User Manual

KinetAsyst™
SF-61DX2
Stopped-Flow System

Prepared by: ..... Date: .....

Validated by: ..... Date: .....

Hi-Tech Limited, Brunel Road, Salisbury SP2 7PU, United Kingdom.
Telephone: 01722 432300. International +44 1722 432300. Facsimile: 01722 432324. International +44 1722 432324.
e-mail: enquiry@hi-techsci.com, support@hi-techsci.com, http://www.hi-techsci.com

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SECTION 1

INTRODUCTION

1.A. NOTICE

Careful observation is required of the Important Notices, Cautions and Warnings herein because of potential hazards to the operator and damage to the accompanying instruments. These instruments are intended for use only by properly trained and supervised research technicians or postgraduate students.

Definitions:

The following symbols are used to indicate areas that present a potential hazard to the user and should be noted:

This symbol, when used alone or in conjunction with other symbols indicates the need to consult the manual provided with the product. A potential risk exists if the instructions are not followed.

This symbol indicates the presence of electric shock hazards. Enclosures and areas of the apparatus marked with this symbol should only be serviced by a suitably trained and informed technician. To avoid risk from electric shock, isolate equipment from the power source before servicing.

This product is CE Marked and as such is certified to meet certain European Directives. Hi-Tech certifies that this product has been tested and found to be in compliance with the appropriate harmonised standards. A copy of the Declaration of Conformity (as produced via the Standards Route) is held on file at Hi-Tech and is available as and when requested.

Limitation of Certification: This certification applies only to the operation of the product (with authentic accessories and options) in the stated configuration and under normal research laboratory conditions. Any modification, misuse or improper or inadequate maintenance by the user voids certification.

1.B HEALTH AND SAFETY AT WORK ACT 1974 - U.K.

In accordance with the above Act, we ensure that all products manufactured or supplied by Hi-Tech Limited are safe and without risk to health when used by suitably trained personnel following our instructions.

It is imperative that all personnel who come into contact with our products have available such of our literature as they require to ensure their safety. If there is any doubt whatsoever relating to the proper use of this equipment, Hi-Tech Limited will be pleased to advise. Hi-Tech can be contacted on the following:

Hi-Tech Ltd.,
Brunel Road,
Salisbury, SP2 7PU,
UK.

Tel: (01722) 432300 (national) +44 (1722) 432300 (international).
Fax: (01722) 432324 (national) +44 (1722) 432324 (international).
e-mail: support@hi-techsci.com

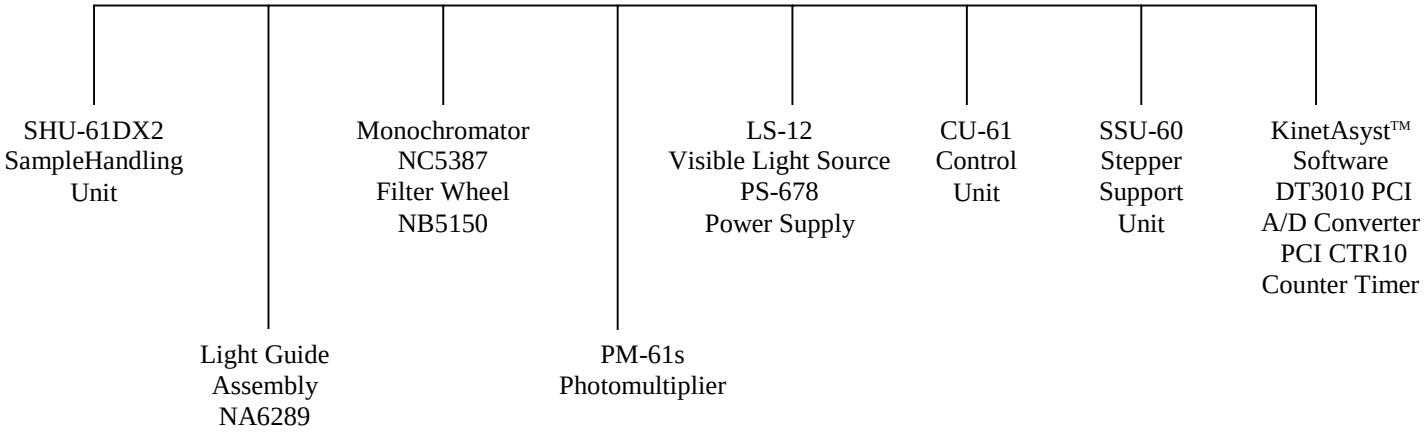
1.C THE STOPPED-FLOW SPECTROFLUORIMETER SYSTEM AND MANUAL ORGANISATION

The chart in Figure 1.C.1 shows the family tree identifying the organisation of the KinetAsyst™ SF-61DX2 system. This shows a basic, core system identified as the " KinetAsyst™ SF-61DX2 Stopped-flow Spectrophotometer/Fluorimeter" together with various options providing excitation and detection units plus other ancillary equipment.

This manual is aimed to provide installation and operation information, as well as describing each equipment and subassembly in the KinetAsyst™ SF-61DX2 system. It is organised in a pattern which tends to group together modules and units in categories relating to sample handling, electronics and optics, with specialised support equipment and attachments dealt with in separate sections.

Figure 1.C.1

KinetAsyst™ SF-61DX2 Double-mixing Stopped-Flow Spectrophotometer/Fluorimeter



Optional Extras

MG-6560 - KinetaScan Photodiode Array
OPT-622 - UV Light Source
OPT-630 - Low Temperature Kit
OPT-642 - Conductivity Cell Attachment
OPT-655 - Circulator & Cooler
OPT-657 - CryoFlo Low Temperature Accessory
OPT-661 - Fluorescence Polarisation Accessory
OPT-667 - Anti-bleaching Shutter
OPT-669 - Extended Pushplate
OPT-670 - Fluorescence Emission Scanning Accessory
OPT-671 - Hg/Xe lamp
OPT-677 - High Intensity Xe Lamp Assembly
OPT-686 - Dual Detection Option
OPT-687 - Dual Fluorescence Detection Option
OPT-696 - Anaerobic Kit

SECTION 2

INSTALLATION

2.A PRE-INSTALLATION

In most cases the instrument will be installed by Hi-Tech personnel or delegates; under such circumstances, the packing crate will be unpacked by the installation technician. However, upon receipt of the crate, any obvious damage or shipping problems should be reported IMMEDIATELY to Hi-Tech Ltd., contact details are to be found in Section 1.B in this manual. A customer report sheet is enclosed with the packing lists for reporting any problems.

Pre-installation: If the instrument is to be installed by Hi-Tech personnel, it is extremely helpful and time efficient to make certain provisions within the laboratory in preparation. A 2 m bench space is normally required to accommodate a complete instrument - this varies from set-up to set-up; the placement of external thermostat baths and the computer should also be considered.

Additionally, electrical power is required at the national voltage with a minimum of five outlets for the system and computer plus additional outlets for options, accessories and thermostating.

A source of compressed air regulated at 7 bar minimum (0.7 MPa or 100 – 110 psi) is also required; the instrument uses 6 mm O/D nylon pneumatic line with a line to hose adaptor available. Other gases such as nitrogen can be used; if in doubt, check with Hi-Tech.

The availability of a suitable computer (PC) is also a necessary prerequisite; a minimum specification for this should have been discussed with Hi-Tech sales personnel at the time of purchase, including the availability of expansion slots for application cards.

If the installation is carried out by Hi-Tech personnel, then an Installation Report will be filled out upon completion of the installation and will detail any problems, shortages and follow-up actions to be taken. Unpacking, location, installation and connection procedures for the instrument system are detailed in the following sections.

2.A.1 UNPACKING

Unpack the instrument and associated units from their respective cartons. Referring to the packing lists shipped with the instrument, ensure that nothing is inadvertently disposed of with the packing material.

Before the instrument is installed, care should be taken to ensure that all packaging materials have been removed and that any ventilation holes are free from obstruction.

2.B LOCATION

This equipment is designed for use on any well-supported laboratory bench with a working height of about 76 cm (30 inches). Care should be taken to ensure that the chosen site be free from excessive shock or vibration that would affect the operation of the instrument. All ventilation holes and heat sink areas must be kept free from any obstruction. Figure 2.B.1 shows the recommended benchtop arrangement of the system; this provides the most convenient interconnecting between units.

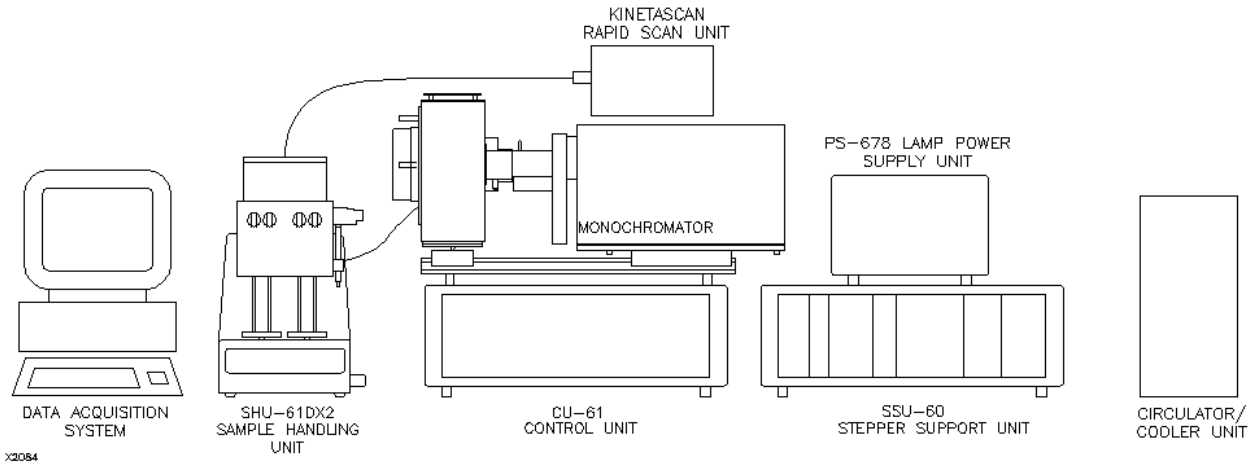


Figure 2.B.1 Typical Benchtop Layout

The Spectrofluorimeter should be used in a laboratory or room where the maximum ambient temperature does not normally exceed 30 ^{O}C (85 ^{O}F). If the user anticipates temperatures in excess of this, then they should contact Hi-Tech Limited. Additionally an atmosphere as free as possible from dust and corrosive vapours is desirable in order to prolong the life of the optical and other components. It is also advisable, but not essential, to keep the room temperature reasonably constant, i.e. within 10 ^{O}C.

The equipment is available for operation from the following electrical power supplies:

220 - 240 V~ at 50/60 Hz
110 - 120 V~ at 50/60 Hz

2.C ELECTRICAL CONNECTIONS

The SF-61DX2 system and associated units, where mains powered, are supplied with a moulded mains power cable that has an IEC plug at the equipment end, and an appropriate national plug at the other. The colour coding of the cable is as follows:

110 V~	LINE	BLACK
	NEUTRAL	WHITE
	EARTH	GREEN
230 V~	LINE	BROWN
	NEUTRAL	BLUE
	EARTH	GREEN/YELLOW

For the schematic detailing the system interconnections, refer to Figure 2.C.1. All cables supplied are identified by part number making it possible to relate them to the diagram in Figure 2.C.1.

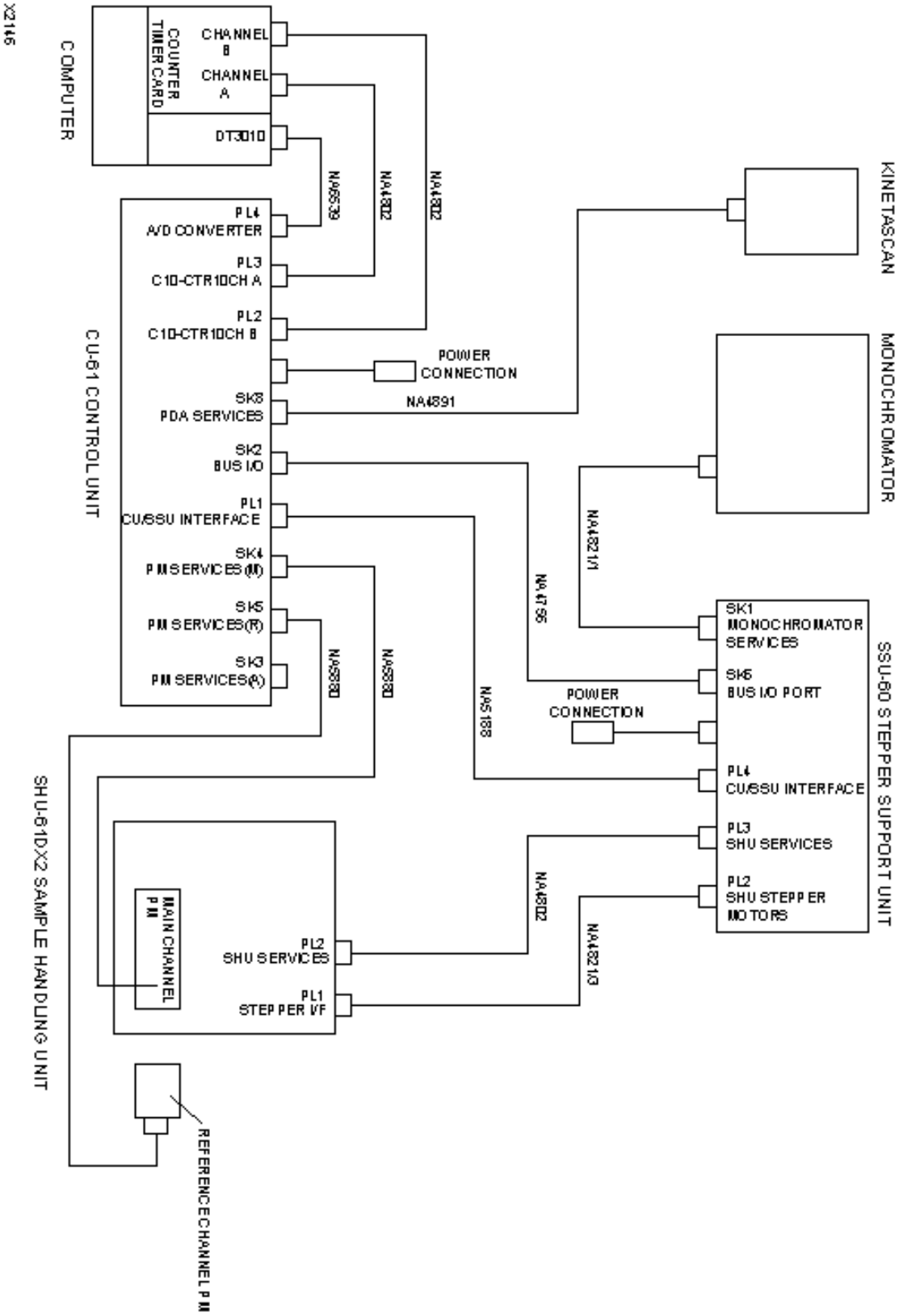


Figure 2.C.1 Interconnection Diagram

2.D THERMOSTAT CONNECTIONS

The SHU-61DX2 sample handling unit should be connected to an external circulator (and cooler) to provide temperature control.

The main section of the sample handling unit encapsulating the flow circuit, including the four drive syringes, drive valves and observation cell together with interconnecting plumbing, can be thermostatted at a constant temperature. There is an option, which allows the reagent reservoirs to be thermostatted, either at the same temperature as the aforementioned by connecting the reservoir thermostat jacket in parallel to the main thermostat jacket, or at a different temperature by using an alternative thermostat supply.

Most users choose not to thermostat the reservoirs; the thermostat lines are simply connected directly to the FLOW-IN and FLOW-OUT spigots. Figure 2.D.1 shows the thermostat connections. Both the FLOW-IN and FLOW-OUT spigots are located at the rear of the sample handling unit. The flow-in spigot connects directly to the internal transfer pipe to the cell block which in turn discharges into the main thermostat jacket.

Connections to the thermostat system are made with stainless steel fittings - the 6mm bulkhead spigots (FLOW-IN and FLOW-OUT) can be connected to the 6mm diameter polythene tubing (supplied as a 3 m length) with flexible tube sleeves eg Tygon.

When filling the system, venting ports are provided at the top of the main thermostat jacket (and at the top of the optional reagent reservoir thermostat jacket). The socket cap head thermostat vent screw shown in Figure 4.B.1.a should be unscrewed about 1 turn to allow displacement of air by filling of the thermostat fluid.

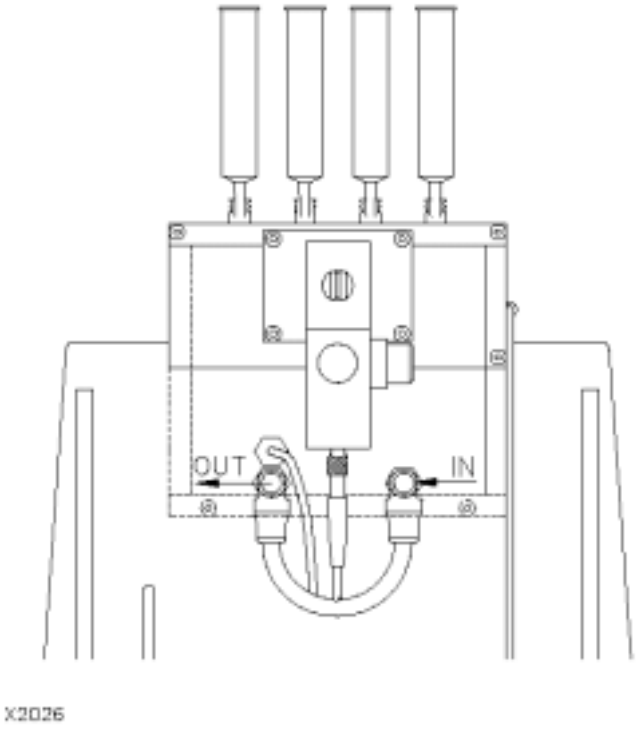


Figure 2.D.1 Thermostat Connections

A polyurethane foam fit can be supplied as part of the thermostat option with the reservoir tank. Two sections provide thermal insulation for both the main thermostat jacket and the cell block. These should be used when working at temperature extremes, in particular at low temperatures to reduce condensation problems and to improve thermal stability. This kit also includes the parts required to purge the optical windows and surfaces with dry gas. Any long pipe runs between the circulator and sample handling unit should also be insulated to improve stability. When using the reagent reservoir jacket the main foam fit has to be cut with a sharp knife to accommodate this; it has been supplied to fit when the reagent reservoirs are not thermostatted.

An external connection to the Pt100 probe can be made for use with circulators whose temperature control system can utilise an external sensor. This type of set-up provides a closed loop control of temperature actually at the sample handling unit - providing the most satisfactory way of ensuring temperature stability. The manufacturer's handbook should be consulted to find out whether this can be achieved with the users' circulator.

2.E. OPTICAL CONNECTIONS

The excitation optics are carried by an optical rail which is ideally located on top of the CU-61 electronics unit. An optical fibre connects this to the beam splitter arrangement at the observation cell on the sample handling unit. Figure 2.E.1 shows the optical rail arrangement and Figure 2.E.2 the optical fibre connection(s) to the splitter.

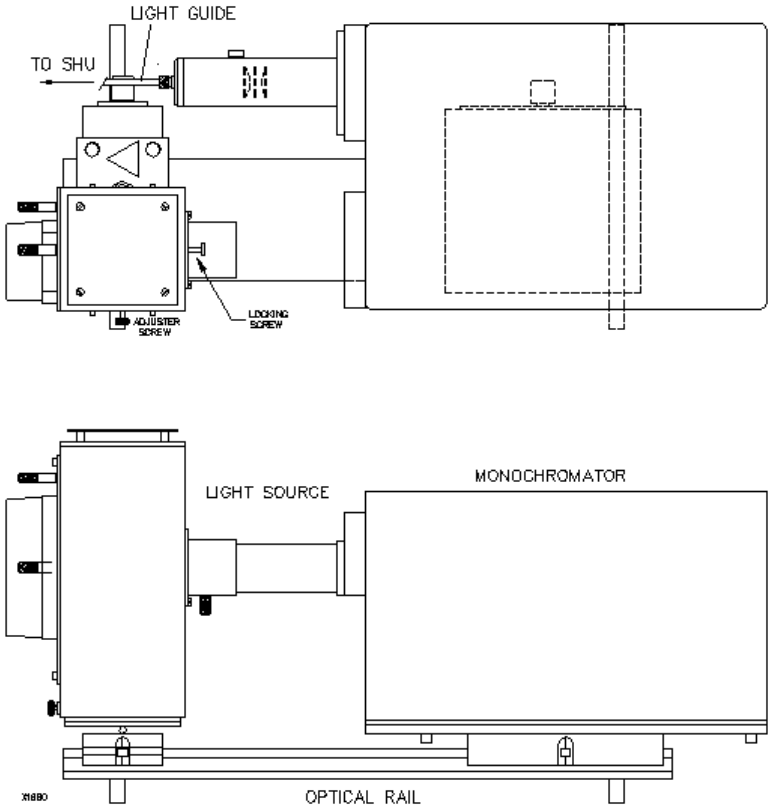


Figure 2.E.1 Optical Arrangement

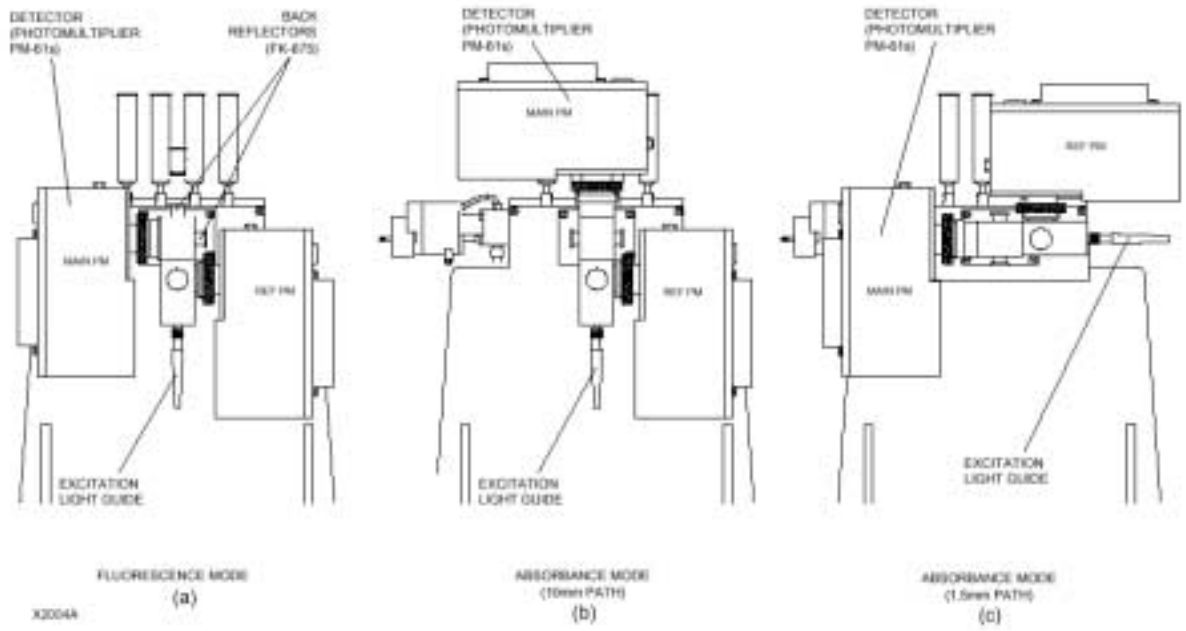


Figure 2.E.2, 3, 4 Optical Configurations

The light sources have to be aligned with the entrance slit of the monochromator; once set the precision optical rail should allow for easy recovery of this alignment. When the light sources are fitted to the rail, some minor lateral adjustment to the lamp housing may be necessary and is easily achieved via the adjuster screw on the lamp housing – the locking screw should be released before adjusting, and subsequently tightened (- see Figure 2.E.1). Should the user have to change a lamp or tube then only realignment of the new device with respect to the optical axes within the housing should be necessary. Two finger screws on the backplate *finely* tune the lamp position with respect to this optical axis (- refer also to Section 3.C.4, Optimising the Optics).

The fibre optic is attached to the optical coupler at the end of the filter wheel mounted at the monochromator exit port with an SMA connector. The other end of the fibre is connected to an SMA connector at the beam splitter.

The beam splitter assembly at the cell block is flange mounted, secured by two M3 screws and sealed by an 'O' ring. If the splitter is ever removed, it will need to be realigned to the optical axis (ie the centre line) of the cell during the replacement. If the user needs further advice, a service procedure is available on request.

The PM-61s detection photomultipliers are fitted to the sample cell block by the appropriate optical couplings. The reference PM-61s photomultiplier (if used) is fitted to the side port of the beam splitter. The optical couplings have a screw ring for connection to a photomultiplier and are flange mounted, each secured by two M3 mounting screws. Both 1.5 mm and 10 mm pathlengths are accessible without reconfiguration of the sample handling unit. Figures 2.E.2, 2.E.3 and 2.E.4 show the optical configurations for absorbance and fluorescence modes.

The photomultipliers are supplied with an integral shutter and a knurled brass screw ring that enables quick and easy connection to the associated coupling. To fit, locate the photomultiplier spigot inside the coupling ring and then rotate the brass screw ring in a clockwise direction (viewed from the photomultiplier). To ensure a tight and secure connection, finish the tightening by rotating the photomultiplier body just a few degrees *with* (in the same direction as) the brass ring. To remove the photomultiplier, the screw ring is rotated in an anti-clockwise direction (viewed from the photomultiplier); if the screw ring is reluctant to move, again rotate the photomultiplier body a few degrees *with* the brass ring.

The optical coupling accommodates standard one inch (25.4 mm) filters which are simply placed in the recess; a vacuum pencil is supplied to facilitate removal of the filter after use. A spring hook is also supplied with the system to facilitate access to the shutter lever. When this shutter lever is up, the shutter is open; conversely when the lever is down, the shutter is closed. Note that it is advisable to close the shutter *before* removing a photomultiplier.

2.F PNEUMATIC CONNECTIONS

The gas supply to the sample handling unit is made via 6mm diameter nylon tubing, which is simply pushed into the slip collet fitting at the rear of the unit. The pressure of the gas supply at source should be set at 7 bar minimum; this is then be regulated down at the sample handling unit.

Locally, the sample handling unit is fitted with its own regulators to set pressures for syringe drives etc.; typically the Drive 2 air pressure, measured on the front panel of the sample handling unit will be 4 bar. Usually the gas supply is from a line or bottle and can be of either air or low purity nitrogen. Where the sample handling unit is located in an anaerobic glove box, high purity nitrogen will be necessary.

Important: Ensure that the gas supply is "clean", free from particles and debris; generally a standard laboratory bottle or tank is fine. If there is any doubt about particulates in the gas supply, a filter should be considered.

2.G COMPUTER INSTALLATION AND ASSOCIATED HARDWARE

For software systems supplied with a computer, the KinetAsyst software is pre-installed on the hard disk.

For installation of the software the user is referred to the KinetAsyst Installation Instructions.

The software installation instructions also include fitting instructions for the associated interface boards.

Ensure that all handling precautions are observed when fitting the boards.

SECTION 3

SYSTEM OPERATION

3.A SWITCHING ON

Generally, prior to switching on the system, it is recommended that the photomultiplier(s) are shuttered and that the photomultiplier high voltage controls on the CU-61 front panel are turned fully anti-clockwise to zero output volts. The exception to this might be when a system has been set up with a scanned baseline and optimised for a particular spectral range or repeated set-up; here the user might choose to leave the setting from day to day when the same mode of operation is to be pursued on a daily basis. Note however that when working in fluorescence mode, the photomultiplier high voltage controls should always be turned down to zero after use.

Periodically, it is advisable to check that all the system interconnection cables are correctly connected and properly seated. It should be noted that the digital circuit board within the CU-61 is powered from the computer. Therefore when reseating any connector in the top row of cables on the rear panel of the CU-61, the computer must be switched off.

Before switching on the system, the lamp needs to be energised. If using an arc lamp, first, ensure that all sensitive electronics and computers in the vicinity are switched off so as to reduce the risk of damage during the ignition. Before switching on the PS-678 Lamp Power Supply unit, ensure that the LAMP SELECTOR control is set for the lamp to be ignited. Switch on the power supply at the mains rocker switch on the rear panel; the POWER ON indicator will illuminate. Press the IGNITE button on the front panel to strike the lamp.

Ensure that the Lamp Selector switch on the PS-678, lamp power supply, is set for operation with the lamp installed *before* the power supply is switched on. Failure to do this could result in damage to the PS-678 as well as the lamp. Do not alter the switch position while the unit is powered.

Beware of the high ignition voltage - 15 kV - generated for an arc lamp. Keep hands away from the lamp housing during ignition … and subsequently when the housing will get hot.

A radiated electromagnetic pulse is generated on ignition of an arc lamp. Certain circuitry can be susceptible to this, causing failure. Computers and electronics in the vicinity should be switched off during ignition of an arc lamp.

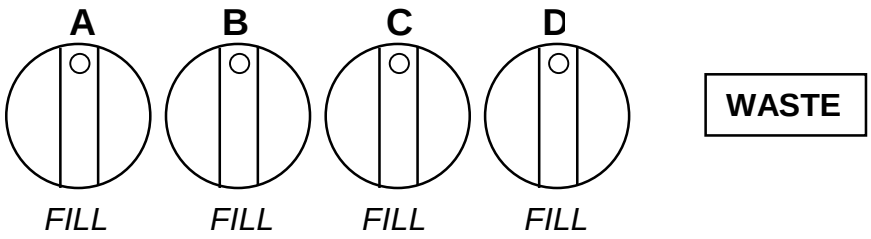
The other units in the system are energised by switching on the mains rocker switches on the rear panels of the CU-61 and SSU-60. The POWER ON indicators will illuminate on these units while the temperature and mode display on the SHU-61DX2 will also become active.

It is necessary to allow about 30 minutes for the lamp to stabilise after switching on, before using the system.

3.B PREPARING THE SAMPLE HANDLING UNIT

3.B.a FILLING

Solutions are introduced into the sample flow circuit through reservoirs or (loading) syringes situated on top of the Sample Handling Unit. The DRIVE/FILL valves are set to FILL to enable transfer of solution from the reservoirs into the drive syringes.

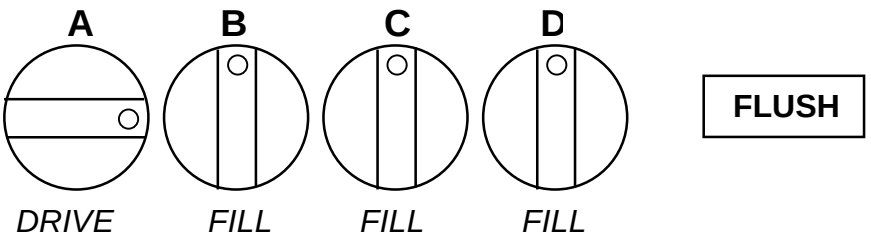


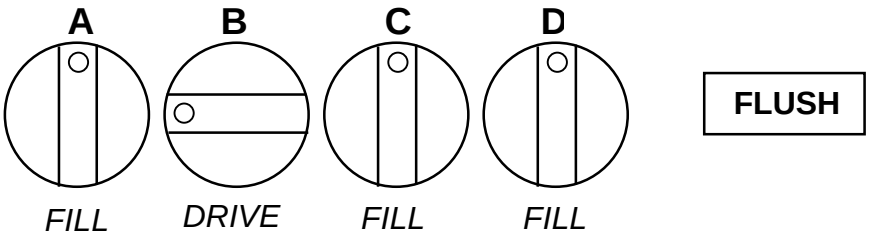
It is important to avoid introducing any air bubbles into the water or buffer solution while filling by pushing on the reservoir syringe more than pulling on the drive syringe - 'more push than pull' is required. Generally it is best to push all solution through to waste, although if water is being used as the buffer solution, careful pumping of the syringes up and down is permissible - it is best to *slowly* draw solution down into the drive syringes but then rapidly expel any bubbles upwards, back towards the filling syringe or reservoir. Having said that, the SHU-61DX2 does not tend to trap air bubbles and any offending bubbles are generally easily pushed through to waste by doing a short series of dummy shots.

3.B.b FLUSHING

It is normal practice to initially fill the sample flow circuit with demineralised water or buffer solution in order to set transmission levels (for absorbance unit reference levels), to exclude air from the system and to avoid pH changes to reagents upon their introduction to the flow circuit.

Push solution through the flow circuit *one valve and syringe at a time.* The STOP/WASTE valve is set to FLUSH by pulling out its stop pin and rotating the handle clockwise, the DRIVE/FILL valves need to be set, *one at a time*, to the DRIVE position and each drive syringe plunger pushed in turn by hand.





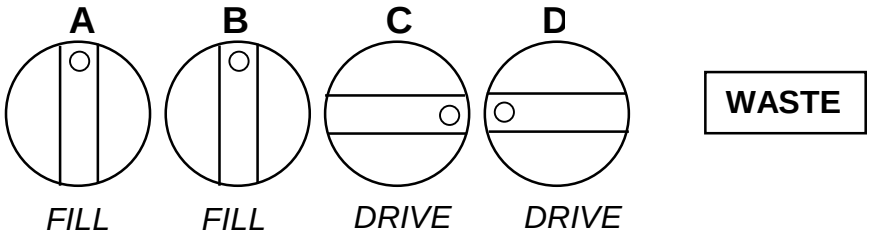
Etc., etc.

Move systematically through the reagent paths from drive to drive, making sure that the whole of the flow circuit is flushed and filled before commencement of any experiments. It should be noted that *all of the flow circuit* must be filled even when using only the single mixing mode.

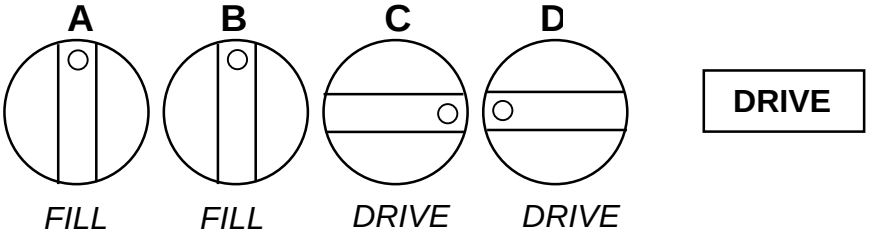
Flushing, particularly if done vigorously, can itself introduce bubbles into the flow circuit; it is therefore recommended that a few manual shots are now done to ensure complete preparation of the flow circuit. This is particularly important when doing absorbance measurements where the water or buffer solution within the observation cell will serve as the reagent blank for the references.

3.B.c DOING A DUMMY SHOT

To do a dummy shot, the Drive 1 (A+B) valves must be set to the FILL position, the Drive 2 (C +D) valves set to DRIVE and, if necessary, the stop syringe emptied. To empty the stop syringe, the STOP/WASTE valve actuator handle is rotated to the WASTE position and the stop syringe plunger is then moved fully upwards, thereby ejecting the syringe contents.



To do the shot, the STOP/WASTE valve must be rotated to the DRIVE position and then the Drive 2 pushplate pulled up; buffer or water will fill the stop syringe.



The DRIVE button on the lower cover can also be used to effect a dummy shot. Note however that the TRIGGER button must be pressed prior to the first shot in order to enable the DRIVE button initially. In this mode, the user will (as with manual shots) have to empty the stop syringe manually and then return the STOP/WASTE valve to the DRIVE position, before pressing the DRIVE button.

After completing a few dummy shots, the user is ready either to optimise the optics and take reference levels for absorbance detection, or to load the reagents for optimisation and the setting of reference levels for fluorescence detection.

Once reference levels have been set, the software ACQUIRE SINGLE SHOT button will become active and can now be used to acquire single shots. Software acquired shots, unlike dummy shots, will automatically attempt to empty the stop syringe prior to the shot as well as acquire data after the shot has completed.

3.B.1 LOADING REAGENTS

To introduce small quantities of reagents into the drive syringes without introducing air and with minimal wastage of solution, while also avoiding dilution (of the reagent) or foaming of protein solutions, requires a certain technique. With the DRIVE/FILL valve in the FILL position, empty the drive syringe water or buffer contents into the reservoir syringe until the syringe plunger is about a finger's width, or about 0.5 cm for standard 1 ml syringes, below its upper travel limit. Remove the reservoir syringe before fully emptying the drive syringe; water or buffer solution will occupy the 'filling volume' above the drive syringe and form a small meniscus on top of the luer connector. The reagent reservoir syringe, from which air has been excluded and with its tip full of reagent, can now be seated in the luer connector and a *small* amount of reagent drawn into the drive syringe; flush this diluted reagent through the flow circuit and repeat. The reagent can now be fully drawn into the drive syringe; remember that to avoid the introduction of air bubbles when filling a drive syringe, "more push than pull" is required. Finally ensure that the STOP/WASTE valve is set to either the DRIVE or WASTE position so that automatic control can be immediately established.

3.C OPTICS SETUP

It is necessary to optimise the optics during the initial setting up of the system for either fluorescence or transmission (absorbance) measurements. Optimisation is required in order to get the best out of the instrument and, in optimising, one strives to maximise the light throughput for a given setup (ie for a given slit width, wavelength range, lamp, detector and type of measurement). This necessitates good alignment of the optics and appropriate separation between the lamp and the monochromator. The higher the light throughput, the lower it is possible to keep the photomultiplier high voltages; this in turn keeps photomultiplier shot noise, the dominant noise contributor of the system, as low as possible and so the signal to noise ratio (- see also sections 5.G, Optical Bandwidth and 5.H, Time Constant) is maximised for the optical setup.

Important: It should be noted, however, that a *minimum* photomultiplier high voltage of 250 V is recommended in order to ensure a linear response of the detector.

3.C.a TRANSMISSION (ABSORBANCE) DETECTION SETUP

For absorbance measurements, the detecting photomultiplier is mounted opposite the incident light entrance, refer to Figure 2.E.3 & 4. The integral photomultiplier shutter must of course be opened before measurements are made and a spring hook is supplied to enable easy access to the lever that opens and closes the shutter. The shutter is open when the lever is *up*. Note that it is advisable to close the shutter by setting the lever down *before* removing a photomultiplier.

It is recommended to select DUAL BEAM operation (- see Section 4.C, CU-61 Control Unit) by the toggle switch on the front panel of the CU-61 when working with an arc lamp.

To optimise the optics in order to maximise the signal response prior to recording the 100 % (maximum incident light) and 0 % (dark condition) transmission levels, either the MANUAL SETUP or AUTO SETUP SCAN BLANK routine (refer to the KinetAsyst software manual) can be used. The MANUAL SETUP option will allow acquisition of data at a single wavelength, whereas the AUTO SETUP SCAN BLANK option, upon completion, will allow data acquisition over a user selected wavelength range.

As with most spectrophotometers, prior to acquiring the 100 % and 0 % transmission levels, it is necessary to introduce a reagent blank to the observation cell, as described in the previous section.

3.C.b FLUORESCENCE DETECTION SETUP

For fluorescence measurements, the detecting photomultiplier is mounted on a dedicated coupling perpendicular to the incident (or excitation) light, refer to Figure 2.E.2.

Before the photomultiplier is screwed onto its optical coupling, the appropriate cut-off or bandpass filter (- to prevent any scattered excitation light from reaching the detector) must be placed in the recess in the optical coupling. While a set of filters, the F-675 Filter Set (- refer to section 4.E.9) is provided with the system, the user may want to use his/her own filters. With this in mind, the recess has been designed for standard 25.4 mm (one inch) filters that can be obtained from any good optics supplier, or indeed the user can cut their own filters from photographic gelatine filters (eg Wratten by Eastman Kodak Company, Rochester, NY, USA). Note that a vacuum pencil is supplied with the system to facilitate removal of the filter after use. Note also that the optical coupling has a protruding lens that will become scratched if care is not taken to store the coupling resting on the threaded spigot, *not* on the lens.

The integral photomultiplier shutter must of course be opened before measurements are made and a spring hook is supplied to enable easy access to the lever that opens and closes the shutter. The shutter is open when the lever is *up*. Note that it is advisable to close the shutter by setting the lever down *before* removing a photomultiplier.

It is usual to select SINGLE BEAM mode by the toggle switch on the front panel of the CU-61 for fluorescence measurements.

Fluorescence intensity, unlike absorbance, is not an absolute quantity and the acquired fluorescence measurement is simply expressed as % response. Setting up is therefore more flexible in that it is not strictly necessary to acquire reference levels unless comparative studies are being undertaken. Typically, simply optimising the fluorescent signal is considered sufficient.

Generally for fluorescence measurements, resolution of the excitation wavelength is not critical and so the user is able to open the monochromator slits wide eg 2-3 mm in order to allow more light through the system. If photo bleaching is a problem however, the slit widths may need to be reduced.

To optimise the optics for fluorescence detection the MANUAL SETUP routine (refer to the KinetAsyst software manual) is used and the excitation wavelength is set. Remember first to install the two mirrored blanking plugs (FK-675) - one is fitted to the rear window of the excitation path, the other to the rear emission window of the observation cell assembly.

With the fluorescent solution in the observation cell, gradually increase the photomultiplier high voltage and then optimise the optics (as described overleaf) until some increase of the live signal is observed. Set the high voltage to give a signal level of about 90 % of full scale. If only a small response is detected, make sure that the optical couplings are indeed optimised, try increasing the monochromator slit widths and 'nudging' the excitation wavelength so as to make sure that the signal is at a peak. Push more solution through to replenish the observation cell if photo bleaching is suspected. It may not always be possible to achieve 90 % full scale, even with the photomultiplier high voltage at a maximum, due to the small fluorescence response; this is not a problem so long as there is a sizeable change that can be distinguished from the noise floor.

3.C.1 LAMP SETUP

The optical rail provides repeatable precision in the alignment of lamp units and the monochromator. Generally the monochromator is not disturbed once set up as described under installation in Section 2.E. However light sources may be changed depending on the nature of measurements to be made (viz. fluorescence or absorbance, UV or visible); they are selected according to suitability in terms of spectral range, intensity and inherent stability. Further advice and information can be obtained from Hi-Tech regarding lamp characteristics. The lamp unit can be fitted to the optical rail in a matter of seconds.

Three lamp backplate assemblies, for visible, deuterium or arc lamps, can be used with the standard lamp housing (- refer to Figure 4.E.1). The four cap head socket screws, one at each corner of the assembly, are removed to allow the backplate to be withdrawn from the lamp housing and a replacement to be fitted.

A xenon, mercury or xenon/mercury arc lamp can be mounted on the standard arc lamp backplate (refer to Section 4.E.1). The optimum position for each lamp and its focusing lens is set during optimisation.

3.C.2 WAVELENGTH SELECTION

Where more than one grating is supplied with different blaze wavelengths then that closest to the wavelength of interest should be selected.

A mechanical digital indicator on the rear of the monochromator gives a reading of the current wavelength as set by the KinetAsyst software. Unless otherwise specified, a direct read-out of wavelength is indicated.

3.C.3 SLIT WIDTH SELECTION

Unless otherwise specified the standard monochromator grating dispersion is 1.8 $nm.mm^{-1}$. Using the micrometer adjusters the slit blades can be set to achieve a desired bandpass; two revolutions of the micrometer barrel gives 1.0 mm (≅ 2 nm). Both entrance and exit slits should have equal settings. Refer also to Section 5G, Optical Bandwidth.

3.C.4 OPTIMISING THE OPTICS

Remember first to open the photomultipler shutter(s) by simply pulling fully out the black knurled knob, located on the filter holder in front of the photomultiplier detector.

To optimise the optics to give maximum signal and improve the signal to noise performance, the signal is viewed on the live display either in the MANUAL SETUP or the AUTO SETUP SCAN BLANK. Each adjustment is made in an attempt to improve the signal level observed on the live display; if as a result the signal saturates (or exceeds the 100 % level), the PM volts need to be reduced. However, remember to keep the PM volts above 250 V (for linearity of the detector response) by reducing the monochromator slit widths as necessary. Optimisation essentially consists of adjusting the lamp position and focusing with respect to the entrance slit to the monochromator, as follows:

Lamp: The whole lamp assembly can be moved on the long optical platform by loosening the clamping mechanism at the far side of the lamp and then sliding it along the rail. To ensure correct alignment of the lamp housing with the monochromator, push the near side of the lamp carrier onto the optical rail while you slide the lamp along the rail and when tightening the clamping mechanism. As a general rule, the lamp needs to be nearer to the monochromator for optimisation of the shorter (UV) wavelengths and conversely, further away for the longer (near IR) wavelengths.

The focusing of the lamp can be altered by unscrewing the black knurled lever (located on the underside of the focusing column) and moving the collecting lens, using this adjusting lever, along the slot. The lamp should be imaging a quite sharp, intense spot at the entrance slit of the monochromator ~ 4 mm in diameter.

Beware of UV radiation. Do not look directly at the lamp output.
Use UV blocking eye protection when necessary.

For fine-tuning, the two finger screws on the lamp backplate alter the lamp position with respect to the lens assembly and hence the optical axis within the housing; the top screw adjusts the tilt or vertical position, the side screw adjusts the rock or horizontal position.

Important: These finger screws are only intended for fine-tuning (- small adjustments either way) and they should generally only need to be adjusted after a new lamp is installed or if the lamp housing is moved. If the spot from the beam on the monochromator entrance slit will not centralise horizontally about the entrance slit, the lamp can be moved laterally across the rail by slackening the locking screw (located beneath the focusing lens assembly at the bottom of the lamp housing) and then turning the front adjuster screw (- refer to Figure 2.E.1). The correct lateral position of the lamp to ensure proper alignment with the monochromator is, however, factory set and any lateral adjustment should not generally be necessary after installation.

Monochromator: The monochromator can be moved on the long optical platform by loosening the clamping mechanism at the rear and underneath the monochromator carrier and sliding it along the rail. For correct alignment, ensure that the near side of the monochromator is pushed back onto the optical rail before tightening the clamping mechanism. At the exit port of the monochromator there is the filter wheel assembly with a focusing attachment (the exit column) that contains a pre-focused lens assembly.

Excitation Fibre: The excitation fibre optic is terminated with SMA connections that ensure optimal positioning. One end of the fibre should be completely inserted into the receptacle on the exit column of the monochromator and tightened by hand. The other end of the fibre is coupled to the beam splitter or an alternative coupling to the observation cell in the same way.

3.C.4.a TRANSMISSION DETECTION

When using the MANUAL SETUP to optimise the optics for a given wavelength, both the 100 % (maximum incident light on detectors) and 0 % (dark condition) transmission levels are recorded by selecting the READ ALL button. Note that it is possible to reread the 0% levels by selecting the READ 0 % button, while individual 100 % levels can be acquired by clicking on the pair of spectacles adjacent to the 100 % reading. FINISH is selected to exit the routine.

If the AUTO SETUP SCAN BLANK route is being used, the monochromator is first moved to a wavelength where the optics are optimised and then moved to a second wavelength where the photomultiplier high volts are maximised. With this completed, the SCAN BLANK button is selected to initiate two scans of the wavelength range - first the 0 % and then the 100 % transmission level is acquired. FINISH is then selected to exit the routine.

Data can now be acquired.

Important: Details of how to use both the setup routines are in the KinetAsyst manual.

3.C.4.b FLUORESCENCE DETECTION

Once the optimisation is complete, it is optional whether you read the signal levels to set references or use the default values by simply selecting the FINISH button to exit the Manual Setup dialogue box.

Data can now be acquired.

Important: Details of how to use the setup routine are in the KinetAsyst manual.

3.D OPERATION

The operation of the SF-61DX2 system involves much interaction with the KinetAsyst software; hence we refer the user to the software manual for "How to do …" certain operations.

3.D.1.a SINGLE MIXING

A minimum stop volume of 100 µl is recommended for single mixing, refer also to Section 5.C, Effect of Stop Volume.

Ensure that water or buffer solution has been gently flushed through from Drive 1 (A+B) syringes (- refer to Section 3.B, Preparing the Sample Handling Unit) so that firstly any "old" D solution that might have diffused back into the A & B paths from mixer 1, see Figure 3.D.1, is removed from the flow circuit and secondly that the entire flow circuit is filled with air-free buffer before any shots are acquired.

The Drive 1 (A+B) valves then need to be set to the FILL position for single mixing operation, so as to create a blocked line in the unused part of the flow circuit.

3.D.1.b DOUBLE MIXING

For double mixing, the stop volume should be set to 240 µl, a nominal 150 µl being supplied by Drive 1 and 90 µl by Drive 2. The flow circuit at the three stages of a double mixing shot is illustrated overleaf in Figures 3.D.1-3.

Important: The default values of 150 µl for both the DRIVE (PUSH) VOLUMES on the Sample Handling Unit page of the Hardware Configuration should not be altered unless syringes other than the standard 1ml are being used; in this situation seek advice from Hi-Tech.

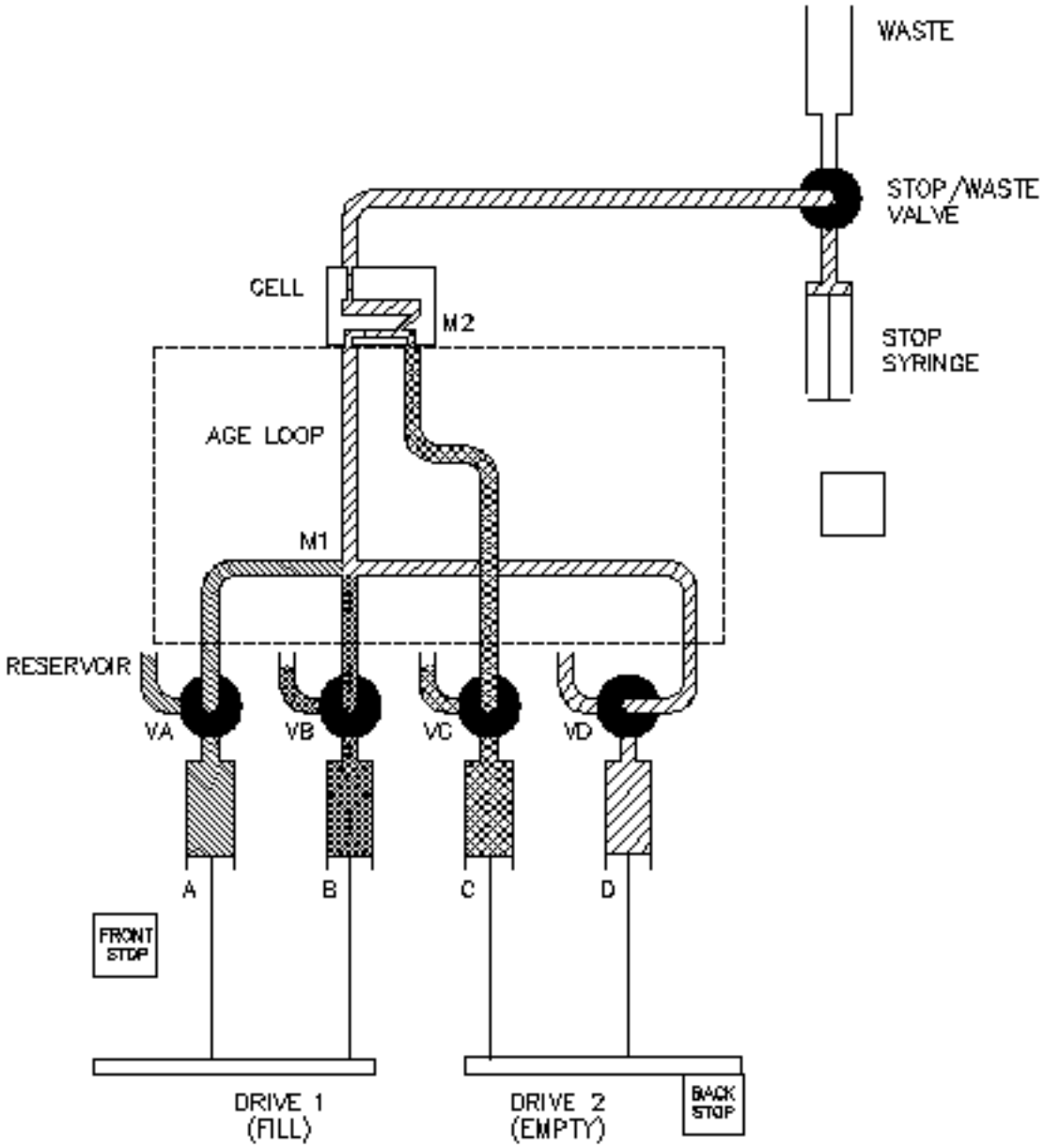


Figure 3.D.1 Double Mixing Operation - Start

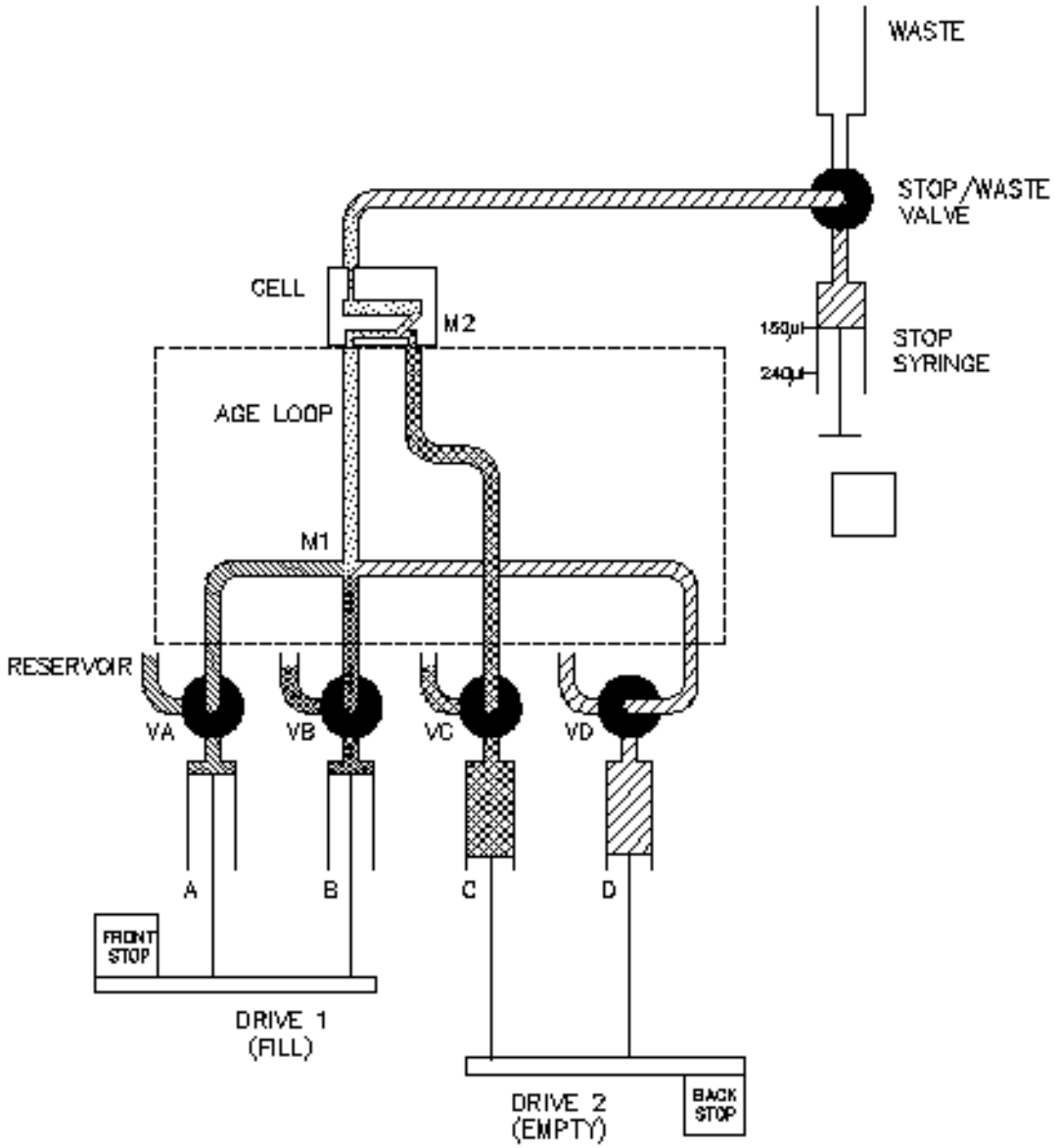


Figure 3.D.2 Double Mixing Operation – First Stop

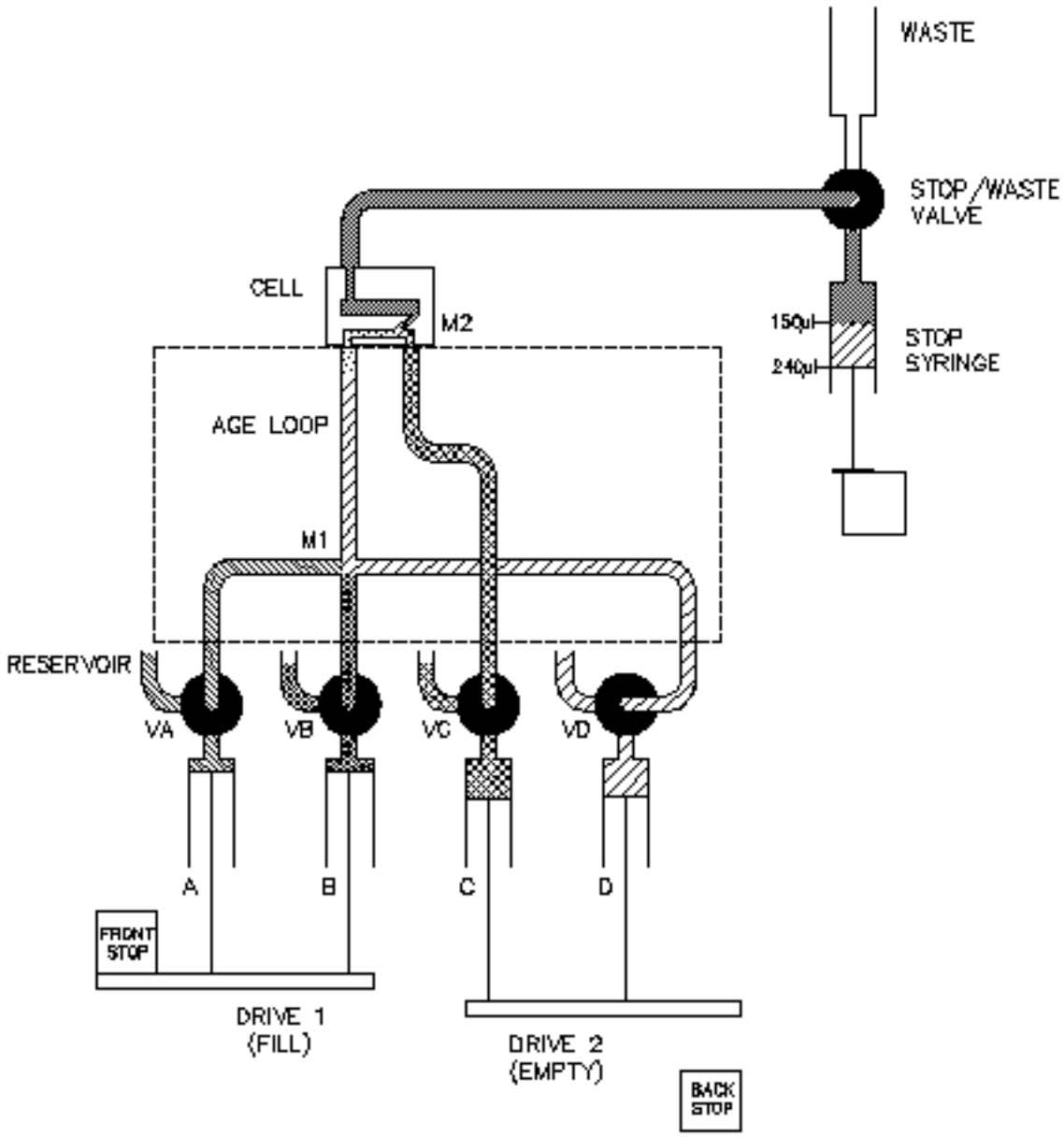


Figure 3.D.3 Double Mixing Operation – Finish

The driven volume for Drive 1 is set by a front stop controlled by a stepper motor. When loading solutions, ensure that the Drive 1 pushplate is *not* pushed down from its reset position in contact with this front stop. Associated with Drive 2 is a back stop, controlled by a second stepper motor; during a shot, this back stop prevents Drive 1 pushing solution back into Drive 2 (rather than into the flow circuit). When loading solutions, ensure that the Drive 2 pushplate is *fully* down on this back stop. These precautions will ensure the correct delivery of solution from each drive on the initial shot after filling or refilling.

The measured flowrates and agetime (refer to Section 4B.6, Sample Handling Unit Electronics) are reported after each single shot or at the end of a sequence of shots. Flowrates from 2 $ml.s^{-1}$ to 8 or even 9 $ml.s^{-1}$ are 'normal' in that a rate above 2 $ml.s^{-1}$ should give good mixing for aqueous solutions. The user should not be overly concerned about some variation in flowrates that will occur as a result of environmental changes and varying viscosity of the solutions as well as the ageing of the unit. It is worth bearing in mind though that a *steady* decline in rates may indicate that the flow circuit is getting clogged up and therefore in need of a thorough clean, while a *sudden* decline could indicate that a leak has developed.

Important: ***Never use cancel acquisition during a DX shot*** – let the shot complete, the air pressure be removed when the stop syringe plunger hits the stop block and the software be reset after the full data set has been acquired. If the drives do not activate or, in particular, Drive 2 does not fire after the prescribed age-time, the stop syringe plunger does not reach the block and hence the shot does not complete, press the TRIGGER button on the SHU lower cover in order to turn off the air pressure (- to relieve the pressure on the flow circuit) and to complete the software data acquisition routine. ***Reset the drives and refill the drive syringes before acquiring another shot.***

3.D.2 RUN TIME

The run time, also known as the sweep time is discussed in Section 5.J.

3.E AFTER OPERATION

When the user has finished a session with the instrument, it is essential that the flow circuit be thoroughly washed through with plenty of water or buffer before shutting down the system. Note that some water or buffer must be pushed through the A & B paths of Drive 1 *even* after only Drive 2 has been used for single mixing.

After work in double mixing mode, it is recommended that the drives be reset at the *end* of a session, before the system is shut down.

Remember to clean the stop syringe as well as the various paths of the stop/waste valve; note that this can be most easily achieved by acquiring a series of shots.

3.F LOW TEMPERATURE OPERATION

It is necessary to reduce the drive pressure at low temperature so as to reduce the pressure in the system that might cause leakage at the syringe seals. It is only necessary to do this for Drive 2 even if double mixing mode is being used; this is because Drive 1 is "front-stopped" and hence will not put undue pressure on the flow circuit components of the SHU. The Drive 2 pressure will need to be turned down to between 2 and 3 bar (0.2-0.3 MPa).

Condensation will generally form on the optical surfaces if there is more than a 15 °C difference between room and operating temperatures. Hi-Tech produces a low temperature kit as an option (OPT-630) that enables dry gas to be blown over the optical surfaces of the cell, the photomultipliers and any filters, so preventing the build-up of condensation. The kit also provides some insulation for the cell block and thermostat bath in the form of foam fits.

It is advisable to tighten the knurled collars (beneath the thermostat bath) that maintain the sealing between the drive syringes and drive/fill valves. This will prevent leakage from around the Teflon syringe tips as a result of a general shrinkage of materials at lower temperatures.

3.G ROUTINE MAINTENANCE

3.G.1.a CLEANING

If regular post-session cleaning of the flow circuit is not sufficient to prevent build-up of contamination, cleaning with 2 M HCl is recommended. The flow circuit can be soaked in the acid for at least half an hour, or overnight if necessary. Ensure that the acid is pushed through all paths in the valves and into the stop syringe before leaving the circuit to soak.

Use plenty of distilled water or buffer to remove the acid and rinse the entire flow circuit very thoroughly with an excess of water or buffer.

It is recommended that if water is used in the thermostat bath, the water be kept clean and 100 ml or so of ethanol be added to help to inhibit the growth of algae etc. or a proprietary bath growth inhibitor be used.

Care should be taken to ensure that the observation cell windows in particular and the optics in general remain clean and free from contamination. It is recommended to use lint-free lens tissue with iso-propanol as the solvent for cleaning any optical surfaces.

3.G.1.b DEGREASING

Grease can trap air bubbles in which case 2 M NaOH may be used to degrease the flow circuit. Ensure that the alkali is not left in contact with the optical cell for more than a few minutes for fear of etching the silica surface.

Use plenty of distilled water or buffer to remove the alkali and rinse the entire flow circuit very thoroughly with an excess of water or buffer.

3.G.2 REGULAR ROUTINE CHECKS

Check that the four knurled stainless steel collars are finger tight; these collars seal the drive syringes to the drive/fill valves and are located beneath the thermostat bath. Note that pliers should not be used to tighten the collars.

Check that the two set screws accessible through the rear window of the DRIVE/WASTE/FLUSH indicator thimble (- part of the stop/waste valve and actuator assembly) are tight. Rotate the valve manually until each screw can be seen and then tighten using an Allen wrench or key (but *not* of the ball-end type) inserted for maximum leverage.

Check that the aluminium collar on the bottom of the stop syringe plunger (that connects the plunger to the linear potentiometer below) will not rotate in a clockwise direction and is therefore tight. Tighten well in a clockwise direction if necessary. Also ensure that all three set screws that secure the collar onto the stop syringe plunger are also tightened well. If the screws are found to be loose, ensure that the plunger is 'bottomed out', i.e. pushed into the collar as far as possible, before tightening very well in a clockwise direction, using an Allen wrench or key (but *not* of the ball-end type) inserted for maximum leverage.

SECTION 4

UNIT DESCRIPTIONS AND SPECIFICATIONS

4.A GENERAL

The SF-61DX2 Stopped-Flow Spectrofluorimeter comprises four major sections; a sample handling unit, two electronics units and a collection of optics for excitation and detection. There is other peripheral equipment such as the cooler and circulator and specialised electronics units such as the lamp power supplies.

4.B SHU-61DX2, SAMPLE HANDLING UNIT

The SHU-61DX2 can be used in classical (single mixing) stopped-flow mode to rapidly mix two reagents, or in a double mixing mode where two reagents are first mixed and then a third reagent is added after some predetermined time. In this latter mode, transient species can be prepared and used as one of the reagents in stopped-flow runs. The second mixing can be effected at tens of milliseconds after the first, out to several hundred seconds. The shortest delay time is limited by maximum flow rates; the longest is limited by diffusion.

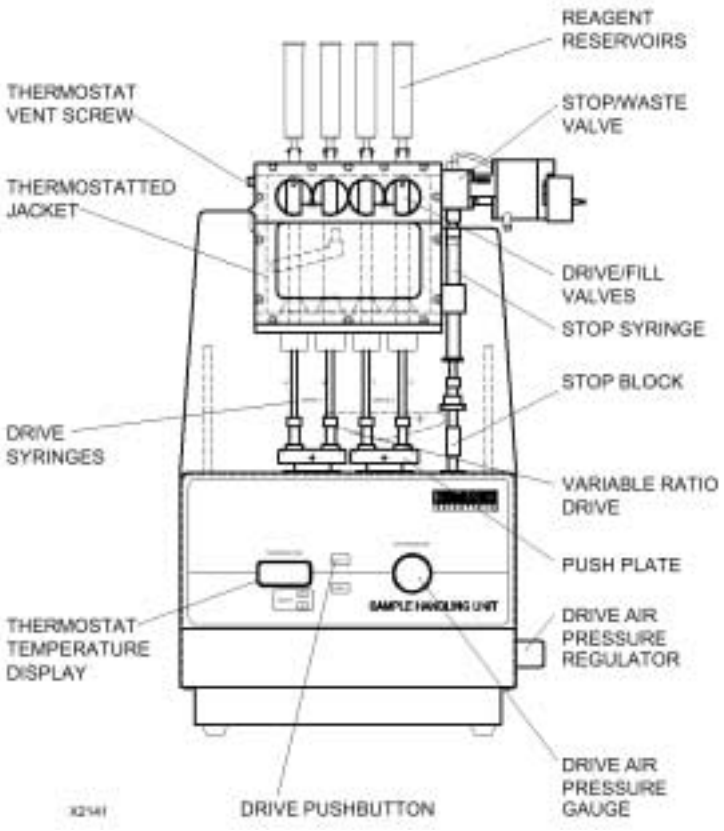


Figure 4.B.1.a Sample Handling Unit – Front View

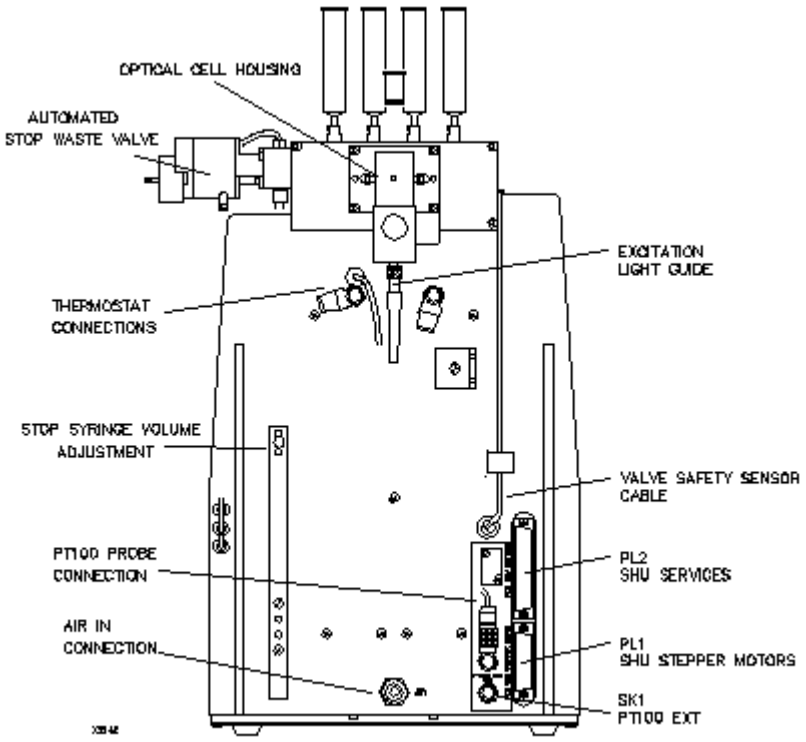


Figure 4.B.1.b Sample Handling Unit – Rear View

The body of this unit is largely constructed in stainless steel providing support and enclosure of the sample flow circuit. All sample flow circuit components are contained within a thermostatable enclosure; a Pt100 temperature probe is fitted within this enclosure. A shrouded enclosure at the base of the unit contains electronic and pneumatic components. A front panel provides temperature, mode and air pressure display and pneumatic drive control.

4.B.1 SAMPLE FLOW CIRCUIT

The sample flow circuit is built around a manifold to which the four DRIVE/FILL valves, the STOP/WASTE valve and observation cell are all close coupled; this arrangement eliminates excessive priming volumes. Furthermore, the manifold maintains great rigidity under conditions of high stopping pressures and is also well suited for anaerobic work.

The four DRIVE/FILL two-position valves are set according to the nomenclature on the cover plate; in the DRIVE position the drive syringe is ported to the sample flow circuit (for operation); in the FILL position the drive syringe is ported to the reagent reservoir (for filling). Sensors are fitted to detect the valve control lever position; this provides interlocking of the air drive preventing driving of the solutions back to the reservoirs causing a "fountain" effect. The four syringes and associated DRIVE/FILL valves are grouped into pairs; each pair of syringes is driven by a common pushplate. The pair on the left are designated A & B and are driven by Drive 1; the pair on the right are designated C & D and are driven by Drive 2. In double (multi-mixing mode) a push-push mode of operation is employed; Drive 1 delivers the product of the mixing of reagents A & B at Mixer 1 into the age loop. After a

delay, Drive 2 uses reagent D (normally buffer in this mode) to displace the (A+B) solution and mix it with reagent C at Mixer 2 (- refer to Figure 4.B.1.1).

The STOP/WASTE valve is pneumatically controlled from software enabling the stop syringe to be automatically emptied prior to the next run. There are three steps to the sequence, firstly the valve is set to the WASTE position, then the stop syringe is emptied (the piston of the stop syringe has a small pneumatic cylinder) and finally the valve is returned to the DRIVE position. A safety sensor is located in the valve actuator assembly to prevent the air drive firing with the valve set in the wrong position. Manual intervention is not necessary, but can be achieved using the actuator handle at the end of the valve.

The STOP/WASTE valve is set according to the thimble indicator; the DRIVE position ports the sample flow circuit to the stop syringe (during a run); the WASTE position ports the stop syringe to waste (for emptying the stop syringe between runs); the FLUSH position ports the sample flow circuit through to waste (for priming and flushing the system). A pin is fitted to the control lever on this valve; with the pin in, toggling between DRIVE and WASTE is enabled (the operational position), allowing easy operation of the unit. To access the FLUSH position, pull the spring-loaded pin and rotate; in this pin out mode, the extremes of travel are limited between DRIVE and FLUSH. Note that these positions are exclusive - mixed, aged solution cannot be back flushed into the sample circuit when emptying the stop syringe between runs.

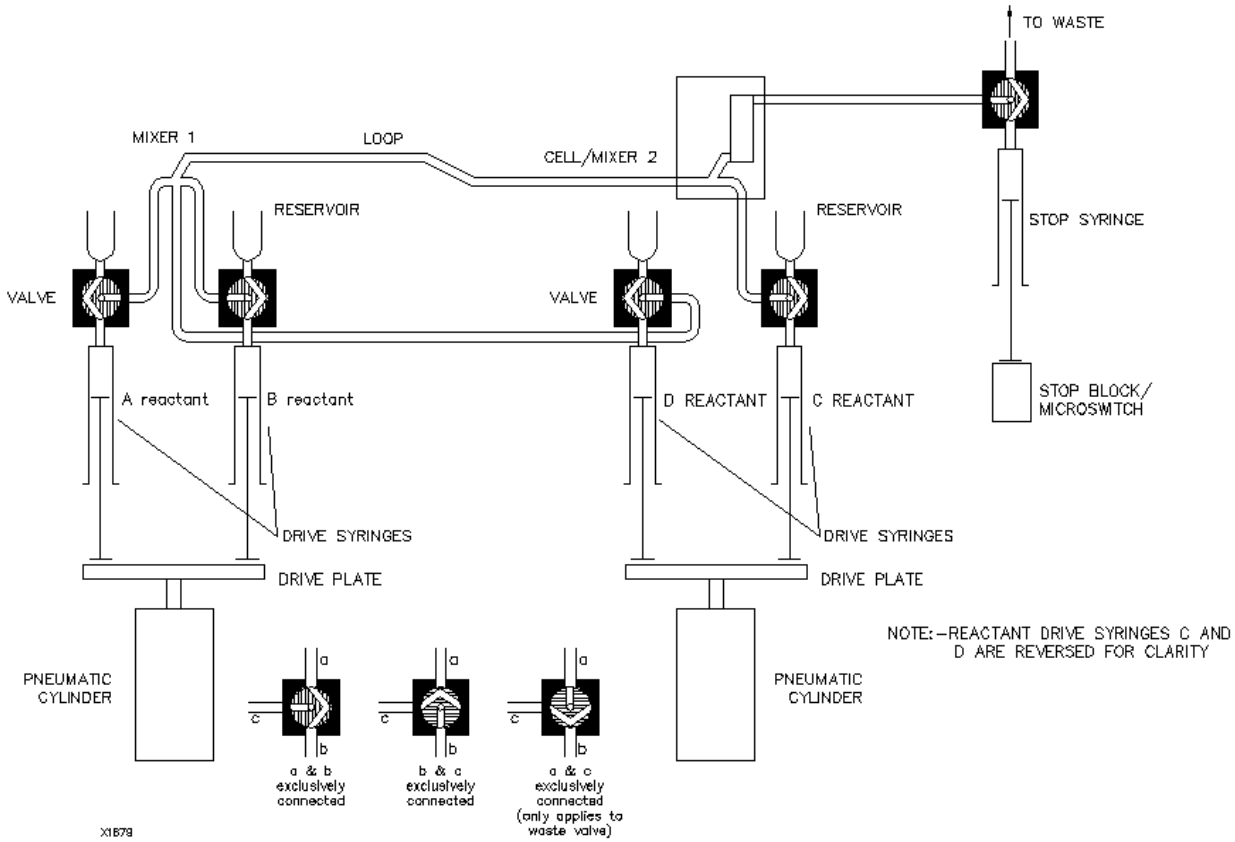


Figure 4.B.1.1 Flow Circuit

Important: The STOP/WASTE valve must be returned to either the WASTE or DRIVE position for automatic control.

4.B.2 OBSERVATION CELL ASSEMBLY

The fused silica cell is mounted in a close fitting aluminium alloy housing. This contains fluidways for thermostating connected to the main thermostat system. The housing also locates and compresses the cell plumbing connections onto the manifold interface. This method of mounting and connecting the cell provides quick and easy removal and replacement (without tedious plumbing to be disconnected and reconnected). Furthermore, specialised cells and mixers can be accommodated and fitted with minimum effort.

With the standard cell, four optical windows are accessible with provisions for coupling to optics; both absorbance pathlengths can be used and there are two fluorescence windows with 90^{o} separation from the excitation path. Figures 2.E.2, .3 and .4 show the optical arrangements.

Care should be taken to ensure that the observation cell windows remain clean and free from contamination. It is recommended to use lint-free lens tissue with iso-propanol as the solvent for cleaning any optical surfaces.

4.B.3 REAGENT RESERVOIRS

The standard sample handling unit is supplied with 5 ml polypropylene disposable syringes as reservoirs. The design is, however, intended to provide flexibility in that the unit can accommodate a user's own method of reagent filling which may result from special experimental conditions (eg anaerobicity) or personal preference.

It is fairly trivial to remove the four standard reservoirs, by twisting and unseating from the luer fittings and to remove the optional upper (reservoir) thermostat enclosure if fitted, by releasing the four M4 socket cap head screws (note the 'O' ring seal on the flange face). This allows access to the four standard luer sockets for the user to connect to with their own filling devices. A step further is the unscrewing of the two luer fittings to access ¼-28 UNF female threads for direct plumbing connections to the FILL ports of the DRIVE/FILL valves.

4.B.4 PNEUMATIC DRIVE SYSTEM

Two pneumatic drive cylinders provide the tractive effort to the drive syringes; the shrouded lower section of the sample handling unit houses all the pneumatic components. The pressure gauge on the front panel indicates the air pressure for Drive 2 and is adjusted from a regulator situated on the right hand side of the unit. Drive 1 air pressure is factory set. The compressed gas supply connection is found on the rear panel of the SHU-61DX2.

4.B.5 STEPPER MOTORS

There are two stepper motors used within the sample handling unit, one for Drive 1 and another for Drive 2; they are used to set the programmed shot volume in double mixing mode. The motors are powered from the SSU-60 Stepper Support Unit.

4.B.6 ELECTRONICS

There is a single front panel circuit housed in the lower shrouded section. The control circuitry for the sample handling unit is housed as a module within the SSU-60, Stepper Support Unit. This circuitry incorporates the air drive control circuitry and safety interlock as well as the data capture trigger circuitry, refer to Section 4.D.6. Within the sample handling unit is a linear potentiometer connected to the stop syringe which follows the flow of the sample through the flow circuit. This potentiometer, in association with electronics on the main SHU circuit board, generates event markers and a data trigger signal which are later utilised by the acquisition software to calculate the drive flowrates and actual agetimes,

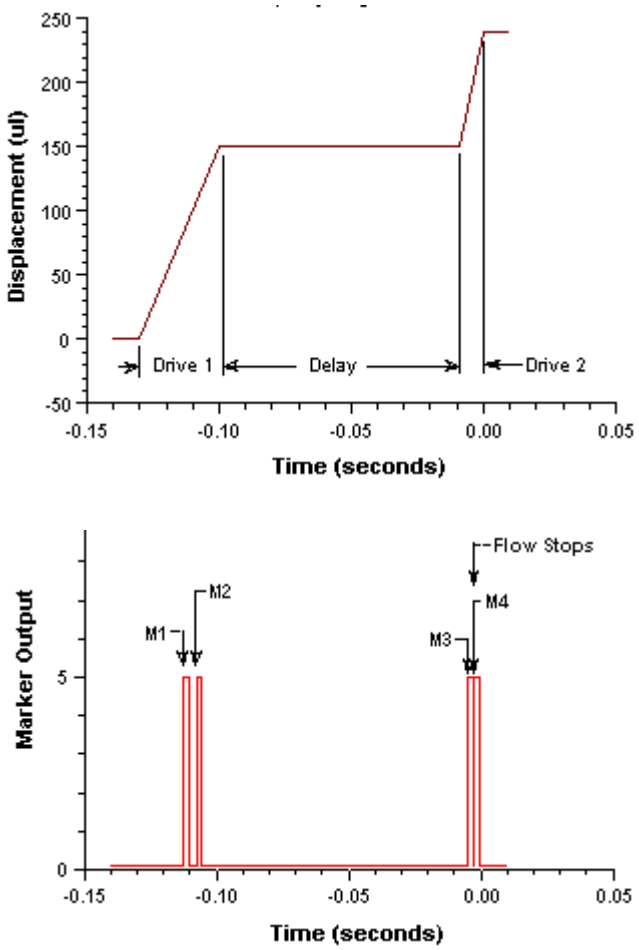


Figure 4.B.6.1 Stop Syringe Movement and Associated Event Markers

Figure 4.B.6.1 shows the output from the potentiometer that follows the movement of the stop syringe, and hence the reagents, through the sample flow circuit. A series of event markers are generated from the potentiometer; each of these is set to identify among other events, the centre of the formation of the aged slug (A + B) and the centre of the formation of the observed slug, C + (A + B). A direct measurement of the time interval between these two slugs is made and reported as the agetime.

Refer to Figure 4.D.3 for the switch/link positions and functions of the SHU control module.

4.B.7 SHU-61DX2 SPECIFICATIONS

Optical Cell (standard version):

Material;	Fused UV silica
Sample dimensions (mm);	10 x 1.5 x 1.5
Pathlengths (mm);	10 & 1.5
Mixer type;	tee

Flow Circuit:

Materials;	Glass, Teflon, Kel-f, silica, polypropylene (standard reagent reservoirs), PEEK
Prime volume (µl);	<100
Drive syringes (standard) (ml)	1
Stop syringe (ml);	1

Temperature Measurement:

Probe type;	1/10 DIN Pt100
Display	3½ digit LCD

Pneumatic System:

Operating Pressure (bar);	4 max. user settable regulator with gauge
External supply (bar);	7 min. recommended, connector at rear panel

Electrical:

Power Requirement;	+15 VDC @ 1 A, -15 V @ 1 A	
Linear Potentiometer;	Resistance:	10k ± 15%
	Electrical stroke:	20 mm
	Power dissipation:	0.4 W @ 20 ^{o}C
	Maximum applied voltage:	28 VDC
Stepper Motors;		
	Type:	040B6 - type 17
	Step angle:	1.8 ^{o}
	Phase current:	0.4 A
	Phase resistance:	17 W

Other features:

The Pt100 output is available for connecting to an external thermostat system (viz. circulator) capable of using a remote sensor.

4.C CU-61 CONTROL UNIT

The CU-61 Control Unit incorporates two main circuit boards, the analogue and digital boards, as well as up to a maximum of three signal conditioning circuit boards, one for each of the main, reference and auxiliary photomultiplier channels, that plug into sockets sited on the analogue motherboard.

Figure 4.C.1 shows the unit front panel, immediately behind which is mounted another, front panel circuit board.

Three ten turn potentiometer control knobs enable high voltage (EHT) adjustment for the main, auxiliary and reference channel photomultipliers. Turning the control knobs clockwise increases the voltage output up to a maximum of about 1000 V. A triple position selector knob sets the channel from which the LCD meter monitors the high voltage.

The dual position toggle switch for either DUAL BEAM or SINGLE BEAM mode enables the user, in dual beam mode, to utilise the reference channel photomultiplier to dramatically reduce the effects of lamp intensity variations by continually tracking any variations. For single beam mode, a dc voltage (4.2 V) is set for the reference channel, enabling the user to observe the raw signal with no compensation.

The other dual position toggle switch sets either X1 or X10 gain for the reference channel. For normal operation the switch is set to X1.

The three LED indicators, POWER ON, 5 V POWER ON and TRIGGERED, respectively show if the CU-61 is powered, the computer is powered and highlight when the system has been triggered. Note that the digital board is powered from the computer.

A push button switch designated RESET is a hardware reset for the data trigger circuitry.

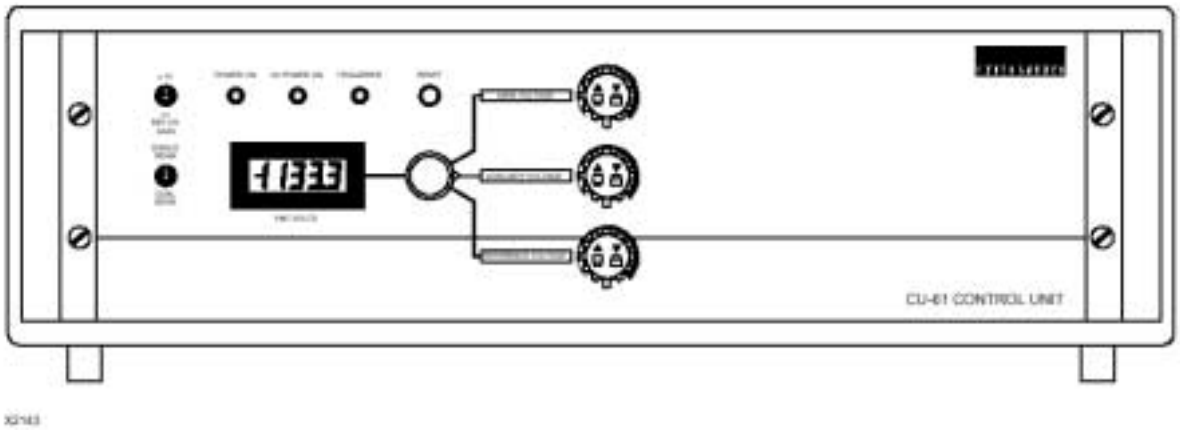


Figure 4.C.1 Control Unit – Front View

Figure 4.C.2 shows the unit rear panel. Note that mains power is connected through the IEC fuse/filter/switch unit; this provides central control to power the entire unit, excluding the digital board.

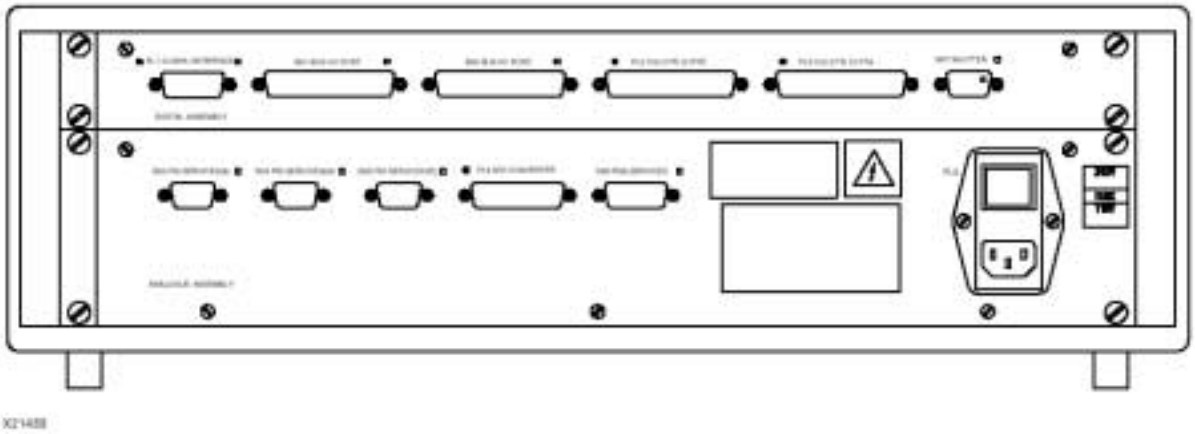


Figure 4.C.2 Control Unit – Rear View

The mains voltage is factory set to either 220/230 V~ or 110 V~ depending on the country of destination; the fuse ratings are as follows:

110 V~	5 A Anti-surge (T)
220/230 V~	5 A Anti-surge (T)

Under no circumstances should alternative ratings be substituted!

The (continuous) current consumption of the CU-61 is 0.3 A at 230 V~.

4.C.1 ANALOGUE CIRCUIT BOARD

The analogue board provides the power supply requirements for the photomultipliers and photo diode array (if supplied) and for the CU-61 unit itself, excluding the digital board. The board also contains interface circuitry for the three photomultiplier channels and the photo diode array control and data acquisition electronics.

4.C.1.a SPECIFICATION

Power Requirement	115 V~ @ 300 mA
Power Supplies	
Internal	+/-12 VDC @ 500 mA
	+5 VDC @ 200 mA
Photomultiplier pre-amp	+/- 12 VDC @ 500 mA
Photomultipler HV power supply	+ 12 VDC @ 500 mA (x3)
Photo Diode Array	+/-15 VDC @ 500 mA
	+5 VDC @ 500 mA
On-board Fuse Ratings	
Internal	100 mA Anti-surge (T)
Photomultiplier pre-amp	100 mA Anti-surge (T)
Photomultipler HV power supply	100 mA Anti- surge (T)
Photo Diode Array	100 mA Anti-surge (T)

4.C.2 DIGITAL CIRCUIT BOARD

The digital board provides the main interfacing between the computer (ie between the software, the analogue to digital (A/D) converter and the counter timer card) and the stopped-flow system hardware electronics. The board also generates the stepper motor clocks for the monochromator and SHU, the external clock for the data acquisition (A/D converter) card and the clock bursts for the shutter control.

4.C.2.a SPECIFICATION

Power Requirement;	+5 VDC @ 200 mA (Counter Timer Card)
Data Inputs;	TTL 0-5 V (Counter Timer Card) CMOS 0-5 V
Data Outputs;	CMOS 0-5 V

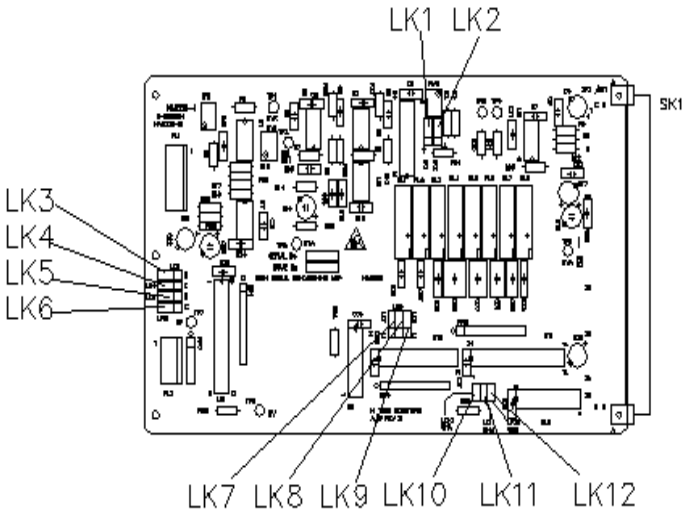
4.C.3 SIGNAL CONDITIONING BOARD, SC-61

The SC-61 signal conditioning board enables offset biases, computer-programmable gains and filter time constants to condition the signal from the photomultiplier detector, before this signal is fed to the A/D (analogue to digital) converter within the computer.

Figure 4.C.3 shows the link settings for the SC-61 printed circuit board which are used to set modes of operation. These settings are factory set and they should only be altered after specific direction from Hi-Tech.

4.C.3.a SPECIFICATION

Signal bias;	200 mV offset (dark condition)
Signal filtering;	RC filter networks 10, 33, 100, 333 µs 1, 3.3, 10, 33, 100 ms
Signal input;	double ended 0 - 10 V
Signal output;	single ended 0 - 10 V
Power requirement;	+5 VDC, ±15 VDC



SIGNAL OUTPUT SENSE:

LK1	1-2*	NON-INVERTED	2-3	INVERTED
LK2	1-2*	NON-INVERTED	2-3	INVERTED

MANUAL/COMPUTER CONTROL OF TIME CONSTANTS:

LK3	1-2*	COMPUTER	2-3	MANUAL
LK4	1-2*	COMPUTER	2-3	MANUAL
LK5	1-2*	COMPUTER	2-3	MANUAL
LK6	1-2*	COMPUTER	2-3	MANUAL

COMPUTER GAIN/UNITY GAIN CONTROL:

LK7	1-2*	COMPUTER	2-3	x1 GAIN
LK8	1-2*	COMPUTER	2-3	x1 GAIN
LK9	1-2*	COMPUTER	2-3	x1 GAIN

CHANNEL SELECTION:

LK10	AUXILIARY
LK11	MAIN
LK12	REFERENCE

PL1 PINS 1-2* LINKED (WITH A JUMPER) FOR COMPUTER CONTROL

* DENOTES DEFAULT POSITION

Figure 4.C.3 Signal Conditioning Circuit Board

4.D STEPPER SUPPORT UNIT, SSU-60

The SSU-60 Stepper Support Unit is an electronics unit consisting of a number of modules which are connected by a backplane motherboard within the SSU-60. All external connections are made at the rear panel of the unit.

Figure 4.D.1 shows an SSU-60 carrying the modules required for use with the SF-61DX2 system. Blank panels are used where modules are not required. It should be understood that module positions are fixed by the backplane motherboard connectors.

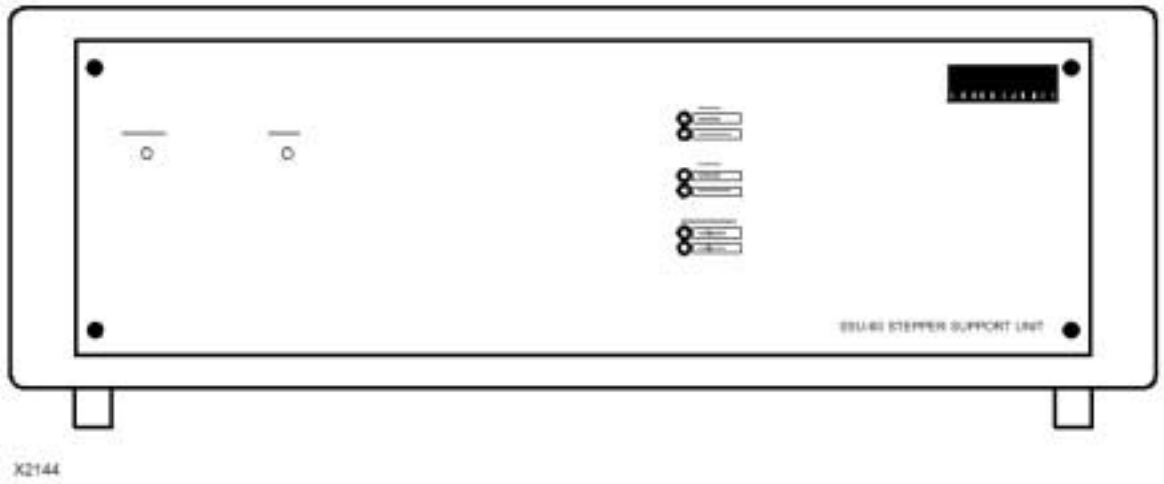


Figure 4.D.1 Stepper support unit – Front View

Figure 4.D.2 shows the SSU-60 rear panel. Note that mains power is connected through the IEC fuse/filter/switch unit; this provides central control to power all internal units as well as the SHU-61DX2 sample handling unit.

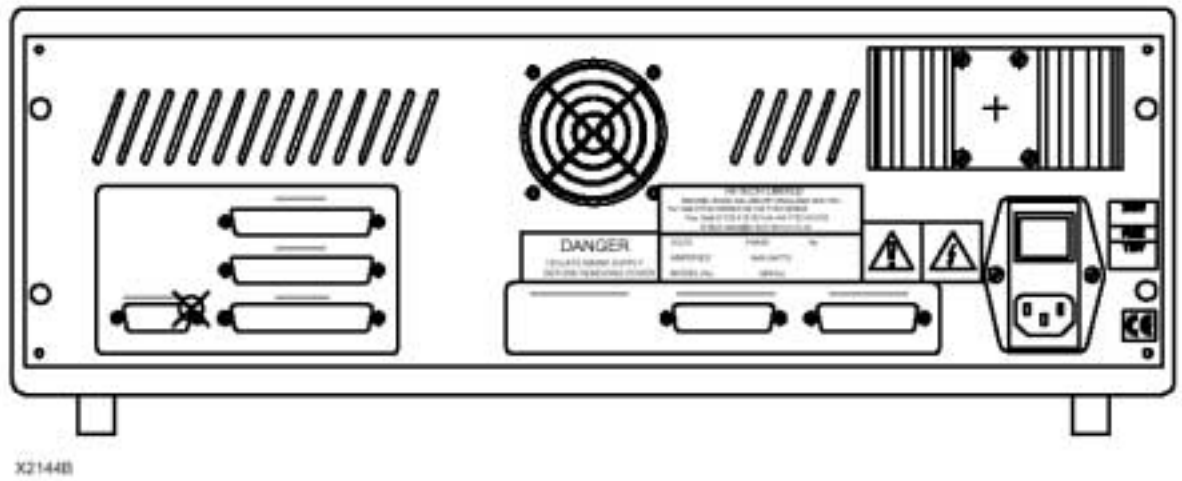


Figure 4.D.2 Stepper Support Unit – Rear View

The mains voltage is factory set to either 230 V~ or 110 V~ depending on the country of destination; the fuse ratings are as follows:

110 V~	5A Anti-surge (T)
230 V~	5A Anti-surge (T)

Under NO circumstances should alternative ratings be substituted!

The (continuous) current consumption of the SSU-60 is 0.5 A at 230 V~.

D.1 SUPPORT POWER SUPPLY, SPS-60

This module provides the dc voltage supply for the other modules within the SSU-60 Stepper Support Unit. It has a front panel LED power on indicator.

4.D.1.a SPECIFICATION

Output;	24 VDC @ 6 A
Power requirement;	110/230 V~ link selectable on the backplane of motherboard (factory set).

4.D.2 SAMPLE HANDLING UNIT POWER SUPPLY, SHU PS-60

This module provides the dc voltage requirement for the SHU-61DX2 sample handling unit. It has a front panel LED power on indicator.

4.D.2.a SPECIFICATION

Output;	±15 VDC @ 1.1 A
Power requirement;	110/230 V~ link selectable on the backplane of motherboard (factory set).

4.D.3 DRIVE AMPLIFIER, DA-60

These units provide the drive requirement for all the stepper motors used within the SF-61DX2 system. They control the stepper motor on each drive of the SHU-61DX2 sample handling unit and also the stepper motor within the monochromator that sets wavelength.

Note that the drive amplifier units are configured on the backplane motherboard for the specific stepper motor being used and therefore the units themselves are interchangeable. From left to right (viewed facing the unit), the drive amplifiers control Drive1, Drive2, and the monochromator, respectively.

4.D.3.a SPECIFICATION

Type;	4-phase Unipolar drive card
Input;	24 VDC @ 4 A
Output;	12 VDC @ 50 mA
	Max. current per phase is 2 A

4.D.4 STEPPER (MOTOR) CONTROL UNIT, SMC-60

The stepper control unit sets the controls for the drive amplifier units from the signals received from the BD-60, Bus Decode Unit. It selects the particular stepper motor(s) to be activated within the SF-61DX2 system and sets the direction and the distance to be moved, as well as the speed of the stepper motor.

On the front panel are six LED indicators which show the status of the various limit (micro)switches used within the sample handling unit and the monochromator.

The module also incorporates the stepper motor disable circuitry, so that in the event of a limit (micro)switch being activated (eg after resetting the drives on the SHU-61DX2) the related stepper motor is inhibited from moving any further in that direction and the relevant LED indicator on the SMC-60 will illuminate.

4.D.4.a SPECIFICATION

Input;	+12 VDC @ 1 A
Output;	0-12 V digital CMOS signals

4.D.5 BUS DECODE UNIT, BD-60

The bus decode unit interfaces between the CU-61 control unit and the sample handling unit, the filter wheel assembly and the scanning monochromator, decoding the computer-generated data received from the CU-61.

The bus decode unit also contains the circuitry for setting the sensitivity of the opto-sensor which is used to calibrate wavelength position within the monochromator.

4.D.5.a SPECIFICATION

Input;	+12 VDC @ 1 A
Output;	0-5 V CMOS digital signal

4.D.6 SHU CONTROL UNIT

The SHU control board provides the control for the sample handling unit functions, incorporating the auto emptying waste cycle and air drive control circuitry. The valve safety sensors are routed to the control board, where they are used to prevent incorrect activation of the air drives. The unit also, in response to the stop syringe (potentiometer) movement, generates flowrate and agetime measurement markers and the data capture trigger signal (- refer to Section 4.B.6 SHU-61DX2 Electronics). There is an AIRON DELAY facility whereby, in single mixing mode, the air pressure to drive 2 may be maintained for a prescribed time – currently set to 1 second - after flow has stopped. The same switch is used to disable a drive sequence check in double mixing mode, which may be a useful facility when the drive volumes are non-standard or very fast agetimes are required.

The circuit board has a group of 8 DIP switches and 3 jumper link settings, as follows:

SW1	Switch 1	OFF - fixed SX mode	ON* - software set SX/DX mode
	Switch 2	OFF* – normal	ON – Air On Delay (SX) /Disable Sequence Check (DX)
	Switch 3	OFF – valve A sensor disabled	ON* – valve A sensor enabled
	Switch 4	OFF – valve B sensor disabled	ON* – valve B sensor enabled
	Switch 5	OFF – valve C sensor disabled	ON* – valve C sensor enabled
	Switch 6	OFF – valve D sensor disabled	ON* – valve D sensor enabled
	Switch 7	OFF – valve S/W sensor disabled	ON* – valve S/W sensor enabled
	Switch 8	unused	

Links	LK1	Temperature	1-2 used for testing	2-3* – normal use
	LK2	Marker	1-2 used for testing	2-3* – normal use
	LK3	Trigger	1-2* positive 10ms pulse	2-3 – negative 10ms pulse

- denotes default position.

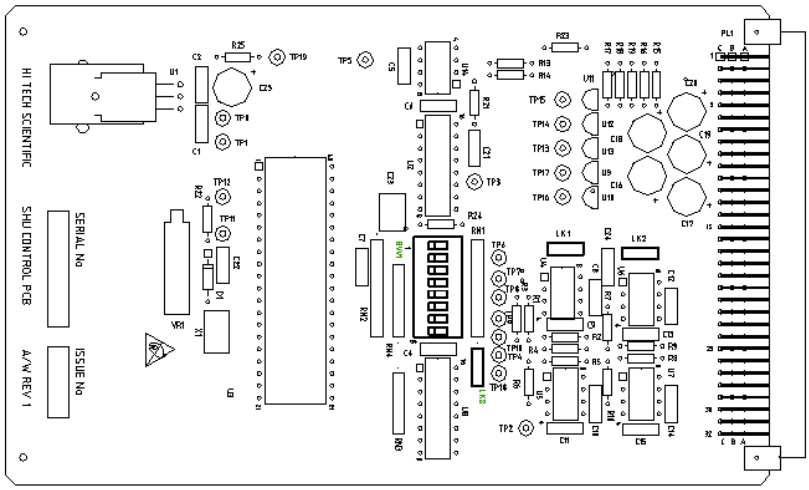


Figure 4.D.6 SHU Control Unit Board

4.D.6.a SPECIFICATION

Input;	+12 VDC @ 1 A
Output;	0-5 V CMOS digital signals 12VDC signals
Trigger signal;	0-5 V buffered TTL (standard setting) User selectable to 5-0 V

4.E OPTICS

The optics comprises both excitation and detection components. The excitation components are mounted on a precision rail, with their output coupled to the observation cell by a fibre optic; the detection components are mounted on the observation cell housing. The standard system is supplied with a single lamp housing - the lamp to be used is mounted on its backplate assembly and fitted into the housing.

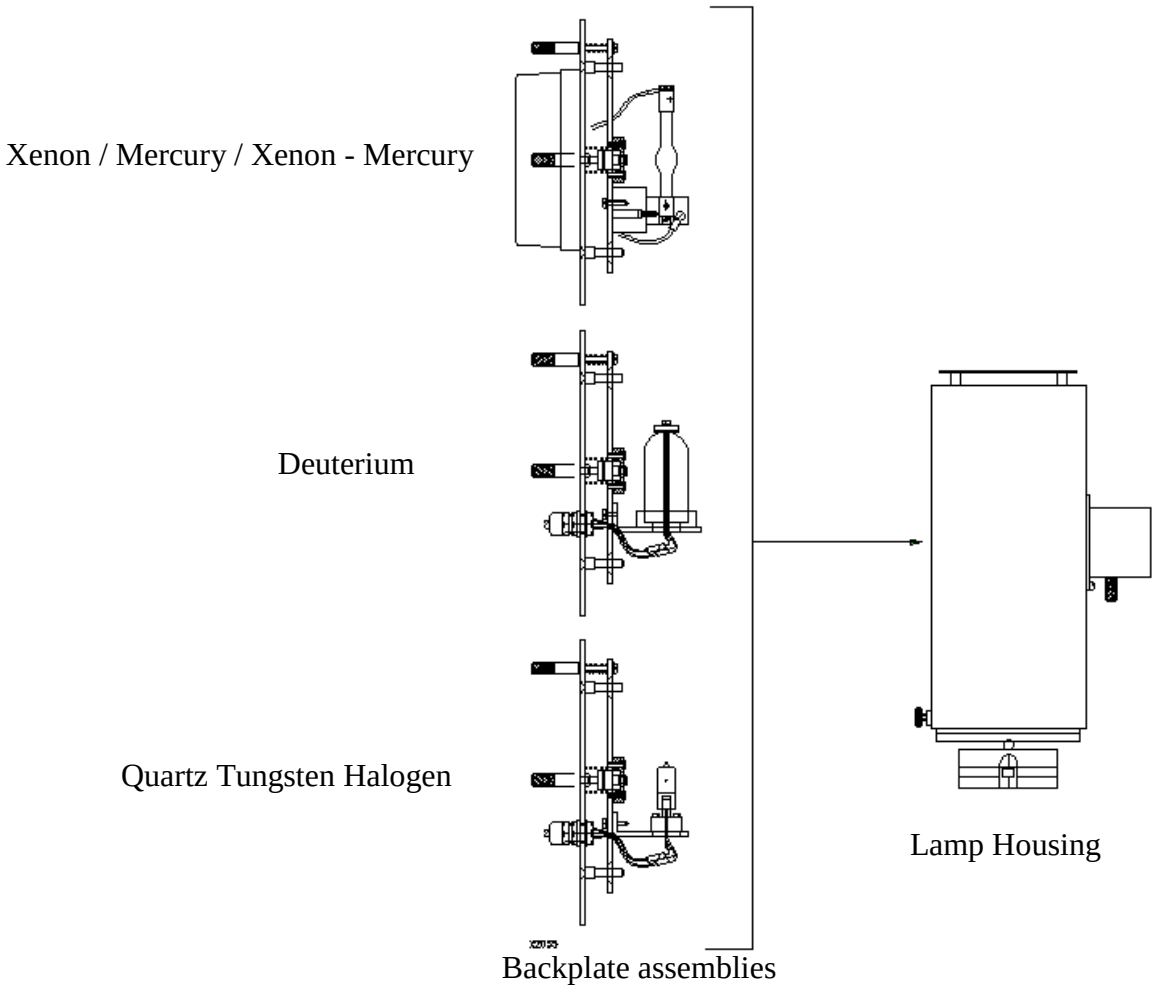


Figure 4.E.1 Lamp Arrangement

The housing is attached to a carrier to enable rail mounting using an adjustable sub-base that permits alignment of the lamp optical axis to the monochromator optical axis. By slackening the locking screw located below the focusing lens assembly, at the bottom of the lamp housing, and then turning the nearside adjuster screw (- refer to Figure 2.E.1), the lamp can be moved laterally across the rail. The correct lateral position of the lamp to ensure proper alignment with the monochromator is, however, factory set and any lateral adjustment should not generally be necessary after installation.

By sliding the lamp housing along the rail, the optimal separation between the focusing lens and the monochromator entrance slit can be set. The position of the collecting lens can be adjusted by unlocking the knurled screw on the underside of the focusing column.

To remove and replace a lamp backplate assembly, remove the four socket cap head screws at the corners of the backplate and carefully pull the assembly clear. Fit the replacement lamp backplate assembly and secure with the mounting screws.

Should the user have to change a lamp or tube then only realignment of the new device with respect to the optical axes within the housing should be necessary. Two finger screws on the backplate *finely* tune the lamp position with respect to the focusing lens located in the column at the front of the lamp housing and hence to this optical axis.

Figure 4.E.1 shows the natural convection-cooled lamp housing and the various backplate assemblies.

4.E.1 ARC LAMP, LS-678

The arc lamp can be either a 75 W xenon super quiet arc lamp, a 75 W xenon/mercury arc lamp or a 100 W mercury short arc lamp. The lamp backplate assembly incorporates the ignitor unit that connects to the PS-678 lamp power supply through a cable. The lamp is mounted by the lowermost ferrule in a socket and retained by two set screws 90 ^{o} apart. The upper lamp ferrule is connected to a terminal lug on a free wire. The mounting socket is secured to a plate that has two adjustable screws - the finger screws mentioned above - used to align the centre of the arc with the optical axis.

The xenon and xenon/mercury lamps are fitted with the anode (+) uppermost connected to the ***Red (+)*** wire lug secured with the terminal nut. The cathode (-), with its terminal nut discarded, is connected directly into the mounting socket, where a spacer ring is also fitted flush with the bottom of the socket to ensure the correct height of the lamp. The ***Black (-)*** wire lug is connected to the mounting block.

Before fitting the mercury lamp, remove the spacer ring from the mounting socket and fit the mercury lamp with the anode (+) placed directly into the socket so that the ferrule is flush with the bottom face of the socket. The two lower set screws are tightened to secure the lamp, and the ***Red (+)*** wire lug is connected to the mounting socket. Fit the supplied clip to the cathode (-) ferrule and attach the ***Black (-)*** wire lug to this clip. If the wires are not long enough, re-route them through the opposite holes in the internal plate.

Figure 4.E.2 shows the mounting arrangement for the xenon and mercury arc lamps.

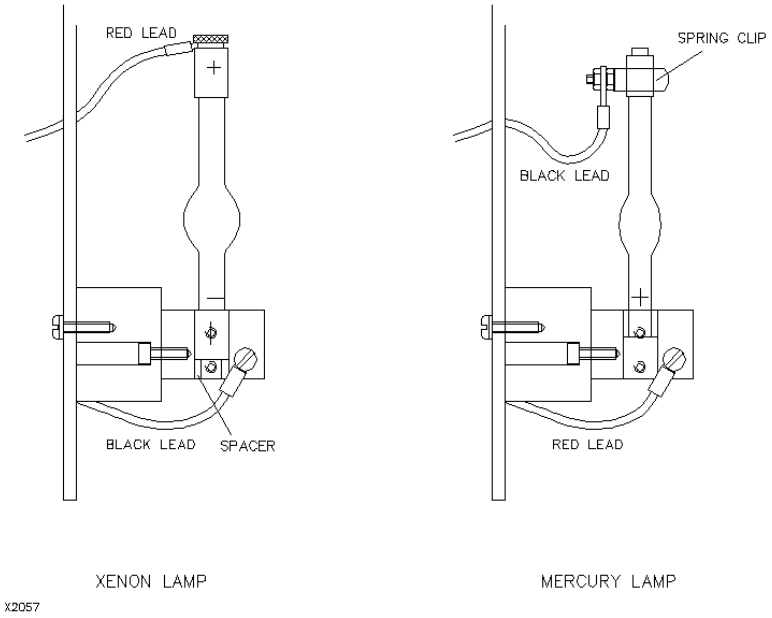


Figure 4.E.2 Xenon and Mercury Lamp Mountings

Detailed manufacturers' specifications for the arc lamps are found in Appendix I.

4.E.1.a SPECIFICATION

Lamp type(s);	75 W super quiet Xenon, Hamamatsu L2194-02 75 W Xenon/Mercury, Hamamatsu L2481-02 100 W short arc Mercury, Wotan HBO 100W/2 (see Appendix I)

The user may substitute their own lamps, but should be aware of the 100 W maximum dissipation rating of the lamp housing and the power supply restrictions. Use only ozone-free lamps unless an extraction system is provided.

Electrical connections;	Built in cable with a 4-pin lemo plug fitted.
Output optics;	Adjustable collecting silica doublet, fixed silica focusing lens providing F/4 aperture focusing at 100 mm approx.

Beware of the explosive potential of arc lamps. Handle the lamp by the ferrules and *only* when cool. *Never* touch the quartz bulb or handle the lamp when hot. Wear facial protection and gloves when handling arc lamps.

Beware of UV radiation. Do *not* look directly at the light output or its reflection. Wear UV blocking eye protection when working with the lamp.

4.E.2 VISIBLE LAMP, LS-12

This lamp backplate assembly is designed to operate with a 50 W quartz tungsten halogen lamp. A visible lamp is fitted by firmly inserting the pins (either way round) into the holes in the ceramic socket until completely home. Take care not to touch the silica bulb. The assembly is fitted with the cable connecting to the PS-678 lamp power supply fed through a grommet entry to the lamp socket. The socket and hence the lamp is carried by a plate which has two adjustable finger screws to align the lamp filament with the optical axis. The UV output of the lamp can be boosted using the VISIBLE + facility by adjusting the variable resistor accessible through the front panel.

4.E.2.a SPECIFICATION

Lamp type;	12 V, 50 W Quartz tungsten halogen (Thorn Type M32)
Electrical connections;	Built-in cable with 4-pin lemo plug connector
Output optics;	Adjustable collecting silica doublet, fixed silica focusing lens providing an F/4 aperture focusing at 100 mm approx.

4.E.3 ULTRAVIOLET LAMP, LS-22

This lamp backplate assembly is designed to operate with a 20 W deuterium lamp. The assembly is fitted with the cable to the Cathodeon power supply fed through a grommet entry. The deuterium lamp is inserted into the PTFE (Teflon) socket on the backplate, ensuring that the window within the lamp is facing forwards. The lamp fitting is completed by carefully tightening the two nuts that bring the clamp plate down onto the top of the lamp bulb. Be careful not to overtighten these nuts nor to touch the silica bulb. The lamp pigtails can now be connected to the bullet crimp connectors as shown in the following table:

Function	Lamp pigtails	Connector to Power Supply
Anode	Red	Red/Pin 1
Cathode/ Heater Rtn (most –ve)	Black	Yellow/Pin 2
Heater	Blue	Blue/Pin 3

The lamp socket is carried by a plate which has two adjustable finger screws used to align the lamp output with the optical axis. The lamp is controlled and powered from a dedicated, stand-alone power supply unit (Cathodeon).

Beware of UV radiation. Do *not* look directly at the light output or its reflection. Wear UV blocking eye protection when working with the lamp.

4.E.3.a SPECIFICATION

Lamp type;	20 W Deuterium Cathodeon J01
Electrical connection;	Built-in cable with 4-pin XCONN 4 MX plug connector
Output optics;	Adjustable collecting silica doublet, fixed silica focusing lens providing an F/4 aperture focusing at 100 mm approx.

4.E.4 MONOCHROMATOR, MG-60

An F/4 Czerny-Turner monochromator is attached to a carrier to enable optical rail mounting. It is secured with dowelled bolts that ensure a set, physical relationship between the optical axis and the optical rail. There are no adjustments to the monochromator mounting available; all adjustments to enable alignment of the lamp to the monochromator are made to the lamp housing or lamp backplate assembly. Only movement of the monochromator along the optical rail is possible, although for correct alignment, it is necessary to ensure that the near side of the monochromator is pushed back onto the optical rail before the clamping mechanism is tightened. The suppliers' manual is included in Appendix III.

4.E.5 FILTER WHEEL ASSEMBLY

The filter wheel is attached to the exit port of the monochromator and comprises a wheel with 6 ‘filter’ positions. It is computer controlled by the KinetAsyst software, although there is a manual override control at the far side of the assembly which, when pressed, steps the wheel to the next position. There is a circular viewing window allowing the user to observe the current filter position.

The wheel positions are as follows:

Position 0 (HOME)	Closed
Position 1	Open
Position 2	BG24
Position 3	WG320
Position 4	HY 1, Holmium Oxide
Position 5	GG495

The filter characteristics are shown in Figure 4.E.9.

4.E.6 FIBRE OPTIC, LG-60

This is a multi-mode single silica fibre terminated with SMA connections in a rugged jacket.

4.E.6.a SPECIFICATION

Fibre:

Type;	550 micron pure silica
Aperture;	0.22 NA
Attenuation;	<12 dB/km (820 nm)

Packaging:

Sheathing;	PVC jacket
Connection;	SMA 905 style connection
Centration;	± 0.1 mm

4.E.7 BEAM SPLITTER

The beam splitter divides the incident light before it reaches the observation cell, so that approximately 80 % converges onto the cell, whilst the remaining 20 % is diverted at a right angle to the side coupler. A PM-61s photomultipler is attached directly to this side coupler for reference channel detection. By splitting off a small proportion of the incident light, variations and fluctuations in lamp intensity are monitored and then compensated for by the use of a ratiometric conversion within the KinetAsyst data acquisition.

When fitting the beam splitter to the observation cell assembly, ensure correct alignment with the detection side of the observation cell - a service procedure detailing how to align the beam splitter is available on request. The angle of the splitter plate within the beam splitter assembly is factory set and should only be adjusted with instruction from Hi-Tech.

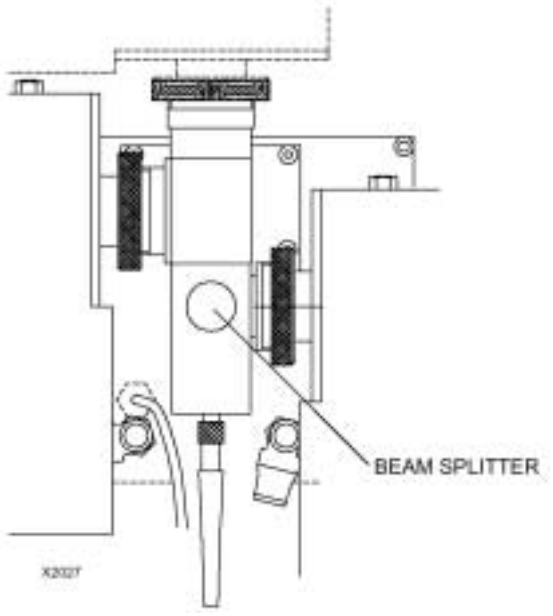


Figure 4.E.7 Beam Splitter

4.E.8 PHOTOMULTIPLIER, SIDE ON, PM-61s

The PM-61s photomultiplier assembly consists of a side-on photomultiplier tube encased in a mu-metal shield, mounted on a socket with an integral dynode chain. It has an integral shutter and is packaged in an aluminium alloy housing, with a single brass screw ring for attachment to the cell block. A pre-amplifier circuit board, including a trans-impedance amplifier, is contained within the housing, connected directly to the socket assembly.

An optical coupling with a single screw ring provides physical mounting of the photomultiplier to the cell block. This coupling is designed to accommodate the F-675 filter set or any other 25.4 mm (one inch) filter. A vacuum pencil is supplied with the system to facilitate removal of the filter after use.

A lever operates the integral shutter and a spring hook is supplied to enable easy access to this lever. When this shutter lever is up, the shutter is open; conversely when the lever is down, the shutter is closed. Note that it is advisable to close the shutter *before* removing a photomultiplier.

A power supply module is attached to the housing and provides the high voltage supply for the photomultiplier dynode chain that is controlled from the front panel of the CU-61.

All electrical connections are made using lemo sockets; note that these are identified by red and blue coloured bands to correspond to their mating connectors.

Detailed manufacturers' specifications for the photomultiplier tube, the dynode socket assembly and the mu-metal shield are to be found in Appendix II.

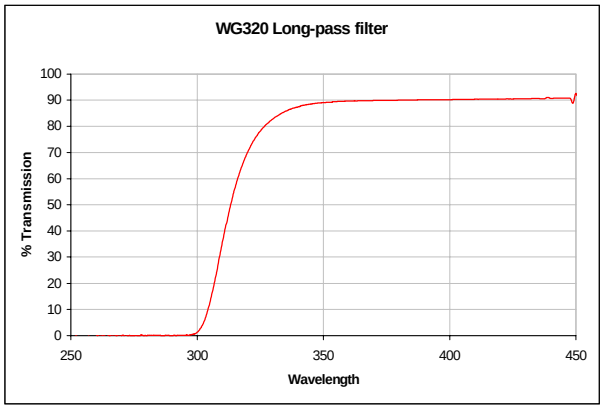
4.E.8.a SPECIFICATION

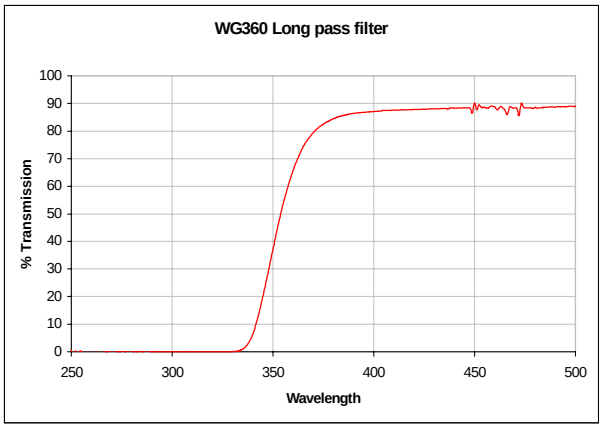
Tube type;	1-1/8" side on Hamamatsu R928 HA (see Appendix II)
Socket/Dynode;	Hamamatsu E 717-63 (see Appendix II)
Mu-metal shield;	Hamamatsu E989 (see Appendix II)
Pre-amplifier PCB	double ended output 0-10 VDC
Power supply module	
Input;	+12 VDC @ 400 mA
Output;	0 - 1000 VDC @ 2.5 mA
Connectors;	lemo, 6 pin (red), HV Control lemo, 5 pin (blue), Signal

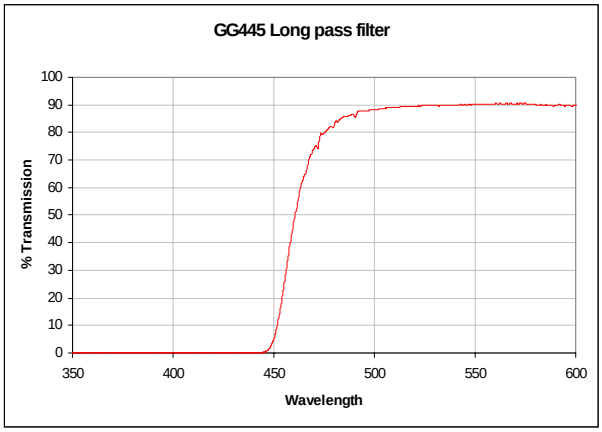
4.E.9 FILTER SET, F-675

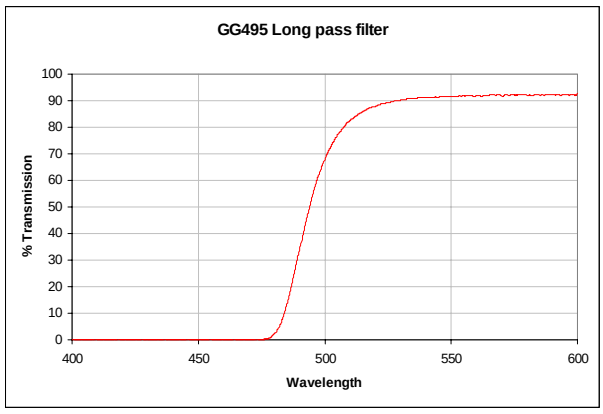
A selection of filters is provided to enable spectral separation of fluorescence emission from the excitation wavelengths.

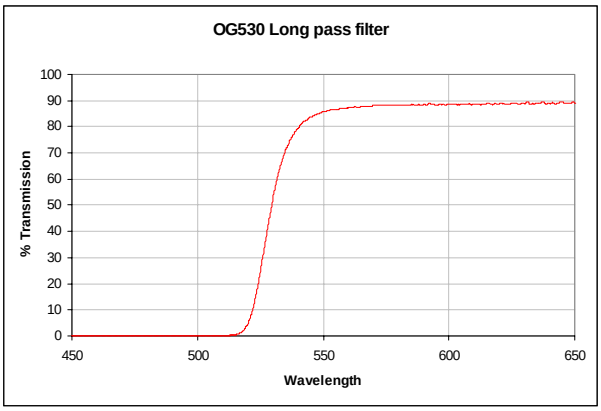
These are mounted in a 25.4 mm (1") diameter bezel that is engraved to identify the filter element. The filter set is presented in a box to ensure safe transit and storage. Keep the filters dust and grease free – use isopropanol as a solvent when cleaning. Handle the filters by the bezels. Figures 4.E.9 show the transmission characteristics of the filter set, also included are the characteristics of those filters used in the filter wheel unit.

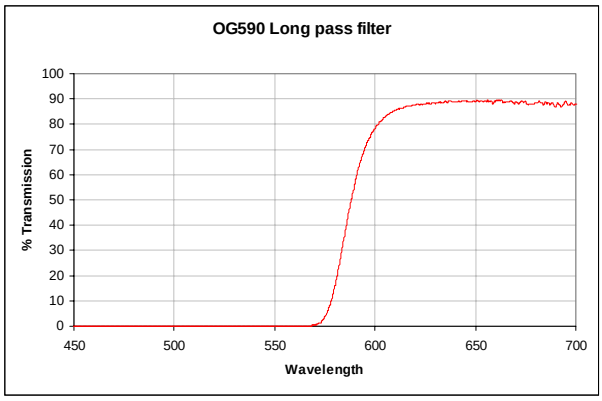


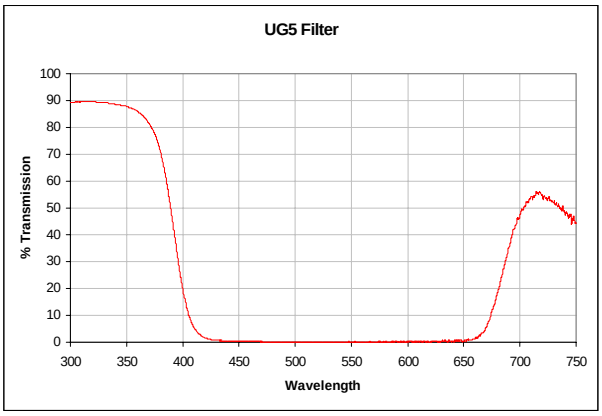


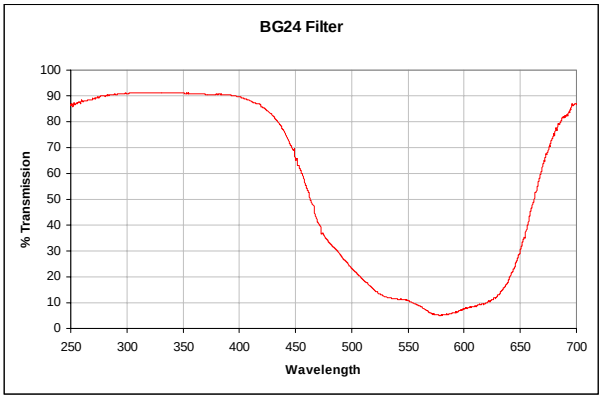












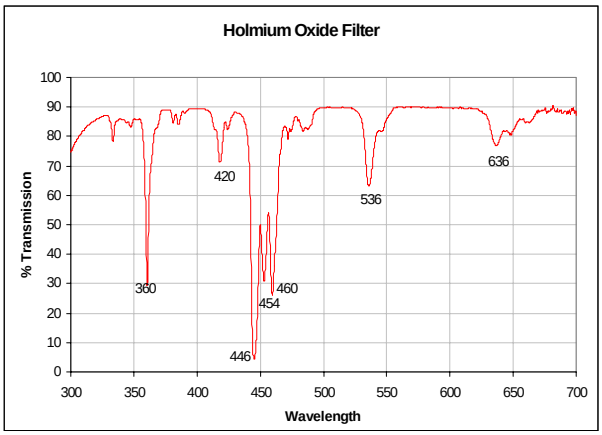


Figure 4.E.9 Filter Characteristics

4.E.10 FLUORESCENCE ENHANCEMENT KIT, FK-675

The FK-675 fluorescence enhancement kit comprises two UV enhanced reflective elements mounted on delrin plugs; one is fitted to the forward excitation window, the other to the rear emission window of the observation cell assembly. The plugs are retained within the window recesses by 'O' seals. The kit is supplied in a special box which is designed to protect the component parts in transit and when not in use.

When installing the two plugs, ensure that all the optical surfaces, including the observation cell windows in the sample handling unit, are clean and free from contamination.

Care should be taken when handling all optical components not to touch the optical surfaces and to use only lint-free lens tissue for cleaning; isopropanol can be used as the solvent for cleaning contaminated surfaces.

4.F.1 LAMP POWER SUPPLY, PS-678

The PS-678 lamp power supply is designed to operate the following types of lamp: the 75 W Xenon Lamp, the 75 W Xenon/Mercury Lamp, the 100 W Mercury Lamp and the 50 W Quartz Tungsten Halogen Lamp. There is a facility to overdrive a tungsten halogen lamp to increase its UV output.

Figure 4.F.1 shows the front view of the Power Supply.

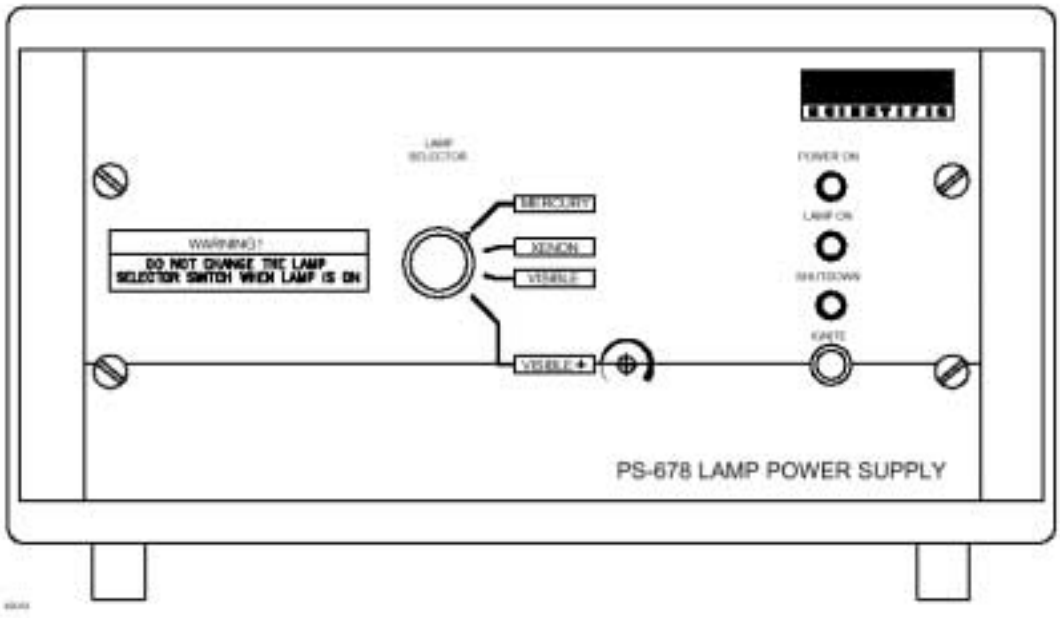


Figure 4.F.1 Lamp Power Supply – Front View

The LAMP SELECTOR control switch is used to configure the power supply for the lamp to be used and ***must be set prior to switching on the unit***.

In the MERCURY position, ***only*** a 100 W mercury lamp can be used.
In the XENON position***, only*** a 75 W xenon or 75 W xenon/mercury lamp can be used.

In the VISIBLE position, ***only*** a 50 W quartz tungsten halogen lamp can be used.

In the VISIBLE + position, ***only*** the 50 W tungsten halogen lamp can be used. In this position, the UV output of the lamp can be boosted by adjusting the variable resistor accessible through the front panel.

The POWER ON LED indicates that the unit is powered and the LAMP ON LED indicates that the lamp is illuminated. The SHUTDOWN LED will illuminate should there be a failure or problem with the power supply.

When pressed, the IGNITE switch will strike or start the lamp.

The lamp assembly is connected to the power supply through the 4-pin lemo socket at the rear of the unit. Figure 4.F.2 shows the rear panel. Note that mains power is connected through the IEC fuse/filter/switch unit.

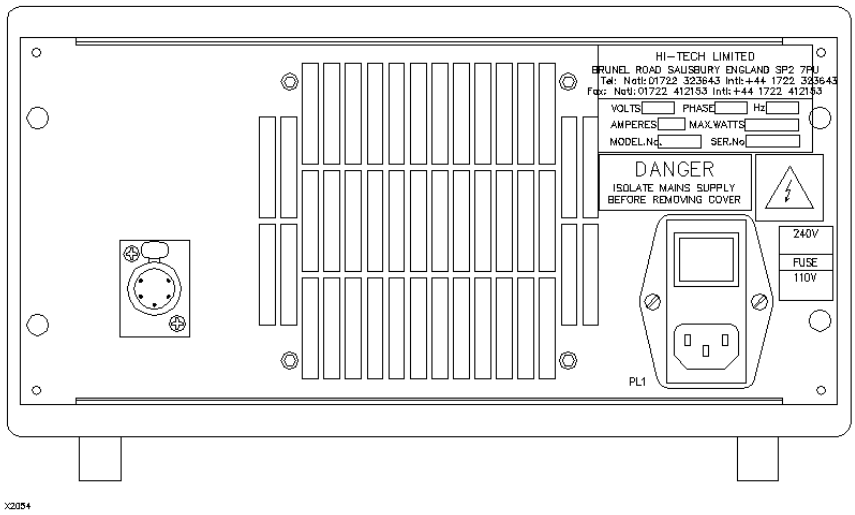


Figure 4.F.2 Lamp Power Supply – Rear View

The mains voltage is factory set to either 230 V~ or 110 V~ depending on the country of destination; the fuse ratings are as follows:

110 V~	2 A Anti-surge (T)
230 V~	2 A Anti-surge (T)

Under NO circumstances should alternative ratings be substituted!

The (continuous) current consumption of the PS-678 is 0.6 A at 230 V~.

4.F.1.a SPECIFICATION

Power Supply:	TX/2- 180 W max switch mode unit with a linear output stage
Output:	Selectable (depending on lamp type used)ghfgfh
Connector:	4 pin lemo

SECTION 5

GENERAL ASPECTS OF STOPPED-FLOW SPECTROPHOTOMETRY

5.A. INTRODUCTION

This section is written in particular for the inexperienced user as a general description and discussion of the stopped-flow technique. It sets out to briefly explain the general aspects of the method and provides more detail of some of the most important aspects and performance parameters.

Further reading on stopped-flow and other rapid reaction methods can be found in:

1. Eccleston, John F, Hutchinson, Jon P, and White, Howard D, (2000) – Protein-Ligand Interactions: Structure and Spectroscopy, Ch.5, Stopped-flow Techniques, Oxford University Press, ISBN 0199637474.
2. Gutfreund, H (1995) - Kinetics for the Life Sciences, Cambridge University Press, ISBN 0 521 48027 2 (hardback), ISBN 0 521 48586 X (paperback).
3. Hiromi, K (1979) - Kinetics of Fast Enzyme Reactions, John Wiley, New York.

5.B. PRINCIPLES OF OPERATION

The stopped-flow spectrophotometer is essentially a hybrid UV/VIS spectrophotometer designed to follow fast signal changes, coupled with a rapid mixing system - the sample handling unit.

The two or more reagents, held in the drive syringes of the sample handling unit, are rapidly driven into the flow circuit, displacing aged solution (from the previous run) as the reaction mix is driven into the observation cell. The linear flow rate of the solutions along with the geometry of the flow circuit effects rapid mixing, initiating the study reaction.

The SF-61DX2 Sample Handling Unit enables both the single mixing of two reagents, using only one of the drives, or the double mixing of three reagents by a push-push mode of operation. This latter mode of operation enables transient species formed by the mixing of two reagents to be subsequently mixed with a third reagent after a delay period.

Flow is stopped by a stop syringe, used to set the driven volume. The stop syringe plunger travel is restricted by a rigid stop block, causing rapid deceleration of the solutions and triggering of the data acquisition system.

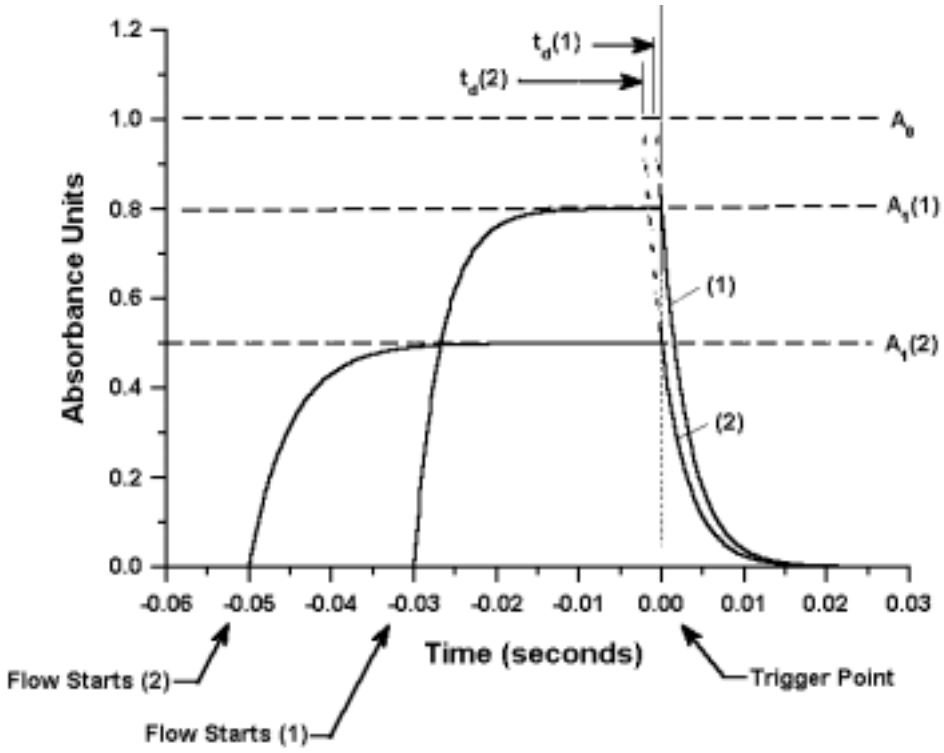
The resulting reaction is followed in the manner associated with classical stopped-flow - optical changes in both absorbance and fluorescence can be detected on both millisecond and second timescales.

5.C. FLOW RATE AND DEAD TIME

The following diagram illustrates the absorbance of the solution in the observation cell for the whole sequence of events of a stopped-flow shot, during which a coloured substance reacts to form a colourless one. Two traces are shown, trace 1 illustrating the use of a higher pneumatic drive pressure, and hence faster flow rate, than trace 2, while A_0 is the total absorbance change of the reaction, i.e. the absorbance observed if the decolourising reagent is absent from the second solution. Note that such traces may be produced in experiments if the pretrigger facility on the data acquisition system is used.

At first, the solution is that left over from a previous run; it is at equilibrium, having reacted completely, and is therefore colourless, with zero absorbance. The air drive is activated and the solutions start to flow. The flow rate rapidly increases to a constant value, dependent on the air pressure in the pneumatic drive and the resistance of the flow circuit. Since the solution takes a finite time to flow from the mixer to the observation point, the mixed solution is already of a certain age and hence has reacted to a certain extent; the age of the solution, and hence the extent of reaction, and hence the absorbance, A_1 or A_2 in the diagram, is constant - this is the period of continuous flow.

The flow stops when the piston of the stop syringe hits the stop block. Data collection is initiated and from then on the absorbance decreases as the solution ages until equilibrium is once more achieved.



The age of the solution at the instant of stopping is defined as the deadtime. To appreciate the effect of deadtime in stopped-flow measurements, consider the observation of a first-order reaction with a rate constant k, a total absorbance change A_{tot} and an observed absorbance change $A_{obs,}$ as seen by an instrument whose deadtime is t_d. These parameters are related by

$$kt_d = \ln(A_{tot}/A_{obs})$$

Let $t_{1/2}$ be the half-life of the reaction, then

$$t_{1/2} = (\ln 2)/k = 0.693/k$$

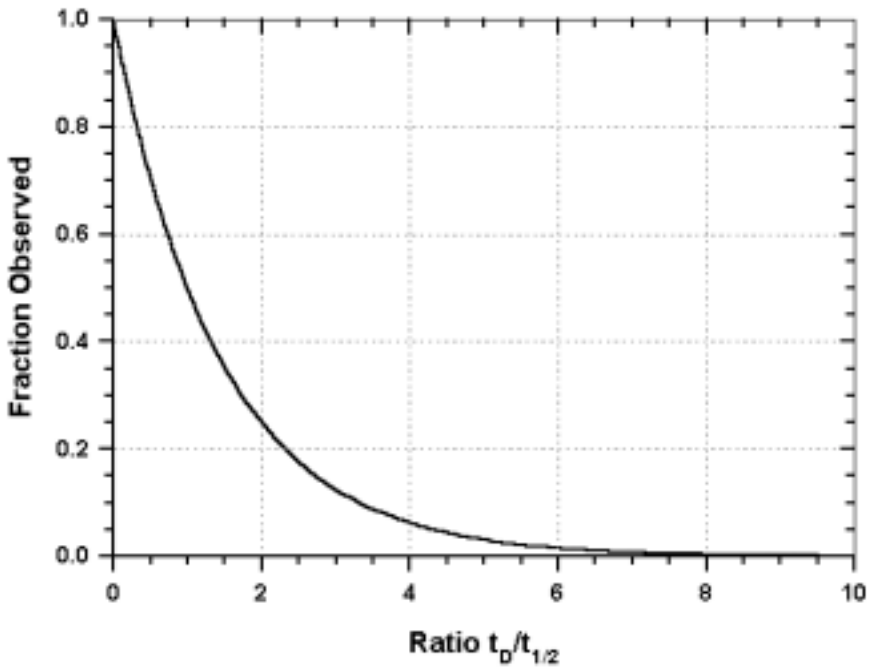
Expressing A_{obs}/A_{tot} as the observable fraction, f_{obs}

$$f_{obs} = (1/2)^{td/t1/2}$$

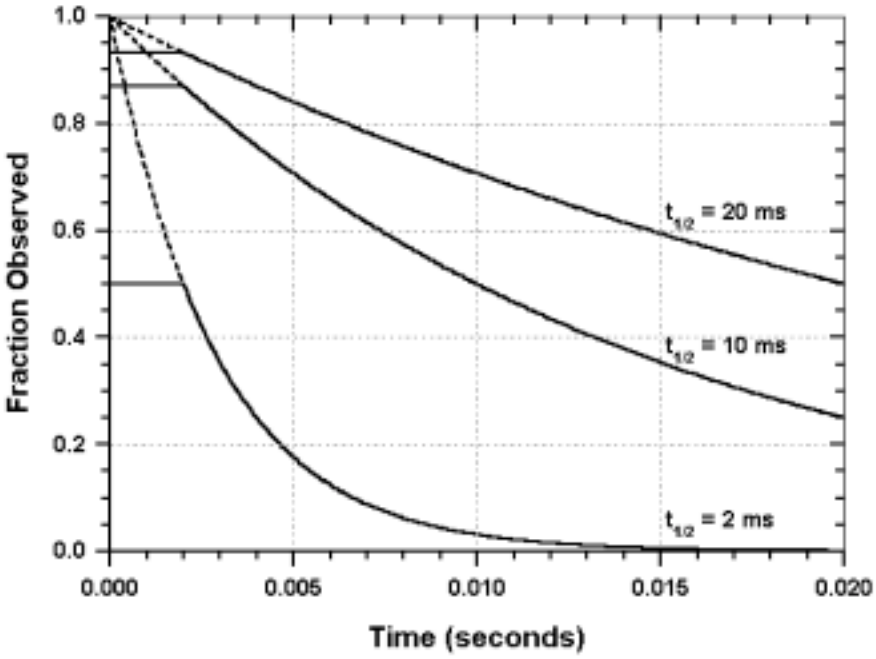
and so for

$$t_d = t_{1/2}$$
$$f_{obs} = 0.5$$

This relationship might be better appreciated by studying the graph below where the observable fraction, f_{obs} and the ratio of the instrument deadtime to the reaction half- life, $t_d/t_{1/2}$, is plotted:



To illustrate this effect on actual observed traces, the observation of three reactions of different rates on a stopped-flow instrument with a deadtime of 2 ms is shown below:



The deadtime may be measured by studying a first-order reaction in which the concentration of a coloured substance falls to zero after mixing with a decolourising solution. If the absorbance is A_1 during the period of continuous flow, and A_0 is the absorbance that is seen if the decolourising reagent is missing from the second solution, then

$$t_d = \ln(A_0/A_1)/k$$

where k is the first-order rate constant.

Suitable reagent solutions are:

Solution A:	12 mg dichloroindophenol ("DCIP") in 100 mL, approx. 2×10^{-4} M
Solution B:	0.02 M ascorbic acid, 0.02 M HCl
Solution C:	0.02 M HCl

On mixing solutions A and B, there is an instantaneous protonation of the DCIP to give a red solution, which is rapidly decolourised by the ascorbic acid. The reaction is followed at λ = 524 nm. The absorbance for zero reaction, A_0, is found by mixing solutions A and C.

The above equation may also be written

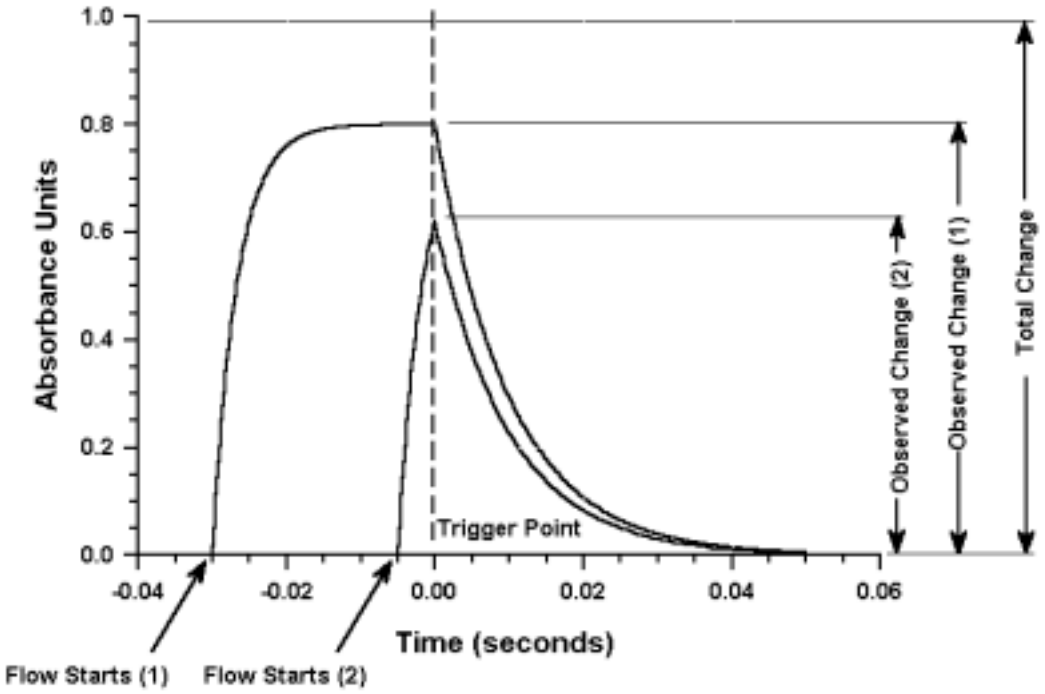
$$\ln(A_1) = \ln(A_0) - kt_d$$

If several solutions, B1, B2, ... of ascorbic acid of various concentrations are used to mix with solution A, the values of k and A_1 found may be substituted into the above equation to give a straight line of gradient - t_d.

5.C.1 EFFECT OF STOP VOLUME

It is necessary to introduce sufficient volume of mixed solution into the sample cell to achieve effective scavenging of old, aged solution as well as to allow sufficient displacement of the drive system to achieve maximum flow rate and hence youngest age of solution in the observation cell before stopping.

The effect of different stop volumes is best illustrated in the following diagram:



Trace 1 shows the pre-stopping absorbance reaching a maximum plateau; trace 2 shows stopping before reaching this same condition. Note how the trace 2 condition reduces the observed fraction of absorbance change, effectively increasing the deadtime.

The stop volume of course relates directly to sample economy since it is the volume (of both reagents together) used for each shot. By checking pretrigger data, it is possible to ensure that enough solution is being pushed, but also to avoid wasting solution (by pushing too much) which will be detected by an excessively long plateau of maximum observed absorbance.

5.D. MIXING TIME

It has so far been assumed that the solutions are thoroughly mixed in the mixer but this is never exactly true. The process of mixing in a stopped-flow instrument can be considered as occurring in two stages. The mixer takes in two liquids, solution A and solution B, and chops each up into small volume elements, so that a region of A is next to a region of B. Mixing is completed by the diffusion of the solute in A into the region of B, and vice versa. Some of this diffusion occurs in the liquid as it flows from mixer to observation point, in the dead volume. The more efficient the mixer, the finer is the subdivision into small volumes, and the more rapid the subsequent diffusion. Diffusion is slower in more viscous solvents, so mixing is slower. Chemical reaction can only occur after the second stage, when the reagents of A and B are in contact at the molecular level. This is why reaction traces for very fast reactions, at the limit of measurability, have a characteristic sigmoid shape. The reaction is going slowly over the first millisecond or so because the mixing is not complete. Hence a short dead time, as measured above, is not necessarily a good thing. The solution at the observation point may be young, not because it was transported rapidly from the mixer, but because it is still being mixed in the observation cell.

It is possible to test mixing efficiency by measuring the rate constants for reactions at limiting rates, but a sounder technique is to use reagents which react effectively instantaneously, giving solutions whose absorbance measures the extent of mixing. This can be done by using as solution A, a buffer solution containing an indicator of pK_a similar to the pH of the buffer. Over a limited range, the absorbance of such a solution is proportional to the amount of acid added as solution B. If no second-stage (diffusive) mixing has occurred, the absorbance is the same as if solution B were pure solvent, since no chemical reaction has occurred. If second-stage mixing has occurred in the dead volume, the absorbance is A_1. If the absorbance for perfectly mixed solutions is A, then the extent of second-stage mixing which has occurred in the dead volume is $(A_0 - A_1)/(A_0 - A)$. Second-stage mixing approximately follows first-order kinetics, and the rate constant k_{diff} gives another measure of mixing efficiency. With more effective first-stage mixing, the finer the subdivision and the greater is k_{diff}. We expect that values of $(A_0 - A_1)/(A_0 - A)$ approaching unity will correlate with high values of k_{diff}. It is therefore possible to produce a quantitative evaluation of a stopped-flow apparatus, and say something like:

> "For a solvent of viscosity of 10 cP, mixing is such that the rate of reaction at the stopping of the flow is 90 % of the true rate, and is 99 % of the true rate 5 milliseconds after".

A high value of $(A_0 - A_1)/(A_0 - A)$ does not in itself signify a desirable mixer, since a value approaching unit can also be found for flow systems with a large dead volume.

For these tests, a suitable solution A is 1 x 10^{-4} M 2,4-dinitrophenol and 0.1 M potassium hydrogen phthalate, and a suitable B is 0.02 M HCl. The absorbance at 360 nm gives the concentration of the anionic form of 2,4-dinitrophenol, which falls as solution B is added. A calibration graph is prepared by measuring the absorbance of solutions each with the same volume of solution A, but with differing volumes of solution B, the final solution being made up to twice the volume of solution A by adding solvent. If, say, the final solution was made from 5 ml of solution A, 3 ml of solution B and 2 ml of solvent, then it has the absorbance characteristic of 60 % mixing.

5.E. MAXIMUM MEASURABLE FIRST-ORDER RATE CONSTANT

It is not necessary to observe the whole course of a first-order reaction to measure its rate constant. If the deadtime is, say, equal to five half-lives, there is still 3 % of the reaction to go when the flow stops. If 100 % reaction gives a large change of absorbance, say one unit, then that final 3 % can still be observed accurately enough to give a good value for the rate constant. The limit is set by mixing. The instrument is designed so that the mixing time is less than the deadtime, but not much less. Experimentally observed rate constants in excess of 1000 s^{-1} are significantly less than expected by linear extrapolation, because mixing is incomplete on this time scale. The extent of the effect depends on the nature of the reaction and on solvent viscosity, and has to be studied for each particular system.

5.F. VIEWING ALONG THE DIRECTION OF FLOW

It has so far been assumed that one observes across the direction of flow, through the 1.5 mm path length. The solution observed is thus all of one age, since the width of the observation beam is only about 1 mm. However it is very common to use the 10 mm path, to increase the change in absorbance, and this requires observation along the path of the flowing liquid. Typically, the mixed solution flows at 10 ml/s, corresponding to a linear flow rate of 4.4 $mm.ms^{-1}$. The solution leaving the cell at the far end is thus 2.25 ms older than the solution which enters. Fortunately, for first-order kinetics, this does not matter. Since the half-life is independent of extent of reaction, the half-life and hence rate constant is uniform down the whole length of the cell. The deadtime is similar to that for solution half way down the cell, observed along the 1.5 mm path.

Because the above argument only applies to simple first-order kinetics, the 10 mm pathlength should not be used for very fast second-order reactions. In general, it is good policy to work under pseudo first-order conditions whenever possible.

5.G. OPTICAL BANDWIDTH

Other things being equal, the more intense the light falling on the detector, the better will be the signal to noise ratio. The signal is proportional to the light intensity, whereas the noise is proportional to the square root of the light intensity. A fourfold increase in light intensity thus doubles the signal to noise ratio. One way of increasing the light intensity is to widen the monochromator slit width. This lets more light through, but at a wider range of wavelengths. This means that the light may no longer be monochromatic enough for the sample to obey Beer's law ($A = \varepsilon cl$, where $A = \log_{10} I_0/I$). Beer's law, which states that the absorbance is proportional to the concentration, is only valid if the extinction coefficient is constant over the range of wavelengths of the incident light. Beer's law is fairly forgiving, especially if the total absorbance is low, or if the absorbance change during the reaction is small. In critical cases, it would be advisable to check by direct calibration, measuring the absorbances of a range of sample solutions, using the slit width in question.

The Beer's law limitation does not apply to fluorescence monitoring, where the more intense the light; the better the signal. The only danger of using a wide slit width is that some of the light incident on the sample may not be absorbed by the cut-off filter for the light monitored at 90 degrees, and will contribute to the background signal. The user should be aware of the relationship between the excitation and the emission spectra to appreciate this. Reference 1 in Section 5A explains this very well and goes beyond the scope of this document.

5.H. TIME CONSTANT (FILTER TIME)

Electronic noise is limited by the response time, or time constant, of the electronic circuitry, also known as the filter time. The noise is inversely proportional to the square root of the response time. Increasing the time constant by a hundredfold reduces the noise by tenfold. The time constants (filter times) are set automatically within the KinetAsyst program by default, although the user does have the option to change this AUTO condition and set the filter time to a selected value within the Hardware Configuration Setup. The drawback of using this method to improve the signal to noise ratio is that the electronics must still be fast enough to provide a faithful record of the changing signal as the reaction occurs. As a guide, the electronic time constant should not be set to a greater time than 10 % of the first half-life of the reaction. It does not matter too much if too low a value of the electronic time constant is selected - any good data collection program, such as KinetAsyst, filters out the noise by digital processing of the data.

The setting of the AUTO filter time (time constant) within KinetAsyst depends upon the rate of data acquisition, i.e.

$$\frac{\text{number of data points} \times \text{number of oversamples}}{\text{run time}}$$

Note that on longer run times, use of the AUTO setting may obscure any fast initial phase.

5.J. RUN TIME

The run or sweep time should be set so that the first half-life of the reaction is over in about 25 % of the acquisition period. It should be borne in mind that the determination of kinetic parameters by fitting routines requires that adequate data are collected to ensure good exponential definition as well as good endpoint definition.

A logarithmic time base is incorporated into the KinetAsyst program; this is particularly powerful in recording complex, multiple exponentials, especially where the user has a limited quantity of reagent available, since one can span milliseconds to minutes in only one or two runs. With a logarithmic distribution of data points with respect to time, one can achieve good definition of fast phases, but also achieve good definition of endpoints for slower phases in a single run.

For further reading on the use of logarithmic timebases the user is referred to:

> Warmsley, A. R., and Bagshaw, C. R. - "Logarithmic timebases for Stopped-Flow Data Acquisition and Analysis", Anal. Biochem. 176, 313-318 (1989).

5.K. FLUORESCENCE

Fluorescence intensity, unlike absorbance, is not an absolute quantity. Setting up is thus more flexible. However the fluorescence signal must not be so large as to go outside the voltage range of the A/D (analog-to-digital) converter, nor so small as to be subject to digitisation error.

Fluorescence intensity, unlike absorbance, is not an absolute quantity and the acquired fluorescence measurement is simply expressed as % response. Setting up is therefore more flexible in that it is not strictly necessary to acquire reference levels; simply optimising the fluorescent signal is often considered sufficient unless comparative studies are being undertaken.

If the fluorescence *increases* during the shot, the "100 %" level is set with the reacted solution at equilibrium in the cell.

If the fluorescence *decreases* during the shot, the levels must be set using a solution whose fluorescence is that of the freshly mixed solution mixture. Water or buffer, free from quenching agent is used as the solution to mix with the fluorescing solution, thus allowing for dilution.

If a familiar system is being studied, experience will give sensible values of wavelength and photomultiplier high voltage. Their exact values are not important.

SECTION 6

KINETASCAN DIODE ARRAY

6.A INTRODUCTION

The KinetaScan Rapid Scanning diode array system provides a high performance spectral scanning system that can be fitted into a Hi-Tech Scientific Rapid Kinetic Spectrophotometer system. In conjunction with KinetAsyst windows software, KinetaScan provides a facility to collect and display a sequence of spectrally resolved data that can be manipulated, analysed and stored; furthermore the data can be rotated to generate time-resolved kinetic traces.

The KinetaScan Rapid Scanning system comprises a KinetaScan unit, a cable assembly and an optical fibre and coupling.

The KinetaScan unit is a compact spectrometer containing a grating and a multi-channel detector, a MOS linear image sensor. The linear image sensor is a self-scanning photo diode array, the scanning circuit using N-channel MOS transistors.

6.B. SPECIFICATION

MOS linear Image Sensor	S3924-512Q Hamamatsu
Number of photo diodes	512
Photo Diode pitch and height	25 um x 2.5 mm
Pixel resolution	0.8 nm
Wavelength range	280 nm – 700 nm
DC Supply	+15 V @ 1 A, -15 V @ 1 A, +5 V @ 1 A

6.C. INSTALLATION

The typical benchtop arrangement is shown in Figure 6.C.

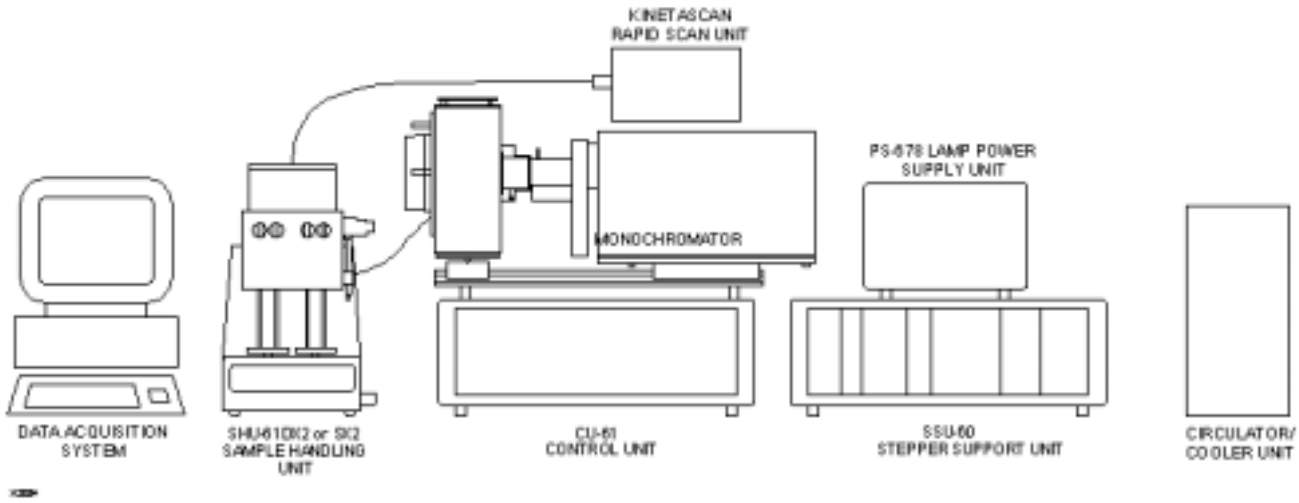


Figure 6.C.1 Typical Benchtop Layout

6.C.1.a ELECTRICAL CONNECTIONS

The CU-61 control unit provides the control and power supply requirements for the KinetaScan unit. The single electrical connection is made with the NA4891 cable from the 15-way D connector on the diode array housing, SK1 PDA SERVICES to a similar 15-way D connector on the rear panel of the CU-61, identified as SK6 PDA SERVICES. Normally this is connected during installation and remains connected during all modes of operation; should the unit be removed and/or reinstalled, remember to switch off the power to the CU-61 before disconnecting or reconnecting the cable.

6.C.1.b OPTICAL CONNECTIONS

As usual for absorbance work, the standard system fibre carries the incident light from the monochromator exit column to the lower port of the observation cell; the second fibre transmits the light from the upper port of the cell block to the diode array. These fibres are terminated with SMA connectors.

The SMA coupling for the second fibre needs to be fitted onto the upper observation cell port. The main photomultiplier and its optical coupling, if already fitted for absorbance work, will first need to be removed and in their place, the optical coupling for the fibre (to the diode array) is fitted. The fibre is then simply inserted between the connector and the diode array and tightened at each end by hand.

6.D OPERATION

The operation of the diode array involves much interaction with the KinetAsyst software; hence we refer the user to the software manual for “How to do …” certain operations.

The diode array can be operated in either SINGLE or DUAL BEAM mode, dependent on the setting of the toggle switch on the front panel of the CU-61. Often single beam mode is quite sufficient, but if dual beam mode operation is required, remember that the pin-hole stop must first be fitted into the reference channel filter holder. In dual beam mode, the reference channel level will be a constant across the wavelengths and should be set to about 80 % using the reference channel high voltage control knob on the front panel of the CU-61.

6.D.1 INTEGRATION TIME

The integration time is selected on the basis of the available signal, which is dependent on the light level at the sample cell. This light level is affected by factors such as the type of lamp being used, optimisation of the optical alignment, setting of the monochromator slits, age of the lamp etc. The user should consider that the minimum integration time allows operation at the maximum scan rates; as a guide, it is quite normal to be able to use the minimum integration time (1.5 ms) with the standard xenon lamp.

6.D.2 OPTIMISATION

In diode array mode, the monochromator is set to zero order and therefore acts simply as a white light reflector. The resolution of 0.8 nm per pixel (photodiode) is governed by the geometry of the diode array and the grating, and hence the monochromator slits can be opened as necessary. Remember that both entrance and exit slits should have equal settings.

It is advisable to start with the slits open generously. Adjust the collecting lens in the focusing column of the lamp housing, using the black knurled lever located on the underside of the column, until the lamp is imaging a quite sharp, intense spot at the entrance slit, approximately 4 mm in diameter.

The lamp spectrum visible in the live display window can be optimised for the wavelength region of interest by altering the separation between the lamp and monochromator; the lamp is moved nearer to the monochromator for optimisation of the shorter (UV) wavelengths, further away for optimisation of the longer (near IR) wavelength region. To ensure correct alignment of the lamp housing with the monochromator, remember to push the near side of the lamp carrier onto the optical rail while sliding the lamp along the rail and when tightening the clamping mechanism. For fine tuning only, the two finger screws on the lamp backplate adjust the rock and tilt of the lamp (see also Section 3.C.4, Optimising the Optics).

The monochromator slits can be used to stop down the light and/or the integration time can be reduced to prevent the signal from saturating. Conversely the signal can be increased by opening the slit widths and/or increasing the integration time.

Important: Data collection only starts when the signal from a photo diode is more than 5 % of that from the photodiode giving the maximum signal and therefore the wavelength range displayed on the scan blank reference will vary with the optimisation.

6.D.3 CALIBRATION

Spectral calibration is built in to the KinetAsyst set up routine and the user is generally referred to the software manual for help in this area of operation. In this way the wavelength to pixel relationship is established as a precursor to all operations.

SECTION 7

EXTENDED PUSHPLATE FOR HIGH RATIO MIXING

OPTION 669

7.A INTRODUCTION

This option provides for a simple modification to the SHU-61DX2, which takes advantage of the double mixing flow circuit but in a single drive action, thus introducing up to four reagents simultaneously into the mixing circuit. When this pushplate is used in conjunction with different size drive syringes, asymmetric mixing can be achieved in two stages, enabling high mixing ratios to be mixed in a manner which is particularly conducive to "difficult" mixing situations, eg protein folding, superoxide dismutation.

7.B INSTALLATION

It is necessary to remove both pushplates from the Drive 1 and Drive 2 air rams; two socket head set screws have to be loosened in each plate. One is found at the front and the other at the side; note that the plates are handed so as to position the side screws outermost.

To remove the drive syringes ..... drain the thermostat system, opening the bleed screw at the top of the thermostat enclosure to allow air to replace the liquid. Remove the buttons from each syringe rod by releasing the setscrews. Next release the knurled locking rings, which retain the syringes; put these to one side and pull down the syringes - it may be necessary to wriggle them slightly in order to break their adhesion to the bulkhead grommets. Carefully withdraw the syringes pulling them out past the grommets. The Teflon seal located at the end of each syringe should withdraw with its associated syringe. If it does not, then it is necessary to remove all four retaining rings and the plate which provides the threaded locations for the aforementioned rings (two screws - one at each end), and then prise out the appropriate grommet so that the seal can be fished out through the exposed hole.

When fitting the replacement syringes, it helps to put a slight smear of silicone grease on the outside of each barrel. Making sure that the seals are in place, offer the syringes through the grommets and locate them in the recesses in each DRIVE/FILL valve. Note that the standard syringes provided by Hi-Tech with this option are: 2.5 mL (2 pieces) and 0.5 mL (2 pieces) - it is strongly recommended that the 0.5 mL syringes are fitted into the A and C positions and the 2.5 mL syringes are fitted into the B and D positions. Replace the four retaining rings and fit the syringe buttons.

The pushplate is fitted to the Drive 2 drive ram; locate it so that the recess locates above the (unused) Drive 1 drive ram. Tighten each of the three socket set screws in the extended pushplate that attach it the Drive 2 ram.

7.C OPERATION

This option is used in a single mixing mode only, albeit with up to four reagents all driven together by the extended pushplate. With the additional syringe piston area introduced in this mode of operation, it is necessary to increase the air pressure to 5 - 6 bar.

With the syringe sizes and arrangement as already described it is possible to achieve a one part in twelve dilution by putting reagent in syringe A or C with buffer in the others. Note that by putting reagent in A an initial dilution is achieved at mixer 1 with solution from syringes B and D rapidly followed by a second dilution at mixer 2 with solution from syringe C. This arrangement is best for experiments where a longer dead time can be afforded in order to avoid mixing artefacts and achieve longer-term stability when observing reactions over several seconds. By introducing reagent at syringe C, its dilution is achieved at mixer 2 with buffer from syringes A, B and D. Here a shorter, closer to standard dead time is possible but without any protection from artefacts.

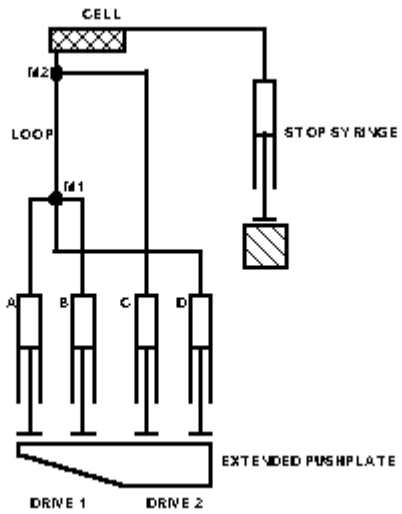


Figure 7.C.1 Extended Pushplate Schematic

SECTION 8

THERMOSTAT CIRCULATOR & COOLER

OPTION 655

8.A GENERAL DESCRIPTION

The thermostat circulator and cooler provide a means of maintaining temperature control of the reagents, by circulating thermostating fluid (eg water) throughout the flow circuit.

The system comprises of:

(i) Fluid circulator and cooler, consisting of a bath with a pump, digital temperature display and temperature controller.

(ii) Connecting tubing, adaptors and hose clamps.

The circulator and cooler are supplied by a third party; if supplied by Hi-Tech, the manufacturer's manual is enclosed in Appendix IV.

8.B INSTALLATION

The user is referred to the Thermostat Connections section (- 2.D) of the Installation chapter.

8.C SPECIFICATION

Liquid circulator: Neslab R*** (see Appendix IV)
(R*** - type depends on variant)

8.D OPERATION

The user is referred to the manufacturer's manual along with Section 2.D, Thermostat Connections.

SECTION 9

FLUORESCENCE DEPOLARISATION

OPTION 661

9.A GENERAL DESCRIPTION

A set of polarisation optics is supplied with this option, enabling the user to collect fluorescence emission signals that are polarised in the parallel and perpendicular planes from the sample cell, which is illuminated with light polarised in the parallel plane. This option necessitates that the system is operated in a T-format with dual channel detection.

9.B SPECIFICATION

Excitation polarizer:	Calcite prism (Glan-Foucault) with control bar for setting // or ⊥ plane excitation
Emission polarizers:	UV Dichroic sheet

9.C INSTALLATION

Figure 9.C.1 shows the rear view of the sample handling unit with the two photomultipliers mounted on the cell block in a T format for fluorescence detection. This arrangement does not use the reference channel and so the reference photomultiplier can be used for the second (Auxiliary) channel. The CU-61 is populated with this second channel, and hence no electronic reconfiguration is necessary, only a re-routing of the photomultiplier cable (NA 5880) from SK5 PM Services (R) to the PM SERVICES (A) connector, SK3 at the rear panel of the CU-61 is required. **Important:** Move the cable with power to the CU-61 switched off.

The two emission polarizers are marked // (parallel) and ⊥ (perpendicular); although these can be fitted to either fluorescence emission window of the cell block, it is necessary to allocate the parallel (//) detector to the Main Channel and the perpendicular (⊥) to the Auxiliary Channel, so that the calculation of anisotropy and polarisation is correctly executed in subsequent data processing. It is also convenient to orientate the emission polarizers so that the nomenclature can be read by looking down onto the instrument.

The excitation polarizer incorporates the optical fibre socket, and is best fitted to the top window of the cell block since access to the // / ⊥ control bar is required during set up. It is necessary to fit the flexible rubber cap supplied with this option to the SMA connector at the beam splitter and also to fit the blanking plug (supplied) to the beamsplitter spigot normally used for the reference photomultiplier.

In order to achieve sufficient intensity of illumination within the excitation beam, it is necessary to use the 75 W mercury-xenon or the 100 W mercury arc lamp.

9.D OPERATION

Set the CU-61 front panel switch to SINGLE BEAM mode - the reference channel will not be present and is not necessary for this mode of operation.

Insert the appropriate long pass filters for the fluorophore present in the reaction. Open the Acquire Control Panel, select dual channel operation by checking the Ch(annel) 2 box and enter the Spectrophotometer Setup. At the first Asystant, select Manual Setup and references. Click the NEXT button and set the excitation wavelength. Ensure that the excitation polarizer is set to // (parallel). With the shutters open, and the fluorescent sample in the cell, increase the high voltage (MAIN VOLTAGE) to the parallel (main) channel until a signal is seen on the live display; now optimise the optics - adjust the lamp position and focusing with respect to the entrance slit to the monochromatic (- refer to Section 3.C.4, Optimising the Optics). "Nudge" the wavelength a nanometer or so in either direction so as to maximise the signal by finding the excitation peak.

Set the signal level so that it is at about 90 % of full scale - close to the fixed reference level observed on the live display. Set the excitation polarizer to ⊥ (perpendicular), (the signal level on the main channel will drop), and increase the voltage to the (second) auxiliary channel so that the perpendicular channel is at the same level as the parallel channel, with an excitation source which is polarised in a perpendicular plane. Switch the excitation polarizer so that it is returned to the // (parallel) position and use the Read All to set all the reference levels - their values will appear in the dialog boxes. Before accepting a final reading for these, ensure that fresh sample is held in the cell - many fluorescent systems are subject to photobleaching that causes a reduction in fluorescence. The main channel will be set to a value close to full scale eg 90; the auxiliary channel will be lower. Since both channels have been "balanced" (the G-factor set to unity) by the setting of the perpendicular channel equal to the parallel with perpendicular excitation, the full scale of the auxiliary channel needs to be manually inserted to be the same as the main. Observe the number set in the 100 % dialog for the main channel and copy this into the 100 % dialog for the auxiliary channel, overwriting the number collected by the auto scanned reference. With this done accept the values set by clicking the FINISH button.

To collect data ..... Stopped flow runs are conducted in the same manner as in normal absorbance and fluorescence modes, except the Data Set up dialogues should be entered to set both channels to fluorescence with a modifier set to polarisation/anisotropy. After data collection, conversion of the data to anisotropy and polarisation is performed by selecting the Polarisation/Anisotropy function found under the Math menu. The data is converted according to the following formulae:

$$\textit{anisotropy, } r = \{(I_{//} / I_{\perp}) - 1\} / \{(I_{//} / I_{\perp}) + 2\}$$

$$\textit{polarisation, } p = \{(I_{//} / I_{\perp}) - 1\} / \{(I_{//} / I_{\perp}) + 1\}$$

$$\textit{total intensity } I_T = 2I_{//} + I_{\perp}$$

When the conversion has been executed, the data set is arranged thus:

Trace 1 - polarisation
Trace 2 - anisotropy
Trace 3 - total intensity
Trace 4 - parallel raw data
Trace 5 - perpendicular raw data.

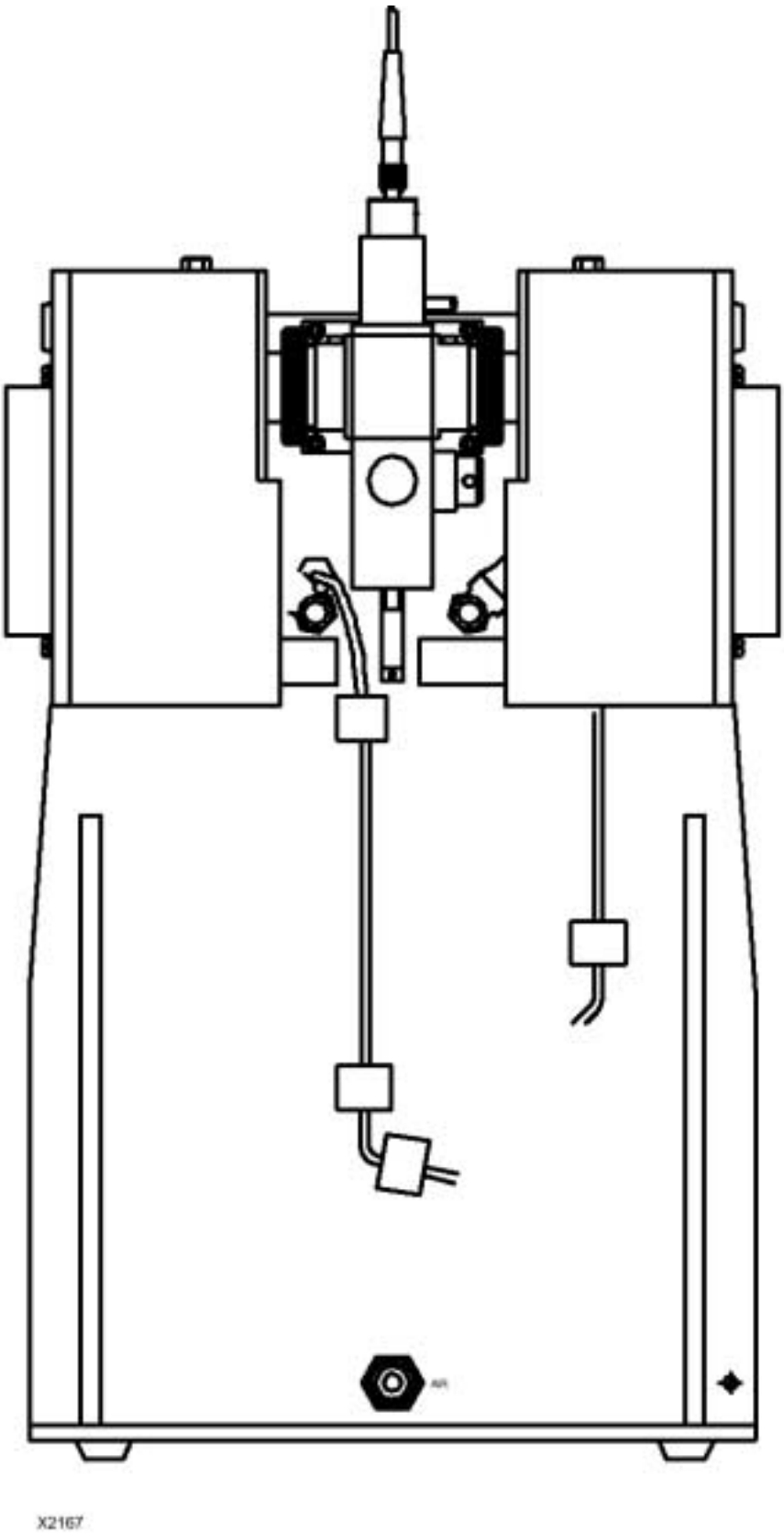


Figure 9.C.1 Sample Handling Unit Rear View

SECTION 10

FLUORESCENCE EMISSION MONOCHROMATOR

OPTION 670

10.A GENERAL DESCRIPTION

A second monochromator used as a manually controlled excitation unit is provided with this option. The standard monochromator supplied with the basic system is used to discriminate the emission signal, thus giving a higher signal resolution than the cut off filters used for broadband fluorescence intensity measurements. By using the features in the SF-61DX2 system and KinetAsyst software, the emission monochromator can be controlled in a manner such that three-dimensional time resolved emission scans are constructed in parascan mode.

10.B SPECIFICATION

Monochromator:	see specification in Section 4.E.4
Emission fibre:	3 mm fused silica bundle fibre with a transformer end

10.C INSTALLATION

Figure 10.C.1 shows a schematic of the optical arrangement incorporating the second monochromator. The benchtop arrangement for this setup is shown in Figure 10.C.2.

The excitation monochromator is located on the standard, long optical rail and the standard fibre connection made to the observation cell. The emission monochromator is rearranged so that the filter wheel/optical coupling is relocated to the entrance slit and is supported on the short optical rail which is conveniently sited on top of the excitation monochromator. The fibre bundle supplied with this option is used to couple a fluorescence emission window to the optical coupling at the entrance to the emission monochromator. The slit termination of this fibre should be aligned with the rectangular emission window of the cell - be careful, however, not to apply excessive load on the fused silica cell when tightening the fibre bundle into place. The photomultiplier detector is fitted to the exit slit of the emission monochromator by removing the filter holder, achieved by simply releasing the four pan head screws and by attaching the adapter plate also supplied with this kit.

The fluorescence enhancement kit, FK-675 should also be installed.

In order to achieve sufficient intensity of illumination within the excitation beam, it is necessary to use the 75 W mercury-xenon or the 100 W mercury arc lamp.

------ Standard system components
___ Fluorescence emission components

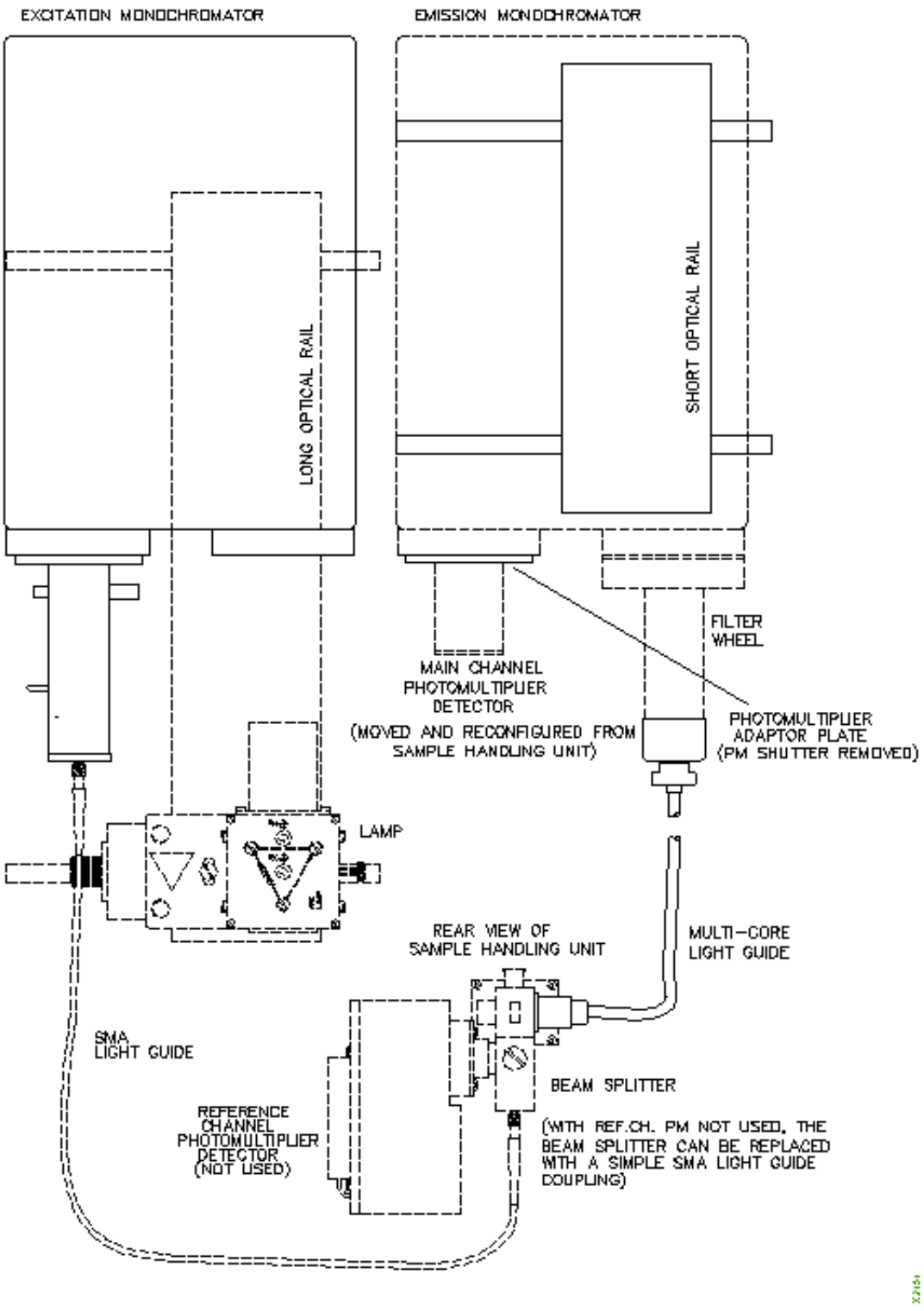


Figure 10.C.1 Fluorescence Emission Monochromator Optical Schematic

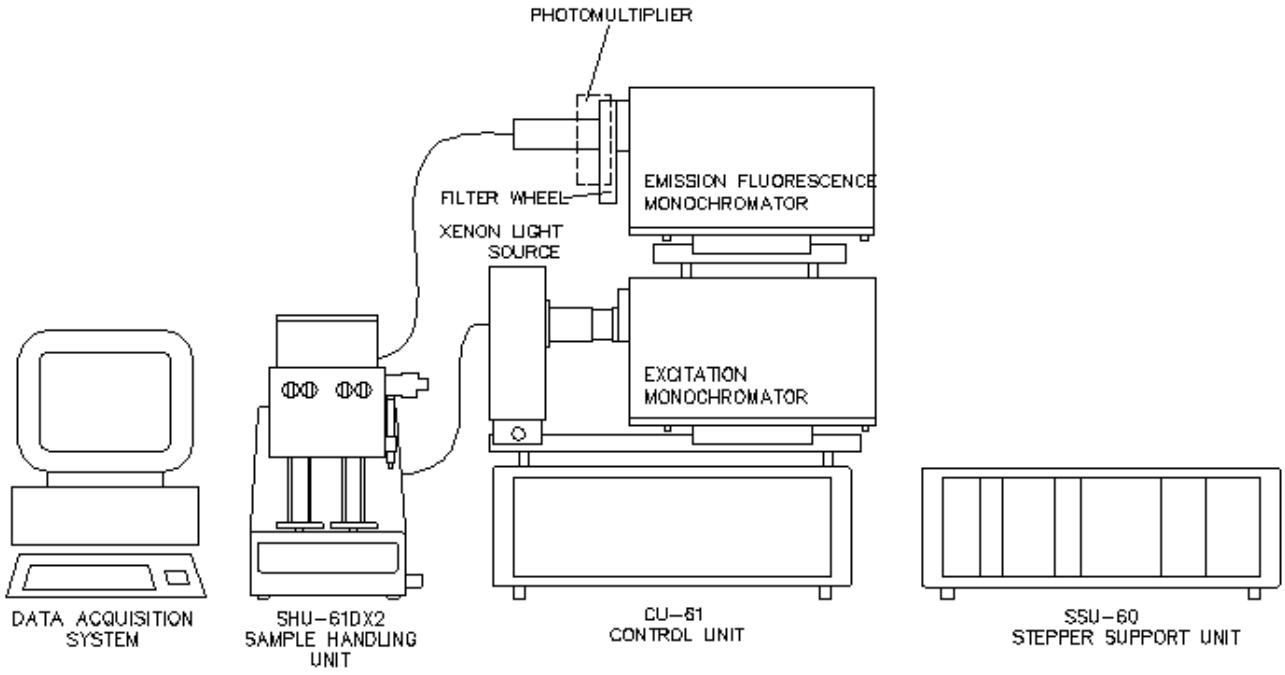


Figure 10.C.2 Fluorescence Emission Monochromator Benchtop Layout

10.D OPERATION

Set the CU-61 front panel switch to SINGLE BEAM mode - it is not appropriate to use the reference channel for this mode of operation.

If the fluorescence *increases* during the shot, the user should adjust the signal level (during setup) with the reacted solution at equilibrium in the cell; one or two dummy shots will fill the observation cell with mixed, fluorescent solution.

If the fluorescence *decreases* during the shot, the setup signal level must be adjusted using a solution free of the quenching agent; use water or buffer as the solution to mix with the fluorescent solution (thus allowing for dilution) and do a dummy shot or two so as to fill the observation cell with this unreacted, fluorescent solution.

Enter the Spectrometer Setup dialog from the Acquire Control Panel and select Manual Setup and references so as to set the emission detection wavelength (- normally this wavelength is set for maximum signal) and a suitable high voltage for the photomultiplier. Having set the emission detection wavelength, adjust the knob on the rear of the excitation monochromator to set the excitation wavelength by hand. With the monochromator slit widths open wide, the fluorescent sample in the optical cell and the excitation and emission wavelengths set, increase the high voltage to the photomultiplier until a signal is seen on the live display; now optimise the optics by adjusting the lamp position and focusing with respect to the entrance slit to the monochromator (- refer to Section 3.C.4, Optimising the Optics). Manually adjust the excitation wavelength a nanometer or so in either direction so as to maximise the signal by finding the excitation peak.

Increase the high voltage until either the maximum voltage of roughly 1000 V is reached or the signal is about 90 % of full scale, close to the fixed reference level observed on the live display. Upon completion, the user should select FINISH to exit the dialogue box - since a stored reference scan is now to be loaded, the user does not read (or set) a reference here.

To load the stored reference scan, select Fluorescence Scanning Mode from the drop down list under the Acquire menu; a stored reference scan, which sets the 100 % reference close to 90 % of full scale, is loaded into the next available buffer (or thumbnail). In selecting this mode, there is also a forced setting of unity gain for all the analogue channels and a reset of the filter wheel sequence, such that no filters are selected during this operation.

Within the Data Setup dialog set the data type for channel 1 to fluorescence. To collect data ..... Stopped flow runs are conducted in the same manner as in normal absorbance and fluorescence modes; the use of the Sequence Setup to acquire data over a range of wavelengths can be used to build a three dimensional data block of emission spectra with respect to time.

SECTION 11

DUAL CHANNEL FLUORESCENCE DETECTION

OPTION 687

11.A GENERAL DESCRIPTION

This option provides the facilities to arrange the photomultiplier detectors so that two fluorescence signals can be monitored. In this arrangement, another auxiliary photomultiplier (or indeed the reference channel detector, supplied as a standard part of the SF-61DX2 system) is used as a second sample detector; the two photomultipliers are attached to the fluorescence windows of the sample cell, so creating a T-format fluorescence system. Thus the monitoring of two fluorophores is possible, providing the user with simultaneous detection of both.

11.B SPECIFICATION

The description and specification for the photomultipliers are found in Section 4.E.8.

11.C INSTALLATION

Figure 11.C.1 shows a view of the rear of the sample handling unit where the two photomultipliers have been connected to the larger, fluorescence windows of the observation cell. The connection of optical components is dealt with in Section 2.E of this manual; with this option, the second photomultiplier is coupled to the cell with an optical coupling, in the same manner as the main, sample channel. The cable associated with this (second) photomultiplier, NA 5880 is routed or, if the reference photomultiplier is being used as the auxiliary detector, rerouted to the connector SK3 PM Services (A), located at the rear of the CU-61. Note that the routing of the cable for the reference channel is to SK5 PM Services (R). **Important:** Ensure that the power to the CU-61 is switched off before connecting the cable.

The excitation fibre is connected to the SMA connector at the beam splitter as usual. It is necessary to fit the blanking plug supplied with this option to the beam splitter spigot normally used for the reference photomultiplier.

Only one element of the fluorescence enhancement kit, FK-675 can be installed.

11.D OPERATION

Set the CU-61 front panel switch to SINGLE BEAM mode - the reference channel is not used

Insert the appropriate long pass or bandpass filters for the fluorophores present in the reaction. Open the Acquire Control Panel, select dual channel operation by checking the Ch(annel) 2 box and enter the Spectrophotometer setup. Select Manual setup and references, click the NEXT button to advance to the Reference Levels page and set the excitation wavelength. With the photomultiplier shutters open and the fluorescent sample to be detected by Channel 1 (Main Channel photomultiplier) in the cell, increase the high voltage to the Channel 1 photomultiplier (MAIN VOLTAGE) until a signal is seen on the live display. Now optimise the optics - adjust the lamp position and focusing with respect to the entrance slit to the monochromator (- refer to Section 3.C.4, Optimising the Optics). "Nudge" the wavelength a nanometer or so in either direction so as to maximize the signal by finding the excitation peak - this is of great importance where a mercury lamp is present.

Set the signal level so that it is at about 90 % of full scale - close to the fixed reference level observed on the live display. Use the Read 100 % button (- denoted by a pair of spectacles to the right of the 100 % box) to set this signal level as the maximum response for the Main Channel. Before accepting a final reading for this, ensure that fresh sample is held in the cell - many fluorescent systems are subject to photo bleaching which causes a reduction in fluorescence.

Next fill the cell so as to introduce the fluorescent condition detected by Channel 2 (Auxiliary Channel photomultiplier). Increase the voltage to the Channel 2 photomultiplier (AUXILIARY VOLTAGE) and observe any increase in signal level. Set the signal level so that it is at about 90 % of full scale - close to the fixed reference level observed on the live display. Use the Read 100 % button (- denoted by a pair of spectacles to the right of the 100 % box) to set this signal level as the maximum response for the Auxiliary Channel. Before accepting a final reading for this, ensure that fresh sample is held in the cell - many fluorescent systems are subject to photo bleaching which causes a reduction in fluorescence. Finally use the Read 0 % button to set the dark condition for both the channels.

To collect data ..... Stopped-flow runs are conducted in the same manner as in normal fluorescence mode. Remember to set the Data Type to Fluorescence for both channels under the Data setup and note that Trace ratioing and subtraction functions are to be found within Data Manipulation under the Math Menu.

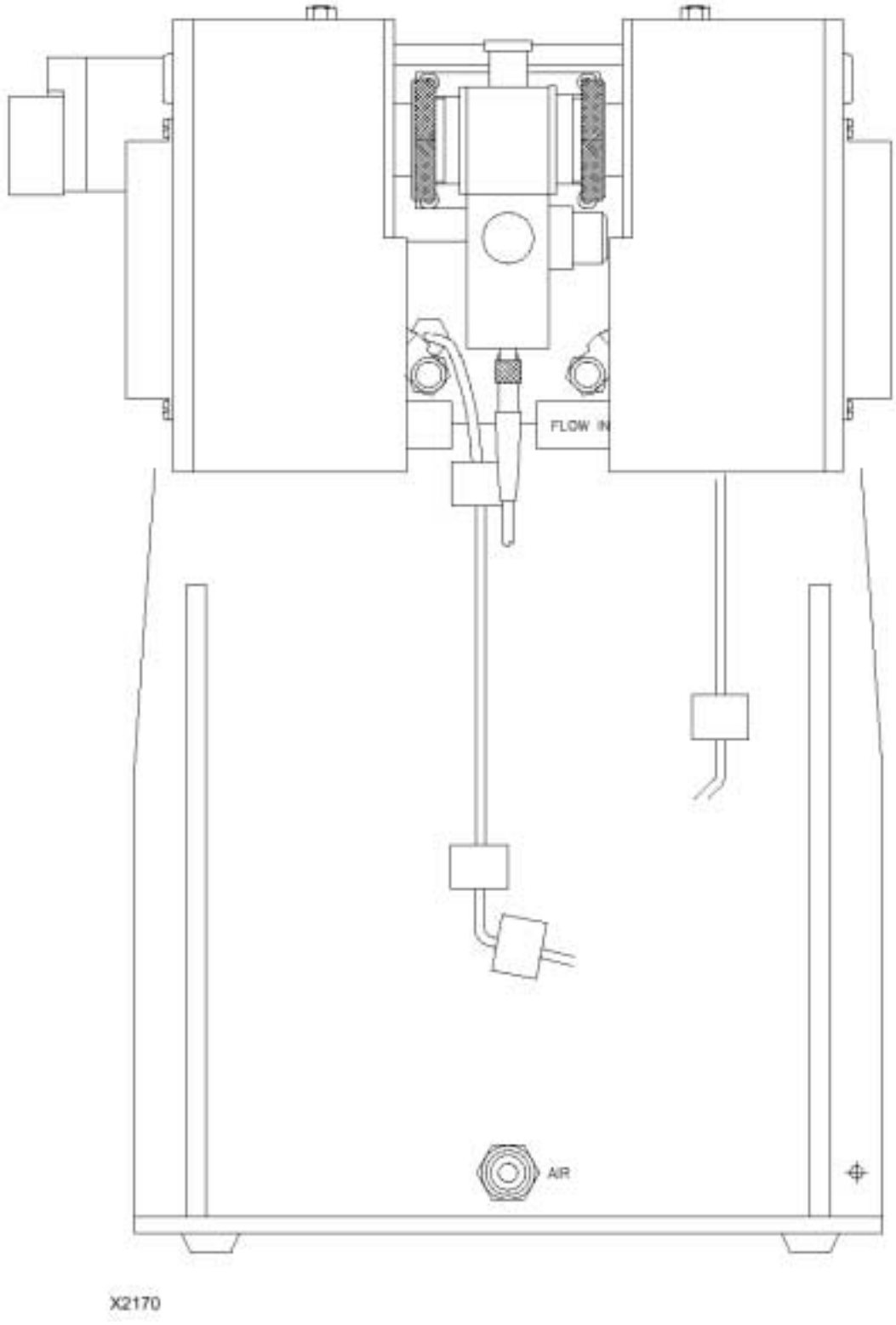


Figure 11.C.1 Sample Handling Unit Rear View

SECTION 12

FLUORESCENCE EXCITATION USING FILTERS

OPTION-679

12A GENERAL DESCRIPTION

The elimination of the monochromator from the excitation path, and the substitution of a suitable bandpass filter, leads to a significant increase in optical throughput, at the expense of resolution. By the introduction of bandpass filters (including narrow bandpass, interference filters) in front of the arc lamp, the fibre is illuminated with light transmitted by the filter, which performs much the same job as the monochromator, but with a broader bandpass. This arrangement can provide some considerable advantage over the more conventional setup with a monochromator, although it will, where photolysis is an issue, also increase the rate of sample decomposition.

12.B SPECIFICATION

The filter set, F-675 supplied with the standard SF-61DX2 instrument contains a selection of long pass and one bandpass filters (- see Section 4.E.9). These are based on the 1" (25.4 mm) diameter industry standard so it is quite easy to find suitable filters in an optics catalogue and/or by special request from Hi-Tech Ltd.

12.C INSTALLATION

The short optical rail is placed on top of the monochromator. It should be noted that although the monochromator is not used, it must remain connected and powered. The arrangement of this rail is shown in Figure 12.C.1. Note that the fibre is fitted with the eccentric ferrule at the fibre support unit. The other end of the fibre is connected to the sample cell through the beam splitter or the simpler, straight coupling. Optimisation of the optical coupling between the light source and the end of the fibre should be performed under the setup routine, with a live display and fluorescent sample in the observation cell.

The fluorescence enhancement kit, FK-675 can be used as normal.

This section addresses only the aspect of fluorescence excitation; emission detection can be through a monochromator, in which case Section 10 should be read, or a filter, or filters in the case of dual channel detection.

12.D OPERATION

Where filters are used in the emission path:

The system is operated in a very similar manner to fluorescence mode with the monochromator. However, when using the Manual setup and references under Spectrophotometer setup, the user should remember that the elimination of the monochromator from the optical path has also eliminated the filter wheel and the automatic shutter, used when taking a dark (0 %) measurement. In order to set up the signal levels in this mode of operation, from the Acquire Control Panel, enter the Spectrophotometer setup and select Manual setup and references; click the NEXT button to advance to the Reference Levels page and introduce the fluorescent sample into the cell. Ensure that the iris shutter (between the lamp and the fibre) is open and the photomultiplier shutter(s) are also open. Increase the high voltage to the photomultiplier(s) and observe any increase in signal. As the signal increases, take the opportunity to optimise the optical separations and alignments. With the signal at about 90 % of full scale, use the Read 100 % button(s) (- denoted by a pair of spectacles to the right of the 100 % box) to record the maximum fluorescence condition for each channel. For the dark measurement, close the shutters in front of the active photomultipliers and use the Read 0 % button to record the dark reference levels. Exit the dialog box by clicking the FINISH button. The system is now ready for operation - remember to open the photomultiplier shutters before attempting to collect data. Note that the wavelength recorded on each data set will merely reflect the position of the monochromator; this is irrelevant information for this set-up.

Where a monochromator is used in the emission path:

For a single emission monochromator, the system behaves as described in Section 10; the filter wheel (on the emission monochromator) automatically introduces the shutter for the dark measurement and so the Read All button can be used as described. If the monochromator is not going to be used for para-scanning, it is possible to treat it just like a filter with an automatic shutter and computer control of emission wavelength.

The use of a second emission monochromator for dual channel fluorescence work introduces the complication that only one monochromator is controlled and hence, only one filter wheel is present. The user should use both monochromators as filters - set them to each of the emission wavelengths and follow the procedure above for dual emission filters.

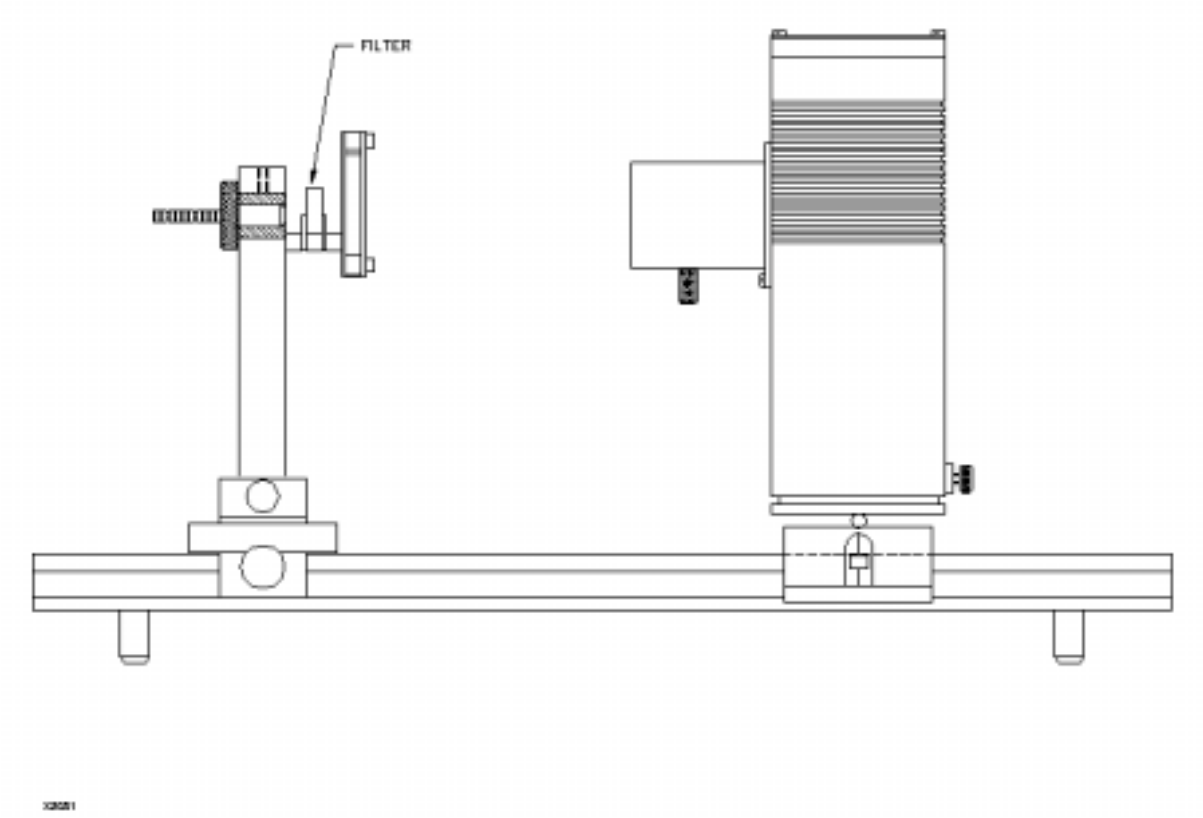


Figure 12.C.1 Fluorescence Excitation with a Filter

SECTION 13

AUTO-SHUTTER

OPTION 667

13.A GENERAL DESCRIPTION

This option provides an electro-mechanical shutter that is controlled automatically by KinetAsyst in the context of certain modes of operation of the SF-61DX2, namely fluorescence and diode array detection over long run times. In the former mode, minimisation of long term photo-degradation (bleaching) of light sensitive biochemical samples is achieved; in the latter mode, white light incident on the reaction volume between scans is shuttered, so reducing any effects due to photochemistry.

The shutter is inserted into the light path; it is connected to a driver box that connects to the CU-61 control unit. The electrical connections and optical arrangement for the two modes of operation are identical (- see Figures 13.C.1 & 13.C.2). The operation varies between the modes, although in both cases shutter control is automated by the KinetAsyst software.

Note that mains power is via the switch/filter/fuse IEC socket unit at the rear of the unit. The mains voltage is factory set to either 220/230 V~ or 110 V~ depending on the country of destination; the fuse ratings are as follows:

110 V~	200 mA Anti-surge
220/230 V~	100 mA Anti-surge

Under no circumstances should alternative ratings be substituted!

The (continuous) current consumption of the shutter control box is 50 mA at 230 V~.

13.B SPECIFICATION

Shutter:	3 mm Teflon coated blade, solenoid operated, 48 Ω coil
Driver box Output:	65 V pulse, 3 ms; 6.2 VDC holding voltage

13.C INSTALLATION

13.C.1 ELECTRICAL CONNECTIONS

Figure 13.C.1 shows the electrical connections for the shutter:

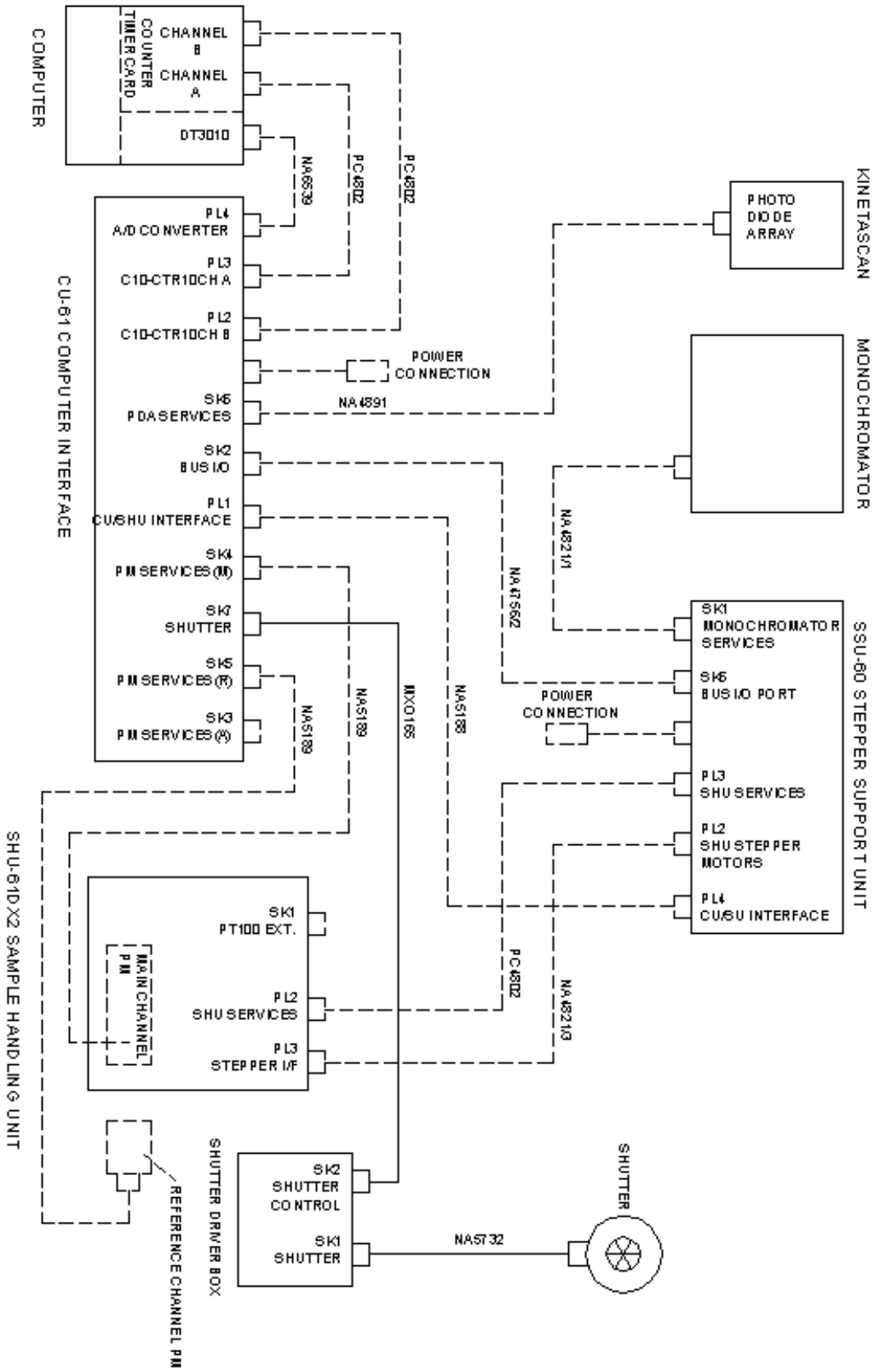


Figure 13.C.1 Electrical Interconnection Diagram

In this diagram the complete SF-61DX2 system interconnection is depicted; however the general system connections are shown in chain dashed line, while those additional connections unique to the shutter option are shown in solid lines. Essentially there is a moulded, 15-way D connection between the rear of the CU-61 (digital unit) and the shutter driver box, and a cable NA 5732 running between the box and the shutter. There is also a mains power cable to the box.

13.C.2 OPTICAL CONNECTIONS

Figure 13.C.2. shows the optical arrangement of the shutter:

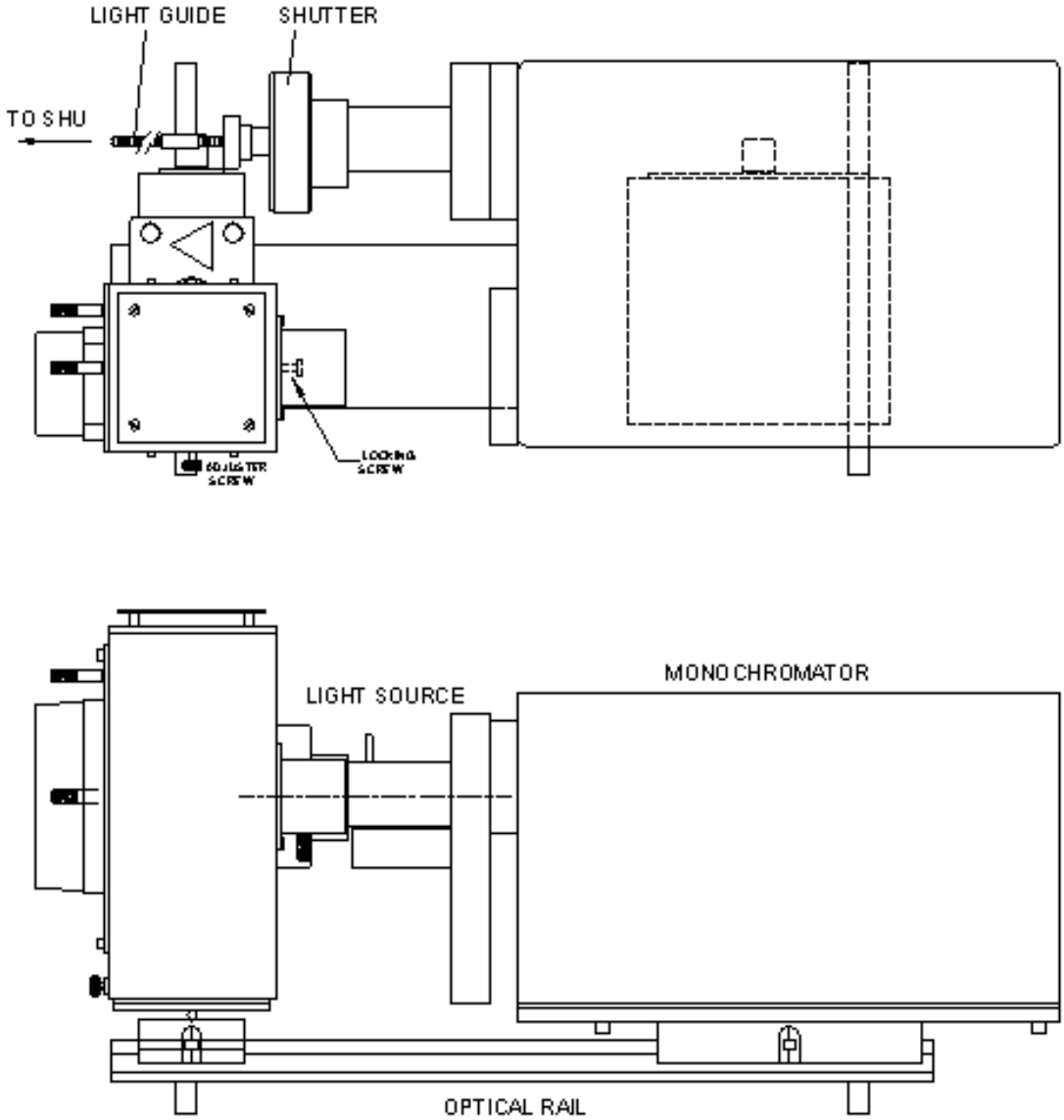


Figure 13.C.2 Optical Arrangement - Top & Side View

The shutter is mounted onto the optical coupling associated with the filter wheel at the exit port of the monochromator. The end boss is removed from the standard coupling by loosening the two securing set screws; the shutter is then mounted in its place, similarly secured by two set screws. The fibre connection is of the standard SMA type.

13.D OPERATION

Important: Due to the longer response time of the reference channel, it is not possible for it to follow the rapid signal changes associated with the "chopping" phase of the shutter; hence in shutter modes of operation, the instrument should always be set to SINGLE BEAM mode on the front panel of the CU-61.

Remember to switch on the shutter unit at the rocker switch on the rear panel; the green LED on the front panel will light up when the unit is powered. Shutter operation is selected by clicking the Auto Shutter box on the Monochromator page of the Hardware setup dialog. Once the shutter is selected, the shutter operation is automatically controlled by the KinetAsyst software. An illustrative timing diagram is shown in Figure 13.D.1.

13.D.a FLUORESCENCE MODE

Generally the operation of the shutter is automated by the KinetAsyst software and the SF-61DX2 system. In fluorescence mode (or more generally, photomultiplier mode) on run times of 10 seconds and longer, a 2.62 second burst of data is initially collected with the shutter open; this is followed by a "chopped" phase, during which time data points are collected between shutter closure periods. The number of data points controls the point spacing and hence the "dark time" and needs to be set within the Data Setup dialog (- see also the definition of the software parameter, Shutter PM First Burst OS).

There are a number of parameters set within an initialisation file, RKWACQHW.INI (under subsection Monochromator Configuration) of KinetAsyst that control the shutter operation and the subsequent data collection in photomultiplier mode. The user should not need to alter the default settings unless instructed by Hi-Tech. The only setting that the user might want to alter is the Shutter PM Gain Equalisation factor; although this is calculated and set during the installation, it will vary according to the light level and may therefore need to be recalculated. However, an understanding of these parameters helps to understand the operation of the shutter and hence an explanation of the software parameters now follows:

Shutter Opening Delay	(default - 0.002 s) this is the time required for the shutter to fully open after being electronically activated.
Shutter PM Data Delay	(default - 0.008 s) this is the time required from the shutter being electrically activated to the collection of the first data conversion (over-sample).

Shutter PM Hold Time (default - 0.0005 s)
this is the additional time that the electrical shutter open signal is held active following the collection of the last data conversion (over-sample).

Shutter Closing delay (default - 0.01 s)
this is the time following the deactivation of the electrical shutter open signal before the shutter can be reactivated. This together with the Shutter Opening Delay determine the minimum shutter period.

Shutter PM First Burst OS (default - 256)
sets the number of data conversions (over-samples) collected for each of the four channels to generate one data point per channel during the initial 2.62 second shutter open phase. The maximum number of over-samples is inversely proportional to the number of points displayed, where the total number collected during this phase should not exceed 32768 samples for each of the four channels. For example, 256 over-samples set results in 128 data points (256 x 128 = 32768). This setting is also dependant on the sample rate/interval; with current hardware this value should remain at 256. **Important:** note that this gives the default value of 128 data points collected in the first 2.62 seconds shutter open phase.

Shutter PM Burst OS (default - 64)
sets the number of data conversions (over-samples) collected for each of the four channels to generate one data point per channel in every shutter open period during the shutter chopping phase. This setting is also dependant on the sample rate/interval; with current hardware this value should remain at 64.

Shutter PM Burst Points Min (default - 10)
sets the minimum number of data conversions (over-samples) that can be allowed with each shutter open period during the shutter chopping phase.

Shutter PM Sample Interval (default - 0.00002 s)
sets the rate at which the data conversions (over-samples) are collected. 0.00002s = 50kHz.

Shutter PM Gain Equalisation (default – 0.95)
sets the relative gain between the initial 2.62 second shutter open phase and the shutter chopping phase, so as to eliminate any step effect from the trace caused by the restricted response of the photomultiplier during the chopping phase. For example, if during the chopping phase the data acquired only reaches

94.8 % of the value of the initial shutter open phase, then the shutter PM gain equalisation factor needs to be set to 0.948.

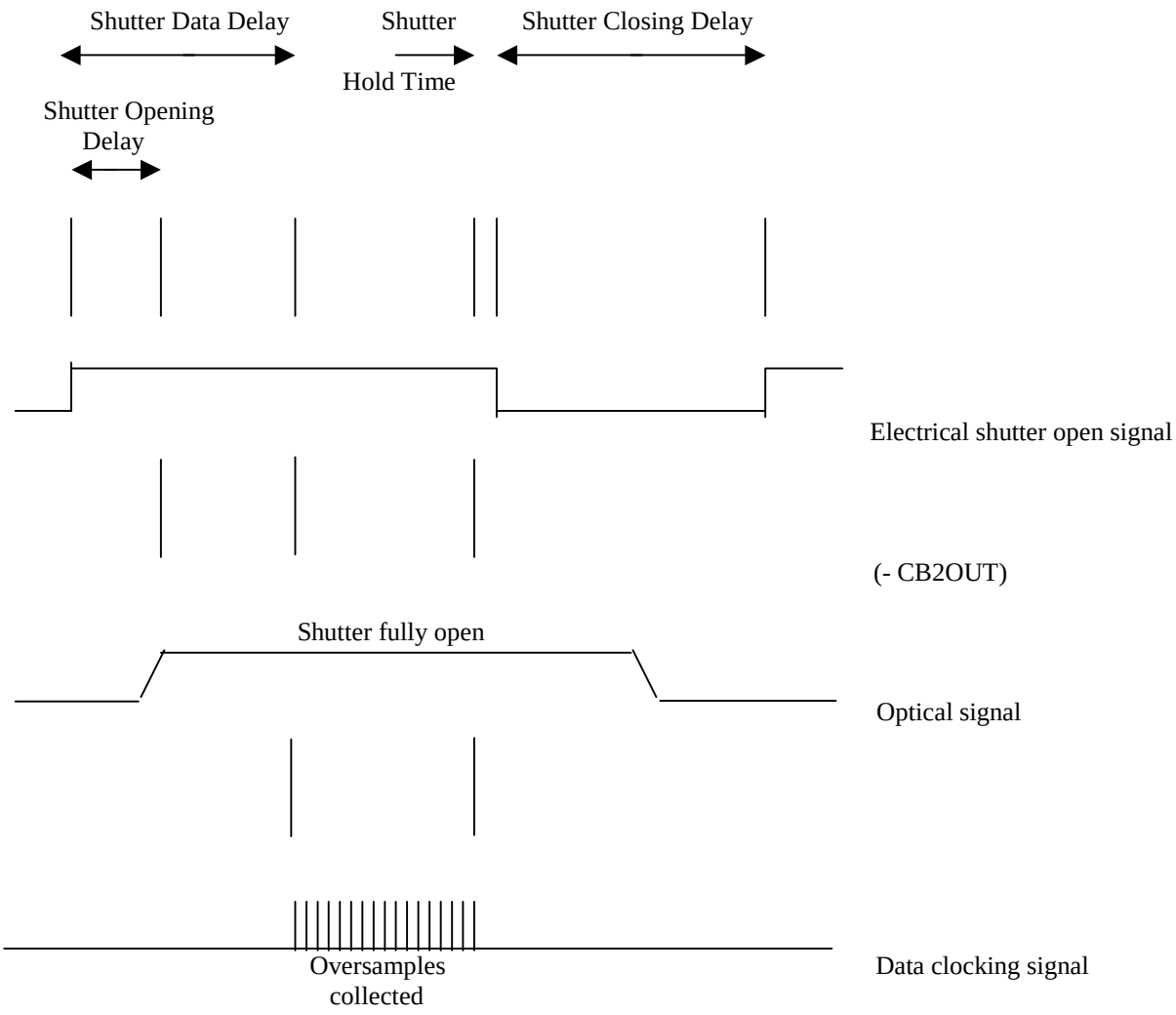


Figure 13.D.1 Shutter Timing Diagram

13.D.b DIODE ARRAY MODE

In photodiode array mode, on run times and with integration times that allow enough time between scans for the cycling of the shutter, white light incident on the reaction volume between scans is shuttered. Thus on relatively long run times, eg 50 s or longer, for a significant portion of this time the sample is masked from the incident white light.

There are a number of parameters set within an initialisation file, RKWACQHW.INI (under subsection Spectrograph Configuration) of KinetAsyst that control the shutter operation in

diode array mode. The user should not need to alter any of these parameters. However, an understanding of the parameters helps to understand the operation of the shutter and hence an explanation of these software parameters is now given:

Shutter Opening Delay (default - 0.002 s)
this is the time required for the shutter to fully open after being electronically activated.

Shutter Data Delay (default - 0.006 s)
this is the time required from the shutter being electrically activated to the collection of the first data conversion of the scan.

Shutter PDA Hold Time (default - 0.000 s)
this is the additional time that the electrical shutter open signal is held active following the collection of the last data conversion of the scan.

Shutter Closing delay (default - 0.01 s)
this is the time following the deactivation of the electrical shutter open signal before the shutter can be reactivated. This together with the Shutter Opening Delay determine the minimum shutter period.

The illustrative timing diagram shown in Figure 13.D.1 also applies to diode array mode, although a single scan, rather than a burst of over-samples is collected each time the shutter opens.

SECTION 14

CONDUCTIVITY DETECTION

OPTION 642

14.A GENERAL DESCRIPTION

If there is no detectable change in the UV-Visible spectrum during the reaction to be studied, but there is a change in the number or type of ionic species, then conductivity detection will be suitable.

Option 642 comprises an observation cell, which is attached to the SHU-61DX2 in place of the standard optical cell, an electronics unit, the CAK-501, which measures conductivity of the observation cell and gives a signal voltage linearly proportional to conductivity, and cabling.

Note that mains power is connected through the IEC socket unit at the rear of the unit. The mains voltage is factory set to either 220/230 V~ or 110 V~ depending on the country of destination; the fuse ratings are as follows:

110 V~	100 mA Anti-surge
220/230 V~	100 mA Anti-surge

Under no circumstances should alternative ratings be substituted!

The (continuous) current consumption of the conductivity meter is 25 mA at 230 V~.

14.B SPECIFICATION

The conductivity cell is a cylindrical cavity in a teflon block, 3 mm long and 3 mm diameter. The ends are closed by platinum electrodes. The nominal cell volume is 21 ul, and the nominal cell constant 4.24 cm^{-1}. Reagents are mixed in a T mixer just upstream of the observed volume.

The CAK-501 meter has the following ranges:

Range 1	0 to 0.5 S
Range 2	0 to 0.05 S
Range 3	0 to 0.005 S
Range 4	0 to 0.0005 S
Range 5	0 to 0.00005 S
Range 6	0 to 0.000005 S

The full scale voltage for each of these is 10 V although Range 1 may be limited to 250 000 uS at 5 V - linearity above this level should not be assumed without calibration. Furthermore, operation at cell conductivities above 250 000 uS should not be attempted at ambient temperatures above 30 ^{o}C in order to avoid excessive dissipation from the amplifier.

The bias control enables the user to "backoff" up to 10 V of signal; this means that signals up to the full scale output of 10 V can be offset so that any signal change can be digitised within a 0 - 5 V analogue channel input range.

14.C INSTALLATION

For the electrical interconnections the user is generally referred to Figure 2.C.1 and accompanying installation instructions; Figures 14.C.1.a and 14.C.1.b supplement this information with cabling details specific to this option. There is an adapter cable supplied with this option that facilitates the introduction of the conductivity meter signal into the appropriate analogue channel. For older systems using the DAS-50 A/D converter, NA 5979 is used; for DT3010 systems, NA 6527 is used. There are subtle differences between these two adapters, but they are, in effect both T adapters for the NA 4330 Signal / Trigger Cable Assembly which incorporate an interface box with a mode selection switch. This "points" the analogue input to either the PHOTOMETRIC analogue signal, or the CONDUMETRIC analogue signal. It is recommended that systems supplied with this option leave this adapter installed for all applications.

The CAK-501 meter has two BNC connections to the CELL electrodes and a BNC connection to the previously referred to interface box within the adapter.

To change the cell from optical detection to conductivity:

1. Ensure that the system is switched off before starting the installation of the conductivity option.

2. Remove the optical components from the optical cell block, eg photomultipliers and fibre optic coupling.

3. Drain the thermostat system from the sample handling unit.

4. Back off the central set screw in the centre of the rear face of the cell block. This releases the clamping and sealing force applied to the silica cell.

5. Remove the four socket cap head screws around the mounting flange of the cell block - loosen each a little at a time, so as to release the cell block. Note the red dot on the top face indicating its orientation and the two special quad seals that seal the thermostat passageways between the SHU and the cell block.

6. Fit the conductivity cell, noting its orientation and the location of the two quad seals - apply a little silicone grease to the seals. Tighten each of the four screws a little at a time in a diagonal pattern. Check for reagent leak. If there is a reagent leak, first use the tool supplied to slacken very slightly – no more than ¼ turn – the cell BNC connectors and then tighten the central sealing set screw, carefully as necessary. Remember to retighten the cell BNC connectors when any reagent leak has been stopped, before checking for thermostat leaks.

When reverting back to optical measurements, remember to switch the signal cable interface box back to PHOTOMETRIC.

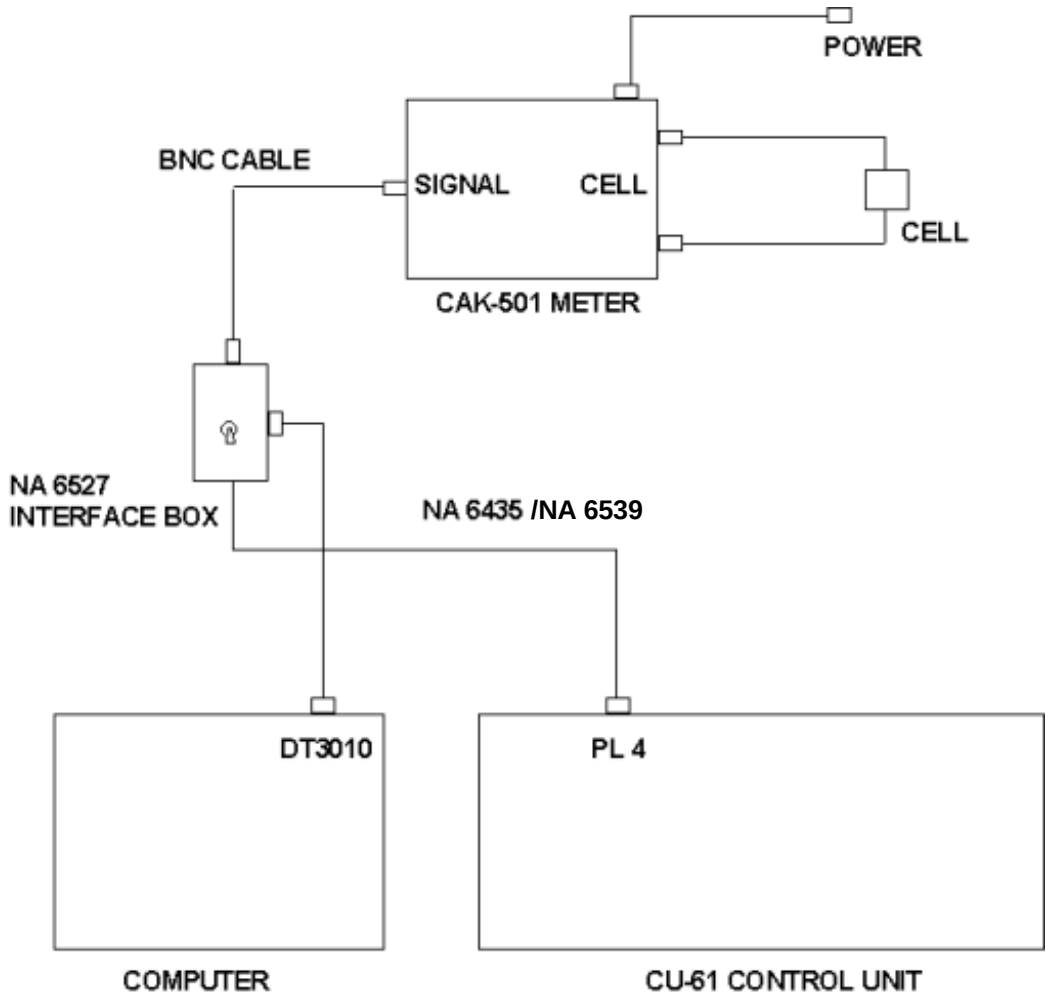


Figure 14.C.1.a Electrical Interconnection Diagram (with DT3010)
Note: general system cabling not shown.

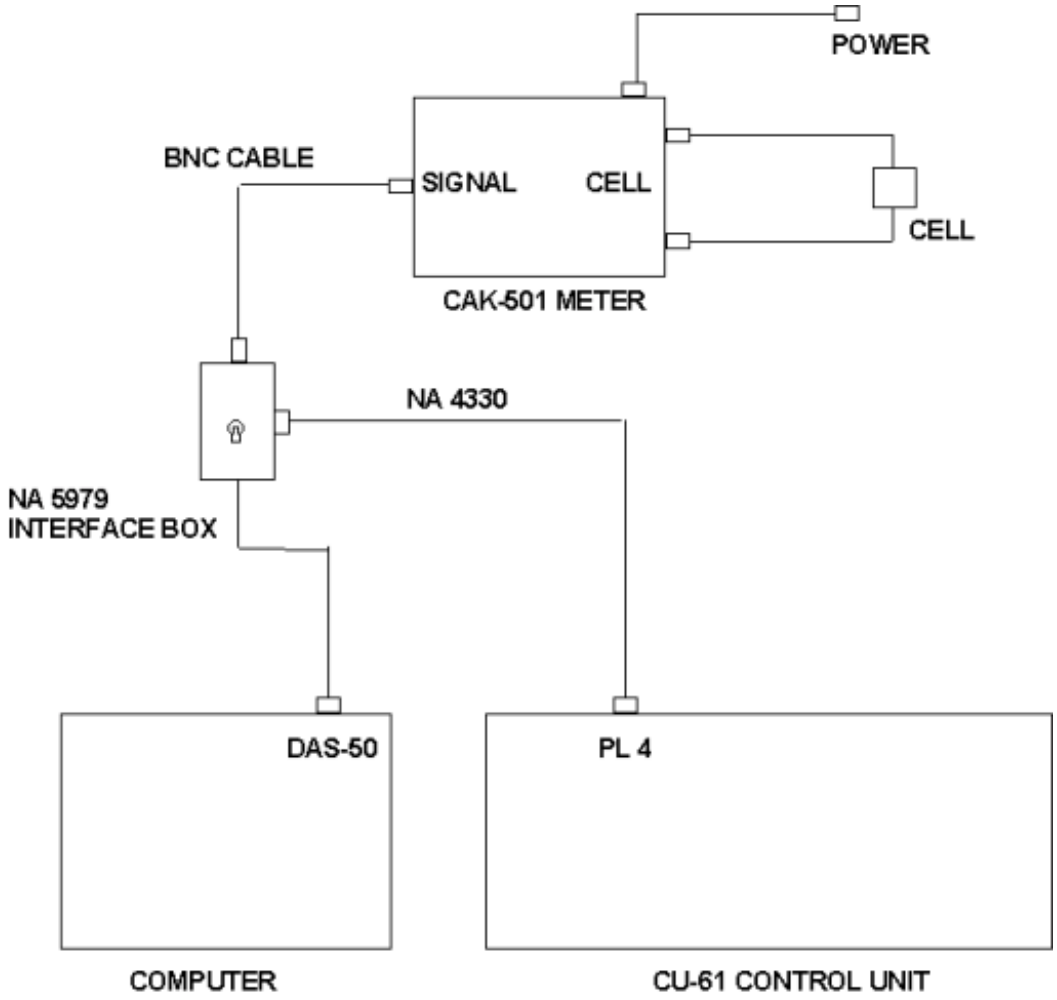


Figure 14.C.1.b Electrical Interconnection Diagram (with DAS-50)
Note: general system cabling not shown.

14.D OPERATION

Set the CU-61 front panel switch to SINGLE BEAM mode - the reference channel will not be present and is not necessary for this mode of operation. The signal cable adapter junction box must be switched to CONDUMETRIC.

For familiarisation, the user is recommended to start with the following experiment:

Solution A: 5 mM NaOH. Pipette 0.5 ml of 1.0 M NaOH into a 100 ml volumetric flask and make up to the mark with water.

Solution B: 1 mM acetylacetone, 8.4 mM KCl. Pipette 1 ml of acetylacetone (pentan-2,4-dione) into a 100 ml volumetric flask and make up to the mark with water. Shake very thoroughly to dissolve the acetylacetone. Pipette 1 ml of this and 8.4 ml of 0.1 M KCl into a 100 ml volumetric flask and make up to the mark with water.

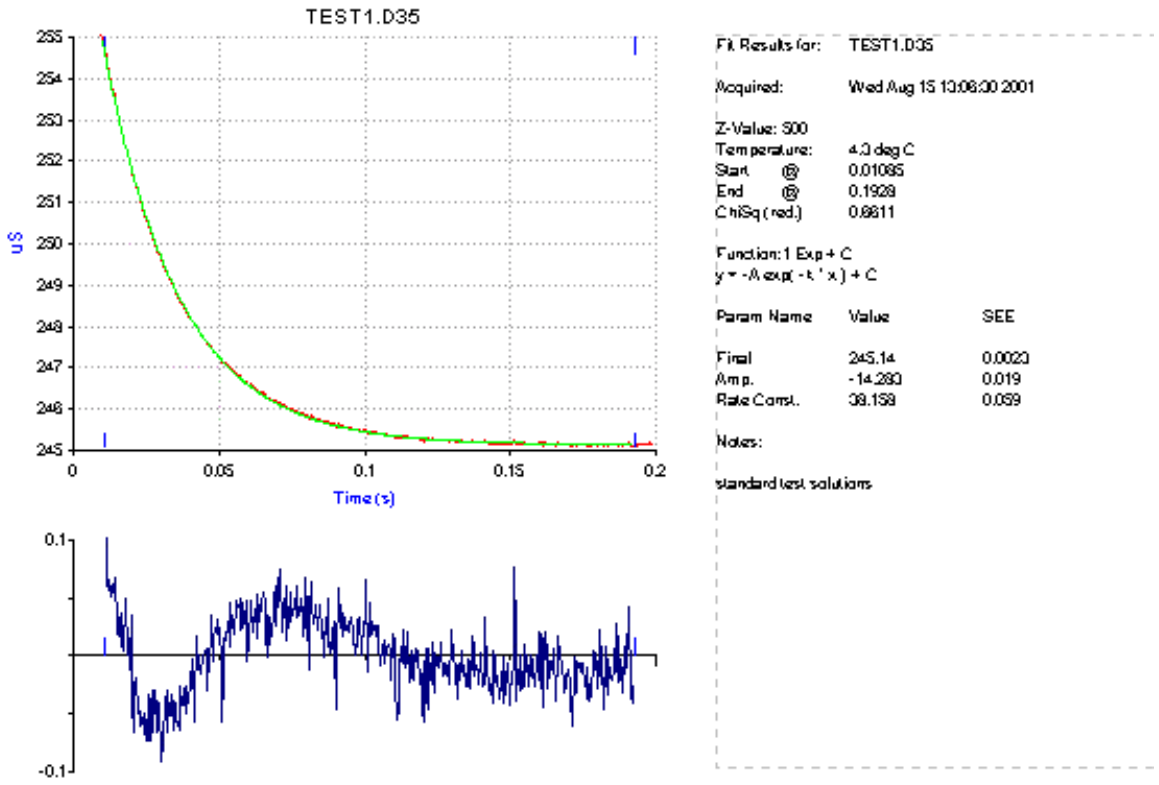
In this reaction, acetylacetone slowly loses a proton to OH-, and the conductivity of the reacting solution falls as hydroxide ion disappears. Note the use of KCl to approximately equalise the conductivities of the two solutions. It is always a good idea to have reagent solutions of similar conductivities and experiments should be designed with this in mind.

To collect data ... ensure that the system including the CAK-501 meter have been switched on for at least 30 minutes to ensure that they have stabilised. Note that although optical measurements are not to be made the monochromator will still be energised and will run through its calibration routine when KinetAsyst starts up. It is not necessary to have the lamp switched on.

In order to enable data collection within KinetAsyst, it is necessary to acquire or at least generate a reference data set. To do this, select Spectrometer setup and then select the Manual setup and references option. At the manual references set up dialogue, use the live display to set the RANGE and METER OFFSET. Make sure that solution is pushed through to the observation cell and start with the RANGE at 1 and the OFFSET at 0. Increase the RANGE and observe the signal - with the test solutions it will go out of range (> 5 V) on RANGE 4. Note that increasing to RANGE 5 causes the signal to fall again and become very noisy in appearance - this is because the amplifier has been driven into saturation. Using RANGE 4 use the OFFSET control to bring the signal level back into the display range - about 2 V of offset will be necessary to set the signal between 70 and 80 % of full scale. Accept the default 100 % and 0 % values (95 and 4 respectively) by exiting the set-up dialogue by pressing the FINISH button. Data acquisition is now enabled.

Collect data as for optical measurements ..... the Y-axis will be scaled in % response. To convert these data to conductance units use the -> Conductivity (µS).... option under the Math Menu. Note that if you wish to store the raw data as % response, you will need to write these to disk, or at least duplicate the data set, before converting. The dialogue boxes that follow the selection of -> Conductivity (µS), enable the user to indicate the RANGE used and choose the appropriate Range Factor (S per volt) and enter the meter OFFSET. The 100 % and 0 % (default) values can also be edited if necessary. The data can then be CONVERTed to µS.

A typical data set with the test system should look like the following:



Note that in single mixing mode, it is possible to hold the air pressure on for a prescribed time after stopping (- set to 1 second) to prevent any artefact from occurring (at approximately 60 ms) when the pressure is by default removed immediately after stopping. This is achieved by setting Switch 2 (SW2) on the SHU control unit within the SSU-60 to the ON position. If this facility is used, sequences should not be set with a run time of less than 1 second.

14.E MAINTENANCE

It is recommended that zero and full scale calibration be checked from time to time as follows:

1. Disconnect both the cell leads from the BNC connectors on the front panel of the CAK-501 meter.
2. Set the OFFSET potentiometer to zero.
3. Select RANGE 1.
4. Connect a digital voltmeter or multimeter set to measure DC voltage to the BNC SIGNAL connector and check that the output is 0.00 Volts.
5. Set the range switch to TEST and check that the output is 10.00 Volts.

If recalibration is necessary, a procedure is available from Hi-Tech on request.

SECTION 15

OPTION 645

AUTO-CYCLING CONTROL ACCESSORY

15.A GENERAL DESCRIPTION

This option consists of an electronic control unit, the ACC-61 Auto Cycling Control Unit and associated cabling. The unit is used to power and control a stand-alone Sample Handling Unit in single mixing mode; this unit can be either the SHU-61SX2, the SHU-61DX2 or the SHU-61.

The auto-cycling control accessory minimizes user intervention by obviating the need to empty the stop syringe after every shot and also improves shot-to-shot reproducibility. It is useful when a technique such as circular dichroism (CD) requires the averaging of data collected from several shots, or when it is necessary to have remote control of the sample handling unit such as in a hazardous environment.

Note that for the ACC-61 to function with the SHU-61, it is necessary that an automatic emptying STOP/WASTE valve be retrofitted in place of the standard manual valve. The variant ACC-61 unit used for the SHU-61 incorporates the pneumatic components, ie solenoids, regulator and gauge, that are necessary to control the automated STOP/WASTE valve.

15.B SPECIFICATION

The (continuous) current consumption of the ACC-61 with the SHU-61DX2 or SX2 connected is 100 mA at 230V~.

Power Input;	110V/230 VAC whichever is applicable (factory set)
Fuse;	250 mA T anti-surge @ 230 VAC 250 mA T anti-surge @ 110 VAC
Output;	+/- 15 VDC @ 1 A
Digital Inputs/Output;	CMOS 0-5 V

Housed within the ACC-61 is the NB6568 control circuit board. The links and switches on the board are factory set as indicated overleaf; should the user wish to change them, please first consult with Hi-Tech.

Link	Name	Position	Description
LK1	TRIGOUT	1-2	TTL 0-5 V - fixed
LK2	TRIGIN	1-2	TTL 5-0 V - fixed
LK3	XSTRT	2-3	TTL 5-0 V (switch closure)

Switch	Name	Position	Description
SW1a	SWVALVE	ON	Stop/Waste valve safety sensor - enabled
SW1b	CVALVE	ON	C Drive/Fill valve safety sensor - enabled
SW1c	DVALVE	ON	D Drive/Fill valve safety sensor - enabled
SW1d	AIRON	OFF	Air pressure maintained after stopping for 1s - disabled
SW1e	SHU61	OFF	Applicable to SHU-61 - disabled

15.C INSTALLATION

The equipment is available for operation from the following electrical power supplies:

220 - 240 VAC at 50/60 Hz
110 - 120 VAC at 50/60 Hz

The ACC-61 is supplied with a moulded power cable that has an IEC plug at one (the equipment) end and the appropriate national plug at the other. The colour coding of the cable is as follows:

110 V~	LINE	BLACK
	NEUTRAL	WHITE
	EARTH	GREEN
230 V~	LINE	BROWN
	NEUTRAL	BLUE
	EARTH	GREEN/YELLOW

The SHU-61SX2 or DX2 sample handling unit is powered and controlled from the ACC-61 by the NA4802/2 cable assembly. This cable is connected between *PL2, SHU Services* on the rear panel of the SHU-61SX2 or DX2 and *PL2, SHU Services* on the rear panel of the ACC-61.

If the unit is to be externally controlled from an alternative source eg a Jasco CD, then a compatible connection is required to the ACC-61 *SK6, Ext.Control* - refer to Section 15.D.

If the data trigger signal (generated as each shot completes) is to be utilised to initiate data acquisition, an appropriate connection is required to the ACC-61, either to *SK5: Trig.Out 1* (3-pin Lemo) or to *SK4: Trig.Out 2* (BNC socket) - refer to Section 15.D for more details.

15.D OPERATION

The ACC-61 enables the user to program and initiate a sequence or cycle of shots. The operator can program the number of shots and the time interval (in seconds) between shots, so as to take into account any data acquisition time. Depending on the stopping volume and the size of the drive syringes, the user should be aware that the drive syringes normally need to be re-loaded after approximately sixteen shots or when the maximum drive span has been reached. At the end of each shot, marginally before the stop syringe plunger hits the stop block, a data trigger signal is generated and this can be used to initiate data collection. If the user wishes to externally control the sequence, ie the number of shots and the interval between these shots, an external control is available at the rear panel; when actioned, this initiates a single shot. At the end of this shot, the data trigger signal initiates a reset of the circuitry in readiness for the next external event.

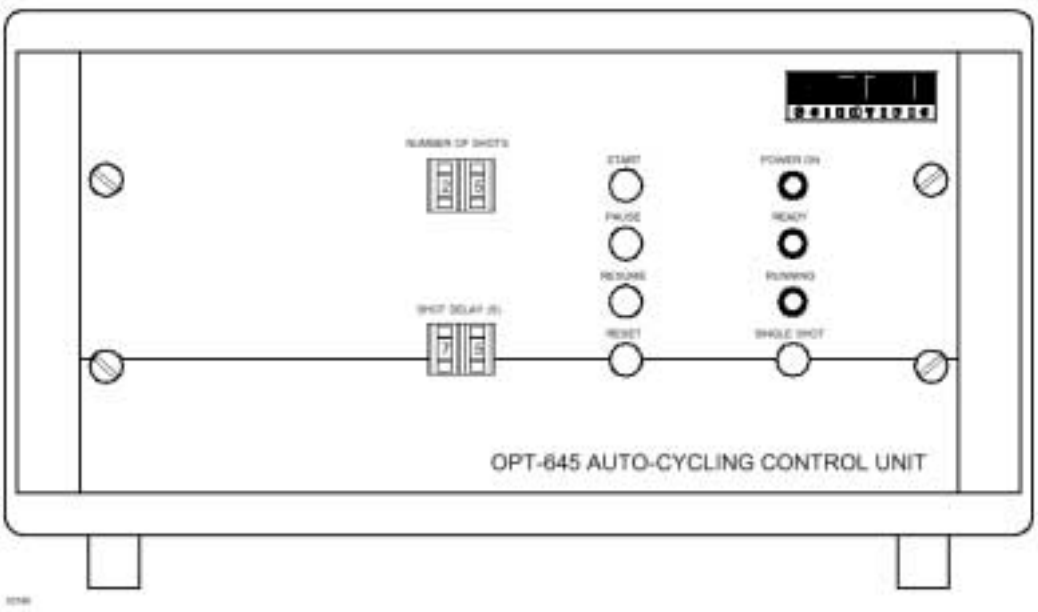


Figure 15.D.1 Front Panel

Figure 15.D.1. shows the front panel and the controls and indicators mounted on it. Each of these controls is described below:

Number of shots - this group of binary-coded decimal switches enables the user to set the number of shots in a sequence, the maximum number of shots being 99.

Shot Delay(s) - this group of binary-coded decimal switches allow the user to set a delay between the end of the last shot and the start of the next shot in a sequence. This is useful when it is necessary to allow for any inherent delays in the acquisition of data, ie longer run times.

Reset - this push button switch should be pressed in order to abort the shot sequence and reset the unit.

Start - this push button switch is pressed to initiate the prescribed shot sequence.

Pause - this push button switch is used to enable reloading of the drive syringes during a shot sequence. During a shot within a sequence of shots, the drive syringes may reach their maximum travel, so preventing the shot from fully completing, ie preventing the stop syringe from hitting the stop block and hence the generation of the data trigger signal that in turn initializes (after any shot delay) the next shot. When pressed, the *Pause* button will de-activate the air drive without triggering the data acquisition; the user is then able to re-load the drive syringes and press the *Resume* button to continue with the sequence of shots. The uncompleted shot is then repeated.

Resume - this push button switch enables the user to continue with a sequence of shots having reloaded the drive syringes after their maximum travel was reached (see *Pause*).

Single Shot - this push button switch enables the user to execute a single shot only.

Figure 15.D.2 shows the rear panel and the rear panel connectors.

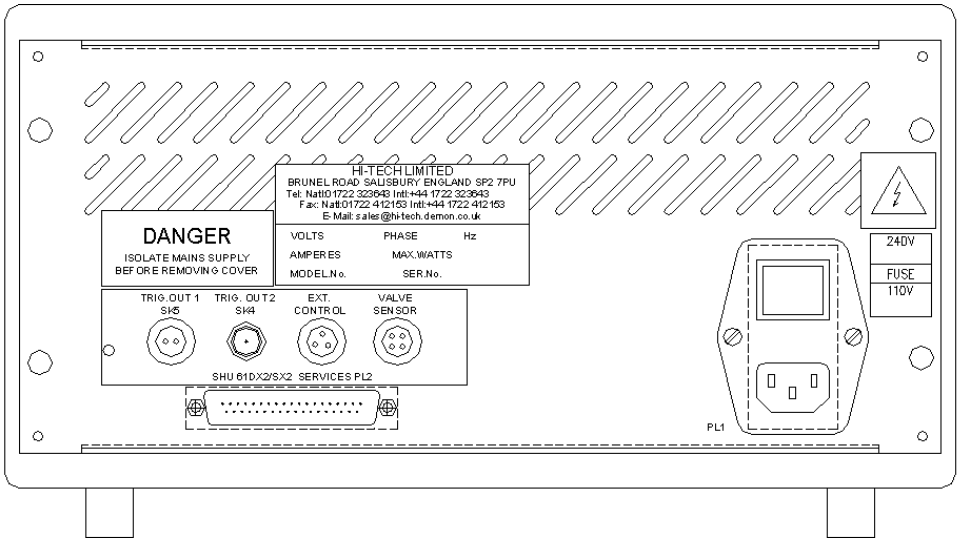


Figure 15.D.2 Rear Panel

The function of each connector is described below:

SK6, External Control - facilitates the control of the shot sequence from an alternative source ie a Jasco CD. A 5-0 V TTL transition or switch closure is required on pin 1 with respect to pin 2 to initiate a single shot. If the ACC-61 is being externally controlled, the user need only use the *Pause* and *Resume* front panel controls for re-loading the drive syringes during a sequence of shots.

SK3, Valve Sensor - the SF-61SX2 & DX2 sample handling unit valves are equipped with safety sensors which are used to disable the air drive activation when any valve is in the wrong (ie FILL) position; this prevents any damage to the valve or the flow circuit and also protects the user, by preventing the driving of solutions back to the reservoirs causing a "fountain" effect. In the case of the SHU-61 sample handling unit, the sensors in the stop/waste valve are connected to SK3.

SK5, Trigger Out 1 & *SK4, Trigger Out 2* - a data trigger signal is generated on completion of each shot and is used to reset the unit and initiate data acquisition. The signal is available at the rear panel via *SK5, Trigger Out 1*, a LEMO type 2-pin socket, (EGG.1B.302.CLL) and *SK4, Trigger Out 2*, a BNC socket. The data trigger output can be set either as a TTL 0-5 V or 5-0 V transition.

PL1 - an IEC filter/fuse/switch unit that accepts a (mains) power cable, on the other end of which is the appropriate national plug.

PL2, SHU Services, is a 37-way `D' type plug that connects to the sample handling unit through the appropriate cable assembly (NA4802/2). It provides the DC voltage supply and all the functionality necessary to control the unit.