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Preface

The software described in this manual is version 4.08 of the Applied Photophysics Ltd SX17.MV Kinetic Spectrometer Workstation Software. The software has been designed to work with all models of Applied Photophysics Ltd Stopped Flow and Sequential Flow Spectra-fluorimeters using computer controlled SpectraKinetic Monochromators and Photometric Controllers. These include the SF17.MV Stopped Flow, the SX17.MV/S/1 Stopped Flow with Manual Stop, the SX17.MV/1 Sequential Stopped Flow with Manual Stop, the SX17.MV/S/2 Stopped Flow with Auto Stop the SX17.MV/2 Sequential Stopped Flow with Auto Stop. It will run on any Archimedes computer with a minimum of 4MBytes of RAM and with either a medium resolution colour monitor or a high resolution multi-sync monitor.

The software will also work on a stand alone Archimedes computer allowing data analysis and manipulation (e.g. producing time sliced spectra from kinetic traces) without tying up the Stopped Flow Spectra-fluorimeter.

The software comes with a 'Cfg' configuration file which contains details of the system hardware along with other relevant information such as the number of points per data acquisition. The features available in the software are governed by this 'Cfg" file. Not all of the features described in this manual are available to all user and the differences between systems are detailed below.

1) Differences Between Computers

Only the Acorn A5000 is capable of reading from, writing to and formatting High Density or DOS format discs. Therefore the only format option available on earlier machines is the ADFS 800K 'E' format. This is also the only format that earlier machines will read from or write to. It is possible to transfer data to DOS format 720K discs from the desktop environment on earlier machines using the blue disc drive icon.

In general the earlier '400' series Archimedes machines were supplied with 2MB of memory. This means that they are not capable of holding fourteen traces each of 4000 points in memory. Therefore software supplied for these machines will only acquire and/or display 400 point data traces. It is possible to alter the 'Cfg" file to allow more points per trace but the total number of traces must be reduced to compensate for this.

Some machines are supplied specifically for data manipulation and analysis and cannot be used for data acquisition. This is because they do not have the necessary expansion cards fitted. The "New Data" context of software running on these machines will only allow keyboard entry data and the normal acquisition screen is not present. Also the Scan context is removed and is replaced with "Draw I/O" as the "Make Draw" task is the only task left available in this section.

2) Differences Between Sample Handling Units.

a) SF17.MV Stopped Flow.

This software is only designed to work with SF17.MV systems fitted with computer controlled SpectraKinetic Monochromators and programmable Photometric Controllers. The features not available for this instrument are:–

SQ Mode. As the SF17.MV is only capable of single mixing stopped flow the sequential mixing mode is disabled. "Delay" and "Profiles" are also removed from the menu.

Kinetic Scans. As the SF17.MV is fitted with a manual stop syringe it is not possible to perform automated Point-By-Point Kinetic Scans. Therefore the "Prompt" attribute is permanently set to allow the user time to empty the stop syringe before prompting to move to the next wavelength. It is necessary to push the fire button on the Stopped Flow Sample Handling Unit to acquire the data at each wavelength. If the button is not pressed within 15 seconds the computer will "time out" and acquire a trace without waiting for a trigger from the Sample Handling Unit. If this happens the trace can be discarded and re-acquired at the same wavelength.

 # Shots. As the SF17.MV is fitted with a manual stop syringe it is not possible to acquire more one trace at a time. Therefore the "Number of Shots" option is set to one and removed from the menu.

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b) SX17.MV/S/1 Stopped Flow with Manual Stop (Capable of being upgraded to sequential stopped flow)

SQ Mode. As the SX17.MV/S/1 is only capable of single mixing stopped flow the sequential mixing mode is disabled. However, if the Sample Handling Unit is upgraded the software will automatically recognise this and allow access to the sequential mixing mode.

Kinetic Scans. As the SX17.MV/S/1 is fitted with a manual stop syringe it is not possible to perform automated Point-By-Point Kinetic Scans. Therefore the "Prompt" attribute is permanently set to allow the user time to empty the stop syringe before prompting to move to the next wavelength.

 # Shots. As the SX17.MV/S/1 is fitted with a manual stop syringe it is not possible to acquire more one trace at a time. Therefore the "Number of Shots" option is set to one and removed from the menu.

c) SX17.MV/1 Sequential Stopped Flow with Manual Stop

Kinetic Scans. As the SX17.MV/1 is fitted with a manual stop syringe it is not possible to perform automated Point-By-Point Kinetic Scans. Therefore the "Prompt" attribute is permanently set to allow the user time to empty the stop syringe before prompting to move to the next wavelength.

 # Shots. As the SX17.MV/1 is fitted with a manual stop syringe it is not possible to acquire more one trace at a time. Therefore the "Number of Shots" option is set to one and removed from the menu.

d) SX17.MV/S/2 Stopped Flow with Automatic Stop

SQ Mode. As the SX17.MV/S/2 is only capable of single mixing stopped flow the sequential mixing mode is disabled. However, if the Sample Handling Unit is upgraded the software will automatically recognise this and allow access to the sequential mixing mode.

d) SX17.MV/2 Sequential Stopped Flow with Automatic Stop

All of the features described in this manual are available.

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!SX.17MV Spectrometer Software

1.0 General Introduction

The !SX.17MV spectrometer workstation software allows both regular stopped flow and sequential stopped-flow to be performed.

The SX.17MV sample handling unit possesses two pneumatic rams and four drive syringes (A, B, C & F (flush) - see hardware manual). In sequential (SQ) mode two primary reagents (A) and (B) are mixed by the first pair of drive syringes and held in a low volume ageing loop (115ul). The second drive is initiated after a pre-determined delay, flushing (using Flush syringe) the loop from the rear, whose contents are mixed with a third reagent (C) before arriving in the observation cell where the subsequent reaction is monitored optically. Reaction ageing times, between 10msecs and 1000secs (for the reaction of A and B in the loop before mixing with C) are available.

In regular stopped-flow mode only one drive is activated, with no delay processing, reagents (C) and (F) being directly mixed and passed to the observation cell.

NOTE for sequential stopped-flow reagents A and B occupy the RIGHT hand pair of syringes while in regular stopped-flow mode they occupy the LEFT hand pair of syringes.

The Applied Photophysics Kinetic Spectrometer Workstation is an extremely powerful and versatile data acquisition and processing facility exploiting advanced 32-bit RISC (reduced instruction set) microcomputer technology. Intended to complement a range of Applied Photophysics products the workstation offers unrivalled performance, demonstrated particularly by stunning colour graphics and data reduction capabilities.

In addition to a proprietary multi-tasking operating system, RISC OS (which provides an advanced windows and icons style user interface) a PC emulation facility allows the computer to run MS-DOS applications when required.

1.1 The RISC Workstation

This section summarises the principal features of the workstation hardware and operating system relevant for understanding later material on software installation and operation. As such it is not intended to be a comprehensive technical specification. The User Guides accompanying the computer provide further information if required.

The standard workstation comes complete with 4 MByte of RAM, a high resolution multi-sync colour monitor, 3.5" floppy disc drive (up to 1.6MB) and 80MByte hard disc. Both parallel (Centronix) and serial ports (RS423/232) are built in. The printer is run from the parallel port. A three button mouse is provided in addition to the extended keyboard. The mouse is used extensively to drive the workstation software.
A portion of the disk may be configured as an MS-DOS partition (for PC applications), the rest being accessed by the RISC OS filing system ADFS.

1.2 Data Acquisition Hardware

Data acquisition is performed via a 12bit 150kHz analogue to digital (A/D) converter mounted within the computer. This also possesses an 8 bit digital I/O bus. Both facilities are accessed via rear panel connectors (D-type and IDC respectively). These are both utilized when operating the SX.17MV stopped flow and are connected via the leads provided to the photometric unit. Further information on the A/D converter can be provided on request.
Peripheral devices, such as the photometric unit, programmable monochromator and the sample head itself are linked to a second IIC communications interface card in the computer.

1.3 The RISC Operating System - RISC OS

The operating system, RISC OS, is an extremely powerful multitasking environment specifically designed to exploit the power of the RISC processor. It is ROM based and therefore does not need loading from disc, nor does it occupy any of the available 4 MByte RAM which is left free for RISC OS applications including the SX.17MV kinetic package. It presents a Macintosh style user interface and desktop program suite. Applications are selected using the mouse to click over icon images on the screen.
Further information on RISC OS and its accompanying desktop facilities (see !Draw, !Paint and !Edit for example) may be found in the User Guide. An extremely powerful version of the BASIC language is also provided (the language in which this software is written!) along with a comprehensive BASIC editor.

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1.4 PC Emulation

PC compatibility is available with a PC emulation package which is installed on the hard disc. The running emulator provides an window environment compatible with an IBM PC with CGA, EGA or VGA colour graphics and 3.5" floppy disc drive (drive A:). The emulation includes Maths Co-Processor emulation. In addition a variable sized MS-DOS hard disc partition (drive C:) can be configured on the resident hard disc.
Note: although a large range of PC applications have been shown to run in this mode, Applied Photophysics cannot guarantee 100% compatibility and execution of any such software.

1.5 !SX.17MV Spectrometer Software

The !SX.17MV software package is a complete data acquisition and analysis system for the Applied Photophysics SX.17MV Sequential Stopped-flow Spectrofluorimeter. The programs are provided both on a single floppy disc (bearing a version number) and installed in an application directory (see below) on the hard disc. A familiarity with the RISC OS operating system, the command line interpreter and the Desktop suite is recommended. This can be gained from the User Guides accompanying the computer.

1.6 Formatting Discs and Making Software Backups

It is advisable to backup the software floppy disc onto another formatted disc as soon as possible. Formatting and backing up discs is described in the User Guides, but briefly can be carried out either directly from the Desktop (simplest) or by using the command line interpreter (CLI).

Formatting: When the desktop screen appears at switch on insert the unformatted floppy disc in the drive. Click the middle mouse button over the floppy drive icon on the menu bar to reveal its associated menu and select the **FORMAT** option. Now follow the prompts (new discs should be formatted with the E option).

Backing up:
Insert the source floppy disc in the drive and select the floppy drive menu as above. Select the **BACKUP** option and again follow the prompts. Note: it is necessary to have previously formatted a destination disc.

Ensure the source disc is write protected ie. the corner hole on the case is uncovered.

Consult the User Guides for information on hard disc formatting if this is ever required.

1.7 The RISC OS Disc Filing System

The filing system used by RISC OS is called the Advanced Disc Filing System or ADFS. It is a sophisticated hierarchical directory rooting system similar to that available under MS-DOS. The principal difference is in the use of . rather than \ to delimit directory paths and the absence of three letter filetype suffixes such as .txt or .bat etc (see User Guide).

A special type of directory is used to contain software applications. These appear in the desktop display of the hard disc root directory (the top level directory, displayed by clicking the left mouse button over the hard disc icon on the menu bar) with a representative icon and a name preceded with an '!'. Such application directories include !Draw and !Paint from the Desktop applications suite.

The spectrometer software adopts the same convention and is contained in the applications directory !SX17MV with an icon based on the appearance of the stopped flow unit.

1.8 Running the Kinetics Software

It is a requirement that the A/D converter and interface are present for the software to run (although analysis only packages can be provided on request). The software can be run from the hard disc according to the startup protocol for other desktop applications, namely by double clicking the left mouse button when the screen pointer is over the !SX17MV icon in the hard disc root directory display.
If programmable peripheral devices such as the photometric unit are not linked and switched on a communications error will be reported at start up. The operator will subsequently not be able to access certain parts of the program associated with data acquisition. All other filing and data manipulation facilities are still available however.

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1.9 Notes on Software Installation

New systems already have the spectrometer software installed on hard disc ready to run. This section is included primarily for future installation of software updates. To perform this installation it is necessary to be familiar with the filing menu system of the RISC OS desktop (see the section Filing Systems in the User Guide).

The spectrometer software to be replaced is contained in the application directory !SX17MV on the hard disc. First rename this directory SX17MV. This will then reappear as a normal directory with a "folder" icon, and act as a software backup in the unlikely event that the replacement software is corrupted. Insert the floppy disc containing the new version of the software and open its director display. Observe the familiar !SX17MV directory and icon. To copy this to the hard disc simply drag* this icon from the floppy display to any point within the hard disc directory window and release the mouse.
The new software can then be run as before.
Note: to delete the backup directory SX17MV it is necessary to first access its contents and unlock them against deletion.

*Point to the icon in the source window and depress the left mouse button. Holding this button down move the pointer to the destination window (an outline of the window entry moves with the pointer) and then release the button to complete the action.

Cfg File Many of the program's parameters are definable and these can be set in the Cfg (Configure) file inside the Application Directory. To gain access to the Cfg file first open the directory containing the SX17MV software, (usually the root directory of the hard drive). Open the SX17MV Application Directory by holding down the shift key on the keyboard, pointing to the SX17MV software icon with the mouse pointer and double clicking the left hand mouse key. Another filler window will open in which you will find a file called "Cfg". Load this file into the text editor, EDIT. (Those with A5000 computers can do this by holding down the shift key and double clicking in the Cfg file with the left hand mouse key. Those with older computers must load EDIT from App1 and dragging the Cfg file to the EDIT icon on the Icon bar.) When the Cfg file is loaded into EDIT you should be able to see the following:–

```
Computer (A400 series = 0, A5000 = 1, A4 = 2)
1
RISC Processor (2,3)
3
Monitor (AKF12 or AKF17 = 0, AKF18 = 1, Taxan775 = 2, LCD = 3)
2
Printer (low resolution = 0, high resolution = 1, turbo = 2)
1
Instrument model (SF17MV = 0, SX17MV = 1)
1
Photometric control unit (manual = 0, programmable = 1)
1
Monochromator (manual = 0, one programmable = 1, two programmable = 2 )
1
Stop-syringe (manual = 0, programmable = 1)
1
Channels (single channel = 1, dual channel = 2)
1
X-shift parameter (µs)
500
Drive ratio A:B (50:1, 25:1, 10:1, 5:1, 5:2, 1:1, 2:5, 1:5, 1:10, 1:25, 1:50)
1:1
Drive ratio C:F (50:1, 25:1, 10:1, 5:1, 5:2, 1:1, 2:5, 1:5, 1:10, 1:25, 1:50)
1:1
Drive pressure hold on (1=1s, 2=2s, 3=5s, 4=10s, 5=all)
4
Max emission PMT voltage (standard instrument = 1000, special apps = 2000)
1000
Software configuration (data analysis only = 0, full system = 1)
1
Initial generation of synthetic data (no = 0, yes = 1)
1
Start up preference (absorption = 0, emission = 1)
0
Mouse step (1 to 5)
5
Data blocks (4 to 15)
15
Points (up to 4000, 400 is standard)
4000
```


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Each line describes an option and the number under that line sets the option. e.g. the above software is set for an A5000 computer with an ARM3 processor and a TAXAN 775 monitor. The printer is set to high resolution and the instrument is a SX17MV with programmable peripherals. It is set for single channel. All the drive syringes are 2.5 ml, (i.e. both drive ratios are 1:1), Some of the options may not apply to your instrument but they should be self explanatory.

2.0 Application features

The spectrometer software is initiated from the RISC OS desktop by double clicking over the !SX17MV icon in the hard disc directory window. During operation other applications are not available ie the current spectrometer software does not support multitasking. If it is subsequently quit, the desktop is restored.

2.1 Summary of Facilities - The principal tasks of the software are outlined below:

Data acquisition in either sequential or regular stopped flow mode.
Data is collected from the photometric controller via the A/D converter into memory. 400 values are collected over timebases ranging from 5 msecs to 1000 secs. In split timebase mode 200 points are acquired at one timebase followed by 200 points at a second timebase. (The number of points is adjustable between 400 and 4000).

The Y-scale has stepped gain adjustment yielding input Y-ranges of +/-5 volts to +/-0.1 volts, or in absorbance mode 2 A.U. to 0.02 A.U. full scale. A real time display of the incoming signal in volts or absorbance is continuously displayed. Up to 15 acquired traces can be accommodated in memory at one time.

Data storage on either floppy or hard discs.
Data can be saved in several formats on disc. Related files can be stored together in a single directory. This is referred to as a **Group**. New groups can be created as required and are all accessed at the root directory level. The action of selecting or creating a group (see below) opens it for subsequent data load/save operations. 5 lines of descriptive text are saved with each data file, four of which are automatically assigned relevant acquisition information.

Approximately 100 data files can be stored on a floppy disc. These can be spread over an arbitrary number of groups, but no single group can contain more than 77 entries (see User Guide).
In addition, when data has been fitted, all results and information necessary to re-display the fit are included in the file when it is saved. A special group (Flibrary) is used for holding user function libraries.

Manipulation and display of data and hard copy output.
Data in memory can be displayed instantly and randomly with a single mouse click. The full colour display is self scaling and data sets can be arbitrarily superimposed or removed. Any area of a screen composition can be inspected with a fast screen zoom facility. A cross hair allows tracking and digital output of absolute data values. Screens may be quickly annotated using a labelling facility before buffered hardcopy to a printer or HPGL plotter. A number of data manipulations are provided including unlimited trace averaging, trace smoothing, trace subtraction, ratioing, and transmission to absorbance conversion.

Fast and robust non linear regression analysis for estimation of kinetic parameters.
A powerful implementation of the Marquardt-Levenberg algorithm is available for non-linear regression analysis of traces to analytical equations. A range of standard mechanisms including multiple exponentials are provided. During a fit any combination of equation parameters may be fitted or held constant allowing previously established values to be imported into complex mechanisms.

The results of a fit are presented as a list of estimated parameters, their standard error and the normalised variance of the data to the fitted curve. Graphically, the theoretical curve is superimposed over the data, and a residual plot displayed. Fitted data is stored on disc with these results. A unique facility allows the operator to supplement the system equations with his/her own user equations, containing up to 10 fitted parameters each. These are entered via a friendly equation editor as direct algebraic expressions. Up to 10 such equations can be entered creating a user set which can be used alongside the system set. These can be stored as re-loadable function libraries on disc, thereby allowing an unlimited range of equations to be generated and accessed. One distinct advantage of this is that different users can independently build up their own libraries of functions without interference.

Data porting to and from other environments.
The A5000 computer can read and write from and to Dos 720k and 1.44MB floppy discs and the SX17MV software takes full advantage of this. CSV (Comma Separated Values) data can be loaded directly into the software, allowing analysis of data generated on other instruments to be analysed in this software package. A built in data editor not only allows inspection and editing of stopped flow data in memory but also entry of data derived elsewhere or

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keyboard entered data. All software facilities including curve fitting may be applied to such files, the length of which is arbitrary, from 1-4000 points. Note: any type of (X,Y) data can be input regardless of units.

Data Format
Data can be saved in a variety of formats. AP-ASCII format is our standard format and described in Appendix IV, CSV for export to other packages, BioKine for export to BioLogic's analysis software and Draw to produce vector graphic file for export to Acorn DeskTop Publishing packages. It is also possible to save data in HPGL file format for porting to and subsequent plotting from MS-DOS machines.

3.0 The user interface and software control

3.1 Use of the Mouse.

The entire software package is graphics based and mouse driven. All operations and selections are initiated by pointing at distinctive regions of the computer screen with the mouse controlled screen pointer (an arrow) and clicking a particular button on the mouse itself. The simplest way to learn about the software capabilities is by experimentation: pointing, clicking a mouse button and observing the result.

3.2 Mouse and Keyboard Entry Protocols

During program execution certain classes of operations require similar response sequences from the operator and where possible these have been given a common format. Such is the case where keyboard entry of names and numbers is necessary. Where operations require intermediate decisions or remain active until user cancellation, brief on-line instructions are provided indicating how completion or termination can be achieved for the task in question.

A number of protocols have been adopted to facilitate more complex tasks by providing groups of common operator actions. These are now described. Note, however that other software applications may adopt different conventions.

Pointing and clicking...an option block or data block
This abbreviated statement is used to denote using the mouse to move the on-screen pointer to within a particular defined screen area and pressing a mouse button to activate the associated task. In general pressing the LEFT mouse button ACTIVATES a task or CONFIRMS a request eg save or load a data file, plotting a trace or switching on a data cursor etc. Conversely pressing the MIDDLE mouse button CANCELS a task. This is frequently used to withdraw from actions or to leave activities eg cancelling a save or load operation or cancelling a screen zoom request. **If in doubt press the middle mouse button to cancel or exit**

It is not always possible to simplify the mouse key actions to these two alone, in which case more complex key combinations can occur. Instructions accompany the activity in such cases eg. cursor cancellation requires simultaneous pressing of both the left and right mouse keys (or in this case pressing the space bar).

Keyboard entry of typed data (eg parameter estimates, file names etc) is always accompanied by presentation of an on screen data entry window which implicitly limits typed entry to the appropriate format,ie number of entries and entry length. Filename entry for example allows a 5 alphanumeric title suffixed by a 2 digit number. If a previous assigned value or name is available this is displayed in the window and can be accepted if appropriate.

When keyboard entry is required a flashing block in the entry window indicates the position of the input cursor. Subsequent editing of values or character strings uses the following rules.

Text is typed from the keyboard in insert mode ie shifting following text to the right. Note, text does NOT wrap around to the next line. The **left** and **right** cursors will move the text entry point in the corresponding direction. The **delete** key can be used to remove characters to the left of the cursor while the **home** key will delete all text on the current line to the right of the cursor. If more than one line of entry is indicated (by the presence of several available prompted input lines) then pressing the **up** or **down** arrows will move between lines. Pressing **return** will drop a line and perform a carriage return. With single line entry or when at the bottom line of a multiple entry input pressing **return** completes the input and closes the window.

In addition, the **left** mouse button will also close the window, the program accepting the currently displayed text values for processing. Conversely the **middle** mouse button closes the window but returns as though the window had not been opened in the first place, ie cancelling the operation and leaving any previously assigned values intact.

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3.3 Special Keys

Function Keys Function keys one to eight mimic the action of the task menu (that is the second menu down). This means that in the 'New Data' section F1 will acquire data, F2 sets the number of shots, F3 performs an Auto PM etc. This action of mimicking the task menu is valid no matter what section of the software you are in.

Number Keys The number keys on the main keyboard, directly below the function keys mimic the action of the Attribute Menu (that is the third menu down). Numbers one to four mimic the left hand four selections and numbers seven to zero mimic the right hand four selections. This means that in the 'New Data' section 1 turns Internal Trigger on or off, 2 turns Split Timebase on or off, 7 results in data being acquired in volts instead of absorption units, 0 switches SQ Flow on or off etc. This action of mimicking the attribute menu is valid no matter what section of the software you are in.

Esc This is used to abort a pre-programmed sequence of events. Examples of these are long time base data acquisition, Screen dumps, Point-By-Point data sequences, multi-shot single wavelength sequences etc, **NOTE the break key duplicates the action of the Esc key.**

Ctrl-Break This performs a system reset and restarts the desktop.

3.4 Screen Layout

A single screen layout is used throughout the software. This can be divided into a number of functional areas.

The **graph area** occupies the bulk of the screen. All graphical displays are presented here. As a rule there is no mouse related activity associated with this region. Instead a display of the mouse pointer position in current graphics units may be displayed digitally in the menu region (see below). The **data in memory** is displayed in a grey backed column of discrete rectangles to the right of the graphics area. This shows the state of occupation of the 15 data buffers available in memory. Each block displays its contents: blank when empty or a data filename when occupied. The colour code to the immediate left corresponds to the colour with which a blocks contents are displayed. **A particular block, whether full or empty is selected by pointing and clicking it with the mouse.**

The action is acknowledged by highlighting of the block and may result in further effects depending on the current program context. In all cases, however, it results in the accessing of the data in the block for a subsequent operation, display, file saving/loading, transformation etc.

The usual purpose of selecting a data block is to display the data it contains in the graphics area.

Immediately below the graphics region are displayed the available program **contexts** (see Section 4.7) in a row of coloured boxes. This is the primary mouse activated menu. The chosen context is shown highlighted with a red background. Only one context can be operational at a time.

Below the context menu in the bottom section of the screen are shown all the **options** available within the chosen context. These comprise two lines of 8 coloured rectangles, each containing the name of their associated function. These are again activated by the mouse. The two rows do differ somewhat in operation, a feature which is discussed later.

These menu bars are not continuously displayed. In the DISPLAY context for example, only when the mouse pointer is brought down towards the region do they appear. Otherwise a digital output of the mouse pointer position in the graphics area is presented. Furthermore during complex tasks they may be temporarily replaced by on-line instructions for the operator. The menu structures and program format are described in more detail in the section 4.7.

3.5 Windows

During execution many activities require input from, or the presentation of information to, the operator. This is carried out using distinctive opening and closing windows. These appear superimposed over the main display for the duration of the task and close down when no longer required, restoring the existing background. All operator keyboard data entry is accepted within such windows. They are used extensively and when open are usually accompanied by instructions (in the menu area) relevant to their purpose.

Often a window will display a choice of objects requiring the operator to make a selection: loading a selected disc file or choosing a fitting equation etc. In this instance a coloured bar highlights one option at a time and moves amongst the alternatives in response to the mouse pointer. When the desired object is highlighted the left mouse button is used to confirm the choice and the program proceeds. Alternatively in the event of an error or a change of mind the middle button will close the window and cancel the task.

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3.6 Program Format

The program format has been structured carefully to allow fast and efficient access to its resources. Related operations and tasks have been grouped together logically into eight program **CONTEXTS** each of which can be entered randomly from any other. Within each a set of submenus allows a comprehensive range of activities. The Contexts are permanently on display as described already and are selected with the mouse pointer in the usual way. The current context is always highlighted with a red background.

The Context titles cover eight functional areas:

New Data Provides options and configuration control for data acquisition from the stopped flow. It also provides access to control of all peripheral devices for centralised signal set-up. In an alternate mode it enables keyboard data entry.

Main This context contains some general mode and review options for example. The software initialises in this context.

Disc All disc accessing operations are carried out from here such as the creation and selection of disc groups and the saving and loading of data or function files. In addition housekeeping tasks include deletion of old files and groups and floppy disc formatting.

Display Options affecting the way data is displayed graphically are provided here. These include the selection of autoscaling, overlay, grids, zoom etc as well as allowing cross-hair tracking of data and graph annotation.

Analyse Curve fitting activity is controlled here. Fitting ranges can be altered, fit equations selected or entered and fits conducted. Results can be displayed and printed out.

Transform This context groups together operations which modify data in memory. Activities include data smoothing, trace averaging and referencing and transmission to absorbance conversion.

Spectra This allows spectra to be generated from sets of wavelength encoded kinetic records, which will have been obtained in the Scan context.

ScanData This provides the facility to either collect accurate steady state sample spectra, or sets of wavelength dependant kinetics.

The menus and options of a context are presented in two rows of mouse activated bars at the bottom of the screen. These two rows accommodate options of a different kind and behave somewhat differently. The **top row** contains **TASKS** while the **bottom row** contains **MODES.**

TASKS are distinguished by the fact that their selection initiates an event or sequence of events which will run to completion either with or without operator interaction, ie they result in a specific and immediate action. Examples include file naming, screen clearing, equation selection, data averaging etc. A task rectangle becomes highlighted whenever the mouse pointer crosses over it indicating that it will be activated with a button press.

MODES on the other hand do not necessarily result in an immediate action, other than the fact that the associated mode rectangle becomes highlighted. Rather they are used as switches to determine the way in which other events, including tasks, are executed. In the Display context for example, Mode options include autoscale, grid and overlay etc all of which can be switched on or off and determine how subsequent display activities are carried out. The on/off action is carried out simply, by successive pointing and clicking the particular option rectangle.

In conclusion two general points are worth emphasising: Any Context can be accessed immediately from any other. For example the operator can pass from Analysis or Disc directly to New Data or Transform with one keypress. There are no complicated routes or key sequences required.

Certain tasks can be carried out in more than one context since they are not associated with a specific menu: data display by memory block selection, for example. This action can be executed in all contexts. The way in which it is carried out, however, is determined by options set exclusively in the display context (grid, autoscale etc), and can only be altered by re-entering this context, to access the menu where these options are available.

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4.0 Regular and sequential stopped flow

The !SX.17MV spectrometer workstation software allows both regular stopped flow and sequential stopped-flow to be performed.

The SX.17MV sample handling unit possesses two pneumatic rams and four drive syringes (A, B, C & F (flush) - see hardware manual). In sequential (SQ) mode two primary reagents (A) and (B) are mixed by the first pair of drive syringes and held in a low volume ageing loop (115ul). The second drive is initiated after a pre-determined delay, flushing (using Flush syringe) the loop from the rear, whose contents are mixed with a third reagent (C) before arriving in the observation cell where the subsequent reaction is monitored optically. Reaction ageing times, between 10msecs and 1000secs (for the reaction of A and B in the loop before mixing with C) are available.

In regular stopped-flow mode only one drive is activated, with no delay processing, reagents (C) and (F) being directly mixed and passed to the observation cell.

It is helpful during system operation if the user is aware of the overall system sample flow circuit since this will help in the interpretation of potential drive volume errors and reduce sample wastage.

Software Support
All necessary software features for supporting sequential mixing are to be found in the 'NEW DATA' context (see below).

Regular or sequential mixing stopped flow operation are selected using the mode key labelled **'SQ Flow'** to the bottom right of the display. When illuminated, by clicking with the mouse, sequential mixing mode is selected. The two softkey options **'Delay'** and **'Profiles'** then become available for programming and interpreting sequential mixing experiments.

Regular stopped flow
By default standard stopped flow is selected, ie only one pneumatic ram (flush ram) is driven. This is the one on the LEFT of the sample handling unit. Reagents should therefore be placed in the corresponding syringes (C and F).

Sequential Mixing
When the 'SQ Flow' mode has been selected, activating the 'acquire' softkey initiates the sequential drive of both pneumatic rams.

Reagents A and B are driven from the right hand pair of syringes and reagents C and F (flush) from the left hand pair. The left hand drive is initiated after the right hand drive according to a pre-set delay. This delay may be adjusted by selecting the **'Delay'** softkey before a drive and typing in that required. Any delay between 0 msec and 1000000 msecs may be entered (in 1msec steps). Note that the entered delay is approximate as drive conditions and sample behaviour influence the actual drive process. However automatic drive monitoring during flow allows the ageing time to be calculated with considerable accuracy after each determination.
Immediately following a drive the calculated ageing time is displayed for a short time. This corresponds to the total time reagents A and B remained in the ageing loop before being mixed with reagent C. It takes full account of both loop filling and emptying times and the portion of the loop that is 'stopped' in the observation cell as well as the software programmed delay.

This calculated ageing time is incorporated along with other experimental conditions (eg. temperature) in the data tag which is saved with data files on disc.

It may well be that one or more errors relating to drive volumes are reported following a run, particularly when setting up at the beginning of new experiments. It is helpful to have a clear understanding of the flow circuit and path taken by the syringe contents to minimise confusion. See below and Appendix III for a description of handling these errors.
More details about the behaviour of a drive are available by selecting the **'Profiles'** softkey.
This displays the precise profiles of the two drives as ram displacement volume versus time (µl vs msecs). This data which is acquired during the actual run, prior to receipt of the stop trigger, is used in the calculation of the exact ageing time of the reagents A and B before mixing with C and subsequent post-trigger observation. Also shown are the total volumes displaced by the two syringe pairs. Test results show that the ideal volumes displayed in the Profiles section are 220µl for Drive 1 and 180µl for Drive 2. The flow volumes should be adjusted as necessary to give these two volumes. Two vertical bars highlight the critical portion of each drive, their separation in time corresponding to the calculated reaction age.

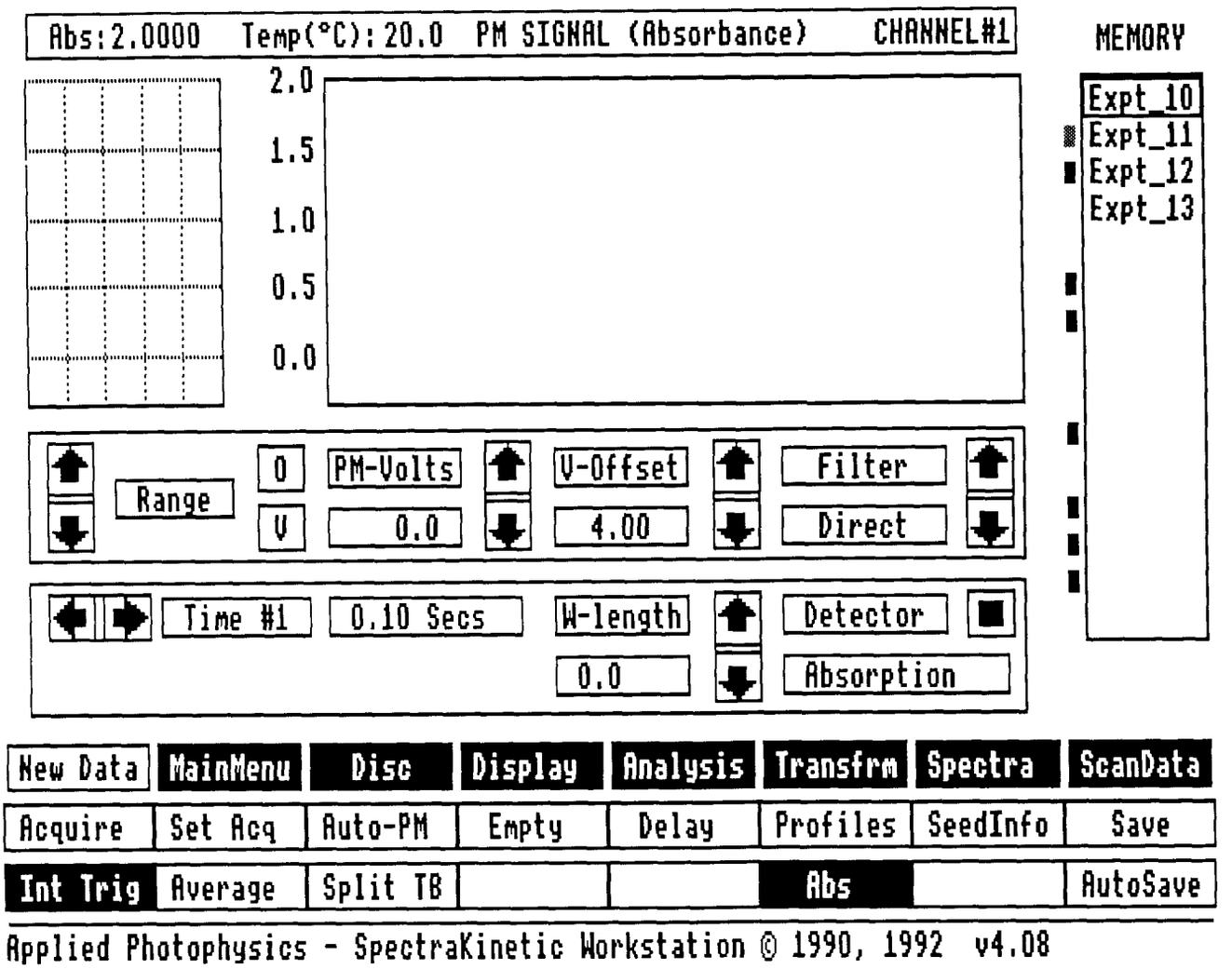
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5.0 Operation Reference

This section provides descriptions of all software operations in each of the eight program contexts. They are covered in the order that they appear across the screen from left to right. **Tasks** are listed first followed by **Modes.** Mathematical methods and data transformations used in certain operations (eg transmission to absorbance conversion) are discussed in Appendix II. Experimental procedures including interactive signal set up with the stopped flow are covered in Appendix I.

5.1 New Data

Because of the high level of peripheral automation in the SX17MV a special control panel supplements the normal softkey options in this context. This is used for centrally adjusting a range of important parameters controlling the input signal:



As you can see from the screen shot above these include:

- Detection mode (Absorption or Emission)
- Photomultiplier voltage
- Signal Offset
- Signal Filter time constant
- Timebase and sensitivity of data acquisition
- Monochromator wavelength

Each of these settings can be simply adjusted using the mouse and are on continuous display.

This centralisation of signal control in the software enables increased system automation and reduces the risk of incorrect signal set-up. In addition all settings are registered with acquired data for later recall.

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To enter the acquisition context select 'New Data' from any other program context. This data acquisition screen has two graphical displays showing both the 'live' incoming signal from the photometric unit and a static display of the last 'acquired' signal. The control panel indicates the settings of the various signal set up and filtering parameters and also the acquisition timebase and sensitivity.

Alongside each displayed value are mouse sensitive arrows which are used to adjust the value up or down. When activated the arrow colour inverts to indicate selection. The particular mouse button pressed affects the speed of the response. The left mouse button allows single step incrementing or decrementing of the parameter or, when held down, slow scanning of the parameter to its upper or lower limit. When operating the mouse incrementally please note that there is a necessary minimum response time to the action. Fast double clicks may be ignored. The right mouse button scans quickly to the upper or lower limit.

The middle mouse button allows typed entry of the required value (for PM volts, Offset and Wavelength only). This is included to facilitate set-up to fixed conditions.

PM-volts: The high voltage to the selected photomultiplier can be adjusted with an accuracy of approximately 0.25 volts between the limits of 0 and 1000 volts. The incremental nature of the control means that on high input sensitivity settings a stepwise alteration of the input signal may be observed. If a reference scan has been performed (see SCANDATA section) and the wavelength has been set to one of those within the scan range it is possible to use the reference value by clicking on V next to the digital readout instead of a entering a numerical value. If the wavelength value is between two used during the Reference Scan the voltage value is interpolated, The PM high voltage can be easily set to zero by clicking on 0 next to the digital readout.

NOTE When scanning the high voltage using the arrow keys the new voltage setting is not sent to the photometric controller until the mouse button is released and therefore not reflected in the incoming signal level until this occurs. This also applies to the offset (below).

Offset: This can be adjusted in steps of approximately 1mv though only 2 decimal places are displayed. The minimum and maximum settings are 0 and 4.99 volts. The recommended offset is 4 volts and this is automatically set by default at software start-up. In absorbance mode the value of the offset cannot be changed as it is used for a reference in absorbance calculations. Note that small deviations between the set value and the live input signal display at the top left of the screen may exist. Less than +/- 10 mvolts is acceptable.

Filter: The time-constant setting can be adjusted from Direct (no in line filtering) through to 1 second.

Detector: The active photomultiplier can be selected using this switch, eliminating the need to change wiring configurations. Absorption selects the side window tube for absorption/transmission work, and Emission the end window tube for fluorescence, light scattering and chemi-luminescence work. Note that switching detectors always resets the high voltage supply to both to 0 volts.

Wavelength: This can be adjusted from 0 to 999 nanometers in steps of 0.1 nanometer.
If 0 is entered the monochromator will automatically perform a self calibration check before ending at this wavelength. While the monochromator is running the wavelength display indicates 'BUSY' . The system will not allow a run to be performed under this condition.

When the wavelength is below 200nm it is not possible to set a PM high voltage.

Timebase and Sensitivity: Set these as required.

Dual Channel Systems
Providing two photometric controllers are connected and both are switched on then the dual channel option can be selected. All set-up procedures are duplicated for the second channel.

Storage of Set-up Parameters
All set-up values are stored with the data following trace acquisition ie PM-volts, Offset, channel etc.

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WARNING

If the user exits to the desktop via the *MAIN menu option* then the PM high voltage is automatically reset to 0 volts. If however the user exits through a *software error condition* or by performing a hard reset this will not be the case. Under these conditions high voltage may remain on the PM outputs. Always switch off the photometric unit(s) before re-configuring or disconnecting cabling.

Other New Data options-

TASKS:

Acquire: In regular stopped flow mode, activates the stopped flow drive (SX17MV only) and causes the workstation to wait for a stopped flow trigger pulse and then acquire a trace from the photometric controller .
In sequential mode sends the pre-assigned delay information to the sample head and performs the required sequential drive. The stopped flow data is acquired on receipt of the stopped flow trigger. Immediately following a successful run the measured delay time is briefly displayed. If the system detects drive volume inconsistencies these errors are reported with suggested corrective action. More details on this may be found in Appendix III.

Set Acq: Brings up a dialogue box which allows the user to set up the following acquisition parameters.

1) The number of traces that will be acquired for a single click on the acquire button may be set, up to a maximum of 9. This is especially useful for signal averaging. If just a number is entered the value will be reset to 1 after the sequence has completed. If an exclamation mark is appended (i.e. 3!) the number will be fixed until deliberately changed by the user or until the software is re-run.

2) The X-SHIFT value may be set, determining by how much acquired traces will be shifted along the X-axis. It results in data being acquired with a correction value applied to the X axis. It is a design feature of this apparatus that there is a small continuation of flow after the trigger point. This is typically in the region of 2 ms. The typical dead time of the instrument is 1.5 ms. If fitted curves were allowed to extrapolate back to the Y axis, i.e. zero time, they would be going to a point approximately 0.5 ms before the mixing time and the calculated starting absorption value would be incorrect. X-shift is intended as a method of shifting the trace so that the zero time axis is as close as possible to the true mixing time. The default shift value is 0µs (ie off) but it is possible to change this value in the Set Acq dialogue box. After having performed your own dead time determination experiments and having decided exactly what value is required.

3) The number of points per data acquisition from this task. The maximum number of points is determined by the amount of available memory and is set in the CFG file.

Auto-PM: (This option is only available when using the Absorption Detector) Pressing this will cause the PM voltage to be increased or decreased automatically until the signal input reaches approximately 0 volts or 0 absorbance. This may be used to balance the signal input against the selected offset voltage at start up, or re-centre the trace when drift has occurred. Note that the iterative method used may sometimes result in a degree of 'hunting' particularly if the signal is noisy. This can be seen as a short period of oscillation of the PM voltage before the process stops. Signal noise and high sensitivities affect the accuracy of the signal zeroing process. If the PM volts change doesn't produce a change in signal due for example to a light blockage the activity will time-out after approximately 8 seconds.
NOTE this facility is an aid only and should not be treated as an accurate method for bringing the signal to 0 volts.

Clicking on the Live display: To further aid pre-run signal set up it is also possible to point and click at a position on the live display screen where it is desired to position the ambient incoming signal level. The PM voltage is adjusted to bring the signal to this point. This is useful when trying to capture a transient over the full input range when its polarity is known. This facility is available for both Absorption and Emission Detectors.

Empty: Automatically empties the stopping syringe ready for a new run.

In sequential mixing mode only:

Profiles: This displays the transducer profiles recorded during the preceding drive. These show the exact syringe displacements vs time of each push and the measured age time of the solution in the observation cell. Any problems with the mechanics of the system will be instantly apparent from this display. For a discussion of these profiles and their interpretation see Appendix III.

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Delay: This allows the operator to request a new delay (age) time for subsequent sequential drives (0-100000msecs). This is approximate since the actual time achieved is measured after the run itself.

Seed Info Allows the operator to input a 'seed' filename and data text 'tag' before commencing acquisition. Windows are provided for this input. Subsequent stopped flow runs adopt this name and tag. The number suffix of the name is automatically incremented for successive runs and the data is placed in sequential memory blocks. The initial destination memory block (1-14) can be selected with the mouse. The default seed name and number is 'Run_01'.

Save This is provided to allow immediate dumping of acquired data to disc under the seeded filename. It is necessary that a disc group has been created or accessed first from the Disc context. (There is also an Autosave facility accessed from the Main Context. See page 14.)

MODES:

Int Trig When set, causes internal triggering of data acquisition. ie the **Acquire** option will not send a signal to the Sample Handling Unit to fire the Rams and the computer will not wait for a trigger from the stopped flow but will result in immediate trace acquisition. This option is useful for assessing signal levels, end points etc. and is on by default.

Average When switched on this will result in multiple trace acquisitions, (set in Set Acq), being automatically averaged together. eg If you have set up to acquire 3 traces and Average is turned on the end result will be one trace in memory which is the average of the three acquired traces.

Split TB Switches on or off the split timebase option. A second set of timebase information is displayed and can be adjusted using the control panel. Note that the total number of points acquired is the same (400-4000) in this mode with half collected over each timebase. The display screen is divided in the X-axis with half being allocated to display each timebase. Note: it is not possible for the first timebase to be set slower than the second. If a conflict arises the second timebase is automatically lengthened as the first is increased.

Dual Selects dual channel mode. Only possible with two photometric units. This will allow either dual wavelength absorption data to be collected or simultaneous detection of fluorescence and absorption at the excitation wavelength. This option is only available if the cfg file has the dual detection option set and you have two photometric control units.

Ch #1/Ch #2 Brings up a dialogue box to allow you to toggle between channels in dual channel mode. Only one at a time can be displayed live for signal set-up. Again, this option is only available if the cfg file has the dual detection option set and you have two photometric control units.

ABS/VOLTS Brings up a dialogue box to allow you to change between acquiring data in absorption units or in volts.

ABS Selects absorbance mode for data collection for current channel. The screen display is changed to show live data in absorbance units (AU) and incoming traces are automatically converted and displayed in these units. The range control now provides sensitivity adjustment directly in AU.

A discussion of the absorbance scales and signal level setting protocol may be found in Appendix I. Note if traces are not collected in AU they can be optionally converted later in the Transform context.

VOLTS Reverses the action of ABS and selects the Volts scale. NB. It directly reflects the photomultiplier signal output and can be treated as a transmission measurement (section 6).

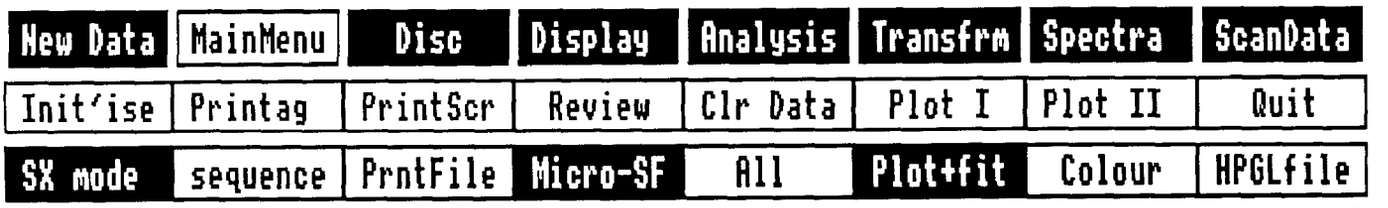
If the Emission Detector has been selected traces can only be acquired as volts. If the Absorption Detector is selected the default is Absorption Units but this can be changed to volts in which case the detector label is changed to Transmission, although the same photomultiplier is being used.

AutoSave Causes newly acquired traces to be automatically saved to disc (in the current group) without operator intervention. This option is provided to eliminate the risk of data loss with valuable samples.

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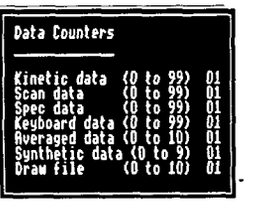
5.2 Main

Entrance and exit point of the software. Pointing and clicking memory blocks can be used for data display.



TASKS:

Init'ise Brings up a dialogue box on the right to allow you to initialise the data counters. When collecting new data the counter increments Run_01, Run_02 etc. Scans, Splines, Averages, Synthetic Data all have their associated counters. All these counter can be set to any value using this task.



Printags Prints out, on the system printer, the tag field of the data block currently highlighted in memory.

Printscr Performs a printer screendump of the current display. Ensure printer is on and on-line.

Review Displays all occupied memory blocks simultaneously as miniature graphs. This can be useful for a quick assessment of the current data in memory. Note that data which has accompanying fit information is labelled with an 'f'.

Clr Data Used to clear data from memory blocks. No check is made whether such data has been stored on disc. The option is cyclic and the user is prompted to point and click further memory blocks for emptying.

Plot I Performs formatted plot to HP-GL plotter via the RS-232 interface, or to an HPGL file if HPGLfile is selected (see below). Only the trace highlighted on the memory buffer is plotted with a fitted curve and residuals if present and if Plot+fit is selected (below). If Colour is selected the fit will be plotted in a different colour to the data. The data tag and accompanying results are also plotted . Refer to appendix V for interface details.

Plot II Performs formatted plot to HP-GL plotter via RS-232 interface, or to an HPGL file if HPGLfile is selected (see below). Reproduces the 'displayed' screen with multiple traces and annotation if present . Successive traces will be plotted in different colours if Colour (below) is also selected (8 pen plotters). Note that the graph limits p1 and p2 of the plotter may be adjusted prior to output to alter the size and aspect ratio of the output. If this task is selected with the right hand mouse button the axis will not be drawn allowing traces to be added to a previous plot.

Quit Exits the software after getting operator confirmation and re-enters the desktop suite. Data in memory is lost. Any photomultiplier high voltage is switched off first.

MODES:

SX mode/SQ mode Brings up a dialogue box to allow the user to switch between Single Mixing Stopped Flow and Double Mixing Stopped Flow, (Sequential Flow). When SQ mode is selected both rams operate according to the delay programmed using the delay softkey (above). When SX mode is selected (the default) the instrument is used for regular stopped flow.**AutoSave** Causes newly acquired traces to be automatically saved to disc (in the current group) without operator intervention. This option is provided to eliminate the risk of data loss with valuable samples.

Sequence Results in Printags printing out the tags of all of the data in the memory blocks in sequence. They will be automatically scaled so that 15 tags can fit on one sheet of A4 paper

PrntFile Results in printouts being redirected from the printer to a file on disc. This results in a much faster printout so analysis time is reduced. Printout files can be dragged as a group to the printer driver in the desktop environment and will queue up so that they can be left to print out when it is convenient. (A printer sheet feeder is an obvious advantage if you wish to print queue.)

Micro-SF/Keyboard Selects the incoming data source. When Micro-SF is selected the stopped-flow sample handling unit is the data source. This results in the acquisition screen presentation when the New Data context is entered. Selected by default. When Keyboard is selected any coordinate data can be entered from the keyboard. In this mode choosing the New data context does not alter the screen presentation as with stopped flow, but instead generates a sub-menu containing the following tasks for data input and editing. Not only can new data files be created, but existing memory records can be edited.
The options available in keyboard NEW DATA are:

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Sortby X .This sorts the currently selected data block in order of increasing X-value. This is necessary if data is appended or out of sequence.

Data A window is opened displaying the contents (if they exist) of the currently selected memory block. Entries are listed as sequential pairs of coordinates (X,Y) separated by a comma. These can be edited in the normal way. It is important to maintain the X,Y format. If more data exists than can be displayed in the window (20 X,Y pairs) further pages can be revealed by pressing the up or down arrows when the input cursor is at the top or bottom line.
To terminate data entry either press the left mouse button or press return until the cursor exits from the window bottom. Data can and should be sorted if out of sequence (above).

The first blank line found in the input window is used to signify the end of a new data file. In the absence of scale information, default values are automatically calculated.

Scales This allows the operator to enter or edit default scale information for data, as X-min,X-max,Y-min,Y-max. If no data exists prior to setting new scales then the data entry window will be automatically opened.

Units This allows scale units (textual) to be entered or edited.

Now returning to the rest of the MAIN context:

MODES:
All Will cause Clr Data to clear all 15 buffer spaces instead of just the highlighted one. This action is irreversible and must be confirmed by pressing the left hand mouse button.

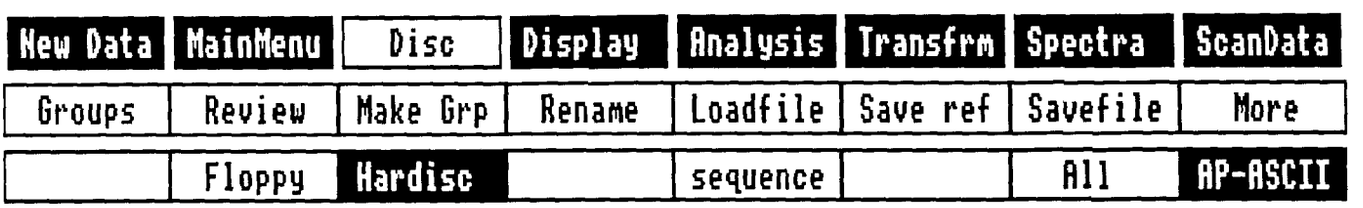
Plot+fit Enables and disables plotting of the fitted curve (where present) over the original data in the HPGL plot II routine. If a multi-pen plotter is used and Colour (see below) is turned on the fit is plotted in a different colour to the data.

Colour When selected chooses new pen colours (if available) for multiple traces when using the Plot II option (up to a maximum of 8). It also results in a fit being plotted in a different colour to the data when using Plot I

HPGLfile Cause Plot I and Plot II to re-direct their data to an HPGL file instead of to the serial port plotter. A box will appear requesting a file name and the file will be saved in the currently active group. This file can then be transferred to a DOS disc and hence to another machine for outputting to any HPGL device.

5.3 Disc

All disc accessing options are provided in this context. Facilities for directing data storage to or from floppy or hard disc are included, as well as utilities for clearing out old files and formatting new discs (floppies only).
Floppy discs can be ADFS 800k or 1.6MByte format or 720k or 1.44MByte DOS format.



TASKS:
Groups This opens a screen window and catalogues the root (top level) directory of the hard or floppy disc according to the mode settings below. From this listing the user is prompted to select a group title to display the data files it contains. All data files are stored within such groups and all groups are accessed at the root directory level.
Note that with the hard disc it is possible to "open" groups which are not created by the kinetic software ie are system software directories. The disc filing system does not attempt to hide these directories and so it is up to the operator not to disturb them.

When the group catalog is opened, a bar highlights one of the entries. This can be moved to a different group heading with the mouse. To open the group and display its contents simply select the left mouse button. The data files are listed in a new window with the available memory for further storage. This selection can be cancelled with the middle mouse button and repeated if required. Alternatively if this group is required for access, simply move the

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mouse pointer down to the menu area to to select a new option.

The act of selecting (or creating) a group and displaying its contents assigns it for load/save operations even if it closed down for the duration of other activities.

Review Displays all occupied memory blocks simultaneously as 15 miniature graphs. This can be useful for a quick assessment of the current data in memory. Note that data which has accompanying fit information is labelled with an 'f'.

Make Group Allows the user to create a new group on the current disc by prompting entry of a new group name (up to 10 characters). A group thus created is automatically opened for data access.

Rename Allows the operator to rename data files **in memory**. This name and number is used as the filename under which the data is subsequently stored to disc. The format requires a maximum of 5 alphanumerics as a file name followed by a 2 digit index number. NOTE that a single number entry will be padded with a leading zero. When displayed in memory blocks or stored on disc the file name and number are automatically bridged with a '_' character. If the sequence mode is on, successive blocks containing data sets which previously had the same starting name, will also be renamed, but with sequential run numbers. This speeds up renaming of groups of data.

Load File Loads a data file from the current group. The group contents window is opened (if it has been closed) and the operator is prompted to first select a data file on the disc with the highlight bar, followed by a memory block destination. The file is then loaded. The sequence can be repeated as required, or cancelled at any time with the middle mouse button. Any data in a destination block is overwritten by incoming files.

If the sequence option (below) is on then then the act of choosing a group entry and a destination will cause all files with the same name to be loaded into consecutive memory blocks . This operation can be cancelled during execution with the middle mouse button. This allows stored sets of related data to be loaded simultaneously.

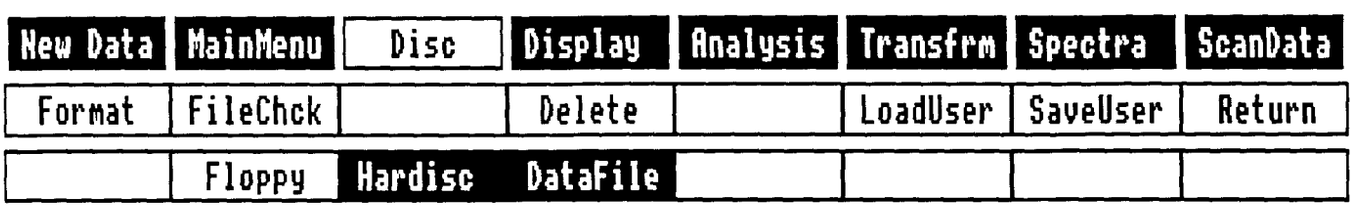
Save ref Allows the user to save a 'Photomultiplier High Voltage/Wavelength lookup table which has been acquired from SCANDATA.

SaveFile This allows files to be saved to the current disc group. NOTE the memory block to save is selected first. When the SaveFile softkey is pressed the user is asked to confirm the action, which then takes place under the filename and number assigned to the data.

Data files with the same name in the current group will be overwritten.

As files are saved the group window display is updated and the available disc memory decremented (approximately 8 kbytes per stopped flow record). If the **all** option is selected (below), then all occupied data blocks are saved automatically.

More This replaces the main Disc menu with a related submenu containing a number of housekeeping options for disc tidying and formatting. These are described here:



Format This option is for initialising floppy discs only and generates an error message if the Hardisc mode is selected. Old floppy discs can be re-formatted with this option.

Discs are initialised to the operator's choice of format. Any data on the disc is lost.

The floppy disc (ensure it is not write protected) should be inserted in the drive before the option is selected. The operator is asked to choose which format he requires. The options are ADFS 800k, ADFS 1.6MByte, DOS 720k and DOS 1.44MByte.The formatting process then takes a minute or two followed by disc verification. The processes are accompanied by continuous drive activity and should not be interrupted. **The user should note that the only format that is guaranteed to be recognised by all machines (particularly older machines) is the ADFS 800k format.**

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FILEchck When this option is clicked on the currently active group is displayed on the screen along with its contents. The User then selects the first file of a sequence in the usual manner. If the file is a kinetic trace a table of file/wavelength for that sequence is produced showing groups of ten files at a time. If the file is a "Spec" or "Spln" file a table of file/time is produced in the same manner. This enables Users to easily identify files in a group without having to load them into the buffers to examine their tags.

Delete This allows flies or groups to be deleted. The type of file or group is controlled by the selection box underneath this option

Load User This allows the operator to load a set of 'User equations' from the special function library group, *Flibrary* (see section 5.5:Analyse). If Flibrary doesn't exist then an error message is returned, otherwise its contents are displayed and a selection made in the usual way. Once loaded the equations can be accessed for curve fitting by choosing User mode in the Analyse context.

Save User This is used to save any user equations currently in memory in the group 'Flibrary' on the current disc. A name for the new file is requested. If the Flibrary group is not found on the current disc it is first created. Such function files can be later re-loaded with the Load User option for use in curve fitting. User equations are created in the Analyse context (section 5.5).

Return This returns the operator from the Utility sub-menu to the main Disc menu.

MODES:
Floppy Selects the Floppy drive for disc access operations.

Hardisc Selects the hard disc for file access. This is on by default.

DataFile/DataGrp/Ref File/TempFile Controls the function of delete.
Data Files – This is used to delete Data files from the currently selected disc group. Files can be removed successively in a cyclic process with the group display being actively updated each time. **Data is not recoverable following such file removal.**
Data Groups – This allows redundant groups to be removed from the disc root directory. The user is prompted to select a title for deletion from the directory display. The activity is not cyclic, ie it must be re-selected for every group to be deleted (unlike Del File).**All data files must be deleted from the group before it can be deleted.**
HV Ref Files – This is used to delete voltage reference files. Files can be removed successively in a cyclic process with the group display being actively updated each time.
Temp Files – This is used to delete temporary files. Temporary files are used to store running averages and generally do not require manual deletion. (Only if a large number of average files (with suffixes pf greater than 09 are used.)

Remaining disc menu options:
MODES:
Floppy Selects the Floppy drive for disc access operations.

Hardisc Selects the hard disc for file access. This is on by default.

Edit Used in conjunction with Tag (described above). When selected this automatically switches on the editing mode when the tag information of a memory block is displayed.

Sequence Selected to allow sets of group files with the same name to be loaded automatically in response to a single Load File command.

All When selected causes all memory files to be saved automatically to the current disc group in response to a single Save File command.

AP-ASCII Brings up a dialogue box which allows the user to select the format in which data will be saved. The present file formats are as follows:–

AP-ASCII – The standard Applied Photophysics data file format.
CSV – Comma Separated Value. The file header and footer are removed and the data is saved in two or three column format (depending on whether a fit is included) with the columns separated by commas.
TSV – As above but with tab separated columns instead of comma separated columns.
Biokine – Biologic's data format. This allows the user to analyse the data using Biologic's BioKine software. (The

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BioLogic curve fitting program uses the Pade-Laplace fitting algorithm whereas Applied Photophysics uses the Marquardt algorithm.)
Draw – A vector graphic format which allows traces to be exported to Acorn Desktop Publishing packages.

5.4 Display

Provides control over data display and provides tools for inspecting specific features of the data. Pointing and clicking memory blocks can be used for data display. But see extra mouse actions for superimposition control in Overlay mode.

New Data	MainMenu	Disc	Display	Analysis	Transfrm	Spectra	ScanData
Printscr	Tag	Clearscr	Cursor	DotStyle	Zoom	Label	Erase
Expanded	Edit		AutScale	Dot Join	Overlay	Edit	Erase 6

TASKS:
PrintScr Performs a printer screendump of the current display. It is essential that the user ensures printer is on and on-line before printing.

Tag Displays the data tag, with or without Edit mode in exactly the same way as described for the Disc context.

Clearscr Clears the current display in preparation for a new graph. Most useful when the Overlay option is selected since the screen is not redrawn as extra traces are added and therefore needs active clearing.
If overlay is off, the screen is automatically cleared when traces are displayed.

Cursor Places a cross-hair on the screen display intersecting the currently selected memory data. The corresponding point number and its absolute X and Y coordinates are displayed in the menu area. The cross-hair can be made to track along the current trace using the mouse keys according to the accompanying instructions. It always appears in the X-position in which it was last displayed (providing this is possible). This is to allow trace amplitude comparison at specific positions on the X-axis. The Space bar is provided as a means of cancelling the cross hair since this allows it to be removed and redrawn on another trace without movement. Note that the mouse pointer is disabled while the cursor is on.

DotStyle When displaying data as discrete dots it is possible to change the size of the dots by clicking on Dot Size, deleting the existing number and entering a number between 1 and 7. 1 is the smallest dot size and 3 to 7 is the largest. Generally 1 would be chosen when a large number of dots are being displayed, 2 when a few dots are being displayed and 3 when a very few dots are being displayed (eg when displaying spectra). The number selected also determines the shape of points output to the plotter. 1 to 6 are individual shapes (triangles, crosses etc), 7 is used to cycle through the six shapes as data is plotted (each trace has points of a different shape).

Zoom Allows any portion of the current display to be expanded to fill the graph area for closer inspection. The display may contain one or several traces (in overlay mode). If the left hand mouse button is pressed over Zoom a 'rubber band' box is windowed over a portion of the screen using the mouse, according to accompanying instructions. (Left button to swap corners, right button to redraw the screen at the required zoom and middle button to cancel the operation without making any changes.) On return the screen is redrawn with coordinate extremes corresponding to those of this window.

When a trace has been zoomed in this way the expanded scales are retained for later display of the memory block even if other traces are displayed in between. The presence of a zoomed scale for a trace is indicated by a +Z in the top right hand corner of the display.

In order to return to a full size display place the mouse pointer over Zoom and press the right hand mouse button. This resets the memory block scales to its original value (depending on the autoscale option).

Label Provides a facility for annotating the screen display. Assuming that the Edit attribute beneath it is turned on up to ten lines of text are entered via a yellow entry window (see right). These lines of text are held in memory and can be used again and again until replaced or deleted. If the Edit attribute is not turned on the same window will appear, but with a blue background and previously entered text can be selected and then positioned using the mouse pointer. The text can be placed anywhere in the



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graphics area, and the process repeated as necessary. This labelling only exists for the lifetime of the current display, but provides a useful facility prior to hardcopy in either this or the analysis contexts.

Eraser This complements the Label option. A small screen block is linked to the mouse pointer. This is used to 'rub-out' mistakes made in annotation (or indeed other parts of the display). Depending on the size of the mistake a 1 or 6 character eraser is available (see below). Erasing text in this manner merely removes it from the graphics window and it would still be printed out as the print routine takes its information from the text buffers. The user must, therefore, either reposition the erased text to place it somewhere else on the screen, (thus resetting the text buffer), or erase all the text using the "Erase all text" option (see below) and start again. (Erasing all text also resets the text buffer).

MODES:

Expanded When selected results in expanded mode screendumps on the dot matrix printer in landscape orientation. When not selected (the default) compact screendumps are produced in portrait orientation.

Edit This is used to switch on tag editing mode when Tag is selected (above), as described previously in the Disc context.

AutoScale This option, which is off by default, causes data to be automatically scaled in the Y-axis prior to display, so that any trends are immediately visible. The data Y-min and Y-max are calculated and extended with 15% of the total Y-range (Y-max-Y-min) at both top and bottom. If autoscale is switched off data is displayed according to its acquisition input range (stopped flow) or the Y-range supplied or edited in Keyboard entry mode. Note that the Autoscale option is overridden when data is expanded with Zoom.

Dot Join If selected data traces are displayed as a continuous line instead of as discrete dots. The default option is Dot Join turned ON.

Overlay This allows superimposition of data blocks over one another using existing graph scales. This seemingly simple task provides some quite confusing situations however until the operator is fully familiar with the display system. When switched on, all subsequent block display activity causes the selected data to be superimposed on the current graph to the existing scale. This, of course, allows direct comparison of data and is a common preliminary to data averaging. In this mode the graph screen area can only be cleared by using the Clearscr option described above. Note that if data does not fall within the scales shown it will not appear even though it is treated by the program as 'on display'. Note, also, that when overlay is on, pressing the left mouse button when selecting a block for display will add it to the graph. Pressing the right mouse button will remove it. This extra control in overlay mode applies to all contexts.

Remember that the scales of the graph relate to the first data block displayed in the sequence. It is this block's scale information that is updated when a zoom operation is carried out even though several traces may be expanded to the new scale. It is therefore necessary to **remember to select the source block when attempting to cancel such a combination zoom.**

Grid-on This adds a 5x5 graticule to the current and subsequent graphs. Note that the presence of a graticule restricts the improved scale presentation available with zoomed displays.

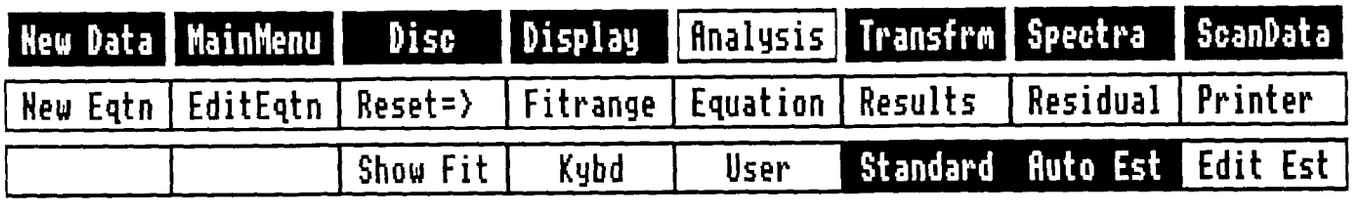
Edit When turned on Label will bring up a dialogue box allowing the user to enter lines of annotation. When turned off Label will bring up a dialogue box containing previously entered lines of annotation for selection and placing.

Erase 6/Erase1/Eraseall Brings up a dialogue box to allow the user to set the size of the erase. The options are 6 characters wide, 1 character wide or erase all the annotation text on the screen.

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5.5 Analyse

Traces on display can be analysed using a robust non linear regression algorithm and the results displayed and printed out. Several fitting equations are provided as standard but can be supplemented by the operator. Pointing and clicking memory blocks can be used for data display. If "show fit" is selected (see modes below), existing fits are automatically superimposed.



Curve fitting: This uses a modernized and robust implementation of the Marquardt algorithm broadly based on the routine Curfit in P.R.Bevingtons Data Reduction and Error Analysis for the Physical Sciences (1969 McGraw-Hill).

TASKS:

New Eqtn This allows the operator to add new analytical equations to which data can then be fitted using the general non-linear regression algorithm provided. Up to 10 such equations can be added each fitting up to a maximum of 10 parameters. Sets of equations added in this way can be saved as function libraries in the group 'Flibrary' on disc and arbitrarily loaded for use at a later date.

To enter equations the operator must first select User mode. An equation editing window is opened which contains prompts for both title and formula input. If a User library file has already been loaded, this will be reflected in appropriate filing information and an index to the position of the forthcoming entry at the top of the window.

The new equation title should be entered first (up to 40 characters). This will later be chosen from amongst other equation titles prior to actual fitting, and should contain sufficient information for this purpose. (eg Exponential + steady state).

Next the **right hand side** of the formula must be **accurately** typed in as a function of X, substituting P(1),P(2),P(3),P(4) where fitting parameters are present (4 in this case):

eg for the exponential + steady state represented:
Y = A0.e(-kt)+mx+c
enter: P(1)*EXP(-P(2)*X)+P(3)*X+P(4)
where P(1) is A0, P(2) is k, P(3) is m, and P(4) is c

The format does use computer/BASIC symbolism to a degree, but essentially reads like the source algebra. Ensure, however, that parentheses in complex expressions are correctly paired. 2 lines of 50 characters are available for formula entry. The entry is terminated as normal by the left mouse button or 'return'. Finally a window is opened to allow the operator to assign names to the parameters (like those listed above:A0,k,m,c). These are used to prompt for parameter estimates before fitting.

Edit Eqtn This allows the operator to edit an existing User equation which has first been selected with the Equation softkey (below). The same window entry sequence is provided as for new equation entry, but now displays the existing function and its details for modification. Since this information can be returned intact, this provides a useful means of checking previous new equation entries.

Reset=> After having selected reset the user is presented with two options. If the left hand button is pressed it will result in the resetting of the fit extremes (ie the data fit range) to the limits of the selected data file (usually points 1 to 400). These are indicated by vertical dotted cursors. This will also zero any set datum (see below). If the right hand mouse button is press it will result in the currently highlighted data block having any fit data stripped from it. (Usually prior to saving as a CSV file for export to another graphing package.)

Fitrange Allows the user to adjust the data range over which subsequent fits are conducted. ie the range over which the normalised variance is minimised (the criterion for best fit used). The minimum and maximum X-values can be set with left and right vertical cursors, whose positions are continuously displayed in the menu area.

It is also possible to set a left hand datum for the fit, by pressing the right (first) and middle mouse button when adjusting the left hand cursor. This provides a 'zero time' offset for data sets and is used if data has been collected with X-shift turned off (see NewData context). It is to this X-datum that amplitude parameters are calculated during

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fitting and is the left limit to which superimposed curves are extrapolated. This datum remains in effect until it is Reset (above). Note that the fit range can be adjusted within the remaining data range to the right of the set datum, but if the left fit marker is set to a point prior to the existing datum, then the datum will be automatically reset to the origin.

Equation This opens a window displaying the functions available for fitting. These will either be the Standard system functions or a set of User functions (when loaded) depending on the User/Standard mode option setting (below). The desired equation is selected with the highlight bar.

In the case of Standard functions, parameter estimates (required by the regression algorithm) are automatically calculated for all but the 2nd order models. In these cases fitting proceeds immediately, with details of parameter convergence being displayed. When successfully completed (<0.1% normalised variance improvement), the calculated theoretical curve is superimposed over the data and the residual screen and results automatically displayed (see below).

Note that the linear fit is simply a direct linear regression analysis (non iterative). Auto-estimation of starting parameters is carried out by default, but estimates can be adjusted routinely, before fitting proceeds, by selection of the Edit estimates mode. In this case, a window is opened containing a list of the parameter names and starting values which can be edited in the usual way. Note that during editing the equation algebra is displayed to facilitate interpretation of each parameters significance.

The option to **edit parameter estimates** can also be taken by pressing the right, rather than the left mouse button, when selecting the fit equation. Apart from the 2nd order case, estimate editing may become necessary with noisier data and complicated functions (where the automatic estimates can be poor) reflected in more iterations (minimisation cycles) of the regression algorithm before convergence and therefore more time will be taken. In extreme cases the fit may fail. This is indicated by an appropriate error message.

Parameter editing is always required when fitting to User equations and should be invoked with the Edit estimates softkey. In most cases, fitting with poor estimates will be with 2nd order calculations where it is necessary to work with Absorbance rather than concentration. The evaluated second order rate must therefore be corrected for this transformation.

The Equation Task can also be used to synthesise curves. If a vacant data block is selected the equation task will bring up the equation menu, as usual. Selecting an equation will bring up a box asking for the parameter values. After these have been entered another box will come up asking for the time base in seconds. After this value has been entered the software will synthesise a curve that fits the parameters entered, will name it SYNTH_01 and will place it into the vacant data block. This is particularly useful for separating Fitted data from the original data as the parameter box will contain the last fitted values as default values.

Parameter Locking Editing parameter estimates provides the operator with the opportunity to lock any combination of the listed parameters for the subsequent fit. Parameters locked in this way will not be adjusted to improve the fit, but treated as constants. **To lock any entered parameter append an '!' to its value at entry.**

A simple example of its use is when a transient end point is accurately known prior to fitting. This can be entered and locked, in place of a floating end point estimate (which will be varied). This improves the subsequent accuracy of the other calculated parameters since they are constrained to a higher degree by this fixed value. Any combination and number of available parameters may be locked in this way, with the proviso that **at least one must be left for adjustment to allow convergence**.

Results This is used for quick inspection of fitted parameters (if they exist) to the currently selected trace. They are displayed in a window with their standard errors (derived from the regression algorithm, see reference cited above).

Residuals This generates the residual screen for the selected data (if it has been fitted), which is the same as that generated automatically on fit completion. It comprises a graph display of residuals in place of the menu area (Yobs-Ycalc) vs X, and the fitted parameters in place of the memory block display (the standard errors are not shown). To the bottom right are shown the fit range together with the normalised variance.

The normalised variance used as the minimisation parameter during fitting is:

$$(Y_{obs}-Y_{calc})2/N_{free}$$

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The entire residual screen may screen dumped to a printer by pressing the left mouse button, or control returned to the Analyse menu by pressing the middle mouse button. If data has been fitted and subsequently stored on disc, all function information and results are saved with it. On recall all fitted parameters and residual screens can be regenerated without having to re-analyse the trace.

Printer This prints out a summary of a fit to the printer (text only). It includes the filename and its disc group origin, tag information and all function details and results.

MODES:

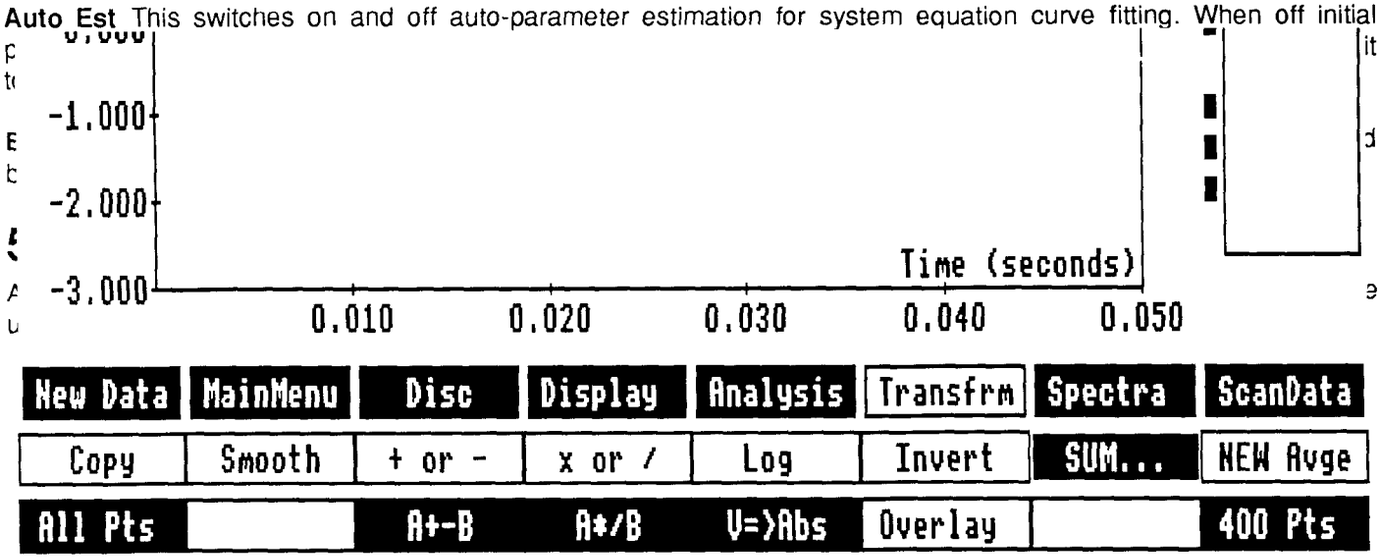
Show Fit When selected, if a data block has associated analysis results then its accompanying theoretical curve is automatically superimposed when it is displayed by the usual point and click method. This allows several fits to be observed on the same screen. This option is off by default.

Kybd Switches on keyboard entry for fitrange values. Instead of using the mouse to enter the start, finish and X-datum values for the fit they can be entered directly from the keyboard. This is useful when fitting data collected using the split timebase option where setting the start and x-datum may be very difficult.

User This selects the currently loaded User Equation set for curve fitting, or equation entry/editing.

Standard This selects the standard (system) set of equations for curve fitting.

Auto Est This switches on and off auto-parameter estimation for system equation curve fitting. When off initial



TASKS:

Copy This is used to create copies of data files in vacant memory blocks. This is sometimes useful when experimenting with transforms, since a copy of the undisturbed data can be left intact. The operator is invited to alternately pick a source and destination memory block for the copy process.

Smooth This allows digital smoothing of data in memory. The data to be smoothed should first be selected (displayed). The algorithm used is based on the Savitzky-Golay least squares procedure (Analytical Chemistry 36 8 p1627). 5 to 21 point smoothes are available. The resulting trace can then be placed in the same or a different memory block. It is then automatically selected and displayed.

+ or - This allows a reference trace to be selected and subtracted from other traces in memory or a constant to be subtracted from or added to traces in memory or for a trace in memory to be subtracted from a constant.
Reference Trace Subtraction. The user is asked to choose a reference trace first, by selecting a data block. It is then possible to observe other traces with this reference subtracted, by subsequent block selections with the left mouse button. Note that the data in these memory blocks is altered by this process. This cycle continues until cancelled with the middle mouse button.
Constant Subtraction. The user is asked for a constant. Selection of traces in memory will result in this constant being subtracted from or added to the trace. This would, in effect, move the trace up or down.
The effect can be reversed, as long as the same reference is present, by pressing the right mouse button.

Ratio This allows either one trace to be ratioed with another or a trace to be multiplied or divided by a constant. It is used in the same way as Difference (see above).

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Log Converts the Y axis of any selected data to the log of its value.

Invert Reverses the polarity of the selected data. ie turns a trace upside down.

SUM.. This is part of the data averaging facility. A running average is first initialised and assigned a vacant memory block using the NEW Avge facility below. The SUM.. softkey is then used to incorporate displayed traces into this average. Traces may be incorporated one or several at a time. This is determined according to whether overlay is selected. When the SUM.. key is clicked, the user is shown the current average title and the current number of contributory traces (ie already averaged). He/she then decides which average to sum the traces into, selecting it by pointing to it with the mouse pointer and confirming by pressing the left hand mouse button. All displayed traces will be incorporated into the selected average by this confirmation.

Thus it is possible to overlay half a dozen traces for inspection, create a new average (NEW Avge), and average them together in one go. Further traces can then be optionally added singly or in groups. The running average can be inspected like any other data block. When a satisfactory average has been completed, it can be saved and analysed. It is possible to simply initiate another average by re-selecting the NEW Avge option. Note that the first trace averaged is used to determine the scales of the new block. It is possible to have more than one current average, the user selecting which of these displayed traces should be summed into

NEW Avge This creates and initialises a new average. The operator is asked to select a vacant memory block to hold the new average. By default the block is titled Avge and numbered sequentially from 01, according to how many averages have already been created in the session.

MODES:

All Pts Allows the user to select which part of a trace copy will work on.
All Pts – Copy will copy an entire trace from one buffer position to another.
1 in every 2 – Copy will copy every other point of a trace from one buffer position to another. The resultant trace will have half as many points as the original.
1 in every 4 – Copy will copy every forth point of a trace from one buffer position to another. The resultant trace will have one quarter as many points as the original.
As per fitrange limits – Copy will copy a region of the original trace to a new buffer position. This region is designated by the position of the ritrange limits in the Analysis context.

A+-B Allows the user to select the action of the "= or -" task key.
A+-B When the task key is activated the user is asked to select a reference trace with the left hand mouse button. Subsequent selection of traces results in the reference being subtracted from or added to the selected trace.
*A+-Constant.*When the task key is activated the user is asked to enter a numerical value. This value can then be subtracted from or added to traces so as to move them up or down the screen relative to other traces. This is very useful for aligning end points or overlaying traces for comparison.
Constant+-A When the task key is activated the user is asked to enter a numerical value. Traces can then be subtracted from this value.

A*/B Allows the user to select the action of the "x or /" task key.
Ax/B When the task key is activated the user is asked to select a reference trace with the left hand mouse button. Subsequent selection of traces results in the trace being multiplied by or divided by the reference trace.
A/Constant* When the task key is activated the user is asked to enter a numerical value. This value can then be multiply or divide into traces.
1/A When the task key is activated the user is asked to select traces which are converted to their reciprocal.

V=>ABS Brings up a dialogue box which allows the user to select the effect that the Log Task has on data. The options are:–
V=>ABS When turned on the log function is changed so as to convert the data from volts to absorption units. This uses the same calculation method used in the New Data ABS mode to convert traces acquired from the DF17MV from volts to absorbance. It is provided to allow the operator to see and analyse data in both units.
Ln Y Axis When turned on the log function will alter the data by performing a natural log of its Y axis.
Log Y Axis When turned on the log function will alter the data by performing a base 10 log of its Y axis.
Log X Axis When turned on the log function will alter the data by performing a base 10 log of its X axis. i.e. it will put the data onto a log time base.

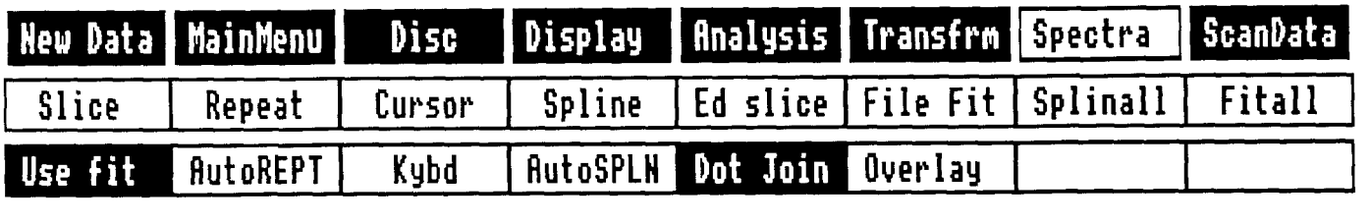
Overlay This switches the overlay option on and off in exactly the same way as it does in the Display context. It is provided here to make immediate inspection of averages and transforms easier.

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400 pts Brings up a box to allow the user to enter the number of points to be assigned to each average file. THe number of points should be the same as the number of points in each file that is going to be included in the average.

5.7 Spectra

This context is designed to allow wavelength encoded data files to be re-compiled into time resolved spectra.



TASKS:

Slice This initiates the process of generating a spectrum from a sequence of kinetic records collected at a series of monochromator wavelengths. The point in time at which the spectrum or 'slice' is taken across these records is first set using the cursor (below). The current disc group is opened (this is selected in the DISC context first) and the operator is invited to choose the first file in the proposed sequence to start the spectrum compilation. This would normally be the kinetic record obtained at the starting wavelength of the set. The destination buffer is then chosen and the spectrum is compiled. Kinetic files are accessed until there is a change in filename encountered in the group. The compiled spectrum can then be accessed from its data block in the normal way.

Repeat This repeats the action of slice at a new cursor position but without the redisplay of the disc group contents. The new slice commences from the same starting source file as was last chosen above. The usual sequence for compiling a set of spectra is to set the cursor and first perform a 'slice', Subsequent spectra derived from the same file set but at new cursor positions can then be compiled using this 'repeat' option.

Cursor This is used to select the time point at which the next spectrum will be taken. Note that spectral compilations from later time points take longer to construct than earlier ones.

Spline This is used to perform a cubic spline fit on a newly compiled spectrum placing a smooth curve through the points available. This is stored in a selected data block. This spline can be saved independently of the original data. The purpose of this feature is to aid interpretation of spectra by improving the clarity of peak and trough positions.

Ed Slice When Auto Repeat is turned on (see below) ten slices are automatically produced. Ed Slice allows the user to edit the times from which these slices are taken. Some of the positions may be left blank if less than ten slices are required.

File Fit This allows the operator to select a disc based file and perform the last used non-linear regression analysis on this and all subsequent like named files in this disc group. Each file is loaded, analysed and re-saved with its fit parameters. The sequence can be terminated by pressing the Esc key once.

Splinall Performs a cubic spline fit to all the spectral data in the memory buffers. After having performed a series of slices the data can all be converted to cubic spline fitted data with one keypress.

Fit All This supplements the previous option and sequentially fits all data in memory possessing a filename in common with that currently selected.

MODES:

Use Fit This is used to force the spectral compilation to use data points from the saved fitted curve (rather than the actual data) where available. This results in the reduction of random photomultiplier noise (present in the original kinetic records) affecting the spectra since these fluctuations are absent from the fit.

AutoREPT Instead of performing a single slice at the cursor position it is possible to perform a series of slices automatically. These slices are taken from the times/points set in the Ed Slice Task. Up to ten slices can be taken automatically and transferred to the memory buffers. Turn AutoREPT on, set the slice positions using Ed Slice, click on "Slice", choose the parent file, choose the first buffer space and the ten slices will be put into the buffers.

AutoSPLN This is used to automatically produce cubic spline fitted data instead of raw spectral data. When a splice is performed with AutoSPLN turned on the kinetic traces are sliced at the required time point and the resulting spectrum is fitted using the cubic spline fit. The result is placed into a memory buffer. AutoSPLN can be combined with AutoREPT to automatically produce a series of time resolved, cubic spline fitted data.

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Dot Join Spectral data that has not had a cubic spline fit performed on it is generally displayed as a series of dots, each dot representing the absorption at a particular wavelength and time. If Dot Join is turned on then spectral data is presented as a series of joined up dots instead of discrete dots.

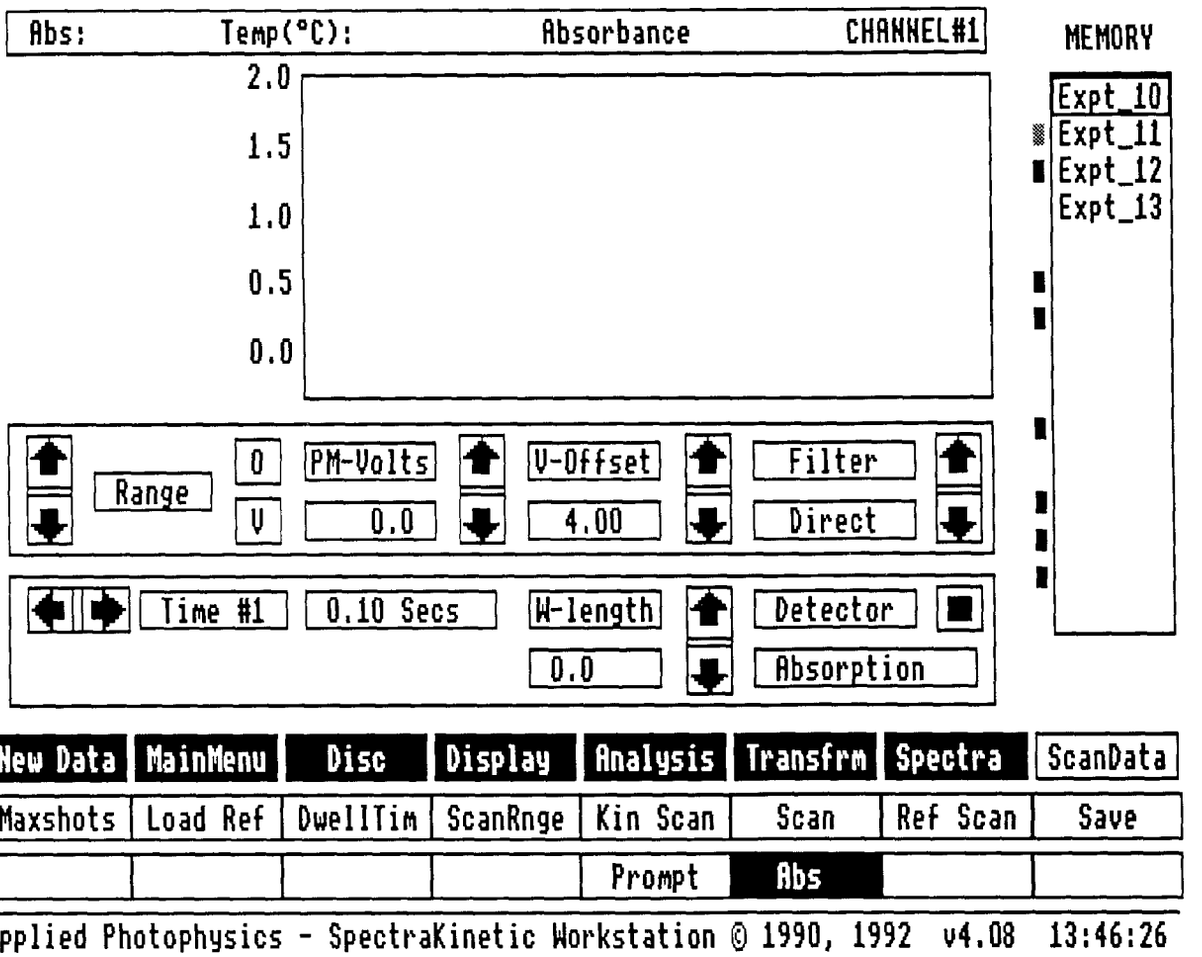
Overlay This duplicates the overlay feature in the DISPLAY context to allow superimposition of new spectra on the display.

5.8 Scan

POINT BY POINT SCANNING

This scanning method can be used to either collect accurate steady state sample spectra or sets of wavelength dependant kinetics. The latter technique allows the subsequent compilation of time dependant spectra in the usec time range.

The method entails scanning a wavelength range in fixed steps and at each wavelength either collecting an average signal value or performing a kinetic run.



SCANNING PRELIMINARIES

Before performing scans the instrument should be well warmed up to ensure signal stability. The Absorbance mode should be selected from the NEW DATA context. The required Absorbance sensitivity should also be chosen, it should be noted that the two most sensitive ranges are not available. It us recommended that AutoPM is selected at a wavelength in the proposed scan range in order to light adapt the photomultiplier prior to scanning itself. The filter setting **must** be **DIRECT**.

TASKS:

Maxshots Allows the user to set the maximum number of shots that will occur before the scan is paused to allow the drive syringes to be refilled. After the syringes have been refilled the scan can be continued. The scan can be halted manually at any time by either pressing the space bar if the halt is temporary or the Esc key if the halt is permanent.

Load Ref Allows a previously saved voltage reference table, obtained from a Reference Scan, to be loaded.

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DwellTim Brings up a dialogue box which allows the user to set the Dwell TIme. This is the time that the spectrometer will wait, after having reached a wavelength and set the photomultiplier high voltage, before measuring the absorption or voltage value.

ScanRnge Brings up a dialogue box which allows the user to set the required wavelength range and step interval (minimum 1 nm).

Kin Scan - for Point-By-Point Spectrakinetic Acquisition This is only available following valid reference collection. Before performing a Kinetic Scan it is wise to conventionally establish the kinetics and appropriate timescale of the sample reaction in the NEW DATA context. Photomultiplier voltage can be varied at this stage since the look up table from the reference is used during the Kinetiic Scan itself.

It is essential that before this task is started a new disc group is created to accept the incoming kinetic runs since they are automatically saved during scan progress (they transiently occupy buffer 1 in memory).

When the task is started the monochromator is moved to the start wavelength. A prompt warns the user that all data in the memory buffers will be overwritten and gives the User an option to cancel the sequence. The User is also warned if the Internal Trigger is switched on in the 'New Data' context as this would stop the rams from firing. If the sequence is continued, then the photomultiplier voltage will be optimised and a kinetic trace taken at each wavelength. The stopping syringe is automatically emptied prior to each drive. A maximum of 77 kinetic runs per scan is allowed.

As the kinetic traces are acquired and saved to disc ten time dependant spectra are compiled in buffer spaces two to eleven. These spectra are sliced from the EdSlice positions (see SPECTRA context.). These positions can be edited prior to acquisition if the default values are not satisfactory. Once the scan is complete, other spectra can be compiled from the stored kinetic runs using the slicing facilities in the SPECTRA context.

Scan - for Steady State Point-By-Point Spectra Initiates a steady state Point-By-Point scan. This is only available following valid reference collection. If water is still in the cell an absorbance baseline can be collected prior to introducing sample.

Selecting "Scan" causes the chosen wavelength range to be scanned and a spectrum to be compiled. The result is placed in the currently selected memory buffer for display and manipulation. Spectrum artifacts can be minimised by subtracting an absorbance baseline from sample spectra.

Ref Scan A reference signal **must** be collected or loaded prior to Point-By-Point scans. This provides 100% transmission levels and a photomultiplier high voltage look up table at each wavelength for use during sample scanning. After having performed a Reference Scan the user has the option of saving the look up table to disc for future use.
NOTE: The wavelength range for the other scans must be within the Reference Scan wavelength range. Providing light source power or slit widths are not adjusted however such a reference can be used many times. The high voltage look up table can be accessed from the New Data context when performing single wavelength absorption runs. As long as the required wavelength is within the scan range clicking on V next to the high voltage digital readout will set the PM high voltage to a value derived from the current high voltage look up table.

For absolute absorbance spectra the reference should be collected with water or buffer in the observation cell.

If the space bar is pressed during the reference run it will be halted and the operator will be given the option of quitting or continuing. If the Esc key is pressed the run will will quit immediately.

Save Duplicates the save task in the New Data Context.

MODES:
Prompt When selected causes the Point-by-Point sequence to pause after each shot and gives the user the option of quitting the sequence, re-taking the shot at the present wavelength or continuing to the next wavelength.

Abs/Trans/Volts Brings up a dialogue box which allows the user to select the type of steady state scan will be initiated when Scan is activated. The option are; Absorption Scan, Transmission Scan and Voltage Scan. The first are referenced against the High voltage look up table and are self explanatory. The third is a non referenced signal against wavelength plot. The photomultiplier high voltage must be manually set at the wavelength where the greatest signal is likely to be. This high voltage will remain constant during the scan. In order for the scan to be presented correctly it is important that the 4V signal offset is removed, (i.e. set to 0V), before the voltage scan is started.

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Appendix I

Signal Level Adjustment

This section describes the procedure for setting up the signal levels from the stopped flow and photometric control unit prior to performing stopped flow runs.

Transmission

A transmission scale, as such, is not provided. Instead the software displays the incoming signal in volts. This directly reflects the photomultiplier signal output. The operator can, however, easily relate voltages to transmission percentages using the 0->100%T / 4->0 volt relationship described below and in Appendix I.

The stopped flow is set up in transmission mode according to the instruction manual. It is assumed that the light sources and all signal electronics are on, and that there is a detectable light signal through the optical cell (shutter open).

The EHT should be at zero and the mode switch on the photometric control unit in the Abs position. In the New Data mode, under these conditions, the live display should indicate a constant signal level input of +4.0 volts.

This +4.0 volts (in the absence of a PM signal) is used as a constant 0% transmission reference.

When the EHT is increased on the photometric control unit the photomultiplier and ancillary electronics produces a negative going voltage proportional to light intensity (transmission). This can be seen as a negative deflection of the trace on the live display screen.

The negative deflection should be increased until it reaches 0 volts (half way down the display screen). This can be achieved in several ways: EHT increase, wavelength, slit width etc. Note if no deflection appears do not use excessive EHT volts (eg >1000 volts), something is wrong!.

0 volts is used as the 100% transmission reference.

Note: it is not necessary that the 0 volts (100% T) level is achieved with water in the cell. A reaction end point is usually more convenient06,3 (but see Absorbance, below).

The PM is now generating a -4 volt light induced signal 'backed off' to 0 volts by a constant +4 volt offset. This allows the signal gain to be increased to observe very small transmission changes around this 0 volt point (see gain If water is used and the sample has a high background absorbance (0.6 AU say) then it will not be possible to use the gain ranges to expand a small reaction transient (0.6 AU = 25% transmission => +3 volts on the display) since the gains can only amplify around the 0 volt position.
ranges, section 5.1). The +/-0.1 volt range for example corresponds to a 97.5% to 102.5% transmission range or 5% transmission full scale.

If the signal drifts out of range, the EHT should be used to bring it back on scale to a suitable position (dependant on transient polarity etc) before further runs. Experiments can now be performed. It is desirable to increase the range sensitivity so that the observed transient occupies a substantial portion of the Y-scale since better use of the A/D resolution is then being made (12 bit or 1 in 4096).

Absorbance

Data collected in the software absorbance mode is automatically converted and displayed in absorbance units (AU). It should be noted here that data can also be independently converted from volts to absorbance in the Transform context. Absorbance mode should be selected. Otherwise all settings are the same as for transmission.

In the absence of a PM signal the live display screen will indicate a +2.0 AU signal level (on maximum range). This is the largest measurable absorbance. All subsequent procedures can be carried out in the same way as described for transmission, except that the EHT should be applied to bring the absorbance signal down to 0 AU.

Note: If absolute absorbances are required, it is necessary to adjust the EHT to bring the signal to 0 AU with water in the observation cell. A problem that then arises is that high background absorbances in the sample prevent more sensitive absorbance ranges being used to resolve small transients (the same as is experienced with transmission measurements) since amplification is centered around 0 AU.

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If absolute absorbances are not required then any portion of the reaction itself (start, end, middle) can be zeroed regardless of background. The resulting scales cannot then be treated as absolute scales ie 0 AU actually includes the background absorbance, but relative differences **are** accurate. ie full scales, and measured amplitudes.

Fluorescence (*or Scattering*)
Operation of the software in the fluorescence mode is similar to operation in the transmission mode except for the following:.

The software is run in the volts mode but the photometric control unit should be set to Fluor. This allows a continuously variable +ve voltage (0 to +5) to be applied to the -ve PM signal using the front panel control. Therefore any background fluorescence level can be precisely backed off. This allows greater control over the acquisition of fluorescence transients with or without background sample fluorescence.

Again all fluorescence signals should be centered around the 0 volts mark to allow optimisation of the input sensitivity range.

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Appendix II

Transmission and Absorbance Measurement

By centering the reaction transient at 0 volts using the background transmission as an arbitrary 100% level it is possible to measure very small optical transients in the presence of substantial background absorbances, provided one is prepared to forego knowing the absolute 100% transmission level (with water in the cell) and therefore the absolute absorbance background.

The absorbance measured and displayed is calculated using the transmission reference information:

+4 volts = 0% Transmission
0 volts = 100% Transmission

Taking for example a steady signal of +1 volt the conversion to absorbance is automatically carried out thus:

from

$$Abs = -\log(T/T100\text{-}T0)$$

$$\Rightarrow \log[(T100\text{-}T0)/(Vin\text{-}T0)]$$

$$= \log[(0\text{-}4)/(1\text{-}4)] = \log -4/-3 = .125 \text{ AU}$$

0% Transmission (actually +4 volts in) cannot be displayed as an absorbance level (=infinity). Also as the incoming signal level approaches 4 volts PM noise may traverse it resulting in -ve transmission values which are forbidden. Thus in Abs mode all input signals are automatically limited to 3.96 volts which gives rise to the 2 AU maximum scale.

0 AU corresponds to 0 volts or 100% Transmission.

At higher absorbance sensitivities the absorbance scales are calculated directly from the corresponding voltage scales according to the above transformation. Since the calculation directly assumes the 0% and 100% transmission values of 4 and 0 volts respectively it is imperative that the photometric unit is in Abs mode. Note the negative absorbances arise from the fact that the input voltage scales extend below 0 volts (ie >100% Transmission). In the Abs mode data runs are acquired in volts but immediately converted to absorbance before display. Data collected in volts mode can optionally be converted to data later, using the V=>ABS option in the transform context. This uses exactly the same method as has just been described.

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Appendix III

Interpretation of the Profile Display

It is important that both profiles rise cleanly and show no major discontinuities in slope during the main period of displacement. Generally speaking their rising phases should not overlap in time since this implies possible dilution errors. The first profile will usually end in a plateau corresponding to its driven limit, while the second should terminate abruptly, corresponding to the stop block being struck by the stop syringe, and post trigger data acquisition taking over. *A 10 msec gap between the end of the flush profile and the extreme right hand side of the display range indicates satisfactory end stop triggering. If this gap is greater than 100msec then correct triggering has failed and a pre-trigger timeout has occurred. Any post-trigger data should be discarded. This may occur if drive 2 is striking its internal ram limiter.*

Possible Errors and Explanations

Fill Drive (A and B)

"Drive 1 not pushing enough:"

The total volume of the A and B drive should at least flush the ageing loop (ie be greater than 115ul) and drive more for more effective flushing. The right hand fill drive which pushes A and B into the ageing loop must be limited by the internal ram displacement limiter, not the stop syringe hitting the stop block, otherwise no flush drive can take place. NOTE that increasing the fill drive displacement by lowering its adjuster and filling its syringes accordingly will correspondingly reduce the volume available for the flush drive, unless the stop block is lowered. (The opposite also applies).

Flush Drive (C and Flush)

Drive 2 not pushing enough, lower stop:
Drive 2 pushing too much, raise stop:

The second drive should displace between about 50 and 200µl total volume (C plus flush). This will displace an uncontaminated portion of the aged reaction of A and B from the ageing loop to be mixed with C and driven on to the observation cell. This drive volume is determined only by the displacement of the fill drive and the position of the stop block.

NOTE that flush drive displacement must not be determined by the its internal stop.

Calculation of Ageing from the Profiles

Each displayed profile represents the time course of the displacement of each drive, starting from its rest position. Observation cell monitoring starts at the precise moment that the stop syringe strikes its mechanical stop/trigger assembly. The !SX17MV software automatically calculates from each drive profile the 20µl of AB + C solution that finally is stopped in the optical detection cell.

Taking a minute volume at the centre of the observation cell (at the moment of triggering) as the point of interest 'p*' it is first necessary to calculate firstly when it passed through the second mixer and before that how long it had spent in the ageing loop. Working back down the second profile from the trigger point (end of drive sequence, start of optical monitoring) it is possible to interpret a final portion of the drive as the volume required to carry 'p*' from the second mixer (where C mixes and terminates ageing) to its resting place at the centre of the cell. This volume is simply equal to the sum of the dead volume (volume from second mixer to cell) plus half the cell volume. These values are known and result in the time index line displayed on the second profile. At this time during the second drive, relative to the end point, our volume 'p*' ceased ageing (AB mixed with C).

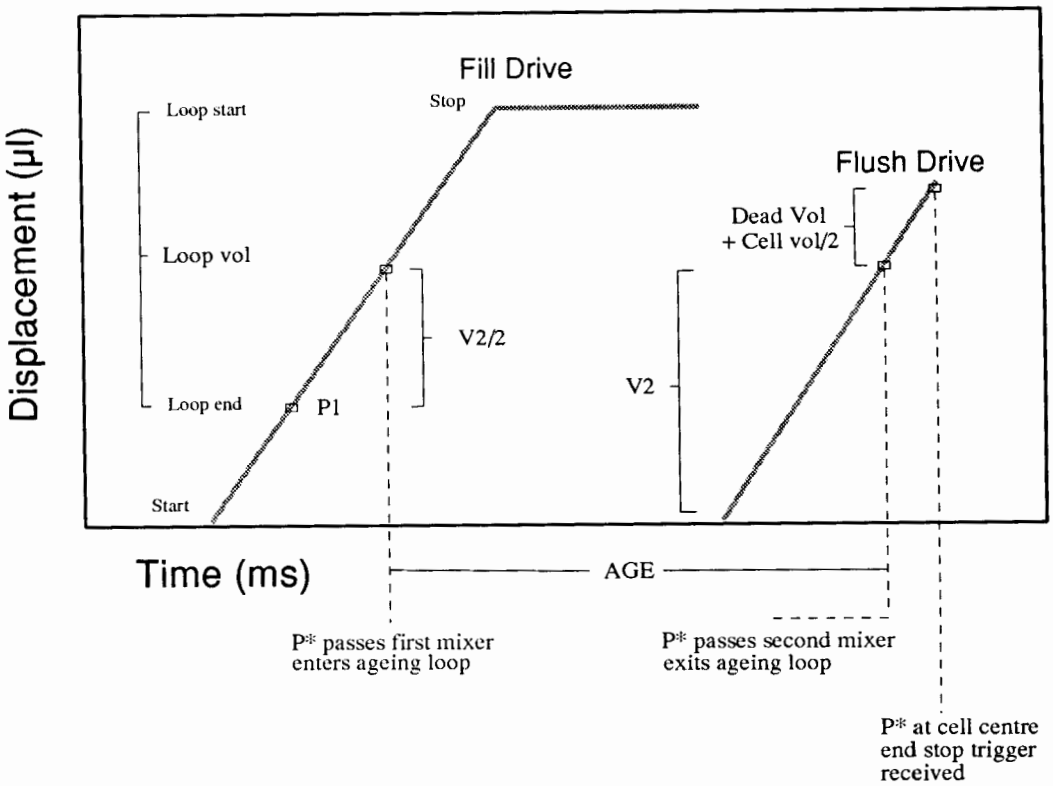
The volume pushed by flush drive preceding this point 'v2' displaced equal volumes from from the ageing loop and of reagent C (see drive circuit, hardware manual). All this solution ends up downstream of point 'p*' at the final trigger mainly filling the stop syringe. However its volume determines where 'p*' originated from in the ageing loop. Turning to the first profile it should be understood that only the final portion of the total displacement equal to the loop volume is relevant, since during this period the aging loop is completely filled with freshly mixed A and B, any excess having been pushed beyond the second mixer.

It is also important to see on this profile that at the end of the fill drive (the **top)** the last of A and B mix and enter the loop, while the A and B sitting at the downstream end of the loop has already aged during its passage down the loop. Therefore counting down this profile by a volume equal to that of the loop gives a reference position 'p1' corresponding to aged A and B sitting immediately next to the second mixer. The total age span down the loop corresponds to the displacement time of this section of the profile. *The second drive must always push volumes from the ageing loop within this range.* It is now possible to relate the 'v2' volume of the second drive back to the first drive to

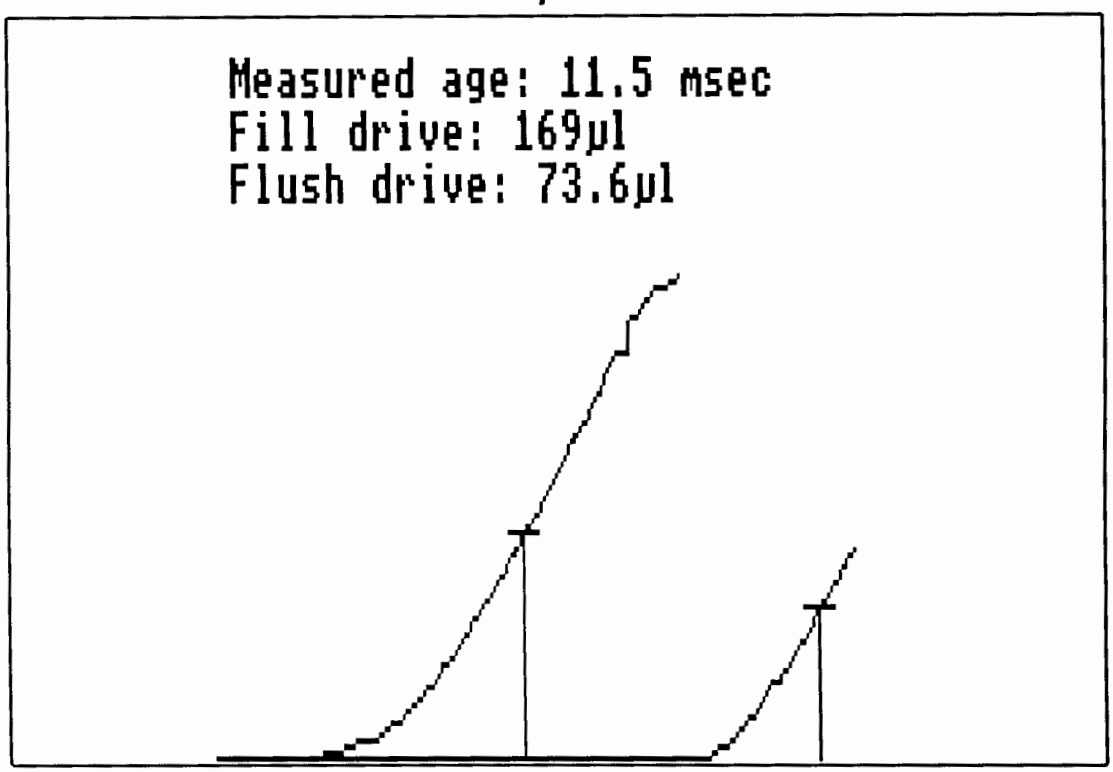
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locate the exact point in the loop which gives rise to point 'p*'. The total age of A and B before mixing with C is given by the difference in time between these points.

Volume 'v2' displaces both flush and 'C' equally therefore only volume 'v2/2' is pushed from the loop by the second drive. Point 'p*' is therefore found by counting a volume v2/2 up the first profile from point 'p1' (the solution already adjacent to the second mixer). The time coordinate of this point provides the position of the left hand time cursor.
The time difference between the two cursors is equal to the accumulated age of A and B including loop fill time, programmed delay and loop empty time.



Screendump of Profiles



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Appendix IV

AP-ASCII File Format

The format of the data files is as follows:

Header:	APL_ASCII			Text string
Coded header:	INFO>.....			Text string**
No of points:	N			Integer
x1(tab)y1{(tab)fit-y*}:	x	y	f	float chr$(9) float {chr$(9) float}
x2(tab)y2:	....			...
...	....			...
xn(tab)yn{(tab)fit-yn}				float chr$(9) float ..
				cr lf only
xscale min				int or float
yscale min				int or float
xscale max				int or float
yscale max				int or float
units x			text string	
units y			text string	
				cr lf only
tag line 1			text string	
2			etc	
3				
4				
5				

All lines are completed by a cr,lf.

*fit-y: if a fit has been performed this third column of data comprises the corresponding fitted curve.
NOTE: these files can be loaded and manipulated directly in the RISC-OS application !Edit which resides in the App1 directory on the hard disc. Non stopped flow datafiles can be loaded an analysed by the workstation software if they adopt this format.

These files can then be simply dragged onto an MS-DOS format disc for exporting to IBM and clone environments.

** The text string following INFO> is made up of three letter fields which provide status information:
ft_ indicates a fitted curve is included
cp_ indicates the file format is as detailed above
wl_nnn.n indicates wavelength information follows, where nnn.n is the wavelength in nm. (to the nearest 0.1nm)

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Appendix V

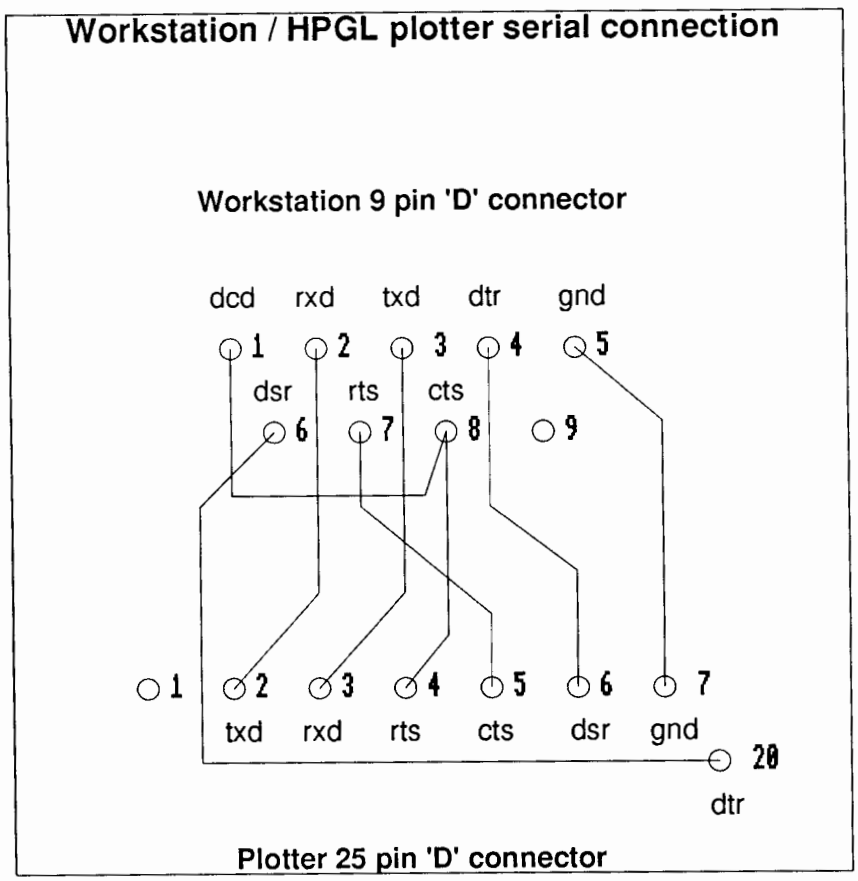
RS-232 HPGL Plotter link

The diagram below shows the required wiring configuration for the serial link.

The recommended operational baud rate is 9600. The computers default baud rate is 1200. To change it type CONFIGURE BAUD 7 at the ' * ' prompt (this can be reached by pressing f12 from the desktop). Then perform a hard reset to preserve the new setting (see the User Guide for further information).

The default data format of the computer is used: 8 data bits, 2 stop bits and no parity.

Adjust your plotter line settings to match these according to its manual.



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Appendix VI

This appendix explains the procedures for collecting absorption and fluorescence data. It should be read in conjunction with the rest of this manual and the hardware manual. Page references are of the form 12-S (i.e. page 12 of the software manual), or 6-H (i.e. page 6 of the hardware manual). These procedures are intended to be a guide for novice users and are not the last word in experimental technique.

Procedure For Acquiring Absorption Data.

Flush through the system with distilled water until there are no more air bubbles appearing in the stopping syringe.....*Page 10-H*
Ensure that the light guide and side window photomultiplier are mounted onto the cell block to give the required pathlength.....*Page 5-H*
Set the required wavelength.....*Page 11-S*
Set required slitwidth/bandpass.....*Page 6-H*
Ensure that the signal offset is set to 4V.....*Page 10-S*
Set the photomultiplier high voltage to give a signal of 0V. (Either by using the AutoPM or by pointing at the 0V line in the Live Display and clicking the left hand mouse button.).....*Pages 10, 11 and 12-S*
Allow the photomultiplier drift to settle whilst periodically adjusting the photomultiplier high voltage to maintain a signal of 0V.
Click on the ABS icon so that the data will be collected in Absorption units.....*Page 12-S*
Adjust the range until the desired scale is obtained.....*Page 11-S*
Check the the signal is still on 0V.
Set the time base to the required value.....*Page 11-S*
Fill the drive syringes with reagent.....*Page 10-H*
You are now ready to start stopped flow mixing and acquiring absorption data.

Procedure For Acquiring Fluorescence Data.

Flush through the system with distilled water until there are no more air bubbles appearing in the stopping syringe.....*Page 10-H*
Ensure that the light guide is fitted into the 2mm pathlength port (port D).....*Page 5-H*
Set the required excitation wavelength.....*Page 11-S*
Set the required slitwidth/bandpass.....*Page 6-H*
Ensure that the correct filter is fitted in front of the emission photomultiplier.....*Page 9-H*
Set the signal offset to 0V.....*Page 10-S*
Set the photomultiplier high voltage to 700V. ✳.....*Page 10-S*
Back off the scattered light signal using the signal offset. (The exact value required is the digital value in the top left of the screen).....*Page 10-S*
Set the time base to the required value.....*Page 11-S*
Fill the drive syringes with reagent.....*Page 10-H*
You are now ready to start stopped flow mixing and acquiring fluorescence data.

If you are looking at a small change in fluorescence on a large fluorescence background you should add the fluorescence background signal to the scattered light signal and offset the combined value to 0V. This will allow you to increase the range sensitivity without the trace going off scale.

✳ The value of 700V is an arbitrary one. It will generally give good results but if your fluorescence signal is going to be very high it can be reduced, say to around 600v. Likewise if your fluorescence signal is very small it can be increased up to a maximum of 1000V

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