Chirascan
SF.3 Stopped-Flow Accessory User Manual

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USE OF THIS DOCUMENT

This document is intended to inform the operator of Applied Photophysics' SF.3 stopped-flow accessory on its design, installation and operation. The SF.3 is used with either the Chirascan™ or Chirascan™-plus circular dichroism spectrometer, and this document should be used in conjunction with the User Manuals applicable to those instruments. It is assumed that the user of this document is familiar with the operation of the Chirascan™ or Chirascan™-plus, and with Applied Photophysics Pro-Data software. In particular it is assumed that the user is familiar with the hazards associated with the operation of the spectrometer, and has read the hazard and other safety information contained in its User Manuals.

Please note that throughout this Manual, the Sequential-Mixing version of the SF.3 is shown in the Figures, with descriptions in the text of how it may be configured for either single-mixing or sequential mixing mode. However, some users will have purchased the Single-Mixing version on which the components for sequential-mixing are not fitted. These users should disregard all references to sequential-mixing.

A series of Quick Guides describing briefly some of the operations of the SF.3 are included as supplements to the main User Manual. These may be detached or printed out and stored in a separate folder.

The information in this document is subject to change without notice and should not be construed as a commitment by Applied Photophysics, who accept no responsibility for errors that may appear herein. This document is believed to be complete and accurate at the time of publication, and in no event shall Applied Photophysics be held responsible for incidental or consequential damages with or arising from the use of this document.

HAZARD SYMBOLS USED IN THIS DOCUMENT

The symbol to the left is used as a general hazard notice indicating that failure to comply with the accompanying instructions may be harmful to personnel or cause damage to the instrument.

The symbol to the left is used as an electrical hazard notice indicating that failure to comply with the accompanying instructions may be harmful to personnel or cause damage to the instrument.

HAZARD TEXT USED IN THIS DOCUMENT

WARNING: in bold red font, accompanied by one of the hazard symbols shown above, indicates that failure to comply with the accompanying instructions could result in death or serious personal injury, or serious damage to the instrument.

CAUTION: in bold blue font, accompanied by one of the hazard symbols shown above, indicates that failure to comply with the accompanying instructions could result in personal injury or damage to the instrument.

OTHER INFORMATORY TEXT USED IN THIS DOCUMENT

NOTE: in bold brown font with no accompanying hazard symbol, is used to clarify an aspect of the instrument usage, or to advise the user on how to improve data quality.

HYPERLINKS

Hyperlinks within the document are shown in underlined blue font. To follow a link, place the cursor over the text and use CTRL+CLICK. To return, use ALT+LEFT ARROW

ESSENTIAL SAFETY INFORMATION

DO NOT PROCEED WITH THE INSTALLATION OR OPERATION OF THE SF.3 UNTIL YOU HAVE READ THESE NOTES AND THE HAZARD AND OTHER SAFETY INFORMATION CONTAINED IN THE MAIN CHIRASCAN™ USER MANUALS.

WARNING: High voltages are used in the Chirascan™ spectrometer. Please exercise care during operation and DO NOT operate units with their covers removed. Remember, high voltages can be LETHAL. The symbol to the left is attached to some units to indicate the presence of high voltage.

CAUTION: Do not disconnect any electrical leads from any of the electronic units without first ensuring that the units are turned off.

CAUTION: the Chirascan™ spectrometer is equipped with a light source (150 W xenon arc lamp or other) that produces intense ultraviolet (UV) radiation. Never look directly at the light source without wearing suitable eye protection.

CAUTION: Ozone is a very reactive gas that is produced when UV light interacts with oxygen. If an ozone producing lamp is used, it essential that the Chirascan™ is purged with clean nitrogen before the lamp is ignited. Failure to purge the instrument will result in the formation of ozone, which is damaging to health and can cause deterioration of the optical components in the instrument.

SF.3 INSTALLATION AND OPERATIONAL REQUIREMENTS

Environmental requirements

The SF.3 has no environmental requirements additional to those of the Chirascan™ or Chirascan™-plus spectrometer.

Bench space

A minimum of 400 mm bench space is required to the right of the Chirascan™ spectrometer without the standard sample handling unit.

Electrical requirements

Operation of the SF.3 is controlled by the Chirascan™ electronics, and there are no additional electrical requirements.

Nitrogen purge gas

Nitrogen purging is only necessary on the SF.3 for measurements at wavelengths below 200 nm, in which case the purge gas requirement is the same as that of the Chirascan™ standard Equilibrium Sample Handling Unit, i.e. 1 litre per minute at a line pressure of 4 to 6 bar.

Pneumatic gas supply

An air or nitrogen gas supply is required to drive the SF.3 pneumatics, at a line pressure of 8 bar (120 p.s.i.). It is recommended that a separate supply is used from the spectrometer purge gas. Coupling to the SF.3 is through a 6 mm diameter push-in type connector, and the user is required to supply all fittings upstream of this connector.

CAUTION: the purge gas filter cartridges supplied by APL are only rated to 100 p.s.i. (6.9 bar). If the same supply is used for the purge and pneumatics, the line should be split upstream of the purge gas filter cartridge, and regulated to between 4 and 6 bar between the split and the cartridge.

Fluid circulator unit

The operating range of the SF.3 is from 5ºC to 40ºC, these limits being set by the stability of the syringes used.

Temperature control of the SF.3 is maintained by circulating water, and a suitable circulator is required. If possible, this should be placed on the floor below the bench on which the SF.3 is situated.

The circulator unit connects to the SF.3 water bath via 8 mm internal diameter tubing. The SF.3 is fitted with quick-fit connectors to allow the circulator to be connected and disconnected rapidly without the need to drain the water bath.

The following circulator units can be controlled from within the SF.3 software when connected to the serial port of the workstation PC by an RS232 cable:

Neslab RTE 200, 300
Fisher Scientific 3016
Thermo RTE7

For further information on circulator unit compatibility please contact the Technical Support Team at Applied Photophysics.

Applied Photophysics recommend the addition of an anti-bacterial agent and antifreeze to distilled water as a suitable thermostatic fluid, provided that these meet with the circulator supplier's specifications.

Servicing

Servicing of the SF.3 should only be undertaken by qualified personnel. If you are in any doubt at all please contact the Applied Photophysics Technical Support Department.

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GLOSSARY

The following abbreviations may be found in this User Manual

AC	Alternating current
APL	Applied Photophysics Ltd.
AU	Absorbance units
CD	Circular dichroism
CPL	Circularly polarized light
CSV	CSViewer or Chirascan Pro-Data Viewer
DC	Direct current
ESHU	Equilibrium sample handling unit
HT	High tension – the same as high voltage
HV	High voltage
KSHU	Kinetic sample handling unit
LED	Light-emitting diode
L min^{-1}	Litres per minute
M	Molar (i.e. mol dm^{-3})
PEEK	Polyetheretherketone
PMT	Photo-multiplier tube
PTFE	Polytetrafluoroethylene
SBW	Spectral bandwidth
SCP	Spectrometer control panel
SF	Stopped-flow
SHU	Sample handling unit
SS	Step size
UHMW-PE	Ultrahigh molecular weight polyethylene
UV	Ultraviolet

CONTENTS

FIGURES

TABLES

Chapter 1: INTRODUCTION

The SF.3 stopped-flow accessory, the single and sequential mixing versions of which shown in Figure 1.1, is designed to be used with either the Chirascan or Chirascan-plus circular dichroism spectrometer. It is based on the Applied Photophysics highly successful PiStar and SX series stopped-flow instruments.

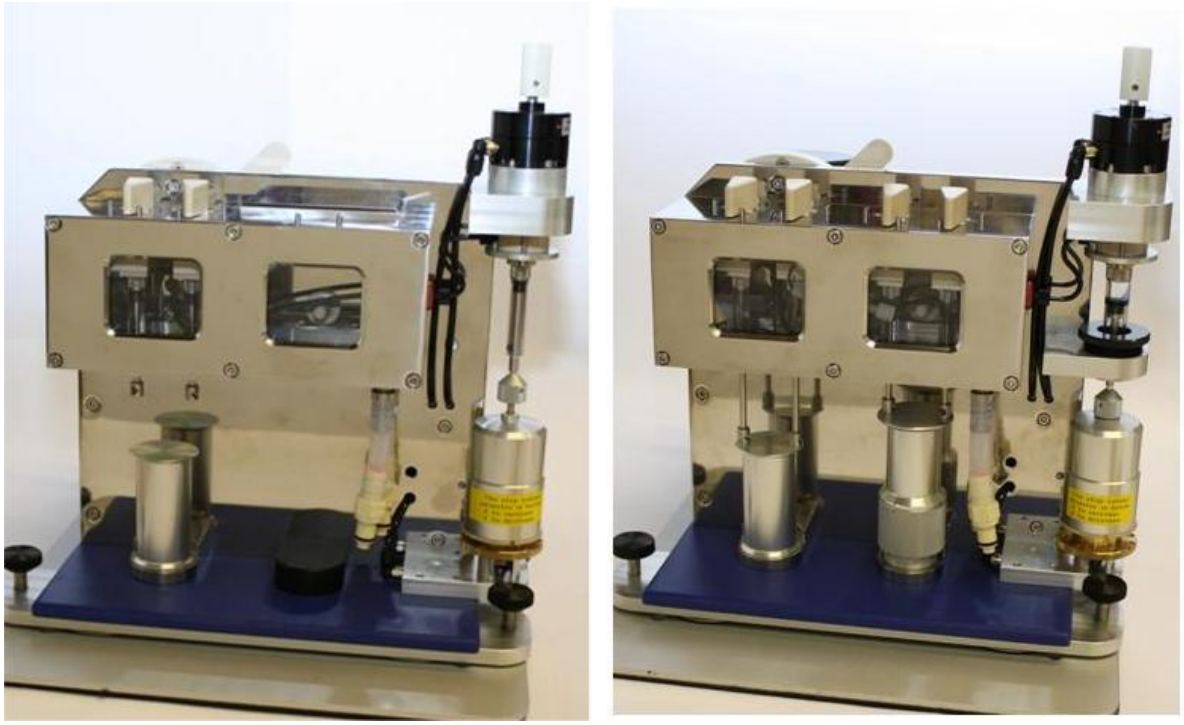

Figure 1.1: The single mixing (left) and sequential mixing (right) versions of the SF.3

Stopped-flow is one of a number of methods of studying the kinetics of reactions in solution. In the simplest form of the technique, the solutions of two reactants are rapidly mixed by being forced through a mixing chamber, on emerging from which the mixed fluid passes through an optical observation cell. At some point in time, the flow is suddenly stopped, and the reaction is monitored using a suitable spectroscopic probe, such as absorbance, fluorescence, fluorescence polarization or circular dichroism. The change in spectroscopic signal as a function of time is recorded, and the rate constants that define the reaction kinetics can then be obtained by fitting the data using a suitable model.

The performance of a stopped-flow instrument is determined to a large extent by its dead time. This is defined as the time between the reactants mixing and the observation beginning, and is essentially the age of the

reaction as the reaction mixture enters the observation cell. The limiting factors in the dead time of a particular stopped-flow apparatus are the efficiency of the mixer, the distance between the mixer and the cell, and the flow rate of the reaction mixture at the instant at which flow is stopped. When its standard observation cell is used, the SF.3 is typically capable of achieving dead times of about a millisecond; when an ultra-small observation cell is used, dead times can be reduced to about half that.

The standard operating mode of the SF.3 is single-mixing. Two reactants are used; these are loaded into syringes, and are forced through the mixer and optical cell by the action of a pneumatically controlled ram which drives the syringe plungers. The reaction mixture emerging from the optical cell enters a third (stop) syringe, and flow ceases when the stop syringe plunger contacts a trigger switch. This simultaneously stops the flow and starts data acquisition.

Normally, the two drive syringes are the same size, to achieve a mixing ratio of 1:1, but syringes of different sizes can be combined to obtain other mixing ratios. This so called asymmetric or ratio mixing is a common requirement in stopped-flow work.

Sequential-, or double-, mixing is a variation of stopped-flow in which two reactants are forced through a pre-mixer into an ageing loop. After a specified delay period, the mixed fluid is forced through a separate mixer with a third reactant, and the subsequent reaction is studied as in single-mixing. Sequential-mixing is used to investigate the behavior of reaction intermediates or short-lived transients.

It is usual to acquire data from a stopped-flow experiment at a single observation wavelength. A xenon-mercury (Xe-Hg) arc lamp is often used; these lamps provide high outputs at the mercury fluorescence emission lines, and one of these is selected as the observation wavelength. Alternatively the wavelength can be scanned, for example to assess the change in structure of a protein over time, in which case a xenon (Xe) arc lamp is preferred.

In the Pro-Data software, the SF.3 is identified as a KSHU (kinetic sample handling unit). The standard Chirascan sample handling unit is identified as an ESHU (equilibrium sample handling unit).

Chapter 2: OPERATING PRINCIPLES

This chapter describes the operating principles of the SF.3 accessory. Single-mixing and sequential-mixing are described, as are the various ways of monitoring the course of the reaction spectroscopically.

2.1 Single-Mixing Operation

A schematic of the set-up for single-mixing operation is shown in Figure 2.1 (below).

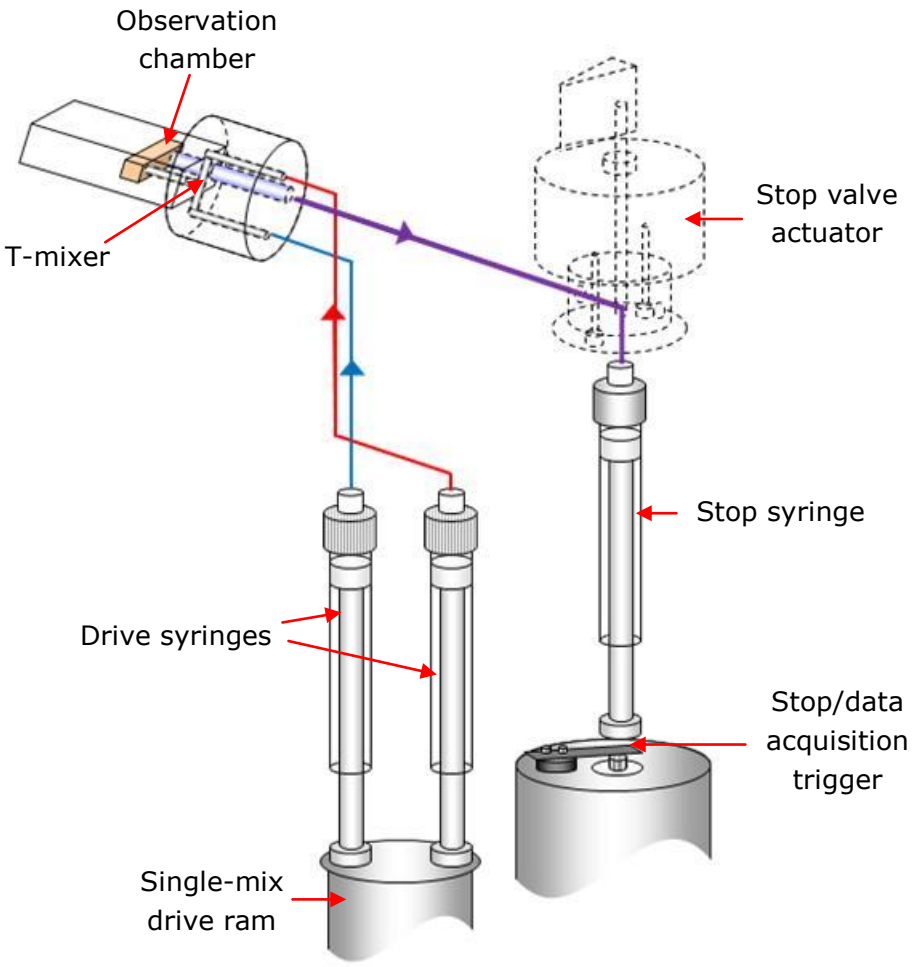

Figure 2.1: schematic of the set-up of the SF.3 for single-mixing operation

Two reactant solutions are loaded into separate drive syringes. A pneumatically driven ram forces each solution from its syringe through

microvolume flow tubing, into an optical cell consisting of a T-mixer and a flow-through observation chamber. The T-mixer produces highly efficient mixing of the two solutions; the reaction occurring in the observation chamber can be monitored spectroscopically. On emerging from the optical cell, the reaction mixture passes through a further flow tube to a stop syringe. When a preset volume of fluid has entered the stop syringe, the syringe plunger hits a hard stop, causing the flow to stop and simultaneously closing an electrical circuit triggering data acquisition.

The two reactant solutions (shown in red and blue in Figure 2.1, previous page) are forced through the T-mixer when the drive ram is fired. The mixed solution (shown in purple), passes through the optical cell to the stop syringe.

The volume of each drive is accurately controlled by the Auto-Stop mechanism. Single or multiple drives can be performed, each drive displacing the same volume of reaction mixture from the observation cell to the stop syringe. Before each drive, the stop syringe is automatically reset, and any fluid it contains is ejected to a waste vessel. Drive syringes of different sizes can be used to produce asymmetric (ratio) mixing of the reactants (Section 5.7.3) .

The optical cell housing contains three observation ports into which detectors can installed for measurement of the change in sample circular dichroism, absorbance or fluorescence as functions of time.

2.2 Sequential-Mixing Operation

For sequential- (double-) mixing operation, two additional drive syringes are used, these being driven by a second, sequential-mix, ram. To study the kinetics of a two stage reaction that proceeds as:

$$A + B \rightarrow \text{product 1} \qquad \text{(premix stage)}$$

$$\text{product1} + C \rightarrow \text{product 2} \qquad \text{(stage 2)}$$

reactants A and B are loaded into the additional pair of syringes, reactant C and a buffer solution are loaded into the original pair. Two drives are then made. On the first drive, the sequential-mix ram forces reactants A and B through a 4-way mixer into an ageing loop. On the second drive, the original (single-mix) ram is driven, forcing the mixture of A and B from the ageing loop into the optical cell, while reactant C is simultaneously driven directly into the optical cell. From that point the sequence is as for single mixing. The buffer solution acts as a flush to

advance the contents of the ageing loop into the cell. A schematic of the sequential mixing set-up is shown in Figure 2.2 (below).

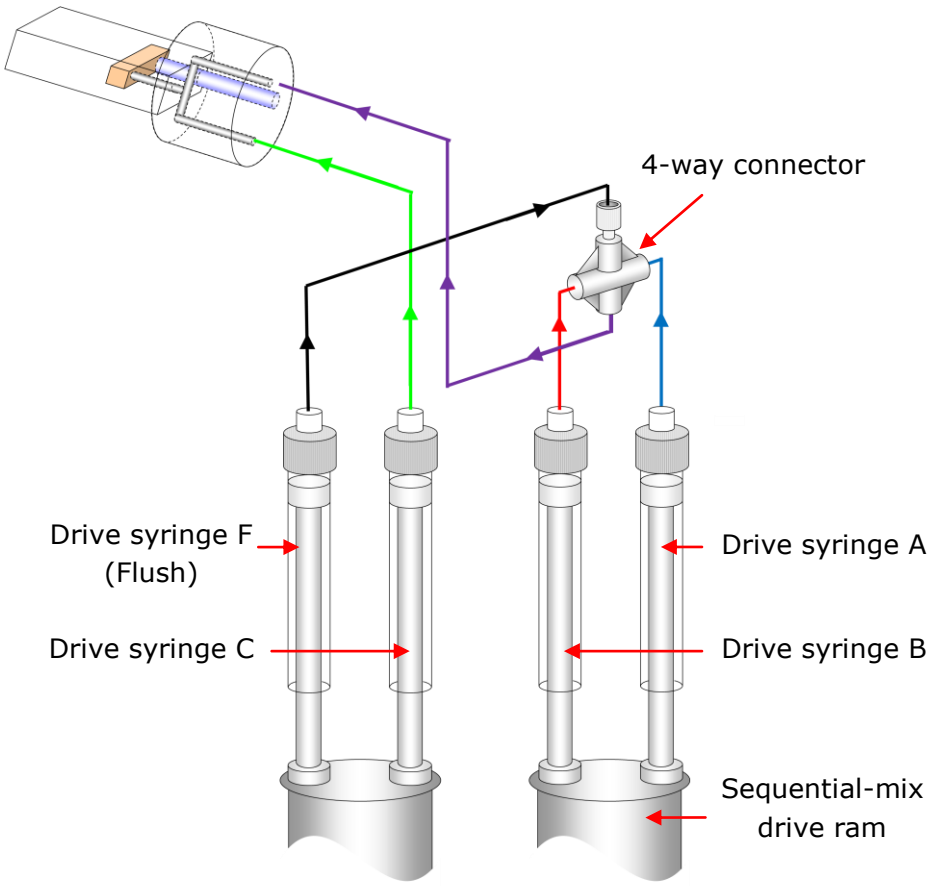

Figure 2.2: schematic of the set-up of the SF.3 for sequential-mixing operation (components of the Auto-Stop assembly are omitted for clarity)

Solutions of A and B (shown in blue and red respectively in Figure 2.2) are loaded into the additional drive syringes. Mixing of these occurs in the 4-way connector when the sequential-mix (or pre-mix) drive ram is fired, the mixed solution (shown in purple) then enters an ageing loop after a user-defined delay time. The mixed solution is forced from the loop by the buffer solution (black) and is further mixed with solution C (green) in the T-mixer when the single-mix (or flush) drive ram is fired. The final mixture (brown) passes through the observation chamber to the stop syringe (not shown in Figure 2.2). It is important for this process that the volumes of each drive is set correctly (Section 7.7.3).

Repeat drives cannot be used for the sequential mixing operation, since both rams need to be reset between drives.

2.3 Ratio-mixing Operation

In its standard configuration, the SF.3 is fitted with drive syringes of equal volume to provide a mixing ratio of 1:1. To achieve other mixing ratios, syringes of differing volumes may be used. For example, where a rapid dilution effect is required, a 1:10 ratio is achieved using 0.25 mL and 2.5 mL syringes. Operation at ratios other than 1:1 is referred to as ratio- or asymmetric- mixing, and the set-up for it is described in Section 5.7.3 of this User Manual.

2.4 Monitoring the Course of the Reaction

The course of the reaction is monitored spectroscopically, by following a change in the absorbance, fluorescence or circular dichroism signal over time.

Normally stopped-flow work is conducted at a single observation wavelength corresponding to that at which the maximum signal change occurs during the course of the reaction. For reactions exhibiting signal changes at more than one wavelength, stopped-flow drives can be conducted at more than one wavelength, and the changes monitored in spectral or kinetic dimensions, a technique referred to as Spectra-Kinetics.

Various lamps and detectors are available to optimize the instrumental set-up in the various modes of operation. These will be described in detail later in this User Manual.

Chapter 3: SF.3 HARDWARE

This chapter provides detailed information on the hardware components of the SF.3, and if relevant on their removal and replacement. Please be sure that you understand the purpose and operation of all the components of the SF.3 before beginning the installation and set-up procedures described in Chapter 5 of this User Manual.

Figure 3.1 (below) shows the fully assembled SF.3 unit. The principle components are labelled for reference; each of these is described in detail later in this chapter.

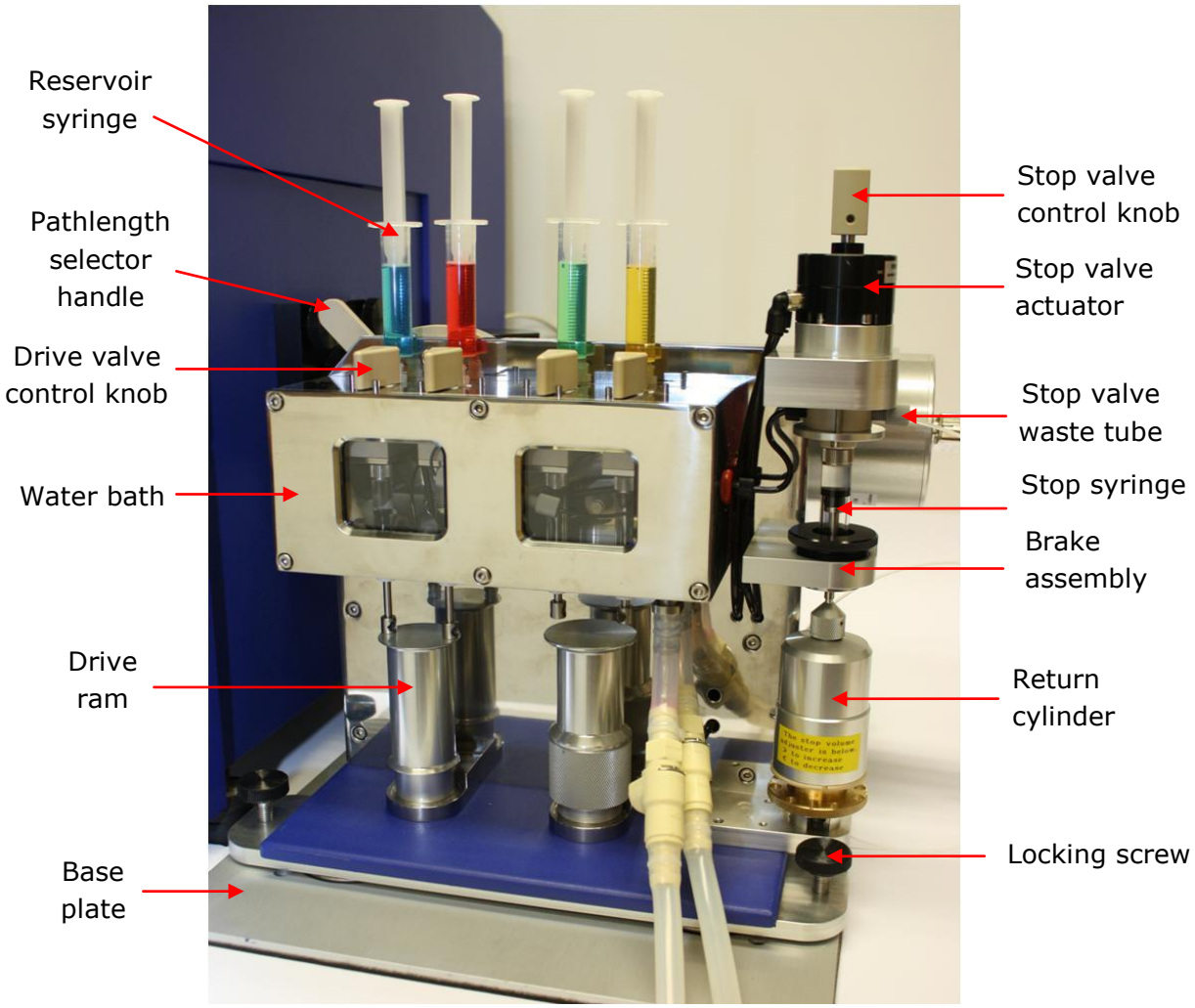

Figure 3.1: the fully assembled SF.3 mounted on the Spectrometer.

3.1 The Flow Circuit

The flow circuit is the route that the reactants follow within the SF.3. The components of the flow circuit depend on whether single- or sequential-mixing is used. All components of the circuit, and hence all materials in contact with the reactants, are biocompatible. The drive and stop syringe barrels are glass, the syringe tips are PTFE, the syringe plunger tips are UHMW-PE, microvolume flow tubing and valves are PEEK, and the optical cell is constructed from quartz.

3.1.1 The flow circuit for single-mixing operation

For single mixing operation the flow circuit can be summarized as:

Reservoir syringe → drive valve → drive syringe → drive valve → flow tubing → T-mixer → observation chamber → flow tubing → stop valve → stop syringe → stop valve → waste

Each reactant is loaded into a drive syringe from a reservoir syringe mounted above it; connection of the drive syringe either to the reservoir syringe or the T-mixer is controlled by a manually operated drive valve. After passing through the T-mixer and observation chamber, the mixed reactants enter the stop syringe through a stop valve which connects the stop syringe either to the observation cell or to waste.

3.1.2 The flow circuit for sequential-mixing operation

For sequential mixing reactants A and B the circuit is:

Reservoir syringe → drive valve → drive syringe → flow tubing → pre-mixer → flow tubing ageing loop → T- mixer and then as for single mixing

For reactant C the circuit is as for single mixing.

3.2 Pneumatic Drive and Drive Rams

The SF.3 uses a pneumatic drive system with two drive rams, shown in Figure 3.2 (below).

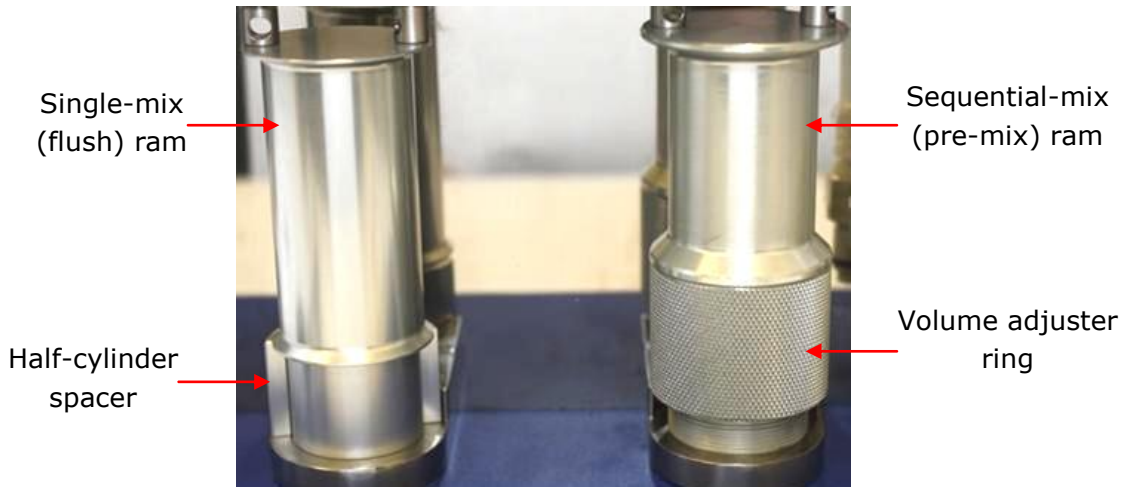

Figure 3.2: single-mix (left) and sequential-mix (right) drive rams. The half-cylinder spacer shown in place on the single-mix ram platform should be removed for single-mixing operation.

The left (single-mix or flush) ram is driven at a pressure of 4 bar (60 p.s.i.), and is designed to perform multiple drives from fully loaded syringes in single-mixing operation. It also functions as the flush ram in sequential-mixing mode. The ram does not control the volume of the stopped-flow drive. The right (sequential-mixing , or pre-mix) ram is driven at a higher pressure than the single-mix ram (8 bar, 120 p.s.i.) and is designed to deliver a constant volume of reactants A and B during the pre-mix step of the sequential-mixing operation.

For single-mixing operation, only the single-mix ram fires; for sequential-mixing the sequential-mixing ram fires first, followed at user defined interval by the single-mix ram. The volume of the first drive of sequential-mixing operation is set by a knurled ring on the sequential-mix ram (Section 7.7.3). The total drive volume is set on the Auto-Stop assembly (Section 7.7.1).

The pressure difference between the rams is to prevent the sequential-mix ram being driven backwards during the second drive. To prevent the single-mix ram being driven backwards during the first drive, it must be resting either on the ram platform or on a half-cylinder spacer. Use of the spacer limits the travel of the ram, thereby reducing the amount of buffer and reactant C required. The spacer should be removed for single-mixing operation.

3.3 Waterbath and Cell Block

3.3.1 Description

The drive syringes and flow tubing described below are contained in a sealed waterbath through which circulating fluid can be passed for temperature control. The circulating water also passes through the rotatable cell block which houses the optical cell, and the temperature is read by a thermocouple within the waterbath. This ensures that the reaction temperature is known accurately. If the appropriate fluid circulators are used (Section 3.10.3), the temperature can be set in the SF.3 Pro-Data control software. The waterbath and cell block are shown in Figure 3.3 (below).

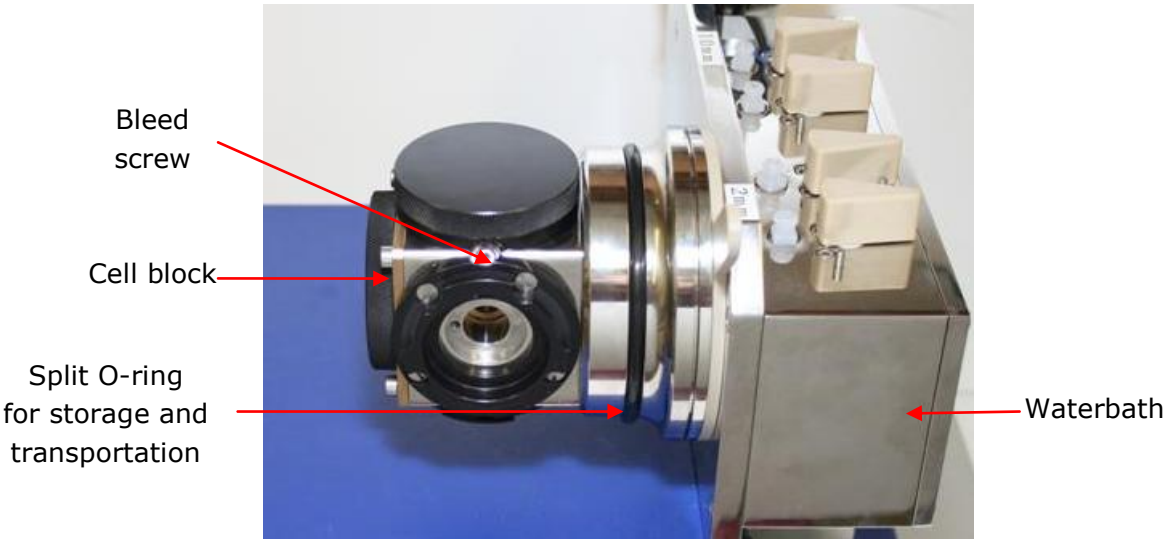

Figure 3.3: the SF.3 waterbath and cell block. The split O-ring should be removed before the SF.3 is installed on the spectrometer

3.3.2 Draining the waterbath and cell block

Drain the waterbath as follows:

1. Ensure that the water circulator unit is switched off (Section 3.10), but is connected to the SF.3 unit.
2. Loosen the bleed screw on top of the cell block using a 2.5 mm hexagonal wrench.
3. Allow the waterbath to drain.
4. Tighten the bleed screw on top of the cell block.

The front panel of the bath can then be removed by unscrewing the six retaining screws.

3.4 Drive syringes

A range of custom designed drive syringes may be supplied by Applied Photophysics with the SF.3 accessory, to suit the application. The syringes are manufactured by Kloehn Ltd, Nevada, U.S.A.

3.4.1 Description of the drive syringes

Drive syringes of various sizes and their fittings are shown in Figure 3.4 (below). The syringe barrels are glass, the syringe tips are PTFE, and the plunger tips are UHMW-PE. The sample may also be in contact with the epoxy adhesives used to cement the syringe ferrule to the barrel.

Figure 3.4: 250 µL, 1 mL and 2.5 mL drive syringes (left to right), with their respective grommets.

For single-mixing operation, two 1.0 mL drive syringes are fitted as standard, although 2.5 mL syringes can also be used. 250 µL and 2.5 mL syringes are also supplied for ratio mixing (a PEEK extender is mounted on the plunger of the 250 µL syringes). For sequential-mixing two additional drive syringes are required; the standard size is also 1.0 mL, although combinations of the other volumes can be used for ratio mixing.

Each syringe screws into a drive valve base plate. The SF.3 waterbath is fitted with rubber grommets to provide a leak-free seal between the drive syringes and the waterbath wall: three sizes of grommet are supplied to match the diameters of the syringes.

The normal temperature operating range of the drive syringes determines the operating range of the SF.3 as being 5°C to 40°C. At higher temperatures, the adhesive used in the syringe construction deteriorates, whereas at lower temperatures leakage may occur between the plunger tip and the syringe barrel.

3.4.2 Removal and replacement of the drive syringes

Drive syringes are shown in position in the waterbath in Figure 3.5 (below). The front of the waterbath and some of the flow tubing have been removed for clarity. The syringes are labeled from right to left as A, B, C and F (flush).

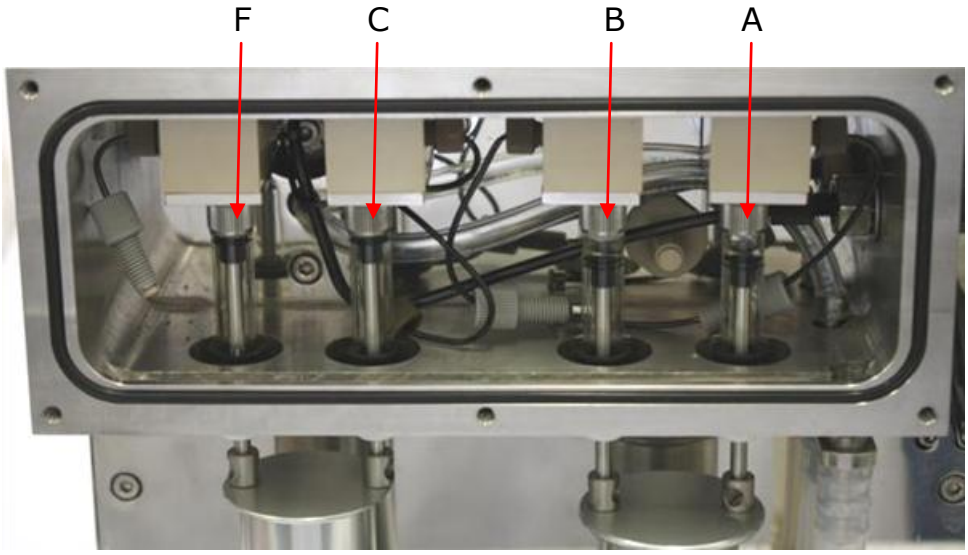

Figure 3.5: the drive syringes in position: for clarity the flow tubing connectors have been removed. Syringes are labelled A, B, C and F (flush) from right to left.

Drive syringes can be removed and replaced either while the SF.3 is connected to the spectrometer, or while it is free standing.

CAUTION: Before attempting to remove the drive syringes, ensure that the waterbath is drained.

A drive syringe should be removed as follows:

1. With the waterbath drained, remove the bath front panel by unscrewing the six retaining screws.
2. Lower or remove the drive ram, and using the fixing tool shown in Figure 3.6 (overleaf), unscrew and remove the retaining ring. The lugs on the fixing tool insert into holes on the underside of the ring.
3. Gripping, the metal fitting, unscrew the syringe from its mounting block, and lower the syringe and grommet out of the waterbath.

Figure 3.6: drive syringe retaining ring (left), and fixing tool (right)

To replace the syringe, first mount the grommet on the syringe. It may be necessary to withdraw the syringe plunger from the syringe barrel to allow this. Then perform in reverse the procedure used for removal. Remember that a leak test should be carried out after installation of the syringes (Section 7.5)

3.5 Reservoir Syringes

Each drive syringe is filled from a reservoir syringe mounted vertically above the waterbath; 1 to 5 mL Luer fitting (slip or lock fit) disposable plastic syringes are recommended. The tips of the syringes are inserted into the female Luer connectors on the waterbath top. The reservoir syringes are shown mounted on the SF.3 in Figure 3.6 (below).

Figure 3.6: reservoir syringes mounted on the SF.3. All four syringes are used for sequential-mixing; only syringes C and F are used for single-mixing

3.6 Drive Valves

Four PEEK drive valves are fitted to the SF.3, to control the flow of solution to and from the reservoir syringes. The valves are controlled manually by rotating the control knobs through 90º between the load and drive positions. When the valve is set to the **Load** position (control knob pointing to the side) the reservoir and drive syringes are connected, when set to the **Drive** position (control knob pointing to the front), the drive syringe and flow circuit are connected (Figure 3.7, below). The A and C drive valves are right-handed (valves rotate clockwise in changing from **Load** to **Drive** positions), whereas the B and F valves are left-handed (valves rotate anticlockwise in changing from the **Load** to **Drive** positions).

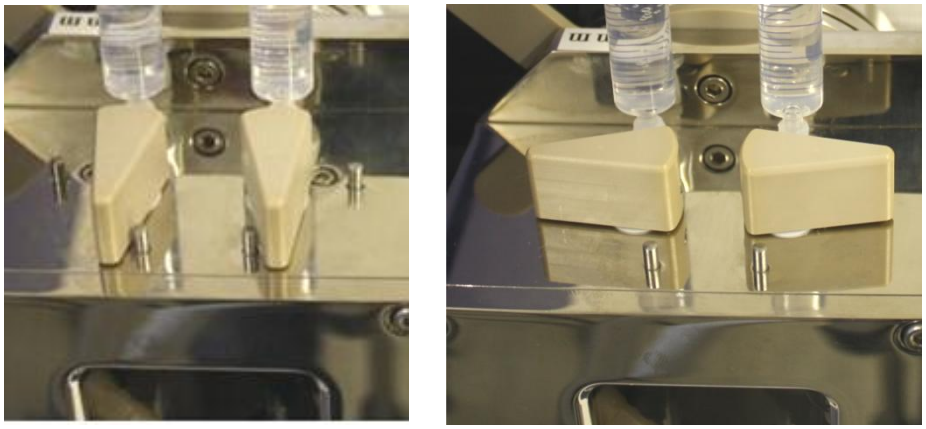

Figure 3.7: drive valves set to the Drive position (left) and Load position (right)

3.7 Flow Tubing

3.7.1 Description of the flow tubing

The flow circuit utilises custom-made PEEK microvolume flow tubing terminated with compression fittings to link the drive valves with the optical cell and the cell with the stop valve. The rigidity and chemical inertness of PEEK make it ideally suited for this purpose. The internal diameter of all the flow tubing upstream of the optical cell is 1.56 mm. The tube connected between the cell and stop valve has an internal diameter of 3.12 mm. The volume of the ageing loop used in sequential-mixing operation is 115 µl.

All the connections between the flow tubing and the various valves and connectors in the flow circuit use threaded fittings with the exception of that between the tubing and the optical cell face. This connection uses a

pressure plate, which creates the seal between the flow tubing and the optical cell face. The arrangement of the pressure plate and flow lines is shown in Figure 3.8 (below), with the pathlength set to 2 mm; when set to 10 mm the plate is rotated clockwise by 90°. The drive valves and syringes have been removed for clarity.

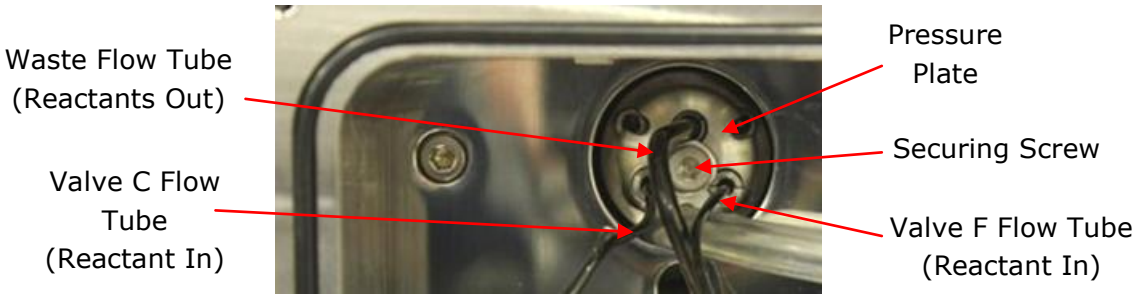

Figure 3.8: the arrangement of the pressure plate and flow lines when the pathlength is set to 2 mm. For clarity the drive valves and syringes have been removed.

3.7.2 Connecting the flow tubing

The configuration of the flow tubing for single and sequential mixing operation is described in Section 5.7.1 and Section 5.7.2 respectively. Tubing connections are made using a linear connector as shown in Figure 3.9 (below) or a similar 4-way connector. When making the connections, tighten to firm finger tightness or use the tightening tool provided (Figure 3.9, below). When the flow circuit is complete, a leak test should be performed (Section 7.5).

CAUTION: do not over tighten the flow tubing connectors, use only firm finger tightness or the tool provided, do not use pliers or other tools.

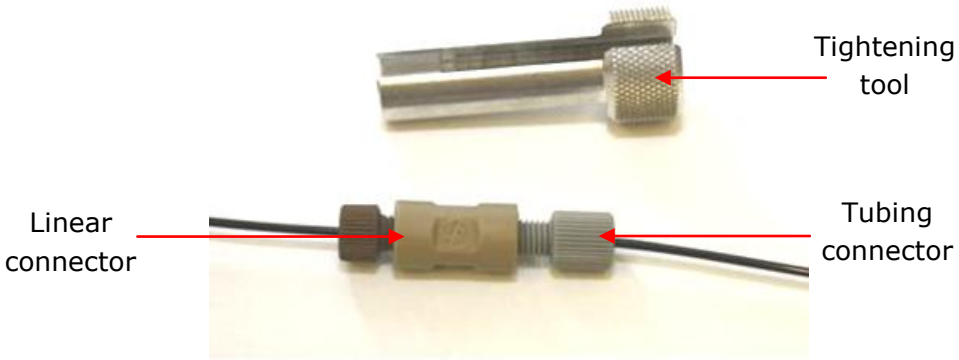

Figure 3.9: tubing connection and tightening tool

3.8 Interchangeable Cell Cartridge System

3.8.1 Description of the cell cartridge system

A 20 µl flow-through optical cell with integral T-mixer is supplied as standard with the SF.3; an optional 5 µl cell with a reduced dead time is also available for rapid kinetics measurements. Two different pathlengths are available for each cell. Pathlength selection is made by rotating the cell through 90º. Each cell also features a fluorescence window. Figure 3.10 (below) shows a schematic of the internal design of the 20 µl optical cell.

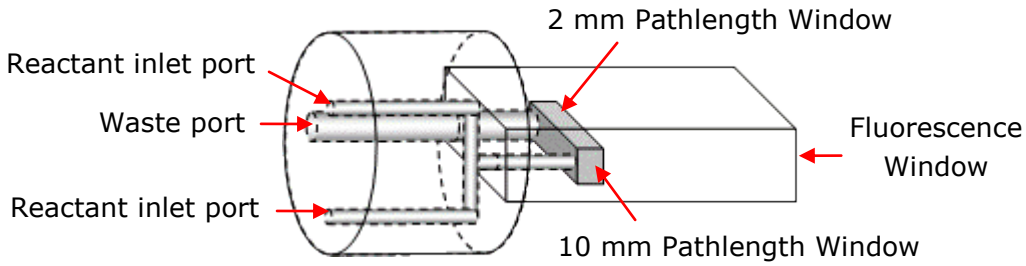

Figure 3.10: schematic of the internal design of the 20 µl optical cell.

NOTE: the 5 µl cell must be operated with the 1 mL stop syringe provided with the cell.

The cells are bonded within cartridges that are designed to be easily interchangeable. A cell cartridge removed from the cell block is shown in Figure 3.11 (below).

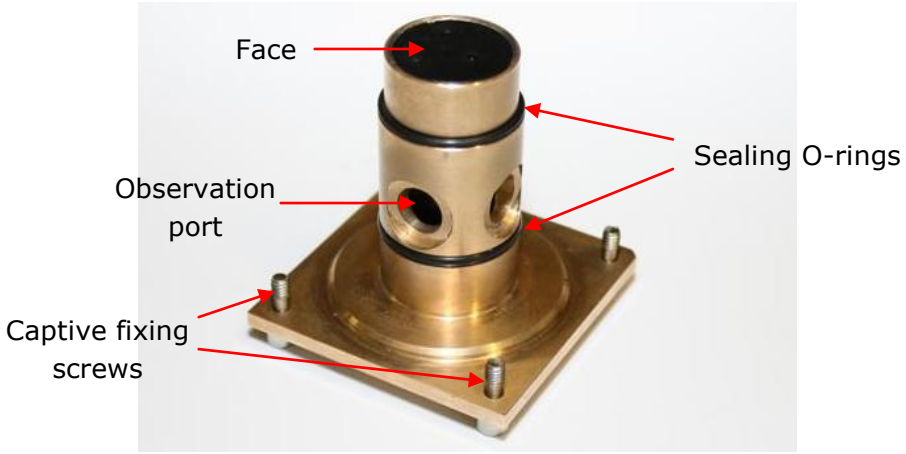

Figure 3.11: the cell cartridge

When mounted in the SF.3, the cell cartridge face presses against the tubing arrangement to the rear of the pressure plate, to form a watertight connection.

3.8.2 Cell pathlengths and dead times

Table 3.1 (below) shows the pathlengths and approximate stopped-flow dead times of the optical cells available from Applied Photophysics.

Cell volume, µl	Pathlengths, mm	Uses	Approximate dead time, ms
20	2 and 10	Standard SF	1.1
5	1 and 5	Rapid SF	0.5

Table 3.1: pathlengths and approximate dead times for the SF.3 optical cell options

The shorter dead time of the 5 µl cell allows it to be used for rapid stopped-flow measurements. It also provides additional pathlength options, but its fluorescence sensitivity is lower than that of the 20 µl cell owing to its smaller fluorescence window.

For fluorescence measurements the choice of pathlength will depend on the absorption and concentration of the sample. Usually the 2 mm pathlength is preferred, since inner filtering, i.e. the partial blockage of the excitation light by absorption, may occur, resulting in an apparent change in the sample fluorescence.

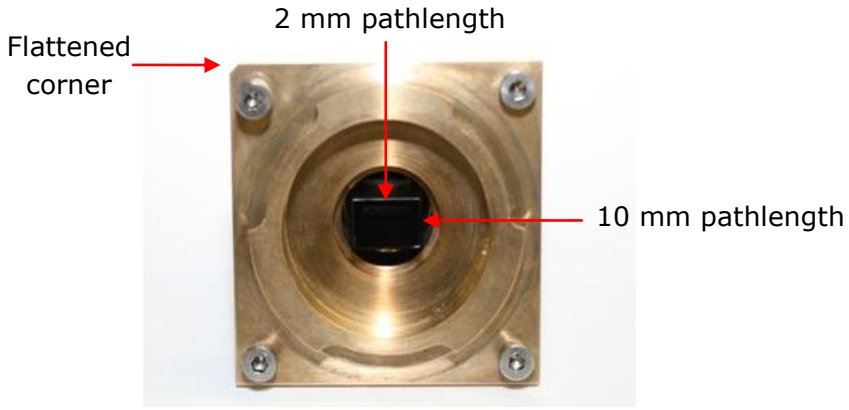

Figure 3.12: detail of the cartridge fluorescence port

Figure 3.12 (previous page) shows detail of the cell cartridge end-plate. The end of the optical cell can be seen in the central cavity. The 10 mm pathlength is with the light beam parallel to the longer cell dimension.

3.8.3 Selecting the pathlength

NOTE: the cell pathlength must be set before the SF.3 is connected to the spectrometer.

Remove the detectors, and disconnect the SF.3 from the Chirascan. The optical pathlength can then be selected by rotating the cell through 90º using the pathlength handle. The shorter pathlength is with the handle to the left when the SF.3 is viewed from the front, and is labelled 2 mm on the waterbath; the longer pathlength is with the handle to the right, and is labelled 10 mm.

3.8.4 Removal and replacement of the cell cartridge system

CAUTION: Before attempting to remove the cell cartridge, ensure that the waterbath is drained.

WARNING: Before disconnecting the detectors, zero the HV voltage by clicking the Zero HV button on the Pro-Data Chirascan control panel.

The cell fitted in the SF.3 should be removed according to the following procedure:

1. Disconnect and remove the detectors from the cell block.
2. Remove the water bath front panel by unscrewing the six retaining screws.
3. Loosen the pressure plate that seals the three flow tubes against the face of the cell cartridge about one quarter turn using the extended 3 mm hexagonal wrench.

NOTE: take care not to unscrew the pressure plate screw too far, as this will cause the tubing attachments to come free. One quarter turn of the screw is normally sufficient.

4. Undo the four captive fixing screws that secure the cell cartridge to the cell block, and carefully withdraw the cell cartridge, as shown in Figure 3.13 (overleaf).

Figure 3.13: removal of the cell cartridge by withdrawal from the rear of the SF.3 unit

The refitting procedure is the reverse of the removal procedure:

1. Ensure that the two O-ring seals on the cartridge and the 55 mm diameter O-ring that seats in a groove on the cell block, are all present.
2. Insert the cell cartridge into the cell block with the flattened corner of the cartridge aligned with the flattened corner on the cell block.
3. Secure the cartridge in position with the four captive fixing screws.
4. Tighten the pressure plate screw to ensure a leak free seal between the three flow tubes and the cell.

CAUTION: do not over tighten the pressure plate as this may result in the quartz cell cracking. A light pressure is all that is required.

5. Re-attach the waterbath cover.
6. Reconnect the detectors.

NOTE: whenever any component of the flow circuit has been changed, it is recommended that after reassembly the flow circuit is checked to ensure that it is leak free (Section 7.5).

3.9 The Auto-Stop Assembly

The Auto-Stop assembly is mounted on the right hand side of the SF.3 Unit. It has three main functions:-

- To control the volume of each stopped-flow drive,
- To stop the flow,
- To trigger data acquisition at the moment the flow is stopped.

The Auto-Stop assembly is shown in Figure 3.14 (below). Its components are described in more detail in the succeeding sections of this chapter.

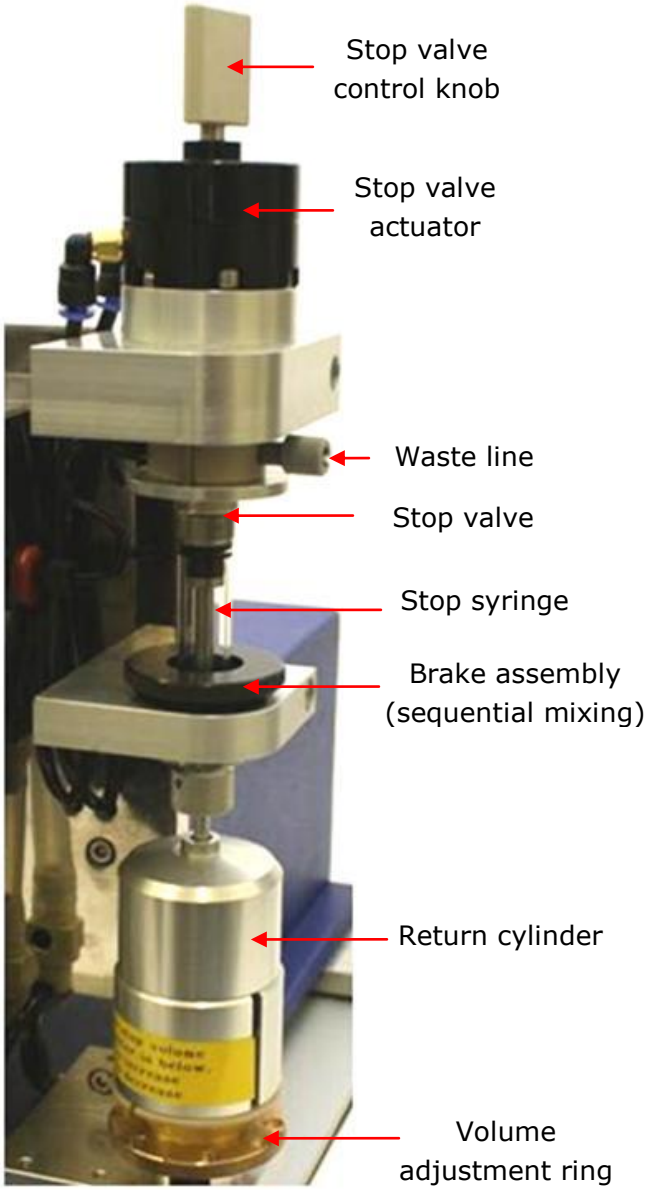

Figure 3.14: the Auto-Stop assembly

3.9.1 The stop valve

The PEEK stop valve controls the flow of solution from the flow circuit into the stop syringe and from the stop syringe to the waste vessel. The valve may be operated either manually or using the software controlled Auto-Stop actuator. A 180º rotation of the valve sets the connection. When the valve is set to the **Drive** position (control knob pointing to the rear) the stop syringe is connected to the flow circuit. When the valve is set to the **Waste** position (control knob pointing to the front) the stop syringe is connected to the waste vessel. These positions are shown in Figure 3.15 (below).

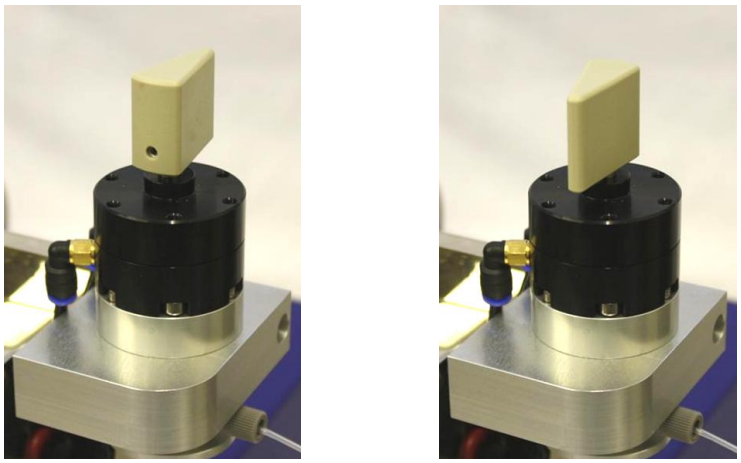

Figure 3.15: the stop valve control knob in the **Drive** position (left) and **Waste** position (right)

A polypropylene tube connects the waste port to a waste vessel.

3.9.2 The Auto-Stop actuator

The Auto-Stop actuator enables the stop valve to be operated automatically by the SF.3 control software. For trouble-free operation of the stop valve it is important that a supply pressure of 8 bar is maintained.

3.9.3 The stop syringe: description

The stop syringe acts as a small reservoir of waste solution from which a volume equal to the total drive volume is discharged prior to each stopped-flow drive. During the drive, the stop syringe is refilled and the

action of the plunger striking the trigger stops the flow and triggers data acquisition.

The stop syringe is screwed into the lower threaded port of the stop valve. The syringes supplied by Applied Photophysics are custom manufactured by Kloehn Ltd. The standard sizes for the stop syringe are 1.0 mL for single-mixing and 2.5 mL for sequential-mixing, where larger total drive volumes are used. The syringe materials are the same as those of the drive syringes: the barrels are glass, the plunger tip is UHMW-PE, and the syringe tip is PTFE.

3.9.4 The stop syringe: removal and replacement

1. If the brake system is in place, first unscrew and raise the knurled retaining ring and remove the brake half-shoes (Section 3.9.5)
2. Unscrew and lower the brake assembly mounting plate and remove the brake shoes.
3. Set the stop valve control knob to the **Waste** (front) position, and manually raise the stop syringe plunger to the fully up position by gripping the knurled plunger handle.
4. Set the valve control knob to the **Drive** (rear) position, and while gripping the plunger shaft to prevent it rotating, if necessary using a pair of fine nosed pliers, unscrew the knurled handle from the shaft.
5. While holding the handle down against the spring, unscrew the syringe from the mounting block, and manoeuvre it free.

To replace the syringe, reverse this procedure.

3.9.5 The brake assembly

The brake assembly is only fitted to instruments equipped with the sequential-mixing option, and is only used for sequential-mixing. For single-mixing, it may be disengaged if a 2.5 mL syringe is used, but should be removed if a 1 mL stop syringe is used.

NOTE: The brake mechanism is provided to improve the results obtained when performing sequential mixing experiments. It imparts friction on the stop syringe plunger and hence prevents over-run after the first (pre-mix) drive, which could otherwise result in cavitation in the flow line and inconsistent results.

Components of the brake assembly are shown in Figure 3.16 (below). Note that within the underside of the retaining ring are a PTFE washer and split retaining ring, and a 26 mm diameter insert washer (Figure 3.17, below). The insert washer seats within the retaining ring.

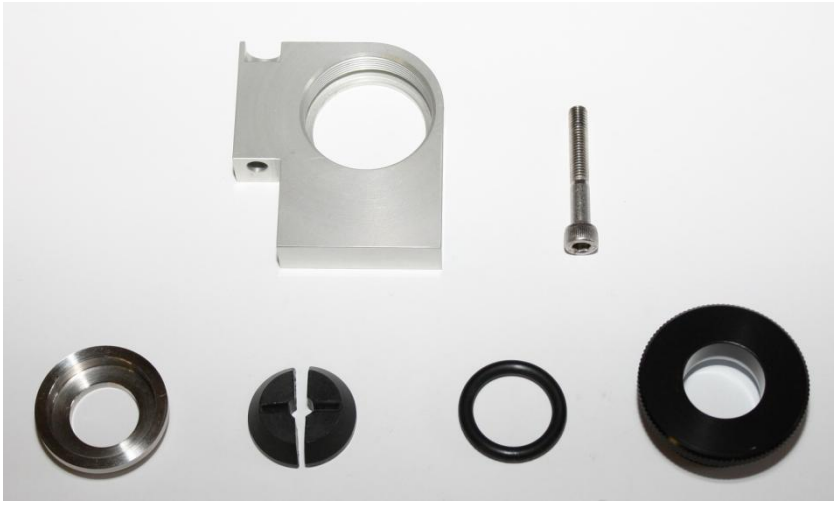

Figure 3.16: components of the brake assembly: left to right, top: mounting plate and screw; bottom: seating ring, brake shoes, O- ring, knurled retaining ring.

Figure 3.17: underside of the retaining ring; the 26 mm diameter PTFE washer seats within the PTFE split ring

The mounting plate is screwed to the SF.3 unit, and the components are then introduced in the following order: seating ring, brake shoes (with the smaller face uppermost), O-ring, and finally the knurled retaining ring, which screws into the mounting plate (Figure 3.18, overleaf).

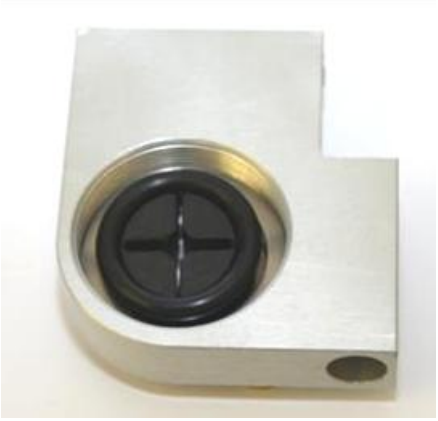

Figure 3.18: position of the seating ring, brake shoes and O- ring within the brake assembly mounting plate

To mount the brake assembly on the SF.3:

1. Remove the stop syringe (Section 3.9.4)
2. Loosely place the mounting plate, seating ring, O-ring and retaining ring over the stop syringe, and remount the syringe.
3. Place the brake shoes in position on the seating ring, and screw the mounting plate to the SF.3 at the threaded hole in the unit side, using the screw provided.

The friction is increased by tightening the knurled screw by rotating it clockwise, thereby engaging the brake shoes more firmly on the syringe plunger. It must be disengaged for single mixing stopped-flow so that it does not impair the dead time of the instrument, but tightened up, as necessary, for sequential-mixing operation.

3.9.6 Stopped-flow trigger

When the stop syringe plunger makes contact with the trigger, the drive stops, and data acquisition is simultaneously activated.

3.9.7 Return cylinder and volume adjustment

This upward movement of the return cylinder automatically discharges the stop syringe. The cylinder is pneumatically driven by compressed gas at a pressure of 8 bar.

The drive volume is set using the knurled adjuster ring mounted below the return cylinder. The zero position for the ring is the fully down

position. To find this, rotate the ring anticlockwise (counterclockwise) when viewed from below, i.e. the front of the ring moves from right to left, until there is no further travel. The desired drive volume is then set by rotating the ring clockwise. Each revolution produces a travel of 1 mm in the stop syringe: this is equivalent to about 33.3 µl for a 1 mL syringe, or about 83.3 µl for a 2.5 mL syringe. The drive volume thus set is the total drive volume, i.e. the volume of the one single-mix drive or of both sequential mix drives.

3.9.8 The Auto-Stop discharge cycle

The Auto-Stop discharge cycle is controlled by three solenoid valves that when activated connect the actuator and return cylinder to the high pressure pneumatics. The first solenoid controls a connection to the stop valve, rotating it to the **Waste** position when activated. The second controls a connection to the return cylinder, when activated raising it by the distance set by the volume adjuster. The third solenoid also controls a connection to the stop valve, returning it to the **Drive** position when activated.

The timing of these operations is critical and is controlled from the **Waste cycle** tab on the **SHU Setup** dialog box (Section 6.4.5). Under normal circumstances the user will not need to alter these timings

3.9.9 Discharging the stop syringe manually

The stop syringe can be discharged manually. This is particularly useful for cleaning the flow circuit before and after measurements. To discharge the stop syringe, use the following procedure:

1. Set the valve control knob to the **Waste** (front) position.
2. Grip the lower part of the stop syringe plunger and push upward expelling the contents of the syringe to the waste vessel.
3. Return the valve control knob to the **Drive** (rear) position.

If the Pro-Data control software has been configured for the SF.3 (Chapter 6), the stop syringe can be discharged using the control software by clicking the **Waste** button on the **KSHU** panel or the **Waste** button on the **General** tab on the **SHU Setup** dialog box (Section 6.4.7).

3.10 Water circulator unit

3.10.1 Temperature control of the SF.3

The sample handling unit is fitted with a waterbath to provide temperature control of stopped-flow experiments, over the range 5°C to 40°C. A fluid circulator may be connected to pump distilled water through the SF.3 waterbath and cell block, thereby ensuring temperature control of the entire flow circuit.

NOTE: Distilled water should be used as the circulating fluid. Applied Photophysics recommend the addition of an anti-bacterial agent and antifreeze to the distilled water, provided that these comply with circulator supplier's specifications.

A thermocouple located in the waterbath reports an accurate temperature measurement to the software. This is recorded with each stopped-flow drive.

3.10.2 Circulator unit connections

The circulator unit is connected to the SHU waterbath via 8 mm tubing. The SHU is fitted with quick-fit connectors to allow the circulator to be connected and disconnected rapidly without the need to drain the waterbath. The front port is the inlet, which connects to the circulator outlet, and the rear port is the outlet, which connects to the circulator inlet.

3.10.3 Software controlled circulator units

Applied Photophysics protocols allow the SF.3 control software to set the temperature of the SF.3 for specified fluid circulators. The circulator should be connected to the serial port of the workstation PC by an RS232 cable. Recommended circulators are:

- Neslab RTE 200, 300
- Fisher Scientific 3016
- Thermo RTE 7

For further information on circulator unit compatibility please contact the Technical Support Department at Applied Photophysics.

Chapter 4: LAMPS AND DETECTORS

The lamps and detectors used with the SF.3 are not considered to be components of the SF.3 accessory, and are described in the main Chirascan™ User Manuals. However, it is important for effective stopped-flow work that the appropriate choice of both is made, and in this chapter the configurations suitable for stopped-flow operation are described.

4.1 Lamps

4.1.1 Lamp types

Selection of the appropriate lamp type is very important for stopped-flow studies. The choice will depend on individual experimental requirements, but lamp output and stability are the critical factors when selecting a lamp for stopped-flow operation. Generally the lamp with the highest output at the particular wavelength of interest is selected to provide the maximum signal to noise ratio. The lamps used may be either xenon (Xe) or mercury-xenon (Hg-Xe) arc lamps; the xenon lamps may be either ozone (O_3) producing or ozone free. The lamps available are listed in Table 4.1, below. All lamps are fitted with breech lock connectors for rapid exchange without the need for alignment and optimization.

NOTE: the standard Chirascan 150 W xenon lamp (Ushio -150SP) used for CD scanning with the ESHU does not exhibit the stability required for stopped-flow measurements.

Lamp	Manufacturer	Uses	Gas purge
150W Hg-Xe (O_3 free)	Hamamatsu L2482	Single wavelength > 250 nm	Not required
150W Xe (O_3 free)	Osram XBO 150W/CR OFR	Spectral kinetics > 250 nm	Not required
150 W Xe (O_3 producing)		Far UV region < 250 nm	Nitrogen

Table 4.1: summary of the properties of the lamps available for use with the SF.3

4.1.2 Xenon and Mercury – Xenon lamps

Stopped-flow kinetics work is usually conducted at a single wavelength, and a mercury-xenon lamp is used since it provides significantly higher output than a xenon lamp at wavelengths corresponding to the mercury emission lines. However, a mercury-xenon lamp cannot be used for spectral kinetics, for which a xenon lamp must be used, as it gives a smoother emission spectrum. The xenon lamp is also preferred when working at single wavelengths away from the mercury emission lines, as it gives higher light intensity in these regions.

The lamp emission profile of the Hg-Xe lamp is shown compared with that of the standard Xe lamp in Figure 4.1 (below)

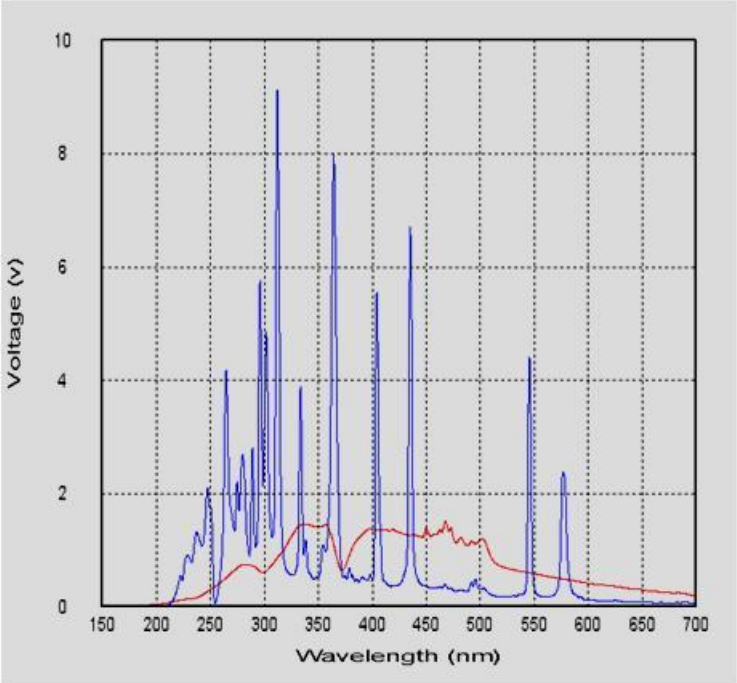

Figure 4.1: comparison of the emission profiles of Hg-Xe (blue) and xenon (red) lamps

The principle mercury emission lines of the Hg-Xe lamp are shown in Table 4.2 (overleaf). A single wavelength corresponding to one of these lines is usually chosen for single wavelength stopped-flow kinetics.

Wavelength, nm	Relative intensity, %	Wavelength, nm	Relative intensity, %
222	6.0	302	52.0
230	9.8	313	100
240	14.2	334	37.1
250	19.2	365	89.5
265	45.6	405	60.8
277	23.9	436	72.8
283	28.8	546	47.9
290	30.7	577	25.4
297	63.1		

Table 4.2: principle emission lines of the Hg-Xe lamp

4.1.3 Ozone-producing and ozone-free xenon lamps

CAUTION: Ozone is a very reactive gas that is produced when UV light interacts with oxygen. If an ozone producing lamp is used, it essential that the Chirascan™ is purged with clean nitrogen before the lamp is ignited. Failure to purge the instrument will result in the formation of ozone, which is damaging to health and can cause deterioration of the optical components in the instrument.

Ozone-free lamps cannot be used at wavelengths in the far UV (< 250 nm), and an ozone producing xenon lamp is available for operation in this region. Ozone producing lamps require constant purging and are generally less economical than the ozone-free alternative. They are not recommended unless it is essential to operate at wavelengths below 250 nm.

4.2 Detectors

The standard range of Chirascan detectors can be used for the corresponding kinetic measurements. The SF.3 is compatible with circular dichroism and fluorescence detectors. For information on the use of two fluorescence detectors with the SF.3 please contact the Technical Support Department at APL.

Chapter 5: HARDWARE INSTALLATION AND CONFIGURATION

This chapter describes the installation and set-up of the SF.3 for single-mixing or sequential-mixing operation. Before beginning the installation or set-up, please be sure that you understand the purpose and operation of the components of the SF.3 described in Chapter 3 of this User Manual. If you are in any doubt, please contact the Technical Support Department at Applied Photophysics for advice.

5.1 General Procedure

This Chapter describes the installation and set-up of the SF.3. The steps involved are:

1. Connecting the SF.3 unit to the spectrometer
2. Electrical connection
3. Connecting the SF.3 pneumatics
4. Connecting the water circulator used for temperature control
5. Connecting the stop valve to waste
6. Setting up the SF.3 for single- or sequential-mix operation
7. Installing the detectors
8. Configuring the control software
9. Setting the temperature

Each of these steps is described in detail in the Sections following.

5.2 Connecting the SF.3 to the spectrometer

The SF.3 is mounted on rollers and can easily be manoeuvred on the bench. Before installing the SF.3, remove the standard Equilibrium Sample Handling Unit from the spectrometer following the procedure described in the main Chirascan™ User Manuals.

CAUTION: before removing the Equilibrium Sample Handling Unit, ensure that the Chirascan is switched off and that the nitrogen purge gas and Peltier cooling water are disconnected.

NOTE: before connecting the SF.3 to the spectrometer, set the required cell pathlength (Section 3.8.3).

Mount the SF.3 locating plate on the spectrometer using the three knurled thumb screws (Figure 5.1, below). The centre of the female connector on the spectrometer should be at a height of between 212 mm and 214 mm above the bench; if necessary alter the height using the adjustment in the spectrometer feet, ensuring that the instrument is level when the adjustment has been made.

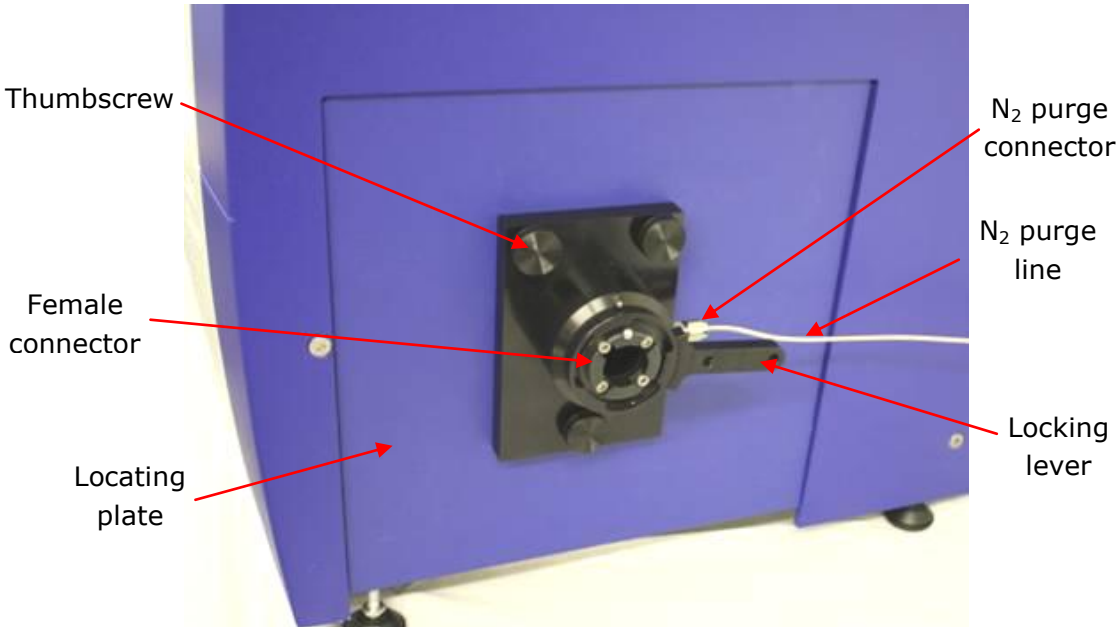

Figure 5.1: the SF.3 locating plate mounted on the Chirascan

1. Place the SF.3 unit on the bench to the right of the spectrometer, and back-off the four locking screws on the base plate by rotating anticlockwise (counterclockwise), until the unit moves freely on its underside rollers.
2. If it is in place, remove the split O-ring from the joint between the waterbath and the cell block (the O-ring is included to relieve stress on the joint during transportation of the SF.3 unit; it should be retained for use when the unit is stored or transported).
3. Select the cell pathlength using the pathlength handle, which rotates through 90º. For the longer pathlength (normally 10 mm) the handle should be to the right when viewed from the front of the unit, for the shorter pathlength (normally 2 mm) it should be to the left.
4. Cap any unused breech lock connectors on the SF.3 using the knurled breech lock cap provided (Figure 5.2, overleaf).

Figure 5.2: breech lock cap for unused connection port

5. While supporting the sample chamber so that its weight is not being taken by the joint with the waterbath, and, with the locking lever in the Unlock position, i.e. about 30º above horizontal, ease the male connector on the SF.3 into the female connector on the spectrometer (Figure 5.3, below)

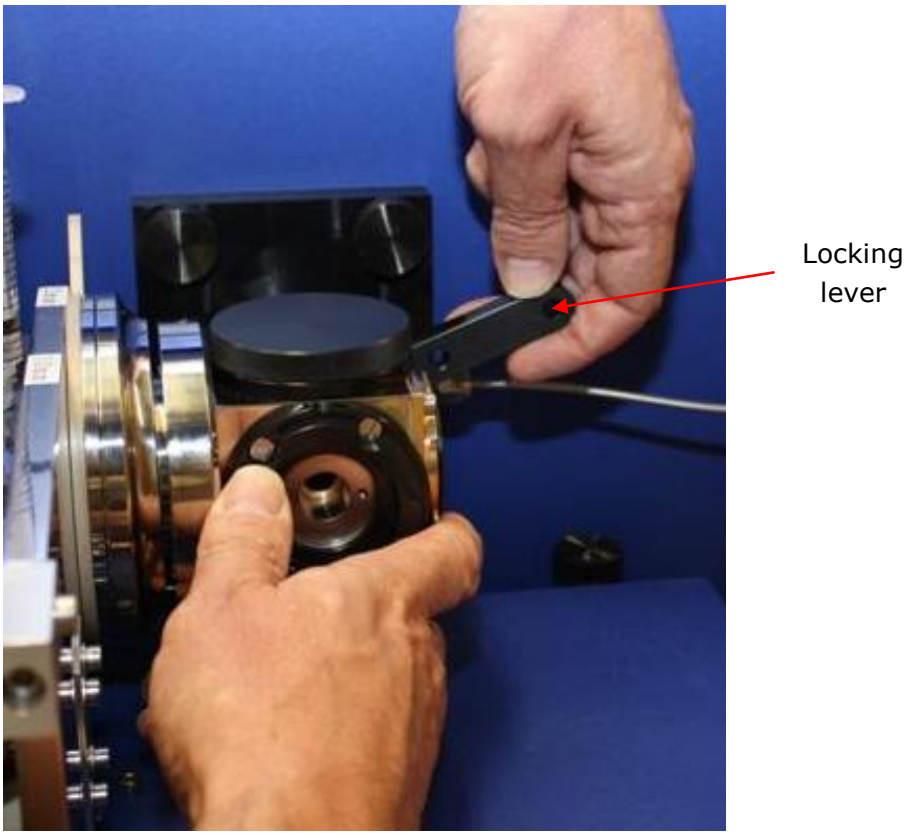

Figure 5.3: the male connector on the SF.3 being eased into the female on the Spectrometer, while the locking lever is held in the Unlock position

6. When the connector has been eased home, lock the unit in place by rotating the locking lever clockwise to the horizontal position. There should be no gap between the front faces of the spectrometer connector and the cell block connector.

7. Ensure that there is no metal-to-metal contact between the inner and outer rings of the cell block, if necessary by adjusting the position of the SF.3 unit slightly, and that no part of the SF.3 other than the connector is in contact with the spectrometer.

NOTE: the seal between the inner and outer rings of the cell block is formed by a rubber bellows, and the joint is designed to prevent the transmission of vibrations from the waterbath to the cell block during a drive. There should be no metal-to-metal contact within the joint, and an equal gap between the rings of 3 to 5 mm.

8. Fix the SF.3 unit in place by turning the locking screws on the plate clockwise until the unit is supported on the plate rather than the underside rollers.
9. If measurements are to be made below 200 nm, connect the nitrogen purge gas supply to the purge gas connector indicated in Figure 5.1.

5.3 Electrical Connection

CAUTION: do not connect the SF.3 to the Chirascan spectrometer unless the spectrometer system is switched off.

Electrical connection of the SF.3 to the Chirascan is from the 37-pin D port labelled **KSHU module** on the rear of the Chirascan electronics rack to the 37-pin D port on the rear on the SF.3 unit (Figure 5.4, overleaf)

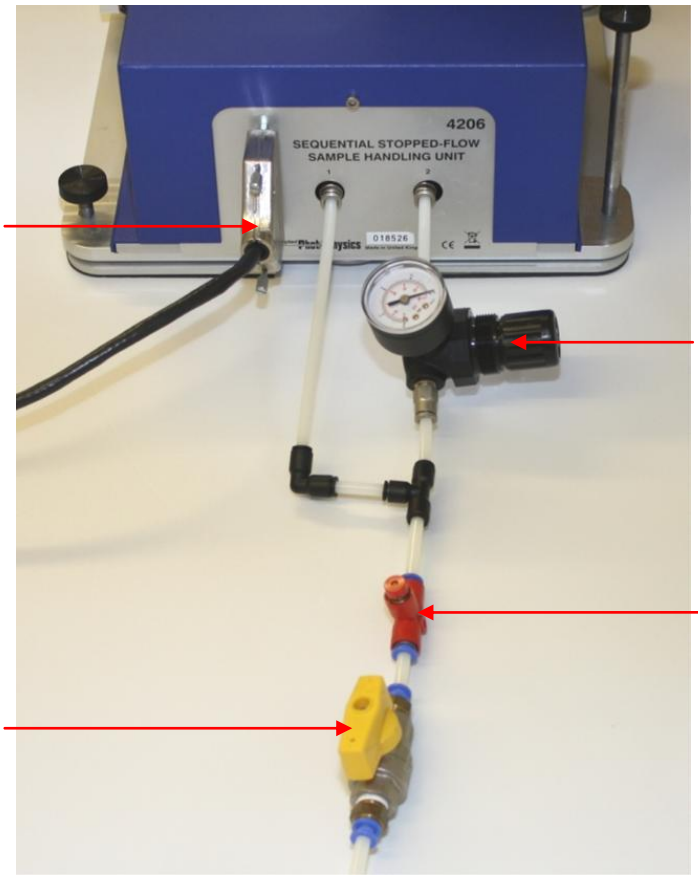

Figure 5.4: electrical and pneumatic connections to the rear of the SF.3 unit

5.4 Pneumatic Connections

The drive rams and Auto-Stop mechanism of the SF.3 are pneumatically driven, and a compressed gas supply at a line pressure of 8 bar is required; the rate of gas consumption is of the order of a few mL per drive. Nitrogen or air may be used, supplied either by a cylinder or a compressor. It is recommended that a separate supply is used from the purge gas. Connection to the SF.3 unit is through 6 mm push-in connectors.

CAUTION: the filter cartridges supplied by APL are only rated to 100 psi (6.8 bar). If the same supply is used for the purge gas and pneumatics, the line must be split upstream of the purge gas filter cartridge, and the supply regulated down to 4 to 6 bar between the split and the cartridge (Figure 5.5, overleaf).

Figure 5.5: pneumatic and filter connections for a single gas source

This pneumatic arrangement is shown schematically in Figure 5.6 (below)

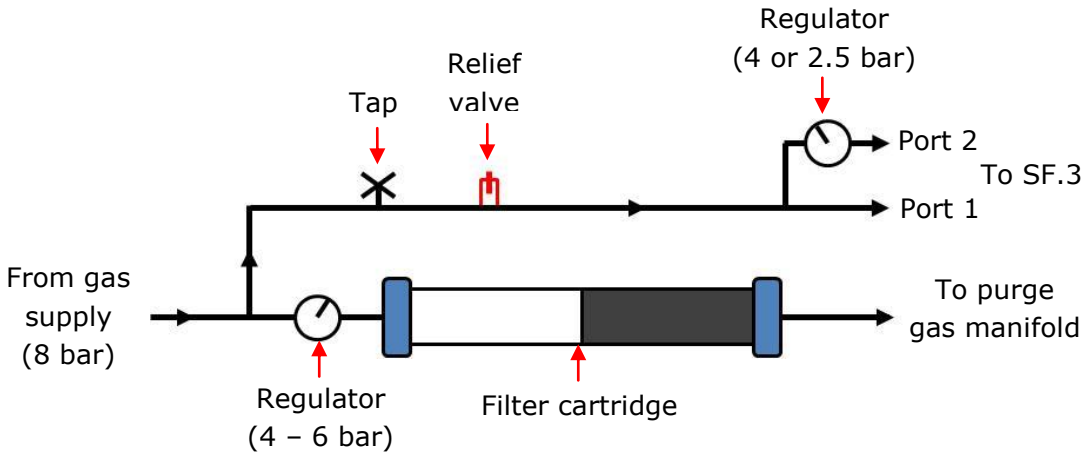

Figure 5.6: schematic of the pneumatic and filter connections

The main pneumatic inlets (labelled 1 & 2) are located on the rear of the SF.3 unit. Port 1 is internally connected to the Auto-Stop assembly and the sequential-mixing drive ram. Port 2 is internally connected to the single-mixing drive ram.

An h-shaped pneumatic tubing arrangement allows both of these ports to be connected to the compressed gas supply (Figure 5.4, previous page). An in-line pressure regulator is included to reduce the drive pressure of the single-mixing drive ram below the 4 bar at which it is set within the SF.3. The regulator cannot be used to increase the drive pressure. A reduced pressure is used for ratio-mixing operation (Section 5.7.3)

CAUTION: the manually operated tap supplied by Applied Photophysics should be included in line upstream of the 'h' arrangement, so that the SF.3 can be isolated from the supply. Include the pressure relief valve in the line between the tap and the 'h' arrangement.

5.5 Connecting the water circulator

The circulator connection to the SF.3 waterbath is through 8 mm tubing. Quick-release stop connectors are fitted to allow the circulator to be connected and disconnected rapidly without the need to drain the waterbath. The waterbath inlet is the front port, which should be connected to the circulator outlet, and the waterbath outlet is the rear port which should be connected to the circulator inlet (Figure 5.6, below).

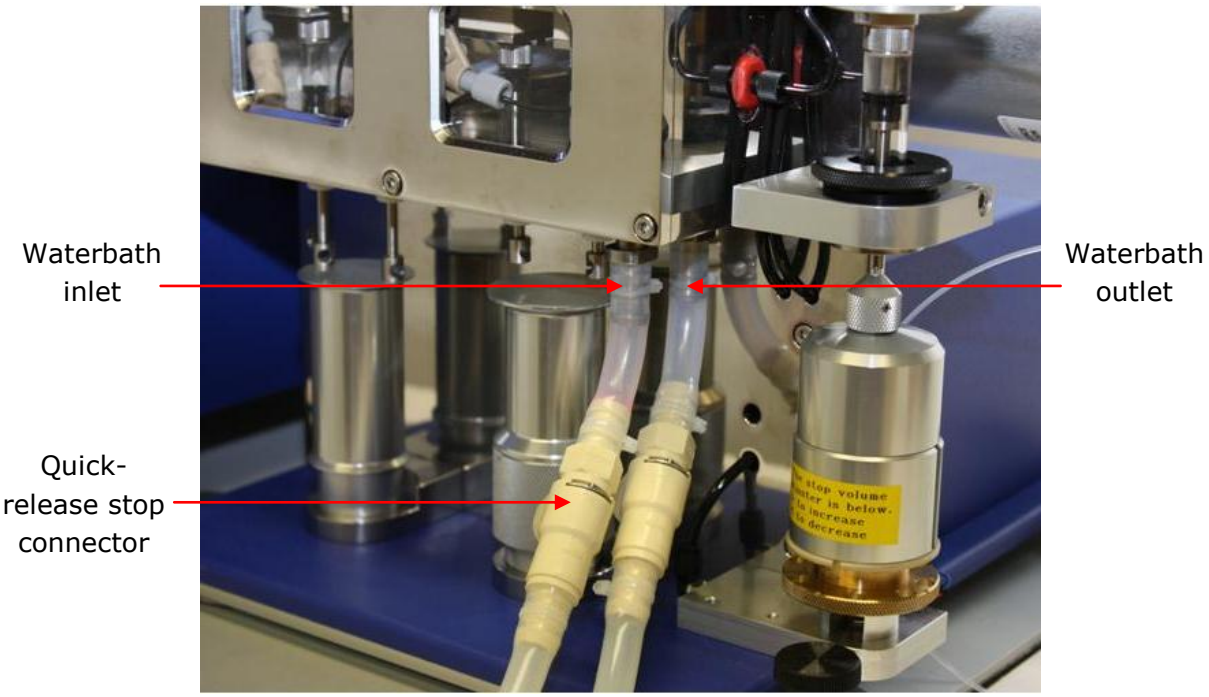

Figure 5.6: waterbath to circulator connections

5.6 Connecting the stop valve to waste

Place the end of the waste tube carrying the PTFE sinker into a suitable receptacle such as a 100 mL bottle.

5.7 Configuring the SF3

5.7.1 Configuration for Single-Mix Operation

The procedure for setting up the SF.3 for single-mixing operation is as follows:

1. Install the required optical cell as described in Section 3.8.4.
2. Install the drive syringes as described in Section 3.4.2. Remember that for single-mixing operation, only the left two syringes (C and F) are used, although the A and B syringes must also be in place to ensure that the waterbath is sealed.
3. Configure the flow circuit as shown in Figure 5.7 (below), and schematically in Figure 5.8 (overleaf), with the F valve connected directly to the pressure plate using the linear connector. The C valve remains connected directly to the pressure plate. The A and B valves, which connect to the 4-way connector, are not used and the free arms of the connector should be plugged.

CAUTION: when tightening the threaded connectors, do not use tools; firm finger tight is all that is required.

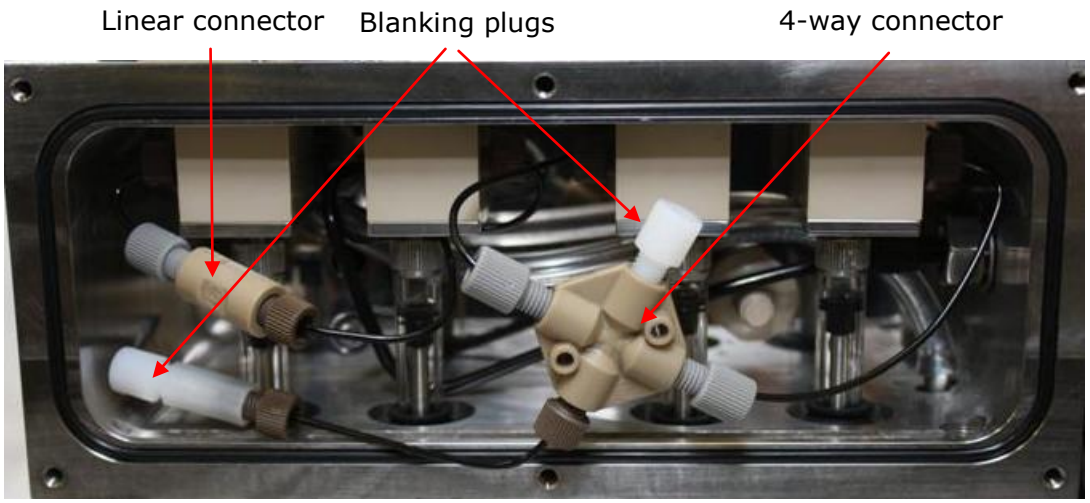

Figure 5.7: flow tubing connections for single-mix operation

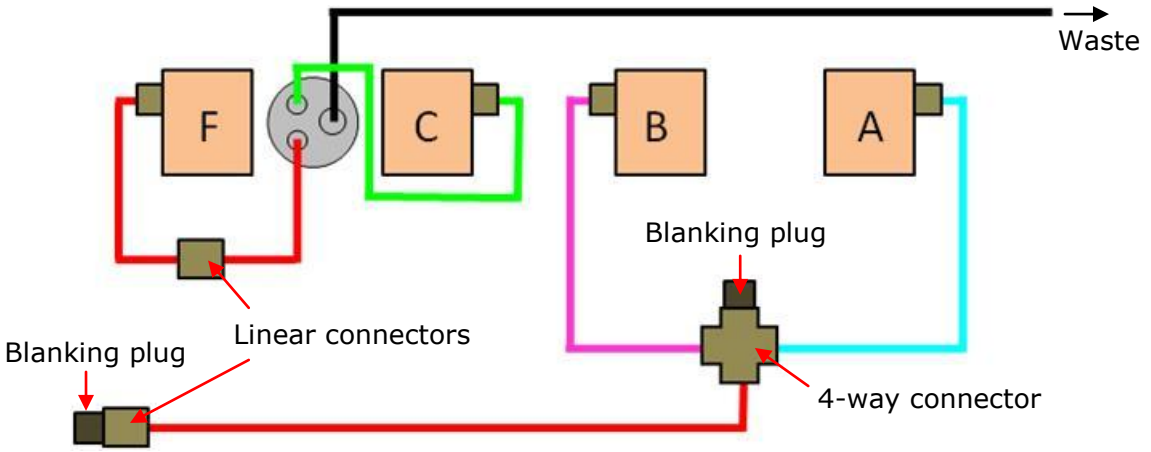

Figure 5.8: schematic of the flow tubing connections for single-mix operation; the tubing sections are colour coded for clarity

4. Remove the brake assembly (Section 3.9.5) and install the stop syringe (Section 3.9.4): normally a 1 mL stop syringe is used for single-mix operation.
5. Remove the half-cylinder spacer from the single-mix drive ram.

5.7.2 Configuration for Sequential-Mixing

The general procedure for setting up the SF.3 for sequential-mixing operation is as follows:

1. Install the optical cell as described in Section 3.8.4.
2. Install the drive syringes as described in Section 3.4.2. Remember that for sequential-mixing operation, the right two syringes (A and B) contain the reactants for premix stage, syringe C contains the additional reactant for stage 2, and syringe F contains the buffer solution.
3. Configure the flow circuit as shown in Figure 5.9 and schematically in Figure 5.10 (overleaf) with valves A and B connected to opposing arms of the 4-way mixer, and valve F to a third arm using the linear connector. The fourth arm connects to the ageing loop. Valve C remains connected directly to the pressure plate.

CAUTION: Do not disconnect any electrical leads from any of the electronic units without first ensuring that the units are turned off.

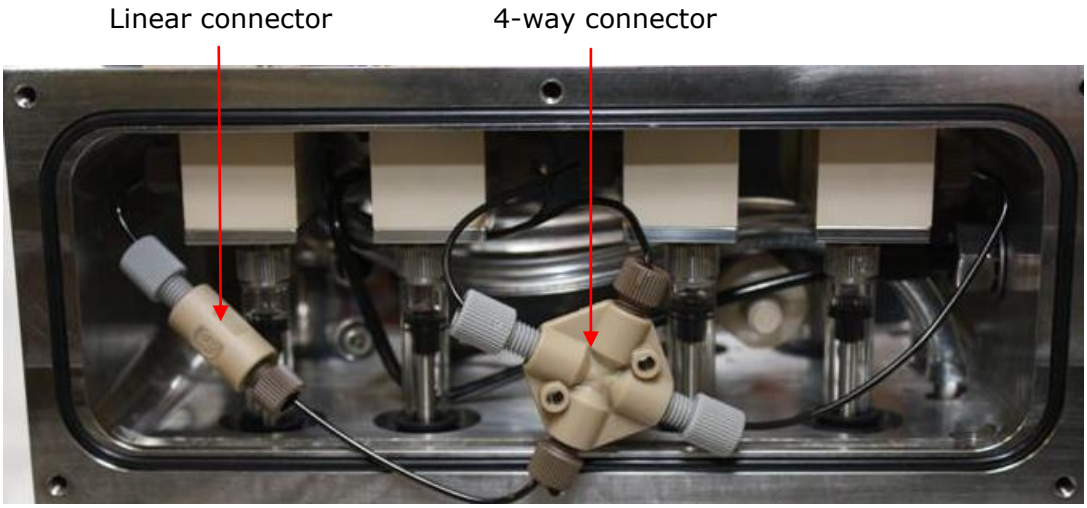

Figure 5.9: flow tubing connections for sequential-mix operation

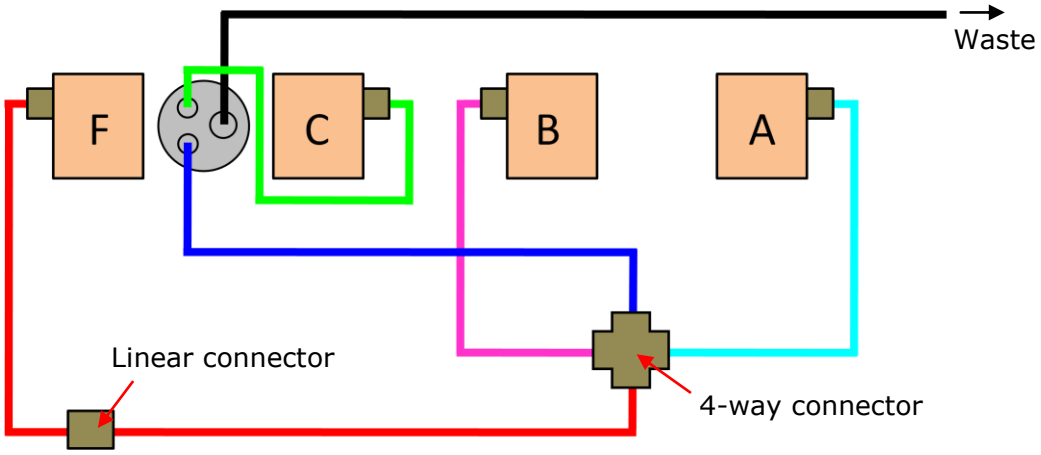

Figure 5.10: schematic of the flow tubing connections for sequential-mix operation; the tubing sections are shown colour coded for clarity

4. Install the brake assembly (Section 3.9.5) and stop syringe (Section 3.9.4): normally a 2.5 mL stop syringe is used for sequential-mix operation.
5. Replace the half-cylinder spacer on the single-mix drive ram

5.7.3 Configuration for Ratio Single-Mixing

The SF.3 is fitted with 1.0 mL Kloehn manufactured drive syringes as standard. This provides a 1:1 stopped-flow mixing ratio. 250 µl and 2.5 mL syringes are also available, and can be used for ratio mixing (other syringes may also be available; please contact Applied Photophysics for further information). The mixing ratios that can be achieved with these syringes are given in Table 5.1 (below)

Mixing Ratio	Syringe Configuration
1:1	1.0 mL + 1.0 mL
2.5:1	2.5 mL + 1.0 mL
4:1	1.0 mL + 250 µL
10:1	2.5 mL + 250 µL

Table 5.1: mixing ratios that can be achieved with the available syringe sizes

The general procedure for setting up the SF.3 for ratio mixing is as for standard 1:1 single-mixing as described in Section 5.7.1. However, the drive pressure must be reduced for ratio mixing operation.

WARNING! To prevent damage to the flow circuit from excessive pressures produced in the drive syringes when ratio mixing is used, the drive pressure must be set to 2.5 bar.

The drive pressure is set at 4 bar for standard 1:1 mixing operation. For ratio mixing this must be reduced to 2.5 bar using the inline regulator connected to the inlet Port 2 on the rear of the SF.3 (Figure 5.4, Section 5.3).

For information on the drive volumes recommended for ratio mixing, see Section 7.7.2.

5.8 Installing the Detectors

5.8.1 General Procedure

There are three available detector ports on the SF.3 cell, one transmission port in line with the light beam, and two fluorescence ports perpendicular to the beam (Figure 5.11, below).

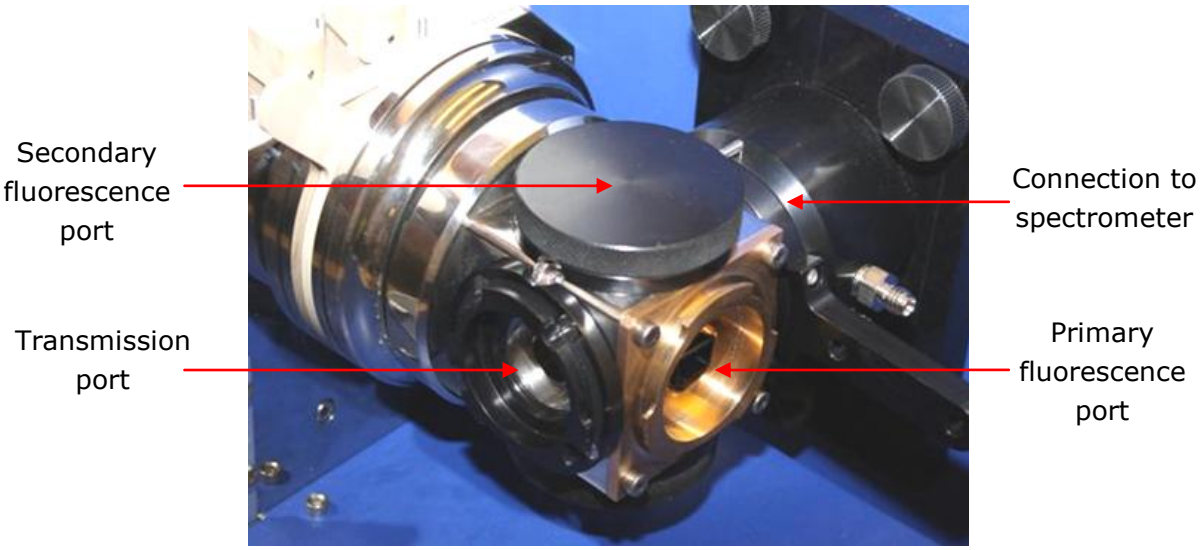

Figure 5.11: detector ports

Breech lock fittings are used between the detector and the cell block (Figure 5.12, overleaf). To install the detector, follow the procedure below:

1. Fit the female breech lock connector on the detector over the male on the port, and lock home by rotating the detector anticlockwise (counterclockwise).
2. Make the electrical connection between the detector and the spectrometer using the cable provided.

To remove the detector, zero the HV supply, and reverse the process for installation.

WARNING: Before disconnecting the detectors, zero the HV voltage by clicking the Zero HV button on the Pro-Data Chirascan control panel.

Figure 5.12: PMT detector showing female breech lock connector and O-ring; the O-ring should be removed if an optical filter is fitted.

5.8.2 CD / transmission detector

The CD detector should be installed in the transmission port, in line with the light beam (Figure 5.13), with the dotted sticker uppermost.

Figure 5.13: a detector installed in the transmission port

NOTE: the CD detector should be installed with the dotted sticker uppermost.

5.8.3 Fluorescence detectors

If a fluorescence detector is used, a 25 mm diameter optical filter should be included to filter out scattered light at the excitation wavelength. A range of filters is available from APL; for details see the main Chirascan User Manuals. The filter holder, a filter and the split ring are shown in Figure 5.14 (below).

Figure 5.14: left to right; filter holder, filter and retaining split ring

1. Remove the O-ring from the face of the detector.
2. Mount the desired optical filter into the holder. The filter should seat down fully into the rebate within the holder, and should be held in position by the split ring, which seats down on the filter.

Figure 5.15 (below) shows the fluorescence detector port with the filter in place and removed.

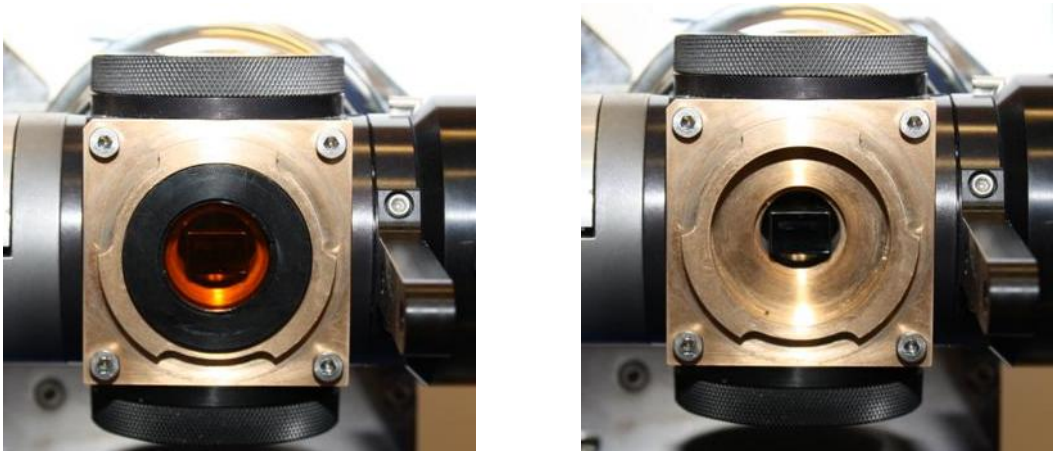

Figure 5.15: primary fluorescence port with the optical filter in place (left) and removed (right).

Chapter 6: SOFTWARE CONFIGURATION

6.1 Introduction

The SF.3 accessory is controlled via the same control panel as all other modes on the Chirascan, although appearance of the panel will alter slightly when the SF.3 is fitted (Figure 6.1, below). The Pro-Data Chirascan software automatically detects whether the standard equilibrium (ESHU) or kinetic (KSHU, i.e. SF.3) sample handling units are connected and displays the corresponding control panel. If both sample handling units are connected, the default SHU option is displayed.

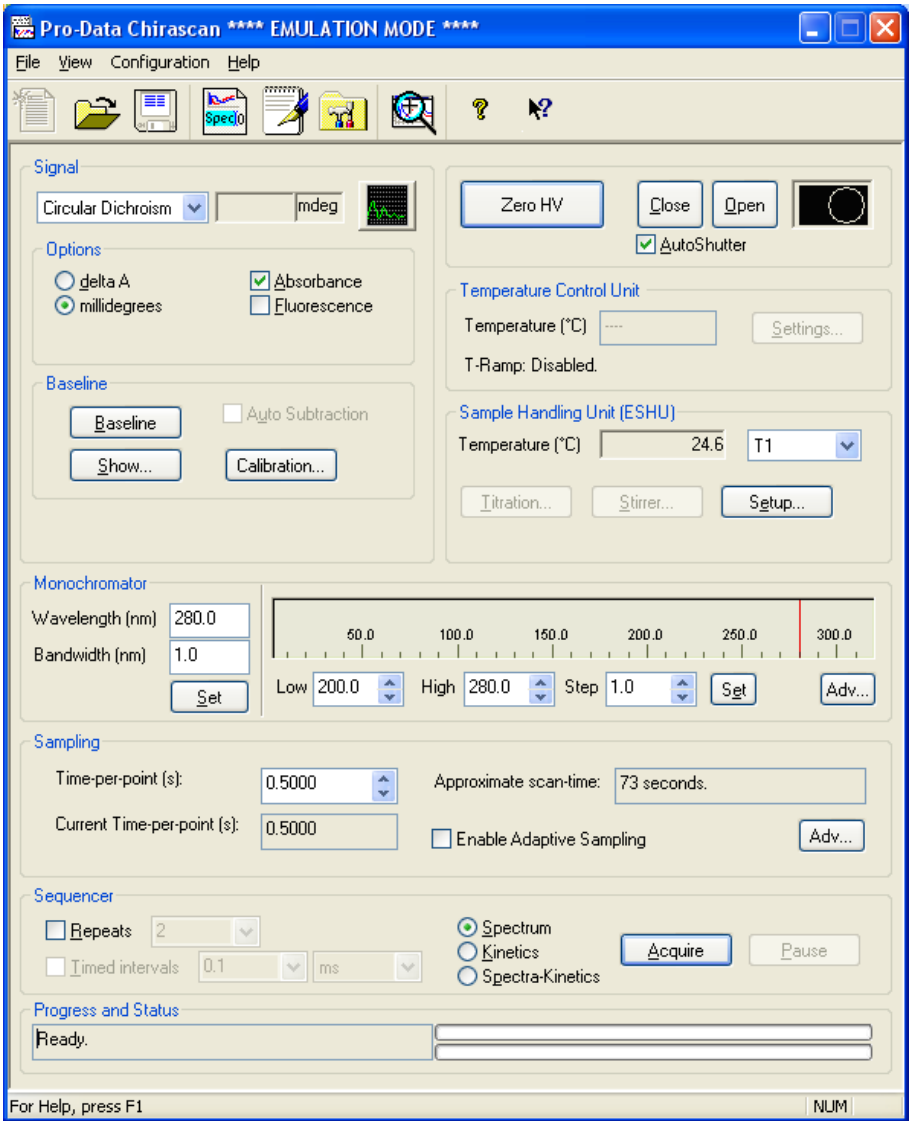

Figure 6.1: the Pro-Data control panel

6.2 The Configuration menu

To reconfigure the active SHU, on the Pro-Data Chirascan control panel, from the **Configuration** menu click **Change Sample Handling Unit SHU** to display the **Select SHU** dialog box (Figure 6.2, below), and select **Kinetics Sample Handling Unit (KSHU)**. This brings up the **KSHU** panel on the Chirascan control panel (Section 6.3). Tick the **Set as default SHU** box to change the default configuration.

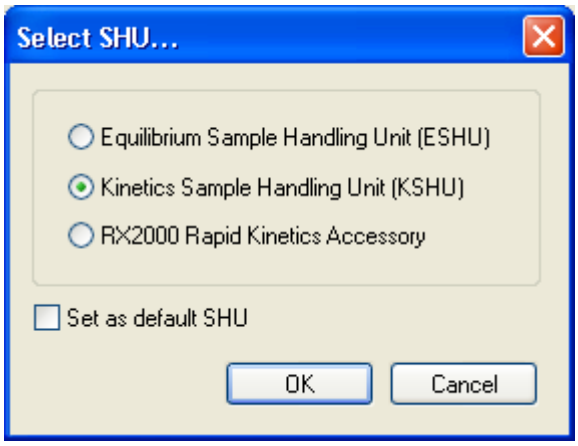

Figure 6.2: the Select SHU dialog box

6.3 The KSHU panel

The **KSHU** panel provides the user with control of the SF.3 unit. The panel indicates whether the software is configured for single- or sequential-mixing operation (Figure 6.3, below).

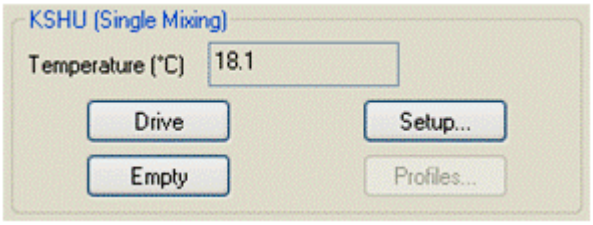

Figure 6.3: the KSHU panel

The **Temperature** box shows the temperature read in the SF.3 waterbath. Clicking each button has the following effect:

- **Drive** completes a stopped-flow drive without data acquisition: this is useful when priming or flushing the flow circuit.
- **Empty** discharges the stop syringe.
- **Setup...** opens the **SHU Setup** dialog box (Section 6.4).
- **Profiles** is only active when the SF.3 is in sequential mixing mode; information collected from the drive ram transducers is displayed (Section 7.10).

6.4 The SHU Setup dialog box

Clicking the **Setup** button on the **KSHU** panel (Section 6.3) opens the **SHU Setup** dialog box. The dialog boxes opened by the various tabs are described below.

6.4.1 The Cell tab

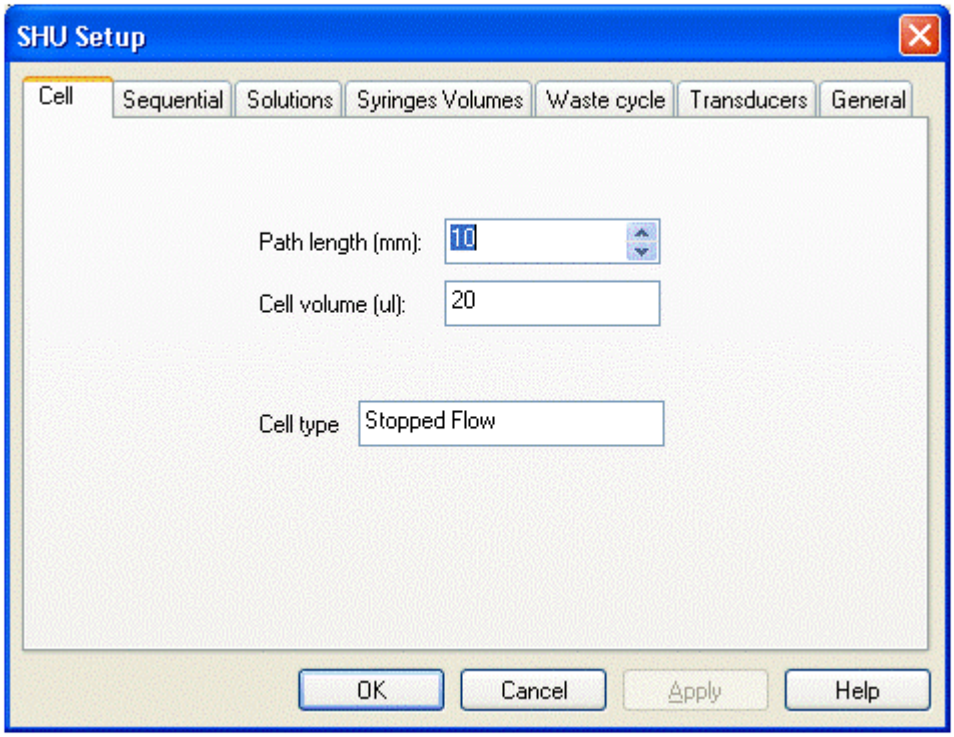

Figure 6.4: the Cell tab

The **Cell** tab is used to enter the details of the stopped-flow cell. The information is stored as a tag on the data file, and is for user reference only; it is not otherwise used by the SF.3 software.

6.4.2 The Sequential tab

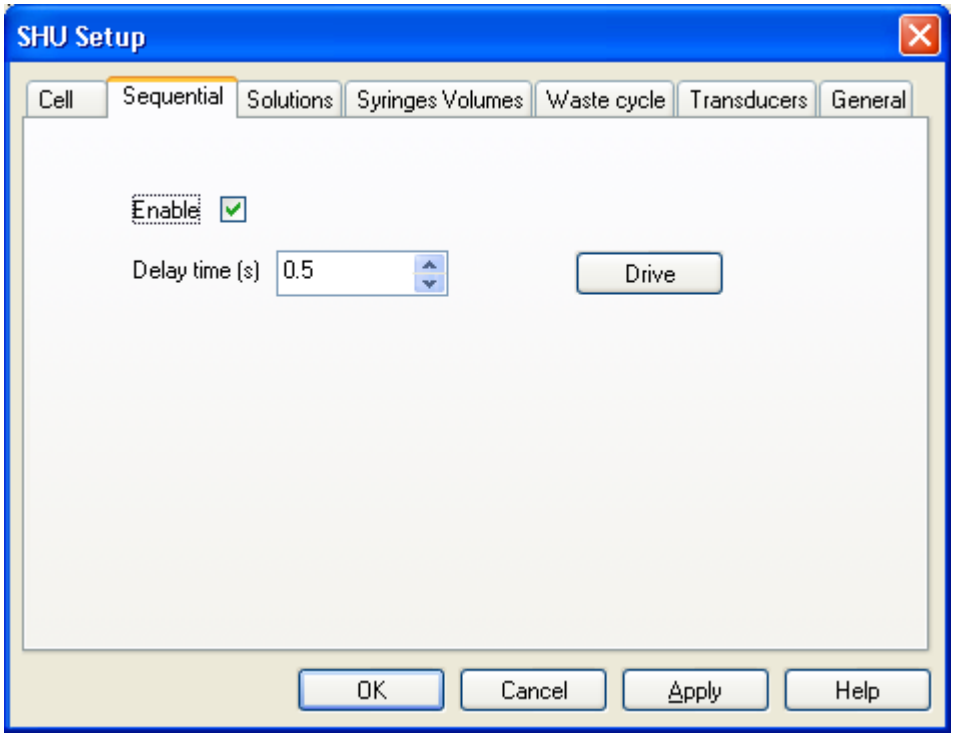

Figure 6.5: the Sequential tab

Sequential mixing operation is set from the **Sequential** tab by ticking the **Enable** box. The **Delay time** sets the time between the first and second drive of the sequential-mixing operation.

Clicking the **Drive** button performs a sequential mixing drive without data acquisition.

6.4.3 The Solutions tab

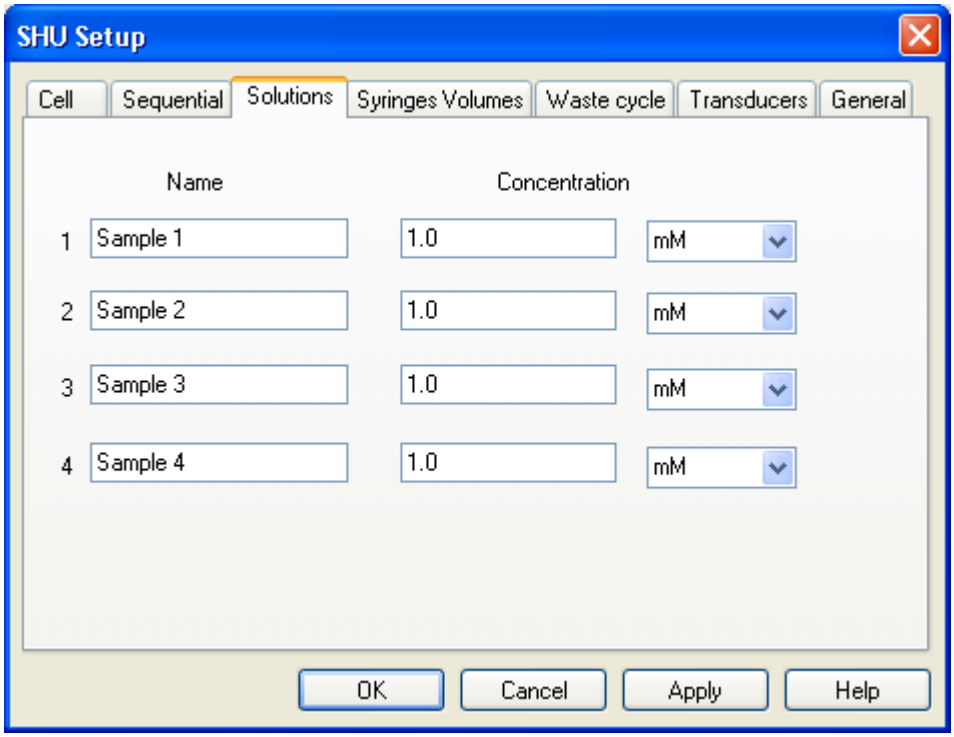

Figure 6.6: the Solutions tab

The names and concentrations of the samples are entered from the **Solutions** tab. The information is stored in the data file, and is for user reference only; it is not otherwise used by the SF.3 software

6.4.4 The Syringes Volumes tab

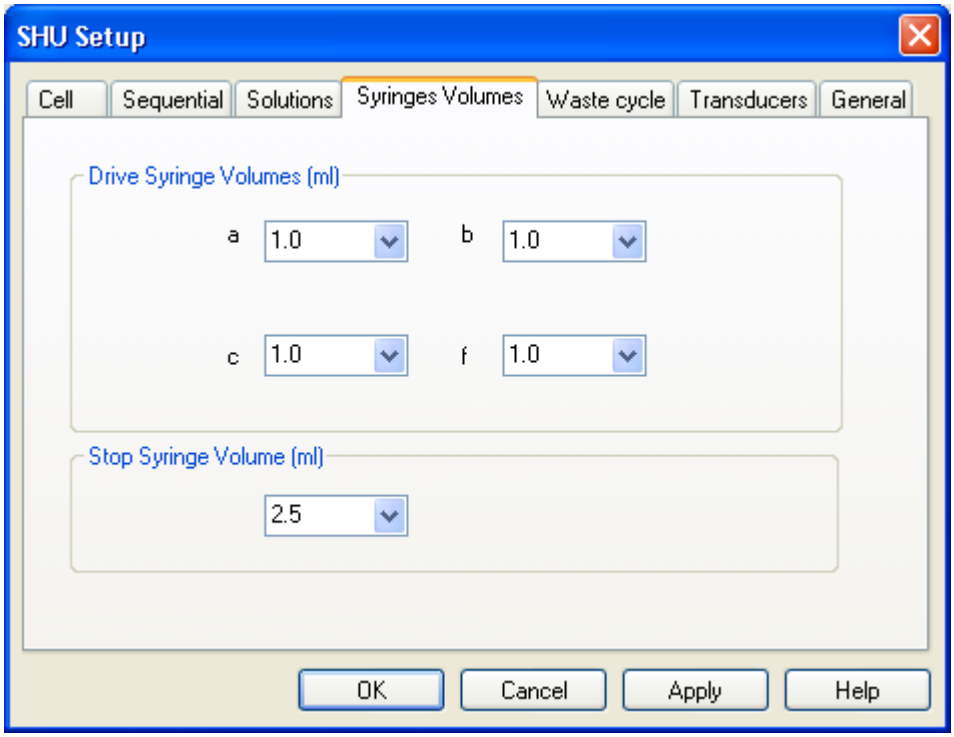

Figure 6.7: the Syringes Volumes tab

The syringe volumes are entered from the **Syringe Volumes** tab. The information is stored in the data file and is used by the **Profiles** function to calculate the drive volumes (Section 7.10)

6.4.5 The Waste cycle tab

The waste timings are shown on the **Waste cycle** tab. The settings are pre-set and should not be changed by the user. If the timings appear to be incorrect, please contact the Technical Support Department at Applied Photophysics.

6.4.6 Transducers tab

Values of the ram transducers are set from the **Transducers** tab: **Ram TX** refers to the drive ram transducer, **Stop TX** refers to the return cylinder transducer. The values are preset and should not need to be changed by the user.

6.4.7 The General tab

The **General** tab shows the online or offline status of the SF.3. The **Drive** and **Waste** buttons perform a drive or discharges the stop syringe respectively, without data acquisition.

The **Save** and **Reload** operations in the **Config** panel are not available to the user.

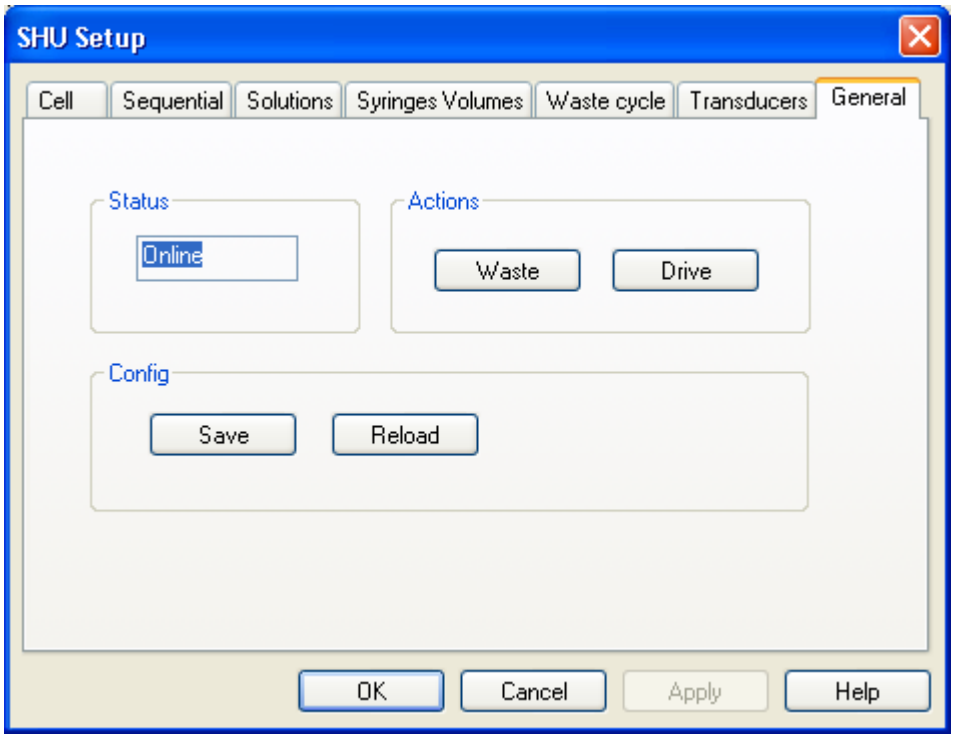

Figure 6.8: the General tab

6.5 The Signal panel

On the **Signal** panel (Figure 6.9, below), select the preferred option from the drop-down list. For stopped-flow work on the Chirascan this is usually **Circular Dichroism** or **Absorbance**, but **Fluorescence** or **Fluorescence Polarisation** can also be selected if the appropriate detectors are fitted.

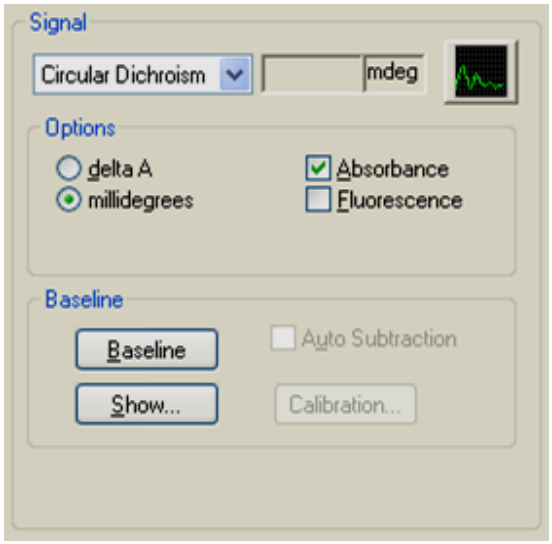

Figure 6.9: the Signal panel

If the **Absorbance** option is selected from the drop-down list, then the absorbance will be measured directly; if using this option, tick the **AutoHT** box which appears in the **Baseline** subpanel. If both the circular dichroism and absorbance are required, tick the **Absorbance** box in the **Options** subpanel, in which case the absorbance is calculated from the HT signal. For the fluorescence to be monitored, a separate detector is required.

6.6 The Sequencer panel

On the **Sequencer** panel, select **Kinetics** for single wavelength operation or **Spectra-Kinetics** for spectral scanning operation (Figure 6.10, below).

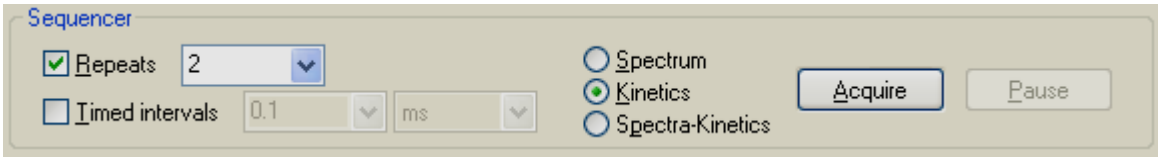

Figure 6.10: the Sequencer panel

If the **Repeats** box is ticked, the SF.3 will perform the number of drives entered in the box sequentially. If the **Timed intervals** box is ticked, the drives will be performed at the intervals entered.

The **Acquire** button is used to perform a drive with data acquisition.

6.7 The Monochromator panel

On the **Monochromator** panel enter the selected wavelength or range and bandwidth (Figure 6.10, below).

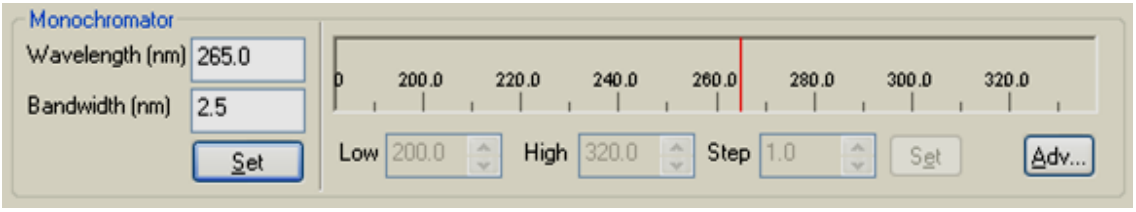

Figure 6.11: the Monochromator panel

If the **Kinetics** option is selected in the **Sequencer** panel, then the range boxes will be inactive and only a single wavelength can be set. The wavelength will depend on the sample and lamp used. If the Hg/Xe lamp is used, a wavelength should be used that corresponds to one of the emission lines shown in Table 4.2 of this User Manual.

If the **Spectra-kinetics** option is selected, then the range boxes will be active, and suitable values should be input.

The bandwidth is an important variable in the set-up of a stopped-flow experiment: the wider the bandwidth, the higher the light throughput, but the lower the spectral resolution. Typically values lie in the range 1.0 to 10 nm, but the optimum value will depend on numerous factors, for example the type of signal being measured and the stability of the

reactants to photochemical degradation (photo-bleaching). A typical setting for absorbance experiments is 1 nm. Fluorescence experiments tend require higher light throughputs using wider bandwidth settings. 4.0 nm and 8.0 nm settings are commonly used for fluorescence or the more challenging CD measurements.

It should be noted that samples which are susceptible to photochemical reaction should be investigated with narrow bandwidth settings to reduce the possibility of photo-bleaching.

When the wavelengths and bandwidths have been entered, click the **Set** button.

6.8 The Timebase panel

In the **Timebase** panel (Figure 6.12, below), **Time** is the time for which data will be acquired; a value can be selected from the dropdown list or entered by the user.

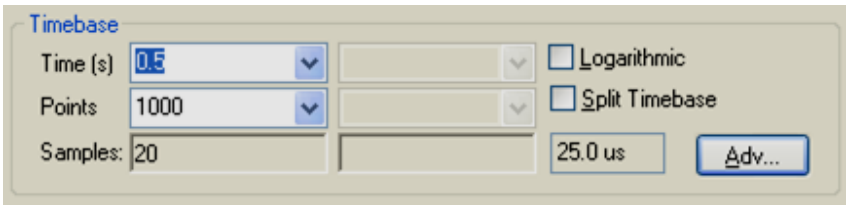

Figure 6.12: the Timebase panel

Points is the number of data points that will be acquired, each point being the average of the number of readings shown in the **Samples** box. This is calculated from the time, number of points and sample period, which appears in the box to the left of the **Adv...** button.

The sample period can be changed by clicking the **Adv...** button, which displays the **Sampling – Advanced** dialog box. The required sample period is entered in the **Sample period** box, and will be accepted by the software when the **Apply** button is clicked.

Unless the **Logarithmic** box is ticked, the data points will be acquired at equally spaced intervals. If the box is ticked, the rate of data acquisition will be very rapid at first, but will decrease logarithmically, in which case the value that appears in the **Samples** box is that of the last data point. Logarithmic data acquisition is useful, for example, to avoid missing the fast phase of a biphasic reaction.
If the **Split Timebase** box is ticked, there will be two steps in the data acquisition, the first step being performed under the conditions shown in

the boxes to the left, and the second step under the conditions shown in the boxes to the right. The total data acquisition time will be therefore the times of the two steps added together.

6.9 The Trigger panel

The **Trigger** panel (Figure 6.13, below) is used to set the origin of the signal used to begin data acquisition.

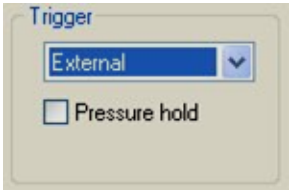

Figure 6.13: the Trigger panel

The dropdown allows the following trigger settings.

- **Internal**: data acquisition will begin as soon as the **Acquire** button is clicked, and no drive will be performed; this function is used mainly for diagnostic purposes, for example to ensure that the computer is receiving a satisfactory signal. For safety reasons, this is the default setting.
- **External** is the usual trigger setting for kinetic data acquisition; when this is set, clicking the **Acquire** button will cause a drive to be performed, and data acquisition will begin when the stop switch is triggered.
- **Remote** allows an external signal to start the data acquisition.
- **Pre-Trigger**: data acquisition will begin immediately the **Acquire** button is clicked, but a drive will also be performed. This is also mainly used for diagnostic purposes, but can be used to perform rudimentary continuous flow experiments.

If the **Pressure hold** box is ticked, the drive pressure will be held after firing until the end of the run. If the box is unticked, the pressure will be released immediately after the stop switch is triggered. However, many experiments are sensitive to the pressure to which they are subjected, and the pressure release will be observed as an artifact in the data. Holding the pressure avoids this, but the extra stress imposed on the KSHU by doing so is undesirable if it is held for more than about 10 seconds, and can reduce the lifetime of the KSHU components.

6.10 The Progress and Status panel

The **Progress and Status** panel (Figure 6.14, below) show the current status of the spectrometer and of data acquisition.

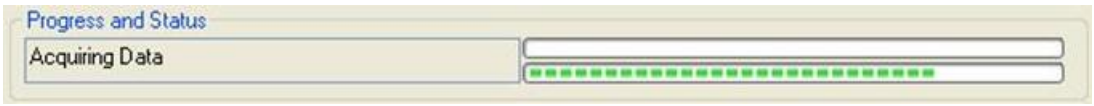

Figure 6.14: the Progress and Status panel

The box to the left of the panel shows the SF.3 status (e.g. **Ready**, **Acquiring Data**); the two bars to the right give a visual representation of the progress. In kinetics mode the upper bar represents the pre-acquisition delay and the lower bar represents the acquisition of data. In spectrum mode the upper bar represents the acquisition at the current wavelength, and the lower the total progress over the whole data set.

6.11 The KSHU dialog box

Access to the SF.3 delays and timer settings is password restricted, and these should only be changed under the guidance of an APL Technical Support Engineer.

From the **View** menu select **Devices** and then on the **Device window** dialog box click the **KSHU** icon  . A password is asked for, which will open the **Kinetic Sample Handling Unit (KSHU)** dialog box shown in Figure 6.15 (overleaf).

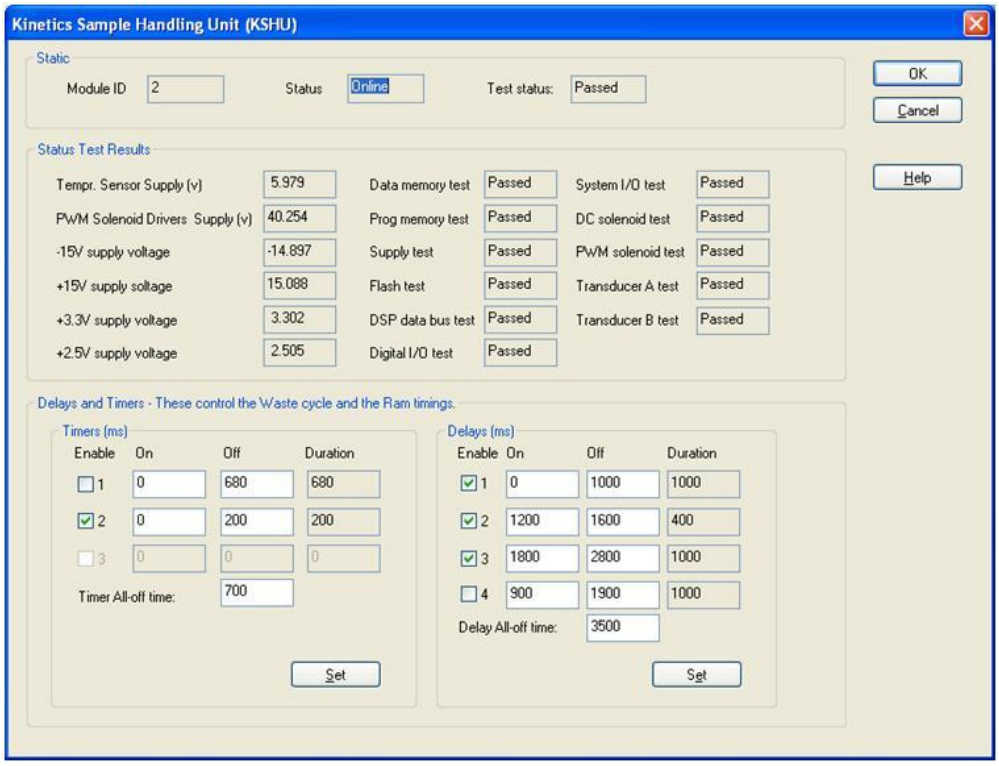

Figure 6.15: the KSHU dialog box

Chapter 7: OPERATION OF THE SF.3

This Chapter describes the operation of the SF.3. It is assumed that it has been installed and set up as described in Chapters 5 and 6 of this User Manual.

7.1 General Procedure

The general procedure for operating the SF.3 is as follows:

1. Configure the SF.3 hardware as required.
2. Configure the Pro-Data software as required.
3. Load the reservoir and drive syringes with distilled water, solvent or buffer solution.
4. Flush the flow circuit and check for leaks.
5. Set and check the drive volumes.
6. Set the temperature
7. Allow to reach thermal equilibrium and run a baseline calibration drive where necessary.
8. Reload the reservoir and drive syringes with reactant, and allow to reach thermal equilibrium.
9. Prime the flow circuit with reactants by performing several drives
10. Collect a series of traces and check for consistent results.

Steps 1 and 2 have been described in Chapters 5 and 6 respectively of this Manual. The operations of loading the drive syringes and running a drive are performed repeatedly during this procedure, and to avoid repetition, they are described in Section 7.2 and Section 7.3 respectively. The other operations are described in the Sections following.

7.2 Loading a Drive Syringe

The procedure for filling a drive syringe is as follows:

1. Set the drive ram is in the fully down position, and the syringe plunger to the fully up position.
2. Fill the reservoir syringe with the required solvent or solution.
3. Insert the reservoir syringe into the appropriate Luer connector.
4. Set the drive valve control knob to the **Load** (side) position.

5. Load the drive syringe by pushing down the plunger of the reservoir syringe; this is preferable to pulling down the drive syringe plunger, as it is less likely to result in cavitation and the introduction of bubbles.
6. If necessary, expel any air bubbles from the drive syringe by working the drive and reservoir syringes up and down together several times; at the end of this process there should be no bubbles remaining in the drive syringe.
7. When the drive syringe is loaded, there are no remaining bubbles, and the drive syringe plunger is in the fully down position, set the drive valve control knob to the **Drive** (front) position.

NOTE: there should be no bubbles remaining in the drive syringe when the syringe is loaded; for good quality data to be collected, it is essential that air bubbles are not present in the flow circuit.

7.3 Running a Drive

CAUTION: when running a drive, keep hands, clothing and other items clear of the SF.3.

A drive can be run either with or without the acquisition of data. For the flushing described below, there is no need to acquire data during a drive.

1. Before the drive is run, the drive syringes should be loaded, and the drive rams and drive syringe plungers in the fully down positions, i.e. there should be no gap between the drive ram face and the syringe plunger.
2. Check that there are no bubbles in the drive or stop syringes. If bubbles are present in the stop syringe, expel them by setting the stop valve control knob to the **Waste** (front) position, and manually raising the syringe plunger.

NOTE: if any bubbles remain in the stop syringe that cannot be expelled by manual pushing, the syringe should be removed and the bubbles expelled.

3. Set the drive valve control knobs to the **Drive** (front) position.

CAUTION: to prevent damage to the optical cell and drive syringes, do not run a drive unless the drive syringe plungers are in contact with the drive rams and the drive valves are set to the Drive positions.

4. To run a drive without data acquisition, click the **Drive** button on the **KSHU** panel of the control software (Section 6.3). To run a baseline calibration drive click the **Baseline** button on the **Baseline** subpanel of the **Signal** panel (Section 6.5), or to run a drive with data acquisition click the **Acquire** button on the **Sequencer** panel (Section 6.6).

7.4 Flushing the Flow Circuit

The flow circuit should be flushed through with whatever liquid is most convenient, for example distilled water, solvent, or the appropriate buffer solution, before any new sample is introduced. Before the first use of the SF.3, or if it has been left to stand for a lengthy period, it should be flushed initially with distilled water.

The flow circuit can be flushed either manually or using the SF.3 control software, but it is recommended that if the flow circuit is reconfigured in any way, it should be flushed and checked for leaks manually before using the control software.

7.4.1 Flushing the single-mix flow circuit manually

If the flow circuit is configured for single mixing, flush manually using the following procedure:

1. If the drive volumes have already been set, this step should be omitted: otherwise with the stop valve control knob set to the **Waste** (front) position, set the drive volume to maximum by rotating the volume adjuster on the Auto-Stop assembly clockwise, i.e. the front edge moving from left to right, until there is no further travel.
2. Load the C and F drive syringes with the flushing fluid (Section 7.2). Ensure that the drive syringe plungers are in the fully down positions and that the C and F drive valves control knobs are in the **Drive** (front) position; set the stop valve control knob to the **Drive** (rear) position.
3. By raising the single-mix ram manually, force the flushing fluid from the drive syringes through the flow circuit and into the stop syringe, until there is no further travel.
4. Discharge the stop syringe by setting the stop valve control knob to the **Waste** (front) position, and raising the stop syringe plunger manually.
5. Repeat steps 2 to 4 until the circuit has been fully flushed.

7.4.2 Flushing the sequential-mix flow circuit manually

If the flow circuit is configured for sequential mixing, the single-mix part of the circuit should be flushed first, followed by the sequential-mix part. The procedure to flush the single-mix part of the circuit is as follows:

1. If the drive volumes have already been set, this step should be omitted: otherwise with the stop valve control knob set to the **Waste** (front) position, set the total drive volume to maximum by rotating the volume adjuster on the Auto-Stop assembly clockwise, i.e. the front edge moving from left to right, and set the second drive volume to maximum by rotating the volume adjustor on the sequential-mix ram anticlockwise (counterclockwise), i.e. the front edge moving from right to left, until there is no further travel.
2. Load all four drive syringes with the flushing fluid (Section 7.2). Ensure that both drive rams and all drive syringe plungers are in the fully down position, and that all drive valves control knobs are in the **Drive** (front) position. Set the stop valve control knob to the **Drive** (rear) position.
3. Flush the circuit by raising the single-mix ram manually until there is no further travel.
4. Discharge the stop syringe by setting the stop valve control knob to the **Waste** (front) position, and raising the stop syringe plunger manually.
5. Repeat steps 2 and 3 until the circuit has been fully flushed.

The procedure for flushing the sequential-mix part of the circuit is the same, except that at step 3, the sequential-mix ram is raised.

7.4.3 Flushing the flow circuit using the control software

It is recommended that the circuit is not flushed using the control software until a leak check has been performed (Section 7.5), and the flow circuit has been found to be free from leaks. The procedure is as described in Section 7.4.1 or Section 7.4.2, except that rather than the rams being raised and the stop syringe discharged manually, the **Drive** button is used as described in Section 7.3. If the circuit is set for sequential mixing, there is no need to flush the two parts of the circuit separately, since both parts are flushed in one sequential drive.

7.5 Checking the Flow Circuit for Leaks

The flow circuit should be checked for leaks regularly, and should always be checked after the flow circuit has been reconfigured in any way, if the SF.3 has been standing for a lengthy period of time, or before first use. It is recommended that if the circuit has been reconfigured, a manual leak check should be made, following the manual flush.

7.5.1 Checking for leaks manually

A manual leak check is best made with the waterbath drained and the front panel removed (Section 3.3.2). All exterior components of the SF.3, and the interior of the waterbath, should be thoroughly dry. The procedure is the same whether the SF.3 is configured for single-mixing or sequential-mixing.
If a leak is found, check that the joint from which the leak is occurring is not loose, otherwise dismantle the circuit and check all components, replacing any that are faulty.

1. When the flow circuit has been fully flushed, and while it still contains the flushing fluid, ensure that the drive syringes are loaded and that the drive rams and drive syringe plungers are in the fully down position.
2. Set the drive valve control knobs to the **Drive** (front) position, and the stop valve control knob to the **Drive** (rear) position.
3. Raise the single-mix drive ram manually until there is no further travel, and maintain firm manual pressure.
4. Visually check that there are no leaks in the flow circuit, and that the drive syringe plungers are not creeping upwards: creep in the plungers is a good indicator of a leak in the circuit.

7.5.2 Checking for leaks using the control software

A check for leaks can be made using the SF.3 control software. However a quick check can be made without draining the bath.

1. After the flow circuit has been fully flushed, and while it still contains the flushing fluid, ensure that the drive rams are fully down, that the drive syringes are loaded, and that the drive syringe plungers are in the fully down position.
2. Set the drive valve control knobs to the **Drive** (front) position, and the stop valve control knob to the **Drive** (rear) position.
3. Tick the **Pressure hold** box on the **Trigger** panel (Section 6.9).

4. Set the time in the **Timebase** panel to at least 10 seconds (Section 6.8). This will ensure that the drive pressure will be held for that length of time.
5. Run a drive using the **Acquire** button on the **Sequencer** panel (Section 6.6), as described in Section 7.3. The **Acquire** button must be used because the pressure hold option is not operative when the **Drive** button is used.
6. Observe closely the plungers on the drive syringes: any creep will indicate a leak in the flow circuit.
7. If the check has been performed with the waterbath drained and dry, visually check the flow circuit for leaks.

7.6 Setting the temperature

If a water circulator is installed that can be controlled through the SF.3 software, the temperature in the waterbath and cell block is set through the **Temperature Control Unit** panel (Figure 7.1, below).

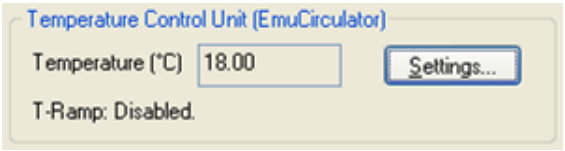

Figure 7.1: the Temperature Control Unit dialog box

Click the **Settings** button to display the **Temperature Control** dialog box, the **Set Point** panel of which is shown in Figure 7.2, below, and enter the target temperature in the **Set Point** box (for other operations controlled through the **Temperature Control** dialog box, see the main Chirascan User Manuals)

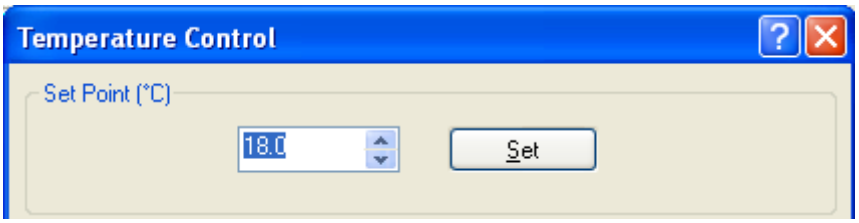

Figure 7.2: the Set Point panel on the Temperature Control dialog box

If the water circulator is not controlled through the SF.3 software, the temperature should be set on the circulator, following the supplier's instructions.

The SF.3 temperature is displayed in the **KSHU** panel (Section 6.3).

7.7 Setting the Drive Volumes

The exact drive volume required for a stopped-flow experiment will depend on a number of factors including the type of measurement, the acquisition time and physical properties of the reagents. It is advisable to set the drive volume and check that it is correct before loading valuable reactants, for example immediately after flushing the flow circuit and checking for leaks.

NOTE: it is recommended that a minimum of 120 µl is set for the total drive volume.

7.7.1 Setting the drive volume for single mixing

1. With the stop syringe valve control knob set to the **Waste** (front) position, set the volume adjuster on the Auto-Stop assembly to the zero position by rotating fully anticlockwise (counterclockwise), i.e. the front edge moving from right to left, until there is no further travel.
2. Set the drive volume by rotating the adjuster clockwise. One full rotation of the adjuster produces a travel of 1 mm in the stop syringe plunger, equivalent to a drive volume of about 33.3 µl per drive if a 1 mL syringe is used, or about 83.3 µl if a 2.5 mL stop syringe is used.

7.7.2 Setting the drive volume for ratio mixing

To obtain dependable experimental results, the total drive volume should be increased as the mixing ratio increases: it is recommended that at least 25 µl of each reactant is used, with a total drive volume of at least 120 µl. Table 6.1, below, gives some suggested drive volumes for ratio mixing. The final column in the table indicates the approximate number of rotations of the volume adjuster required to achieve the quoted drive volumes if a 1 mL stop syringe is used.

Mixing Ratio	Drive Volume	Adjuster rotations
1:1	130-140 µl	4
2.5:1	160-170 µl	5
4:1	200-210 µl	6
10:1	250-260 µl	7.5

Table 7.1: suggested drive volumes for ratio mixing

7.7.3 Setting the drive volumes for sequential mixing

The key to calculating the drive volumes required for sequential mixing is that the volume of the ageing loop is 115 µl. It would therefore be wasteful to use a total drive volume of greater than 230 µl for the first drive.

For the second drive, it is critical that no buffer from the flush syringe should enter the observation cell. Since the further downstream (i.e. further from the drive ram) the reaction mixture is in the loop, the older it will be, and it is desirable that youngest mixture should be used for the second reaction, the flush volume should be about 90 µl. If 1:1 mixing is used, the total volume of the second drive should therefore be about 180 µl. A total drive volume of about 400 µl is therefore recommended. Since it convenient to set the volume adjusters in quarter turn increments, the following procedure can be used:

1. With the stop valve control knob set to the **Waste** (front) position, set the adjuster on the sequential-mix drive ram to the zero position by rotating fully clockwise, i.e. the front moving from left to right, until there is no further travel. Set the adjuster three and a quarter rotations anticlockwise (counterclockwise). This produces a travel of 3.25 mm in the A and B drive syringes, equivalent to a total first drive volume of about 220 µl if 1 mL drive syringes are used.
2. Rotate the volume adjuster on the Auto-Stop assembly fully anticlockwise (counterclockwise), i.e. front edge moving from right to left, until there is no further travel, then rotate it four and three quarter rotations clockwise. This produces a total drive volume of approximately 400 µl for a 2.5 mL stop syringe, and therefore a second drive volume of about 180 µl.

7.7.4 Checking the drive volumes

When the SF.3 is used in sequential mixing mode, it is essential that the drive volumes are correct. These can be checked following a drive.

Ensure that the drive and stop syringes are full and free from bubbles, and that the drive rams and drive syringe plungers are in the fully down positions, then click the **Drive** button on the **KSHU** panel of the SF.3 control software (Section 6.3).

The drive volumes calculated by the SF.3 software are shown on the **Drive Profiles** window, which is displayed by clicking the **Profiles** button

on the **KSHU** panel (Section 6.3). Small adjustments will probably have to be made if exact drive volumes are required.

7.8 Running a Baseline Calibration

The reason for running a baseline calibration and the procedure for applying the calibration are described in the main Chirascan User Manual.

A baseline calibration is normally performed with a reference material in the observation chamber of the optical cell. Usually the reference is the reactant medium, for example distilled water, solvent or buffer, but other materials may be used. One option is to use one of the reactants (usually colourless); another is to use the chemical end-point of the reaction under investigation. If water used as the reference, the absorbance has absolute units, otherwise the units are relative.

It is essential that in all other respects the conditions for the baseline calibration drive are identical to those that are to be used for the reaction drive: no alterations to the SF.3 hardware or software configuration should be made, the temperature should be the same, and so on.

The reference material can be introduced into the observation chamber in a number of ways, for example it can be loaded into both drive syringes and a drive run as described in Section 6.3. Alternatively, just one drive syringe (preferably syringe C) can be loaded with the reference material, which is introduced to the optical chamber manually using the following steps.

1. Set the stop valve to the **Waste** (front) position, and raise the stop syringe plunger manually to eject the contents.
2. Set the stop valve to the **Drive** (rear) position.
3. Set the drive valve of the syringe containing the reference material to the **Drive** (front) position, and the other drive valves to the **Load** (side) positions.
4. Raise the plunger of the syringe containing the reference material manually until the stop syringe plunger reaches the end of its travel.
5. Repeat steps 1 to 4 until the observation chamber contains only the reference material.

When the observation chamber contains only the reference material, click the **Baseline** button on the **Baseline** subpanel of the **Signal** panel to collect the baseline calibration data (Section 6.5).

7.9 Loading Reactants and Running a Reaction Drive

After the baseline calibration, has been run, the drive syringes should be emptied, and reloaded with the reactants. A reaction or series of drives can then be run.

1. Empty the drive syringes by setting the drive valve control knobs to the **Load** (side) position, and manually raising the syringe plungers to their highest positions, so that the fluid contained by the syringes is ejected to the reservoir syringes.
2. Replace the reservoir syringes with ones containing the reactants, and reload the drive syringes.

NOTE: for single mixing operation, the solution with the higher density should be loaded in the F drive syringe, to ensure better mixing If the two samples are of equal density, use the C syringe for the more valuable sample as it has the lower priming volume. Remember that for sequential mixing, the F syringe must contain the buffer solution.

3. Set the drive valve control knobs to the **Drive** (front) position, and click the **Acquire** button on the **Sequencer** panel (Section 6.6).
4. Repeat until reproducible results are obtained.

NOTE: remember that for single mixing, successive drives can be carried out immediately, but for sequential mixing operation, the drive syringes must be reset between drives.

7.10 Viewing and Adjusting the Drive Profile

The drive profile can be viewed after completion of the drive by clicking the **Profiles** button on the **KSHU** panel (Section 6.3); this function is particularly useful when setting the drive volumes for sequential mixing. Figure 7.3 (overleaf) shows a sequential mix drive profile with 500 ms sequential delay, and Figure 7.4 (overleaf) shows a drive using the same set-up, but with the sequential delay time reduced to 50 ms.

The drive profile should show a sharp transition between the drive (sloping part of the profile) and volume hold (horizontal part of the profiles. Use the brake assembly (Section 3.9.5) to achieve the optimal profile without overshoot, increasing or decreasing the friction imparted by the brake as required by rotating the knurled nut.

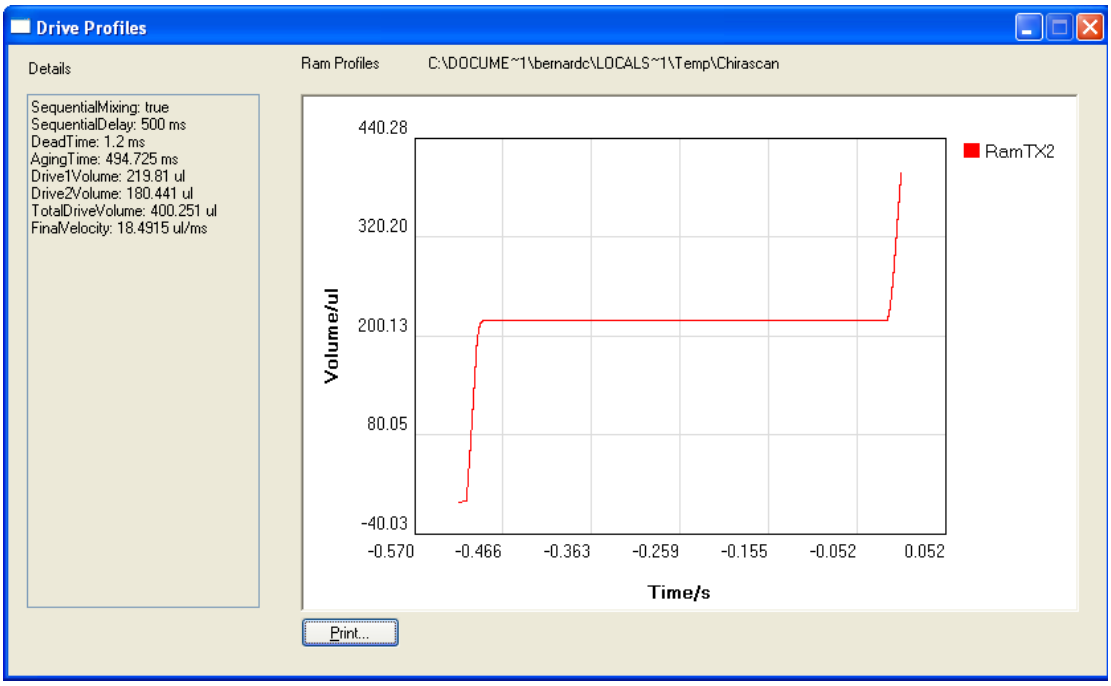

Figure 7.3: sequential-mix drive profile with 500 ms delay time

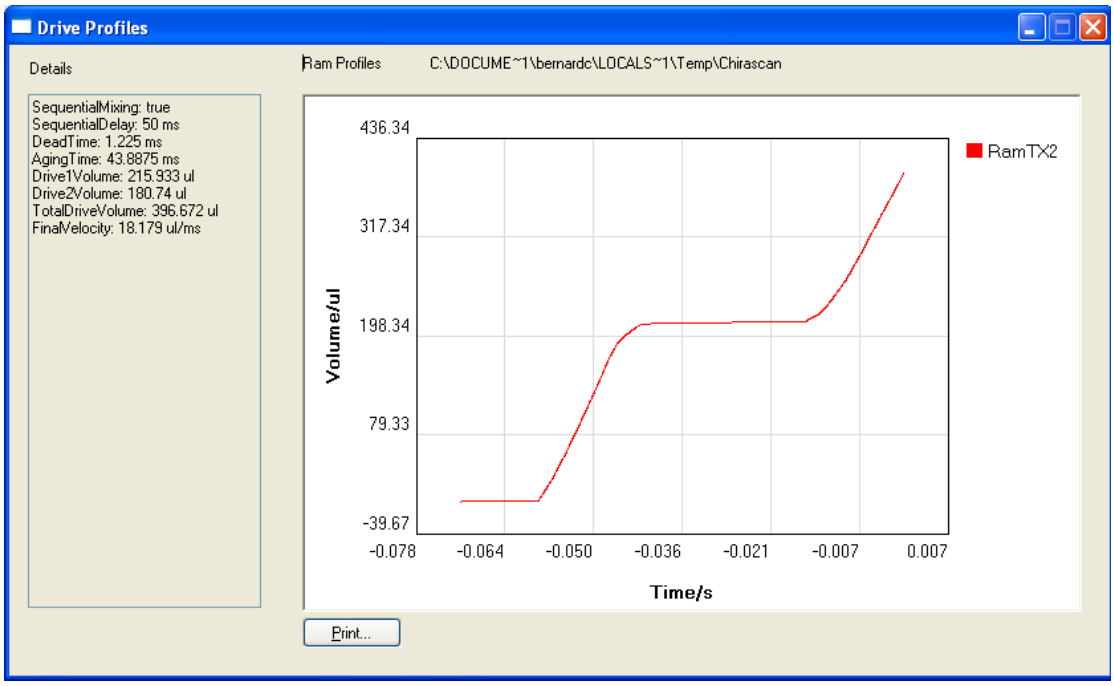

Figure 7.4: sequential-mix drive profile with 50 ms delay time

7.11 Shut Down Procedure

After the experimental work is complete, the system should be shut down.

1. Flush the flow circuit with solvent or distilled water to remove the reactants (Section 7.4), and if necessary continue to flush with compatible solvents until distilled water can be used as the flushing fluid.
2. Isolate the SF.3 from the high pressure supply by closing the manual tap installed in the pressure line, and relieve the pressure by manually depressing the vent valve.
3. Switch off the fluid circulator and drain the SF.3 waterbath (Section 3.3.2).
4. Shut down the spectrometer as described in the main Chirascan User Manual.

7.12 Cleaning and Maintenance

We recommend that your SF.3 unit is cleaned and checked regularly. Clean the flow circuit by flushing with solvent or distilled water (Section 7.4). Clean the exterior of the unit by wiping with a soft damp cloth. Clean any spillages from the external surfaces of the unit immediately, remembering to take the appropriate safety precautions when handling any chemicals.

Check the unit visually, in particular the syringes to make sure they are not leaking or cracked, and the flow tubing to make sure it is not kinked. Run regular leak tests (Section 7.5), and

For long term storage, flush with ethanol to facilitate drying, remove the syringes (Section 3.4.2 and Section 3.9.4) and store them in their original containers. Cover the unit and store in a clean, dry environment.

Appendix 1: MATERIALS

The materials used for the SF.3 flow circuit, and with which the reactants come into contact are all biocompatible. The drive syringes have glass barrels, UHMW-PE plunger tips and PTFE syringe tips. The flow tubing and valves are PEEK, and the T-mixer and optical cell block are constructed from quartz.

PEEK is a thermoplastic material that is compatible with a wide range of organic solvents, acids and bases making it an ideal choice for the stopped-flow circuit.

The compatibility of PEEK with various reagents encountered in stopped-flow measurements is outlined in Table A1, below.

Reagent	Compatibility
1,1,1 Trichloroethane	A
1,2 Dichloroethane	B
Carbon Tetrachloride	A
Chloroform	A
Dichloromethane	B
Dichlorobenzene	A
Ethylene Dichloride	A
Trichloroethylene	A
Aliphatic Esters	A
Butyl Acetate	A
Ethyl Acetate	A
Diethylether	A
Dioxane	A
Petroleum Ether	A
Tetrahydrofuran	A
Benzene	A
Toluene	A
Xylene	A
Hexane	A
Phenol (dilute)	A
Phenol (conc)	C
Dimethylsulphoxide	B
Diphenylsulphone	B
Acetonitrile	A

Reagent	Compatibility
Urea	A
Ammonium Chloride	A
Calcium Salts	A
Copper Salts	A
Iron(II) Chloride	B
Iron(III) Salts	A
Manganese Salts	A
Magnesium Salts	A
Nickel Salts	A
Potassium Salts	A
Silver Nitrate	A
Sodium Salts	A
Tin(II) Chloride	A
Sulphites	A
Hydrogen Peroxide	A
Bleach	A
Soap Solution	A
Sodium Hydroxide	A
Nitric Acid (Conc)	B
Sulphuric Acid (Conc)	B
Hydrochloric Acid	A

A – No attack – no or little adsorption B – Slight attack C – Severe attack

Table A1: compatibility of PEEK with various reagents

Please note that the information provided in this Appendix is provided for guidance only, and that Applied Photophysics cannot accept responsibility for damage to the SF.3 system produced by the action of samples.

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