, so you could learn the operation of the system without reference to detailed descriptions of the software in this manual. Prior to conducting any experiment, be sure the proper excitation wavelength is set and emission filters are installed.

1. Turn on Equipment
2. Double click on the KinTek Stopped Flow icon to start the program
3. Set monochromator wavelength
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

As described in Section I, the KinTek Stopped-Flow system has three basic components, the light source, the motor control box and the computer. It is important that the computer NOT be on when the lamp is ignited. The high frequency pulse from the lamp igniter may crash the computer program if it is on. It is necessary to turn on the equipment in the following sequence.

1. Turn on the cooling water for the lamp.
2. Ignite the Lamp **with computer and controller turned off**.
3. Turn on the Motor Control Box
4. Boot up the Computer and enter the program

The lamp should be cooled while in operation. We recommend using the supplied circulating water bath at ambient temperature.

1. Turn on the arc lamp power supply. Press the light green button to ignite. Be certain lamp has been aligned (*D. Lamp Installation & Adjustment*). Allow lamp at least fifteen minutes to warm up.
2. Turn on the power to the Motor Control Box.
3. Turn on the computer and run the KinTek Stopped Flow software. If the message, "No Connection to Instrument" comes up, either the power was not turned on to the Motor Drive Box or the connections have not been made properly. 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.

When the program begins, it causes the drive motor to back up to the "Home Position". It uses this as a reference point to calculate the number of shots remaining and can easily be returned to this position. 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, DIODE Signal, Valve Open/Closed and Shots Left.

The following section provides a description of the commands needed to set up the instrument to collect data. The items within the menu selections shown at the top of the window are explained in further detail in Section III.

C. Setting the Wavelengths and Selecting Detectors

To set the excitation wavelength on the monochromator enter the desired wavelength in the *Monochromator wavelength* field in the *Channel Setup* menu.

Either the photodiode or the photomultiplier tube (PMT) can be used for absorbance or transmittance measurements. However, only the PMT can be used for fluorescence or light scattering because of the requirement for higher sensitivity. 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 (Fluorescence Anisotropy) data. For fluorescence measurements, the excitation wavelength is selected on the output monochromator and emission filtering is used in front of the PMT. We supply a 320 nm long-pass filter for use of protein measurements.

Filters (1.0 inch/25.4mm diameter) can be mounted in front of the PMTs (*Figure-7)*. Make sure the power is off to the PMT before disconnecting the PMT. It is best to have the room lights turned off and to work under reduced light when removing the PMT. Exposure of the PMT to room lights (with the HV disconnected) will not damage the PMT, but higher dark current readings will persist for several hours after exposure of the PMT to room light.

D. Loading the Samples

A single valve is used to load each of the three drive syringes. The instrument is initially shipped in the two-syringe position (conventional mixing) because it is recommended that you learn to collect and analyze data in a simple two-syringe experiment prior to attempting a three-syringe experiment (double mixing). Procedures for putting the instrument into double mixing are described in Section II-J. Double Mixing Experiments.

Below the syringe chamber on the instrument you will find the LOAD/FIRE valve. The positions are color coded with “LOAD” marked yellow, and “FIRE” is colored red. In the “LOAD” position each syringe is attached to its loading port. In the “FIRE” position, the syringes are connected to the lines leading into the mixing chamber. The computer monitors the position of the valve by using two optical position sensors. This prevents the computer from firing a shot in the wrong valve position.

Turn the knob to the “LOAD” position. This setting is noted on the main screen at the valve position indicator. The valve should always be in either the “LOAD” or “FIRE” position. A question mark (**?**) appears at the valve position indicator if the valve is between positions. If both sensors are active, an "ERROR” message will occur. The sensitivities of the sensors can be adjusted electronically to correct any problems (Appendix C).

It is recommended to flush and practice with distilled water or buffer prior to loading precious samples. To load samples move the selector knob to the “LOAD” position. Conventional mixing uses syringe “A” and syringe “C”. DO NOT LOAD SOLUTION INTO SYRINGE “B” WITHOUT CHANGING THE DOUBLE MIXING VALVE TO THE OPEN POSITION. This is described in the last part of this section. Load water into drive syringe “A” by pushing solution in. If syringe is tight, you may want to lift plunger shaft of the drive syringe slowly up with one hand while pushing water in from the loading

syringe with the other. After syringe is loaded, look into the drive syringe inside of chamber and be certain no bubbles are present. If bubbles are present, push vigorously back and forth between the drive and load syringes, pausing to allow air bubbles to rise in the load syringes. Once bubbles are purged from drive syringe, repeat for drive syringe "C". There are two ways to flush. The first way to flush is to load syringes with water or buffer, click Valve Closed to open the valve. You will hear a click and the screen will display **Valve Open**. Next push solutions through by hand. You will see the waste syringe filling. The second way to flush is to utilize the motor drive. The computer via the *Motor Control* menu controls the position of the drive plate. Home sends the motor to its uppermost position while Goto Endpt sends the drive plate to the endpoint of the drive motion that has been set at the factory. Up and Down buttons can be used to position the drive plate at any intermediate position. Click Goto Endpt to push solutions through observation cell. Repeat the above procedure 2 additional times to ensure any old reactants as well as air has been flushed out of the system.

To load samples, attach syringes containing fresh solution to the loading ports located beneath each drive syringe and ensure the valve in "LOAD" position. If care is taken to remove all of the air bubbles in load syringes prior to loading drive syringes, sample loading becomes more efficient. Degassing of solutions is desirable, but may not be necessary in most cases.

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, place the sample loading valve in the "FIRE" position and flush fresh solution through the observation cell.

The knob on the photodiode controls the sensitivity of the photodiode. Turn the knob clockwise to increase sensitivity and counterclockwise to decrease sensitivity. A continuous readout of the signal is given on the main screen of the Stopped-Flow program. You can also click Scope Off in the oscilloscope section under the *Data Collection* menu to examine the incoming signal. It is usually best to work in the middle of the scale.

The PMT sensitivity is set by adjusting the internal 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 fluorescence increase is expected, it is recommended to set the PMT high voltage manually, using the Auto Set feature sets the voltage at half scale and there is the possibility that the trace could go out of the scale.

After the sensitivity of either detector is changed, 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.

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 absorbance.

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. The two 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. A total of one million data points per second can be distributed between two time domains and all channels collected. The system defaults to 2000 points for data collection per channel. The time of data collection should be such that the reaction is monitored for 6 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,C) as well as any experimental comments to be saved as part of the file.

Finally under *Channel Setup*, select which detection channels you wish to use for the experiment: PMT-1, PMT-2 (if the optional second PMT channel was purchased with the system), and/or DIODE. 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 

, and then take a reference reading at the desired wavelength. To set the dark current value press 

. 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. The LOAD/FIRE valve must be in the "FIRE" position to initiate data collection.

H. Saving and Recalling Data

Before manipulating any data, it is recommended to save it. Click on Save or Save As under the *File Menu*. Additionally, by pressing Export, data can be saved as a .txt file to allow users to import it into data fitting software or spreadsheets. 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 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 Analysis

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 bow next to a desired trace. The trace colors change 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.*

J. Double Mixing Experiments

The principle behind a double mixing experiment and the required setup configuration are illustrated in Figure-8. Solutions from syringes "A" and "B" are first mixed and then flow through a delay line before being mixed with solution from syringe "C". The delay line is CNC machined into the valve housing and has a typical volume of 50µl.

The first shot clears the delay line of aged material, waits for the specified first reaction time entered by user, then pushes mixed "A" + "B" with "C" into the observation cell. This triggers data for collection over the period of time specified in the Data Collection panel.

The total reaction time covers both the time for the solutions to flow through the delay line, plus an explicit pause, which must be at least 10 msec. Given the typical flow rates (8.0 mls/second) and delay line volume, the flow through the delay line will take 10-15 msec, and so the minimum allowed first reaction time will be about 20-25 msec. The software will limit any entered time smaller than this to this minimum.

The shot volume for double mixing experiments is calculated automatically, based on the specified delay line volume, and is not user adjustable. The range of practically usable volumes is small, as the first push must be large enough to fill the delay line, with no point in much larger pushes, while the second push must be small enough so that only aged, reacted, A+B mix with solution C in the reaction cell. The software automatically calculates a shot volume in the middle of the usable range.

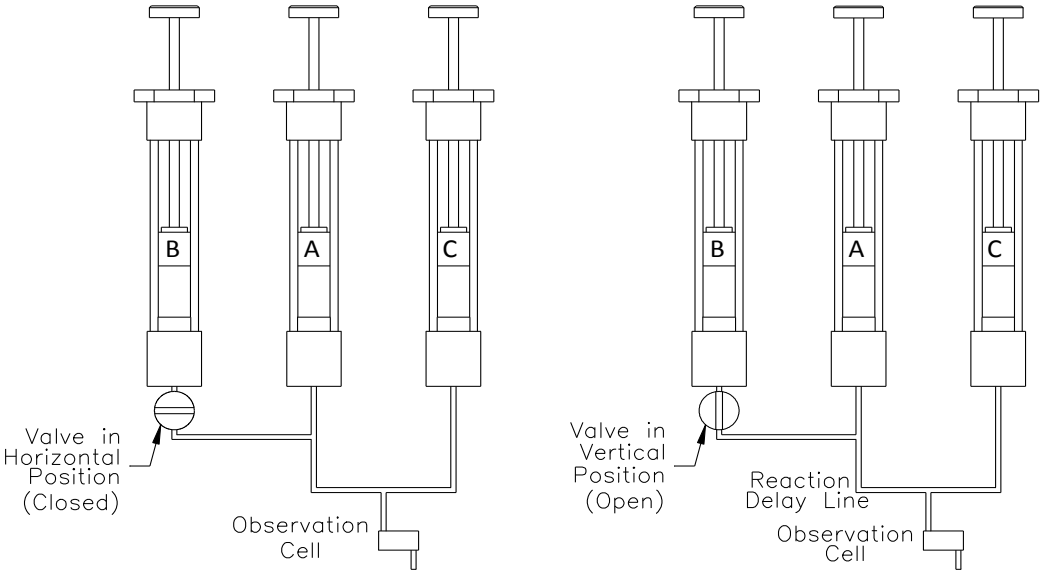


Figure-8: ***Double Mixing Experiment Setup***. Tubing connections are shown for experiments involving 2 or 3 syringes.

1. Changing Between Single Mixing and Double Mixing

To change between single mixing and double mixing simply turn the single mixing double mixing valve shown in *Figure-2*. If the slot in this valve is in the vertical position, it opens the line in syringe "B", and then allows the system to perform double mixing. If the valve position is in the horizontal position, then the system is in the single mixing configuration.

2. Measurement of the Delay Line Volume

The delay line volume must be calibrated by direct measurement. The measurement procedure is based upon loading the delay line with a solution of known absorbance and then calculating the volume by the dilution observed after flushing the delay line with a known total volume of solution. The load/flush sequence is described as follows:

1. Load the standard known absorbance solution into syringe "A", with buffer in syringes "B" and "C". Take care to load each solution excluding bubbles and forcing solution to flush out all of the lines leading through the cell.
2. Open the Stop-Valve and force sufficient solution from syringe "A" to fill the delay line with fresh Solution A.
3. Open the Stop-Valve and force solution from syringe "C" to flush out the line from the second mixer to the exit point. This will clear any of Solution A from the second mixer to the end.

4. Flush the contents of the delay line out by pushing from syringe “B” (containing buffer). Collect the sample and determine the volume and absorbance. Calculate the delay line volume according to the simple dilution:

Delay line volume = Collected Volume * Final Absorbance/Initial Absorbance

3. Computer Setup for a Double Mixing Experiment

The syringe drive computer must be programmed to do a double push for each data collection. This is done under the Setup Instrument menu. Select the checkbox 'Double Mix Experiment?' to turn on double mixing experiment mode. Then enter the appropriate value for the volume of the delay line just below this.

The time for the reaction of A+B is set in the Data Collection menu, as 'First Reaction Time'.
This field will be shown once 'Double Mix Experiment' is selected. This reaction time will be limited to be at least long enough for the flow time plus at least a 10 msec pause, a limit of about 20-25 msec.

You are now ready to do a double mixing experiment. The motor will drive twice and trigger data collection at the end of the second push.

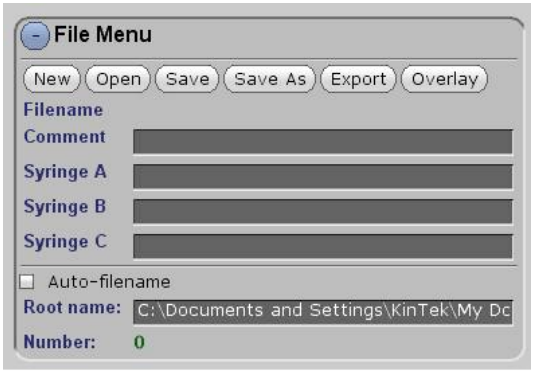
K. Instrument Shutdown

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

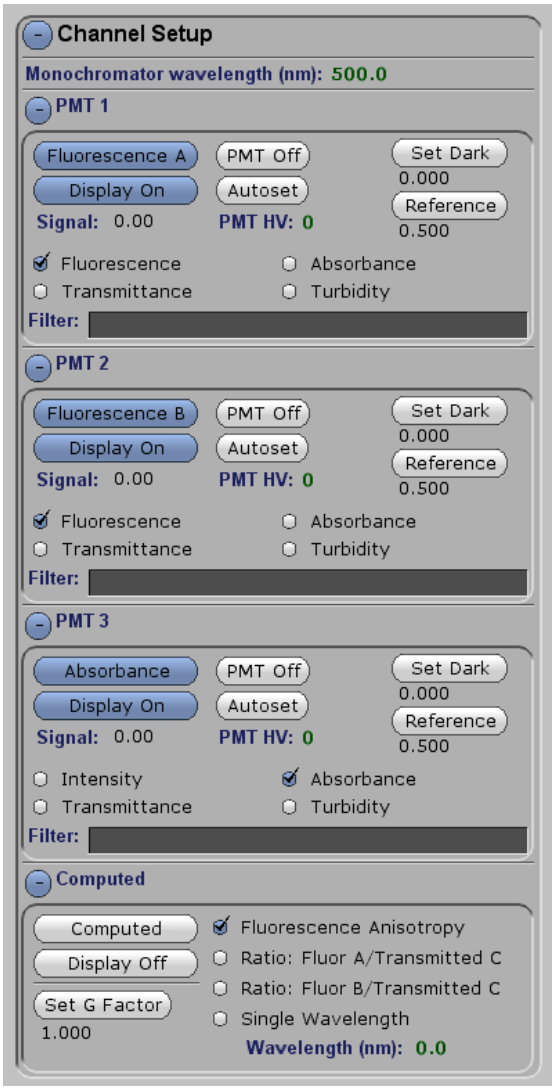
Syringe B – A text box for the contents of syringe B

Syringe C - A text box for the contents of syringe C

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.

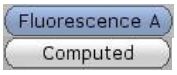
Number – The current count for auto filename. The user can change this field, normally the count will increase by 1 each time a file is saved and **Auto Filename** is selected.



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.



- 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 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.

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

- 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.

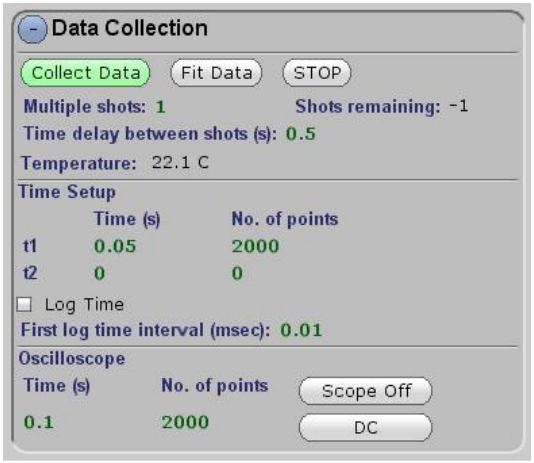
Data Type – Each detector has a list of types of data that can be collected. Fluorescence, Absorbance, Transmittance or Turbidity.

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.

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.

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 which you fit (or open a previously saved data file), and then take additional data pressing New will select only the second set of data.

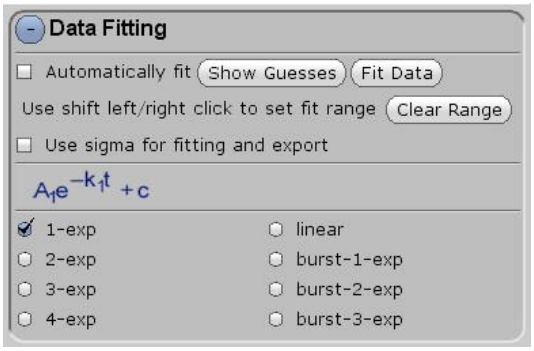
Invert - Inverts the selected traces. Any traces that are selected will be deselected and any traces not selected will be selected.

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.

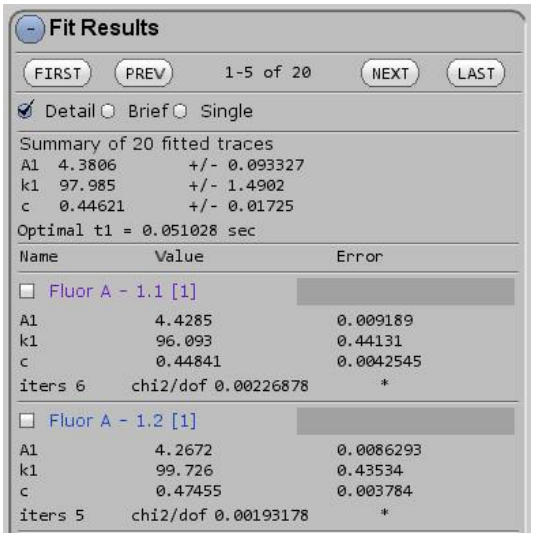
Clear Range - Clears any data fit range manually selected by the user.

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 sigmas 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 sigmas 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 – 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 sigmas as translucent bars, Guesses for fits as white curves, and Fits as black curves.

Point Size/Line Thickness/Num Sigmas: 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.

Set Endpt - Sets the current drive position as the end point. The end point is the position past that the drive will not move during a collection and is used to calculate the number of shots remaining.

Goto Endpt - Moves the drive plate to the position currently set as the end point (typically the position where the syringes are empty).

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

Manual Shutter - Displays the status of the auto shutter accessory if the system is equipped. If the system is not equipped with the auto shutter "Manual Shutter" is displayed. If the system is equipped with the shutter "Shutter Open" or "Shutter Closed" will be displayed.

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" and "Dead time offset" can be set here and will be saved in the instrument configuration file.

Double Mix Experiment? – Select this box if performing a double mixing experiment. The Delay Live Volume defaults to 50 µl but can be adjusted by the user if the calibrated volume of your delay line differs significantly.

Syringe Types – Select the syringe volumes in this section, the software will use the syringe volume to determine the number of shots remaining and the distance needed to achieve the "Total volume per shot" entered above.

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

Load/Fire Sensor – Select this check box to have the software ignore the load/fire sensor position. **Caution:** Damage may occur is the drive is moved with the load/fire valve in the wrong position. Only experienced users should ever choose to ignore the sensor.

Wavelength Drive – The wavelength drive is standard on SF-300X instruments and 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 auto this box should be selected to activate its features.

Titration – To be selected if you are performing an experiment with the optional titration module.

Communication Port – Select the serial port used on the PC for motor control. Typically this will be COM1.

Selected Instrument – Multiple instruments can be run from a single PC (not simultaneously) and the current system is selected here. Also a "simulated" system can be run.

Stop Delay – Displays the current stop valve delay in msec., this value is static and is set at the factory.

Discard Time – Displays the current data discard time in msec., this value is static and is set at the factory.

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

Appendix

A. Test Reactions

Principle of the method:

Reaction of N-bromosuccinamide 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 μM^{-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 SF-2004 syringe and observation cell assembly, and should be done as needed to maintain stable data for your SF-2004. 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 drive syringes with DI Water.
    a. With the syringe control valve in the LOAD position fill syringes with DI water.
    b. Open the STOP valve using the KinTek SF program.

c. Turn syringe valve to FIRE position.
d. Manually drive the DI water through the cell and into the waste syringe.
e. Close the STOP valve and empty the waste syringe.

2. Flush system with 2N NaOH solution by forcing it backwards from the waste syringe.
   a. Fill a syringe with the 2N NaOH solution and attach it to the waste collection line.
   b. Open the STOP valve in the SF program.
   c. With the syringe valve in the FIRE position drive solution up through the cell from the waste syringe into the sample syringes.
   d. Close the STOP valve and allow solution to soak for 5-10 minutes.
   e. Open the STOP valve and manually drive the solution back to the waste syringe.
   f. Close the STOP valve and empty the waste syringe and reconnect.
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 a syringe with 2N HCL solution at attach it to the waste collection line.
   b. Open the STOP valve in the SF program.
   c. With the syringe valve in the FIRE position drive solution up through the cell from the waste syringe into the sample syringes.
   d. Close the STOP valve and allow solution to soak for 5-10 minutes.
   e. Open the STOP valve and manually drive the solution back to the waste syringe.
   f. Close the STOP valve and empty the waste syringe and reconnect.
5. Flush with DI water (see step 1).

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-10* 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 

. The other channels are adjusted the same way.

3. Valve Position Sensor Adjustment

The sensitivities of the sensors for the valve “FIRE” and “LOAD” positions can be adjusted with resistors labeled FIRE and LOAD on the board. The Fire LED lights up when the “FIRE” sensor is active, while the Load LED lights up when the “LOAD” sensor is active. To adjust, turn both sensors clockwise until either LED light up. Next, place your valve in the “LOAD” position, turn the Load Resistor counterclockwise until the Load LED lights and “LOAD” is displayed on your upper right hand corner under “Valve Position”, then give about a quarter turn extra. Finally, place your valve in the FIRE position, turn FIRE Resistor counterclockwise until the Fire LED lights and “FIRE” is displayed on your screen, then give a quarter turn extra. If both sensors sensitivity is over

adjusted, an “ERROR” is noted on the computer screen under “Valve Position”. If one of the sensors sensitivity is not set a “?” will appear on the screen.

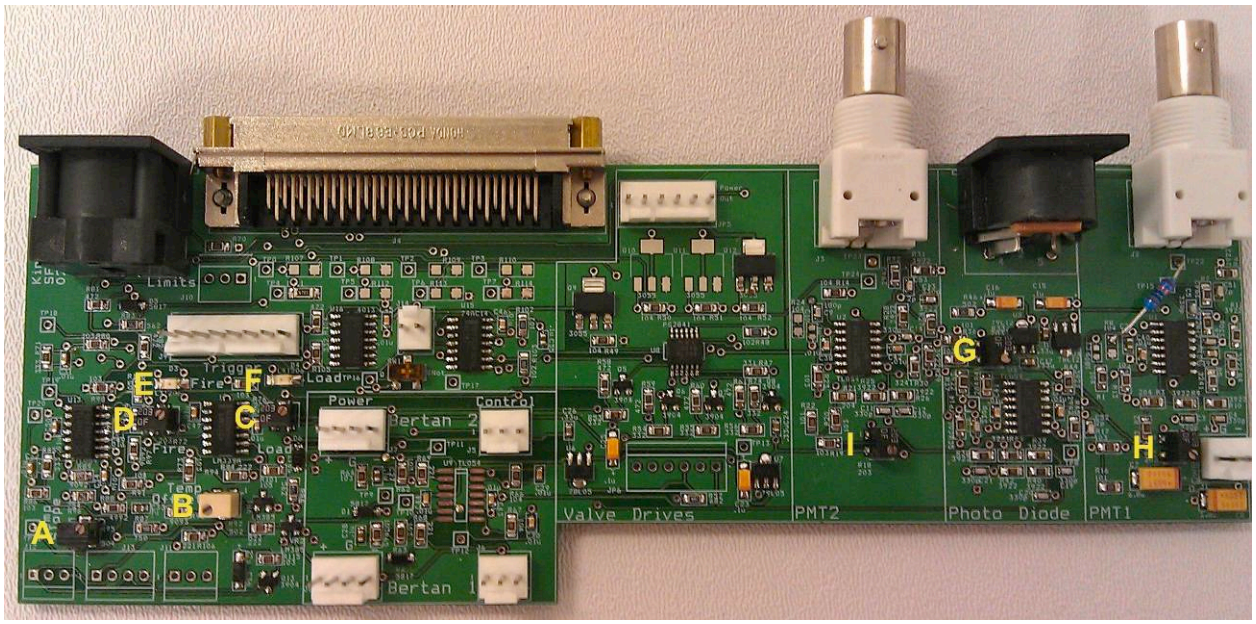


Figure-10: ***Printed Circuit Board.*** A. Temp Slope B. Temp Offset C. Load Sensor Adjustment D. Fire Sensor Adjustment E. Fire LED F. Load LED G. Photodiode Zero Adjustment H. PMT-1 Zero Adjustment I. PMT-2 Zero Adjustment

D. Parts and Accessories

Syringes: Syringes can be ordered from KinTek Corporation. They are not available from any other supplier because they are modified for the Stopped-Flow. The standard syringes are 2.5 ml. Smaller and larger syringe sizes are available on request. Smaller syringes require an adapter to fit into the syringe holder.

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

Part Number	Description
86-105	0.5 ml Drive Syringe
86-110	1.0 ml Drive Syringe
86-120	2.5 ml Drive Syringe
86-150	5.0 ml Drive Syringe

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
semrock@idexcorp.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 Glan-Taylor polarizer can be adjusted using two setscrews that are visible when the position lever is midway between vertical and horizontal positions (*Figure-11.b*). These screws can be loosened and the filter can then be turned in the fitting to finely tune its rotation. *NOTE: The prism alignment is set at the factory and changing its position is not recommended except by experienced users.*

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.

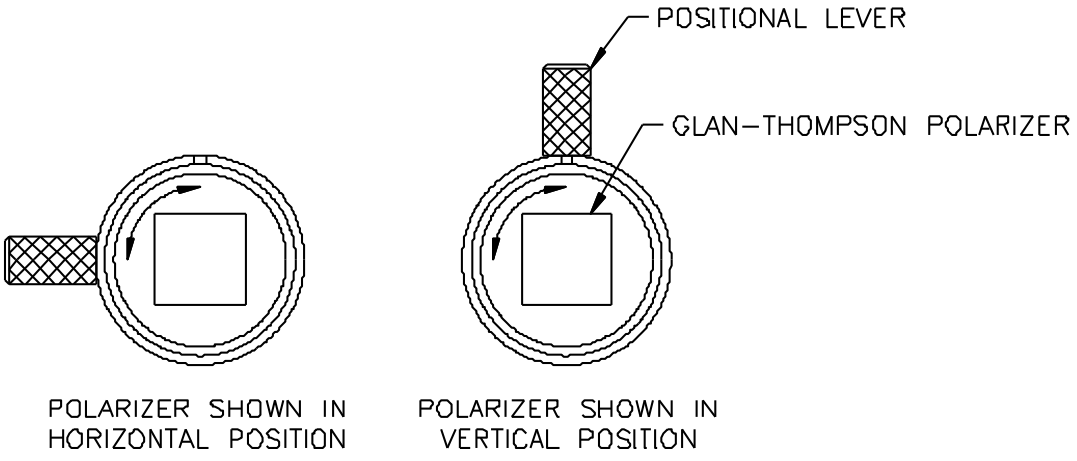


Figure-11.a: ***Polarizer Position.***

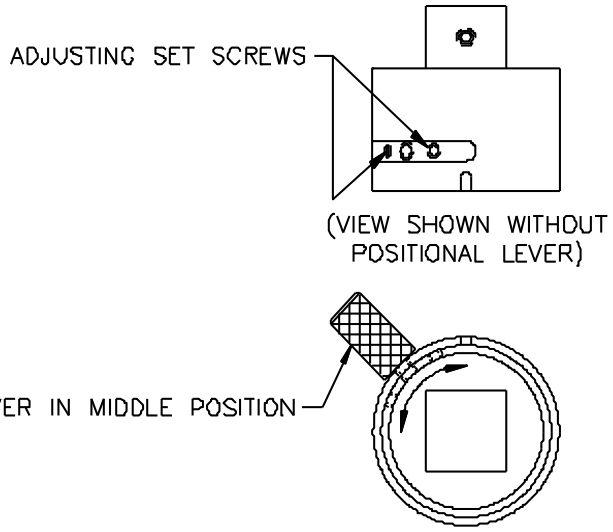


Figure-11.b: ***Polarizer Adjustment.***

F. Removing Tower Assembly

If for some reason your Tower Assembly should leak, it is recommended to send it back for evaluation. Our trained staff will evaluate the circulating cooling/heating system and check for any leaking or inconsistency in the mixing.

To remove the tower, detach the detectors (Photodiode & PMT/s) from the cube (*Figure-2*). Please attach supplied cover to the photomultiplier tube after removing. In the rear of the stand (*Figure-3*), unplug the temperature probe and fiber optic cable adapter. There are three screws that hold the Tower Assembly to the stand. Once the tower is removed disconnect the exit line. Use caution not to lose the plastic ferrule that seals between the line and cell holder (*Figure-1*). Ship back to the factory address listed on the cover of the manual.

G. Changing Syringes

To change the syringes see above instructions to remove the Tower Assembly. Once the Tower Assembly is removed you will need to pull out the syringe adapters. To do this you must first unscrew the Phillips head screw in the center of the plungers and remove the circular plastic piece holding the syringe adapters in place. Next pry up the syringe adapters with a small blade screwdriver or a pair of tweezers. The acrylic housing will pull off; it may be tight because of the O-ring seal at the bottom. Once this has been removed, all three of the syringes are exposed. Unscrew the syringe/s that will be changed. Screw in the new syringe/s ensuring they are tight enough to properly seal. Reassemble the tower assembly, and attach back onto the stand in the reverse order of removing. If you have chosen to replace the syringe with one of a different volume you must select the appropriate syringe volume in the *Setup Instrument* menu.