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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.

EARTH/GROUND

The chassis of all units MUST be well grounded, preferably via the earth accompanying the mains supply. Allowing a chassis to float with respect to ground potential may result in the chassis acquiring a dangerous potential.
All of the following units must be plugged into the 4-way power distribution board(s) supplied. If more than one board is supplied then they should be connected to each other in series, that is, one board is connected into the next.

05-109 Monochromator
04-122 Power Controlled Lamp Supply.
13-110 Sequential Stopped-flow Sample Handling Unit (SHU).
13-111 Photometric Controller.
01-109 Auto-transformer* .

PNEUMATIC DRIVE RAMS

The maximum input pressure is 10 bar (150 psi) and for normal operation the minimum is 8 bar (120 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.**

ASYMMETRIC MIXING

To avoid irreparable damage to the sample flow circuit the input pressure to the drive ram MUST BE adjusted in proportion to the change in combined cross-sectional area of the drive pistons. Contact Applied Photophysics for full instructions, Fax# (44) 372 386477.

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 fill.

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

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DX.17MV Sequential Stopped-flow Spectrofluorimeter.

1.0 System Description

The DX.17MV Sequential Stopped-flow Spectrofluorimeter allows chemical, biochemical and biological fast reactions initiated by stopped-flow and sequential-mixing stopped-flow to be followed using spectrophotometric and fluorimetric detection methods. For an introduction to the stopped-flow method refer to Gibson,Q.H. and Milnes,L. (1964) Biochem. J., 91, 161.

The standard DX.17MV comprises

Sample Handling

13-110 Sequential Stopped-flow Sample Handling Unit With Auto-Stop Mechanism

Spectrometer Workstation

RISC-processor Unit (32-bit Archimedes).
AKF-18 Multisync Colour Monitor or TAXAN 775 Multisync.
LC-20 Printer NLQ.
13-111 Photometric Controller.
01-109 Auto-transformer 250W (supplied as standard when required - N. America and Japan).

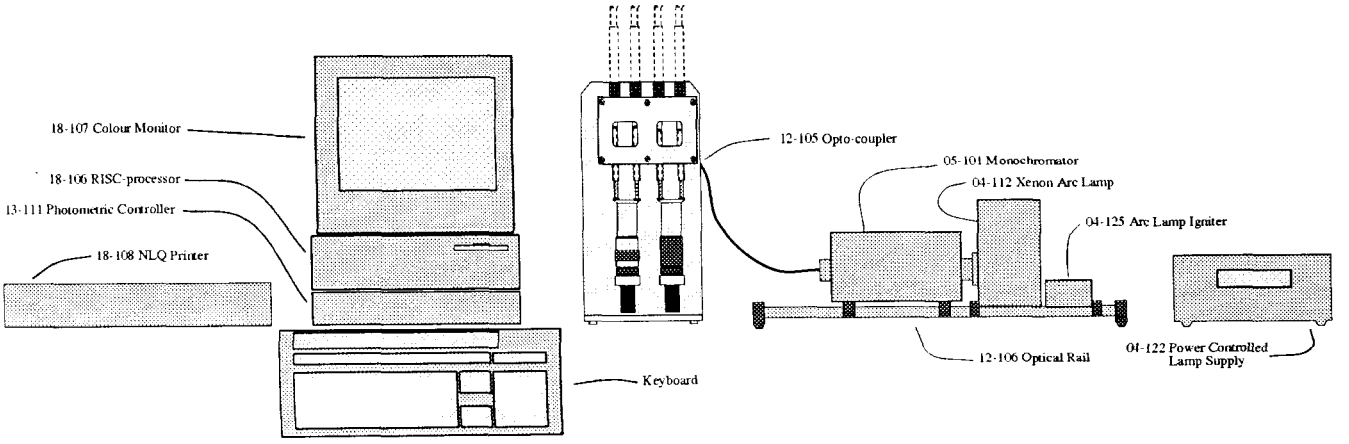
SpectraKinetic Photometrics

04-112 Arc Light Source (150W xenon).
04-122 Power Controlled Lamp Supply.
04-125 Lamp Igniter.
05-109 SpectraKinetic Monochromator.
12-105 Opto-coupler (flexible silica fibre optic).
12-106 Optical Rail (0.65m).
21-110 Absorption Photomultiplier.
21-112 Emission Photomultiplier.

2.0 Instrument Set-up

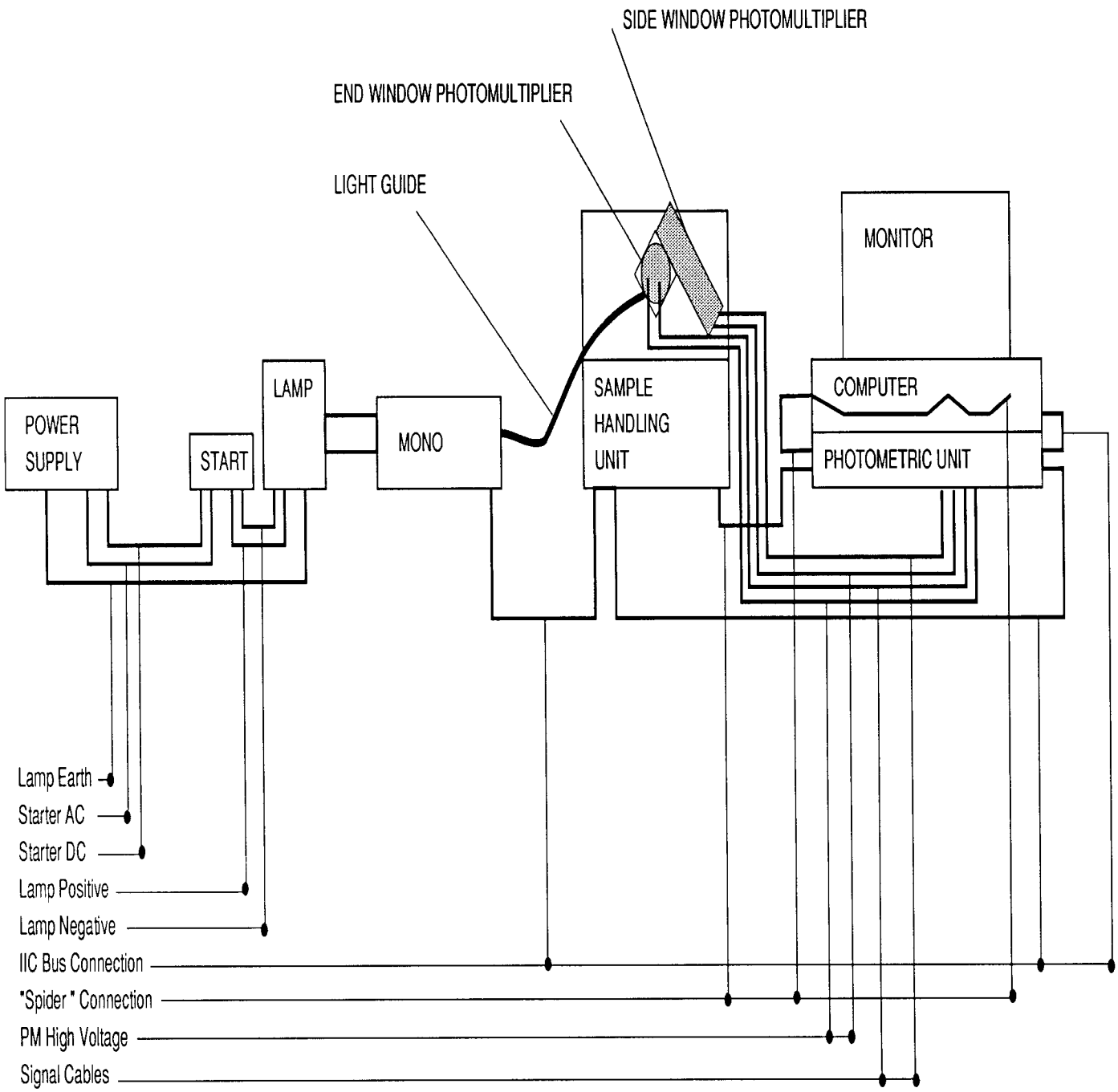
Assemble the DX.17MV spectrofluorimeter as indicated below, with the 04-112 Arc Light Source and 05-109 SpectraKinetic Monochromator positioned on the optical rail to the right of the sample handling unit. Ensure that the lamp housing snout is pushed fully into the flange attached to the monochromator entrance port.

Push the bigger 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 sample handling unit. The 2 mm pathlength is accessed from the lower right hand port and the 10 mm 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 2 mm pathlength port so that the full length of the optical cell is evenly illuminated. The securing screw (with nylon washer) for the opto-coupler mounting flange ensures that it is mounted in the correct orientation. Attach the 21-112 Emission Photomultiplier to the rear port (dedicated to emission measurements) and the 21-110 Absorption Photomultiplier to the port opposite that used for light input.



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DX17MV Electrical Connections (Rear View)



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2.1 Instrument Connections

04-112 Arc Light Source.
One loose cable (green/yellow) provides the earth link between the 04-112 and the 04-122 Power Controlled Lamp Supply. The igniter cables connect to the lamp housing itself and the 04-122 Power Controlled Power Supply (follow the colour codes RED PLUG to RED SOCKET, etc).

05-109 SpectraKinetic Monochromator.
A peripheral communication cable (black coiled) connects to either the IIC Interface or the Photometric Controller.

13-110 Sample Handling Unit.
Connect a source of dry gas (air or nitrogen) to the gas inlet using the pneumatic supply adaptor supplied. Alternatively 6 mm OD nylon pressure tubing can be directly connected in the place of the pneumatic supply adaptor. The Auto-Stop Mechanism should be placed onto the unit case with the valve to the left as seen from the front of the Sample Unit. The trigger black twisted flying lead connects to a socket on the back panel. The Earth connector connects onto the terminal post on the back of the Sample Handling Unit. Three 4 mm OD nylon pressure tubing connect the air to the Auto-Stop Mechanism, note correct sequence A, B & C. The sample flow line with a stainless steel tube fitting on one end should be connected into the back of the Sample Handling Unit thermostatted housing, (the stainless steel fitting goes to the Sample Handling Unit, the black plastic connector goes to the Autostop Mechanism valve.)

13-111 Photometric Controller.
Two sets of photomultiplier cables (set = signal cable + high voltage cable) connect the 21-110 Absorption Photomultiplier and 21-112 Emission Photomultiplier to the Photometric Controller. A multi-plugged control cable (known as a "spider cable") connects the Sample Handling Unit and RISC-processor Unit to the Photometric Controller. A peripheral communication cable (black coiled) connects the Photometric Controller to the IIC Interface fitted in the back of the RISC-processor Unit.

21-112 Emission Photomultiplier (end-window).
Fit assembly to back of cell block using the three finger screws and nylon washers provided. There is provision for a cut-off filter.

IIC Bus.
The IIC Bus allows for communication between the different, computer controlled, units and the computer. The connection between the units is by means of black 'curly cords'. As it is a parallel bus it does not matter in which order the units are connected.

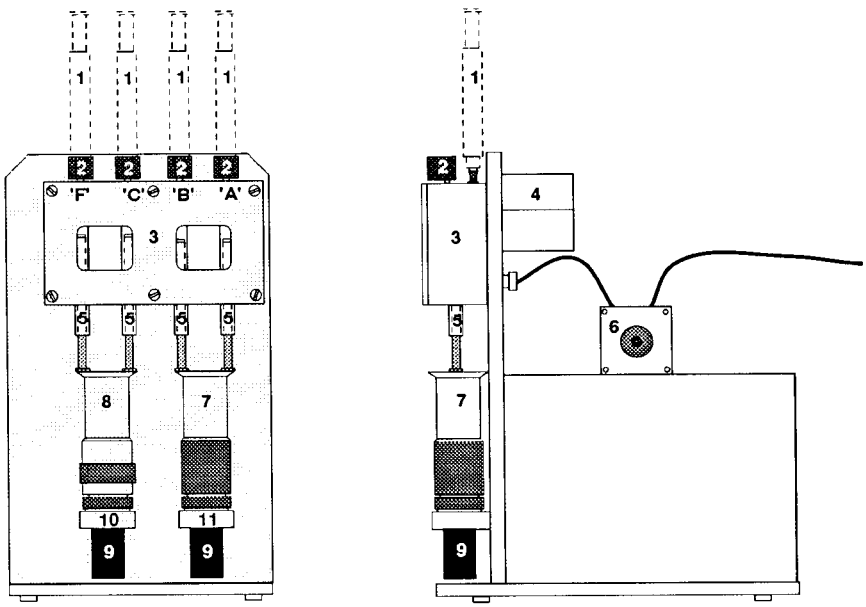
Optional extras

22-101 Circulator Bath Cooler.
Connect the tubing provided to the inlet and outlet ports located on the rear of the sample handling unit (the inlet port is the lower of the two nozzles). An air vent is located at the top of the stainless steel block that houses the sample handling unit observation cell.

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3.0 General Information and Notes

DX-17MV SAMPLE HANDLING UNIT



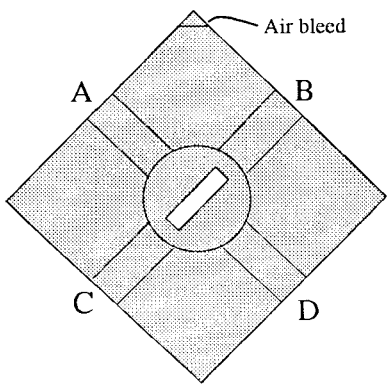
1 Reservoir Syringes
2 Control Valve Knobs
3 Thermostat Bath
4 Cell Block
5 Drive Syringes
6 Auto-Stop Arrangement
7 Pre-Mix Drive Ram
8 Flush Drive Ram
9 Transducers
10 Flush Ram Shelf
11 Pre-Mix Ram Shelf

3.1 Chemically inert sample flow circuit.

The flow circuit is chemically inert and free of stainless steel. It is constructed of glass (syringe barrels), silica (optical detection cell), LAFT (sample flow circuit tubing) and Teflon (syringe piston seals and valves).

3.2 2 and 10 mm spectrophotometric pathlengths.

The optical detection cell has orthogonal viewing ports that gives a choice of 2 mm (ports A-D) and 10mm (ports B-C) pathlengths. 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.



Cell block optical ports. Ports A-D and B-C give 2mm and 10mm pathlengths respectively. The D port is used for the excitation light (fluorescence).

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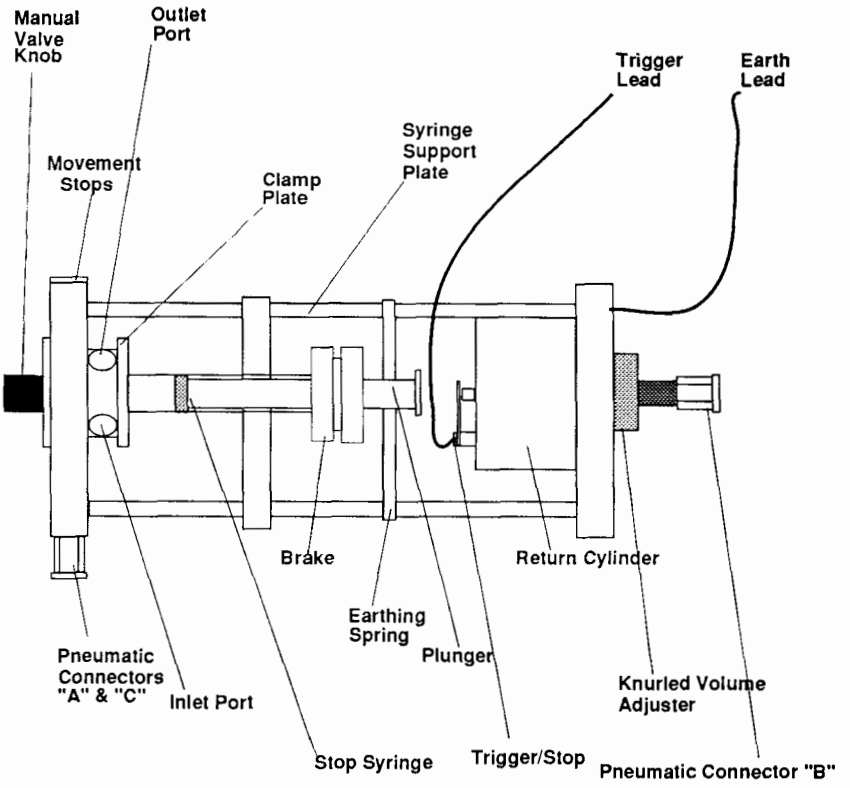
3.3 Emission port.

The fluorimetric port 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.

3.4 Pneumatic Drive Rams.

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 of between 8 to 10 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 irreparable damage. FOR ASYMMETRIC STOPPED-FLOW MIXING EXPERIMENTS THE INPUT PRESSURE MUST BE REDUCED (see asymmetric section of manual).

3.5 Auto Stop Mechanism.

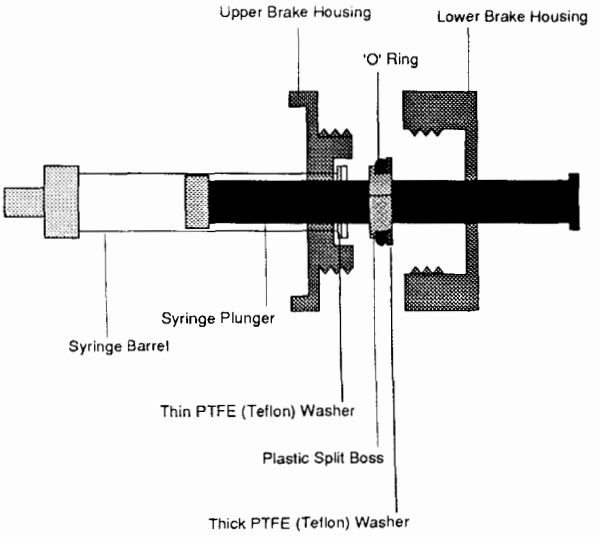


The Auto Stop mechanism is fixed to the side of rear cover of the Sample Handling Unit. The valve and the return cylinder are both controlled from the workstation, although both can be operated manually (when flushing the system, for instance). The three pneumatic inlets, marked 'A', 'B' and 'C' are connected to the three outlets on the back of the Sample Handling Unit. The trigger lead should be plugged into the socket on the back of 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.

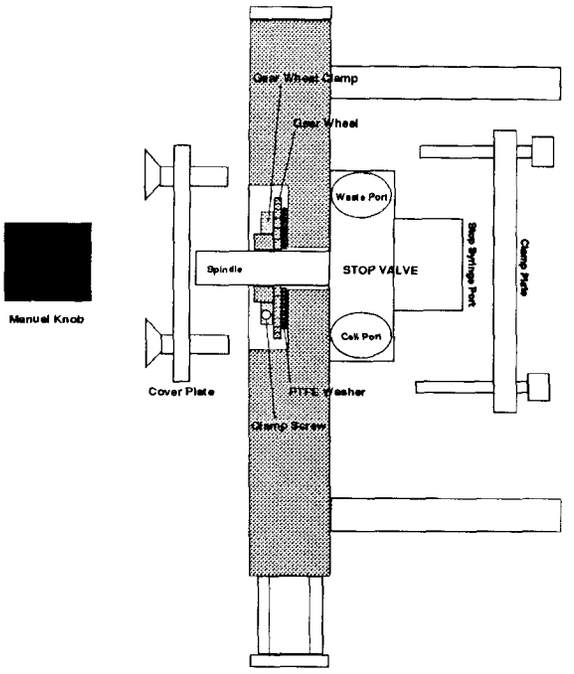
When collecting kinetic traces at different wavelengths to produce time resolved spectra the instrument will reset itself before each run, minimising the operator's involvement.

EXPANDED VIEW OF BRAKE MECHANISM



The brake mechanism, (see view to left), 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 increase by tightening the two housings together and decreased by loosening them. They should be loose for single mixing stopped flow so that they do no reduce the instruments dead time, but tightened up, as necessary, when performing sequential flow experiments.

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PROCEDURE FOR CHANGING STOP VALVE

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

When changing the stop valve it is vitally important that the new valve is in the correct orientation. In order to ensure this you must have the AutoStop set to the drive position before it is disassembled.

1) Disconnect all liquid, pneumatic and electrical connections from the AutoStop to the Sample Handling Unit and remove the AutoStop to a suitable work bench.

2) After having ensured that the AutoStop is set to the DRIVE position remove the cover from the end of the Manual Knob, loosen its retaining nut and remove the Manual Knob.

3) Remove the drive syringe.

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

5) Loosen the clamp screw which secures the Gear Wheel Clamp and remove the Gear Wheel Clamp (This requires a 3/36 inch Allen Driver).

6) Undo the two screws holding the Clamp Plate in position and remove the Clamp Plate.

7) Remove the old Stop Valve by pushing on the Valve Spindle. Be very careful not to rotate the Gear Wheel. (NB The Valve Spindle may be a very tight fit in the Gear Wheel and it may require a considerable amount of force to push it through.)

8) Set up the new Stop Valve so that the flow hole through the Valve Spindle is aligned with the Cell Port. This may be done by eye but many people find it easier to do by blowing through the cell port whilst rotating the Valve Spindle. When the least amount of force is required to blow through the cell port the spindle is correctly aligned. **Do not confuse the cell port with the waste port.**

9) Align the new Stop Valve with the AutoStop so that the two ports are visible on the top of the valve. Refer to the diagram if you are unsure about the orientation of the new valve.

10) Whilst holding the Gear Wheel in place fit the new Stop Valve. Many people find a short length of metal tubing a useful aid for holding the Gear Wheel in place (a socket driver from a socket set is ideal). It is important that the Gear Wheel is not rotated. Again the spindle may be a tight fit and a considerable amount of force may be required to push it through the Gear Wheel.

11) Refit the Clamp Plate and tighten up its screws so as to clamp the valve in place.

12) Refit the Gear Wheel Clamp and tighten up the Clamp Screw.

13) Refit the Cover Plate.

14) Align the mark on the Manual Knob and refit the Knob to the Spindle.

15) Refit the AutoStop arrangement to the Sample Handling Unit and reconnect all of the connections. Be especially careful that the pneumatic connections are connected to the correct pneumatic ports.

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3.6 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.

3.7 05-109 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 diffraction grating giving a useful operating wavelength range of 200 to beyond 600nm. 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.

3.8 04-112 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 04-122 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.

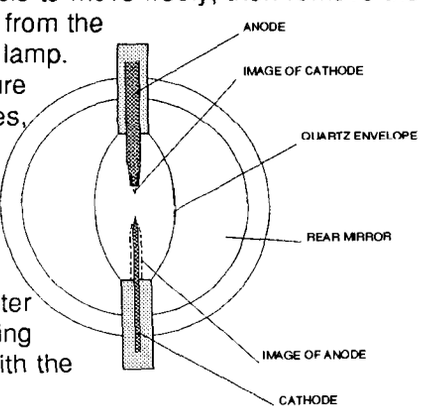
There are a number of potential HAZARDS associated with any arc light source

- The latest Applied Photophysics NON-INDUCTIVE Starter Unit uses a D.C. 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, care should be taken, especially when the back of the lamp housing is open, (under which circumstances the lamp power supply should be removed from the mains electrical supply).
- 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.

Replacing The Xenon Bulb.

The high stability 150 W xenon bulb (OSRAM XBO 150W/GS OFR) is available from Applied Photophysics or can be sourced in the United States from Osram Corporation, 110 Bracken Road, Montgomery, NY 12549. (tel. 914 457 4040 and fax. 914 457 4142).

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 is worn. Fit the alignment mirror to the back of the housing. Open the hinged rear door by first unsrewing the two thumb screws located at the top corners. Loosen the clamp at the top of the lamp so that it is able to move freely, then remove the cable from the bottom of the lamp. It should now be possible to remove the lamp from the top clamp. Unscrew the extender fitted to the top of the lamp and fit it to the new lamp. When fitting the replacement follow the bulb manufacturers instructions. Ensure that the bulb is in the correct position by checking that the image of its electrodes, formed by the rear mirror, is coincident with the electrodes themselves (see diagram right). Move the lamp towards and away from the mirror until it is in its focal position. (i.e. when the electrodes remain aligned with their images no matter what angle you look at them from). Remove Remove the alignment mirror and return the lamp housing to the optical rail. Check the alignment of the lamp housing optics by using the real time display provided on the spectrometer workstation. A mirror adjustment is located on the igniter side of the lamp housing and this should be used to optimise the optical alignment of the lamp housing with the monochromator.



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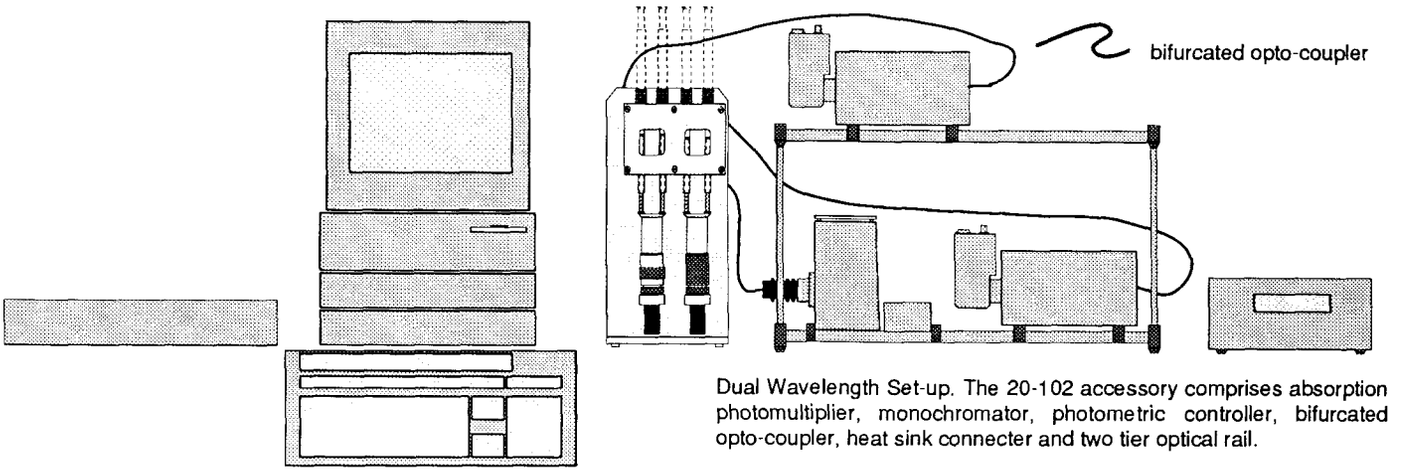
XENON ARC BULBS CAN EXPLODE IF MISHANDLED

As a rule the arc light source does not require any special attention. 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. To align the lamp, gently rotate the knurled knob on the right hand side of the lamp housing whilst watching the live display on the workstation monitor (See software manual). This knob adjusts the horizontal angle of the output beam and should be adjusted so that the live display shows maximum signal. (At 480 nm, in absorption mode, using the 2 mm pathlength, with water in the cell and a slit width of 1 mm the P.M. volts should be around 450 to 480 volts.) It is recommended that the lamp alignment is checked weekly.

3.9 04-122 Power Controlled Lamp Supply.

The 04-112 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`s front panel carries the pertinent operating instructions. The 04-122 has thermal switches and a cooling fan to protect the unit from over heating. The 04-122 should therefore be placed in a well ventilated position on the bench with at least 150cm between the fan outlet at the back of unit and a vertical surface.

3.10 20-102 Dual Wavelength Accessory (optional extra)



Dual Wavelength Set-up. The 20-102 accessory comprises absorption photomultiplier, monochromator, photometric controller, bifurcated opto-coupler, heat sink connecter and two tier optical rail.

Refer to the layout figure when setting up the DX.17MV to perform dual wavelength experiments. The second 13-111 Photometric Controller is placed under the spectrometer workstation. A heat sink connector is fitted to the xenon arc lamp output snout, fix in position with the grub screw, and push fit in the regular opto-coupler. There is provision for an absorption photomultiplier on each monochromator exit slit assembly. The bifurcated opto-coupler provided fits into either of the ports normally reserved for an absorption photomultiplier, however, it is recommended that the 10 mm path length is used for dual wavelength work. The bifurcated ends should be push fit into the monochromator entrance slit assemblies.

3.11 Spectrometer Workstation

- for full operational details refer to the DX.17MV SOFTWARE MANUAL.

The spectrometer workstation comprises photometric controller, 32 bit RISC-processor unit, 12 bit analogue-to-digital converter (ADC), IIC peripheral communication interface, hard disc, colour monitor, matrix printer (laser printer or plotter - optional extras). 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

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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. Two offset modes are provided, one for spectrophotometric (transmission/absorption) studies and one for emission and light scattering measurements. 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 offset corresponds to the signal difference obtained when the lamp housing shutter is opened and closed). 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.

3.12 13-111 Photometric Controller

POWER	Check the fuse fitted and voltage selector are correct for the line voltage (100/110/120V = 1 A anti-surge fuse or 220/240V = 500 mA anti-surge fuse). Connect power cord to one of the 4-way plug boards supplied (guarantees a common earth/ground).
HV OUTPUT	HV.ABS high voltage socket to supply the absorption photomultiplier. HV.FLUO high voltage socket to supply the fluorescence photomultiplier.
SIGNAL INPUT	SIGNAL (ABS) 5 pin DIN socket for connection to the absorption photomultiplier signal output. (FLUO) 5 pin DIN socket for connection to the fluorescence photomultiplier signal output.
COMPUTER	15 pin D-connector (female)

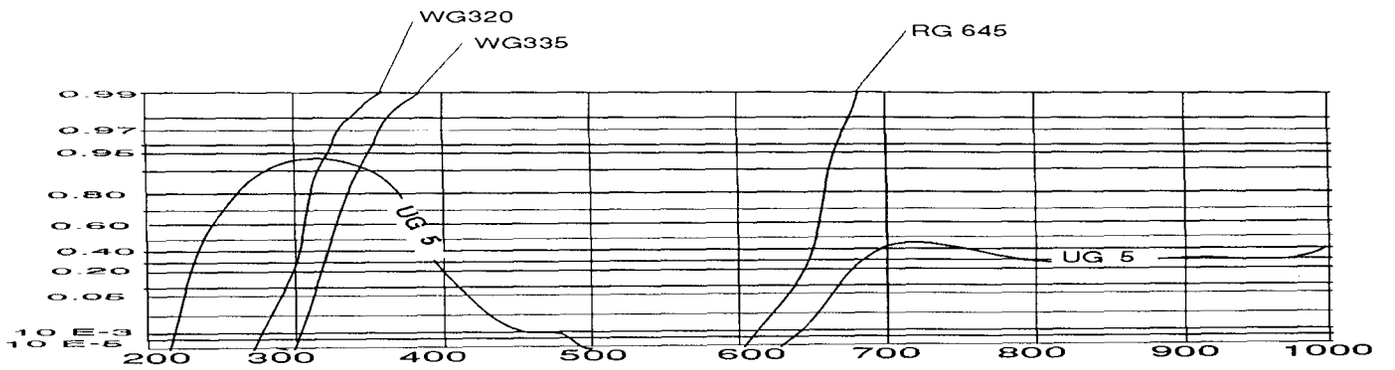
4.0 Use of optical filters.

In some circumstances it is advisable to use an optical filter in the space provided between the absorption photomultiplier and the optical detection 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. A RG645 filter is supplied to remove all wavelengths below 645 nm. It should be noted, however, that the photomultiplier supplied as standard only goes up to 650nm. In order to detect light above this wavelength it is necessary to fit an R-928 photomultiplier tube.

UV ABSORPTION MEASUREMENTS. When operating at wavelengths below 320 nm, it is possible to experience interference from white stray light. This stray light is caused by scatter of the intense white light from the monitoring lamp when it strikes the grating and other optical surfaces. The problem is made worse when a high background absorbance is experienced at the wavelength where measurements are being made. A significant improvement in performance can be achieved by using the type UG5 bandpass filter (240 to 400 nm) to block the unwanted stray light.

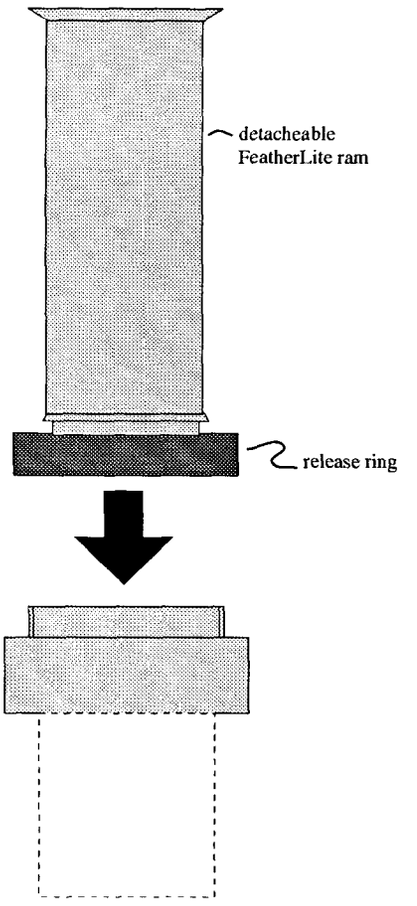
FLUORESCENCE MEASUREMENTS. When measuring fluorescence it is important that the excitation light does not get to the photomultiplier and swamp the fluorescence signal. In order to do this it is important to place a cutoff filter between the photomultiplier and the sample cell. This cutoff filter should 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 335nm and the other, which cuts off below 320nm. Other filters are available.



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5.0 Operating Procedures - STOPPED-FLOW

The following procedure describes the use of the 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).

Turn the 'A' and 'B' control valves to the FILL position (if they are fitted).

Screw the knurled volume adjuster on the Auto Stop fully clockwise (i.e. until the pneumatic connection starts to rotate), then screw it two 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 position. Ensure that the FeatherLite 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 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.

Turn the 'F' control valve to the FILL position and the 'C' 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.

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 position. Replace the 'C' and 'F' reservoir syringes with ones containing your samples. 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).

Set the 'C' and 'F' control valves to the DRIVE position. Ensure that the 'A' and 'B' valves (if fitted) are in the fill position.

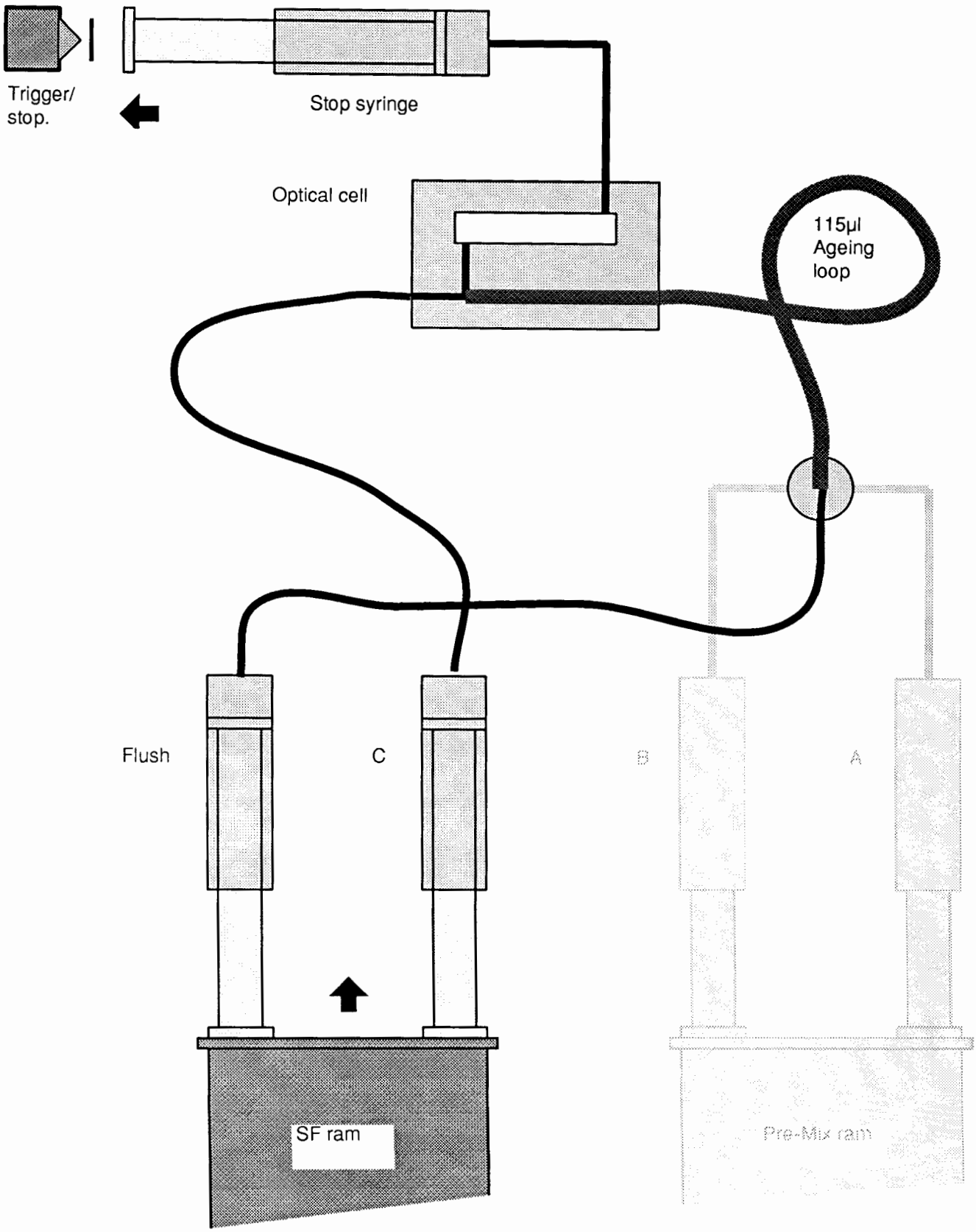
THE SAMPLE HANDLING UNIT IS NOW READY
(now refer to the Software Manual)

Thirty determinations or more can be made in quick succession before the drive syringes will need to be refilled.

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DX.17MV sample flow circuit

Stopped-flow determinations are made using the 'simple' stopped-flow ram in the "flush ram" position.

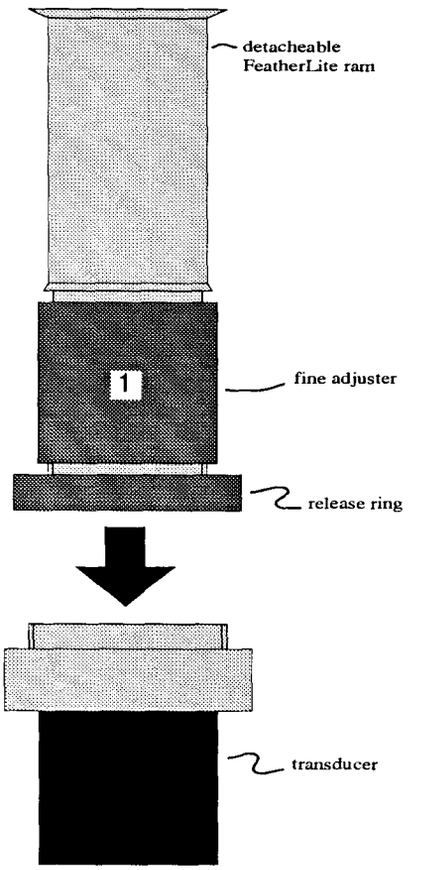


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5.1 Operating Procedures - SEQUENTIAL STOPPED-FLOW

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 double- or multi-mixing). An 115µl ageing loop is used for all determinations above 10ms and below 1,000,000ms. Regardless of desired ageing time, the time taken to fill the ageing loop and then fill the optical cell with a good reaction mixture is approximately 10ms. For longer ageing times an operator entered time is used to delay the reaction mixture in the ageing loop.

Sequential stopped-flow mixing can be now readily used by the non-expert. There are no cells or ageing loops to routinely change, no changing of experimental parameters such as sample flow rate, pneumatic pressure / step rate, ageing method or complicated calculations or calibrations.



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

Fit the flush ram (fitted with a plain spacer in position 1:- see diagram to right) 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 position 1:- see diagram to right) to the right hand (PRE-MIX) side of the Sample Handling Unit so that it can drive the 'A' and 'B' syringes.

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

Screw the knurled volume adjuster on the Auto Stop fully clockwise (i.e. until the pneumatic connection starts to rotate), then screw it eight turns anti-clockwise (counter-clockwise). This equates to a total flow volume of approximately 400 µl.

Push the FeatherLite 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. (As the thread pitch is 0.5mm each turn of the adjuster equates to 0.5mm of stroke and this equates to 20.8 µl/syringe). Larger volumes are unnecessarily wasteful with sample.

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 FeatherLite 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.

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

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 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).

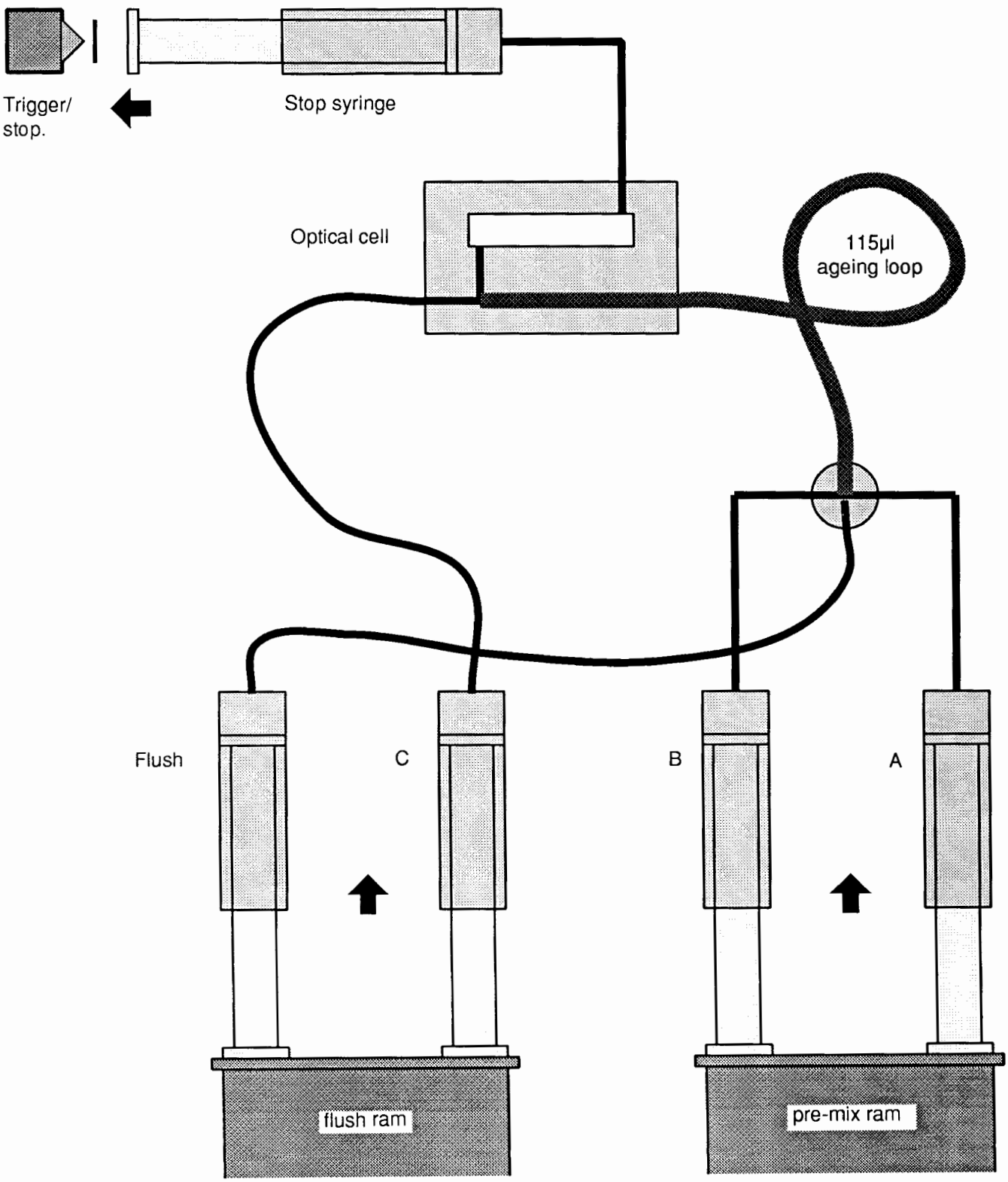
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Set the control valves to the DRIVE position.

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

After each SF determination refill the drive syringes. Allow time for the samples to reach temperature.

DX.17MV sample flow circuit
The flush is used to displace the reaction mixture from the ageing loop.



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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. Without the following precautions, carefully prepared deoxygenated samples will take up oxygen from the material used in the sample handling unit.

Prepare a pH 8.0 Tris/HCl buffer (5x10-2M) and purge thoroughly with oxygen free nitrogen or argon.

Fill the closed circuit thermostat system with some of this buffer solution having first made provision to continue purging with inert gas once it is in place.

Prepare a 5x10-3M sodium dithionite solution using the pH 8.0 buffer and purge thoroughly.

Flush out thoroughly the complete flow circuit including reagent reservoirs, drive syringes, stopping syringe and sample flow lines using deoxygenated buffer.

Replace the buffer in the sample handling unit with the dithionite solution and leave the instrument to stand for twelve hours.

Dissolve 1 gm of sodium dithionite in 5 ml of pH 8.0 buffer and introduce this into the closed circuit thermostat medium shortly before the anaerobic stopped-flow measurements are to be made.

Before introducing the reagents to the sample handling unit, thoroughly flush (at least four times) the sample flow lines and syringes with deoxygenated buffer to remove all traces of sodium dithionite.

For extreme anaerobic operation, it is necessary to use the 22-101 Circulator Bath/Cooler (or equivalent). The 22-101 has provision for the purging of the thermostat medium with oxygen-free nitrogen. This can be important for the more stringent experiments where oxygen in the thermostat fluid passes through the walls of the sample flow circuit and influences the experiment.

7.0 Asymmetric mixing

By fitting drive syringes of different volumes, it is possible to mix the samples together in different ratios. The Hamilton AccuDil syringes listed below are compatible with the DX.17MV and, by using the appropriate combinations, the following ratios can be readily set up 1:1, 2.5:1, 4:1, 5:1, 10:1 and 25:1.

Capacity	100µl	250µl	500µl	1000µl	2500µl
Hamilton Part#	201050	201100	201150	201200	201250

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. 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 to 10 bar and is connected to the 'T' connector on the back of the sample handling unit. When asymmetric mixing is to be performed this 'T' connector must be removed and two separate pneumatic supplies connected. Looking from the back of the unit an 8 to 10 bar supply is connected to the left hand connector and a 2 bar (30psi) supply is connected to the right hand connector. 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.

8.0 Working in the Far-UV

The normal system, as supplied, will allow absorption measurements to be made down into the near-UV, that is down as far as 250nm. Below that point factors such as lamp output, photomultiplier sensitivity, and stray white light combine to such a level that the data collected may be suspect. In order to effectively and efficiently collect data in the far-UV it is necessary to attend to the following points:-

1) Replacing the Xenon lamp with a far-UV lamp will extend the useful UV output range of the lamp to 200nm and provide the potential for the instrument to work down to this wavelength.

2) Replacing the photomultiplier detector with one designed specifically for work in the far-UV will improve the quality of the data collected at 200nm by reducing the effect of stray white light.

3) Although following the above two points will extend the working range to 200nm the instrument stray light will still be around 3%. This can be reduced to 0.5% by replacing the ruled grating in the monochromator with a holographic grating. This final point is only really necessary if you are planning to perform extremely demanding far-UV absorption spectroscopy.

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9.0 Test reactions

9.1 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

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.

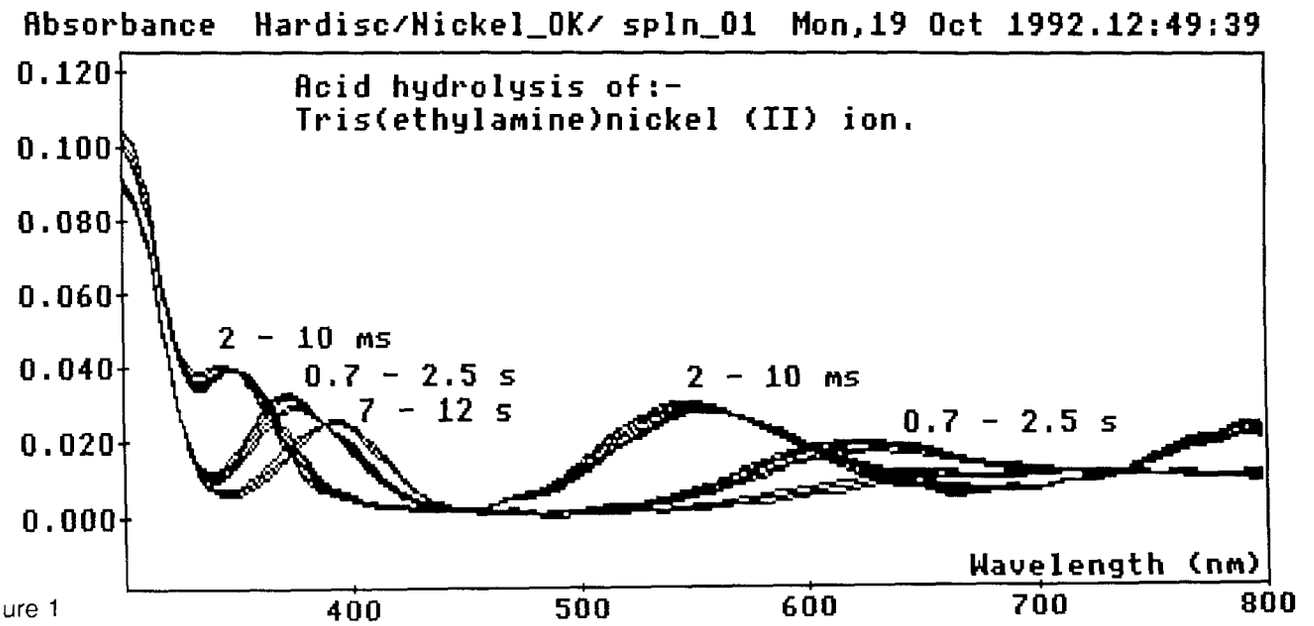


Figure 1

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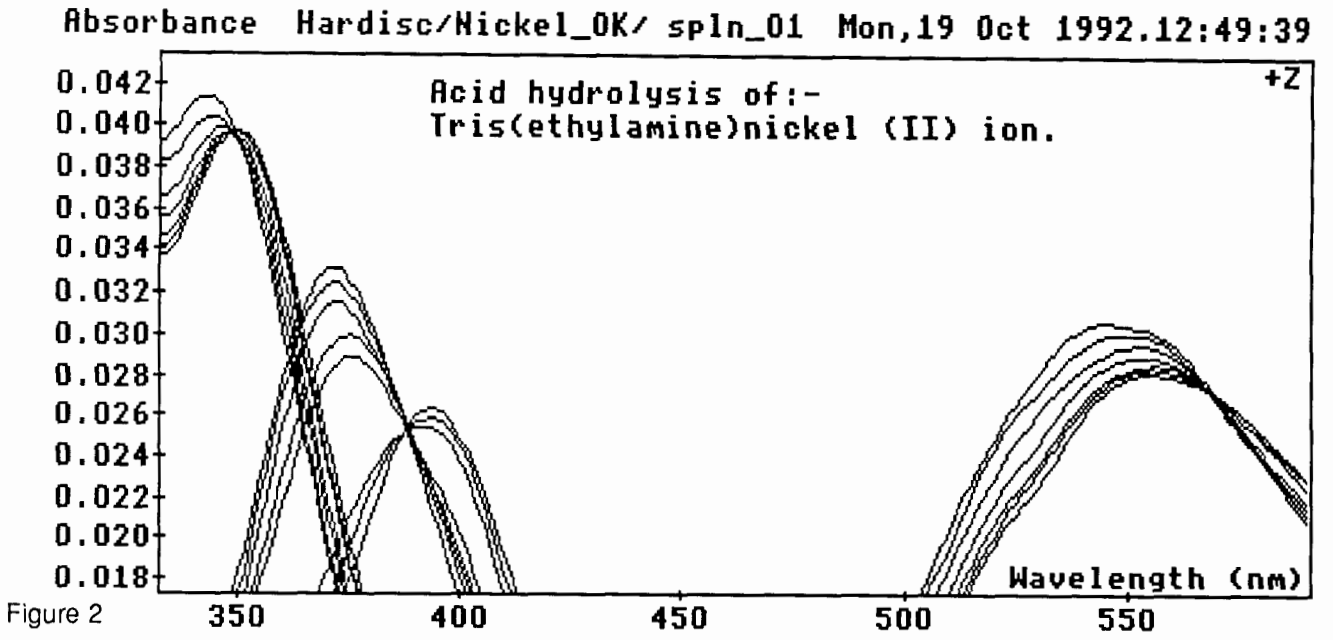


Figure 2

Data imprecision due to light source fluctuation is of the order of 0.001 AU (0.25% 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 DX.17MV host program, the same kinetic data can also be analysed within the separate 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.

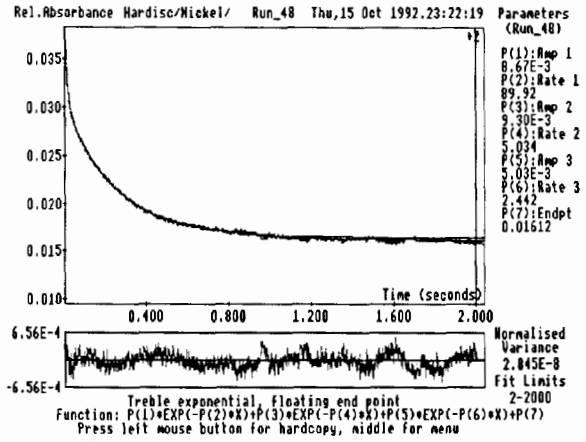


Figure 3

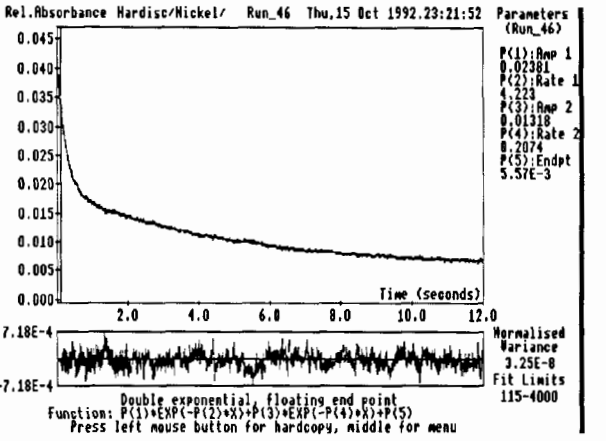


Figure 4

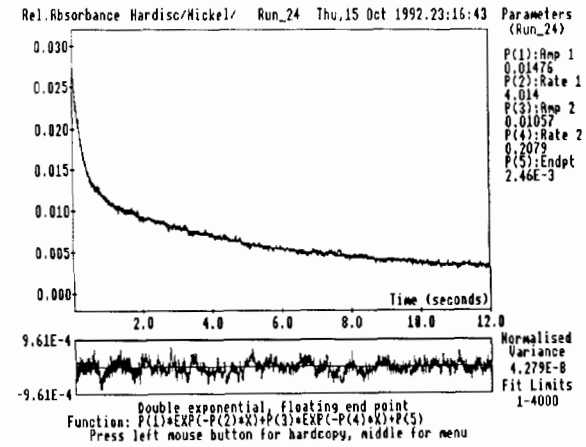


Figure 5

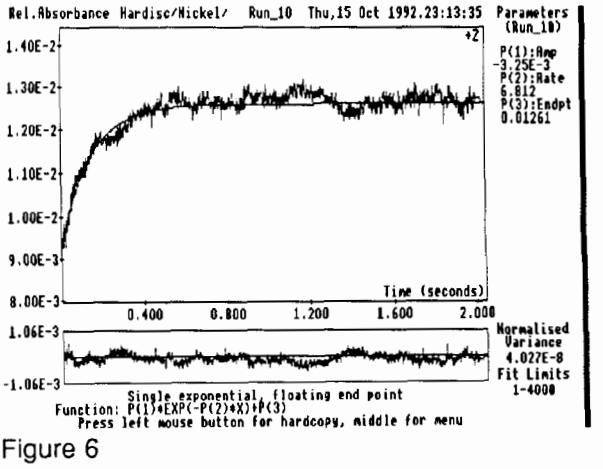


Figure 6

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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 100ms. 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 35 acquisitions.

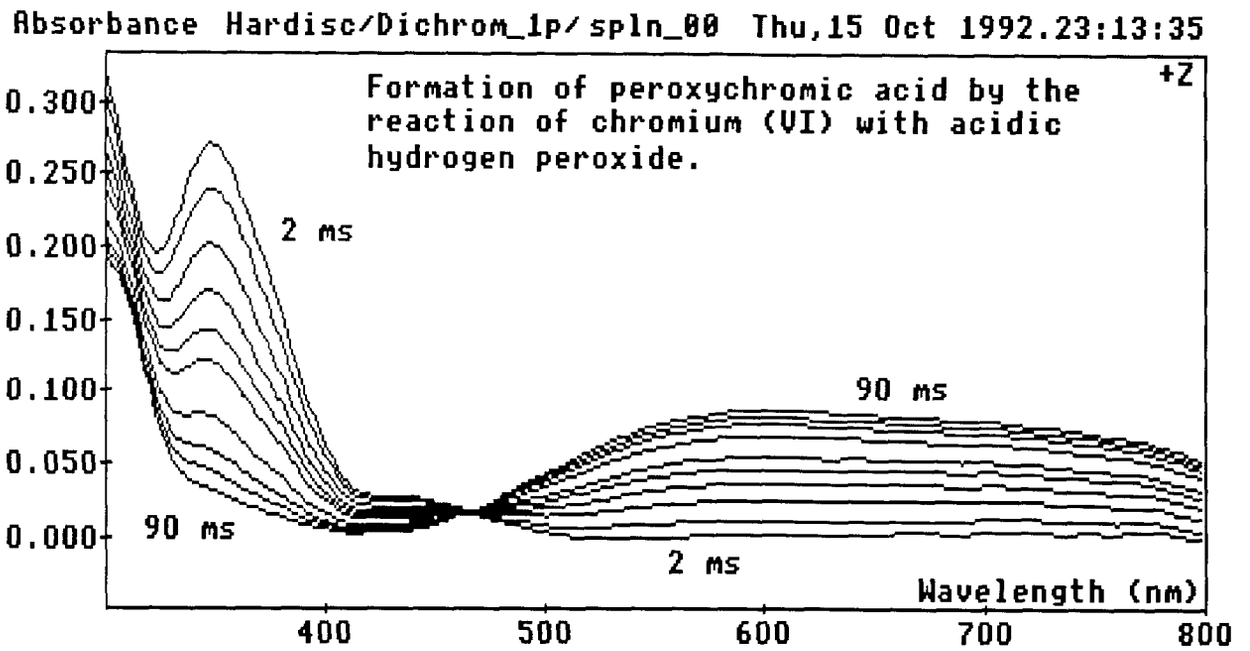


Figure 7

9.2 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 (10-3M) dichromate against the undiluted (10-1M) H2O2 reagent (trace 1), undiluted dichromate against 50% diluted H2O2 (trace 2) and 50% diluted dichromate against undiluted H2O2 (trace 3). This reference data is shown below in Figure 8.

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

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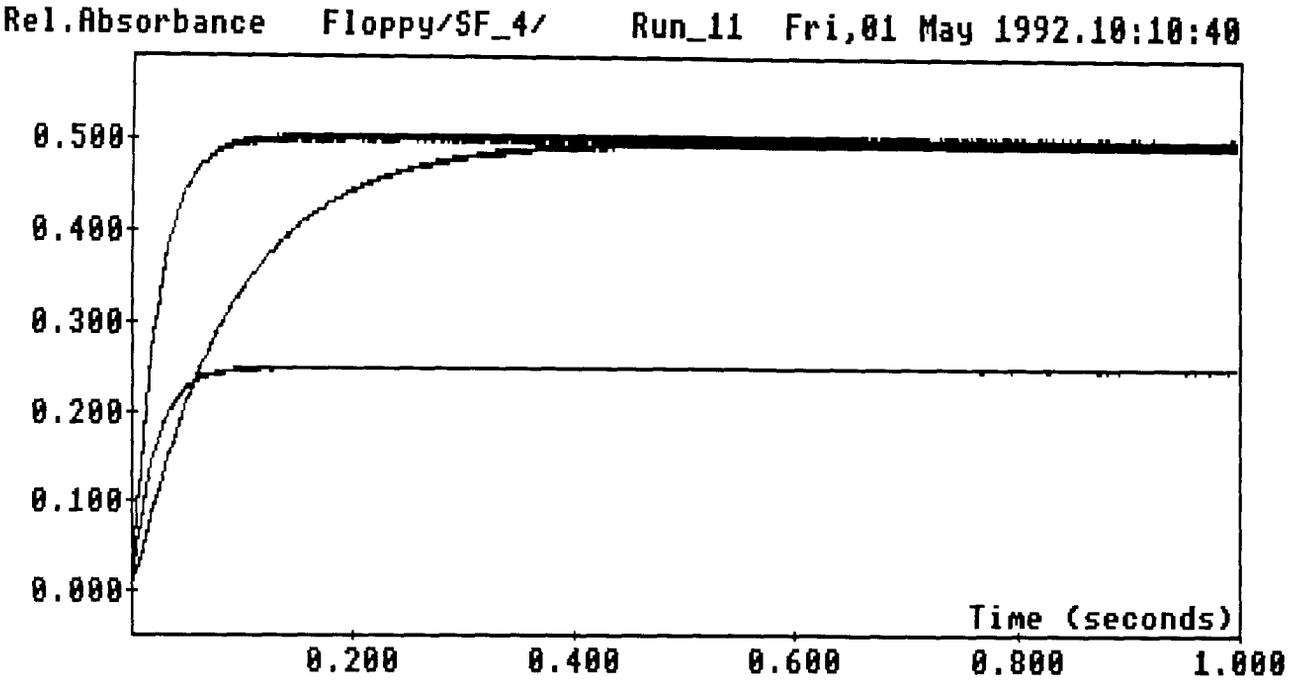


Figure 8

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.

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 = water; C = dichromate; F(flush) = water

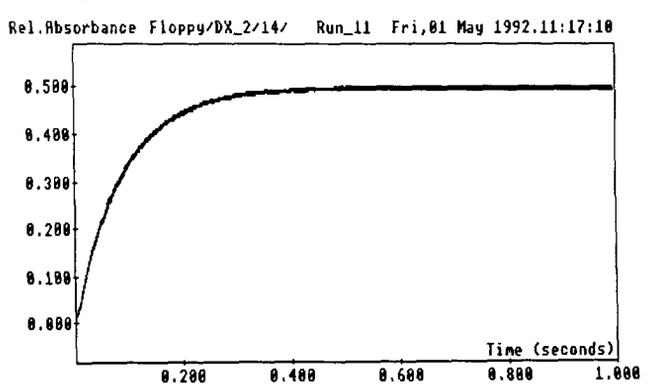


Figure 9

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

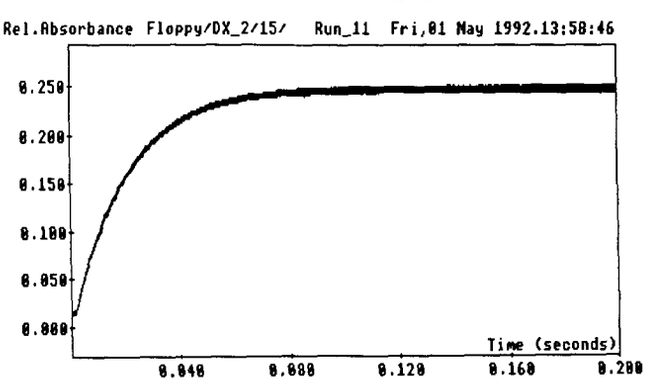


Figure 10

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9.3 Dead-time determination

9.31 Introduction

The determination of the dead-time performance of the DX.17MV 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.

9.32 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.

9.33 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.

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This equation transforms to :-

$$DA_{obs} / DA_{tot} = e^{-k_{app}.t_d}$$

and

$$\ln(DA_{obs}) = \ln(DA_{tot}) - k_{app}.t_d.$$

With the equation in the final form above, a plot of ln(DAbsorbance) 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.

9.34 Results

The following results are quite typical for the DX.17MV 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

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10.0 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.

10.1 Arc Light Source

Fault	Possible Causes	Remedy
Arc lamp fails to strike	1) Starter unit spark gap has worn increasing spark gap.	1) Remove the starter unit from its mounting platform. Remove the cover from the starter unit. Loosen the locking nut which secures the top electrode in the spark gap. Screw the top electrode down until it just touches the bottom electrode. Unscrew it 3/4 of a turn. Tighten the locking nut, replace the cover and replace the starter unit.
	2) Arc lamp requires replacing.	2) 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 7). 9 NB The lamp supplied with the system will have been used in the testing of the system and will have a shorter life than subsequent lamps.
	3) High voltage start pulse is escaping to earth.	3) Check the routing of the wiring from the starter unit to the bottom of the lamp. If the wiring has been damaged replace it. If it comes into contact with 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.
	4) A high resistance in the path to the bottom of the lamp is blocking the start pulse.	4) 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.

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10.1 Arc Lamp (Continued)

Fault	Possible Cause	Remedy
Lamp output appears low.	1) The lamp is misaligned	1) Check the alignment of the lamp using the live display in the New Data context of the software and the adjustment knob on the back of the lamp housing. If this does not improve the lamp output turn off the lamp, allow it to cool, open up the back of the lamp housing and check that the image if the lamp electrodes in the rear mirror is in alignment with the actual electrodes. (This procedure is detailed more fully at the end of section 3.8.)
	2) The lamp is being run at the wrong power.	2) Check that the lamp is being run at 150W. Ensure that while the meter on the power supply is being read the power control knob is pushed fully in so that the meter is reading power.

10.2 Monochromator

Fault	Possible Causes	Remedy
Computer gives "Monochromator not present" error.	1) Bad or incorrect IIC bus connection.	1) Check that the black, curly 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 in 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)

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10.3 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 black, curly 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.
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 approximately 10 seconds).	1) Trigger/Stop mechanism has become corroded due to oxidation or chemical attack.	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.
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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10.3 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.
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.	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 P.T.F.E. (Teflon) tips have worn to the extent that they cannot be rejuvenated.	2) The syringe plungers or the entire syringe should be replaced with new Hamilton syringes. For the standard 2.5ml syringes the Hamilton part numbers are: 201270 for the plunger alone and 201250 for the entire syringe.
Syringe Plungers Corrode.	1) The syringe plungers are made from aluminium and are coated, during manufacture, with a thin layer of P.T.F.E. (Teflon). 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. They can be replaced when next required. As long as the valves are left in the "Fill" position in air should enter the flow circuit.

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10.3 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 a pair of long nosed pliers. It should be tight enough that it cannot be loosened by hand, but be wary of damaging the flanges at the end of the tubes.

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