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Hardware Section

This section includes descriptions of the SX.18MV-R stopped-flow spectrometer and accessories.

It covers setting up the instrument and the hardware aspects of operating the instrument.

It also covers hardware fault finding and remedies.

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ESSENTIAL READING, SAFETY NOTES

HIGH VOLTAGES



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

LAMP IGNITION

The Xenon lamp uses a SafeStart igniter so that sensitive electronic equipment will not be damaged.

Under no circumstances should the igniter be activated, by pressing the ignite button on the lamp power supply, if the leads from the igniter are not connected to the lamp housing. Failure to observe this requirement will result in damage to the igniter and will void the warranty.

LAMP ADJUSTMENT

The red insulated hexagonal driver must be used to align the lamp after replacement. Under no circumstances should a non-insulated hexagonal driver be used when the lamp is running. (see chapter 3.5)

EARTH/GROUND

The chassis of all units MUST be well grounded using the grounding straps supplied with the system. Allowing a chassis to float with respect to ground potential may result in the chassis acquiring a dangerous potential.

All of the electronic units MUST obtain their mains power from the filtered distribution bar supplied with the system.

PNEUMATIC DRIVE RAMS

The input pressure should be set to 8 bar (125 psi).

To avoid irreparable damage to the sample flow circuit the pneumatic drive ram must not be operated with a gap between the syringe drive and the syringe pistons. Also both drive syringe pistons must be in contact with the drive ram. i.e. The drive ram should not be used to fire a single drive syringe.

ASYMMETRIC MIXING

To avoid irreparable damage to the sample flow circuit the input pressure to the drive ram MUST BE adjusted to 2 bar (30 psi) when syringes of different sizes are being used to give mixing ratios of other than 1:1.

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FILL SYRINGE DRIVE (Sequential Version)

The fill syringe drive is fitted with an internal stop to limit its stroke.

To avoid irreparable damage to the sample flow circuit this stop MUST be adjusted to ensure that the travel of the syringe drive is limited by this internal stop and NEVER EVER stopped by the stop syringe mechanism. If in doubt, test the adjustment by manually performing a shot.

TEMPERATURE CONTROL

For obvious reasons, the cell temperature should not be allowed to drop below 0°C if you are using aqueous chemistry. The maximum temperature for the sample handling unit is 50°C. Above this temperature seals will start to leak.

DISCONNECTING LEADS

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

FOR CUSTOMERS WITH 110V ELECTRICITY SUPPLIES ONLY!

Contrary to the instructions given on page 5 of the Acorn computer Welcome Guide, **the computer must be connected to a 110V AC power supply outlet.**

ENVIRONMENTAL REQUIREMENTS

The various units of the SX.18MV-R stopped-flow spectrometer require the following environmental conditions:-

Operating:-	Temperature, +5°C to +35°C: Humidity, 20% to 80% non condensing
Storage:-	Temperature, -20°C to +50°C: Humidity, 5% to 80% non condensing

Servicing should only be undertaken by qualified personnel.

If you are in any doubt at all please contact the Technical Support Coordinator at Applied Photophysics Ltd
EMail TechSup@apltd.demon.co.uk
Fax +44(0) 1372 373893

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1.0 System Descriptions

The SX.18MV-R Stopped-flow Spectrofluorimeter allows fast chemical, bio-chemical and biological reactions initiated by stopped-flow and sequential-mixing stopped-flow to be followed using absorption, fluorescence and circular dichromism detection methods. For an introduction to the stopped-flow method refer to Gibson,Q.H. and Milnes,L. (1964) Biochem. J., 91, 161.

You will have ordered one or more of the following and should check what you have received against the following list.

1.1 SX.18MV-R.

This is the basic single wavelength, single mixing, absorption, fluorescence stopped-flow spectrometer. It consists of the following components:-

a) Power controlled Xenon lamp power supply.

b) 150W Xenon lamp housing fitted with your choice of ozone free Xenon lamp (cuts off at 250 nm) or ozone producing Xenon lamp (cuts off at 205 nm). Mounted behind the Xenon lamp housing is the 'SafeStart' igniter.

c) Monochromator fitted with a 250 nm holographic grating.

d) Optical rail to mount the lamp housing and monochromator onto.

e) Optical coupler, a light guide which connects the output from the monochromator to the sample handling unit's optical cell.

f) Sample handling unit, fitted with 20µl cell cartridge.

g) 5µl cell cartridge and 1ml stop syringe for short (500µs) dead time work.

h) 9 stage side window absorption photomultiplier (Hamamatsu R928) with high voltage and signal leads and red high pass filter (190nm to 850nm).

i) 12 stage end window emission photomultiplier (Hamamtsu R6095) with high voltage and signal leads and 305nm & 320nm cutoff filters (280nm to 600nm). An alternative tube (Hamamatsu R1104) is available if extended wavelength range is required (200nm to 900nm)

j) Photometric control unit. (A cream coloured rectangular box with connectors on the back.)

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k) Acorn computer fitted with IIC communication card and 12 bit ADC card.

l) Multiscan monitor.

m) Colour bubblejet printer.

n) The following accessories:- A set of shielded IIC bus connecting cables; a set of analogue control cables; a long length of high pressure hose with two hose clamps and a metal pneumatic connector to connect the compressed gas bottle to the sample handling unit; an h connector to connect the high pressure hose to the two ports on the back of the sample handling unit, a pressure regulator and gauge for reducing the pressure on the stopped-flow drive ram when performing variable ratio mixing, two glass reservoir syringes; a bag of spare fuses; a set of metric hex drivers; a filtered main electrical distribution board; a set of power leads; a set of manuals and back up software.

1.2 SQ1

This is the sequential flow accessory and consists of the following components:-

a) The sample handling unit will have four drive syringes instead of two and will also have extra drive valves, plumbing etc.

b) Two extra drive rams. One with a knurled spacer and one with a plain spacer.

c) A stop syringe brake assembly.

d) Two extra glass reservoir syringes.

1.3 SK1

This is the scanning option that allows steady state and time resolved absorption spectra to be collected automatically. This option involves changes to the electronics and software and does not involve any extra components.

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1.4 SK1E

This is the extended scanning option. It allows steady state and time resolved emission spectra to be collected automatically. As an alternative it allows for the two monochromators to be used on the excitation/absorption side giving much better stray white light inhibition. This allows reliable performance in the far UV (250 nm to 205 nm) and accurate measurements of large absorptions (1.5 to 2 AU). It consists of the following components:-

a) An extended optical rail which is long enough to have the lamp and two monochromators mounted in series on it. A shorter rail is mounted on extension rods, above the main rail, to support the lamp and monochromator when performing monochromatic emission work.

b) An emission light guide to couple the emission port of the sample handling unit optical cell to the second monochromator. This optical coupler differs from the absorption/excitation coupler in that the monochromator mount (the large end) has a round aperture in the absorption/excitation coupler and a slit aperture in the emission coupler.

c) A channel two monochromator along with its mains electricity cable and IIC bus cable.

d) A monochromator coupler to attach the exit of monochromator one to the entrance of monochromator two with minimal light losses.

1.5 DD1.

This is the Dual Detection option. It allows absorption and fluorescence data to be collected simultaneously. It consists of a photometric control unit with Channel 2 printed on the front.

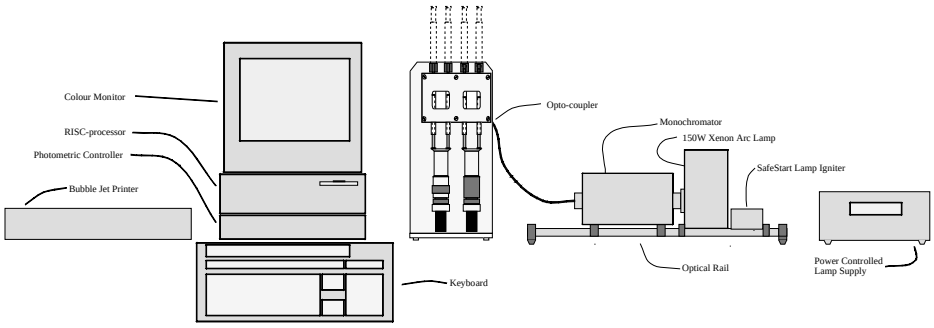
1.6 AN1.

This is the anaerobic accessory. It increases the anaerobic capabilities of the instrument. It consists of the following components:-

A set of 4 three-way valves with luer fittings.
An anaerobic manifold.

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2.0 Instrument Set-up



2.1 Basic SX.18MV-R Instrument Set Up (Mechanical)

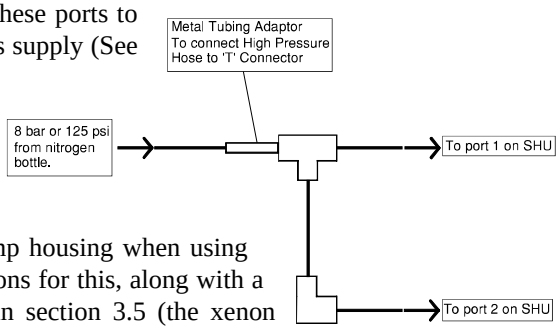
Assemble the SX.18MV-R spectrofluorimeter as indicated above, with the 150W Xenon arc light source and SpectraKinetic monochromator positioned on the optical rail to the right of the sample handling unit. (The sample handling unit is shown with the SQ1 option fitted.) Ensure that the lamp housing snout is pushed fully into the flange attached to the monochromator entrance port. On certain workbenches vibration from the fan in the lamp power supply may be transmitted to the optics. This will show up as a 50 or 60 Hz ripple on your traces. If this should be the case mount the lamp power supply on a separate bench or on a shelf behind the bench. If neither of these options are suitable isolate the lamp power supply from the bench by placing it on a foam base with a rigid top surface (Thick computer mouse pads work quite well). Ensure that the ventilation holes on the bottom of the PSU are not obstructed.

Connect the waste tube to the stop valve outlet. Put the free end of the waste tube into a suitable receptacle.

The main pneumatic inlets (labelled 1 & 2) are on the back of the unit. Port 1 connects to the right hand drive ram, used for sequential mixing, and to the AutoStop. Port 2 connects to the left hand drive ram.

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An 'h' connector allows both of these ports to be connected to a compressed gas supply (See Diagram to right). The input gas pressure must be set to 125 psi (8 bar). Compressed Nitrogen is usually used. This



for supplying nitrogen to the lamp housing when using ozone producing lamps. Instructions for this, along with a connection diagram are shown in section 3.5 (the xenon lamp section)

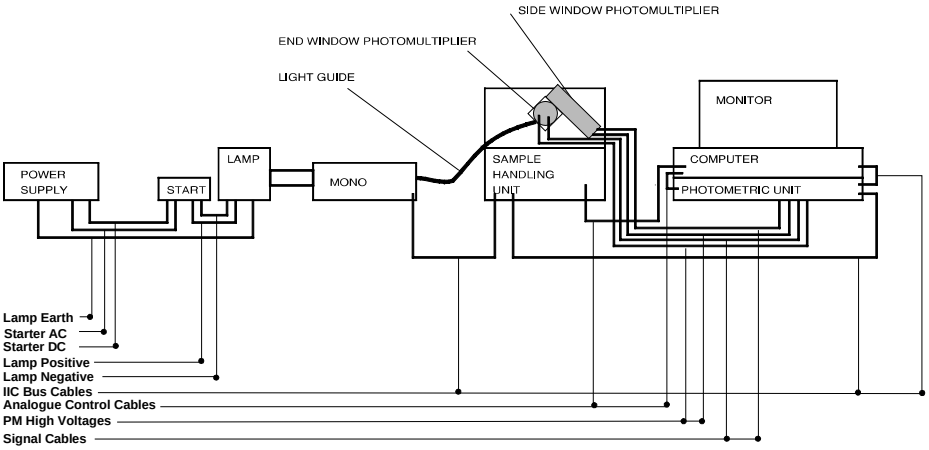
Push the larger end of the opto-coupler into the exit port of the monochromator and the other end to one of the two lower ports available on the cell block on the back of the sample handling unit sample handling unit. The short pathlength is accessed from the lower right hand port and the long pathlength is accessed from the lower left hand port. When the unit is shipped two of the ports have plastic blanking plugs fitted to them. These plugs should be fitted to the two ports on the unused pathlength. When making fluorescence measurements, the fluorescence signal is maximised by bringing the excitation light into the short pathlength port so that the full length of the optical cell is evenly illuminated. This also results in the best linearity due to the lower internal re-absorption. The securing screw (with nylon washer) for the opto-coupler mounting flange ensures that it is mounted in the correct orientation. Attach the emission photomultiplier to the rear port on the cell block. (NOTE: Check the front surface of the photomultiplier before fitting it as some tube are shipped with a protective black plastic cover over the front which must be removed before use.) Fit the absorption photomultiplier to the port opposite that used for light input.

The two tubing connections on the lower right of the sample handling unit water bath are for connecting a circulating thermostatic water bath. The front port is the inlet and the rear port is the outlet. It is important that the inlet and outlet are connected correctly otherwise the sample handling unit thermostat area will not fill correctly. It is suggested that the circulating thermostatic water bath is positioned below the sample handling unit so that it is easy to drain the sample handling unit into it. Draining is best done by removing the small bleed screw on the very top of the cell block on the back of the sample handling unit. If the sample handling unit is to be used at low temperature then the cell block must be purged with nitrogen to prevent condensation forming on the optics. An attachment for connecting a nitrogen line to the cell block is available from Applied Photophysics Ltd or one can be made by pushing a short hypodermic needle through a solid rubber stopper that will fit into an unused port on the cell block. The nitrogen line can then be connected to the hypodermic needle and gently flowed into the cell block.

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2.2 Basic SX.18MV-R Instrument Set Up (Electrical)

SX.17MV Electrical Connections (Rear View)



2.21 Mains Electricity Supply

All of the units should be set for the mains electricity voltage of your particular country. All of the units are provided with mains cables terminated in IEC plugs. These plugs should be connected to the distribution bus provided. The distribution bus should be terminated by you with a plug suitable for your country. This means that only one socket is required to power the entire system. The printer is a 240 volt printer. In countries that do not have a 240V electrical supply the printer must be supplied via its step-up transformer. The printer mains cable plugs into the transformer, the transformer is then plugged into the distribution bus. If the supplied printer is ever replaced with a printer purchased locally, then the step-up transformer must be removed. The mains electricity connections are not shown in the diagram above so that it can be more easily understood.

2.22 IIC Communication Bus

The computer communicates with the photometric control unit, the sample handling unit and the monochromator by means of an IIC communication bus. All of the units on the bus have two small rectangular connections that look like telephone connections. These are the sockets for the bus. The units must be connected together in the following order; computer to photometric control unit to sample handling unit to monochromator. The two sockets on each unit are identical, there is no in and out. This means that it does not matter which connectors are used.

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2.23 Arc Light Source.

One loose, braided cable provides the earth link between the lamp housing and the optical rail. The SafeStart igniter has two black inlet cables and two outlet cables, one white and one red. The outlet cables plug into the back of the lamp housing. The white cable is the negative and also carries the ignition pulse to start the lamp, hence the heavy insulation. The red cable is the positive. The two inlet cables connect to the back of the lamp power supply. The lamp power supply and igniter should be treated as one electrical entity with the following characteristics:-

Supply voltage	*100V, 115V, 120V, 200V, 220V and 240 Volts*
Supply frequency	*50/60 Hz*
Power rating	*330 VA*
Fuses (110V - 120V)	*5 A (T)*
Fuses (200V - 240V)	*2.5 A (T)*

2.24 SpectraKinetic Monochromator.

One of the IIC sockets on the side of the control box on the rear of the monochromator is connected to one of the sockets on the sample handling unit.

Supply voltage	*120/240 Volts*
Supply frequency	*50/60 Hz*
Power rating	*20 VA*
Fuses (120V)	*500 mA (T)*
Fuses (240V)	*250 mA (T)*

2.25 Sample Handling Unit.

One of the IIC sockets is connected to the monochromator, the other IIC socket is connected to the photometric control unit. The 9 way analogue control socket is connected to the ADC card in the back of the computer.

Supply voltage	*120/240 Volts*
Supply frequency	*50/60 Hz*
Power rating	*100 VA*
Fuses (120V)	*1.6 A (T)*
Fuses (240V)	*800 mA (T)*

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2.26 Photometric Control Unit.

Two sets of photomultiplier cables (set = signal cable + high voltage cable) connect the absorption photomultiplier and emission photomultiplier to the photometric control unit. The sockets on the back of the unit are clearly labled. The analogue control socket connects to the ADC in the back of the computer. One of the IIC sockets is connected to the sample handling unit, the other is connected to the computer.

Supply voltage	*120/240 Volts*
Supply frequency	*50/60 Hz*
Power rating	*20 VA*
Fuses (120V)	*1 A (T)*
Fuses (240V)	*500 mA (T)*

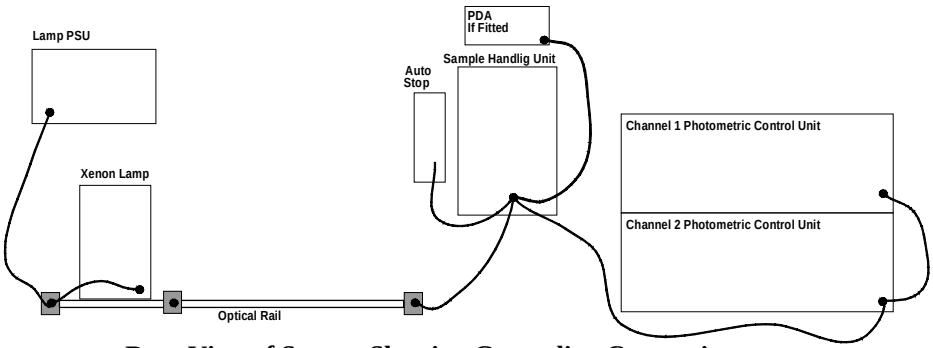
2.3 Grounding (Earthing) Connections

The electronic circuitry used in the stopped-flow spectrometer is very sensitive and must be properly grounded. All of the discrete electronic units, except the computer and the monochromators, have grounding posts on the back. A set of braided grounding straps is provided with the system. The units should be connected together to provide a common ground base.

The actual grounding of the system is through the mains supply ground. It is vital that the mains electrical supply in your laboratory is properly grounded.

The diagram below shows the basic configuration of the grounding connections.

Note: The computer and monochromators are separately grounded.



Rear View of System Showing Grounding Connections

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2.4 SQ1 Set Up

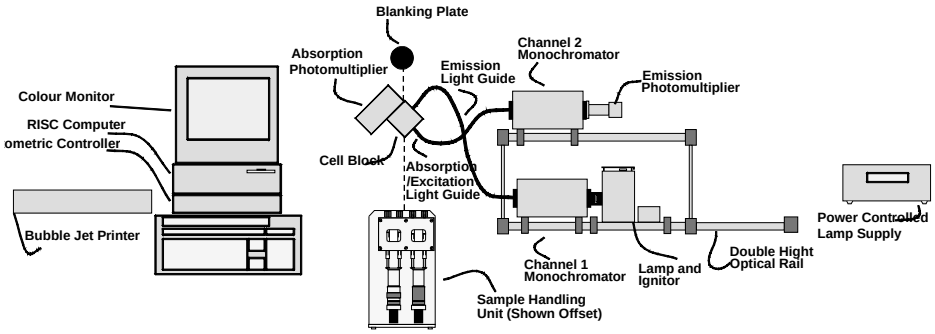
The sequential mixing option is described fully in its own chapter later in this manual.

2.5 SK1 Set Up

The basic scanning option does not involve any extra hardware and so requires no extra setting up instructions.

2.6 SK1E Set Up

This option allows the user to choose between two different instrument configurations. The first and more general configuration allows for emission wavelengths to be selected by the channel 2 monochromator. Absorption/excitation wavelengths are selected by the channel 1 monochromator. The instrument layout for this configuration is shown below.



The general assembly of the components is as for the basic system. The following points, however, should be noted.

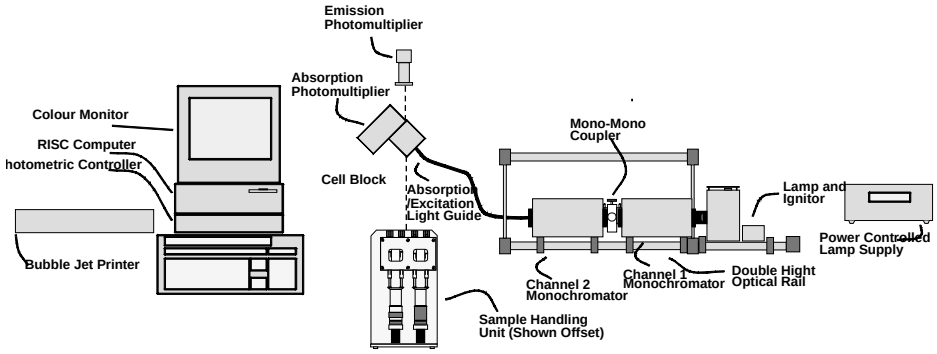
The sample handling unit is shown in a partially exploded form so that the cell block attachments may be seen clearly. The blanking plate fits over the port usually used by the emission detector and stops external room light from entering the cell.

The easiest way to differentiate between the two light guides is to look at the ends with the large black plastic couplers, which fit into the two monochromators. The absorption/excitation light guide has a round aperture on this end whilst the emission light guide has a slit aperture on this end.

The lamp is always connected to the channel 1 monochromator and **never** to the channel 2 monochromator.

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The only additional electrical connection required is that the IIC socket on the channel 2 monochromator is connected to the spare IIC socket on the channel 1 monochromator.
The second configuration allows for better stray white light elimination. It is used when studying absorption in the far UV, (below 260 nm). It is also used when studying high absorptions, (greater than 1.5 AU). Emission work must now be performed in the traditional manner using cut-off filters. The layout of this configuration is shown below.



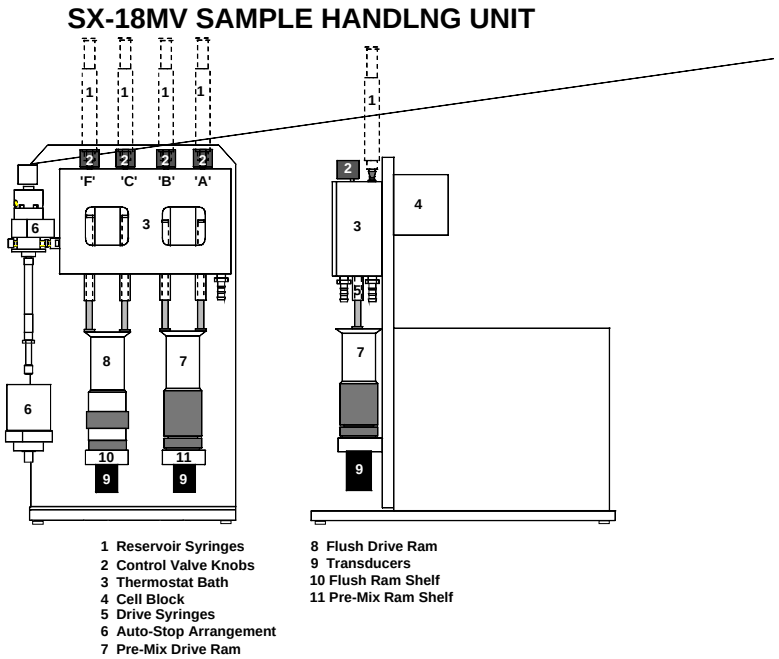
2.7 DD1 Set Up

The channel 2 photometric control unit that comes with the DD1 option should be placed under the channel 1 photometric control unit. The analogue out socket is connected to the CH-2 socket on the ADC card in the back of the computer. The channel 1 photometric control unit IIC socket that is normally connected to the sample handling unit should be connected to one of the IIC sockets of the channel 2 photometric control unit. The other IIC socket on the channel 2 photometric control unit is connected to the IIC socket on the sample handling unit.

The absorption signal and high voltage leads are connected to the channel 1 photometric control unit's sockets. The emission signal and high voltage leads are connected to the channel 2 photometric control unit's sockets. Absorption data can now be collected on channel 1 and emission data can be collected on channel 2. If the dual channel option is selected in software then the two channels can be set up independently and data can be collected from both channels simultaneously.

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3.0 General Information and Notes



3.10 Sample Handling Unit

The diagram above shows the sample handling unit fitted with the sequential mixing option. The drive syringes, flow lines and optical cell are surrounded by a thermostat bath. The flow circuit is chemically inert and free of stainless steel. It is constructed of glass (syringe barrels), silica (optical detection cell), PEEK (sample flow circuit tubing and drive valves) and Teflon (syringe piston seals). NB Kloehn drive syringes are now fitted as standard. These can be obtained from Kloehn Ltd., 10000 Banburry Cross Drive, Las Vagas, NV−89134, Telephone 702 243 7727, Fax 702 243 6036. The part number for the 2.5 ml syringe is 18196.

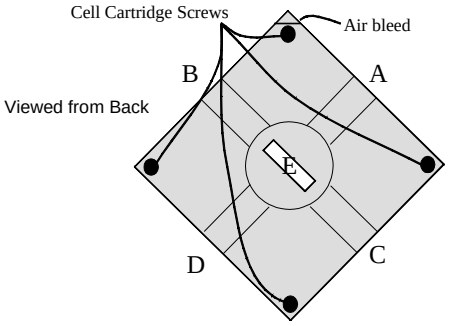
To satisfy the differing needs of the stopped-flow mixing and sequential stopped-flow mixing techniques three drive rams are supplied on the full sequential-flow machine. On the single-mixing instrument only one drive ram is supplied. A supply of compressed gas, usually nitrogen or air (pressure 8 bar for symmetric mixing), is required to operate the rams. Internal factory preset regulators set the working pressures and on no account should these settings be changed, as excess or insufficient pressure is likely to cause damage. FOR ASYMMETRIC STOPPED-FLOW MIXING EXPERIMENTS THE INPUT PRESSURE MUST BE REDUCED (see asymmetric section of manual).

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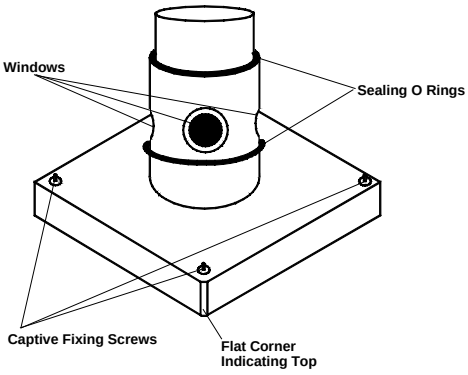
3.11 Cell Block

The SX.18MV-R uses a novel cell cartridge system that allows cells to be changed with a minimum of work. The standard SX.18MV-R comes fitted with 20µl cell, giving pathlengths of 2mm and 10mm. This cell can readily be changed for cell with different pathlengths, ie a 5µl cell giving pathlengths of 1mm and 5mm. The larger cell has a dead time of around 1ms with very high sensitivity. The smaller cell sacrifices some sensitivity for a much faster dead time around 500 µs.

All cells have a long and short pathlength. The long pathlength is between ports B and C. The short pathlength is between ports A and D. The optical pathlength is selected by moving the Absorption Photomultiplier (A or B ports) and Opto-coupler (C or D ports) to the respective ports on the cell block.



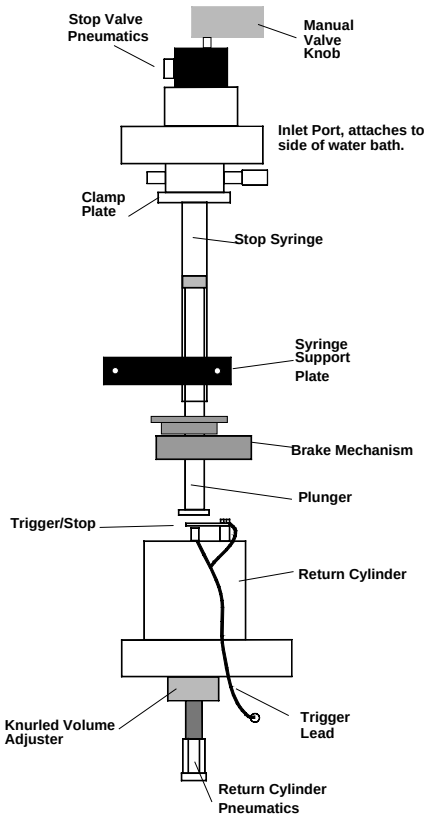
The fluorimetric port, E, is at the back of the cell block and the Emission Photomultiplier (high sensitivity) should be mounted using the rubber light seal, finger screws and nylon washers provided. When measuring fluorescence the excitation light guide should be connected to the D port to give the best illumination and lowest inner filter effect.



Cells are mounted in a cartridge, shown in the diagram to the left. To change the cartridge, first remove the light guide from the cell block. Then drain the thermostat water from around the drive syringes and remove the water bath front plate. Next, loosen the tubing pressure plate that forces the flow tubing onto the cell. (See section 3.23 for more information regarding the pressure plate.) Finally undo the four captive fixing screws and remove the cell cartridge. When fitting the new cell cartridge, ensure that the flat corner of the cartridge is at the top. Fix the cartridge in place with the four captive fixing screws. Tighten the tubing pressure plate to ensure a good seal between the three flow tubes and the cell. Replace the light guide and the thermostat bath cover. Flush the flow circuit with distilled water until you are confident that all of the air bubbles have been expelled.

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3.20 Auto Stop Mechanism.



The Auto Stop mechanism, shown to the left, is fixed to the front left hand side of the Sample Handling Unit. The valve and the return cylinder are both controlled from the workstation, although both can be operated manually as long as the pneumatic drive pressure has been removed, (when flushing the system, for instance). The three pneumatic inlets are connected to three solenoids inside the Sample Handling Unit.

The Stop Syringe is emptied by clicking on "Empty" in the "New Data" section of the software, or automatically when "Acquire" is clicked to acquire data.

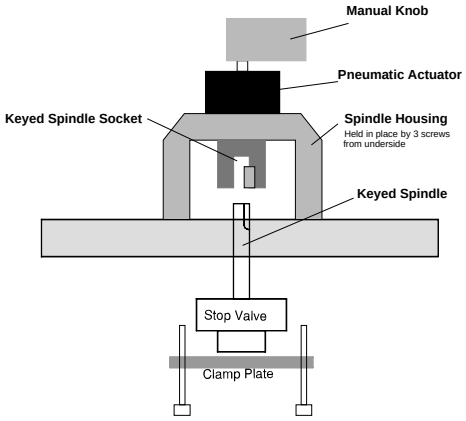
When collecting kinetic traces at different wavelengths to produce time resolved spectra the instrument will reset itself before each run, minimising the operator's involvement.

3.21 Stop Syringe Brake Mechanism

The brake mechanism, (see view above), is provided to improve the results obtained when performing sequential flow experiments. It is used to provide friction on the stop syringe plunger and hence prevent over-run after the first drive. This would result in cavitation in the flow line and hence poor results. The friction is increased by tightening the mechanism decreased by loosening it. It must be loose for single mixing stopped-flow so that it does not impair the instrument's dead time, but tightened up, as necessary, when performing sequential flow experiments. It is unlikely that the brake assembly will be required on the most up to date systems which are fitted with lightweight PEEK piston stop syringes. The very low inertia of these PEEK pistons means that they are unlikely to over-run.

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3.22 Procedure For Changing Stop Valve



When following the instruction for changing the stop valve please refer to the diagram on the left.

1) Disconnect all connections from the stop valve.

2) Ensure that the Manual knob is in the DRIVE position

3) Remove the stop syringe.

4) Undo the two screws holding the clamp plate in position and remove the clamp plate.

5) Pull the old stop valve out of the stop assembly

6) Gently insert the spindle of the new valve into the stop assembly. Rotate the valve and spindle until the keyway lines up and push the valve fully into the stop assembly.

7) Ensuring that the manual knob does not rotate, turn the valve until one of the ports faces to the front of the stop assembly. (As the ports are 180 degrees apart and are identical, it does not matter which port is facing forwards.)

8) Use a torch (flashlight) to check that the port in the valve body is lined up with the port in the valve spindle.

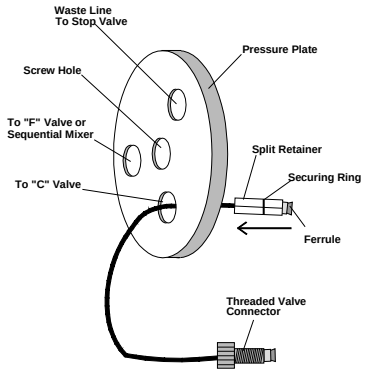
9) Replace the clamp plate and secure it with the two clamp plate screws. These screws need to be tightened so that they will stop the valve from rotating when its spindle is turned, but not so tight as to distort the valve.

10) Replace the stop syringe.

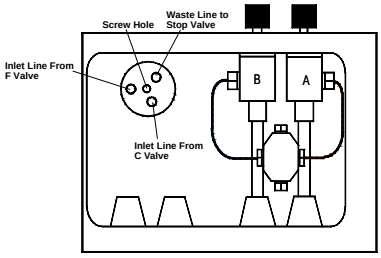
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3.23 Flow Tubing.

As of January 1998 Applied Photophysics is using custom made Polyetheretherketone (PEEK) tubing terminated with compression fittings. PEEK tubing allows for smoother curves and is more robust than Teflon tubing. Compression termination has the advantage of being easier to make than flanged termination, is able to withstand considerable higher pressure and is generally more robust. This tubing is supplied made up and ready to be fitted into the sample handling unit. The tubes are attached to the cell using a pressure plate, which enables both inlet tubes and the outlet tube to be tightened or loosened simultaneously.



The three tubes that connect to the cell must be assembled into the pressure plate as a complete set before being attached to the cell or to their respective valves. The diagram on the left shows the pressure plate with one of the three tubes. One end of the tube is fitted with a threaded valve connector. This is the end that will screw into the valve. The other end of the tube has a compression ferrule fitted to it. To fit a tube, pass the ferrule end through the hole in the pressure plate; fit the split retainer (a two part stainless steel retainer) around the tube and secure it in place with the securing ring; push the tube, along with its split retainer in the direction of the arrow so that it secures into its place in the pressure plate.



After assembling the three tubes into the pressure plate the assembly can be fixed to the cell block from inside the sample handling unit water bath. (See diagram to left.) This is most easily done by removing the “C” and “F” valves to allow good access to the rear of the cell block.

The full list of tubes are as follows:

Single Mix	**Sequential Mix**
	PK18 - A Valve to SQ Mixer
	PK17 - B Valve to SQ Mixer
PK25R - F Valve to Cell	PK19 - F Valve to Connector
	PK22 - Connector to SQ Mixer
	PK16R - SQ Mixer to Cell or Connector to Cell
PK15R - C Valve to Cell	PK15R - C Valve to Cell
PK20R - Cell to Stop Valve	PK20R - Cell to Stop Valve

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3.3 Light Source Assembly

The light source assembly comprises a short optical rail upon which the monochromator and the arc light source are mounted. The opto coupler is attached to the exit slit of the monochromator and is used to couple the probe light for absorption measurements and/or the excitation light for fluorescence measurements to the observation cell of the sample handling unit. The individual components are described in more detail below.

3.4 SpectraKinetic Monochromator

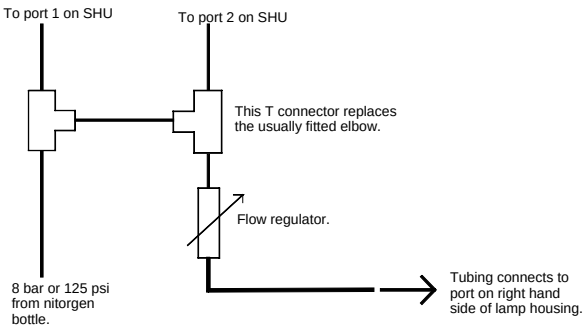
This is a precision instrument that has been accurately aligned and calibrated in our laboratories using a He-Ne laser and mercury emission lines. It is fitted with a 250nm holographic diffraction grating giving a useful operating wavelength range of 200 to beyond 700nm. The entrance and exit slits are continuously variable and allow the bandpass to be varied from below 0.5 nm up to 37 nm. The slit blades are ground to a close tolerance and mechanically linked to a multiturn dial calibrated in mm. Select a slit width (typically in the range 0.25 to 2 mm) compatible with the required light throughput and spectral requirements. When choosing a slit width the operator should always bear in mind how susceptible to photo-degradation his samples are. The optical system delivers so much light that, for example, Tryptophan exhibits photo-degradation when the slit width is set to greater than 0.5 mm (2.3 nm bandpass). The wavelength bandpass is calculated by multiplying the slit width readings (in mm) by 4.65. Bandpasses below 2 nm are most accurately set using feeler gauges. The final bandpass adjustment should always be set by moving the dial in a clockwise manner, this procedure will give maximum accuracy and reproducibility.

3.5 Arc Light Source

The light source uses a 150W gap shortened xenon bulb and includes a housing, a Safe-Start Igniter and is powered by the power controlled lamp supply. The rectangular image (approx. 1.5 x 10 mm) of the lamp arc obtained from the housing owes its shape to an unconventional optical arrangement in which the phenomenon of astigmatism has been utilised.

If you have specified a far UV light source then the action of the UV light on the oxygen in the atmosphere will produce ozone. Ozone fumes are hazardous to health if breathed in and will also damage the light seal of the monochromators. It is necessary, therefore, to prevent ozone production. This is achieved by purging the lamp housing with inert gas, usually nitrogen. A tubing attachment is located on the right hand side of the lamp housing for this purpose.

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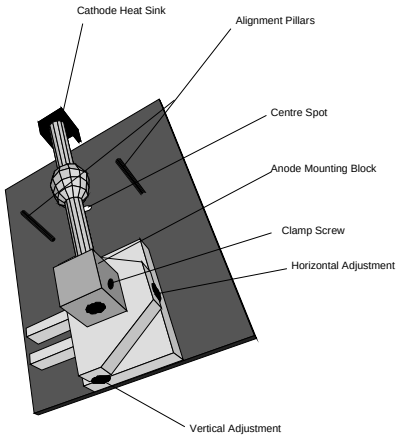
If nitrogen is being used as the main drive gas supply then the elbow on the 'h' connector used to connect the gas supply to the back of the sample handling unit can be replaced with the supplied flow regulator assembly as shown in the diagram to the left. The tubing on the outlet of the flow regulator should be connected to the purge inlet on the right hand side of the lamp housing. Before starting the lamp the flow regulator should be opened to allow a minimal steady flow of gas through the lamp housing. This flow should be allowed to continue for at least 15 minutes prior to starting the lamp. This will ensure that the lamp housing is completely flushed through and that all the air in the lamp housing is replaced with nitrogen. The nitrogen flow **must** be maintained whilst the lamp is running.

There are a number of potential HAZARDS associated with any arc light source

- Never, under any circumstances, ignite or run the lamp outside of the lamp housing.
- The latest Applied Photophysics NON-INDUCTIVE Starter Unit uses a D.C. ramp voltage to ignite the lamp. Whilst it will not affect sensitive electronic units or computer programs running in the vicinity of the lamp during lamp ignition, it is still good practice to start the lamp before any other electronic units.
- During ignition the operator should as a precautionary measure keep at a distance (say 0.5 m) from the cables that connect the lamp housing and the igniter.
- The intense light output from the lamp should not be directly observed, it contains a considerable amount of ultraviolet radiation.
- When running, the arc envelope contains several atmospheres of pressure. It should NEVER be uncovered or handled whilst hot and the operator must wear safety goggles/visor when changing the lamp.
- If the quartz envelope is accidentally touched by bare skin, it is important that the envelope is carefully cleaned using a soft cloth moistened with ethanol.

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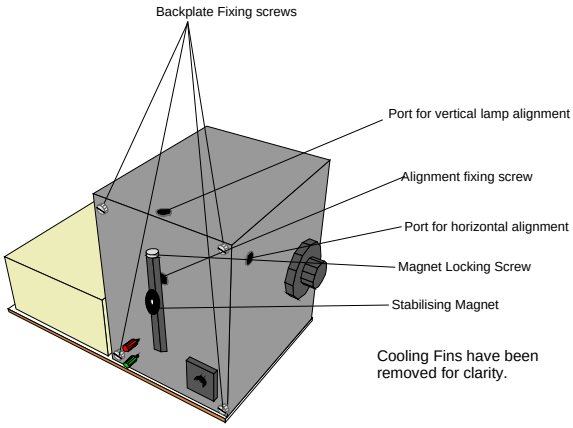
Replacing The Xenon Bulb.



Two types of xenon lamp are used in Applied Photophysics stopped-flows. The ozone free lamp which gives very high stability but cuts off at 250nm is manufactured by Osram and is part number XBO 150W/CR OFR. It is available from Applied Photophysics or can be sourced in the United States from Osram Sylvanian Inc. 100 Endicott Street, Derse, MA−01923, Telephone 508 777 1900. The ozone producing bulb is not quite as stable as the ozone free lamp but goes down to 200nm. It is manufactured by Hamamatsu and its part number is L2273. Note that for all xenon lamps the positive end (anode) is the end with the larger electrode.

XENON ARC BULBS CAN EXPLODE IF MISHANDLED

Disconnect all the cables and remove the lamp housing from the optical rail. As the lamp operates at a very high internal gas pressure it is very important that it is only handled when cold and protective equipment (especially a face visor) is worn. Loosen the magnet locking screw and remove the stabilising magnet. Remove the rear panel by undoing the four large corner screws. (See diagram to the right.) Loosen the clamp screw on the anode mounting block so that the top of the lamp can move freely, then remove the cable and the cathode heat sink from the bottom of the lamp. (Before removing the heat sink make a note of its orientation.) It should now be possible to remove the lamp from the top clamp. Loosen the alignment fixing screw on the back of the plate and adjust the horizontal and vertical adjusters so that they are approximately in the centre of their travel. Fit the positive end of the new lamp into the anode mounting block but do not tighten the clamp screw yet. Fit the cathode heat sink (in the correct orientation) and cable to



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the negative end of the lamp. Adjust the vertical position of the lamp so that the gap between the electrodes is level with the two alignment pillars.. Now tighten up the clamping screw on the anode mounting block. Check that the gap between the electrodes is vertically level with the two alignment pillars and horizontally level with the centre spot. If this is not the case adjust the horizontal and vertical position of the lamp using the adjusters shown in the above diagram. Make sure that you remember to tighten up the fixing screw on the back of the lamp backplate when you have finished to clamp the lamp adjustment assembly into place. The rear panel can now be refitted to the back of the lamp housing using the four corner screws.

Fine tune the alignment of the lamp housing optics by using the real time display provided on the spectrometer workstation. This is done by slightly loosening the alignment fixing screw, removing the adjuster screw plastic cover plugs and carefully adjusting the vertical and horizontal alignments whilst watching the live display on the workstation monitor. The lamp should be adjusted to give the maximum signal (ie the lowest absorption)

It is essential that the adjustment is made using the red insulated hexagonal driver, so that there is no risk of electrical shock. It is also important that the alignment fixing screw is not loosened too much. If it is too loose then the X-Y adjustment block will wobble as you try and align it making accurate alignment almost impossible. Generally loosening the alignment fixing screw by less than one quarter turn is enough.

The lamp should be adjusted so that the live display shows maximum signal. First set the wavelength to 450nm and the monochromator slits to 0.5mm. Ensure you are using the absorption detector. Use the AutoPM so that the software sets the PM high voltage to give a 0AU signal. Now use the X and Y lamp adjusters to make the absorption signal as negative as possible. If the signal disappears from the bottom of the display, click on AutoPM again to reduce the PM high voltage.

Once everything is aligned and locked the lamp should not require re-aligning. Use the insulated hexagonal driver to lock the lamp into position as well as for adjusting it. After aligning the lamp remember to replace the adjuster screw plastic cover plugs and the stabilising magnet (if fitted).

The lamp will take up to 30 minutes to stabilise after ignition and the lamp should be replaced every 1000 operational hours or when its output becomes unstable. The shutter is closed when it is pulled to the front. The alignment of the lamp is critical, as any misalignment will result in excess noise, due to both a lower light level and, more importantly, increased vibrational noise.

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3.6 Power Controlled Lamp Supply.

The lamp power supply is a purpose-designed unit of the very highest specification. The excellent short-term stability of this unit is such that the stability of the xenon arc source is close to that of filament light source. This is a major achievement and means that for all but the most demanding spectrophotometric experiments the xenon arc light source is the preferred source. The unit has thermal switches and a cooling fan to protect the unit from over heating. It should therefore be placed in a well ventilated position on the bench with at least 150mm between the fan outlet at the back of unit and a vertical surface.

3.7 Spectrometer Workstation
- for full operational details refer to the SX.18MV SOFTWARE MANUAL.

The spectrometer workstation comprises of a photometric controller, 32 bit RISC-processor unit, 12 bit analogue-to-digital converter (ADC), IIC communication interface, hard disc, colour monitor and inkjet printer. The spectrometer workstation is supplied with a complete data acquisition and processing software package with a graphics based file and screen management system. The 12 bit ADC, used to digitise the signal output from the photomultiplier, is located within the RISC-processor unit. The workstation features a live display of the incoming signal to assist in setting up the spectrometer and to allow real time viewing of all kinetic traces. The ADC sampling rate can be selected as required. The shortest sweep time is 5 ms and the longest provided is 1000 s.

In addition to signal conditioning circuits, the photometric controller provides the variable high voltage used to control the gain of the photomultiplier tubes. The photomultiplier output signal enters the photometric controller through a differential amplifier to minimise electrical noise. The signal then passes through a Sallen-Key analogue filter circuit with switchable time constants so that photomultiplier shot noise can be reduced by the maximum amount compatible with the particular reaction rate being measured. The Sallen-Key filter results in a twofold improvement in noise reduction compared to a simple low pass filter design. A signal offset (null) facility allows small changes in signal level to be amplified. When making absorption measurements, a fixed (4.0 volt) offset is provided and normal operation requires that the gain of the photomultiplier be adjusted so that the total signal output from the photomultiplier is equal to the fixed offset. This procedure greatly simplifies the setting up and use of the instrument. For fluorescence and light scattering measurements where variable background levels are encountered due to stray light, the variable offset facility is used.

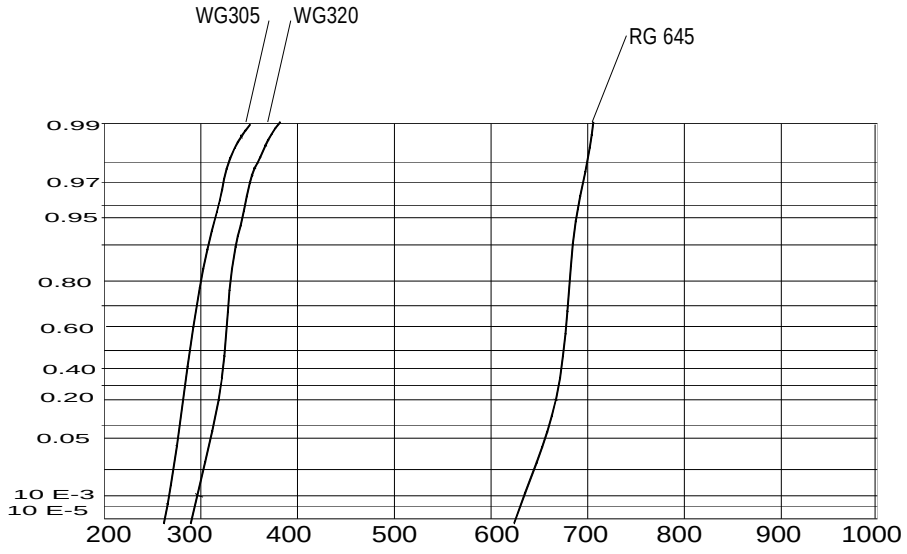
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4.0 Use of optical filters.

In some circumstances it is advisable to use an optical filter in the space provided between the photomultiplier and the cell block to remove unwanted wavelengths.

NIR Absorption Measurements. When operating at wavelengths in excess of 650 nm it is important to be aware that the light leaving the monochromator will also contain a small proportion of light with a wavelength equal to half that set on the monochromator wavelength dial (second order stray light). This phenomenon is common to all monochromators fitted with diffraction gratings. In order to remove this second order light an RG645 filter is supplied to remove all wavelengths below 645 nm.

Fluorescence Measurements. When measuring fluorescence it is important that the excitation light does not get to the photomultiplier and swamp the fluorescence signal. In order to do this it is important to place a cutoff filter between the photomultiplier and the sample cell. This cutoff filter should be of a value higher than the excitation wavelength (so that it cuts it off), but lower that the emission wavelength (to allow it to pass). Two such filters are provided with the system, one which cuts off below 305nm and the other, which cuts off below 320nm. Other filters are available.

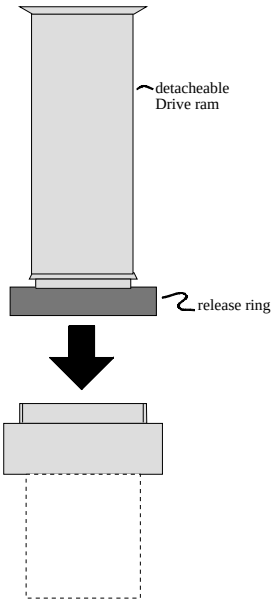


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5.0 Operating Procedures

Single Mixing Stopped-Flow

The following procedure describes the use of the syringes and the various valves during sample loading, sample flow circuit flushing and stopped-flow mixing. *NOTE – for shipping purposes the sample flow circuit is flushed with anti-freeze solution. It is important that this circuit is well flushed with distilled water before use.*



Attach the waste tube to the stop valve on the automatic stop and place the free end in a waste bottle.

Fit the stopped-flow ram to the left hand (FLUSH) side of the Sample Handling Unit so that it can drive the 'C' and 'F' syringes.

Fit the black plastic dust cover to the PRE-MIX ram shelf (if fitted).

Screw the knurled volume adjuster on the Auto Stop fully clockwise (i.e. front edge moves from left to right), then screw it two and a half turns anti-clockwise (counter-clockwise). This equates to a total flow volume of approximately 100 µl.

Fit two reservoir syringes filled with distilled water to the 'C' and 'F' Luer fittings. It is recommended that 10 ml plastic disposable syringes are used.

Turn the 'C' and 'F' control valves to the FILL (side) position. Ensure that the drive ram is pushed fully down. Fill the 'C' and 'F' drive syringes by pushing down the plungers of the reservoir syringes. Expel any air bubbles by flushing backwards and forwards between the drive syringes and the reservoir syringes several times.

Turn the 'F' control valve to the DRIVE (forward) position and manually push the water from the drive syringe, through the flow circuit and into the stopping syringe.

Empty the stopping syringe by clicking on empty in the "NEW DATA" context of the software.

Repeat the above two steps until the drive syringe is empty.

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Turn the 'F' control valve to the FILL (side) position and the 'C' control valve to the DRIVE (forward) position and manually push the water from the drive syringe, through the flow circuit and into the stopping syringe.

Empty the stopping syringe by clicking on empty in the "NEW DATA" context of the software.

Repeat the above two steps until the drive syringe is empty.

You may find that is difficult to expel the last few bubbles from the stopping syringe. If this is the case then empty the syringe, unscrew it from the valve, fill the syringe with distilled water (from a beaker), hold the syringe vertically over the beaker and gently expel the bubbles, empty the syringe into the beaker, screw it back into Auto Stop valve. It is now ready for use.

Ensure that all the control valves are set to the FILL (side) position. Replace the 'C' and 'F' reservoir syringes with ones containing your samples. If one of your samples is more dense than the other then the densest solution **must** go into the "F" drive syringe. If both of your samples are of the same density then the most expensive sample, usually the protein solution, should go into the "C" syringe as it has the smaller priming volume. Fill the drive syringes by pushing down the pistons of the reservoir syringes (this ensures that there is no cavitation inside the drive syringes and, therefore, no bubbles).

Prime the sample handling unit by manually pushing 200 µl from the right hand "C" syringe and 300 µl from the left hand "F" syringe.

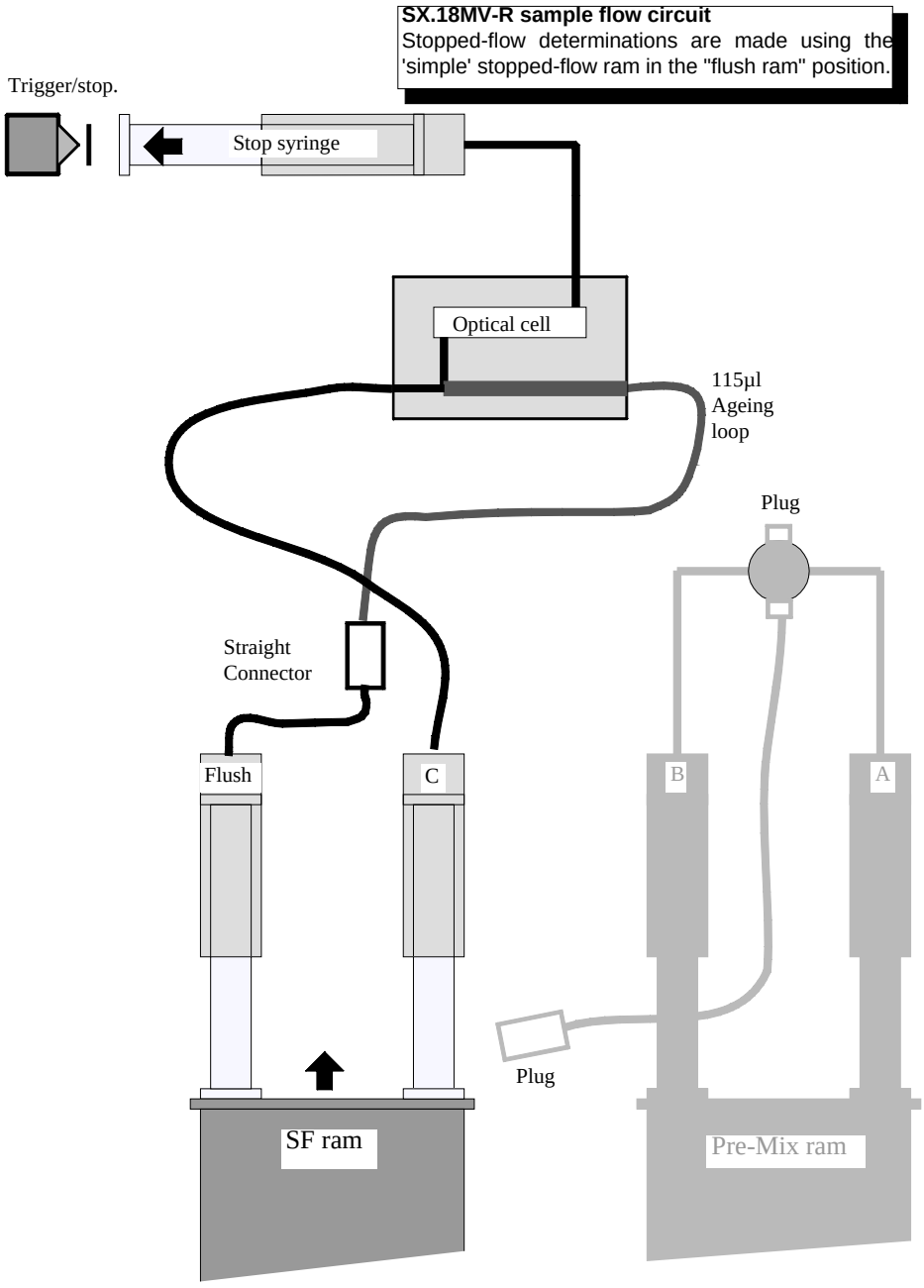
Completely fill both of the drive syringes and ensure that there is no gap between the drive ram and the drive syringe plungers.

Set the both control valves to the DRIVE (forwards) position.

THE SAMPLE HANDLING UNIT IS NOW READY
(now refer to the Software Manual)

Twenty five determinations can be made in quick succession before the drive syringes will need to be refilled.

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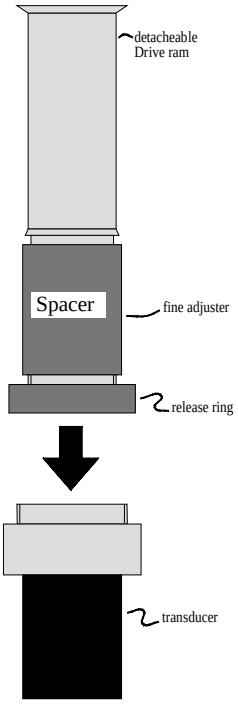


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5.1 Operating Procedures

Sequential Mixing Stopped-Flow

The introduction of the Zero Interrupt Facility has revolutionized the ease, economy, time resolution, precision and productivity of the Sequential Stopped-flow Mixing Method (sometimes called multi-mixing). An 115µl ageing loop is used for all determinations from 10ms to 1,000,000ms.



The following procedure describes the use of the syringes and the various valves during sample loading, sample flow circuit flushing and sequential-flow mixing.

Drain the thermostat solution from the water bath by removing the bleed screw from the top of the cell block. Remove the front of the water bath. Referring to the flow diagram on the previous page, remove the aging loop from the straight through connector which connects to the flush line. Remove the plugs from the pre-mixer and the end of the extension tube. Connect the extension tube to the straight through connector on the end of the flush line and the aging loop to the pre-mixer. Pressure test the flow line to make sure that it is not leaking. Replace the front of the water bath and the bleed screw.

Fit the flush ram (fitted with a plain spacer in above diagram) to the left hand (FLUSH) side of the Sample Handling Unit so that it can drive the 'C' and 'F' syringes.

Fit the pre-mix ram (fitted with a knurled adjuster in above diagram) to the right hand (PRE-MIX) side of the Sample Handling Unit so that it can drive the 'A' and 'B' syringes. Push the drive ram on the pre-mix side upwards until it hits its internal stop. Screw the fine adjuster on the pre-mix ram fully upwards so that it is touching the ram. Screw it back down five complete turns. This gives a pre-mix volume of 110 µl/syringe. ie the total drive 1 volume should be 220 µl ± 10 µl. Larger volumes are unnecessarily wasteful with sample.

Screw the knurled volume adjuster on the Auto Stop fully clockwise, (i.e. front edge moves from left to right, until the pneumatic connection starts to rotate), then screw it eight and a half turns anti-clockwise (counter-clockwise). This equates to a total flow volume of approximately 400 µl and will result in a drive 2 volume of 180 µl (±10 µl).

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Turn the 'A' , 'B' , 'C' and 'F' control valves to the FILL position.

Fit four reservoir syringes filled with distilled water to the 'A' , 'B' , 'C' and 'F' Luer fittings. It is recommended that 10 ml plastic disposable syringes are used.

Ensure that the drive rams are pushed fully down. Fill the drive syringes by pushing down the plungers of the reservoir syringes. Expel any air bubbles by flushing backwards and forwards between the drive syringes and the reservoir syringes several times.

Turn the 'F' control valve to the DRIVE position and manually push the water from the drive syringe, through the flow circuit and into the stopping syringe.

Empty the stopping syringe by clicking on empty in the "NEW DATA" context of the software.

Repeat the above two steps until the drive syringe is empty.

Flush from the other three drive syringes in the same manner as described above.

Fill all four drive syringes with water and turn all of the control valves to the DRIVE position. Put the software into sequential mixing mode. Set up the photometrics (wavelength, PM volts, timebase etc) as for conventional single mixing stopped-flow. Set a delay time of 1000 ms. Turn off internal trigger and acquire a sequentially mixed trace.

Note the age time that appears on the screen. Refill the drive syringes and repeat the acquisition until reproducible age times are obtained with flat absorption or fluorescence traces.

Click on PROFILES and check that the drive volumes are 220 µl ±10 µl for Drive 1 and 180 µl ± 10 µl for Drive 2. Adjust the Drive 1 and total volumes if necessary.

Ensure the all the control valves are set to the FILL position. Replace the reservoir syringes with ones containing your samples, the flush reservoir syringe should be filled with a neutral buffer solution. Fill the drive syringes by pushing down the pistons of the reservoir syringes (this ensures that there is no cavitation inside the drive syringes and, therefore, no bubbles). Ensure that there are no air bubbles in the drive syringes.

Set the control valves to the DRIVE position.

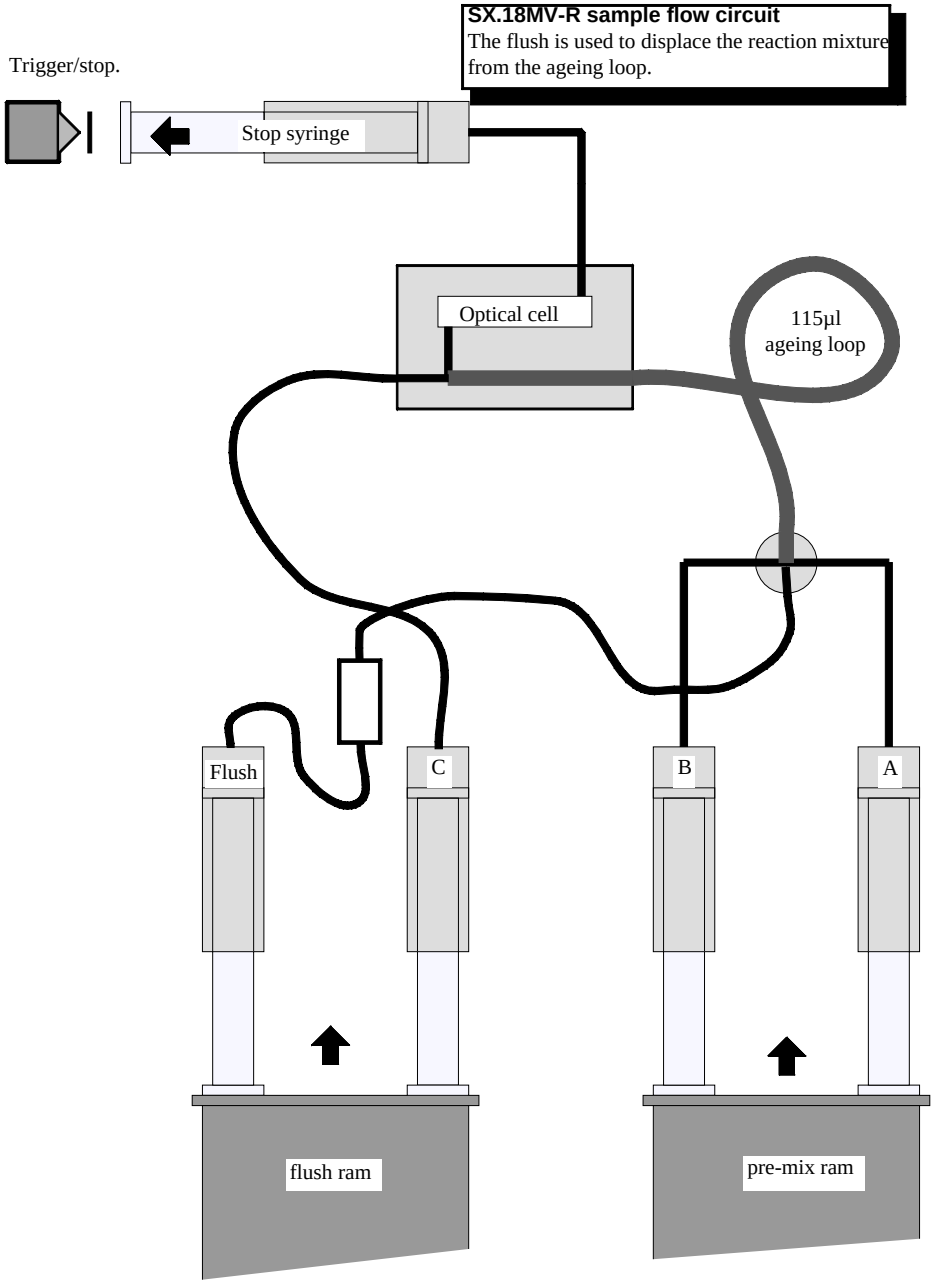
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THE SAMPLE HANDLING UNIT IS NOW READY
(now refer to the Software Manual)

The first trace that you acquire with chemistry will prime the flow lines and should be discarded.

The drive syringes must be refilled after each acquisition.

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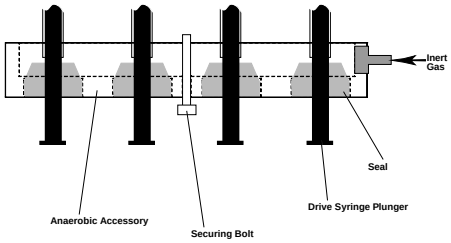
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6.0 Anaerobic operation

To operate under anaerobic conditions does require particular care with respect to eliminating oxygen dissolved in the thermostat medium and adsorbed into the wall material of the sample flow circuit.

There are three places where oxygen can get from the atmosphere to your samples and all of these must be dealt with to provide a suitable anaerobic environment.

The first is between the plunger and barrel of the drive syringe. Although the syringes are gas tight the tips of the plungers are made of Teflon which does not act as a barrier to oxygen. In order to stop oxygen from passing between the barrel and plunger the end of the syringe barrel needs to be in an inert environment.
This is achieved using the anaerobic accessory supplied with the AN1 accessory. To fit the accessory push it over the drive syringe plungers and bolt the accessory to the underside of the sample handling unit water bath so that the ends of the drive syringe plungers are enclosed. Connect an inert gas supply, (either nitrogen or argon), to the connector on the side of the accessory and gently purge so that the ends of the drive syringes are in an inert environment. The diagram above shows a cut away view of the assembled accessory.



The second opportunity for oxygen contamination is from the flow tubing. Current system are shipped with PEEK tubing and PEEK drive valves as standard. This minimises the danger of oxygen contamination from the tubing and the valves.

The thermostating liquid in the circulating water bath should be purged of oxygen by gently bubbling nitrogen through it at 25°C ± 1°C. 1g of sodium dithionite should be added to the thermostat liquid. The circulator must be left running for the duration of anaerobic operation. A solution of 600ng/ml Glucose Oxidase and 10mM glucose in 100ml of sodium acetate, pH5, should be flushed through the internal flow lines of the sample handling unit. After 1 hour deoxygenated buffer should be used to flush out the glucose oxydase solution. The sample handling unit is now ready for anaerobic use.

The third opportunity for oxygen contamination is at the point where the samples are introduced into the drive syringes. For basic anaerobic work the use of gas tight luer tip reservoir syringes is recommended. Prepare the anaerobic samples as usual, draw them into gas tight syringes, bring them quickly to the sample handling unit, as you

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are about to fit them to the luer connections expel a small amount of the sample to prevent an air bubble from being trapped between the reservoir syringe and the fitting.

Advanced anaerobic work requires the use of three way valves to introduce the samples into the system, These are supplied as part of the AN1 accessory.

Method.

1. Flush the drive syringes and flow circuit of the SX.18MV-R with anaerobic buffer, after flushing you should leave some of the anaerobic buffer in the drive syringes.
2. Prepare your sample(s) in a glove box and load this into a gas-tight (luer lock) syringe with a 3-way stop valve on the end of the syringe, ensure this valve is closed.
3. The syringe and 3-way valve should be transferred to the stopped-flow instrument.
4. Now connect an empty disposable syringe on to the unused port on the 3-way valve. With the 3-way valve in the same position (i.e. closed) you should now turn the drive valve to the 'open' or 'fill' position. Now push up on the drive syringe and expel the anaerobic buffer from the drive syringe into the empty disposable syringe on the side of the 3-way valve. By doing this you are 'scrubbing' both the drive valve, the luer fitting and the part of the 3 way valve that has yet to be in contact with the anaerobic sample − thus eliminating any remaining oxygen in these components.
5. The 3-way valve can now be turned through 90 degrees − thus opening it, now introduce a small amount of the anaerobic sample into the drive syringe.
6. Turn the 3-way valve back to the closed position and then push this small volume of anaerobic sample out through the 3-way valve to the disposable syringe (that contains anaerobic buffer). In this way you further scrubbing the valve, syringe and luer fitting with sample.
7. The 3-way valve can once again be turned through 90 degrees to open it and then sample can be loaded in the normal way.

The above procedure can also be used when replacing the glucose/glucose oxidase solution (in the flow circuit, valves and syringes) with anaerobic buffer, prior to flushing the system with your anaerobic sample; i.e. first flush with glucose/glucose oxidase, then with anaerobic buffer and then finally with a small volume of your anaerobic sample.

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7.0 Asymmetric mixing.

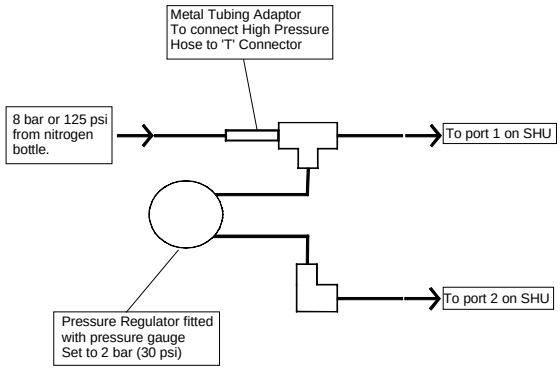
(Variable Ratio Mixing)

By fitting drive syringes of different volumes, it is possible to mix the samples together in different ratios. The Hamilton Salt Line syringes listed below are compatible with the SX.18MV-R and, by using the appropriate combinations, the following ratios can be readily set up 1:1, 2.5:1, 5:1, 10:1 and 25:1.

Capacity	2500µl	1000µl	500µl	250µl	100µl
Hamilton Part#	203340	203330	203320	203310	203300

IMPORTANT

The changing of a standard drive syringe for one of a smaller capacity means that it is possible to generate excessive pressures within the sample flow circuit. In order to avoid damaging the sample flow circuit it is important that one of the drive syringes is always a 2.5ml syringe. This can be either a Hamilton syringe or a Klohne syringe. The various ratios are obtained by altering the size of the other syringe. It is also necessary to reduce the input pneumatic pressure to the sample handling unit. Under normal circumstances the pneumatic supply is 8 bar and is connected to the 'h' connector on the back of the sample handling unit. When asymmetric mixing is to be performed the join between the two ports is replaced with a pressure regulator fitted with a pressure gauge. This can be used to reduce the pressure going into port 2 to below the internally regulated pressure. The pressure regulator should be set for 2 bar, irrespective of the drive ratio being used. In short, looking from the back of the unit an 8 bar supply is connected to the left hand connector (port 1) and a 2 bar (30psi) supply is connected to the right hand connector (port 2). This ensures that the stopped flow ram is driven under reduced pressure and hence the pressure in the sample flow circuit does not build up to dangerous level.



The diagram to the right shows the input pressure connections set up for asymmetric mixing.

Finally, the total drive volume should be increased as the ratio increase in order to obtain good reproducible results. We would recommend 200µl for 2.5:1 250µl for 5:1, 300µl for 10:1 and 350µl for 25:1 ratios.

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8.0 Test reactions

8.1 General performance tests

The following reactions are used to check the general performance of the spectrometer with respect to single wavelength and wavelength scanned operation.

1) Acid hydrolysis of tris(ethylamine)nickel (II) ion.

This is a useful reaction, having several isosbestic points, for demonstrating the generation of time-resolved spectra from kinetic data acquired over a broad wavelength range. The first reaction step has a time course covering 10 to 20 ms. Other changes take place over a period of several seconds.

Solution 1

0.05M	Nickel nitrate
0.16M	1,2-diaminoethane

Solution 2

1M	Nitric acid

Absorption maximum:	348, 550, 624, 700 nm
Absorbance range:	0 to 0.05A
Scanning range:	300 to 800 nm
Wavelength interval:	10 nm
Optical pathlength:	2 mm
Reagent volume:	50 µl

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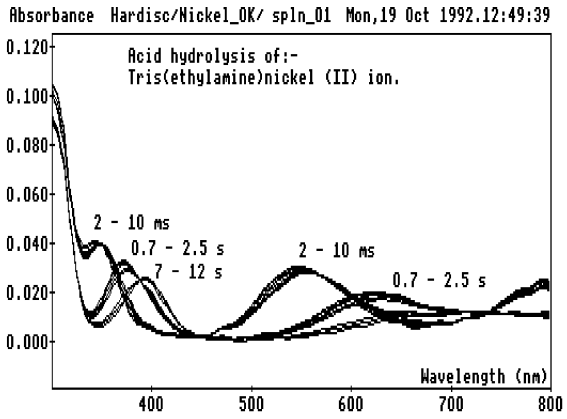


Figure 1

The spectra illustrated in Fig. 1 were constructed from a series of kinetic files produced following data acquisition over the wavelength range 300 to 800 nm. The overall reaction has more than one step with the first being completed in about 10 to 20 ms. The final stage of the reaction is still taking place after several seconds. In order to cover the complete time course of this reaction, the spectrometer was programmed to acquire data using two timebases and and a total of 4000 data points. The duration of the first timebase was 2 seconds and over this period data was collected at every ms. The final 2000 points were collected over the period 2 to 12 seconds. Time resolved spectra were constructed and those which highlighted the isosbestic points were plotted. Figure 2 shows the effect of expanding the above data using the zoom facility to allow closer examination of the isosbestic points.

Data imprecision due to light source fluctuation is of the order of 0.001 AU (0.25%

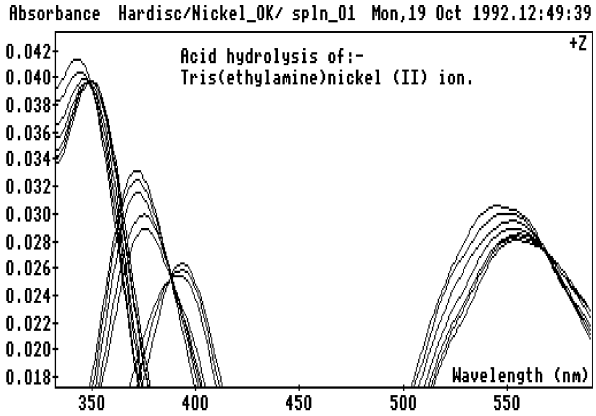


Figure 2

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transmission change) and can be seen as small ripples on the spectra. The following figures show the time profiles of data collected at the isosbestic wavelengths ie. 330, 350, 570 and 710 nm. Besides using the data manipulation facilities within the SX.18MV-R host program, the same kinetic data can also be analysed within the "Pro-K" Global Analysis program.

The complete data set comprised 51 kinetic traces with a total volume of just under 3 ml for each reagent. These kinetic traces were obtained under complete software control with one reload of the drive syringes after 35 drives.

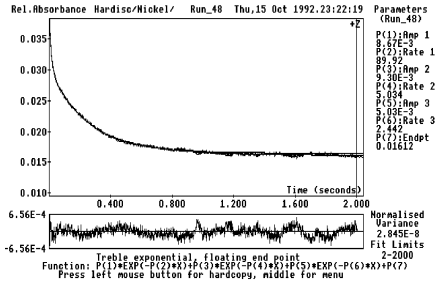
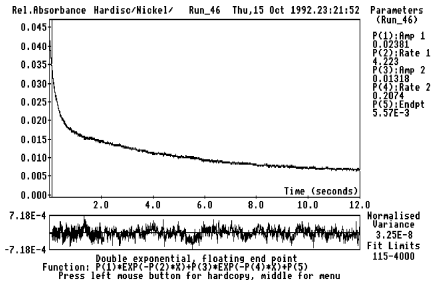


Figure 3



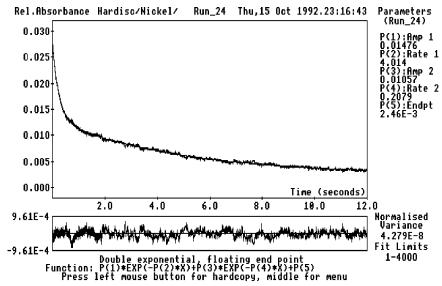


Figure 5

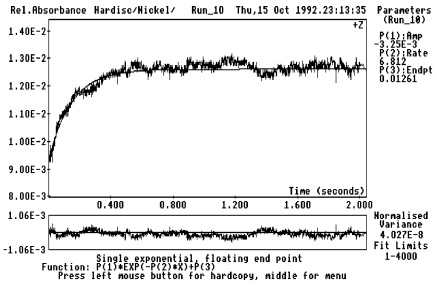


Figure 6

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2 Reaction of chromium (VI) with acidic hydrogen peroxide forming peroxy chromic acid.

The formation of peroxychromic acid takes place in about 200ms. Within this time period, the reaction shows a very well defined isosbestic point at 466 nm.

Solution 1

0.001M	Potassium dichromate
0.1M	Potassium nitrate

Solution 2

0,1M	Hydrogen peroxide
0.1M	Nitric acid

Absorption maximum:	580 nm (peroxychromic acid)
Absorbance range:	0 to 0.3A
Scanning range:	300 to 800 nm
Wavelength interval:	10 nm
Optical pathlength:	2 mm
Reagent volume:	50 µl

Figure 7 shows the time resolved spectra constructed from kinetic data acquired with a 100 ms timebase. Data collection was automatic with a single refill of the drive syringes after 25 acquisitions.

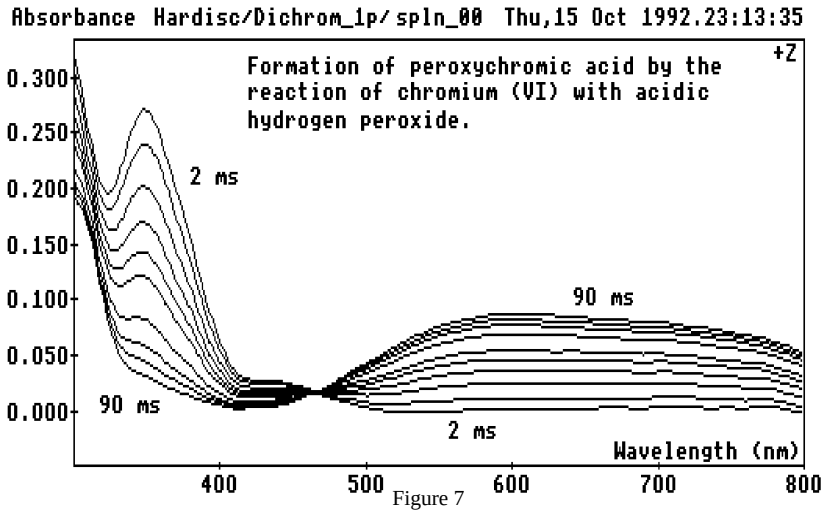


Figure 7

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8.2 Performance test specific to sequential-mixing mode

The sequential mode of operation has been extensively evaluated using the reaction of dichromate with acidic hydrogen peroxide mentioned above. Initially, this reaction was run in single mixing mode in order to obtain reference data for subsequent comparison with data obtained using the sequential mode of operation. The set of reference data was obtained by running undiluted (10^{-3}M) dichromate against the undiluted (10^{-1}M) H_2O_2 reagent (trace 1), undiluted dichromate against 50% diluted H_2O_2 (trace 2) and 50% diluted dichromate against undiluted H_2O_2 (trace 3). This reference data is shown below in Figure 8.

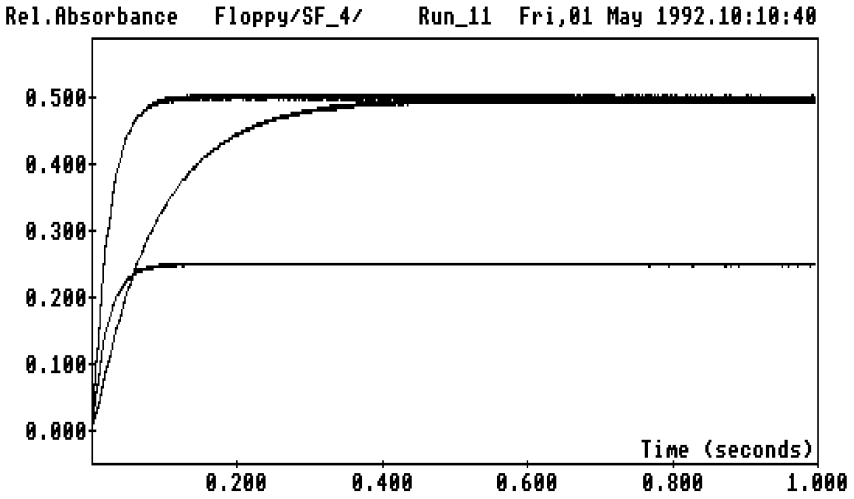


Figure 8

The integrity of mixing etc. for the sequential mode of operation was performed using the following two procedures.

Procedure 1

The reagents were loaded into the instrument as follows:- acidic hydrogen peroxide in syringe A, water in syringe B, dichromate in syringe C and water in syringe F (the flush syringe). A and B were mixed and pushed into the ageing loop for different age times (10, 15, 20, 25, 50, 100 and 1000 ms). For each age time, eight traces were obtained and all of these were superimposed on the same plot (Figure 9), together with the appropriate reference data curve.

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Procedure 2

The reagents were loaded into the instrument as follows:- dichromate in syringe A, water in syringe B, acidic hydrogen peroxide in syringe C and water in syringe F (the flush syringe). Data was then collected and plotted in the manner outlined above (Figure 10).

Both plots have at least 60 overlaid kinetic records demonstrating the very acceptable level reproducubility when operating in the sequential mixing mode.

A = H_2O_2; B = H_2O;
C = dichromate; F(flush) = water

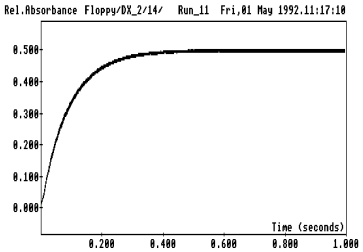


Figure 9

A = dichromate; B = H_2O;
C = H_2O_2; F(flush) = water

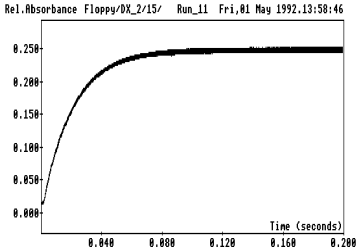


Figure 10

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8.3 Dead-time determination

8.31 Introduction

The determination of the dead-time performance of the SX.18MV-R stopped-flow spectrometer has been performed according to the methodology as published by Tonomura et al, in Analytical Biochemistry **84**, 370-383 (1978). The reduction of 2,6-Dichlorophenolindophenol by L-ascorbic acid is discussed in considerable detail in this paper (including the effect of pH) with respect to its use as a test reaction for dead-time determinations on stopped-flow instrumentation. At the end of the paper, the authors suggest certain "recommended conditions" which we have followed.

The following summarises the operating conditions :-

i. DCIP prepared in water containing 10% propanol.
ii. L-ascorbic acid solution in dilute hydrochloric acid.
iii. The concentration of HCl is chosen so that the pH becomes 2.0 after mixing with the DCIP solution.
iv. The ascorbic acid concentration is varied from 1mM to up to 100 mM final concentration.
v. The concentration of DCIP concentration does not exceed 1 mM.
vi. Kinetic data acquired at 524 nm.

8.32 Solutions prepared

i. **Hydrochloric acid (0.02N) / sodium chloride (0.2M)**
100 ml of 0.1N HCl + 5.8g of NaCl diluted to 500 ml with water

ii. **DCIP (500µM) DCIP**
14.5mg of DCIP dissolved in 10 ml of propanol + 1.16g NaCl diluted to 100ml with water.

iii. **Stock ascorbic acid solution (200mM)**
3.52g ascorbic acid diluted to 100 ml using solution 1 above (hydrochloric acid / sodium chloride).

iv. **Working ascorbic acid concentrations**
From the stock ascorbic acid solution, the following concentrations were prepared using the HCl/NaCl solution for dilution:- 2mM, 5mM, 10mM. 15mM, 20mM, 30mM, 40mM, 60mM, 80mM, 100mM and 120mM.

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8.33 Data analysis

Kinetic data was collected for each of the ascorbic acid concentrations. In each case, the data used for analysis was the average of 8 runs. When analysing the kinetic data for each concentration, the **FitRnge** option in the APL software was used to restrict data analysis to data collected 2ms from the time of triggering. After overlaying the kinetic traces for each of the ascorbic acid concentrations together with their appropriate kinetic fits, each of the kinetic fits was found to intersect at a common point and this point was taken to be zero time (t_0).

The dead-time was calculated according to the follwing equation :-

$$t_d / t_{½} = \ln(x_{tot} / x_{obs}) / \ln 2$$

where
t_d = dead-time
$t_{½}$ = half life
x_{tot} = theoretical total initial absorbance
x_{obs} = observed initial initial absorbance.

This equation transforms to :-

$$\Delta A_{obs} / \Delta A_{tot} = e^{-k_{app}.t_d}$$

and

$$\ln(\Delta A_{obs}) = \ln(\Delta A_{tot}) - k_{app}.t_{d.}$$

With the equation in the final form above, a plot of ln(ΔAbsorbance) against rate constant produces a straight line graph, the slope of which gives an accurate measure of the instrument's dead-time performance. The measured rate constant was also plotted against the final ascorbate concentration (mM) in order to establish at which rate constant, deviation starts to occur.

Measurements have been carried out on several systems using standard equipment (ie. light guide etc.) for both 2mm and 10 mm pathlength configurations. In addition, measurements have also been carried out using a special light guide with a small active diameter (1.5mm aproximately) positioned so that the light passes through only the first part of the flow tube where observations are made (2mm pathlength). In this way only the most freshly mixed reagents are observed.

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8.34 Results

The following results are quite typical for the SX.18MV-R spectrometer :-

2mm pathlength (regular) light guide :-	1.34 ms to 1.37 ms
2mm pathlength (special light guide) :-	0.85 ms to 1.02 ms
10 mm pathlength (regular light guide) :-	1.18 ms to 1.23 ms

The approximate dead time obtained with the 5µl cell is 500µs.

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9.0 Trouble Shooting

Servicing should only be undertaken by qualified personnel.

If you are in any doubt at all please contact the Technical Support Engineer at Applied Photophysics Ltd

HIGH VOLTAGES

High voltages are used in this spectrometer. Please exercise care during operation and DO NOT operate units with their covers removed. Remember, high voltages can be LETHAL.

9.1 Arc Light Source

Fault	Possible Causes	Remedy
Arc lamp fails to strike	1) Arc lamp requires replacing.	1) If the lamp has been running for more than 1000 hours (less if started and stopped frequently) it should be replaced with a new one following the instructions in this manual (see page 12).
	2) High voltage start pulse is escaping to earth.	2) Check the routing of the wiring from the starter unit to the bottom of the lamp. If it comes within 2.5cm of any earthed surface (such as the side of the lamp housing) re-route it. Particular attention should be paid after replacing the lamp as the wiring to the bottom of the lamp is often moved.
	3) A high resistance in the path to the bottom of the lamp is blocking the start pulse.	3) There is the possibility that oxidation or other corrosion at one of the wiring junctions will impede the start pulse an consequently make the lamp difficult to start. This is especially so of the connection to the bottom of the lamp. If there is evidence of corrosion the connection should be taken apart, the contact surfaces cleaned and the connection re-assembled.
Lamp output appears low.	1) The lamp is misaligned	1) Check the alignment of the lamp as described earlier in this manual.
	2) The lamp shutter is partially closed.	2) Check that the shutter on the front of the lamp housing is fully open. ie pushed away from you.

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9.2 Monochromator

Fault	Possible Causes	Remedy
Computer gives Monochromator not present error.	1) Bad or incorrect IIC bus connection.	1) Check that the IIC bus cable is plugged into the monochromator and that it is connected to another unit on the bus.
	2) Monochromator not powered up.	2) Check that the monochromator is switched on. If it is, but the indicator lamp is not illuminated check the fuse in the back of the unit just below the mains socket. The value of this fuse for your input voltage is printed on the back of the unit.
	3) Monochromator is busy when accessed by computer.	3) When the system is first switched on the monochromator resets itself to 0nm. If it is in the process of doing this when the software is run it is possible to get this error message. Wait for the monochromator to finish then attempt to set the wavelength. If the error message persists check 1 and 2.
Low Monochromator throughput.	1) Slits are closed.	1) The unit is shipped with the slits closed to minimise the amount of dust that can enter. Set the slit widths to give a bandwidth suitable for the experiments you are running. (1mm = 4.65nm)

9.3 Sample Handling Unit

Fault	Possible Causes	Remedy
Computer gives Sample Head ID Not Found error.	1) Bad or incorrect IIC bus connection.	1) Check that the IIC bus cable is plugged into the Sample Handling Unit and that it is connected to another unit on the bus.
	2) Sample Handling Unit not powered up.	2) Check that the Sample Handling Unit is switched on. If it is, but the indicator lamp in not illuminated check the fuse in the back of the unit just below the mains socket. The value of this fuse is 800mA for 240V systems and 1.6A for 110/100V systems. **N.B.** If the computer gives this error on power up it is necessary to reboot the software after correcting the fault.
Rams do not fire when computer is ARMed.	1) Low or no pneumatic pressure applied to back of Sample Handling Unit.	1) Check that you have opened all the valves between the compressed gas supply and the Sample Handling Unit. Check also that the compressed gas bottle is not empty.

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9.3 Sample Handling Unit (continued).

Fault	Possible Causes	Remedy
Valves leak or valve stems 'pop out' when rams are fired.	1) Valves have been damaged by either:–	1) The leaking valve must be replaced and the following born in mind:-
	a) Over-pressuring of system.	a) Over-pressuring of the flow circuit can be caused in two ways. The first is due to performing asymmetric mixing without lowering the input pressure to the drive rams. Read section 7.0 of this manual. The second is if, during the pre-mix in sequential mixing the flow is stopped by the stopping syringe instead of the internal front stop inside the pre-mix ram. If this is the case the flow volumes should be adjusted. (see section 5.1)
	b) Shock to system caused by rams being fired with gap between ram and syringe plungers.	b) As has been described earlier in this manual it is absolutely imperative that the rams are never fired whilst there is a gap between them and the syringe plungers. One or more of the control valves may be damaged and will need replacing.
	2) Connection between valve and flow tubing is loose.	2) Remove front of water bath and carefully tighten connections. These should be tightened just past finger tight.
Sample Handling Unit fails to trigger computer for data collection. (Indicated by long pause after ARM, even on short time bases, followed by "Time Out" data collection after approx-imately 10 seconds).	1) Trigger/Stop is corroded.	1) Remove trigger leaf from Trigger/Stop mechanism. Clean spring and stop with emery paper or fine file. Reassemble and test.
	2) Poor cable connection, either to back of Sample Handling Unit or to ADC card in computer.	2) Unplug the multi way 'Spider' connection from the back of the Sample Handling Unit and the ADC Card. Check that the pins inside the connectors are still in place and have not worked loose. Pay particular attention to pins 6 and 19 on the 20 way IDC connector which plugs into the I/O port on the ADC card in the computer.
	3) Faulty ADC board.	3) Set the data acquisition timebase to 0.1 seconds. Short out pins 17 and 18 on the 20 way IDC connector which plugs into the back of the Sample Handling Unit. Arm the Computer. It should wait for a trigger. Remove the short from the connector. The computer should trigger immediately. If the above happens as described the problem is in the sample handling unit. If the unit still fails to trigger repeat the above but bypass the cable altogether. i.e. remove the 'spider' cable connection from the ADC card I/O socket, short out pins 6 and 19 and repeat as above. If the system now triggers as it should the cable is at fault, otherwise the ADC card is at fault. In either case contact the Technical Support Engineer at APL.

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9.3 Sample Handling Unit (continued)

Fault.	**Possible Causes.**	**Remedy**.
Thermostat solution does not fill water bath.	1) Thermostat in and out connections wrong way round.	1) The design of the thermostat flow circuit allows for the thermostat solution to flow in one direction around the water bath and cell block. If the tubing connections are the wrong way round the thermostat solution will not flow correctly and the thermostat bath will not fill. The tube delivering thermostat solution from the circulating bath to the sample head should be connected to the lower thermostat connection on the back of the Sample Handling Unit. The return tube should be connected to the upper thermostat connection on the back of the Sample Handling Unit.
Auto-Stop valve functions incorrectly.	1) Pneumatic drive pressure too low.	1) The Auto-Stop valve is driven by compressed gas bled from the pneumatic ram supply. If this pressure is reduced below 6 bar (90 psi) the valve will fail to function and, instead of emptying the stop syringe to waste, the return cylinder will back flush solution into the flow circuit. If this happens increase the input pneumatic pressure.
Tubing connection from Sample Handling Unit to Auto-Stop leaks.	1) Connection not tight enough.	1) The connection on the back of the Sample Handling Unit from which the flow tube to the Auto-Stop comes generally needs to be tightened up using the supplied tool. It should be tight enough that it cannot be loosened by hand, but be wary of damaging the ferrules at the end of the tubes.
Drive syringe plungers leak.	1) The P.T.F.E. (Teflon) tips have 'cold flowed' so that they no longer form a good seal with the barrel of the syringe. 2) The P.T.F.E. (Teflon) tips have worn to the extent that they cannot be rejuvenated.	1) The tips of the syringe plungers can be rejuvenated by boiling them in water for five minutes and then replacing the plungers in the syringe barrels whilst still hot. This allows the tips to re-form and provide better sealing. 2) The syringe plungers or the entire syringe should be replaced with new Hamilton syringes. For the standard 2.5ml syringes the Klohne part numbers are: 18197 for the plunger alone and 18196 for the entire syringe.
Syringe Plungers Corrode.	1) Older syringe plungers are made from Teflon coated aluminium If the plungers are not removed and dried at the end of every session this layer can break down and flake off, exposing the aluminium below.	1) This corrosion is largely cosmetic and, in general, has very little effect on the operation of the instrument. In order to minimise the corrosion it is advisable to remove the plungers from the syringes (with the reservoir syringes removed and the valves in the "Fill" position) and dry them with paper towels. Alternatively the piston can be replaced with the latest PEEK pistons. See above for the part number.

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9.4 Computer

The computer is unable to display the desk top screen or the monitor display is not synchronised: ie. the picture is fuzzy because it is scrolling at a fast rate.	The battery backed RAM or CMOS RAM is responsible for the computer configuring itself correctly. If for some reason the CMOS RAM has become temporarily corrupted then the computer will not boot up properly. This can result in the computer selecting an unsuitable screen mode. Hence the un-synchronised screen display.	Usually the way to correct this fault is to reset the computer's configuration in the CMOS RAM. There are two ways of doing this. The two methods are referred to as: **'R-power on'** and **'Delete-power on'** resets. It is advisable to perform the 'R-power on' reset first as this is the least severe of the two types of reset. Only perform the 'Delete-power on' reset if the 'R-power on' fails to work. The two types of reset are described below.

The 'R-power on' reset

1. Hold down the **R** key as the computer is switched 'on'. Do not release the key until text has appeared on the screen. Hopefully the computer will have booted-up and is displaying the desk top screen. Assuming that the desk top is being displayed you may notice that the screen size or mode is a little different. You might have to alter the screen height to see the icon bar. Now open up the hard disc root directory, as normal. Inside this directory you will find a file called 'Configure'. Double click on this file. This should cause the 'Configure' icon to appear on the right hand side of the icon bar. Now perform a **[Ctrl] [Break]**. This is done by holding these two keys down simultaneously. The **[Break]** key can be released almost immediately but the **[Ctrl]** key must be held down until text has appeared on the screen. The computer should now boot-up and should be displaying the desk top screen in the correct screen mode.

The 'Delete-power on' reset

Hold the **[Delete]** key down as the computer is switched 'on'. Again do not release this key until text has appeared on the screen. The computer should be displaying its desk top screen. Where necessary make adjustments to the screen height so that the icon bar can be viewed. You will notice that the hard disc icon has not appeared on the icon bar. This will have to be re-installed. To do this: Click on the 'Apps' file on the left hand side of icon bar. This will display a directory window called 'Resources'. Within this window is a file called '!Configure'. Double click on this '!Configure' file. A 'Configure' icon will now appear on the right hand side of the icon bar. Click on this icon. A window titled 'Configuration' will appear. Within this window is a file called 'Discs'. Click on the file called 'Discs'. This will bring up a new window titled 'Hard disc drives'. Within this window there are sections. The section we are interested in is the second section titled 'IDE hard discs'. Inside this section (IDE hard discs) are two white arrows on a blue background. Click just once with the left mouse button on the arrow pointing upwards. A single hard disc icon will appear inside the 'IDE hard discs' section. Now click with the left mouse button on 'OK' at the bottom right of the window. A warning box will appear: 'Warning from !Configure'. Don't worry about this warning. Click with the left mouse button on 'OK' inside the warning box. The hard disc icon 'IDEDisc4' will appear at the very left of the icon bar. The hard disc is now re-installed. Open up the hard disc root directory , as usual, by clicking on the hard disc icon. Inside the root directory is a file called 'Configure'. Double click on this Configure file. Now perform a **[Ctrl] [Break]**. This is done by holding these two keys down simultaneously. The **[Break]** key can be released almost immediately but the **[Ctrl]** key must be held down until text has appeared on the screen. The computer should now boot-up and should be displaying the desk top screen in the correct screen mode.

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