KinTek AutoSF-120 Stopped-Flow Setup Summary

1) Turn on the lamp water coolant pump and lamp power supply.
2) Ignite the lamp by momentarily pressing the square green button on the lamp power supply. It will light up when the lamp has ignited. Allow 15 minutes for the lamp to warm up and stabilize.
3) Turn on computer and power for the AutoSF (toggle switch on back right corner).
4) To start the software, double click on *KinTek StopFlow* icon on the desktop.
5) Place the appropriate bandpass or cutoff filter in holder for fluorescence measurements.
6) Check the physical readout of the wavelength on the monochromator and make sure it agrees with the readout on the computer. If there is a discrepancy, enter the correct wavelength of the monochromator at the bottom of the menu under Instrument Setup.
7) In the software, select the channels for data collection. Up to three channels can be collected simultaneously.
8) FLUSH the cell using the *Flush* commands (pipette the appropriate volume (set by user) of solution into each sample cup, the press *Flush*).
9) LOAD Samples: Pipette your samples into the sample cups, then click on *Load New*. Note the load volume can be set; a typical volume is 200 µL, but as little as 120 µL can be used.
10) Adjust the photomultiplier high voltage (PMT HV) for each channel. You can either enter the PMT HV manually or use the *AutoSet* function, where the instrument will increase the HV until a signal of half of the maximum range is reached. By default, the instrument is setup with a 1-10 volt scale, but for very small light levels, a 0-1 volt scale can be set under the Instrument setup menu.
11) Optional: Close shutter (slider on the monochromator) and read the dark current. Then open the shutter. This is usually a small number and can be neglected.
12) If performing absorbance measurements you need to read a reference voltage. Here you have two choices: a) read the reference based upon the endpoint of the reaction, in which case you will record the change in absorbance; or b) flush the cell with buffer (or appropriate reference blank solution), then read the reference voltage, in which case you will then record absolute absorbance units.
13) SET THE TIME for data collection and the number of data points that you want to save to disk. The instrument will collect data at the fasted rate possible (1 megahertz for one channel), then average to get the number of points desired for the time range selected. Generally 2,000 points are more then adequate, but perhaps 8,000 points can be stored if you are concerned that the reaction is faster than the time selected allows. Alternatively, the log timescale function allows data to be collected with a higher density of points at shorter times; this allows fast and slow reactions to be observed.
14) COLLECT DATA. Click on the *Collect* icon. The displays the number of shots remaining based upon the volume per shot and total volume remaining.
15) FIT DATA to the appropriate function - see the *Data Fitting Menu*.
16) SAVE DATA. All of the multiple traces are saved. It is often convenient to select the best traces, eliminate outliers, and then average and save the average trace in a separate file. You can also export the data to a simple text file for importing in to other data fitting programs, such as KinTek Explorer.