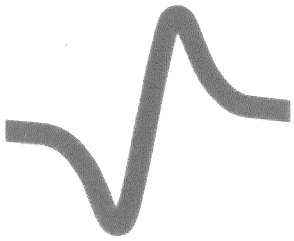

SFM-4 User's Manual

WARRANTY

BIO-LOGIC WARRANTS EACH INSTRUMENT IT MANUFACTURES TO BE FREE FROM DEFECTS IN MATERIAL AND WORKMANSHIP UNDER NORMAL USE AND SERVICE FOR THE PERIOD OF ONE YEAR FROM DATE OF PURCHASE*. THIS WARRENTY EXTENDS ONLY TO THE ORIGINAL PURCHASER.

THIS WARRANTY SHALL NOT APPLY TO FUSES OR ANY PRODUCT OR PARTS WHICH HAVE BEEN SUBJECT TO MISUSE, NEGLECT, ACCIDENT, OR ABNORMAL CONDITIONS OF OPERATION.

IN THE EVENT OF FAILURE OF A PRODUCT COVERED BY THIS WARRENTY, THE PRODUCT MUST BE RETURNED TO AN AUTHORIZED SERVICE FACILITY FOR REPAIR AND CALIBRATION AND TO VALIDATE THE WARRANTY.

THE WARRANTOR MAY, AT THEIR DISCRETION , REPLACE THE PRODCUT IN PLACE OF REPAIR. WITH REGARD TO ANY INSTRUMENT RETURNED BECAUSE OF DEFECT DURING THE WARRENTY PERIOD, ALL REPAIRS OR REPLACEMENTS WILL BE MADE WITHOUT CHARGE. IF THE FAULT HAS BEEN CAUSED BY MISUSE, NEGLECT, ACCIDENT, OR ABNORMAL CONSITIONS OF OPERATION, REPAIRS WILL BE BILL AT NORMAL COST. IN SUCH CASES, AN ESTIMATE WILL BE SUBMITTED BEFORE WORK IS STARTED.

IN CASE ANY FAULT OCCURS :

NOTIFY BIO-LOGIC OR NEAREST SERVICE FACILITY, GIVING FULL DETAILS OF THE DIFFICULTY, AND INCLUDE THE MODEL NUMBER, TYPE NUMBER , AND SERIAL NUMBER. UPON RECEIPT OF THIS INFORMATION, SERVICE OR SHIPPING INSTRUCTIONS WILL BE FORWARDED TO YOU.

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* EXCEPTION : ARC LAMPS SOLD BY BIO-LOGIC ARE ONLY WARRENTIED FOR A PERIOD OF 3 MONTHS FROM DATE OF PURCHASE.

I. GENERAL SECTION

TABLE OF CONTENTS

1 INTRODUCTION AND SPECIFICATIONS

1.1 General description

The Bio-Logic stopped-flow module SFM-4, consists of a mechanical subsystem and a power supply MPS-51/4.

The mechanical sub-system consists of four machined syringes, one valve block with four 3-way valves, the possibility to include one to three mixers and up to two ageing loop.

The SFM-4 syringes, valves, delay lines and cuvettes are enclosed in a water jacket to allow temperature regulation of the reactants containers. The syringe plungers of the stopped-flow are driven by four stepping motors via four ball screws.

THE MECHANICAL DESIGN.

The mechanical part of the SFM-4 module is carefully constructed, the parts in contact with the biological sample and the buffers are all machined out of materials selected for their inert characteristics: stainless steel, teflon, Kel-F and quartz.

Millisecond dead time can be achieved with the SFM-4 instrument due to the combined effects of high-performance control of the stepping motors, and a drastic reduction of the dead volumes.

Ageing lines of various volumes can be used. The ageing line of the instrument can be replaced and made tight in a few minutes.

INTELLIGENT POWER-SUPPLY.

The high performance of the SFM-4 unit and the high speed of the stepping motors can be achieved only because of the quality of its power-supply MPS-51/4. The MPS-51/4 unit contains four independent constant current power supplies all driven independently by their own microprocessors.
The sequence of impulses to be sent to the stepping motors are stored in the memory of each board. One main microprocessor board synchronizes the four power supplies, and performs the communication with the microcomputer via serial interface.

MICROCOMPUTER COMMANDS.

The SFM-4 module is controlled by the user with the keyboard of a PC/XT/AT or compatible microcomputer. Various screens permit the user to :
- know the volumes of solution contained in the four syringes
- perform manual or automatic movement of the syringes
- create a sequence of reaction with complete control of time and volume delivered by the four syringes
- save or recall the sequences
- program the synchronization pulse used to trigger the acquisition system
- load the data acquisition software: Bio-Kine.

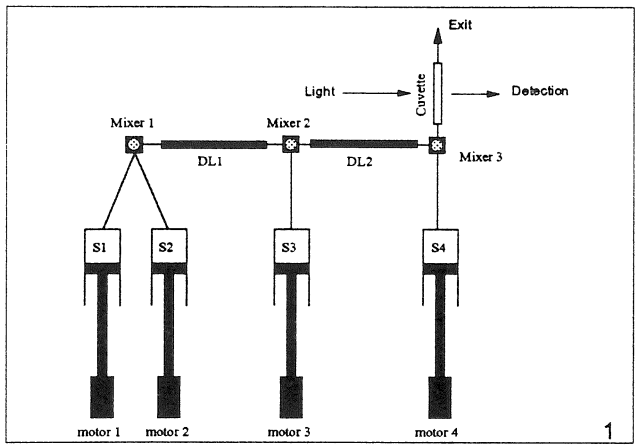
1.2 Modes of operation

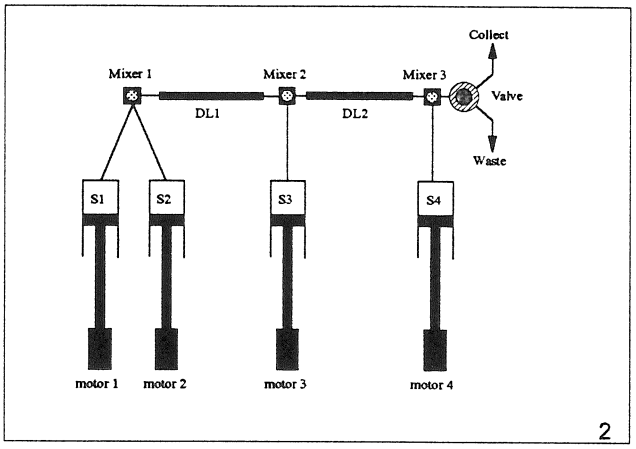
The SFM-4 has two main operating mode that are briefly described below. More detail can be found in other sections of this manual.

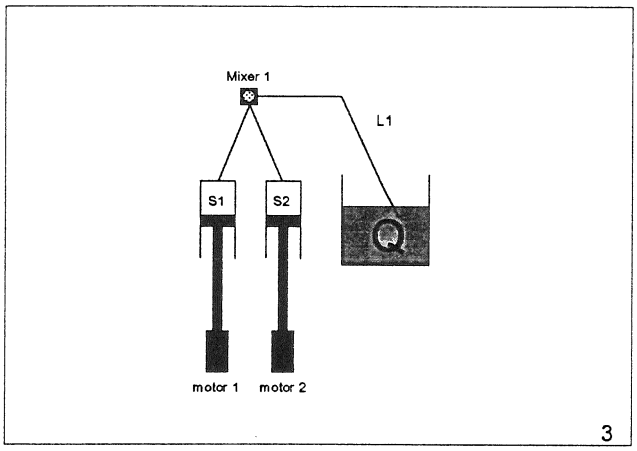
i) SF mode (commercial reference SFM-4/S). In this configuration the SFM-4 is a full stopped-flow instrument with an optical observation chamber.
This is described in the panel N°1 in figure 1 below. In this configuration the SFM-4 has unique features for a stopped-flow instrument. Four solutions can be mixed and injected in the cuvette, two delay lines can be installed with three mixing chambers.
The speed capability of the SFM-4 instrument with its 4 syringes running gives a dead time below 1 ms in the observation cuvette.

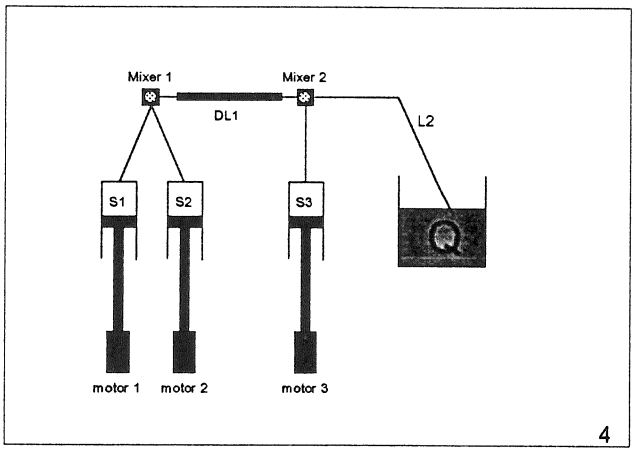
ii) QF mode (commercial reference SFM-4/Q). In this configuration the SFM-4 features a complete quench-flow instrument. In this mode of operation, various mode of operations are possible possible as described in the figure 1 below. It can be used as a four syringe quench-flow instrument with two delay lines, three mixers and a diverting valve for waste and collect (panel N°2). Alternatively, an external flow line can be connected for direct injection of the mixture into a quenching solution. This mode may be used with or without an additional delay line as shown in panel N°3 and 4 below.
It can be used in a simple 3 syringe mode and direct collection in a syringe as described in panel 5.
In another mode the mixture can be injected onto a filter at the same time as it is mixed with a flow of washing buffer (panel N°6).
Flash quenching with a photoreactive reagent is also a mode that can be easilly implemented to the SFM-4.
Many other configurations are possible, you are invited to inquire about the feasibility.

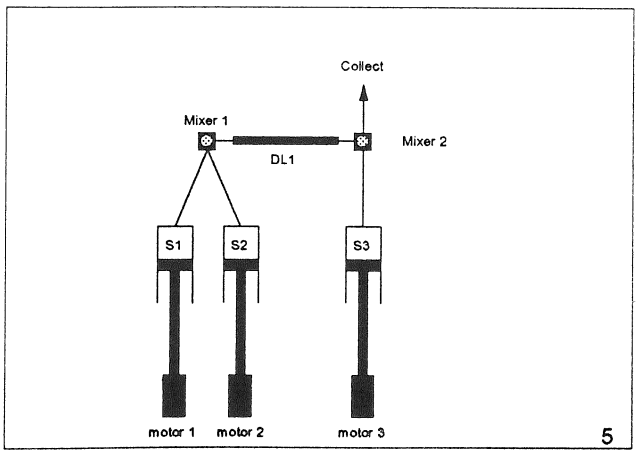
The commercial reference SFM-4/QS has all the components for the two applications. A SFM-4/S or a SFM-4/Q can be updated to SFM-4 /QS.

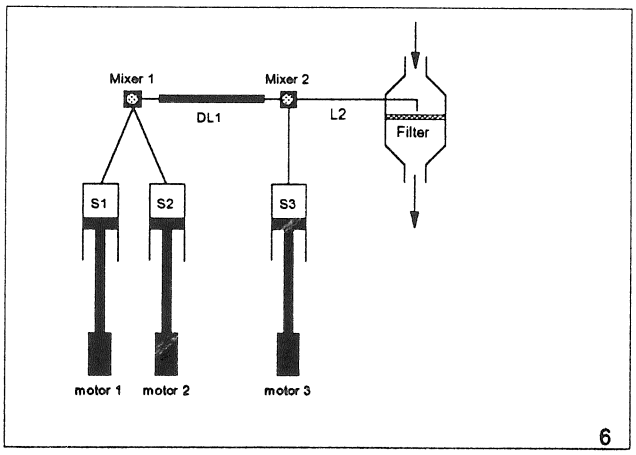

Exit
Light
Cuvette
Detection
Mixer 1
Mixer 2
Mixer 3
DL1
DL2
S1
S2
S3
S4
motor 1
motor 2
motor 3
motor 4
1


Collect
Mixer 1
Mixer 2
Mixer 3
Valve
DL1
DL2
Waste
S1
S2
S3
S4
motor 1
motor 2
motor 3
motor 4
2


Mixer 1
L1
S1
S2
motor 1
motor 2
3


Mixer 1
Mixer 2
DL1
L2
S1
S2
S3
motor 1
motor 2
motor 3
4


Collect
Mixer 1
Mixer 2
DL1
S1
S2
S3
motor 1
motor 2
motor 3
5


Mixer 1
Mixer 2
DL1
L2
Filter
S1
S2
S3
motor 1
motor 2
motor 3
6

1.3 Specification

Table A

SPECIFICATIONS for SFM-4	
Number of syringes	4
Driving mechanism	one stepping motor per syringe
Stepping motor drive operating at 6400 steps per motor turn	
Number of mixers	1 to 3
Ageing line between the two mixers	25 to 1000 µl
Programmable trigger for data acquisition and synchronization of accessories	
Filling range of the drive syringes	500 µl to 15 ml
Minimum injection volume per shot	20 µl
Flow-rate range	0.05 to 6 ml/s/syringe (up to 8 ml/s with acceleration phase)
Variable ratio range	1/1 to 1/20, larger than 1/100 with double dilution
Minimal dead time	0.7 ms at 12 ml/s total flow rate with FC-08 cuvette in SF mode 1.0 ms in QF mode with the minimal volume delay line and 20 ml/s
Material	stainless steel (or Kel/Fon special order)
Syringe volume	18 ml (other volumes on special order)
Volume per step	0.14 µl
Duration of flow	adjustable from 1 ms to 9999 ms per phase
Power requirement	300 Watt, 110/220 Volt, 50/60 Hz
Total weight	12 kg

1.4 Principle of operation

The syringes of the SFM-4 are driven by four independent stepping-motors. The stepping-motors are of hybrid technology of 200 steps per revolution and 4 phases, each phase being powered by a constant current supply (2.9 A per phase). The power supply of each motor is microprocessor controlled. A complex impulse sequence enables micro-positioning of the motor's rotor with an accuracy equivalent to 1/32 of the mechanical step. This gives an effective number of steps of 6400 per revolution, or a volume quantification of 0.14 μl per micro-step, when standard syringes are used.

With the damping produced by the rotor inertia, this results in an almost continuous, linear movement of the syringe even at very low flow rates.

The motors can be activated manually or automatically. The manual mode is mainly used to refill or wash the syringes; the four syringes can be driven independently and their speed adjusted using the microcomputer with a very simple menu (see SOFTWARE INSTRUCTIONS in SFM-4/S and SFM-4/Q sections for more details). The automatic mode is used in the actual stopped-flow experiments.

The motor impulses are counted in the positive direction (refilling), or negative direction (emptying), so that the contents of each syringe can be continuously displayed. Zero volume corresponds to the upper position of the syringe and zeroing can be done using the keyboard of the microcomputer.

The movements of the syringes being completely controlled by the microprocessor, there is no need for a stop syringe. Thus, the stop artifact present in most conventional stopped-flow systems is absent in the SFM-4. The observation system can be synchronized with the syringe "start" or "stop" by using the trigger pulses available on the front panel of the MPS-51/4 power supply.

The independence of the four syringes allows a higher versatility of the injection sequence (e.g. injection of one syringe only, unequal filling of the syringes, variable ageing times, variable concentration, variable ratios, etc...)

The reproducibility and regularity of the linear translation of the syringes and the absence of pressure artifact allow optical recording during the drive sequence. These features greatly facilitate the determination of the initial phase of the reaction being monitored and make the equipment suitable for very accurate, continuous flow experiments.

1.5 Description of the mechanical design

The observation chamber and the syringes of the SFM-4 stopped-flow module are mounted vertically, facilitating purging off bubbles, which are evacuated during refilling by a few up and down movements of the drive syringe. The maximum tilt angle of the instrument is 20° from the vertical.

The syringes, valves, and observation chamber are very carefully thermo-regulated. This thermo-regulation prevents the occurrence of temperature artifacts on a very wide temperature range, and permits rapid kinetic studies even at temperatures below 0°C.

1.6 The ageing loops

The SFM-4 instrument has ageing loops, permitting various delay to be obtained between two mixers. The ageing loops are machined into stainless steel spacers. These spacers can be inserted between two mixer blocks or between one mixer block. See INSTALLATION OF THE STOPPED-FLOW COMPONENTS in SFM-4/S and SFM-4/Q sections for full description of ageing lines installation and calculation of volumes.
Replacement of the ageing loops is an easy operation which usually takes only a few minutes.

Ageing loops of nominal volumes up to 1000 μl are available.

Standard equipement of SFM-4/S version does not include ageing lines as described in INSTALLATION OF THE STOPPED-FLOW COMPONENTS section. SFM-4/Q and /QS versions are delivered with two sets of ageing lines up to 200 μl. Ageing lines of 500 μl and 1000 μl can be obtained as additional accessories.

To evaluate the ageing time, the entire volume between the two mixers has to be taken into account. This volume includes the ageing line plus the dead volumes volumes between the both sides of the delay line and the mixers. The complete description of the volumes are described in the sections VOLUME OF FLOW LINES SFM-4/S and SFM-4/Q.

2 GENERAL INSTRUCTIONS FOR INSTALLATION

This section of the manual contains information on the installation and preliminary operation of all versions of SFM-4. It is recommended that the contents of this section be read and understood before any attempt is made to operate the instrument. In case of difficulties please contact Bio-Logic or its nearest representative.

2.1 Operating features

Figure 2

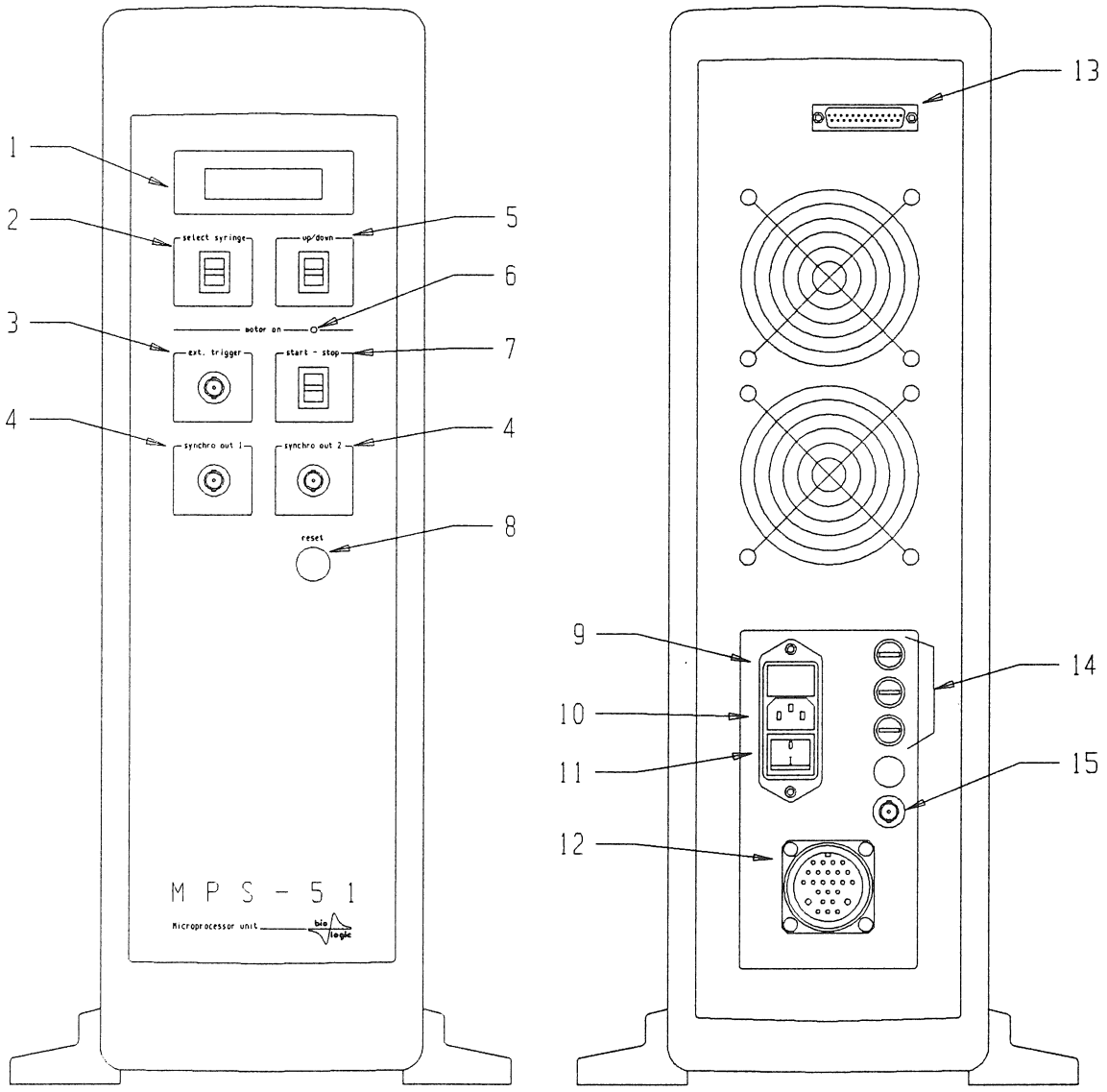


Table B

	NAME	FUNCTION
1	LCD DISPLAY	Used to display messages (selected syringe, auto mode...)
2	SYRINGE SELECTOR	Selects the syringe for the manual control (5)
3	TRIGGER INPUT	Input for an external signal to trigger the drive sequence
4	SYNCHRO PULSE OUTPUT	Pulse output to trigger the recording system, or any electronic device to be synchronized with the instrument
5	MANUAL MOVEMENT	Manual control of the syringes
6	"MOTOR ON" INDICATOR	Lights when, at least one of the motors, is activated
7	START/STOP	Initiates (or stops) the programmed sequence in the automatic mode. The instrument may also be started and stopped using the keyboard of the PC.
8	PROGRAM RESET	Resets the MPS-51 main program
9	MAIN POWER FUSE	3 A for 220 V, or 6 A for 115 V
10	AC LINE CONNECTOR	
11	MAIN POWER SWITCH	
12	MOTOR POWER CONNECTOR	Sends the power pulses to the stepping motors
13	LOGIC CONNECTOR	Connects the MPS-51 controller to the PC
14	MOTOR FUSES	5 A
15	HARD STOP (SF) or OUTPUT VALVE (QF) BNC CONNECTOR	

2.2 AC power and connections

Before connecting the SFM-4 to the local AC line, verify that the setting of the instrument matches the local line voltage. Prepare the SFM-4 for operation by connecting the mechanical subsystem to the power supply unit and the power supply unit to the controller. Plug the controller into the appropriate AC line. Prepare the SFM-4 for operation by connecting the mechanical subsystem to the MPS-51 unit. Connect the MPS-51 to the serial port of the microcomputer.

2.3 Water circulation

The SFM-4 module may be connected to a circulating water bath for temperature regulation. The coolant flows through two internal circuits: one around the injection and reservoir syringes, and the other through the valve block and observation head.

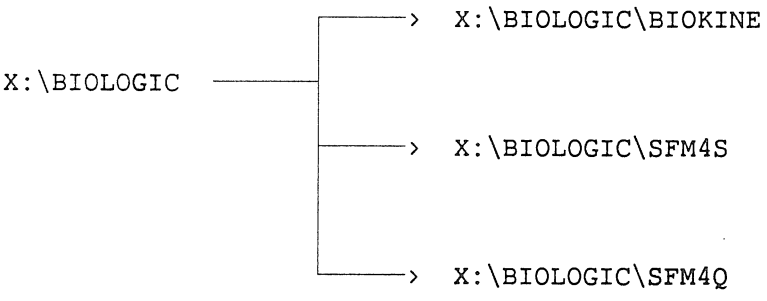
2.4 Installation of the software on the hard disk

Note: at this point we assume that you are familiar with the DOS files structure See your DOS manual for more details.
Make a backup copy of your original diskette set and put it in a safe place.
Note: The following procedure is available if you ordered the rapid kinetic software BIOKINE software together with SFM-4/QS, if it is not the case, then do not read the instructions concerning BIOKINE.

Insert disk 1 in your diskette drive and run: **INSTALL**

Follow the instructions displayed.

After the INSTALLATION is done, a BIO.BAT program is copied onto the root directory of the hard disk and the following directories are created:



(Where X is the name of the disk drive [C,D,E,...])

If you wish the software to run automatically when your computer is turned on, use the DOS editor EDLIN (or any other TEXT EDITOR) to add the following line to the AUTOEXEC.BAT file.
BIO.BAT

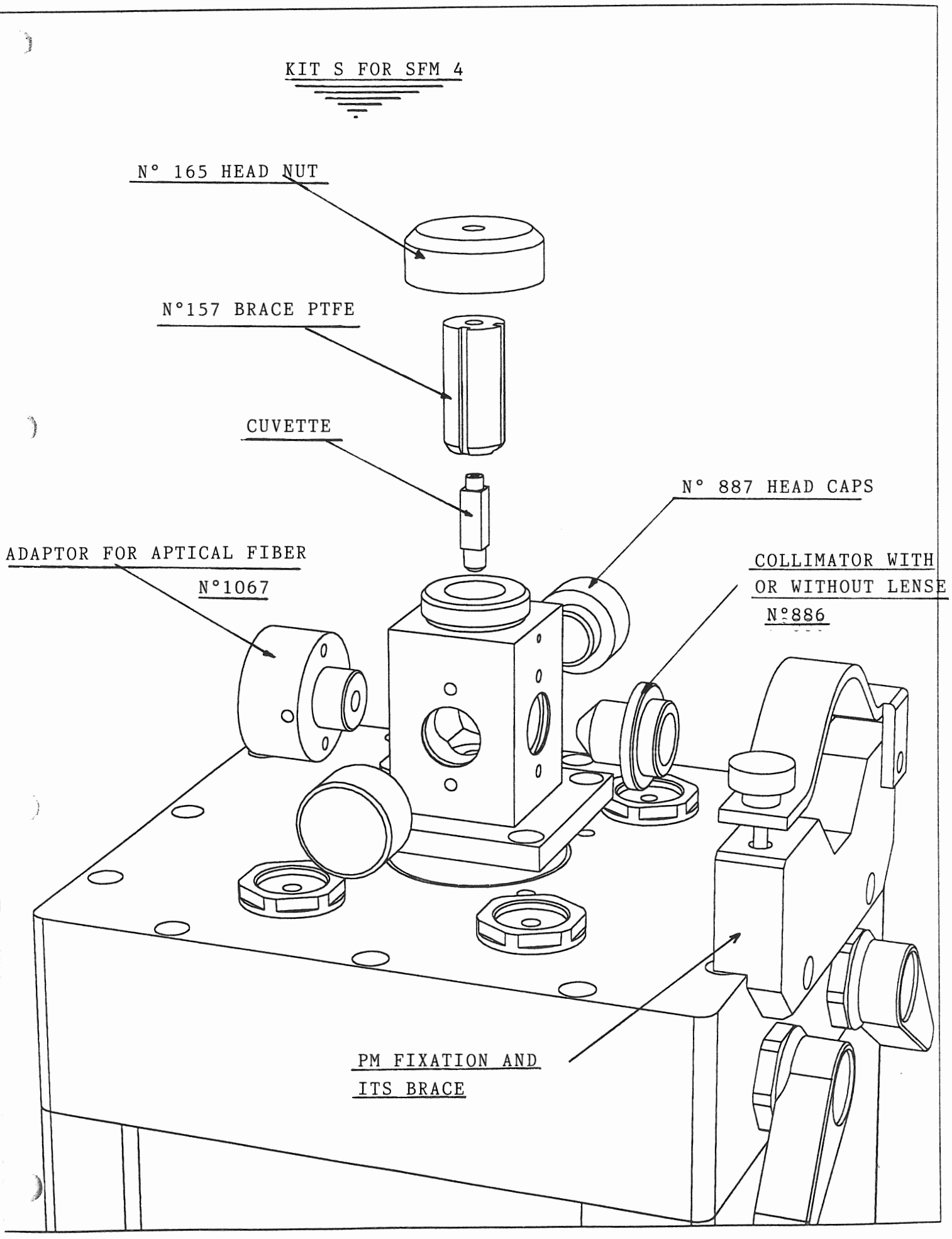
THE NEXT INSTALLATION STEPS ARE SPECIFIC OF THE VERSION OF THE SFM-4 VERSION USED. PLEASE CONSULT THE PART OF THE MANUAL CORRESPONDING TO THE VERSION THAT HAS BEEN ORDERED OR INSTALLED

SECTION II

SFM-4 INSTRUCTION MANUAL

STOPPED-FLOW MODE (SFM-4/S)

KIT S FOR SFM 4



1 INSTALLATION OF THE OPTICAL SYSTEM

The Bio-Logic stopped-flow module can be adapted with any good quality optical system. However, higher performance can be achieved with the Bio-Logic optical and light detection components.
Examples of installations and equipment are listed in the table below:

Table I

Equipment :	Main applications, advantages, or limitations :
SFM-4 + fibre optics for illumination + fibre optics for light detection + a host spectrophotometer or fluorometer. No BioKine software.	OK if connected to a spectrophotometer for absorbance measurements.
	Probably insufficient for fluorescence. Except in case of very high fluorescence signal, connection to a fluorometer will lead to an insufficient sensibility in detection.
	Since no BioKine is in the package, data analysis will have to be provided by the spectrometer used.
As above + BioKine	Same as above for the optics. BioKine can be included only if one can solve the problem of compatibility between the host spectrophotometer and BioKine. This option is feasible for most cases.
SFM-4 + fibre optics for illumination + host spectrophotometer + detection channel (PMS-200 + PM tube) + Bio-Kine + computer + A/D board.	The host spectrophotometer is just used as a light source. As the detection is made directly on the SFM with Bio-Logic detection channel, light will be sufficient to enable absorbance and fluorescence experiments. This configuration only saves the price of the light source.
	Illumination may be a little weak for low intensity fluorophores.
Complete set-up : SFM-4 + MOS-200 or MOS-200/S + Bio-Kine + A/D board + computer	Completely independent set-up for fluorescence and absorbance. Very high intensity light source with MOS-200/S.
Complete set-up :SFM-4 + MOS-400/CD + Bio-Kine + A/D board + computer	As above, with CD capability in addition.
Complete set-up : SFM-4 + MOS-400/DW + Bio-Kine + A/D board + computer	As above, with dual wavelength absorbance or dual wavelength excitation fluorescence

Please refer to the specific brochure for optical system installation.

2 INSTALLATION OF THE STOPPED-FLOW COMPONENTS.

2.1 The observation chamber

The observation chamber has three observation windows allowing measurements of transmittance, single or double wavelength fluorescence, and light scattering or fluorescence polarization, without adding reflecting or beam splitting elements. The two windows at right angles to the incoming light can be equipped with lenses to increase the efficiency of light detection.
The observation head can be equipped with several types of cuvettes, described in the next page.

Cuvettes TC.xx/10 have a 1x1 mm^2 section, cuvettes TC.xx/15 have a 1.5x1.5 mm^2 section. Replacement of the observation cuvette is described in section xxx.

The FC type cuvettes have blackened edges to reduce light scattering in fluorescence configuration. FC-15 and FC-20 are the best choices for CD experiments in the far UV. Their large aperture facilitates low noise recording at these wavelengths.

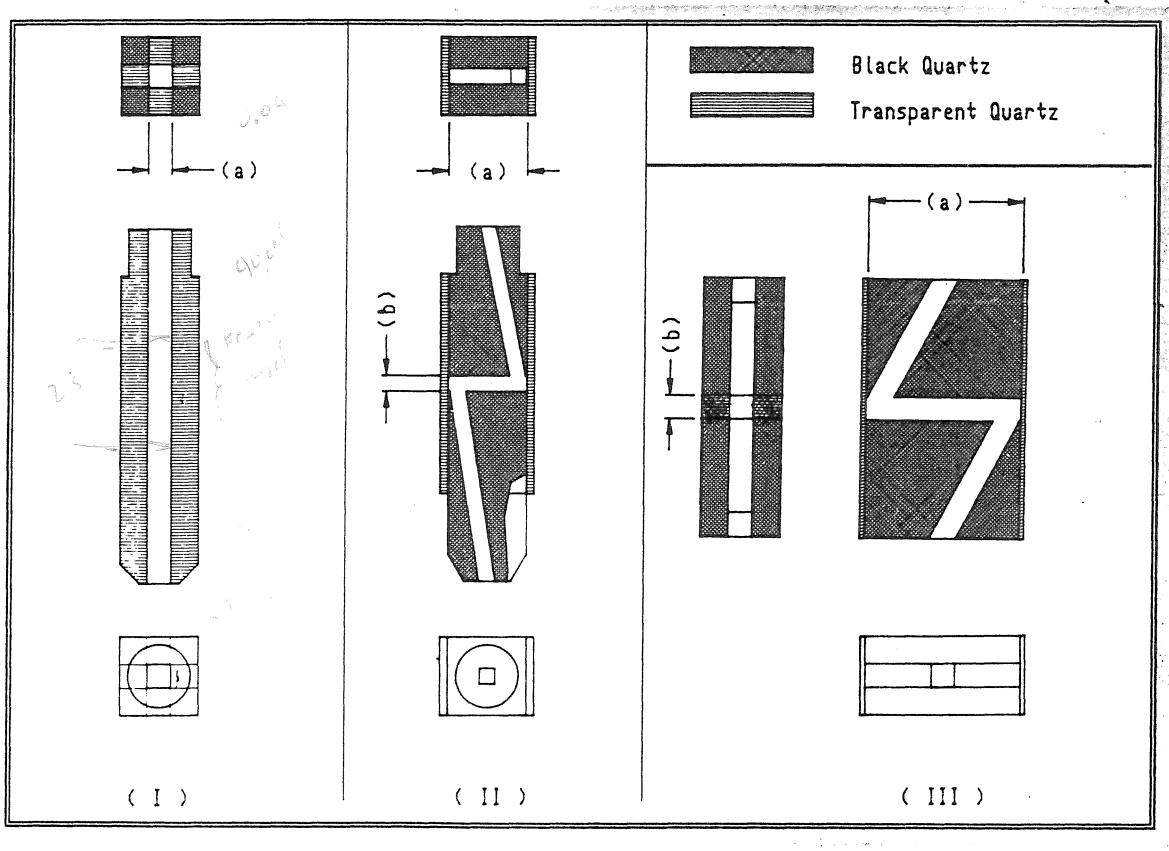
The TC-100 models have been primarily designed for absorbance, however both sides of the light path are transparent and can be used for fluorescence. This will be a primary choice for fluorescence experiments using dilute samples and excitation with a laser or any other low divergence source.

SFM-3 Cuvettes

The cuvettes available for the SFM-3 unit have continually evolved to fulfill the requirements of our users. Here is a list of presently available cuvettes. You are invited to inquire about any special need.

MODEL (1)	Part N°	Drawing N°	Light path (mm) (a)	Aperture (mm) (b)	Volume of cuvette (µl) (2)	Dead Time at 10 ml/s total flow rate (ms) (3)	Holder Model	Application
FC-08	54-08	I	0.8	-	19	0.9	1	Fluorescence Light scattering Absorbance, CD
FC-15	54-15	I	1.5	-	31	3.1	1	
FC-20	54-20	I	2.0	-	54	5.4	1	
TC-50/10	54-51	II	5.0	1.0	15	1.5	1	Absorbance, CD
TC-50/15	54-55	II	5.0	1.5	33	3.3	1	
TC-100/10	54-60	III	10.0	1.0	23	2.3	2	Absorbance
TC-100/15	54-61	III	10.0	1.5	40	4.0	2	CD

(1) All cuvettes are in suprasil (transparent from 185 to 2500 nm)
(2) From mixer to center of the cuvette.
(3) Dead time is inversely proportional to the flow rate



The FC type cuvettes have blackened edges to reduce light scattering in fluorescence configuration. FC-15 and FC-20 are the best choices for CD experiments in the far UV. Their large aperture facilitates low noise recording at these wavelengths.

The TC-100 model has been primarily designed for absorbance, however both sides of the light path are transparent and can be used for fluorescence. This will be a primary choice for fluorescence experiments using dilute samples and excitation with a laser or any other low divergence source.

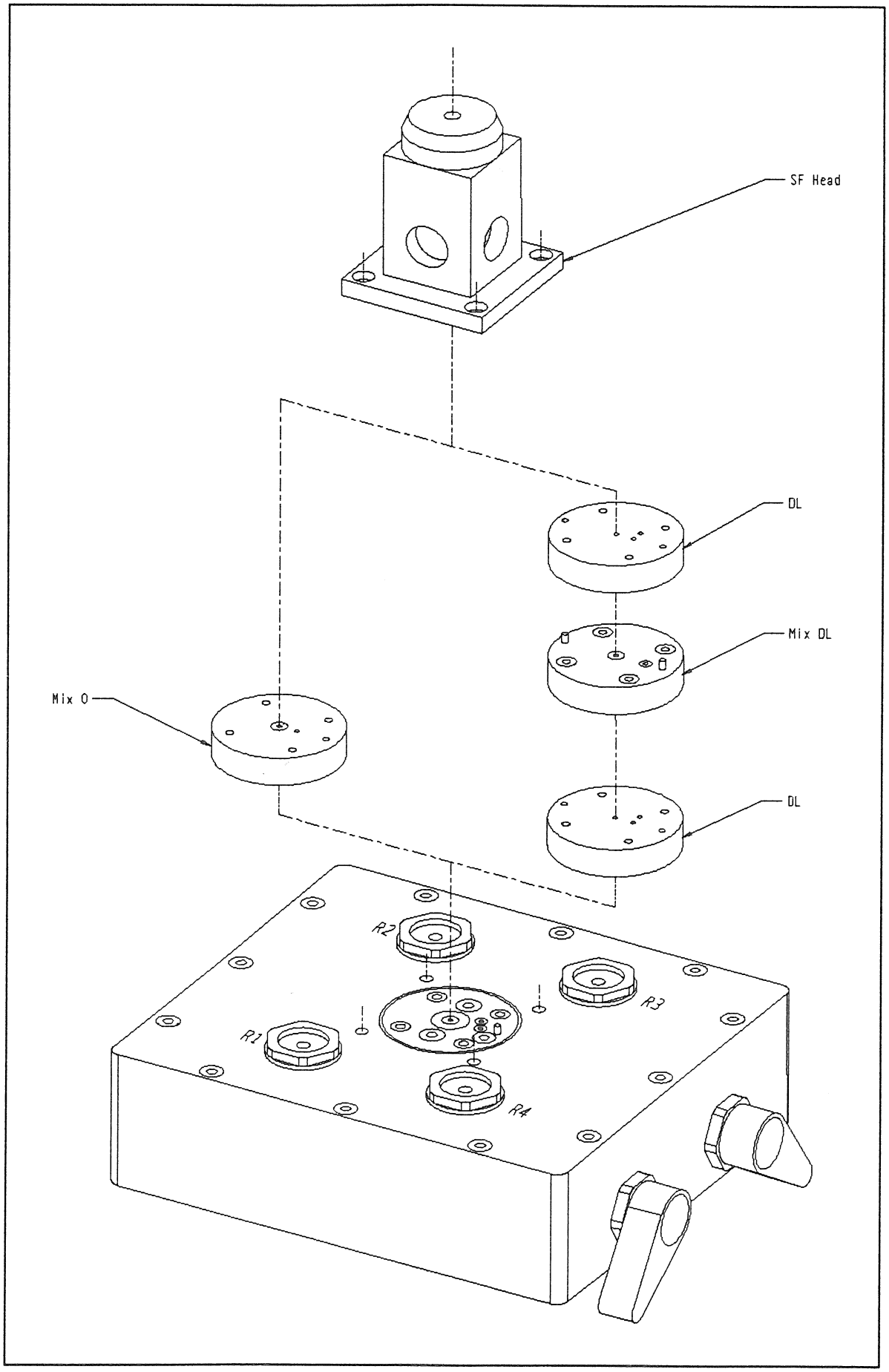
2.2 Installation of the mixer blocks and of the delay lines

In the stopped-flow mode, the four syringes of the SFM-4 can be used to performe several goals. It is difficult to list all the possibilities here. Some are described below :

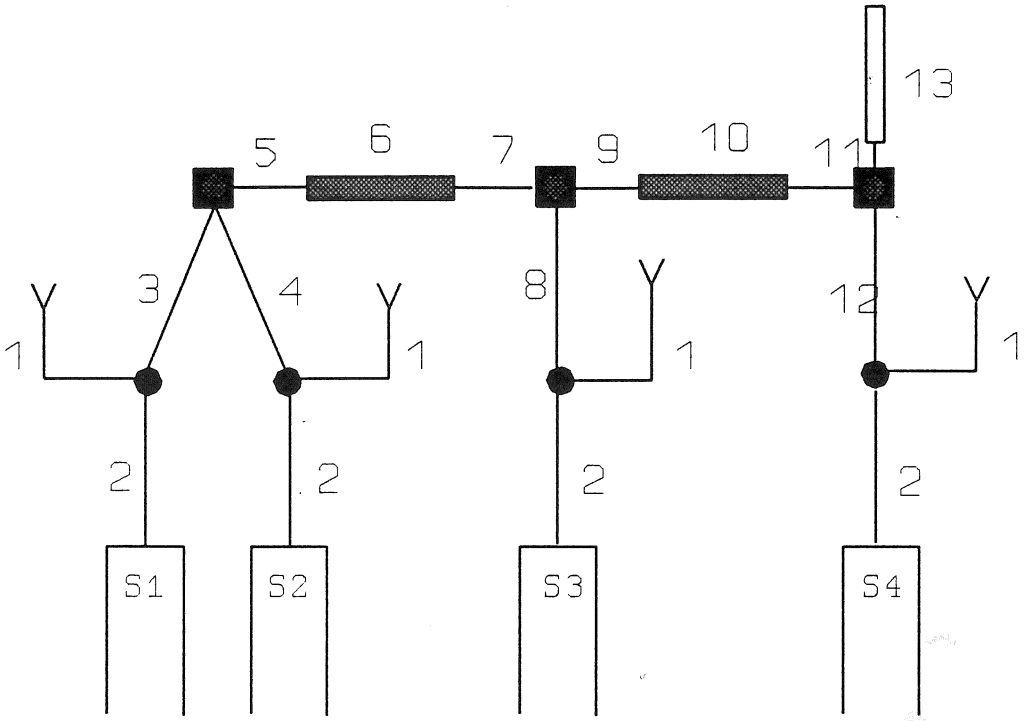
a) To load up to three reagents and to mix it in different shots with the content of the syringe N°4.
b) To vary the concentration of one or two substate and mix the result with the content of syringe N°4.
c) To perform sequential mixing and delays between up to 3 reagents before they are mixed with the content of syringe N°4.

For the first two applications the observation head will be installed on the SFM-4 body through one mixing block labeled **MIX 0**, and with no additional delay lines. (see figure on the next page)

For the last application the observation head will be mounted on the SFM-4 body through three spacers consisting of : one delay line, mixing block labeled **MIX DL** and a second delay line. (see figure on the next page)


SF Head
DL
Mix DL
Mix 0
DL
R2
R3
R1
R4

2.3 Volume of the flow lines.



The numbers in the table below refer to the numbers on the figure above :

	Without delay lines (MIX 0)	With delay lines (MIX DL)
Line number	Flow line volume (μl)	
1	80	80
2	41	41
3	151	151
4	151	151
5	6	6
6		Delay line
7	11	11
8	156	172
9	6	6
10		Delay line
11	16	16
12	188	227
13	Cuvette	Cuvette

2.4 Intermixer volumes

The table below summarizes the mechanical volumes between the mixers. The ageing time in each of these flow line will be given by :

Ageing time between two mixers = (Intermixer volume)/(flow rate)

	Delay line used							
Flow line	No	17	40	90	140	190	500	1000
Mixer 1 to 2	17	34	57	107	157	217	517	1017
Mixer 2 to 3	22	39	62	112	162	212	522	1022
Mixer 3 to center of the cuvette	Dependent on the cuvette model See chapter 2.1							

In the "No delay line" mode the "**MIX 0**" mixing block has to be used, in the "Delay line mode " the "**MIX DL**" mixing block has to be used.

The volumes indicated above are the mechanical volumes. The hydrodynamical volume may vay slightly around these values. It is recomended to calibrate these volumes with known reactions using procedure similar to that used in the Quenched-flow mode.

Also similarly to this mode two ageing mode may be used here : continuous flow or interrupt mode.

2.5 Liquid outlet system

During the injection phase, the liquid in the cuvette can reach linear velocities greater than 20 meters per second. At the flow stop, the liquid column has to be immobilized in a fraction of millisecond. Depending on the stop mode this can result in overpressure or underpressure that are potential sources of stop artefact. In the SFM-4, there are several procedures for the output flow.

2.5.1 Free flow-system

In this mode the outlet is continously open and connected to a tube. This procedure may to be used in case of pressure sensitive organelles or in case of the existence of pressure artefact on the cuvette material (as in CD).
Care should be taken to allow the liquid column to be interrupted as close as possible to the SFM-4 exit.

To do this an exit tube is provided in the standard equipment. This tube has a vent permitting entry of air. It is recomended to connect this tube to a larger PVC tube to permit further air entry. If these precautions are not taken, and for example if a long continuous tube is connected to the outlet, a long column of liquid will be pushed during the flow. At motor stop the inertia of this liquid column will inevitably generate underpressure in the cuvette and leads to cavitation.

2.5.2 Hard-stop system

In this mode flow will be immobilized by a combination of two mechanisms : firstly, from the stepping motors stop, secondly, by a high speed electrovalve which closes the output of the SFM-4 cuvette. This electrovalve is actuated by the programmable power-supply of the SFM-4. No overpressure is developed in the observation cuvette because of the perfect synchronization with the motor halt. The result is the elimination of the stop and overpressure artifact giving an incomparable quality of the fastest stopped-flow traces.

Operation of the valve is as follows:

1. SFM-4 is in manual mode : The valve is always open.

2. SFM-4 is in automatic mode.
   - Between two shots : the valve is always closed
   - During a run :
     a) the valve opens at the beginning of the flow.
     b) the valve closes at a designated number of miliseconds before the flow stops.

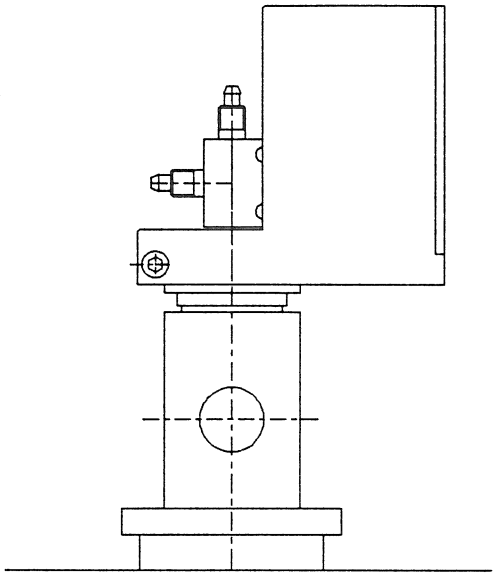
2.5.3 Installing the hard-stop valve

Install first the appropriate cuvette in the observation head, tighten the upper screw.
Connect the hard-stop assembly to the MPS-51 power supply (see general instructions section I)

Note: Connect a small tube to the horizontal exit of the hard stop device for waste.

The functions of the hard-stop valve are programmed from the Installation menu of the software. (see section 4.3).

Hard-stop may be turned on or off (see section 4.3). When on, the current pulse used to close the hard-stop valve is actuated with a few milliseconds lead to compensate for electrical and mechanical delays. Correct value for this lead is around 4 ms.



2.5.4 Exit in a syringe

In this method a syringe is plugged at the SFM-4 exit. The linear momentum of the liquid flowing out of the cuvette will be dissipated in the liquid contained in the syringe. This procedure gives clean stop signal.

For a better result, it is recomended to use high quality 10 to 20 ml glass syringes with teflon piston (Hamilton). Plastic syringes are too soft and do not give good results.

Before the runs it is recomended to fill the syringe with about 1 ml solution and to carefully eliminate the bubbles.

3 SPECIAL ACCESSORIES OR INSTALLATIONS

3.1 Small drive syringe

The SFM-4 stopped-flow works with a high driving speed range. In particular, different speeds can be programmed on each of the syringes leading to a mixing ratio different from 1 to 1 in each of the mixers. Ratios as high as 1 to 20 can be obtained by programming different flow rates in the syringes without syringe exchange.

For continuous operation with high dilution ratio, we advise installation of a syringe of smaller volume for injecting the solution to be diluted. This will enable the motor pushing this syringe to run at a faster and smoother rate. Syringes of 4 ml are available from stock. Other volumes may be designed on customer request.

This model of syringe is installed in the SFM-4 module as the standard version. Please refer to the technical section of this manual for syringe disassembly and reassembly.

See section 4.3, for software modification associated with this syringe replacement.

3.2 A mixer for high-density solutions

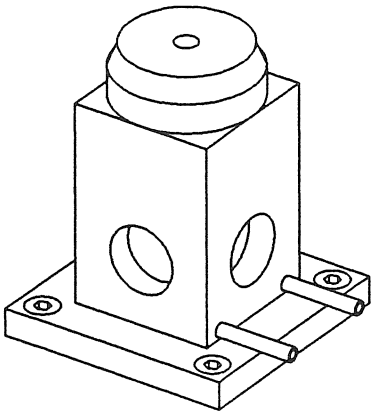
3.2.1 Description

Mixing solutions of different densities proffers a formidable challenge for stopped-flow instruments. In typical folding/unfolding experiments, heavy solutions of urea or guanidine chloride are mixed with pure aqueous buffers. The result is an unavoidable convection reaching the observation cuvette 10 to 30 seconds after mixing creating a massive artefact ruining definitively the kinetics being recorded. The SFM-4 module can be equipped with a specially designed mixer (model HDS) that includes an internal siphon-like frame that allows complete blockage of convection created by density or temperature differences. Using this mixer stopped-flow traces produced by mixing high density solutions with water can now be recorded from the first millisecond to several 100 seconds.

3.2.2 Installation

Installation procedure is identical to that of a standard mixer. Please refer to the technical section for description.

3.3 Observation head with separate cooling part



This feature permits a temperature regulation of the observation head different from the main body of the instrument. This may be used in cases where mixing of the solution produces a temperature change of the solution flowing into the cuvette.

4 SOFTWARE OPERATION

4.1 Introduction

This section of the manual contains operating instructions and a description of the experimental procedure. It is assumed that you have run the installation softawre as described in section I

4.2 Installation of the SFM-4/S version of the software

Run **BIO** from Root directory. You will get the following screen:

```
                                    ----------

 SFM-4/S  |  SFM-4/Q  |Exit|
 ---------|-----------|----

 Driving Software
 Kinetic Software
 ----------------
 Config Driving Software
 Config Kinetic Software
```

```
 Application Driver Software  V3.0

          Copyright  (c)  1991
    Bio-Logic Company  -  All right reserved

    Z.A. de FONT RATEL - 38640 CLAIX - FRANCE
     Ph. 76.98.68.31         Fax  76.98.69.09
```

```
                                    ----------
```

4.3 SFM-4/S driving software configuration

The SFM-4/S configuration program is allready set at the default values. It can be used to install or change the syringe model, set different flow-rate limitations, enable or disable the hard-stop mode, and select serial port number.
After validating "Config Driving software", the following screen will appear:

Syringe and RS232 installation V3.02

Volume per step (µl)	Flow Rate (ml/s) Lower limit	Higher limit	Syringe Vol. (µl)
4.540	0.045	10.000	20500
4.540	0.045	10.000	20500
4.540	0.045	10.000	20500
4.540	0.045	10.000	20500

Serial port (COM1)	1
Hard stop lead	4 ms
Hard Stop status	Enabled

Type <CTRL P> to change serial port number
Type <CTRL S> to change hard stop lead
Type <ALT S> to enable/disable Hard Stop
Type <ESC> when installation is done

4.3.1 Syringe installation

Unless otherwise specified, the SFM-4 stopped-flow module is equipped with standard 18 ml syringes (⌀17 mm) giving 4.54 µl per mechanical step. The flow rate is limited by the minimum duration of the drive impulses giving a maximum flow rate of 6 ml/s with the standard 18 ml syringes.

The 4ml syringes (⌀8 mm) give 1.005 µl per mechanical step.

4.3.2 Installation of the hard stop

Typing <**ALT-S**> will enable or disable the hard-stop mode in (SF). <**CTRL-S**> will select the hard-stop lead (from 1 to 5 ms).

4.3.3 Installation of the serial port

The program detects the number of available serial ports.
Type <**CTRL-P**> to select which serial port you would like to use.

4.4 SFM-4/ driving software

By validating "Driving Software", the SFM-4 menu will soon appear on the screen:

```
BIO-LOGIC                          SFM-4/S  V3.20                    Help : <F1>
 NONAME.SF4
                                Stopped-Flow Parameters                Syringe Vol.
 Phase          1   2   3   4   5   6   7   8   9
 Time           0   0   0   0   0   0   0   0   0   ms
 S1:  .....     0   0   0   0   0   0   0   0   0   µl              S1:    0 µl
 S2:  .....     0   0   0   0   0   0   0   0   0   µl              S2:    0 µl
 S3:  .....     0   0   0   0   0   0   0   0   0   µl              S3:    0 µl
 S4:  .....     0   0   0   0   0   0   0   0   0   µl              S4:    0 µl
 Sync.  1       0   0   0   0   0   0   0   0   0
 Sync.  2       0   0   0   0   0   0   0   0   0
 ------------------------------------------------------------------------
   Files        Clear data        Auto        Manual        Exit
Load, Save.
```

(Note : the second Sync. line will be absent when the hard-stop mode is enabled)

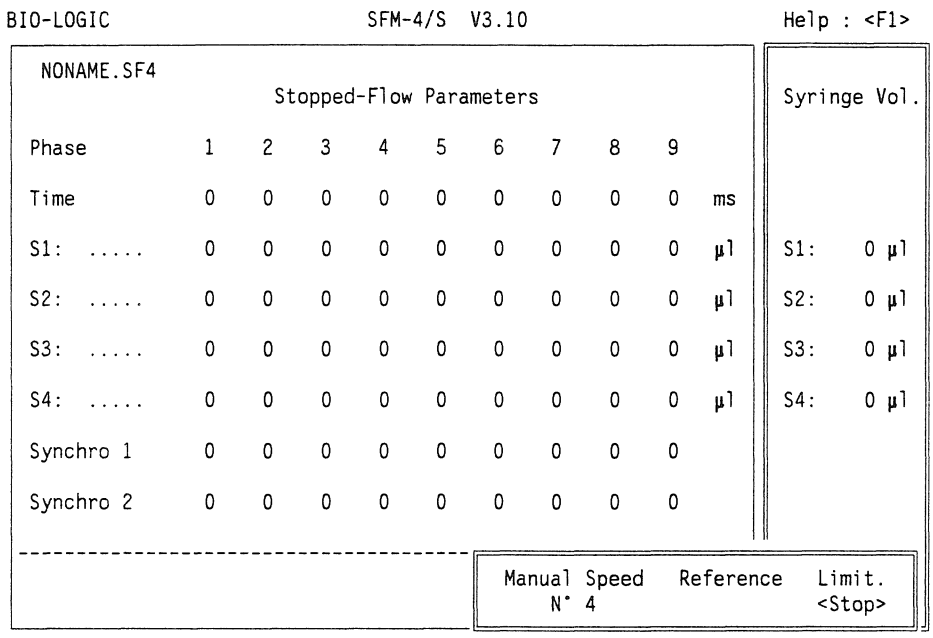
An action can be performed by placing the cursor of the desired function and validating with the <**Enter**> key. Displacement of the cursor is performed by using the **space bar**. Alternatively, execution of a function can be obtained by typing the first letter of the function. The "**Syringe Volume**" box on the right side of the screen indicates what volume is being displayed in the syringe counters. If no number is shown, this indicates that the communication with the MPS-51 power supply is not established. Turn the power supply on, check the connection, or eventually reset the power supply by pressing the reset button on its front panel.

Type <esc> or Exit to exit from the SFM-4 program:

NOTE : In the absence of connection with the computer (or with the PC turned-off), the MPS-51 unit is automatically set in the manual mode. It is therefore possible to drive the syringe and wash the instrument without being connected.

4.5 Manual control of the syringes

From the main menu control line type <**M**> or type <**Enter**> with the cursor on the [**Manual**] function. This results in display of the [**Manual**] menu:



From low speed N°1 to high speed N°5

At this point it is possible to:

- Control the manual speed of the motors
- Drive the motors from the keyboard
- Initialize the syringes counters

The motors can be controlled by the front panel of the MPS-51 except when the automatic mode is turned on. They can also be controlled by the computer keyboard when the [**Manual**] menu is turned-on. To select the motor, use the vertical arrow. Use <**PageUp**> or <**PageDown**> to raise or lower the piston of the syringe respectively. The speed of the manual movement can be selected by: typing <**S**>, by selecting the [Manual Speed] function with the Space-Bar and typing <**Enter**>, or by using the horizontal arrows.

4.6 Initialization of the syringes

The microprocessor of the SFM-4 integrates the movements of the 4 syringes so that the actual residual volumes can be displayed at all times on the screen. When the instrument is turned on, the counters show a nonsense value and have to be initialized.

Select the syringe to be initialized with the vertical arrows. Use the keyboard or the manual switch of the MPS-51 power supply to raise the plunger of the syringe to be initialized UNTIL THE SYRINGE REACHES ITS UPPERMOST POSITION.
There is no real danger in raising the plunger to the very end. Once approaching the end of its course, the motor will oscillate and vibrate; as it becomes out of phase with the driving pulses, it will completely lose its torque. (Nevertheless, there is no reason to unnecessarily prolong this treatment either). Type <**R**> or select [**Reference**] to set to zero volume the counter of the corresponding syringe.

CAUTION: measurement of the residual syringe volume is made by counting the logic pulses from the controller. Thus, if for any reason, a syringe is blocked during a run, the value measured may become erroneous. This may occur in the case of incorrect positioning of the valves.

The [**Limit**] function: if this function is set to [**Stop**], the motor's plungers cannot be driven from the keyboard above their upper limit (less than zero volume). This function is disabled by setting it to [**Pass**]. Type <**L**> or validate the function [**Limit**] to enable or disable this limitation.

4.7 Filling the syringes

Utmost care should be exercised during this operation. Normal operation of the system requires that no bubbles are present in the injection syringe: should this occur, the buffer flow through the observation chamber will not be correctly controlled by the plunger movement.

The four syringes of the SFM-4 stopped-flow module can be driven and refilled independently in the manual mode. Filling may be performed with disposable plastic syringes inserted into the four cylindrical receptacles. The thermostat jacket allows equilibration of the buffer before the filling sequence. Filling is performed with the valves pointing to the (**R**) labels.

While refilling, exert a slight manual pressure on the plunger of the reservoir syringe, this will prevent a negative pressure in the reservoir during pumping, which could result in bubble formation.

Bubbles in the drive syringes may be eliminated simply by driving up and down several times the drive syringe when they are connected to the reservoir. One or two shots in the observation cuvette will then be sufficient to definitively eliminate any bubbles remaining in the valves and the mixer. In all cases it is strongly recommended that buffers be degazed and filtered.

IMPORTANT ! Precautions when only two syringes are used (in particular in SF mode):

- *The mixing has to occur in the last mixer, so use of syringe 4 is mandatory. Use Syringes 1 and/or 2 and/or 3 with the syringe 4.*
- *The unused syringes and their flow lines should be filled with buffer as carefully as the other syringes. Turn the valve of the unused syringe to the (C) position (cuvette) and make a few manual pushes in the upward direction to ensure that the flow line between the valve and the mixer is correctly filled with buffer. Turn the valve back to the (R) position.*

The Stopped-Flow-Module is now ready for operation.

4.8 Creating a drive sequence

This is performed from the main menu. The drive sequence has been divided into 9 phases. In each phase the operator has to enter the duration of the phase in milliseconds and the volume in microliters delivered by the four drive syringes in each of the phases. Selection is made by displacing the cursor with the arrow keys.

In the left of the screen, a field is reserved to enter four letters identifying the contents of the four syringes. Enter in this field by depressing the **<Tab>** key. Return to the data field in the same way.
If SFM-4 is used with Bio-Kine software, all the informations entered will be saved with acquired data.

With ordinary aqueous solutions the motor can drive the syringes up to at least 6 ml/s without acceleration phase. This means that motors will not stall if the following sequence is programmed:

BIO-LOGIC SFM-4/S V3.10 Help : <F1>

TEST

Stopped-Flow Parameters

Phase	1	2	3	4	5	6	7	8	9	
Time	100	50	0	0	0	0	0	0	0	ms
S1:	0	300	0	0	0	0	0	0	0	µl
S2:	0	0	0	0	0	0	0	0	0	µl
S3:	0	0	0	0	0	0	0	0	0	µl
S4:	0	300	0	0	0	0	0	0	0	µl
Sync. 1	1	0	0	0	0	0	0	0	0	

Phase # 2
Flow (ml/s)
Tot.:12.000
S1 : 6.000
S2 : 0.000
S3 : 0.000
S4 : 6.000
Tot. Vol (µl)
600

Files Clear data Auto Manual Exit

Transfers parameters and sets MPS-51 in auto mode.

Each time parameters are entered in the table a window opens on the right side of the screen and indicates the flow rate per syringe at the time and the total flow rate in the cuvette at the time of the current phase, and the total volume injected during this phase.
To eliminate this window type "Tab"

4.9 Acceleration phases

It is possible that your instrument will be able to push solutions at a faster rate than 6 ml/s, but this is not guaranteed in all cases and eventually one of the motors can get stalled, giving a completely flawed reaction mixture. To program flow rates up to 8 ml/s before the actual fast flow rate, you must enter an acceleration phase. An example is given below :

BIO-LOGIC SFM-4/S V3.10 Help : <F1>

TEST

Stopped-Flow Parameters

Phase	1	2	3	4	5	6	7	8	9		Syringe Vol.
Time	100	10	50	0	0	0	0	0	0	ms	
S1:	0	50	400	0	0	0	0	0	0	µl	S1: 2995 µl
S2:	0	0	0	0	0	0	0	0	0	µl	S2: 3200 µl
S3:	0	0	0	0	0	0	0	0	0	µl	S3: 1795 µl
S4:	0	50	400	0	0	0	0	0	0	µl	S4: 3200 µl
Sync. 1	1	0	0	0	0	0	0	0	0		

Files Clear data Auto Manual Exit

Transfers parameters and sets MPS-51 in auto mode.

We recommend that you test the sequence on inexpensive buffer solutions of same viscosity as that which will be used in the real experiment.

4.10 Programmable synchronizing pulses

The MPS-51 power supply can be programmed to deliver a synchronizing pulse (trigger). This pulse is delivered from connector #4 (see section I of this manual) at any stage of the drive sequence. The default value is "**0**" for all the phases, which means that 0 Volt is always present on the "synchro out" BNC connector. Use the horizontal arrows to place the cursor on the phase to be used to trigger the recording device, type any number to validate; the number "**1**" will appear at this location (Type <**0**> to reset).
More than one phase can be active for triggering, during these phases the MPS-51 controller will deliver a 5-Volt signal. If Bio-Kine software is being used, acquisition will start on the falling edge, i.e. at the end of the active phase. For example, with the sequence shown in section **4.12**, acquisition will start at the end of phase 1 (beginning with phase 2). In case the hard-stop is desabled, a second trigger pulse can be programmed for another application.

4.11 Saving or loading the experimental parameters

The created data can be saved on files. A series of experimental conditions can thus be prepared before the experiment, and subsequently recalled while the program is being run. At the command level, type <**F**> for Files. Save or load the parameters by following the instructions displayed on the screen. For help at any point during operation, type <**F1**>. The data on the screen can be erased by typing <**C**>.

4.12 Running the automatic mode

The sequence created in the microcomputer will be transferred to the MPS-51 unit to be executed in the automatic mode.
Verify that the manual valves corresponding to the active syringes are set in the right position (i.e. pointing to (**C**)).
Type <**A**> or select [**Auto**] to enter the automatic mode.

The screen will show the following messages:

BIO-LOGIC SFM-3 V3.10 Help : <F1>

TEST

Stopped-Flow Parameters

Phase		1	2	3	4	5	6	7	8	9	
Time		0	300	5	10	0	0	0	0	0	ms
S1:	AA	0	15	30	80	0	0	0	0	0	µl
S2:	EAU	0	0	0	0	0	0	0	0	0	µl
S3:	....	0	0	0	0	0	0	0	0	0	µl
S4:	DCIP	0	15	30	80	0	0	0	0	0	µl
Sync. 1		0	1	0	0	0	0	0	0	0	

Syringe Vol.

S1: 4001 µl
S2: 3200 µl
S3: 2801 µl
S4: 3200 µl

Start/Stop <Esc> Exit
22 shots to go

Start or Stop according to the status of unit

When ready, type <**S**> to run. The run can also be activated by depressing button #5 on the front panel of MPS-51 or from an external trigger signal. In case of necessity the sequence can be terminated by typing the <**S**> key again.

IMPORTANT !
The power supply has been designed to deliver high power to the motors over a short time (low duty cycle), it is turned-off shortly after completion of the run. Permanent activation of the power supply may result in components overheating. The red LED #2 on the front panel indicate when at least one of the motor power supplies is activated. Reset the program (button # 3) if this LED remains permanent, turn-off the power supply in case the trouble persists. Start the sequence again. This type of trouble should never occur under normal operation. This warning has, nevertheless, been included here to prevent failure under abnormal environmental conditions.

5 DATA ACQUISITION SOFTWARE

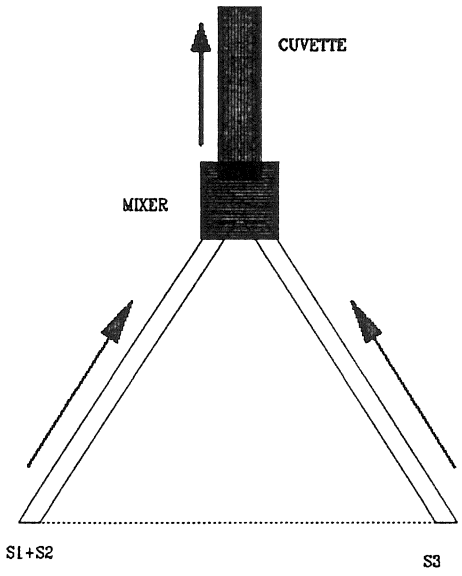
With the BioKine software, the same microcomputer may be used both to control the SFM-4 stopped-flow, and perform data acquisition/analysis. Once the parameters have been loaded in the memory of the MPS-51 unit, switch to the automatic mode by typing <**A**>, and return to the Bio-Kine software by typing <**E**> to exit.
The Bio-Kine menu will soon appear and will then be ready for data acquisition.
Direct access to the data acquisition software from the main menu will be obtained by validating "kinetic software" (see section 4.2)

Please refer to the BioKine Manual for Software Operation.

6 A SHORT STOPPED-FLOW PRIMER

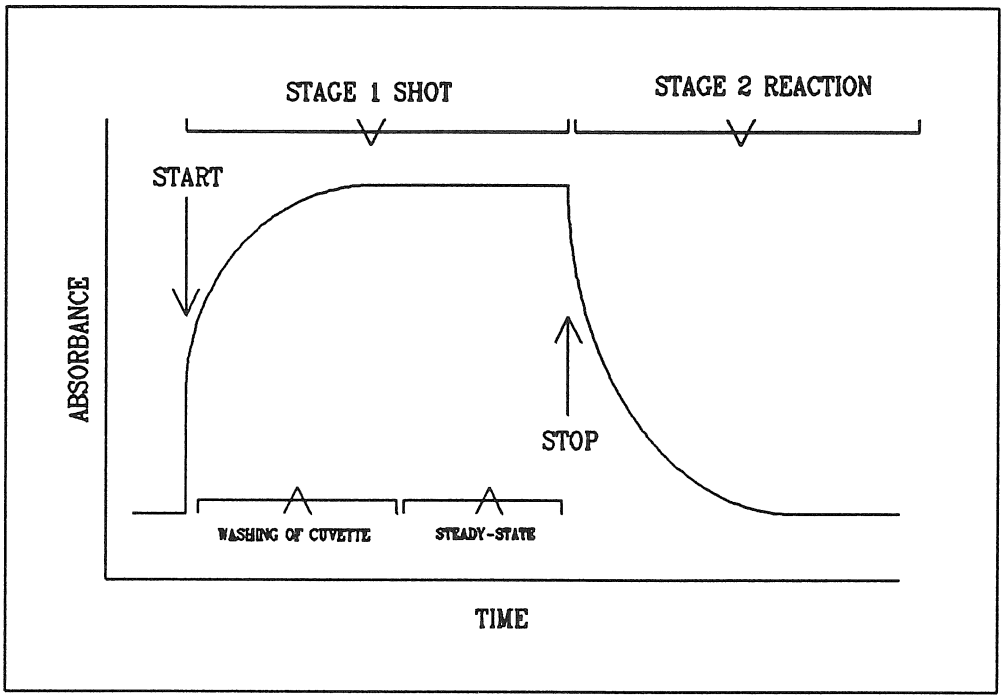
6.1 The general Principle of Experiments with the SFM-4 Stopped-Flow Module

There are many variations on the stopped-flow experiment, such as multiple mixes, continuous-flow, and accelerated flow. However, the simplest stopped-flow experiment occurs in two stages.
In the first stage, flow is initiated by two plungers forcing liquid through a mixer and along a flow path into an observation cuvette. The mixture ages as it travels along this path, the rate of ageing depending on the flow-rate of the mixture and the volume of the flow path. In this first stage, the mixer, flow path, and cuvette are initially washed by the constantly refreshed mixture, until a steady-state condition arises in which the age of the mixture tracks linearly with distance along the flow path; during the steady-state condition, at any particular point in the flow path, the mixture is of a particular age. Furthermore, the age of the mixture in the cuvette during the shot is the theoretical dead-time, which is the time before which observation of the mixture is impossible.



The second stage of the experiment begins when the flow is stopped. At this point, the mixture in the cuvette (and elsewhere) becomes stationary and continues to age. Observation of the mixture in the cuvette after the stop, therefore, shows a timecourse of the reaction from the dead-time onward.

The figure below shows an example reaction, in which reagent A, in the presence of B, reacts to form product C. A has a strong absorbance, while B and C do not. Therefore, as a reaction proceeds, the absorbance of a mixture of A and B should fall, as A is diminished.



6.2 Design and execution of stopped-flow experiments

Experiments are designed using the Bio-Kine/SFM-4 driver software on the computer. This is accomplished by filling-in elements of a (spreadsheet-like) table which describes the actions of the device. A sample experiment is shown here:

BIO-LOGIC SFM-4/s V3.10 Help : <F1>

TEST

Stopped-Flow Parameters

Phase	1	2	3	4	5	6	7	8	9	
Time	100	50	0	0	0	0	0	0	0	ms
S1:	0	0	0	0	0	0	0	0	0	µl
S2:	0	200	0	0	0	0	0	0	0	µl
S3:	0	0	0	0	0	0	0	0	0	µl
S4:	0	200	0	0	0	0	0	0	0	µl
Sync. 1	1	0	0	0	0	0	0	0	0	

Syringe Vol.

S1: 2995 µl
S2: 3201 µl
S3: 1795 µl
S4: 3200 µl

Files Clear data Auto Manual Exit

Transfers parameters and sets MPS-51 in auto mode.

The sample experiment shown utilizes two phases. The first phase is used solely to trigger data acquisition, so that the shot may be observed. The second phase defines the shot. Over fifty milliseconds, syringe 2 and syringe 4 each push 200 microliters through the mixer. Syringe 1 remains dormant in this experiment.

Once the experiment is designed, the MPS-51 is programmed with the experimental parameters. Experimental design is passed to the MPS-51, over the serial/RS-232 cable. Next, during the actual experiment, the MPS-51 controls the syringes, and the computer is used to collect data using Bio-Kine software and the the Data Translation Card inside the computer.

6.3 General advice

6.3.1 Achievement of fastest dead-times

The dead-time of a stopped-flow experiment is the time before which observation of the mixture is impossible. The dead-time depends on a number of factors, only some of which the experimenter can control. Ideally, the dead-time depends only on the rate of flow of the mixture from the mixer and the volume of the cuvette. As the flow rate increases, the dead-time falls. As the cuvette volume falls, so does the dead-time.

Nevertheless, an effective stopped-flow experiment depends on a number of other inter-related factors, such as adequate signal, complete washing of the

cuvette, prevention of cavitation, and prudent use of valuable reagents. The relationships between these factors requires careful consideration and experimentation; balanced trade-offs are often necessary to achieve successful stopped-flow experiments.

6.3.2 Washing

It is necessary to completely wash the cuvette during the shot, so that observation after the shot is only of the recently mixed samples. To accomplish this, sufficient volume of mixed samples needs to pass through the cuvette during the shot. This volume varies with flow rate and viscosity of the sample.

6.3.3 Cavitation

Cavitation occurs when turbulence creates regions of low enough pressure in a liquid that a "cavity" is formed (which fills with the liquid's vapour). These cavities collapse incompletely, leaving behind small bubbles of vapour which interfere with optical observation methods. As the flow rate increases through a mixer, so does the likelihood of cavitation. The probability of cavitation also increases with increasing viscosity at a given flow rate. De-gassing of solutions decreases the probability of cavitation by lowering the total vapour pressure available to fill the cavities.

6.3.4 Signal amplitude

The signal amplitude is generally proportional to the path length of the cuvette and the construction of (signal-generating) agent. A required increase in signal could be accomplished by an increase in path length, an increase in the concentration of agent. However, the experimenter is limited by practical concerns such as value of sample, viscosity of sample, dead-times, and inherent limitation of signal (such as inner-filter effect).

6.3.5 Flow rate

The flow rate is limited by the speed with which the stepping motors can push. At the design nominal flow rate of 6ml/s with all four syringes and using the smallest cuvette, sub-millisecond dead-times may be accomplished. However, solutions of increased viscosity will lower the obtainable syringe speed. Also, at colder thermostated temperatures, the speed often falls. The limitation to syringe speed may sometimes be defeated by the use of ramping, which allows each syringe to come to some intermediate speed for a short time before jumping to the higher speed.

The dead time may be decreased by decrease of cuvette volume, which may have adverse impact on signal amplitude. The dead-time may also be decreased by increased flow rate. Increased flow rate beyond the use of two syringes at their natural limit. 6ml/s may be achieved in two ways:

a) Use two or three syringes to simultaneously push the first reagent (through the first mixer and/or into the second mixer, with each syringe at its maximum flow rate. Syringe 4 also pushes at its maximum rate, generating mixing in mixer 3 at a total flow rate of 18 or 24 ml/s.

b) Use ramping to try to exceed the natural limit to the stepping motor velocities.

Again, careful trade-offs are required to balance each of these factors against each other in order to accomplish fully successful stopped-flow experiments:

In order to...	one should...	but at risk is...
lower dead-times	increase flow rate	stalled motors
		cavitation
		overuse of reagent
		inadequate washing
	decrease cuvette volume	loss of signal
increase signal	increase pathlength	overuse of reagent
		increased dead-time
		inadequate washing
	increase [reagent]	overuse of reagent
		increased viscosity causing cavitation
		increased viscosity leading to stalls
		increased viscosity leading to inadequate washing

7 TEST REACTIONS

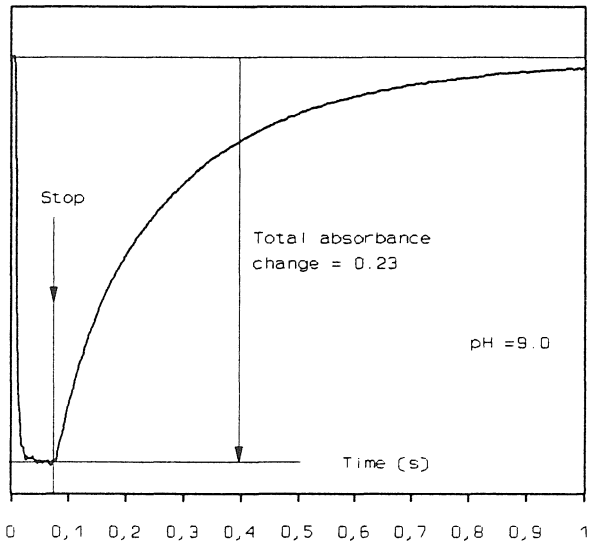
7.1 Evaluation of the dead time

The reaction used is the reduction of 2,6-Dichlorophenolindophenol (DCIP) by ascorbic acid (AA).

A complete description of this reaction and its use can be found in *Tonomura et al, Analytical Biochemistry (1978), 84, 370-383.* DCIP has a strong absorbance at 524 nm, and reduction by ascorbic acid results in a nearly complete decoloration. The second order reduction rate constant is highly dependent on pH, and varies from about $10^{4.6}$ $M^{-1}.s^{-1}$ at pH 2.0 to $10^{2.5}$ $M^{-1}.s^{-1}$ at pH 8.0. If the concentration of DCIP is sufficiently smaller than AA, the reaction can be treated as a pseudo first-order reaction whose rate constant will be directly proportional to the AA concentration.

All these properties make this reaction a very useful tool for stopped-flow calibration. We use the acid pH fast reaction to calibrate the dead time of the SFM-4 instrument, then we use the neutral pH slow reaction to check the quality of the stop, to evaluate the washing of the observation cell, and to test the variable ratio mixing capabilities.

Syringe 1, 2 or 3 : 10 mM Ascorbic Acid (AA)
Syringe 4 : 0.5 mM Dichlorophenolindophenol (DCIP)

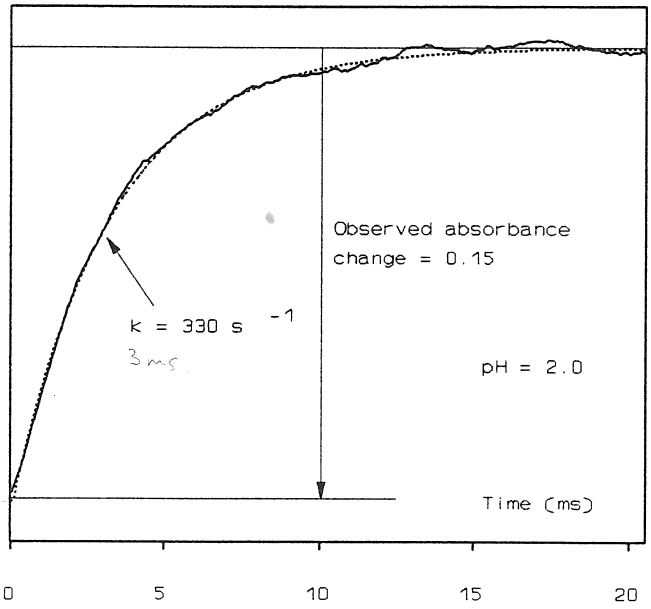


The decoloration of DCIP was followed by measuring the transmittance at 524 nm. We used the TC-50/10 transmittance cell. The reaction was followed at pH 9.0 and pH 2.0.

Reaction at pH 9.0 is shown in the figure above. Data acquisition was made using BioKine software, and acquisition was started at the beginning of the drive phase.

Collected data show a transition with a half time around 100 ms. Using the slow reaction conditions ensures that 100 % of the reaction is observed. At the same time, smooth kinetics enable an easy detection of any stop artifact.

The reaction at pH 2.0 is shown in the figure of this page: it gives a much faster transition with a half time close to 2 ms.



Measurement of the rate constant and amplitude of the transition at pH 2.0 permits an evaluation of the dead time of the instrument. Calculations are described below.

Results of the analysis using the BioKine software :

pH	Total flow rate (ml/s)	Obs. Absorbance	Rate constant (s-1)
9	8	A0 = 0.23	n.d.
2	16	A1 = 0.15	k1 = 330

Dead time was evaluated according to :

DT = 1/k1 * Ln (A0/A1) = 1.3 ms

Theoretical minimal dead time with this cuvette and this flow rate is 0.9 ms (see section **2.1** of the manual).

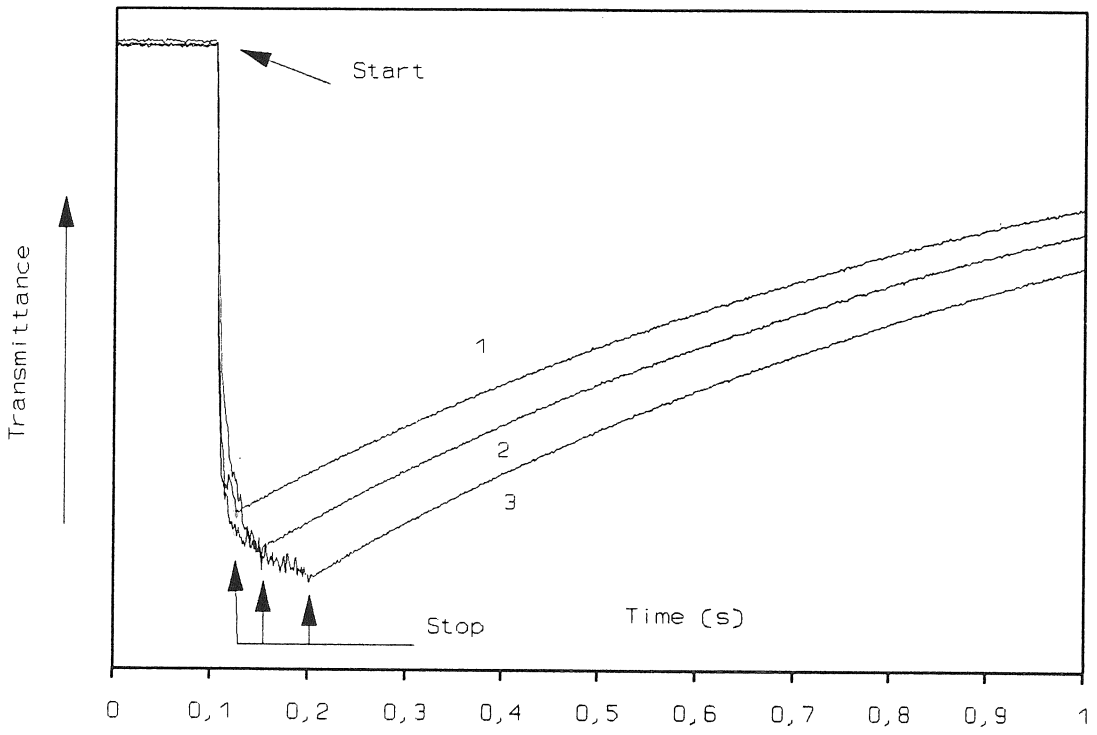
7.2 Evaluation of the cuvette washing and quality of stop

Reaction used is as described in section **7.1.**
Experiments described in the figure below show one method of evaluating of the quality of the cuvette washing. This experiment, again, enables one to check the absence of stop artifact. The data acquisition was started 100 ms before initiation of the drive sequence.

Only syringes 1 and 4 were used. Syringe 1 contained Ascorbic Acid at pH 9.0 and syringe 4 was filled with 100 μM DCIP; the mixing ratio was 1 to 1. The cuvette used has a 5 mm light-path and total volume of 15 μl.

Three experiments are shown using the following parameters :

	Duration of drive sequence (ms)	Volume pushed per syringe (ll)	Total flow rate (ml/s)
1	25	75	6.0
2	50	150	6.0
3	100	300	6.0



The results shown indicate, first, the absence of a stop artifact and, second, how washing is improved by increasing the volume of mixture passing through the cuvette.

7.3 Variable ratio mixing

The possibility to obtain variable mixing ratio by a simple programming of the instrument (i.e. without changing the syringes) is one of the major advantages of the Bio-Logic stopped-flow instruments. The microprocessor control of the stepping motors giving 6400 steps per revolution of the motor gives a smooth and quasi continuous movement of the syringe over a very large range of flow rate. A few exemplary experiments using the instrument are described below.

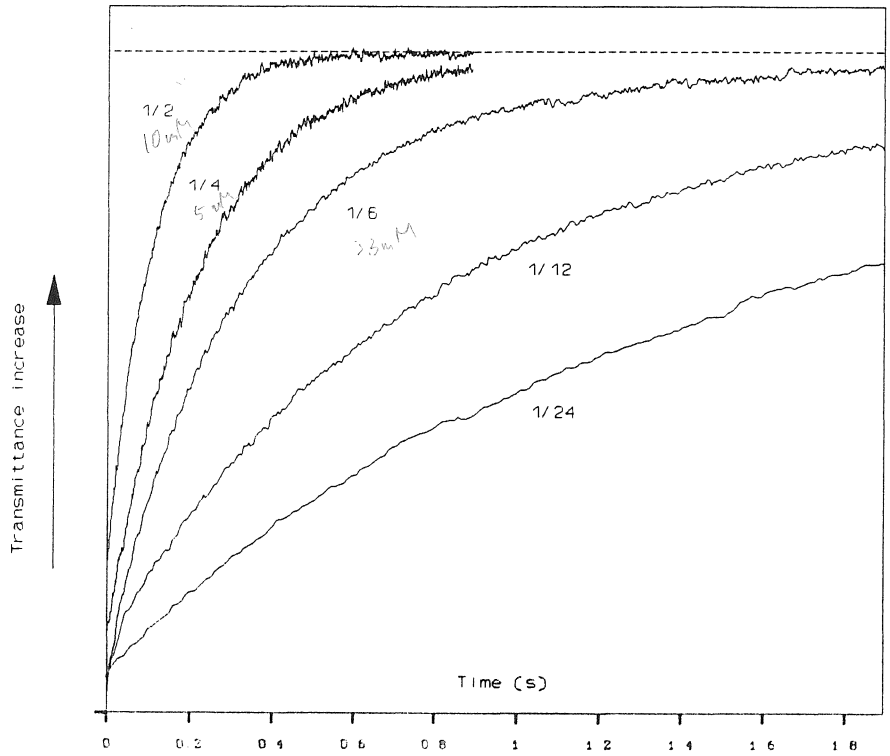
7.3.1 Experiments using DCIP and ascorbic acid

Contents of the syringes :

Syringe 1 : Ascorbic acid (20 mM)
Syringe 2 : Buffer
Syringe 4 : Dichlorophenolindophenol (100 μM)

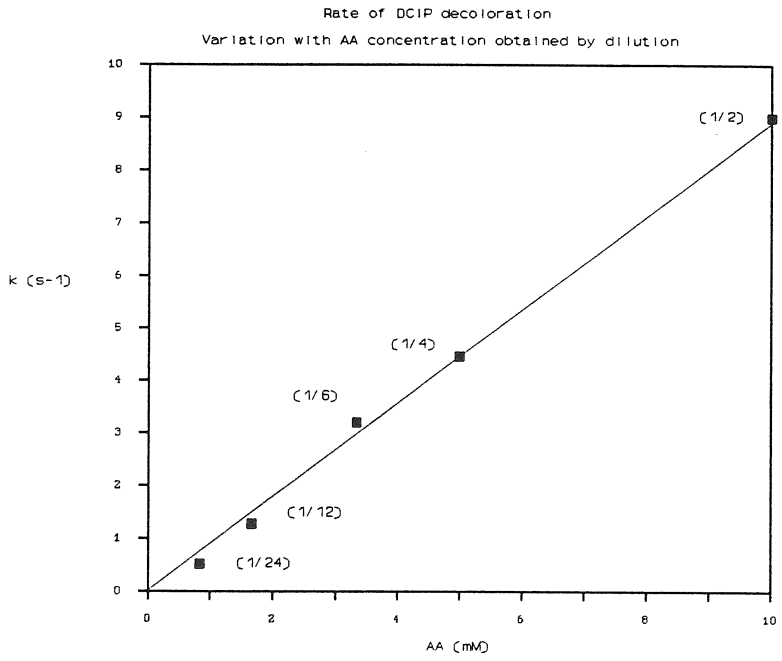
During the drive sequence the four syringes are programmed to deliver equal volumes to V1, V2 and V4 respectively.
The SFM-4 stopped-flow is programmed so that the volume of DCIP, V4, is constant, as is (V1 + V2). (V1+V2)/V4 is then constant and equal to 1/1, giving a constant final DCIP concentration 1/2 that of the DCIP in syringe 4.

In a series of experiments V1 and V2 are varied, giving a changing final concentration of ascorbic acid equal to V1/(V1+V2+V4). By this method, a



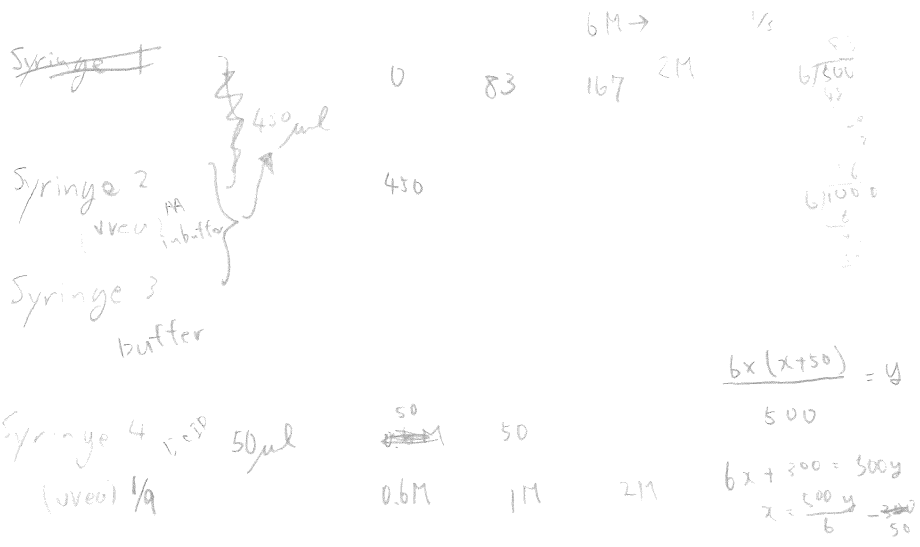
high dilution ratio of one component (here AA) can be obtained without changing the concentration of the other (DCIP).
Resulting data are shown in the figure above, where dilutions up to 1/24 were used.

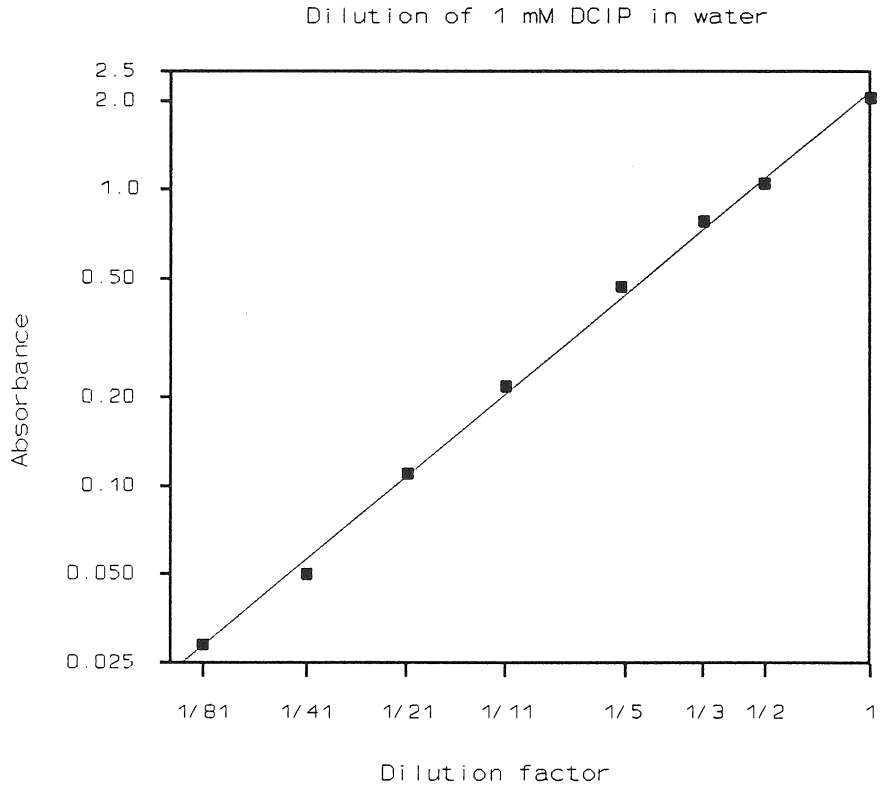
The rate constants measured using the BioKine software show a satisfactory linear relationship as a function of AA concentration. Numbers in parentheses represent the degree of dilution of AA.
Final concentration of DCIP was 50 μM.



Dilution factors of 1/50 or higher can be obtained. This is demonstrated in the next experiment where we mixed 1mM DCIP in syringe 4 with buffer in syringe 1. There is no reaction to follow in this case, the only goal of this experiment is to test final absorbance after mixing.

The results shown in the figure below indicate a satisfactory linear relationship between the absorbance measured after mixing in the SFM-4 stopped-flow and the final DCIP concentration which was calculated according to the ratio of the volumes delivered by syringes 1 and 4.





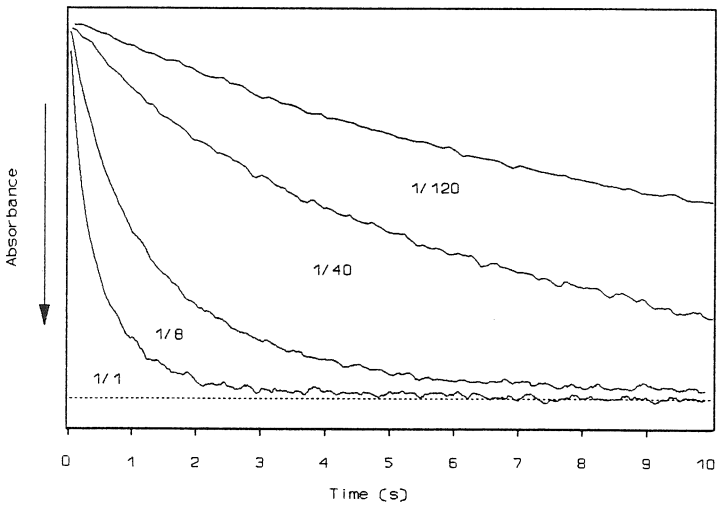
7.3.2 Measurement of variable ratio mixing by following alcohol dehydrogenase activity

Buffer used was : 100 mM Tris-Cl, 1 mM EDTA, 5 g/l of semicarbazyde-Cl and 25 mM ethanol.

Contents of the syringes :
S1 = Buffer
S2 = Buffer + 1 mg/ml alcohol dehydrogenase (ADH)
S4 = Buffer + 1 mM NAD

As described for the DCIP experiment above, each experiment is a mixture of volumes V1, V2 and V4 delivered at the same time by the three syringes.

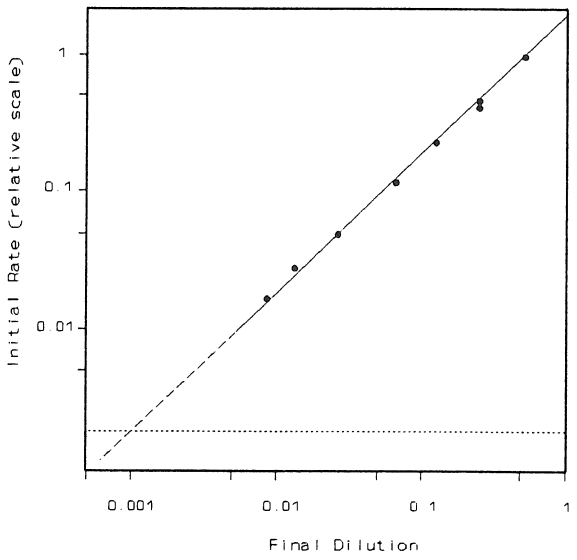
Ratio (V1 + V2)/V4 was kept constant and equal to 1. Varying V1 and V2 give a final dilution of ADH equal to V2/(V1 + V2 + V4).



The advantage of this technique is that the final concentration of NAD is constant whatever the final ADH concentration. Ratio of 1/2 to 1/120 were used.

Transmittance was measured at 340 nm with a 5 mm cuvette and the rate of formation of NADH was observed by following the decrease of the transmitted light. Here are shown some of the traces obtained.

The initial rate of the reaction was measured for each of the traces. These rates are plotted as function of the dilution factor on a log-log scale. This plot shows a reasonable alignment on a line of slope 1 indicating a linear relationship between the initial rate and the dilution factor.

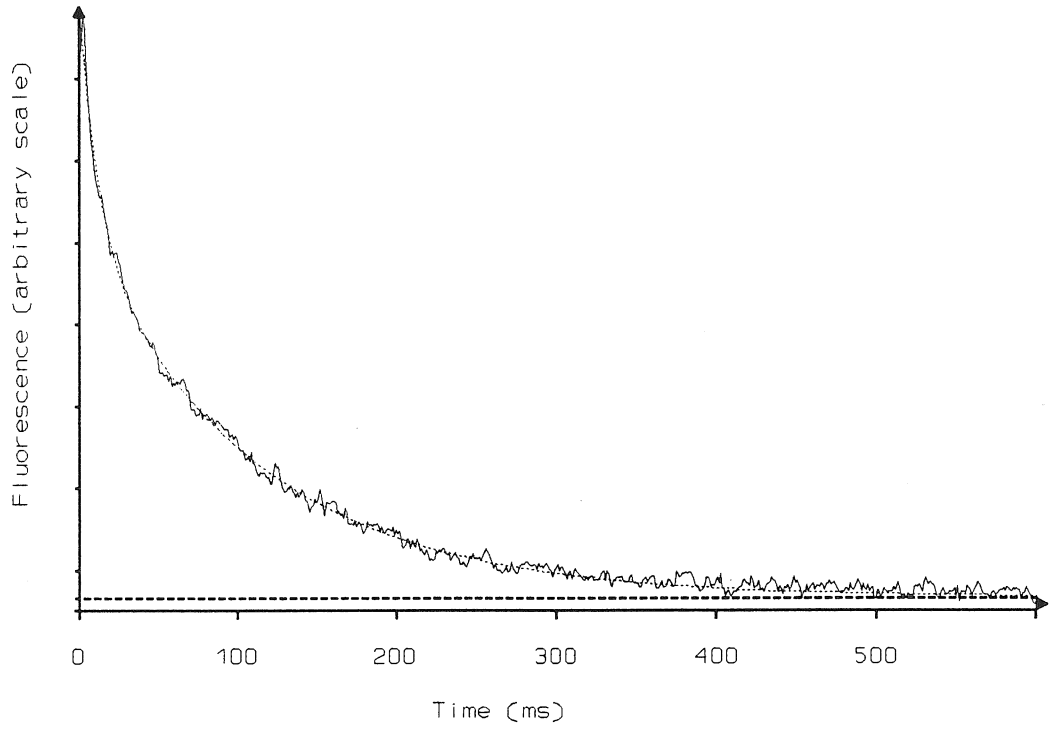


The horizontal dotted line shows the remaining ADH activity which was measured after two washings of the cuvette with V2 = 0 (no enzyme), following a shot with V2 = V4 (1/2 dilution of ADH). Further washing would have reduced this contaminating activity which corresponds to a concentration of 1/1000 of ADH. This, however, sets a limit for the reasonable dilution which can be obtained with the stopped-flow.

7.4 Mixing solutions of unequal density and viscosity

This situation is commonly found when the kinetic of enzyme renaturation is to be measured.

As a test reaction, we used cytochrome-c denatured in 5.5 M guanidine. This solution was mixed with buffer in large dilution ratio using the SFM-4 stopped-



flow, so that rapid decrease of guanidine concentration result in refolding of the enzyme. The kinetics of renaturation was followed by monitoring the cytochrome-c intrinsic fluorescence.

Conditions were :

Syringe 1 = 100 mM NaCl and 20 mM MOPS, pH 7.5
Syringe 2 = not used (filled with buffer)
Syringe 4 = 50 μM cytochrome-c in 5.5 M Guanidine-Cl and 20 mM MOPS, pH 7.5

Excitation wavelength was at 290 nm and emission was recorded at right angle through a low-pass cut-off filter with 50% transmission at 320 nm. Temperature was 25 °C.

Various mixing ratio were used, the result shown above have been obtained for a 1 to 10 dilution of the enzyme solution contained in syringe 4, giving a final enzyme concentration of 5 μM and a final guanidine concentration of 550 mM. At this concentration of salt, the enzyme shows rapid renaturation.

The experimental curve was fitted with two exponentials of rate constants 83 s^{-1} and 9 s^{-1} and amplitudes of 38 % and 62 % of the total transition respectively. The fit is shown as a dotted line under the experimental trace.

Convection artifact.
These artifacts are due to the slow rise of light buffer from the last mixer and subsequent entry into the observation chamber after mixing. This entry is detected by a sudden and reproducible change in absorbance or fluorescence at 10 to 100 seconds (or more) after the mixing. The existence of this artifact and the time at which it is observed are dependent on the relative densities and viscosities of the mixture and of the light buffer.
In the above example, a large dilution ratio was used so that the final mixture has a density not too different from that of the NaCl buffer. As a consequence no convection artifact was visible when data acquisition was prolonged for more than 100 seconds.
On the other hand, if a 1/1 mixing was used, the high concentration of guanidine in the cuvette (2.75 M) would have resulted in the formation of a large gradient of density at the last mixer. Under these conditions, if no precautions are taken, rapid rise of NaCl buffer in the observation cuvette can be observed about 20 s after mixing.
A method to completely eliminate the convection artifact has been proposed by Blond-Elguindi et al. (1988) in their work referenced at the end of this manual. These authors used heavy water in the light buffer for it to match the density of the mixture in the cuvette.
However, the best solution is to use the high density mixer model HDS developed by Bio-Logic. This mixer is described in section 3.2.

SECTION III

SFM-4 INSTRUCTION MANUAL

QUENCHED-FLOW MODE (SFM-4/Q)

1 GENERAL INSTALLATION

1.1 Introduction

This section of the manual contains information on the installation and operation of the SFM-4/Q. It is recommended that the content of this section be read and understood before any attempt is made to operate the instrument. In case of difficulties please contact Bio-Logic or its nearest representative.

1.2 Installation

The basic equipments are:

- A stable surface to ensure correct vertical positioning of the SFM-4/Q
- A PC or compatible microcomputer and one RS-232 serial interface

If radioactive reaction mixture is collected through the upper collect port with the aid of a disposable plastic syringe, make sure that the syringe is inserted tight to avoid projection of any material.

1.3 Water circulation

The SFM-4/Q module may be connected to a circulating water bath for temperature regulation.

1.4 AC power and connections

Before connecting the SFM-4 to the local AC line, verify that the setting of the instrument matches the local line voltage. The connections of the SFM-4/Q are described in the general section of this manual. Prepare the SFM-4 for operation by connecting the mechanical sub-system to the MPS-51 unit. Connect the MPS-51 to the serial port of the micro-computer.

2 INSTALLATION OF THE SFM-4/Q COMPONENTS

2.1 Introduction

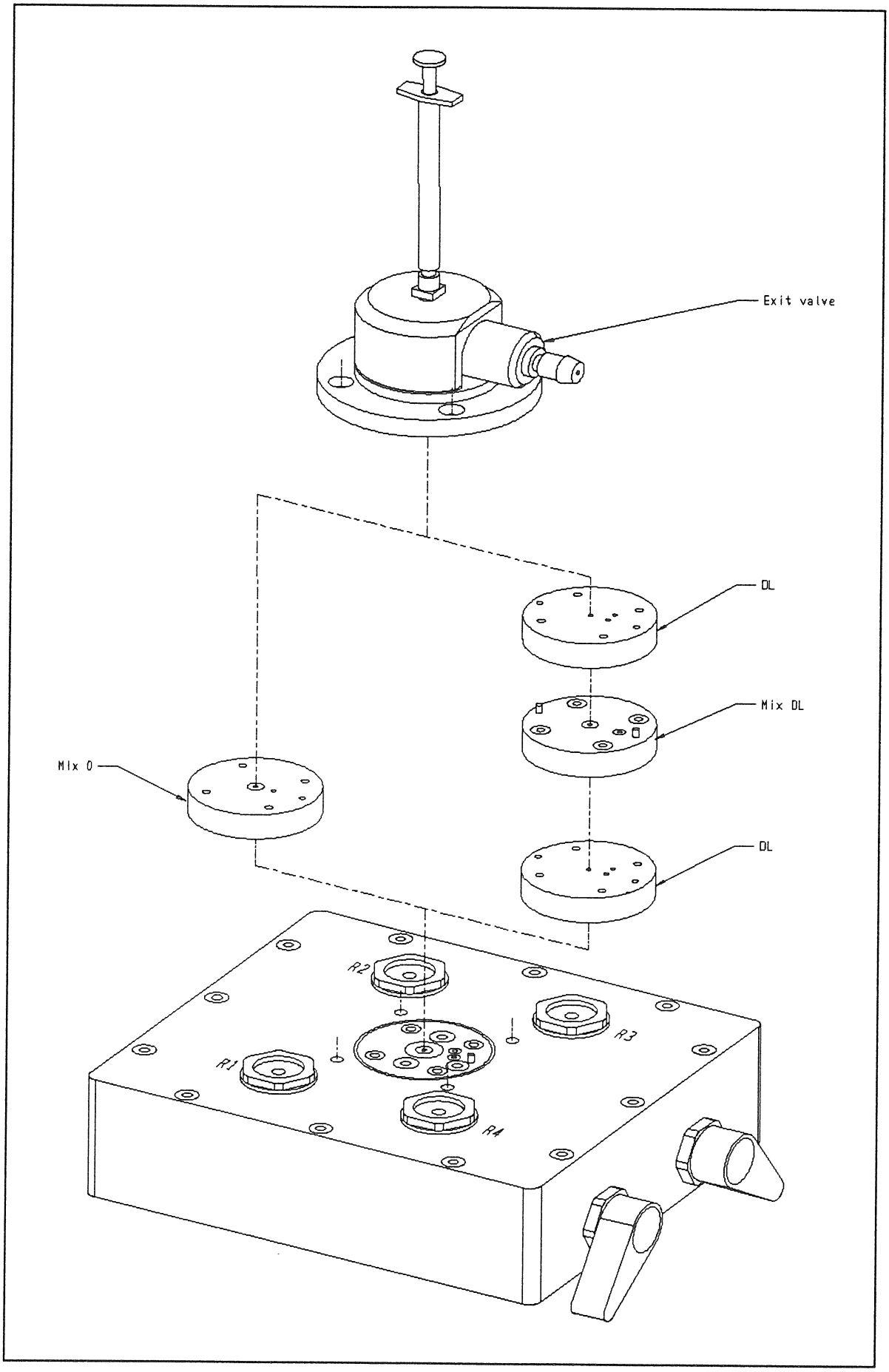
This section of the manual contains operating instructions and a description of the experimental procedure. Please read the previous section of this manual before attempting any operation of the apparatus. Before shipping, the unit has been thoroughly checked and all movable parts lubricated and so can be used immediately.

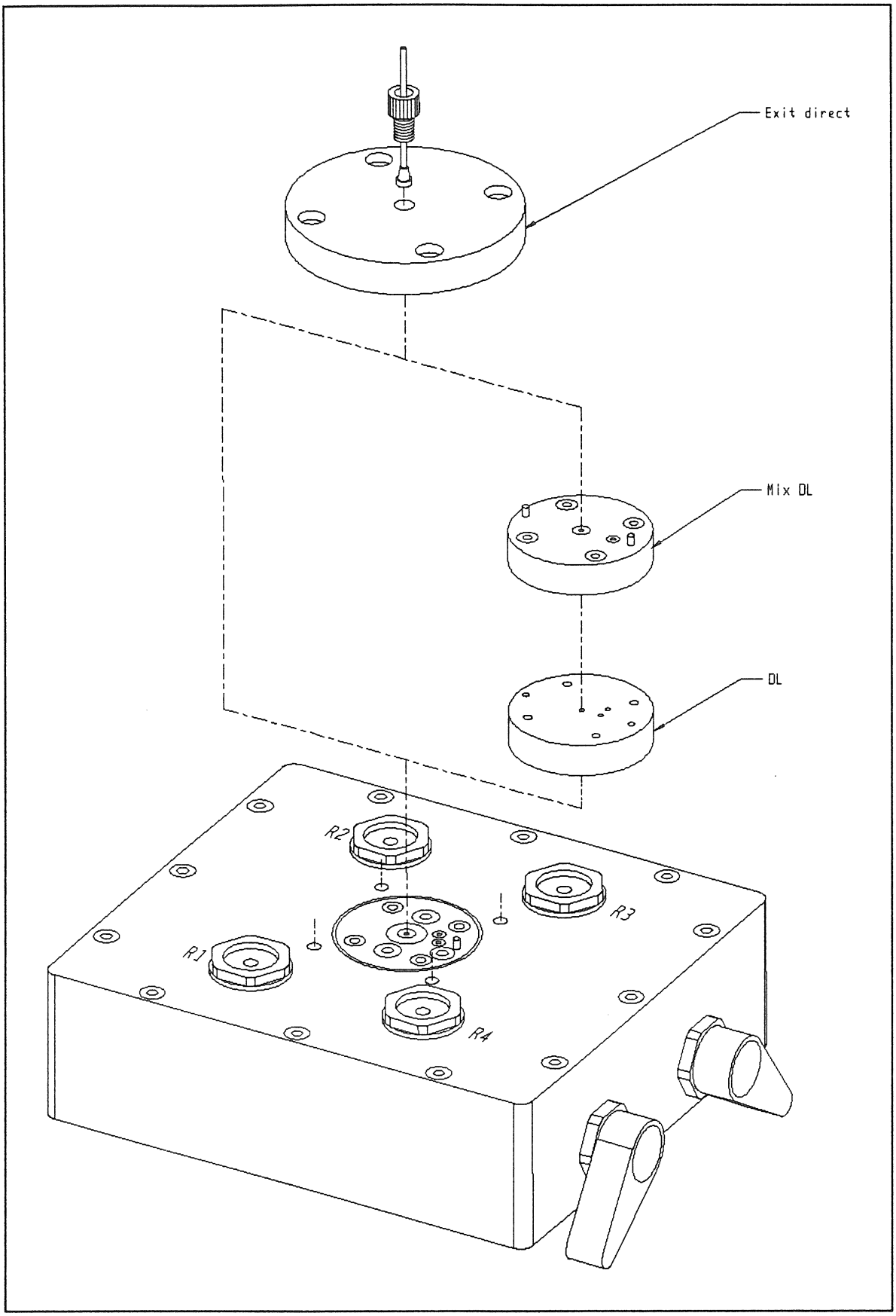
2.2 Installation of the mixer block and of the delay lines

In the quenched-flow mode, the four syringes of the SFM-4 can be used to perform several goals. It is difficult to list all the possibilities here. The most usual being described below :

It can be used as a four syringe quench-flow instrument with two delay lines, three mixers and a diverting valve for waste and collect
This assembly is illustrated in the figure of page 5. The diverting valve is mounted on the SFM-4 body through three spacers consisting of : one delay line, mixing block labeled **MIX DL** and a second delay line.
For very short dead times the delay lines can be omitted permitting millisecond ageing time to be reached. In this case the intermediate mixer block to use is the one labeled **MIX 0**. (See figure page 5)

Alternatively, an external flow line can be connected for direct injection of the mixture into a quenching solution. This mode may be used with or without an additional delay line as shown in the figure page 6.

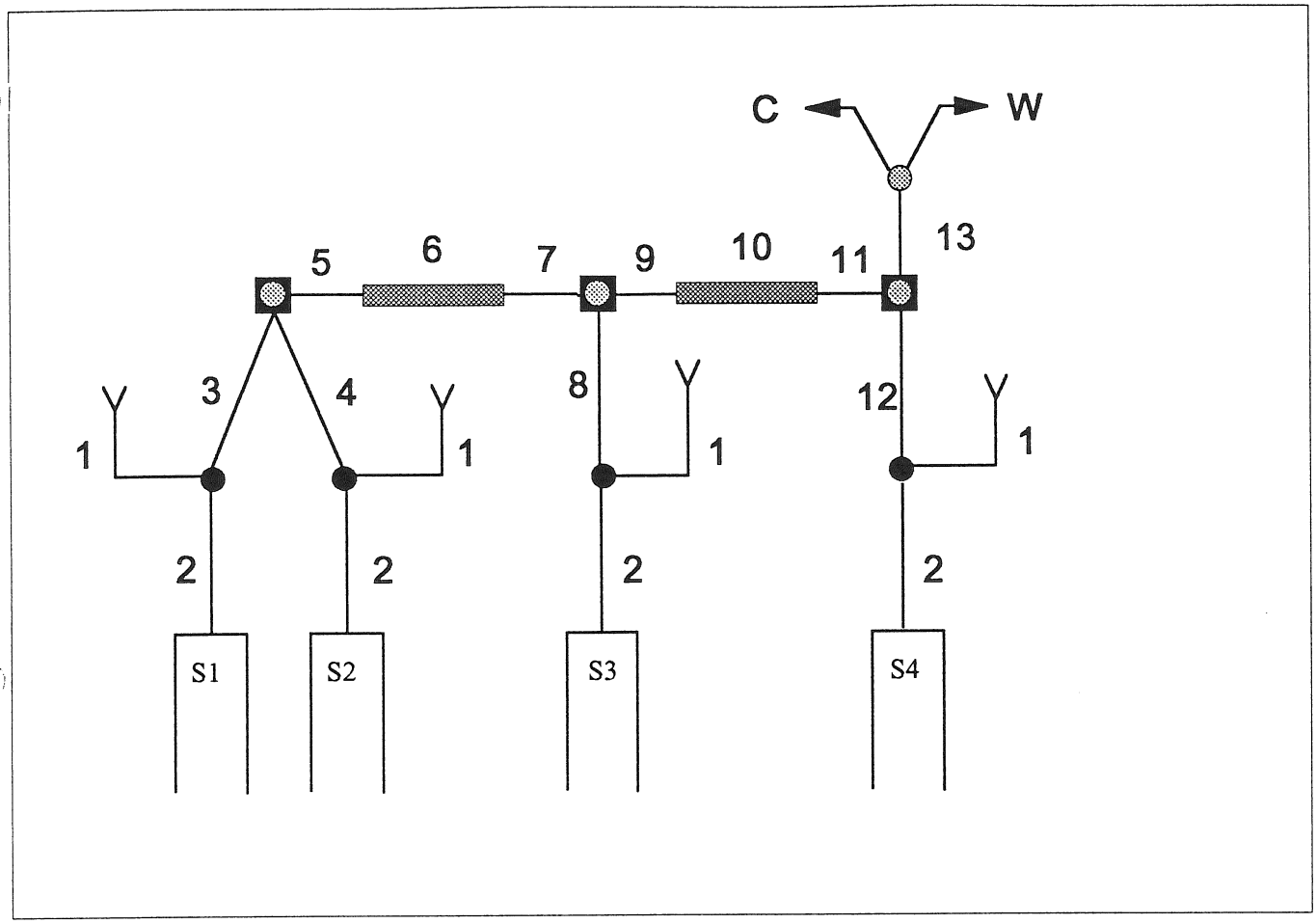

Exit valve
DL
Mix DL
Mix 0
DL
R2
R3
R1
R4

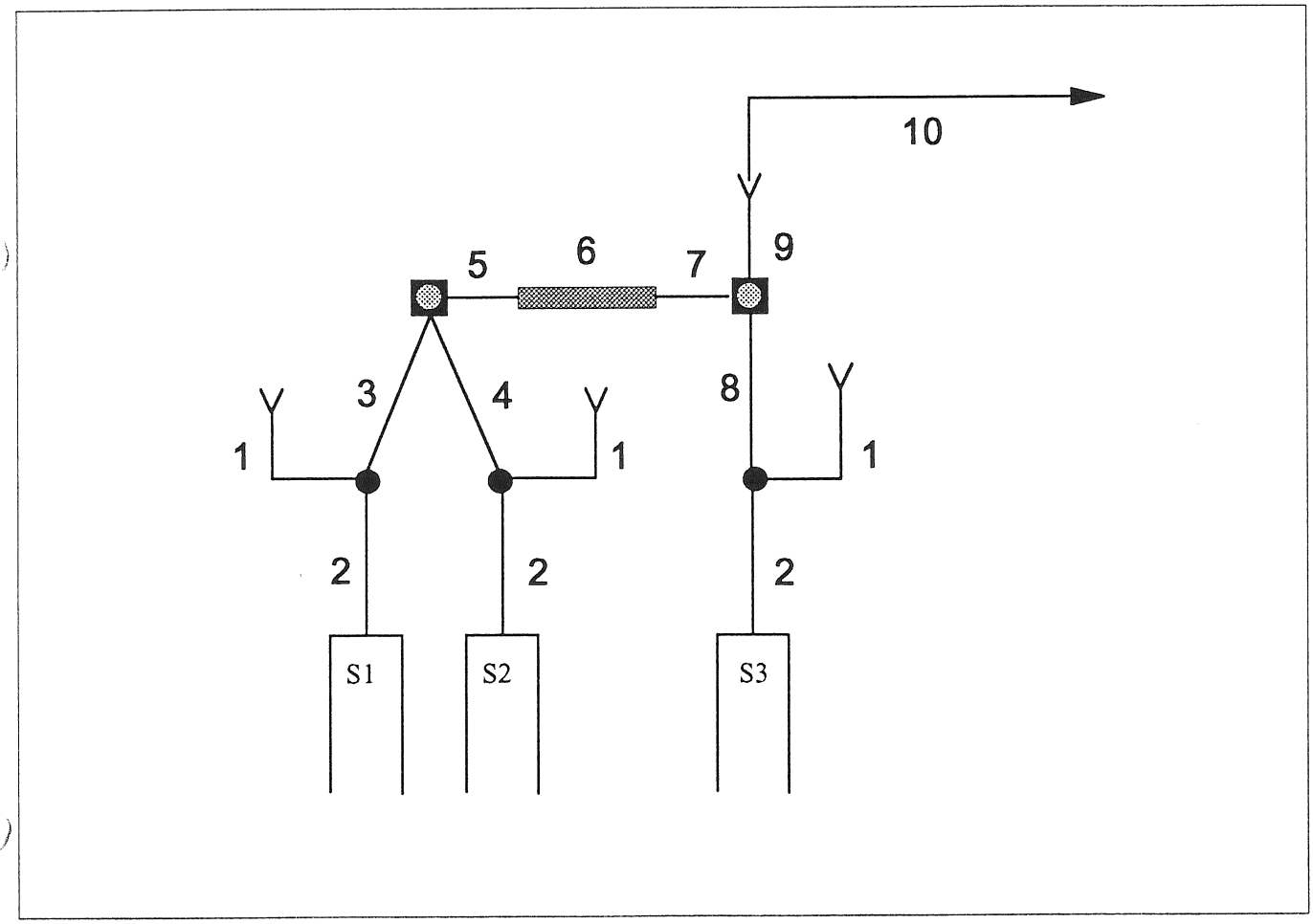

Exit direct
Mix DL
DL
R2
R3
R1
R4

2.3 Volume of the flow lines.

The numbers in the table below refer to the numbers on the figure of the next page :

	With 3 mixers and exit valve		With 2 mixers and direct exit	
	Without delay lines (MIX 0)	With delay lines (MIX DL)	Without delay line (MIX 0)	With delay line (MIX DL)
Line number	Flow lines volumes (µl)			
1	80	80	80	80
2	41	41	41	41
3	151	151	151	151
4	151	151	151	151
5	6	6	6	6
6		Delay line		Delay line
7	11	11	11	11
8	156	172	156	172
9	6	6	9	9
10		Delay line	External tube	External tube
11	16	16		
12	188	227		
13	45	45		


C
W
13
5
6
7
9
10
11
3
4
8
12
1
1
1
1
2
2
2
2
S1
S2
S3
S4


10
9
5
6
7
3
4
8
1
1
1
1
2
2
2
S1
S2
S3

2.4 Intermixer volumes

The ageing lines can be used two ways :
i) to store the mixture between two flow period. This is the interupt mode, it will be used for ageing time larger than 100 ms.
ii) in the continuous flow mode. In this case the ageing of the mixture is given by the time it takes to flow from one mixer to the other. The age will be given by the formula below :

Ageing time between two mixers = (Intermixer volume)/(flow rate)

The mechanical volume between two mixers is given below :

	Delay line used (volumes in µl)							
Flow line	No	17	40	90	140	190	500	1000
Mixer 1 to 2	17	34	57	107	157	207	517	1017
Mixer 2 to 3	22	39	62	112	162	212	522	1022
External tube	Two models provided : 0.76 mm dia. = 4.54 µl/cm 1.58 mm dia. = 19.61 µl/cm							

In the "No delay line" mode the "**MIX 0**" mixing block has to be used, in the "Delay line mode " the "**MIX DL**" mixing block has to be used.

The volumes indicated above are the mechanical volumes. The hydrodynamical volume may vay slightly around these values. It is recommended to calibrate these volumes with known reactions using procedures described in the chapter 4.3.
Also similarly to this mode two ageing mode may be used here : continuous flow or interrupt mode.

2.5 Liquid outlet system

Two mode of liquid ejection can be used : free-flow system of diverting valve system.

2.5.1 Free flow-system

In this mode the outlet is continously open and connected to a tube. The mixture is ejected into a test tube or a becher containing a quenching solution. This tube forms the last delay line and its length can be adjusted at will by the user. In case the volume collected is not very large as compared with the tube volume it is recomended to wash the old solution out of the tube during two shots.

2.5.2 Exit in a syringe

In this method a syringe is plugged at the SFM-4 exit.
This may be used in case the delay line volume is small as compared to the volume collected. When using this method it is recomended to wash the delay lines and the mixers with buffer between two shots (using one of the syringes).
During the actual run this buffer contained in the delay line will dilute the collected sample and will have to be taken into account during sample evaluation.
This mode of operation will be used mostly for short ageing times when the delay line volumes are small.

The shortest distance betwee mixers are in the range of 20 µl. The maximum flow rate of the SFM-4 instrument is 6 ml/s without acceleration and 8 ml/s with acceleration. Thus with two syringes flowing at the same time through a delay line, ageing times in the range of 1.5 ms may be reached. This time can come very close to 1 ms with three syringes flowing at the same time.

2.5.3 Diverting valve output.

This is the classical quenched-flow mode. It may be used when the delay line volume becomes too large as compared to the volume collected. This will be usually the case for long ageing times in continuous flow mode and in the interupt mode.
The diverting valve can operate correctly up to a certain pressure. Because of this its operation is not guarantied above 15 ml/s .

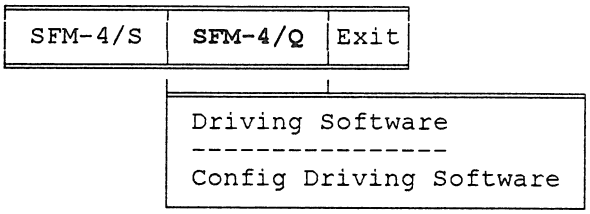
3 SOFTWARE OPERATION

3.1 Introduction

This section of the manual contains operating instructions and a description of the experimental procedure. It is assumed that you have run the installation softawre as described in section I

3.2 Installation of the SFM-4/Q version of the software (DOS version)

Run **BIO** from Root directory. You will get the following screen:
Use the arrow keys to select the menu option you wish to run:




```
        Application Driver Software  V3.0

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   Bio-Logic Company  -  All right reserved

  Z.A. de FONT RATEL - 38640 CLAIX - FRANCE
   Ph. 76.98.68.31         Fax  76.98.69.09
```

3.3 SFM-4/Q driving software configuration

The SFM-4/Q configuration program is allready set at the default values. It can be used to install or change the syringe model, set different flow-rate limitations, enable or disable the hard-stop mode, and select serial port number.
After validating "Config Driving software", the following screen will appear:

Syringe and RS232 installation V3.02

Volume per step (ul)	Flow Rate (ml/s) Lower limit	Higher limit	Syringe Vol. (ul)
4.540	0.045	10.000	20500
4.540	0.045	10.000	20500
4.540	0.045	10.000	20500
4.540	0.045	10.000	20500

Serial port (COM1)	1
Exit valve lead	3 ms

Type <CTRL P> to change serial port number
Type <CTRL S> to change hard stop lead
Type <ESC> when installation is done

3.3.1 Syringe installation

Unless otherwise specified, the SFM-4 stopped-flow module is equipped with standard 18 ml syringes (⌀17 mm) giving 4.54 µl per mechanical step. The flow rate is limited by the minimum duration of the drive impulses giving a maximum flow rate of 6 ml/s with the standard 18 ml syringes.
The 4ml syringes (⌀8 mm) give 1.005 µl per mechanical step.

3.3.2 Installation of the exit valve

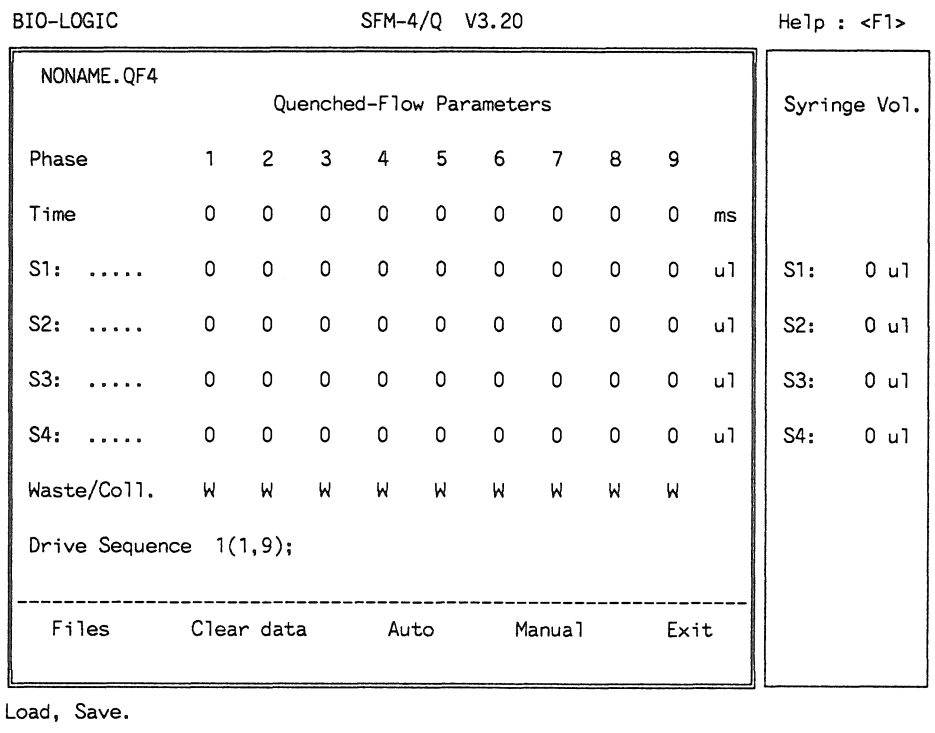
<CTRL-S> will select the exit valve lead (from 1 to 5 ms). This lead is used to compensate for the electromagnetic lag that may affect the valve. The default setting is 3 ms.

3.3.3 Installation of the serial port

The program detects the number of available serial ports.
Type **<CTRL-P>** to select which serial port you would like to use.

3.4 SFM-4/ driving software

By validating "Driving Software", the SFM-4/Q menu will soon appear on the screen:



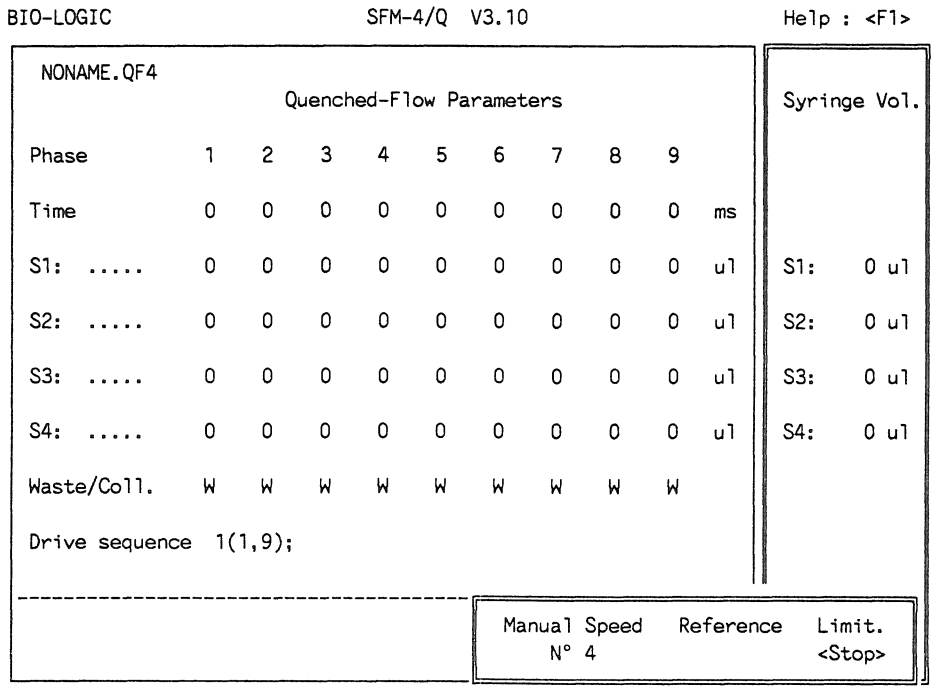
An action can be performed by placing the cursor of the desired function and validating with the <**Enter**> key. Displacement of the cursor is performed by using the **space bar**. Alternatively, execution of a function can be obtained by typing the first letter of the function. The "**Syringe Volume**" box on the right side of the screen indicates what volume is being displayed in the syringe counters. If no number is shown, this indicates that the communication with the MPS-51 power supply is not established. Turn the power supply on, check the connection, or eventually reset the power supply by pressing the reset button on its front panel.

Type <**esc**> or **Exit** to exit from the SFM-4 program:

***NOTE** : In the absence of connection with the computer (or with the PC turned-off), the MPS-51 unit is automatically set in the manual mode. It is therefore possible to drive the syringe and wash the instrument without being connected.*

3.5 Manual control of the syringes

From the main menu control line type <**M**> or type <**Enter**> with the cursor on the [**Manual**] function. This results in display of the [**Manual**] menu:



From low speed N°1 to high speed N°5

At this point it is possible to:
- Control the manual speed of the motors
- Drive the motors from the keyboard
- Initialize the syringes counters

The motors can be controlled by the front panel of the MPS-51 except when the automatic mode is turned on. They can also be controlled by the computer keyboard when the [**Manual**] menu is turned-on. To select the motor, use the vertical arrow. Use <**PageUp**> or <**PageDown**> to raise or lower the piston of the syringe respectively. The speed of the manual movement can be selected by: typing <**S**>, by selecting the [Manual Speed] function with the Space-Bar and typing <**Enter**>, or by using the horizontal arrows.

3.6 Initialization of the syringes

The microprocessor of the SFM-4 integrates the movements of the 4 syringes so that the actual residual volumes can be displayed at all times on the screen. When the instrument is turned on, the counters show a nonsense value and have to be

initialized.
Select the syringe to be initialized with the vertical arrows. Use the keyboard or the manual switch of the MPS-51 power supply to raise the plunger of the syringe to be initialized UNTIL THE SYRINGE REACHES ITS UPPERMOST POSITION. There is no real danger in raising the plunger to the very end. Once approaching the end of its course, the motor will oscillate and vibrate; as it becomes out of phase with the driving pulses, it will completely lose its torque. (Nevertheless, there is no reason to unnecessarily prolong this treatment either). Type <**R**> or select [**Reference**] to set to zero volume the counter of the corresponding syringe.

CAUTION: *measurement of the residual syringe volume is made by counting the logic pulses from the controller. Thus, if for any reason, a syringe is blocked during a run, the value measured may become erroneous. This may occur in the case of incorrect positioning of the valves.*

The [**Limit**] function: if this function is set to [**Stop**], the motor's plungers cannot be driven from the keyboard above their upper limit (less than zero volume). This function is disabled by setting it to [**Pass**]. Type <**L**> or validate the function [**Limit**] to enable or disable this limitation.

3.7 Filling the syringes

Utmost care should be exercised during this operation. Normal operation of the system requires that no bubbles are present in the injection syringe: should this occur, the buffer flow through the observation chamber will not be correctly controlled by the plunger movement.
The four syringes of the SFM-4 stopped-flow module can be driven and refilled independently in the manual mode. Filling may be performed with disposable plastic syringes inserted into the four cylindrical receptacles. The thermostat jacket allows equilibration of the buffer before the filling sequence. Filling is performed with the valves pointing to the (R) labels.

While refilling, exert a slight manual pressure on the plunger of the reservoir syringe, this will prevent a negative pressure in the reservoir during pumping, which could result in bubble formation.
Bubbles in the drive syringes may be eliminated simply by driving up and down several times the drive syringe when they are connected to the reservoir. One or two shots in the observation cuvette will then be sufficient to definitively eliminate any bubbles remaining in the valves and the mixer. In all cases it is strongly recommended that buffers be degazed and filtered.

IMPORTANT ! *Precautions have to be taken to fill all the flow lines of the usnused syringes (if any).*

The SFM-4/Q Module is now ready for operation.

3.8 Creating a drive sequence

This is performed from the main menu. The drive sequence has been divided into 9 phases. In each phase the operator has to enter the duration of the phase in milliseconds and the volume in microliters delivered by the four drive syringes in each of the phases. Selection is made by displacing the cursor with the arrow keys.
In the left of the screen, a field is reserved to enter four letters identifying the contents of the four syringes. Enter in this field by depressing the <Tab> key. Return to the data field in the same way.

With ordinary aqueous solutions the motor can drive the syringes up to at least 6 ml/s without acceleration phase. This means that motors will not stall if the following sequence is programmed:

```
BIO-LOGIC                    SFM-4/Q  V3.20                    Help : <F1>
╔═══════════════════════════════════════════════════════════╗ ┌──────────────┐
   TEST                                                      │              │
                        Quenched-Flow Parameters             │ Phase # 2    │
Phase          1    2    3    4    5    6    7    8    9     │ Flow (ml/s)  │
Time         100   50    0    0    0    0    0    0    0 ms  │ Tot.:10.000  │
S1: .....      0  300    0    0    0    0    0    0    0 ul  │ S1  : 6.000  │
S2: .....      0  300    0    0    0    0    0    0    0 ul  │ S2  : 0.000  │
S3: .....      0    0    0    0    0    0    0    0    0 ul  │ S3  : 0.000  │
S4: .....      0    0    0    0    0    0    0    0    0 ul  │ S4  : 6.000  │
Waste/Coll.    W    W    W    W    W    W    W    W    W     │ Tot. Vol (ul)│
                                                             │     600      │
Drive sequence 1(1,9);                                       └──────────────┘
-------------------------------------------------------------┌──────────────┐
  Files       Clear data       Auto       Manual       Exit  │              │
╚═══════════════════════════════════════════════════════════╝ └──────────────┘
Transfers parameters and sets MPS-51 in auto mode.
```

Each time parameters are entered in the table a window opens on the right side of the screen and indicates the flow rate per syringe at the time and the total flow rate in the cuvette at the time of the last phase, and the total volume injected during this phase.
To eliminate this window type "Tab"

3.9 Acceleration phases

It is possible that your instrument will be able to push solutions at a faster rate than 6 ml/s, but this is not guaranteed in all cases and eventually one of the motors can get stalled, giving a completely flawed reaction mixture. To program flow rates up to 8 ml/s before the actual fast flow rate, you must enter an acceleration phase. An example is

given below :

```
BIO-LOGIC                          SFM-4/Q  V3.20                    Help : <F1>

   TEST
                              Quenched-Flow Parameters                 Syringe Vol.

Phase          1   2   3   4   5   6   7   8   9
Time         100  10  50   0   0   0   0   0   0  ms
S1: .....      0  50 400   0   0   0   0   0   0  ul                 S1: 2995 ul
S2: .....      0   0   0   0   0   0   0   0   0  ul                 S2: 3200 ul
S3: .....      0   0   0   0   0   0   0   0   0  ul                 S3: 1795 ul
S4: .....      0  50 400   0   0   0   0   0   0  ul                 S4: 3200 ul
Waste/coll.    W   W   W   W   W   W   W   W   W
Drive sequence 1(1,9);

-------------------------------------------------------------------------------
  Files        Clear data        Auto        Manual        Exit

Transfers parameters and sets MPS-51 in auto mode.
```

We recommend that you test the sequence on inexpensive buffer solutions of same viscosity as that which will be used in the real experiment.

3.10 Programming the exit valve

The exit valve action is to divert the exit flow either to waste or to collect. The waste mode will be operated during all the washing phases that are preliminary to the collection of the usefull sample.
The programming is performed by entering a label on the 6th line of the control screen. If a "W" is entered the valve will not be activated during this phase and the flow during will be eliminated through the Waste port of the valve.
If a "C" is entered on a given phase, the valve will be activated and the liquid will flow out of the collection port of the valve.

3.11 Incubation sequence

An incubation phase between two flow phase is simply programmed by typing the time of incubation in the first line and typing zero in all of the syringe volumes.

3.12 Drive sequence

The programmed phases will be executed according to the drive sequence. The default

sequence is **1(1:9)**; which indicates that all phases 1 to 9 will be executed once in series (if one of the phases has a time value of 0 it will just be skipped in the sequence).
Other type of sequences can be created as in the following examples : A subsequence as : **2(2:5)** indicates that phases 2 to 5 will be executed two times as follows : 2, 3, 4, 5, 2, 3, 4, 5.
A sequence as : **2(2:5);6;8** indicates that phases 2 to 8 will be executed as follows : 2, 3, 4, 5, 2, 3, 4, 5, 6, 8

3.13 Saving or loading the experimental parameters

The created data can be saved on files. A series of experimental conditions can thus be prepared before the experiment, and subsequently recalled while the program is being run. At the command level, type <**F**> for Files. Save or load the parameters by following the instructions displayed on the screen. For help at any point during operation, type <**F1**>. The data on the screen can be erased by typing <**C**>.

3.14 Running the automatic mode

The sequence created in the microcomputer will be transferred to the MPS-51 unit to be executed in the automatic mode.

Verify that the manual valves corresponding to the active syringes are set in the right position (i.e. pointing to (**C**)).

Type <**A**> or select [**Auto**] to enter the automatic mode.

The screen will show the following messages:

BIO-LOGIC SFM-4/Q V3.10 Help : <F1>

TEST

Quenched-Flow Parameters

											Syringe Vol.
Phase	1	2	3	4	5	6	7	8	9		
Time	0	20	200	20	500	100	0	0	0	ms	
S1:	0	100	0	0	0	0	0	0	0	ul	S1: 4001 ul
S2:	0	100	0	100	0	0	0	0	0	ul	S2: 3200 ul
S3:	0	0	0	100	0	100	0	0	0	ul	S3: 2801 ul
S4:	0	0	0	0	0	400	0	0	0	ul	S4: 3200 ul
Collect.	W	W	W	W	W	C	W	W	W		
Sequence	1(1,9);										

Start/Stop <Esc> Exit
22 shots to go

Start or Stop according to the status of unit

When ready, type <S> to run. The run can also be activated by depressing button #5 on the front panel of MPS-51 or from an external trigger signal. In case of necessity the sequence can be terminated by typing the <S> key again.

IMPORTANT !
The power supply has been designed to deliver high power to the motors over a short time (low duty cycle), it is turned-off shortly after completion of the run. Permanent activation of the power supply may result in components overheating. The red LED #2 on the front panel indicate when at least one of the motor power supplies is activated. Reset the program (button # 3) if this LED remains permanent, turn-off the power supply in case the trouble persists. Start the sequence again. This type of trouble should never occur under normal operation. This warning has, nevertheless, been included here to prevent failure under abnormal environmental conditions.

Type <**F1**> to obtain more details about the syntax used to enter and correct the drive sequence.

Identification : On the left of the screen a field is reserved to enter four letters to identify the contents of the three syringes. Set the cursor in this field by depressing the <**Tab**> key. Return to the data field by the same action.

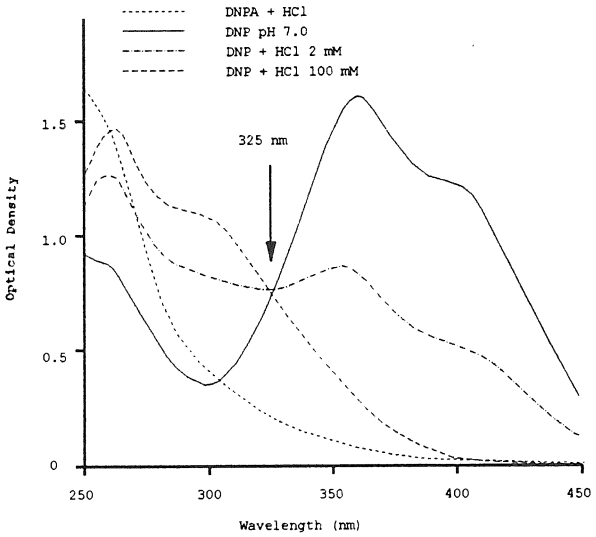
4 CALIBRATION OF THE INSTRUMENT

This part of the manual describes some test reactions which may be used to calibrate and test the performance of the SFM-4 quench-flow. Details about the manipulation of this instrument are given in the previous chapters of the user's manual; please refer to it for instructions.
There are a large number of references that may be used to help the user to understand more about the basics of rapid mixing and quench technique. We recommend three of them, as well as the many references that they include:

- Barman, T.E. and Gutfreund, H. (1964) in, Rapid Mixing and Sampling Techniques in Biochemistry. (Ed. B. Chance, R.H. Eisenhardt, Q.H. Gibson and K.K. Lonberg-Holm, Eds.). Academic Press, London, pp. 339-344.
- Gutfreund, H. (1969), Methods in Enzymology, 16, 229-249.
- Barman, T.E. and Travers, F., Methods of Biochemical Analysis (1985), Vol. 31, 1-59.

4.1 Description of the test reaction

The test reaction described here is the Alkaline hydrolysis of 2,4-dinitrophenyl acetate (DNPA; Gutfreund, 1969). Later we describe the study of sarcoplasmic reticulum Ca-ATPase phosphorylation/dephosphorylation to illustrate an experiment of real biochemical interest. (Courtesy of P. Champeil and S. Orlowsky, CEN Saclay, France).



Alkaline hydrolysis of 2,4-dinitrophenyl acetate (DNPA)

The absorbance spectrum of DNPA in HCl is shown in figure 4.1. After a short exposure to NaOH, 2,4-dinitrophenol (DNP) is produced. The spectrum of DNP under various pH conditions is also shown, revealing an isobestic point at 325 nm.

The test reaction will be as follows :
DNPA in acidic conditions ---> exposure to NaOH for time t, leading to formation of DNP ---> Quenching of the hydrolysis reaction with excess HCl.
The quantity of DNP formed during the exposure to alkali will be followed at 325 nm (In acid, the molar extinction at this wavelength is 5.6 10^3 $M^{-1}.cm^{-1}$).
At 20°C, the reaction DNPA + OH^- ------> DNP has a second order rate constant in water of 56 $M^{-1}.s^{-1}$. Conditions can easily be set to make the concentration of OH

sufficiently larger than that of DNPA to create a pseudo first-order hydrolysis reaction of apparent rate constant kapp = 56.[OH^-] s^{-1}.
These properties make this reaction a very useful tool for calibration of any quench flow instrument, and the reaction can simultaneously be checked in a conventional optical stopped flow.

4.2 How to get various ageing times

The SFM-4 instrument can be operated in the continuous or interrupted mode.
In the continuous mode, the delay between two mixers is adjusted by the delay line volume and the flow rate. The use of stepping motors enables setting a flow rate which is independent of the solution viscosity. This is not feasible with an instrument based on a pneumatic drive. The continuous mode is used to study reactions from one to one hundred (or so) milliseconds.
In the interrupted mode, the reaction mixture is held in the delay line for a programmed time and then mixed with quencher in the last mixer. This mode is used for incubation times of a few ten milliseconds to several seconds or more.

The critical decisions in the use of the quench-flow instrument are the choice of delay lines, flow rates, and operational mode described above. The calibration experiments which follow are intended to give some examples of these choices.

4.3 Measurement of flow lines volumes and of efficiency of washing.

Knowledge of the exact volume (V) between the two mixers is critical. In the continuous mode, the age of the reaction (T) is equal to:

$$T = V/F \quad \text{(F being the programmed flow rate).}$$

Because of the use of stepping motors, there is no need to calibrate the flow rate; it will be as specified by the user.
In the table 1.6.2 above, an estimation was given about the total volume (V) for each of the delay lines used. It is recommended these volumes to be checked and calibrated by the user.
Below is an example of the calibration of the smallest delay between mixers 2 and 3. This will be obtained by inserting no delay line between mixer 2 (Block labelled MIX 0) and mixer 3. This is supposed to give a total inter-mixer volume of 22 µl when inserted in position N°2.
No delay will be inserted between mixer 1 and mixer 2.

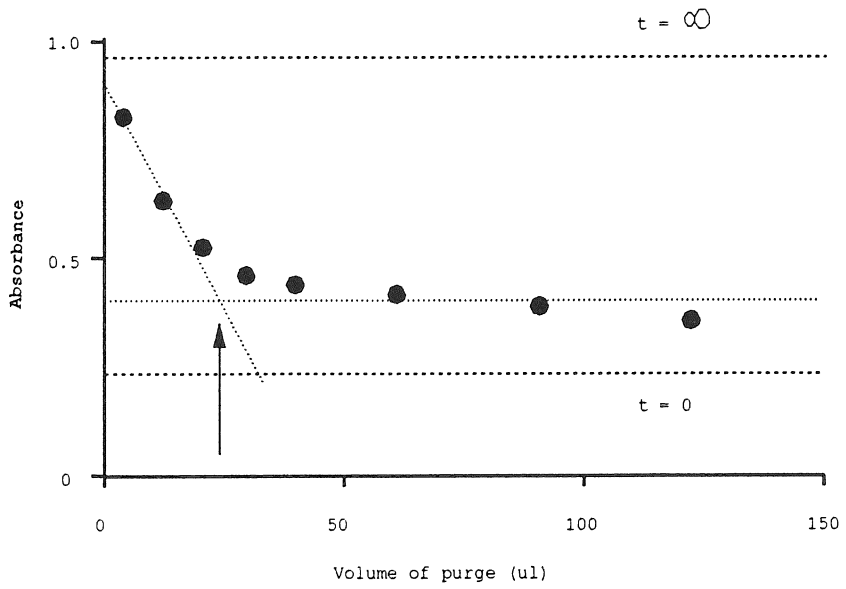
The reaction proceeds as follows: DNPA in syringe 2 is mixed in the second mixer with alkali from syringe 3, the solution flows through Dl_2, and the hydrolysis reaction is stopped in mixer 3 with excess acid flowing from syringe 4.
The grid below shows a sample SFM-4 program, any other choice of phase number

being valid.
Phase N°2 is used to purge the delay line, during which time all solution is evacuated into waste. After phase N°2, the mixture is collected in phase N°4 and the amount of DNP formed during the flow through Dl_2 assayed.

	PHASE N°	1	2	3	4	5	6	..
	TIME (ms)	0	t	0	30	0	0	0
SYRINGE NUMBER		VOLUMES (ul)						
Not used	1	0	0	0	0	0	0	0
DNPA 1.5 mM	2	0	V	0	60	0	0	0
NaOH 400 mM	3	0	V	0	60	0	0	0
HCl 500 mM	4	0	V	0	60	0	0	0
Valve		W	W	W	C	W	W	W

Delay line N°1 = 0 ul
Delay line N°2 = 0 ul

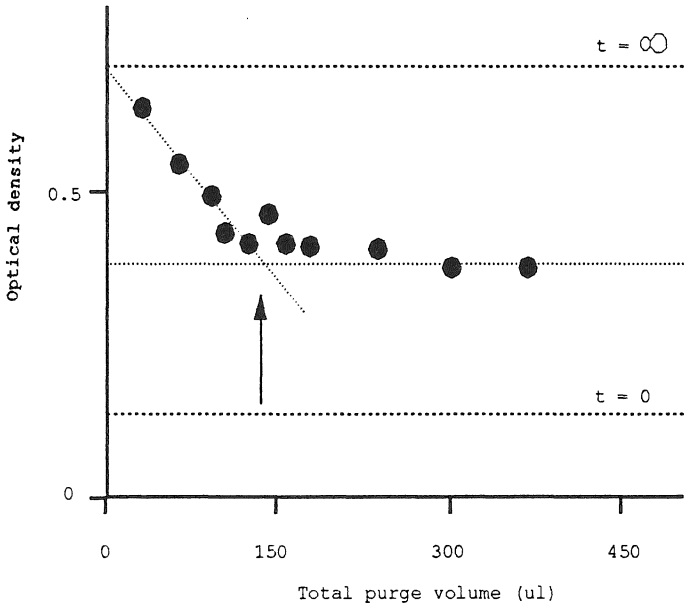
The purge volume is the volume flowing into the delay line before collection, and is equal to 2xV. Figure below shows the results as a function of purge volume. Absorbance for zero and infinite time of incubation respectively, were measured independently.



It is obvious that the reaction products collected from the delay line after using a purge volume of zero are completely contaminated by the old reaction mixture standing in the delay line before the experiment was started. The curve shows a clear break around 25 µl. It is at this point that Dl_2 has been washed once.
This first experiment confirms that the real volume between mixer 2 and 3 when no delay ligne is installed is around 24 µl as indicated in table of chapter 2.4.

The same curve shows that contamination is still present after the first washing and that the delay line needs to be washed with at least 100 µl to be reasonably free of contaminating reactant.

A similar experiment with a delay line of 100 µl is shown in figure 4.3.(b). It shows that the total dynamic volume of this delay line is close to 120 µl, a volume very close to the 122 µl indicated in table 1.6.2.



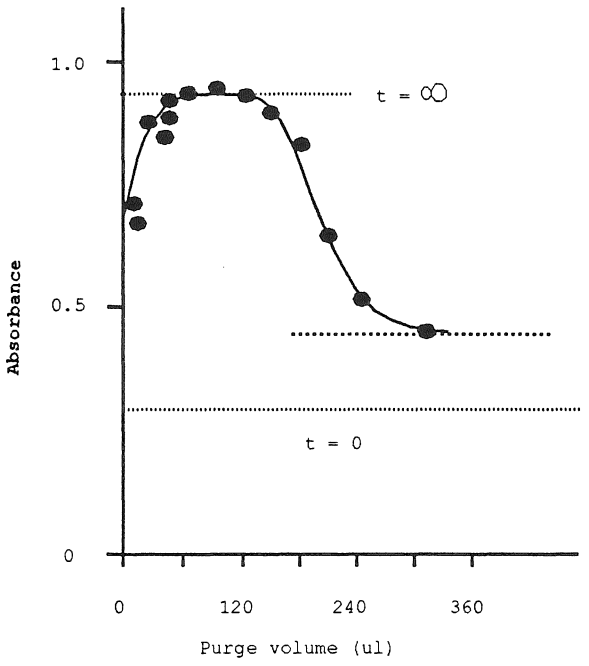
4.4 Recovery of the material contained in the delay line.

In the interrupted mode, the reaction mixture is transiently stored in the delay line. During this incubation period, unwanted mixing occurs at both ends of the delay line so that only a fraction of the mixture can be recovered from the delay line. The experiment described below is intended to give an estimate of that fraction.

	PHASE N°	1	2	3	4	5	6	..
	TIME (ms)	50	50	3000	0	Tp	20	0
SYRINGE NUMBER		VOLUMES (ul)						
Not used	1	0	0	0	0	0	0	0
DNPA 1.5 mM	2	150	0	0	0	V	30	0
NaOH 100 mM	3	150	0	0	0	V	30	0
HCl 240 mM	4	0	150	0	0	V	30	0
Collect		W	W	W	W	W	C	W

Delay line N°1 = 25 ul
Delay line N°2 = 200 ul

This experiment is designed to test delay line N°2. In the first phase, DNPA and NaOH are pushed through the delay line and wasted. The second phase is used to wash the last mixer with HCl. The reaction mixture is then allowed to age for several seconds in the delay line (phase 3).
Phase N°5 corresponds to the purge of the delay line, the solution being pushed and evacuated. Purge volume is again equal to 2xV. After this purge, 60 µl of the reaction mixture are collected and measured. Tp is the purge duration, selected to give a constant flow rate during the purge phase of 1.5 ml/s for each syringe.



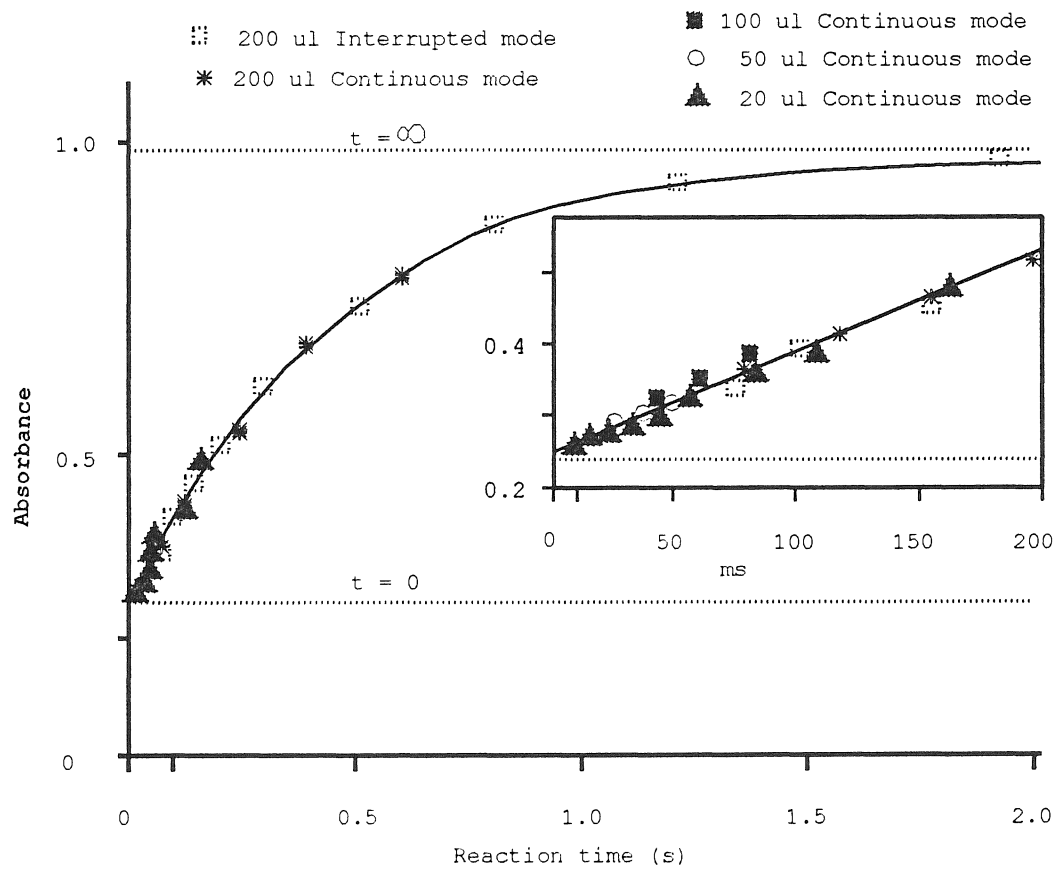
Results of this test are shown above as a function of purge volume. Due to the long ageing time in phase N°3, the solution collected in the last phase should correspond to the full reaction (t = ∞). Contamination on the leading edge of the liquid column contained in the delay line is observed when the volume of the purge is zero. Contamination on the trailing edge is observed for overly large purge volumes, when the fresh reactants pushing the liquid column are collected.
These results demonstrate that, for a delay line of 200 µl nominal volume (corresponding to a total approximate volume of 220 µl, the first 20 to 30 µl and the last 30 to 40 µl should be discarded to avoid contamination.

4.5 General checking of the instrument

Experiments shown below have been obtained under the following conditions:
Syringe 1 : (not used)
Syringe 2 : 1.5 mM DNPA in 2 mM HCl.
Syringe 3 : 100 mM NaOH
Syringe 4 : 240 mM HCl
Reaction times were obtained by varying the delay line volume, the flow rate, and the operation mode. The actual delay line volumes were obtained according to the procedure described above.

Delay line volume (ul)	Volume to use (ul)	Mode	Time frame (ms)	Flow rate per syringe (ml/s)
0	17	Continuous	1 to 20	8 to 0.4
17	34	Continuous	2 to 40	8 to 0.4
40	57	Continuous	5 to 70	6 to 0.4
90	107	Continuous	9 to 140	6 to 0.4
190	207	Continuous	17 to 600	6 to 0.18
190	-	Interrupted	100 to 2000	-
500	-	Interrupted	250 to >>	-
1000	-	Interrupted	500 to >>	-

The important point here is to observe agreement, on one hand, between experiments using different delay lines and on the other hand, between experiments using the same delay lines but operated under different modes (continuous or interrupted). The results show that the data obtained under these various conditions are reasonably consistent.
The apparent rate constant for the DNPA-DNP reaction is 2.3 sec^{-1}, which yields a second order rate constant of 46 $s^{-1}.M^{-1}$.
In chapter 4.10 below are listed some parameter guidelines for the various delay lines and modes. These parameters were used in the experiments described in this section and in section below.



4.6 Minimum ageing time

The minimal ageing time for the SFM-4 is obtained using the shortest intermixer volume (no additional delay line and MIX 0 block) operating in the continuous mode. The total intermixer volume using this delay line has been evaluated (above) to be 22 µl. The minimum possible ageing time depends on the achievable flow rate. With the valve a maximum flow rate of 15 ml/s is recomended. So that the flow that can be reached in the second delay line can be in the range of 12 ml/s giving an ageing time in the range of 2 ms. An example is shown below :

	PHASE N°	1	2	3	4	5	6	..
	TIME (ms)	10	40	20	0	0	0	0
SYRINGE	NUMBER	VOLUMES (ul)						
Not used	1	0	0	0	0	0	0	0
Reactant 1	2	60	240	0	0	0	0	0
Reactant 2	3	60	240	0	0	0	0	0
Quencher	4	30	120	0	0	0	0	0
Collect		W	C	0	0	0	0	0

Delay line N°1 = 0 ul
Delay line N°2 = 0 ul

In case the valve is not used and direct collection is performed, higher performances can be reached. If two syringes can be used to push one solution flow rates can reach higher values. An example is shown below :

	PHASE N°	1	2	3	4	5	6	..
	TIME (ms)	30	40	20	0	0	0	0
SYRINGE	NUMBER	VOLUMES (ul)						
Reactant 1	1	180	0	0	0	0	0	0
Reactant 1	2	180	0	0	0	0	0	0
Reactant 2	3	180	0	0	0	0	0	0
Quencher	4	90	0	0	0	0	0	0
Collect		W	W	0	0	0	0	0

Delay line N°1 = 0 ul
Delay line N°2 = 0 ul

Flow rate will reach 18 ml in the flow line between the last two mixers giving an ageing of around 1.2 ms.

4.7 Unequal ratio mixing

One of the major advantages of the SFM-4 instrument is the ability to vary the mixing ratio by simply modifying the stepper motor program. This does not require any syringe change. The quasi-continuous movement of the motor down to very low speeds permits efficient mixing to large ratios.

4.8 Double mixing

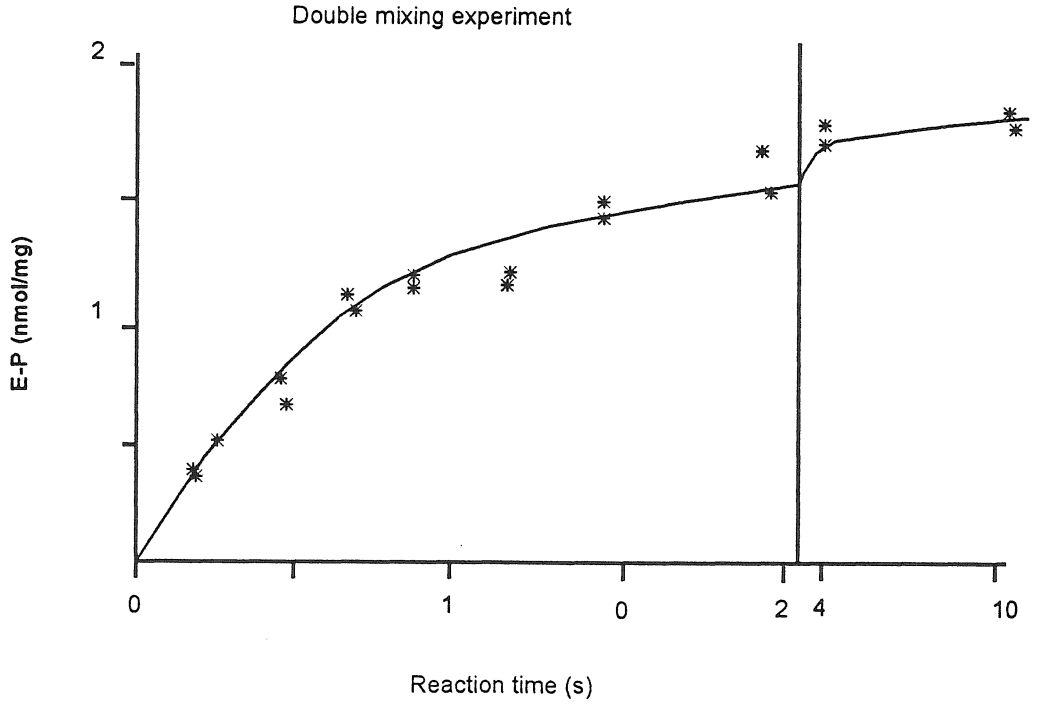
We performed a double mixing experiment to measure the rate constant of the calcium-induced transition in the absence of ATP. The enzyme incubated in EGTA is mixed with calcium in the first mixer of the instrument, after which the solution is allowed to age in the first delay line (interrupted mode). After this incubation, the enzyme is mixed with ATP in the second mixer and allowed to age 23 ms in the second delay line (continuous mode). Finally, the reaction is quenched with perchloric acid (PCA) in the last mixer.

The SFM-4 parameters are set as follows :

	PHASE N°	1	2	3	4	5	6	..
	TIME (ms)	0	420	T	100	60	0	0
SYRINGE NUMBER		VOLUMES (ul)						
ATPase + EGTA	1	0	210	0	50	30	0	0
Calcium	2	0	210	0	50	30	0	0
ATP-32P	3	0	0	0	200	120	0	0
PCA	4	0	0	0	150	90	0	0
Collect		W	W	W	W	C	W	W

Delay line N°1 = 200 ul
Delay line N°2 = 70 ul

The amount of phosphorylation as a function of calcium incubation time is shown in the figure below. These results indicate that the rate constant of the change induced by calcium alone is slower than that induced by calcium in the presence of ATP.



4.9 General guide lines for creation of the drive sequences

All of the parameters below are for 1/1 mixing ratio.

CONTINUOUS MODE

	PHASE N°	1	2	3	4	5	6	..
	TIME (ms)	0	Tp	0	Tc	0	0	0
SYRINGE NUMBER		VOLUMES (ul)						
Not used	1	0	0	0	0	0	0	0
Reactant 1	2	0	Vp	0	Vc	0	0	0
Reactant 2	3	0	Vp	0	Vc	0	0	0
Quencher	4	0	Vp	0	Vc	0	0	0
Collect		W	W	W	C	W	W	W

Delay line N°1 = 0 ul
Delay line N°2 = variable

Phase 2 is the purge phase. Tp must be varied to give the same flow rate as in collection phase N°4, at least up to about 6 ml/s. Above this, an acceleration phase has to be included between the purge and the collect phase.
Ageing time will be calculated according to :

$$T_{age} = (dl).Tc/(2.Vc)$$

The collection volume is set according to what should be obtained at the collection port. (**dl**) is the measured volume of the delay line.

The purge volume depends on the delay line used, as indicated below :

Delay line Nominal Volume (ul)	Purge Volume Vp (ul)
17	40 to 90
40	60 to 100
90	150 to 200
190	200 to 300

INTERRUPTED MODE

The 190 µl delay line and above are generally used with this mode. Here is an example sequence; many others may be created.

	PHASE N°	1	2	3	4	5	6	..
	TIME (ms)	0	150	Ta	15	40	0	0
SYRINGE NUMBER		VOLUMES (ul)						
Not used	1	0	0	0	0	0	0	0
Reactant 1	2	0	200	0	25	60	0	0
Reactant 2	3	0	200	0	25	60	0	0
Quencher	4	0	200	0	25	60	0	0
Collect		W	W	W	W	C	W	W

Delay line N°1 = 0 ul
Delay line N°2 = 200 ul

This corresponds to the collection of 180 µl of solution. The reaction age is

$$T = Ta + (dl).40/(2.60) = Ta + 73 \text{ ms.}$$

SECTION IV

SFM-4 TECHNICAL INSTRUCTIONS

CAUTION !

THESE INSTRUCTIONS ARE INTENDED FOR QUALIFIED PERSONNEL ONLY. TO AVOID ELECTRIC SHOCKS DO NOT PERFORM ANY SERVICING OTHER THAN THAT DESCRIBED BELOW UNLESS YOU ARE QUALIFIED TO DO SO.

1 INTRODUCTION

This section of the manual contains information about factory service, maintenance.

Careful cleaning with distilled water is required at the end of a series of experiments or before a change of buffer.

2 GENERAL MAINTENANCE

2.1 Disassembly of the SFM-4 water jacket

This operation permits an overhaul of the entire upper part of the SFM-4. This part contains the many important components of the instrument : the syringe body, the valves and the first mixer.

- Empty the coolant.
- Set the syringe plugers at their lowermost position
- Unscrew the upper cover.
- Lift the upper cover.
- If necessary lift out the lower part of the water jacket.
- The syringe bodies can be removed and unscrewed at that point.
  Note : syringe removal can be performed without opening the water jacket (see chapter 24 below).

  Follow the instructions of chapter 24 below.
- To prevent corrosion of the water jacket, two magnesium blocks are screwed (one on each part of the water jacket). It is normal that these magnesium blocks get rapidly corroded. They have to be exchanged only before they become completely destroyed.

Note : The upper part can be screwed back onto the SFM-4 frame without the lower

part of the water jacket. The instrument will be functional except for the temperature regulation.

- Reassemble in the reverse order.

2.2 Valve inspection or replacement

After prolonged and extensive use of the Sfm-3 instrument, the SFM-4 valves may need to be inspected or replaced. To this, use the special flat wrench delivered with the instrument

1) Remove coolant from the device.

2) Use the wrench to loosen the stainless steel nut which surrounds the valve shaft and plugs the bulkhead of the device. In this operation, keep the handle attached to the valve shaft. Remove the entire assembly consisting of the valve, the spring, the handle, and the nut.

3) Inspect the valve, check for the presence of scratches. Wash, and reinstall in the reverse order.

4) If replacement is felt necessary, replace with an entirely new assembly. Return the used one to the factory or to your distributor.

NOTE : although the valve have been machined with extreme great care permutation is not recomended.

2.3 Plunger disassembly and reassembly

- Lower the plunger with the manual control down to its lowest position.
- Unscrew the two screws holding the plunger onto its two vertical guides.
- Remove the plunger and its holder.
- Wash and/or exchange the teflon head.
- Reassemble in the reverse order.

Note : Drive syringe leakage
A slight amount of leakage past the teflon syringe tips is normal and necessary, and increases with the speed of the syringes. This slight leakage is the result of design trade-offs regarding the sealing quality and rigidity of the syringe tips. Excessive leakage can often be alleviated by a number of remedies. The amount of leakage which is considered excessive is an operational definition, depending

on the nature of the experiments being performed, etc.

Examples of experiments which put high demands on the sealing quality would be those with higher flow rates and/or those using more viscous solutions. The following are tips to help minimalize syringe leakage:

1) In general, the SFM-4 should be stored between usages (i.e. overnight or longer) with the drive syringes dry and in the fully withdrawn position, such that the white teflon syringe heads are outside of the drive syringe barrel. This allows the teflon to expand slightly between uses, allowing a better seal during experiments.

2) If leakage occurs which exceeds several drops per syringe run, empty the guilty syringe, then withdraw it completely from the drive syringe barrel. Inspect the white teflon tip both visually and with your fingers. Scratches and other defects which allow leakage will often be invisible in the white teflon due to low contrast, but apparent by touch. If there are defects in the tip, order new ones. If the tip is dirty, clean it with an ethanol-dampened wiper.

3) If leakage persists after observing steps 1 and 2 above, the tips may be removed and boiled to expand the teflon. Teflon will contract upon cooling slightly less than it expands upon heating, allowing a better seal with the syringe barrel. To remove the tip, fully withdraw the syringe shaft from the barrel, loosen the Allen screw at the base of the syringe shaft, and lift the shaft from the crossbar. Then, remove the teflon tip by unscrewing it from the steel shaft. Boil the tip for ten to fifteen minutes, making sure the boiling flask does not go dry. When the tips are cool, replace them by reversing the removal procedure described above.

4) If leakage persists, order new tips.

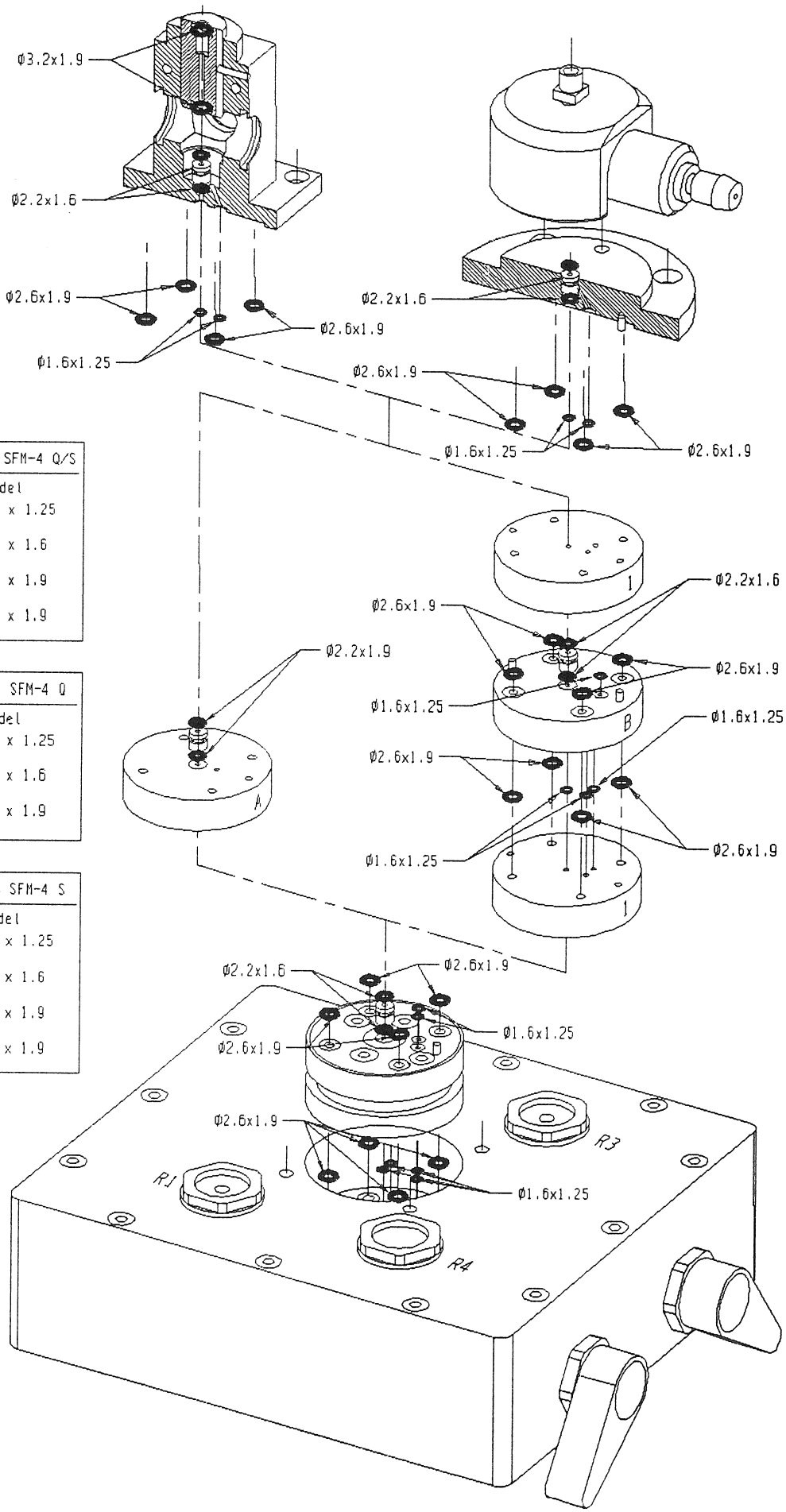
2.4 Syringe disassembly and reassembly

- Remove the syringe plunger and its holder as described above.
- Unscrew the syringe from the bottom of the water jacket using a fork wrench or pipewrench, dimension 24 mm.
- Pull and remove the syringe body from below.
- Wash or replace.
- Check the Kel-F sealing on top of the syringe.
- Carefully wash the outer surface of the syringe body.

- Grease the O-ring with silicon grease.
- Screw the syringe back in place by hand.
- Tighten with the fork wrench.

2.5 Disassembly and reassembly of the observation head or delay lines blocks.

- Stop the coolant circulation.
- Unscrew the four screws holding the head.
- Pull the head out.
- Replacement of the cuvette and of the mixer are described in 2.8
- Replace or wash.
- Replace the two O-rings. (see next page for O-ring description and dimensions).
- Reassemble.



Kit O-Rings SFM-4 Q/S	
Qty:	Model
14 :	ø 1.6 x 1.25
10 :	ø 2.2 x 1.6
24 :	ø 2.6 x 1.9
2 :	ø 3.2 x 1.9

Kit O-Rings SFM-4 Q	
Qty:	Model
12 :	ø 1.6 x 1.25
8 :	ø 2.2 x 1.6
20 :	ø 2.6 x 1.9

Kit O-Rings SFM-4 S	
Qty:	Model
8 :	ø 1.6 x 1.25
6 :	ø 2.2 x 1.6
12 :	ø 2.6 x 1.9
2 :	ø 3.2 x 1.9

2.6 Lubrication

- Clean the instrument periodically to remove moisture, dust and grease.
- Unscrew and clean the valves when the instrument is not to be operated for a long period of time.
- Lubricate the driving screw with mineral oil periodically. Access to it is obtained by removing the front cover.

2.7 Replacement of observation cuvette

- Replacement of the observation cuvette is a routine operation which does not necessitate removal of the observation head.
- Disconnect the exhaust tube.
- Unscrew and remove the black cover of the observation head.
- Slowly pull and remove the upper teflon cylinder.
- Remove the observation cuvette.
- Replace the new observation cuvette.
- Reassemble in the reverse order.
- Replace and tighten the black head cover by hand.

2.8 Exchange of mixers

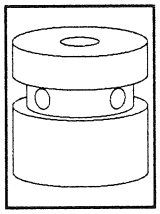
Exchange of Mixer 3:

- Remove parts # 887 and 886.
- Unscrew part # 165.
- Remove parts # 164,165,157 and the cuvette.
- Unscrew and remove part # 160.
- Remove O-Ring (2.2 x 1.6) of part # 160.
- Remove mixer 2.

- Screw part # 160.
- Insert new mixer (groove upside).
- Insert O-Ring (2.2 x 1.6).
- Insert cuvette and parts # 157, 165,164.

Mixer position:

- UP -

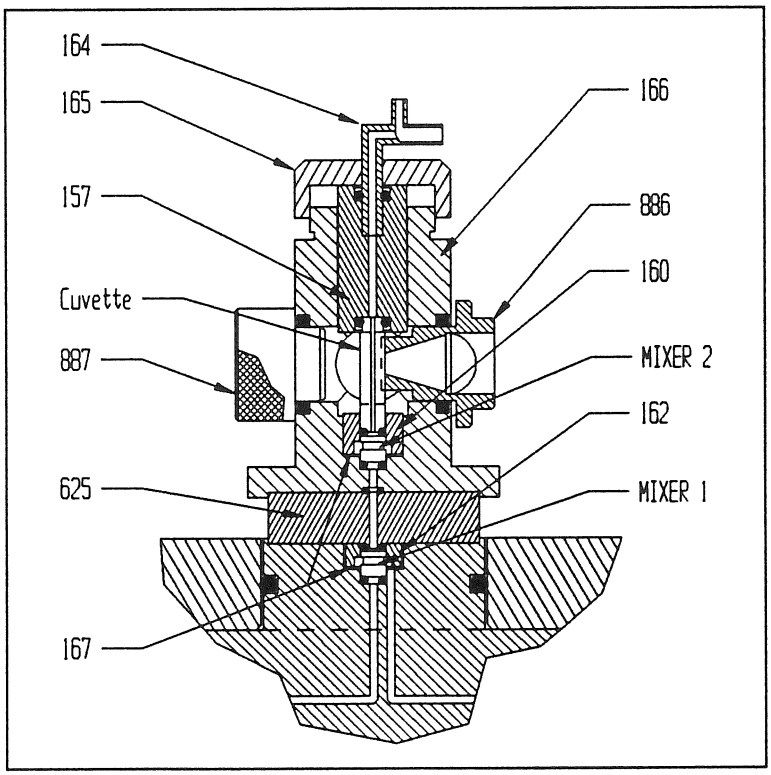


- DOWN -

Exchange of Mixer 1:

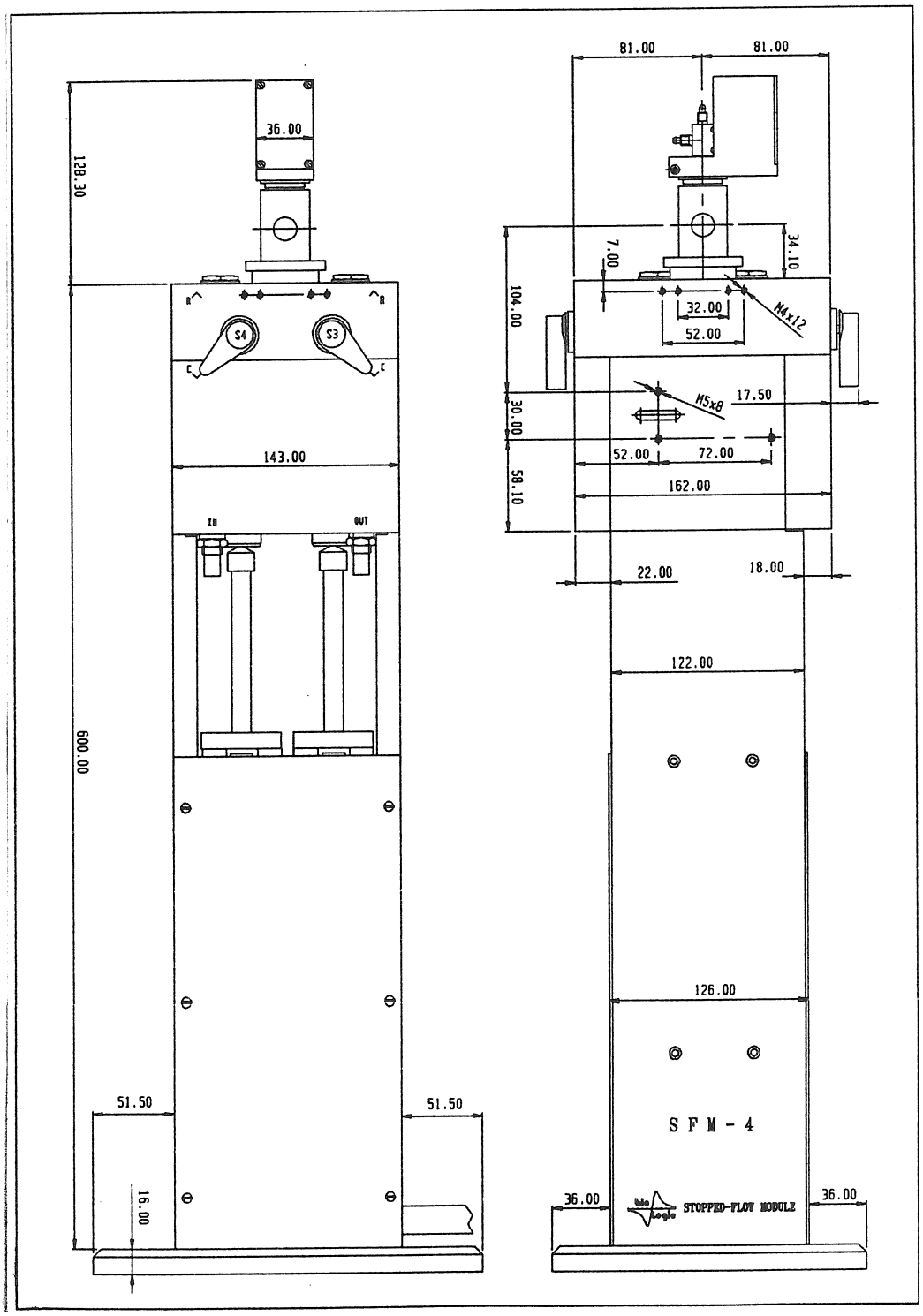
- Unscrew the 4 screws of the head # 166 Remove ageing loop.
- Unscrew part # 162.
- Remove O-Ring (2.2 x 1.6) of part # 162.
- Remove Mixer 1.

- screw part # 162.
- Insert new Mixer (groove upside).
- Insert O-Ring (2.2 x 1.6).
- put again ageing loop and head # 166 and screw it again.

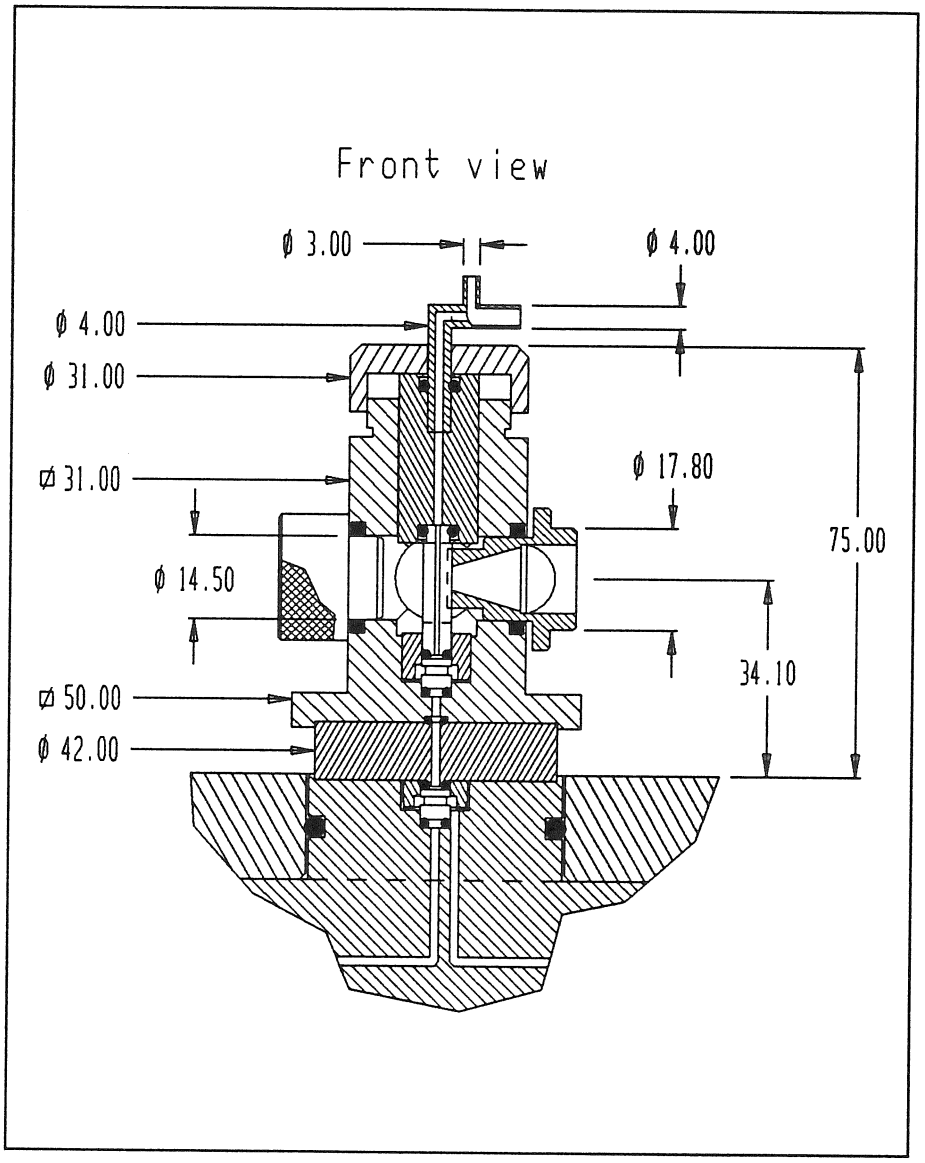


3 MECHANICAL DRAWINGS

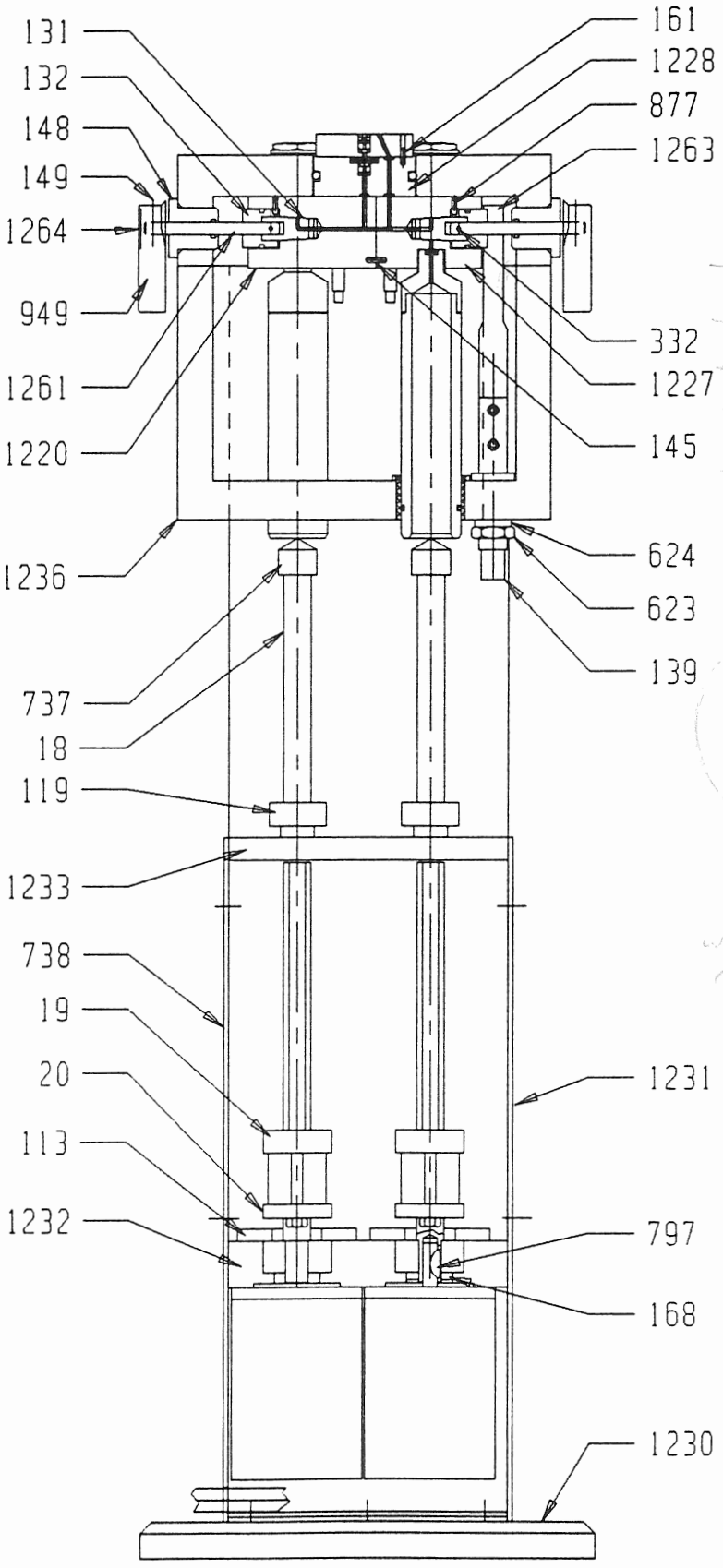
3.1 General dimensions



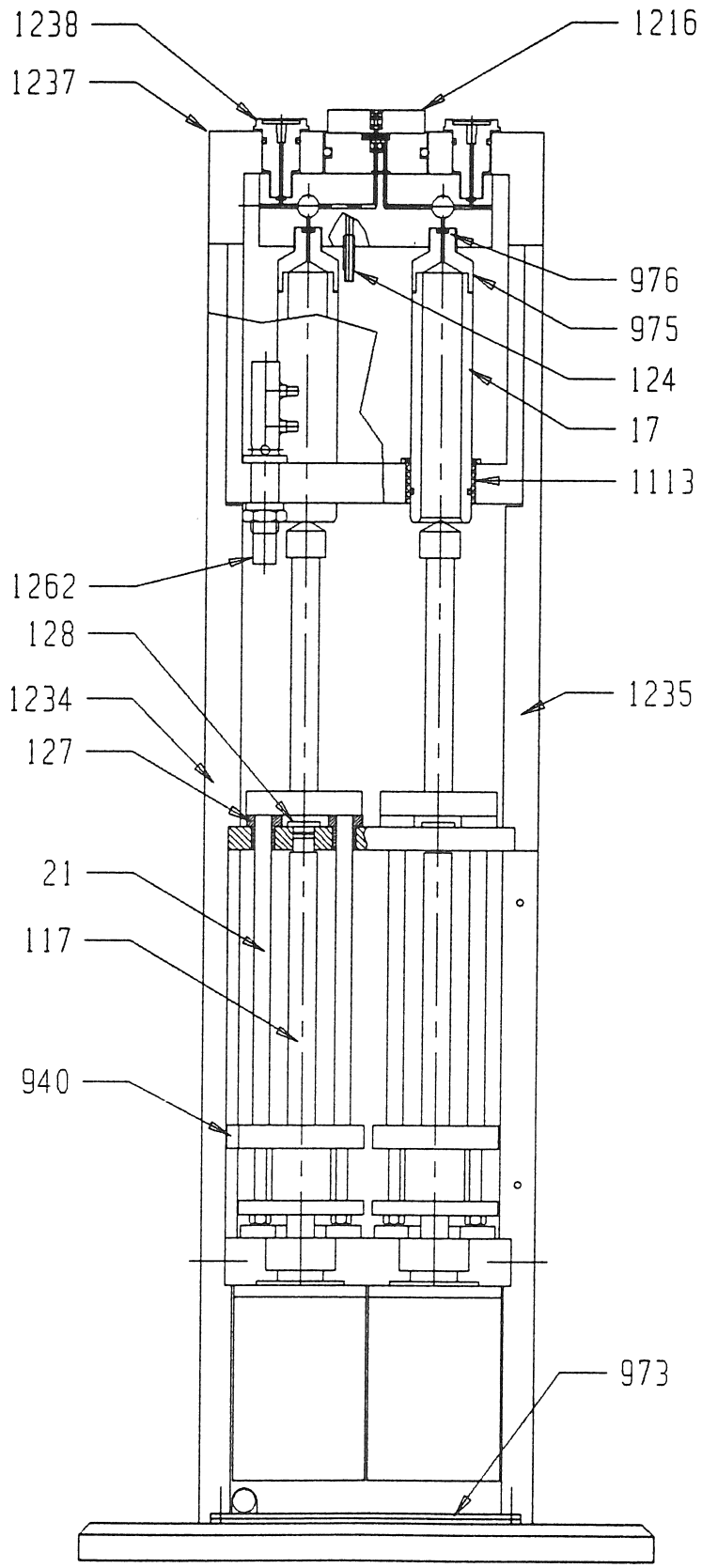
3.2 Dimensions of the head



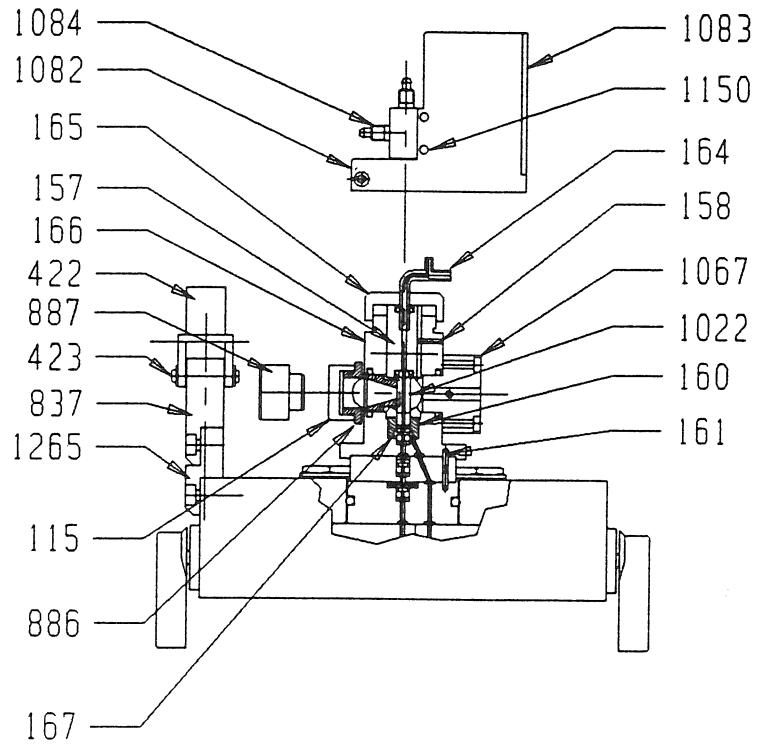
3.3 Front view references

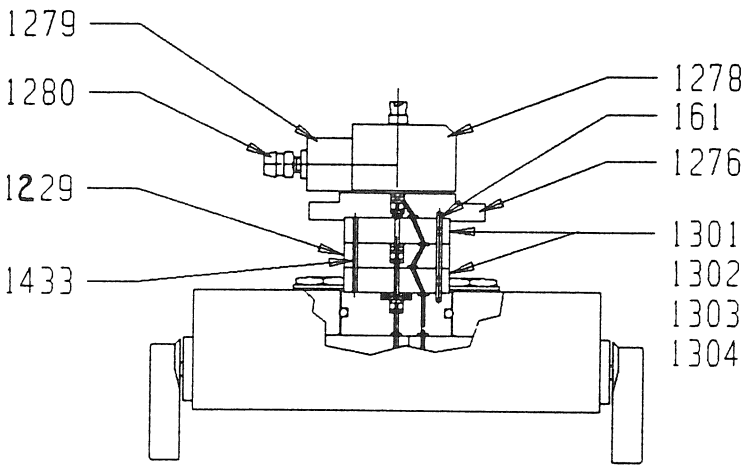


3.4 Side view references



3.5 Top view references





4 SCHEMATIC DIAGRAMS

9.1 - CPU schematics
9.2 - CPU components
9.3 - CMP02 (logic part) schematics
9.4 - CMP02 (logic part) components
9.5 - CMP02 (Analogic part) components
9.6 - CMP02 (Analogic part) schematics
9.7 - Front panel schematics
9.8 - Front panel components
9.9 - Power supply schematics
9.10 - RS-232 Pinout
9.11 - Serial port configuration of MPS-51 power supply

9.11 - Serial port configuration of MPS-51 power supply

The serial port of MPS-51 uses standard 25-pin RS-232C data line configuration. It can be connected to the serial communication card of the microcomputer via a 25-pin/25-pin cable or via 25-pin/9-pin, the later being used in most AT computers.

Pin number		Signal name
25-pin connector	9-pin connector	
1	-	Chassis ground
2	3	TxD (transmit data)
3	2	RxD (Receive data)
4	7	RTS (Request to send)
5	8	CTS (Clear to send)
6	6	DSR (Data set ready)
7	5	SG (Signal ground)
8	1	DCD (Data carrier detect)
20	4	DTR (Data terminal ready)
22	9	RI (Ring indicator)

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