µSFM
Micro Volume stopped-flow
User's Manual
Version 1.1

- Equipment installation

WARNING!: The instrument is safely grounded to the Earth through the protective conductor of the AC power cable.

Use only the power cord supplied with the instrument and designed for the good current rating (10 Amax) and be sure to connect it to a power source provided with protective earth contact.

Any interruption of the protective earth (grounding) conductor outside the instrument could result in personal injury.

- General description

The equipment described in this manual has been designed in accordance with EN61010 and EN61326 and has been supplied in a safe condition.

- Instructions for use

To avoid injury to an operator the safety precautions given below, and throughout the manual, must be strictly adhered to, whenever the equipment is operated. Only advanced user can use the instrument.

Bio-Logic SAS accepts no responsibility for accidents or damage resulting from any failure to comply with these precautions.

GROUNDING

To minimize the hazard of electrical shock, it is essential that the equipment be connected to a protective ground through the AC supply cable. The continuity of the ground connection should be checked periodically.

ATMOSPHERE

You must never operate the equipment in corrosive atmosphere. Moreover if the equipment is exposed to a highly corrosive atmosphere, the components and the metallic parts can be corroded and can involve malfunction of the instrument.

The user must also be careful that the ventilation grids are not obstructed on the right and left sides and under the chassis. An external cleaning can be made with a vacuum cleaner if necessary.

Please consult our specialists to discuss the best location in your lab for the instrument (avoid glove box, hood, chemical products, …).

AVOID UNSAFE EQUIPMENT

The equipment may be unsafe if any of the following statements apply:

- Equipment shows visible damage,
- Equipment has failed to perform an intended operation,
- Equipment has been stored in unfavourable conditions,
- Equipment has been subjected to physical stress.

In case of doubt as to the serviceability of the equipment, don't use it. Get it properly checked out by a qualified service technician.

LIVE CONDUCTORS

When the equipment is connected to its measurement inputs or supply, the opening of covers or removal of parts could expose live conductors. Only qualified personnel, who should refer to the relevant maintenance documentation, must do adjustments, maintenance or repair.

EQUIPMENT MODIFICATION

To avoid introducing safety hazards, never install non-standard parts in the equipment, or make any unauthorised modification. To maintain safety, always return the equipment to Bio Logic SAS for service and repair.

GUARANTEE

Guarantee and liability claims in the event of injury or material damage are excluded when they are the result of one of the following.

- Improper use of the device,
- Improper installation, operation or maintenance of the device,
- Operating the device when the safety and protective devices are defective and/or inoperable,
- Non-observance of the instructions in the manual with regard to transport, storage, installation,
- Unauthorized structural alterations to the device,
- Unauthorized modifications to the system settings,
- Inadequate monitoring of device components subject to wear,
- Improperly executed and unauthorized repairs,
- Unauthorized opening of the device or its components,
- Catastrophic events due to the effect of foreign bodies.

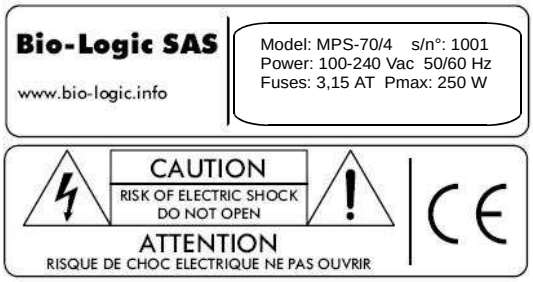
IN CASE OF PROBLEM

Information on your hardware and software configuration is necessary to analyze and finally solve the problem you encounter.

If you have any questions or if any problem occurs that is not mentioned in this document, please contact your local retailer (list available following the link http://www.bio-logic.info/distributors/france.html). The highly qualified staff will be glad to help you.

Please keep information on the following at hand:

- Description of the error (the error message, picture of setting or any other useful information) and of the context in which the error occurred. Try to remember all steps you had performed immediately before the error occurred. The more information on the actual situation you can provide, the easier it is to track the problem.
- The serial number of the device located on the rear panel device.



- The software and hardware version you are currently using. On the Help menu, click About. The displayed dialog box shows the version numbers.
- The operating system on the connected computer.

General safety considerations

 Class I	**The instrument is safely grounded to the Earth through the protective conductor of the AC power cable.** **Use only the power cord supplied with the instrument and designed for the good current rating (10 A max) and be sure to connect it to a power source provided with protective earth contact.** **Any interruption of the protective earth (grounding) conductor outside the instrument could result in personal injury.**
 	**CAUTION :** • Do Not Open, Risk of Electric Shock • **Mechanical hazard:** moving part (Motorised µSFM)
 	**WARNING ! ! ! :** **If dangerous fluids are using, adapted protections should be applied (face, hand):** Risk of projection
	**CAUTION, Risk of Electric Shock** • To Avoid Electric Shock Ensure you the protective earth integrity • Do Not Open

Servicing considerations

Guarantee and liability claims in the event of injury or material damage are excluded when they are the result of one of the following.

- Improper use of the device,
- Improper installation, operation or maintenance of the device,
- Operating the device when the safety and protective devices are defective and/or inoperable,
- Non-observance of the instructions in the manual with regard to transport, storage, installation,
- Unauthorised structural alterations to the device,
- Unauthorised modifications to the system settings,
- Inadequate monitoring of device components subject to wear,
- Improperly executed and unauthorised repairs,
- Unauthorised opening of the device or its components,
- Catastrophic events due to the effect of foreign bodies.

ONLY QUALIFIED PERSONNEL should operate (or service) this equipment.

EQUIPMENT MAINTENANCE

WARNING !: Before performing any maintenance, disconnect the line cord and all test cables.

Ventilation:

The user must carefully check that the ventilation grids are not obstructed under the chassis. An external cleaning can be made with a vacuum cleaner if necessary.

Cleaning:

Ventilation grids: external cleaning can be made with a vacuum cleaner if necessary.

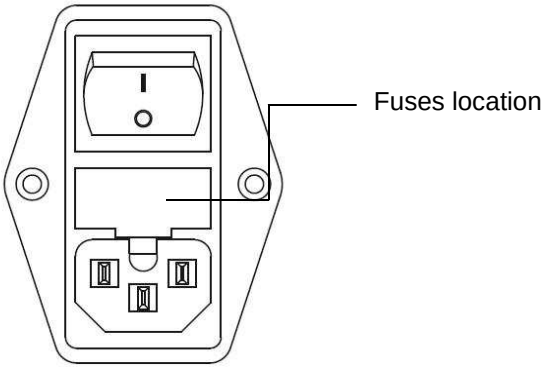
Use a damp cloth or mild, water-based cleaner to clean the instrument. Clean the exterior of the box only, never the circuit board. Do not apply cleaner directly to the box or allow liquids to enter or spill on the box.

Fuses:

WARNING !: To maintain protection from electric shock and fire, replace fuses, with the same rating and type.

Rating: 3,15 AT / 250 Vac

Size: 5-20 mm



EQUIPMENT RATING

Electrical

- Input (MPS-70):

  Input voltage range: 90 to 264 Vac

  Power: 250 W max

  Frequency: 47 to 63 Hz

  Fuses (Neutral +Phase): 2 x 3,15 AT, 250 VAC (5x20 mm)

See "µSFM specifications" chapter for detail

Environmental

- **Indoor use**
- **Operating Temperature:** 10°C to +40°C Indoor use
- **Storage Temperature:** 0°C to +50°C
- **Pollution degree:** 1 (no pollution or only dry)
- **Altitude:** <2000 m above sea level
- **Humidity:** 10% to 80% non-condensing

- **Case protection:** IP20 for MPS-70
- **Warm-up:** 1 hour to rated accuracy
- **Cooling:** Internal DC Fans for MPS-70, Natural convection for µSFM
- **Vibration:** not specified
- **Choke:** not specified

Mechanical

MPS-70/2:

- **Size**: 148(H) x 430(W) x 298(D) mm
- **Weight**: 8 kg without cable

µSFM:

- **Size**: 650(H) x 200(W) x 140(D) mm
- **Weight**: 8.7 kg with HardStop

Safety complies with EN61010-1.

EMC complies with EN61326.

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CE

DECLARATION OF CONFORMITY

N°: CETR_SFM-4000_Rev. A

We,

Bio-Logic SAS
1, Rue de l'Europe
38640 Claix France

declare under our sole responsibility that the products,

SFM-2000 / SFM-3000 / SFM-4000 with cables
MPS-70/2 / MPS-70/3 / MPS-70/4 with cables

are in conformity with the following standard(s) in accordance with the provisions of the Electromagnetic Compatibility Directive 2004/108/CE and the Low Voltage Directive 2006/95/CE:

Security: IEC 61010-1
EMC: IEC 61326

Emissions
- EN 55022: Conducted Class B
- EN 55022: Radiated Class A
- EN61000-3-2: Harmonic Current

Immunity
- IEC 61000-4-2: ESD
- IEC 61000-4-3: EM field
- IEC 61000-4-4: Burst
- IEC 61000-4-5: Surge
- IEC 61000-4-6: Conducted RF
- IEC 61000-4-8: Magnetic Field
- IEC 61000-4-11: Voltage Dip/Short Interruptions

Date: November 21, 2011

J-P Dardouillie, Compliance Manager

François Guy, President

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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. USING A NONCOMPATIBLE SOLVENT WITH THE SFM IS NOT COVERED BY THE WARRANTY.

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 PRODUCT OR REPAIR. WITH REGARD TO ANY INSTRUMENT RETURNED BECAUSE OF A 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 BILLED AT NORMAL COST. IN SUCH CASES, AN ESTIMATE WILL BE SUBMITTED BEFORE WORK IS STARTED.

IN CASE ANY FAULT OCCURS:

NOTIFY BIO-LOGIC OR THE 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.

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

1 INTRODUCTION AND SPECIFICATIONS

1.1 General Description

The Bio-Logic stopped-flow module µSFM consists of a mechanical subsystem and a power supply (MPS-70/2).

The mechanical sub-system consists of two syringe driving systems based on stepping motors and drive screws, an observation cuvette, and a valve system allowing isolation of the cuvette.

The µSFM uses commercial gas-tight syringes made out of glass with PTFE pistons.

The syringes, valves, and cuvette are enclosed in a water jacket to allow temperature regulation of the reactants. The syringe plungers of the stopped-flow are driven by two stepping motors via two ball screws.

1.1.1 The mechanical design

The mechanical part of the SFM module is carefully constructed. The parts in contact with the sample and the buffers are all machined out of materials selected for their inert characteristics: stainless steel, PFFE, VITON, FFKM, PEEK, and quartz.

Millisecond dead times can be achieved with the µSFM due to the combined effects of high-performance control of the stepping motors and low dead volumes.

1.1.2 Intelligent power supply

The high performance of the SFM and the high speed of the stepping motors can be achieved only because of the quality of its power supply. The MPS-70/2 unit contains independent constant current power supplies for each syringe, all driven independently by their own microprocessor.

The sequences of impulses to be sent to the stepping motors are stored in the memory of each motor board. One main microprocessor board synchronizes all the power supplies and performs the communication with the microcomputer via a USB interface.

1.2 Specifications

The general specifications of each SFM are listed in **Error! Reference source not found.**.

Table 1– µSFM Specifications

GENERAL µSFM SPECIFICATIONS	
Number of syringes	2
Driving mechanism	One stepping motor per syringe (6400 steps per motor turn)
Number of mixers	1 Berger Ball
Trigger	Programmable trigger for data acquisition and synchronization of accessories
Priming volume	24 µl
Minimum injection volume per syringe	3µl
Minimum amount of sample to load	50 µl
Flow-rate range of syringes	0.03 – 1.8 ml/s/syringe
Minimum flow rate for efficient mixing	1 ml/s (total flow rate through the mixer)
Variable ratio range	Continuously variable from 1/1 to 1/9.
Minimal dead time	1 ms
Numbers of shots from a 100 µl stock	6 using 1:1 mixing ratio 10 using 1:10 mixing ratio
Material of flow lines	PEEK
Syringe volume	500 µl.
Volume per µ-step	10 nl
Power requirement	300 Watt, 110/220 Volt, 50/60 Hz
Total weight	8,7 kg

1.3 Principle of Operation

The syringes of the µSFM are driven by independent stepping-motors. The stepping-motors are of hybrid technology with 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 0.010 µl per micro-step (µ-step).

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 syringes can be driven independently and their speed

adjusted using the microcomputer with a very simple menu. The automatic mode is used for actual experiments.

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

The movements of the syringes are completely controlled by the microprocessor, which eliminates the need for a stop syringe. Thus, the stop artefact present in most conventional stopped-flow systems is absent in the µSFM. 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-70/2 unit.

The independent control of each syringe allows for high versatility of the injection sequence. It is possible to make an injection of one syringe only, unequal filling of the syringes, variable ageing times, variable concentration, variable mixing ratios, and other complicated actions with only a few keystrokes.

The reproducibility and regularity of the linear translation of the syringes and the absence of pressure artefact allows for optical recording during the drive sequence. This feature greatly facilitates the determination of the initial phase of the reaction being monitored and makes the equipment suitable for very accurate continuous flow experiments

1.4 Description of the Mechanical Design

Figure 1 shows the µSFM. The observation chamber and the syringes of the µSFM are mounted vertically. This allows for easy purging of bubbles, which are evacuated with a few pushes of each drive syringe.

The syringes, valves, and observation chamber have been carefully designed to be thermo-regulated (section **Error! Reference source not found.**). This thermoregulation prevents the occurrence of temperature artefacts on a very wide temperature range and permits rapid kinetics studies even at temperatures near 0°C.

Figure 1: µSFM

The drive syringes used in the µSFM are 500 µl gas tight glass syringe type (Hamilton 1750C, piston diameter is 3.26 mm)

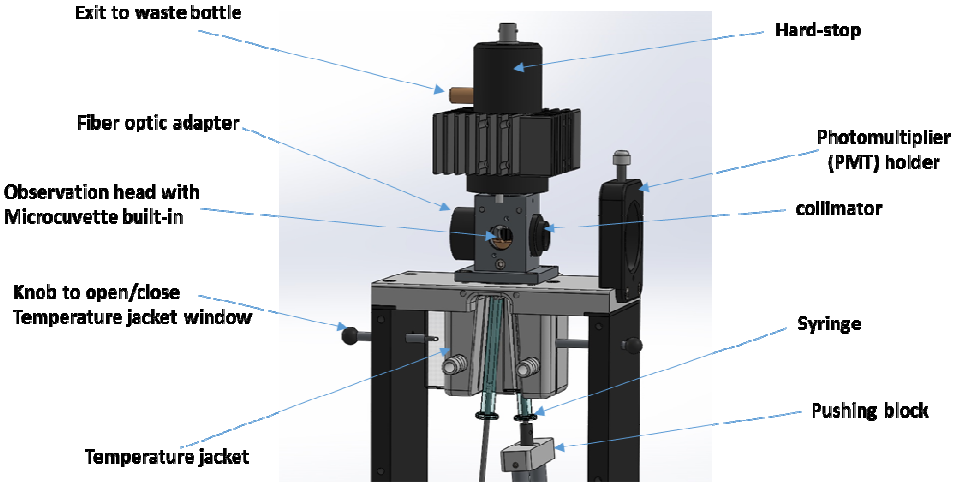


Figure 2 : µSFM details (in a absorbance configuration)

2 GENERAL INSTRUCTIONS FOR INSTALLATION

This section of the manual contains information on the installation and preliminary operation of the µSFM instrument. 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. The µSFM can be connected to the MPS-70/2. Please refer to the appropriate following operating features.

2.1 Operating Features MPS-70

The connection of the MPS-70/2 with the PC is done through a USB connector by plugging the USB cable on the rear panel of the MPS-70/2 and by installing the driver (provided with Bio-Kine software installation CD).

The selection of the syringes individually or simultaneously can be done manually by pressing the buttons (2) on the front panel (select buttons).

- By pressing one button, syringe 1 or 2 is selected while the corresponding led is lighted on.
- By pressing the two buttons at the same time, the two syringes are simultaneously selected while the led are lighted on.
- The up/down buttons allow you to move all the pistons of the stopped flow up and down.

The general features of the MPS-70/2 are shown below in Figure 3, Figure 4 and Table 2.

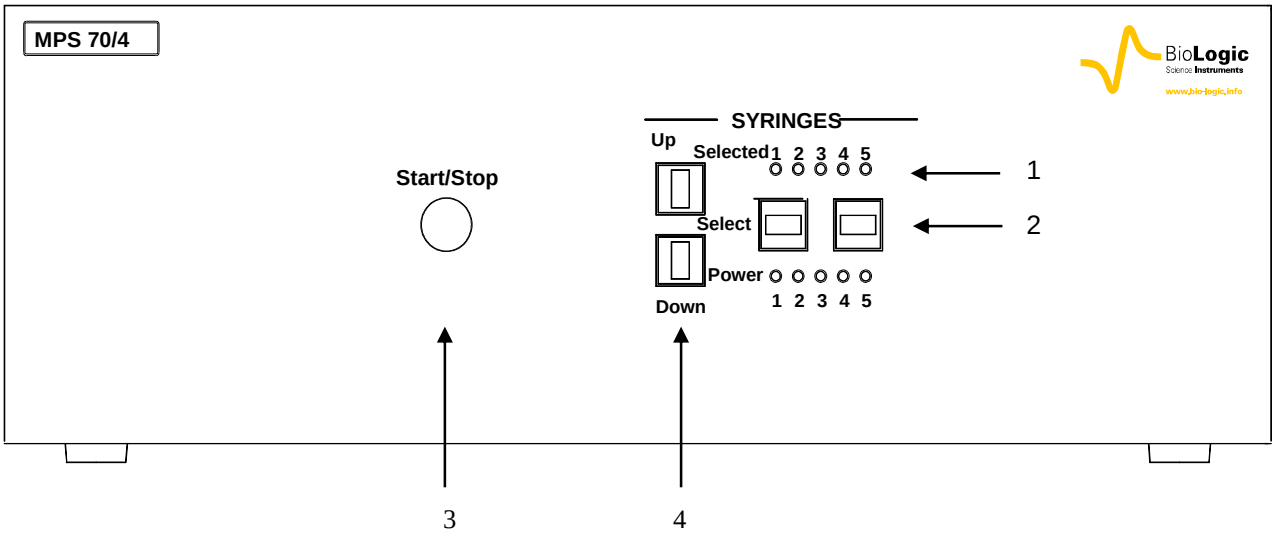


Figure 3: MPS-70/2 Front Panel

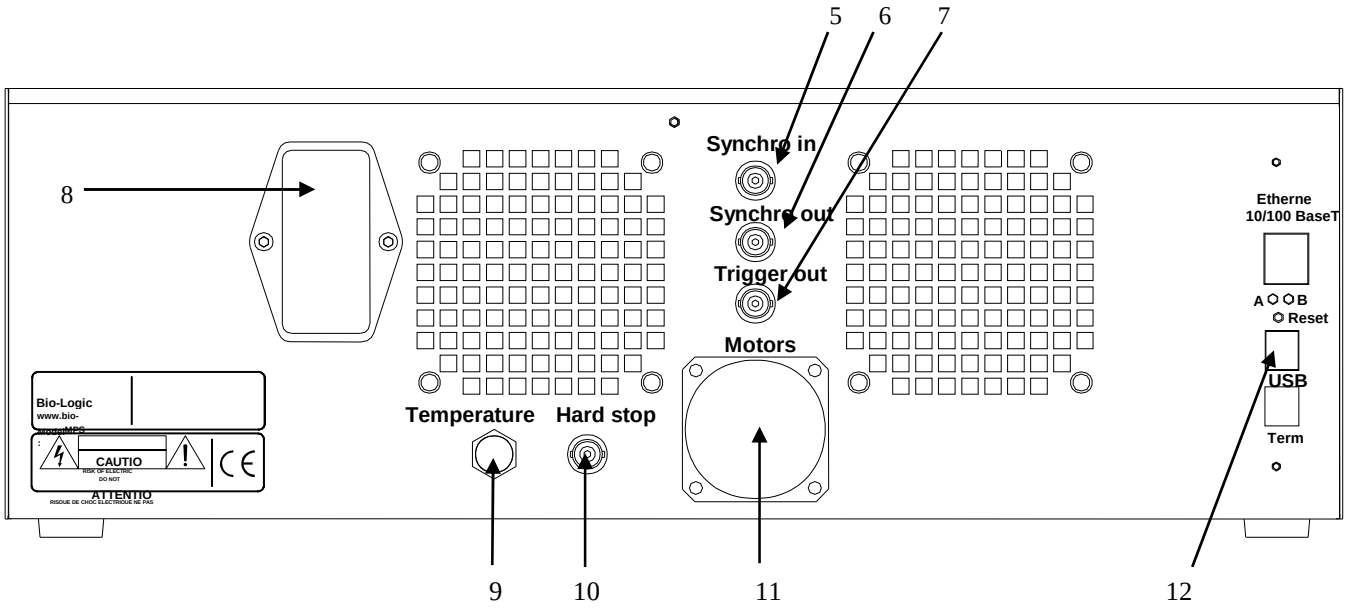


Figure 4: MPS-70/2 Rear Panel

Table 2- MPS-70/2 Panel Descriptions

	NAME	FUNCTION
1	SYRINGES	Used to display syringe selection
2	SYRINGE SELECTOR	Selects the syringe for the manual control
3	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.
4	MANUAL UP/DOWN MOVEMENT	Up and down movement for manual control of the syringes
5	SYNCHRO IN	Input for an external signal to trigger the drive sequence
6	SYNCHRO OUT	TTL Pulse output for special application
7	TRIGGER OUT	TTL Pulse output to trigger the recording system, or any electronic device to be synchronized with the instrument
8	MAIN POWER SWITCH	
9	TEMPERATURE PROBE CONNECTOR	Connects to temperature probe
10	HARD STOP (SF) BNC CONNECTOR	Connects to Hard Stop Valve
11	MOTOR POWER CONNECTOR	Sends the power pulses to the stepping motors
12	USB CONNECTOR	Connects the MPS-70/2 controller to the PC

2.2 AC Power and Connections of MPS-70/2

Before connecting the µSFM to the local AC line, verify that the setting of the instrument matches the local line voltage. Prepare the µSFM for operation by connecting the mechanical subsystem to the MPS-70/2 unit. Connect the MPS-70/2 to the USB port of the microcomputer. Finally, plug the MPS-70/2 into the appropriate AC line.

2.3 Temperature Regulation

The syringes, valves, and observation chamber of the SFM are designed to be temperature regulated. Organic oil (like Perfluorinated oil) may be preferred for temperature regulation to avoid corrosion, but the user should check compatibility with stopped-flow materials beforehand. Mixtures or water and glycol are also ideal for temperature control. Careful temperature regulation minimizes any occurrence of temperature artefacts. The µSFM module may be connected to a circulating temperature bath for temperature regulation. The coolant flows through two internal circuits: around the injection syringes and through the isolation valve block and observation head. With careful temperature regulation, temperature artefacts can be avoided over a very wide temperature range (between 0°C and 50°C).

3 INSTALLATION OF THE STOPPED-FLOW COMPONENTS

3.1 Syringe installation

The drive syringes used in the µSFM are 500 µl gas tight glass syringe type. Remove carefully the syringe barrel and the piston from the shipping box. Install the piston in the barrel of the syringe. To install the syringes on the µSFM make sure the pushing blocks are in loading position to have enough space to insert the syringe in the temperature jacket, tighten the syringes manually to avoid any leakage.

3.2 The Observation Head and cuvette

The stopped-flow observation head (Figure 5) is installed on the top of the µSFM. The observation head has four optical windows: one window for illumination and three for observation. This allows measurements of absorbance, transmittance, circular dichroism, single or double wavelength fluorescence emission, light scattering or fluorescence polarization without adding any 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.

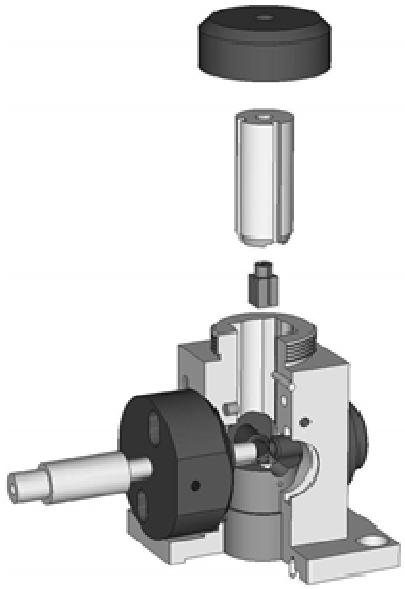


Figure 5: µSFM Observation Head

The observation head consists of a built-in micro Berger Ball mixer combined to a µFC-08 cuvette. µFC-08 has a 0.8 mm light path in both absorbance and fluorescence position, its dead volume is varying from 1 to 3 µl. Minimum dead time obtained with the µSFM cuvette is 1 ms.

3.3 The micro mixer

Each µSFM comes from the factory with a mixer installed. The mixer is located in the observation head to be as close as possible to the observation cuvette. The mixer itself cannot be removed by the user as it is embedded in a holder. If the mixer needs to be

replaced at one point it is then necessary to change the entire holder and mixer so it can be assembled and tested in factory.

As in all stopped-flow systems, the mixer is one of the most delicate pieces of the instrument. It is recommended to follow rigorously the cleaning procedure to avoid a blockage of the mixer. Instructions for removal and replacement of the mixers are described in section 8.2.

3.4 Flow Line Volumes

Figure 6 below indicates the volumes of the SFM flow lines. It should be noted that the volumes given in the table are the *mechanical* volumes. The hydrodynamic volumes may vary slightly around these values. The table below indicate the volumes of the µSFM flow lines.

µSFM FLOW LINE VOLUMES	
Line Number	**Flow Line Volume (µl)**
1	26 µl
2	24 µl
3	2-3 µl

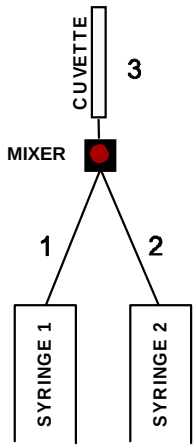


Figure 6 : µSFM flow lines volumes

3.5 Liquid Outlet System: Hard Stop Valve

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 must be immobilized in a fraction of a millisecond. Several different stop modes can be used to immobilize the liquid column. The stop mode used can result in overpressure or underpressure conditions that are potential sources of stop artifacts. The mode chosen by Bio-Logic is presented below: the hard stop system.

In this mode, the flow will be immobilized by a combination of two mechanisms: first, from the stepping motors stop and second, by a high speed electrovalve (hard-stop) which closes the output of the µSFM cuvette. This hard-stop is actuated by the programmable power-supply of the µSFM. No overpressure is developed in the observation cuvette because synchronization of the hard-stop with the motor halt. The result is elimination of the stop and overpressure artifact giving high quality stopped-flow traces with the lowest dead times.

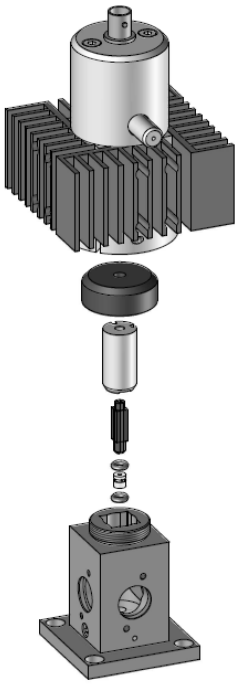


Figure 7: Hard Stop installation

The hard-stop can be driven under different modes where the valve keeps open or closed between shots. Please refer to the Bio-Kine manual for more details.

The installation of the hard-stop on the observation head is shown in Figure 7.

4 SOFTWARE CONFIGURATION IN STOPPED-FLOW MODE

The µSFM is controlled by Bio-Kine software which is also used to control acquisition parameters. This section precisely describes the configuration of the software. Please note that the procedures and examples have been generalized, and configuration choices should be made based upon the equipment purchased and intended experiments.
This section assumes that the user has already installed Bio-Kine software on the host microcomputer.

4.1 SFM installation with MPS-70/2 using Bio-Kine 4.80 (or higher)

Once Bio-Kine is loaded, choose **Install, device installation** in the main menu (Figure 8). The stopped-flow communication is established from this window by checking the **stopped-flow device** box and choosing the **USB port** for **MPS-70**. Accept the parameters using the **OK** button.

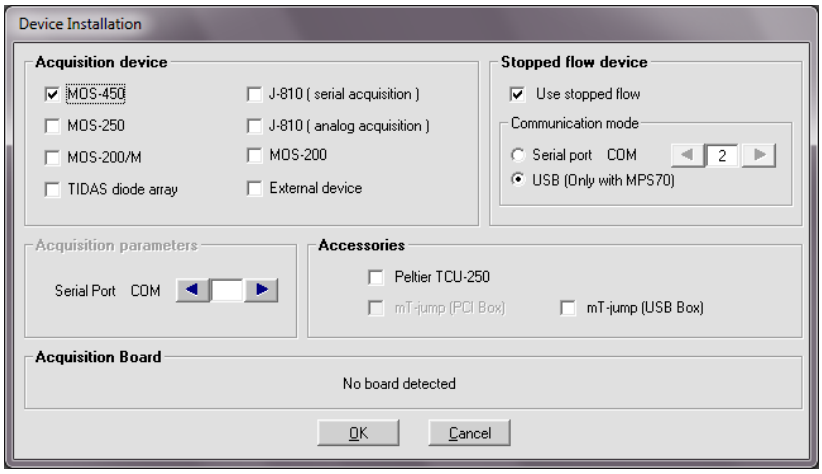


Figure 8: device installation.

4.2 Stopped-flow Configuration

Once the stopped-flow device and its USB port are selected in the **device configuration** menu, choose **the Install, stopped-flow configuration** menu (Figure 9).

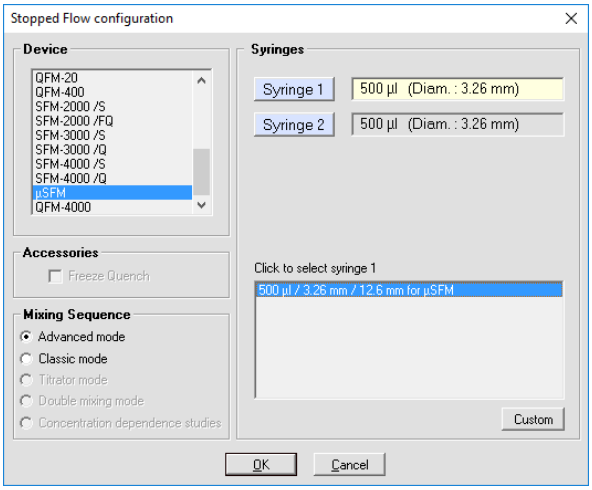


Figure 9: stopped-flow configuration.

Select the **µSFM** in the list of instruments that can be controlled by Bio-Kine. Syringe configuration is made in the same window. The active syringe is displayed in yellow. Select the volume of the syringes that have been installed in each syringe position of the µSFM by clicking on the right one.

The µSFM comes equipped with standard 500 µl syringes, and these are the default syringes installed in the software. Changes only need to be made in the software when syringes of different volumes have been installed in the µSFM.

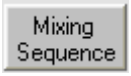
Use the 

button to enter syringe specifications if you have a custom syringe. In this condition the window shown in Figure 10 is displayed. It is then necessary to enter volume, piston diameter and screw pitch of the custom syringe to add it to the standard ones.

Figure 10: custom syringe

WARNING: Incorrect syringe configuration will cause volume and flow rate calculations to be incorrect!

4.3 Stopped-flow status area

A vertical menu bar on the left of the screen is dedicated to the stopped-flow device. This menu bar gives access to the syringe control window using the  button. The button 

gives an access to the mixing sequence (refer to section 5.6. When the mixing sequence is ready and activated, a new window appears (Figure 11) with a shot control window displayed in the area.
At any time, information about the configuration of the stopped-flow can be found in this bar such as device, number of shots available, cuvette type and temperature (in case the temperature probe is connected to the MPS-70/2).

Figure 11: stopped-flow menu bar

5 INSTRUMENT OPERATION IN STOPPED-FLOW MODE

5.1 Manual Syringe Control

The syringes of the µSFM can be controlled either manually or automatically. Automatic control of the syringes is strictly used for experiments. The manual control of the syringes is used for initialization, filling, and emptying the syringes. Manual movement of the syringes can be made either directly from the MPS-70/2 or through Bio-Kine. Both methods are described in the following sections.

5.1.1 MPS-70/2

Syringe control directly from the MPS is made through the use of the buttons on the front panel of the MPS (Figure 4).

The "selected" buttons are used to select the syringe to be moved. The left button selects syringes from number 1 to 2, the right button selects syringes from number 2 to 1. A simultaneous selection of the syringes is possible by pressing at the same time the left and right buttons.

The (up) and (down) buttons are used to empty and fill the syringes. The corresponding light at the front panel will indicate which syringe has been selected.

5.1.2 Biokine Software

Syringe control from Bio-Kine is made through the  button in the stopped-flow status area. The window shown in Figure 12 is displayed. The MPS is then initialized, and communication is established between Bio-Kine software and the MPS unit. The message 'MPS on line' is displayed in a green window in the stopped-flow status area

The syringe to be moved is selected by clicking on the corresponding frame or by pressing the <**Left>** or <**Right>** arrow keys on the keyboard. The new selected syringe will be surrounded with a red rectangle.

Syringes are emptied or filled using the  ,  ,  , and  buttons or with the <**Up**> arrow and <**Down**> arrows. The  button or the  button and move a syringe upwards or

downwards, respectively by one elementary movement. The button or the button move the piston upwards or downwards by 10x elementary movements.

The size of the elementary steps and syringe movement speed is controlled in the Manual Speed section of the window. The and buttons are used to change the manual speed. It could be very useful to reduce cavitations during the loading of sample for example. The display shows the speed in flow rate based on the syringe installed and moved.

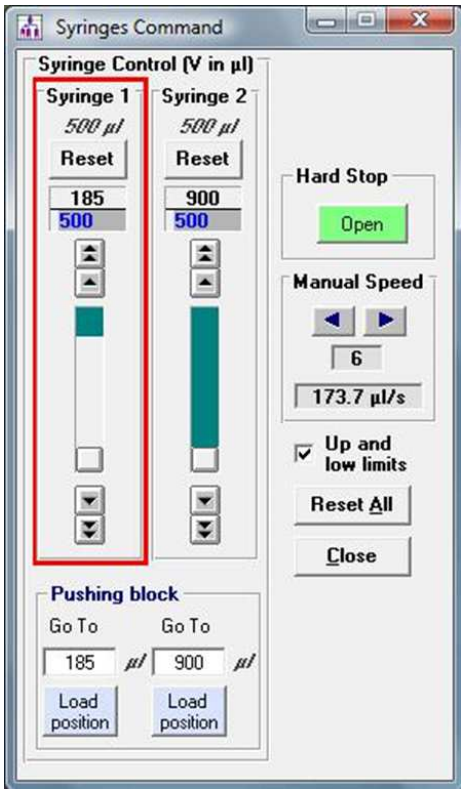


Figure 12: syringe control window

5.2 Syringe Initialization

The MPS-70/2 that controls the µSFM follows the movements of the syringes so that the actual residual volumes are displayed at all times (see Figure 12). When the MPS is turned on and the software started, the syringe volume counters show **Ref ?** and must be initialized.

The syringes are initialized by setting the syringes to their uppermost (empty) position and resetting the syringes in Bio-Kine. The syringes can be selected and their pistons moved to their uppermost positions either directly with the MPS or from Bio-Kine. Once a syringe has reached its uppermost position, the syringe motor will oscillate and vibrate as it becomes out of phase with the driving pulses. Before reaching the very top of the machine reduce the manual speed to speed 1 or 2 so vibration do not stress the glass syringes. At this speed there is no danger for the µSFM when this occurs, but there is no reason to unnecessarily prolong this treatment either.

<u>The Syringes initialization procedure is given below</u>

1) Install the gas tight syringe 1.

2) Make sure the hard-stop is open Open (Figure 12)

3) Raise the syringe 1 push-block with manual speed 1 or 2 using the command described in section 5.1 until the syringe is in its uppermost position; the syringe motor will oscillate and vibrate as it becomes out of phase with the driving pulses.

4) Repeat steps 1 to 3 for S2.

5) Reset (initialize) the syringes individually by pushing the Reset button for each syringe or all at once by pushing the Reset All button in the syringe control window (Figure 12).

If, by accident, a syringe is returned to its uppermost position the syringe volume counter will again show Ref ?, and the syringe must be reinitialized.

CAUTION: Measurement of residual syringe volume is made by counting the logic pulses from the controller for each syringe. If, for any reason, a syringe is blocked during a run, the pulses will not correspond to the true volume delivered, and the value displayed may become erroneous. In this case, it is advisable to reinitialize the syringes.

5.3 Loading solutions in the µSFM

WARNING: Utmost care should be exercised during this operation especially when working with precious solutions. Normal operation of the system requires that no bubbles are present in the injection syringes. If this occurs, the buffer flow through the observation chamber will not be correctly controlled by the plunger movement and artifacts may be observed. For best results it is recommended that all solutions be degassed and filtered before filling the µSFM.

The reference is supposed to be done for both syringes.

1) Click on the Load position button so the pushing blocks go to their loading position.

2) Close the hard-stop in the syringe command window**.**

3) Open the temperature jacket windows.

4) Unscrew the syringe manually and remove the syringe to the gas tight syringes to be used.

5) Install the loading adapter on the syringe or use a commercial needle.

6) Load the gas tight syringes with the sample and carefully eliminate any bubbles inside the syringes.

7) Remove the loading adapters (or needle) from the gas tight syringes and install the syringes in the SFM. Close the temperature jacket window

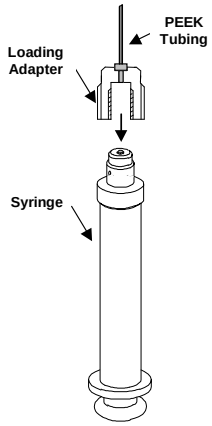


Figure 13– Loading Adapter

8) Open the hard-stop Open in the syringe command window

9) Check visually the volume of liquid you have loaded in the syringe (e.g. 170 µl)

10) Select Manual speed 4 or 5 and enter this volume + 15-20µl in Biokine (eg 185 µl). Click enter so the pushing block goes automatically to the 185 µl position.

(in case you enter the exact volume you loaded at this stage make sure the piston of syringe fits well into the pushing block)

11) Reduce the manual speed to speed 1 and 2 and Enter 170 in the volume box so the pushing blocks gets in contact with the piston.

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

5.4 µSFM Cleaning and Storage

After each day's experiments the µSFM should be cleaned. A thorough cleaning of the µSFM will ensure that it has a long functional life and diminish any chance of sample contamination for the next user of the instrument. The procedure below is the recommended daily cleaning procedure to be done before shutting off the instrument.

1) Click on the Load position button so the pushing blocks go to their loading position.

2) Close the hard-stop

3) Remove the syringes and wash them free of any remaining samples or buffer.

4) Fill the syringes full with water and reinstall them in the SFM.

5) Make sure the hard-stop is Open (Open)

6) Push the piston by hand or move it from MPS-70/2 to wash the flow line and cuvette until S1 is empty.

7) Repeat step 1 to 6 three times

8) Repeat step 1 to 7 three times using ethanol (70 – 100%).

9) Repeat step 1 to 6 once with air

10) Remove syringes from the instrument, remove the piston and store syringes body and piston in their shipping box.

IMPORTANT: Do not forget this step! The syringe plunger tips are made of Teflon. Removing the syringe plungers allows the plungers to expand each night and make a tight seal during the next use, minimizing any chance of leaks.

5.5 Long-term Storage of the SFM

If the µSFM will not be used for a long period of time (more than several weeks), it should be cleaned as explained in section 5.4. If the µSFM is connected to a circulation temperature bath, the temperature bath should be disconnected from the µSFM and the µSFM drained completely of all cooling liquid. Afterwards, is recommended that the µSFM cooling circuits be flushed with ethanol followed with air. The µSFM is now ready for storage.

5.6 Creating a sequence using the advanced mode

5.6.1 µSFM options

An advanced menu was created to improve the friendliness of the design of the stopped-flow sequence and to optimise experimental settings in order to get the best quality results. This

mode can only be used for a single mixing experiment. The advanced mode must be selected in the stopped-flow configuration (refer to section 4.2) then click on Mixing Sequence button in the stopped-flow status area. The window shown in Figure 14 appears.

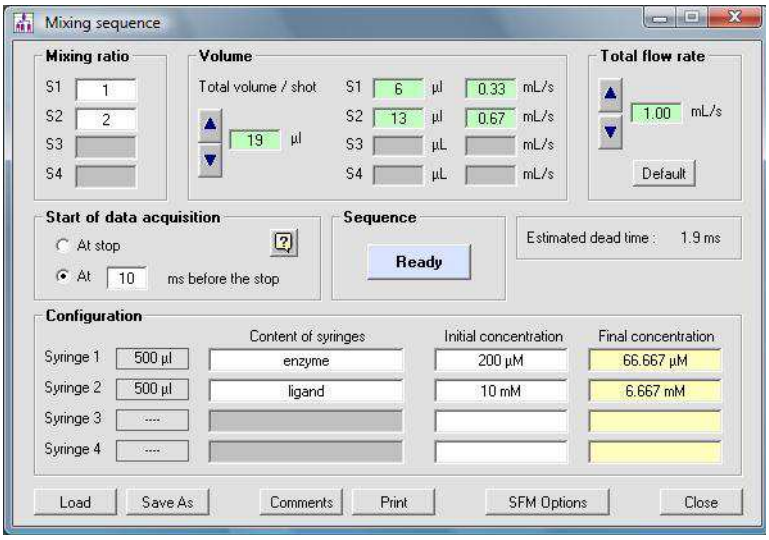


Figure 14 : driving sequence in advanced mode

First operation should be to check the configuration of the stopped-flow by clicking on the SFM Options button.

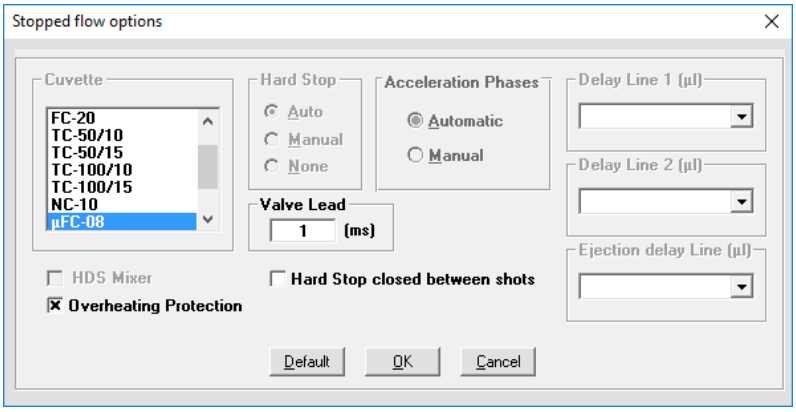


Figure 15 : SFM options in advanced mode

• the µFC-08 is the only cuvette available for µSFM and there is no possibility to select the HDS mixer, for application requiring such mixer user needs to consider our SFM-2000 series.

• **Valve Lead:** This section of the window allows entering the number of milliseconds before the flow stops that the hard stop starts closing. The default value is 2-3 ms. The lead time may be adjusted (from 0 – 5 ms) to fine-tune the quality of the stop. The precision of the setting is 0.1 ms.

• **Overheating Protection:** The default mode is checked. It is a protection against electronic overheating after a long working day.

• **Hard-stop closed between shots**: in advanced mode, the configuration of the hard-stop is automatically set: the hard-stop closes at the end of the pushing phase (or few milliseconds before if a **lead time** is selected) and opens at the end of the acquisition. If the

user needs to keep the hard-stop closed at the end of the acquisition (to run a spectrum for example), then it is necessary to check the corresponding box.

5.6.2 Design of stopped-flow sequence

The window shown in Figure 14 is separated into six areas: mixing ratio, volume, total flow rate, start of data acquisition, shots, and configuration. These different areas are respectively described below.

- **Mixing ratio**: it is the first parameter set. It is possible to enter a decimal value for the ratio.
- **Volume**: it is necessary to set the total volume of the reactants pushed into the cuvette using the 'up' or 'down' arrows. We recommend to push a minimum total volume of 24µl to achieve a proper washing of the flow lines between each shot. The volume is proportional to a micro-step volume in order to improve the reproducibility of results. Once the total volume is selected, the volume to be pushed for each syringe is calculated. The total volume selected should be big enough to wash the cuvette efficiently between two shots (refer to the color code for the limits).

IMPORTANT: The volume boxes of individual syringes do not show decimal value so the real pushed volume may be slightly different from the displayed volume. The µSFM always push the total volume indicated and will consider decimals for individual syringe in the hardware/sowftare.
For example in the sequence shown in Figure 16: for a total volume of 26µl Biokine displays 21 µl and 5 µl for syringes (which may not correspond on a 1:2 mixing ratio at a first glance). Real shot will be 5.2 µl and 20.8 µl so mixing ratio is correct and this can be controlled doing dilutions experiments. The number of shots is also calculated using the 5.2 µl value so you get as much data as possible from your stock solution.

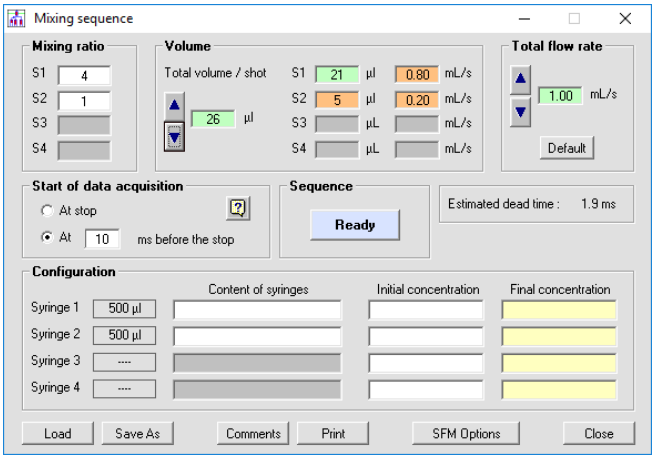


Figure 16: example of sequence

- **Total flow rate**: total flow rate must be selected using the 'up' and 'down' arrows. Once the total flow rate is selected, the flow rate for each syringe is automatically calculated. 1 ml/s is considered the minimum value to get efficient mixing. There are also limits for a single syringe according to their respective volume (refer to colour code), so total flow rate gets limited when using asymmetric ratio.
- **Start of data acquisition**: Using the 'stop' option, only the kinetics will be recorded. The acquisition is started when the hard-stop closes. To make sure the cuvette is well washed and the stationary state is reached, it is advised to start the acquisition a few ms before the stop.

• **Configuration**: In this area, it is possible to find the volume of the syringe installed and the type of cuvette. The content of the syringes can be entered with initial concentration; the final concentrations are calculated using the mixing ratio selected

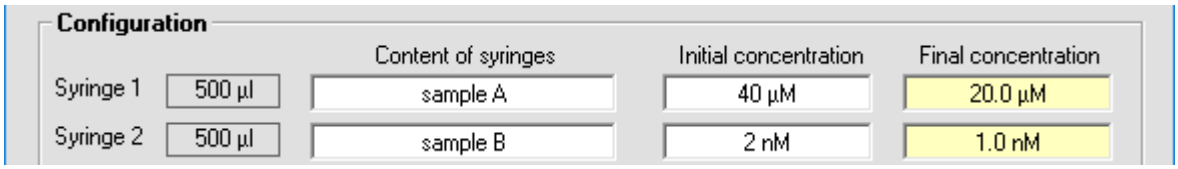
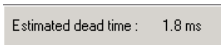


Figure 17: syringe content

• **Sequence**: Once the sequence ready, click on the  button. The shot window is now activated on the left panel of the screen.

• The **estimated dead time** of the reaction is given in ms. The dead time is calculated using the cuvette and mixer selected (dead volume) and the total flow rate



$$\text{Dead Time} = \frac{\text{Cuvette Dead Volume}}{\text{Total Flow Rate}}$$

Figure 18 –Estimated dead time

A color code is used to warn the user about the choice of the parameters selected:

	Colour code	message
Total volume	green	OK but we advise using at least 24 µl
	yellow	total volume may be insufficient for washing the cuvette
	red	Total volume too low for correct washing of cuvette
Volume per syringe	green	OK
	yellow	Syringe volume may be insufficient for washing of mixer
	red	Syringe volume too low
Total flow rate	green	OK
	yellow	Flow rate too low for correct mixing (< 1 ml/s) or close to maximum speed
	red	Flow rate may be difficult to achieve for this cuvette (>1.8ml/s)
Flow rate per syringe	green	OK
	yellow	Flow rate close to safety limitations
	red	Flow rate out of range (too low or too high)

Standard operations can be made from the same window:

- Load a sequence using the Load button.
- Save a sequence using the Save As button.
- Print a sequence using the Print button.
- Close a sequence using the Close button.

- **Comments**: a text window is opened by clicking on the Comments button. Comments will be saved with the sequence.

5.6.3 Programmable synchronization trigger using the MPS-70

Only Trigger can be used in the advanced mode. In this mode the hard-stop cannot be controlled manually from **Synchro out.**

Trigger is a falling edge (5→0 V). The acquisition will start at the end of the pushing phase or few milliseconds before according to the configuration chosen by the user in the driving sequence.

5.7 Minimum volume per shot

To wash the µFC-08 correctly and ensure the stationary state is reached, a minimum of 24 µl must be injected at each shot. It means doing a 1:1 mixing ratio user will mix 12 µl of each reactant minimum. Doing a 1:2 mixing ratio user can push 8 µl with S1 and 16 µl with S2. The minimum volume that can be push will define the number of shots you can run from a precious stock solution.

	Stock	300 µl	200 µl	100 µl	50 µl	Volumes injected
Mixing ratio						
1:1		24	14	6	2	12- 12 µl
1:2		x	20	10	3	8 - 17 µl
1:4		x	x	14	5	5-21 µl
1:9		x	x	x	9	3-29 µl

5.8 Running a shot

Once a driving sequence has been entered or loaded, it is transferred to the MPS-70/2 by pushing the **Single** or **Multiple** buttons (in classic mode) or **Ready** (in advanced mode).

The MPS is now in automatic mode and the **shot control** window appears in the stopped-flow status (as shown in Figure 19). The **Shot control** window shows the number of shots possible based the current volumes in the µSFM syringes. It also indicates whether the µSFM is running a driving sequence or ready for the next shot. A driving sequence is executed by pushing the ▶ button or the **start-stop** button on the front panel of the MPS-70/2. The ■ button can be used to stop an experiment prematurely if necessary.

If the **Single** button was used to transfer the driving sequence to the MPS-70/2, only a single shot can be made. The End button must then be pushed to return to the driving sequence and the **Single** button must be pushed again to re-transfer the driving sequence to the MPS-70/2 for a subsequent shot.

If the **Multiple** buttons was used to transfer the driving sequence to the MPS, the ▶ button can be used to execute shots until the **shot** window shows that 0 shots remain. The End button is then pushed to return to the driving sequence.

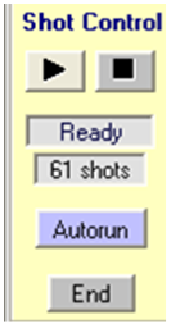


Figure 19: shot control window

6 A SHORT STOPPED-FLOW PRIMER

6.1 General Principle of Experiments with the SFM

There are many variations on the stopped-flow experiment, such as multiple mixing, continuous-flows and accelerated flow. However, the simplest stopped-flow experiment occurs in two stages.

In the first stage, the flow is initiated by two plungers. The plungers force liquid through a mixer and along a flow path into an observation cuvette. The resulting mixture ages as it travels along the flow path and into the cuvette. The amount of ageing depends on the flow-rate of the mixture and the volumes of the flow path and cuvette. In this first stage, the mixer, flow path, and cuvette are initially washed by the constantly refreshed mixture. This continues until a steady-state condition arises in which the age of the mixture is completely linear with respect to the distance along the flow path. Once the steady-state condition is reached, any particular point in the flow path represents the mixture at a particular age. Furthermore, the age of the mixture in the cuvette at the point of observation during the shot is the theoretical dead-time (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 but continues to age.

Observation of the mixture in the cuvette after the stop, therefore, represents a time course of the reaction from the dead-time onward.

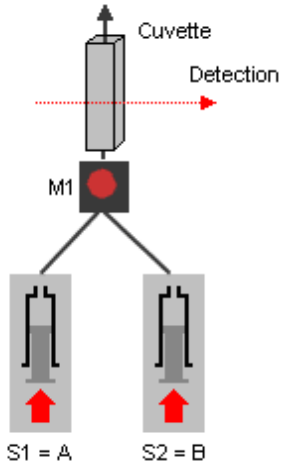


Figure 20: A Simple Stopped-Flow Experiment

Figure 20 shows a schematic of a simple stopped-flow experiment. In the experiment, reagents A and B are pushed into a mixer where they react to form product C. Reagent A has a strong absorbance, while reagent B and product C do not. Therefore, as the reaction proceeds, the absorbance of a mixture of A and B should decrease as A is diminished. Figure 21 shows a cartoon of the experiment over time. Note the two stages of the experiment as described above.

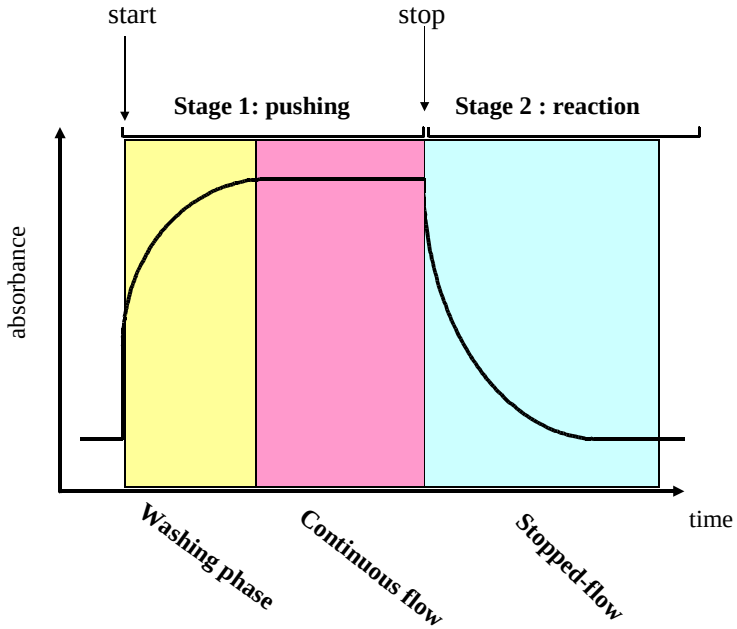


Figure 21: Stopped-Flow Experiment Time course

IMPORTANT: In every stopped-flow experiment enough liquid must be pushed to wash the flow path and cuvette and achieve a steady-state condition. If this is not done, all samples are contaminated and the resulting signal trace does not represent the true time course of the reaction! Typically the rate constant measured would be ok but a loss of amplitude may be observed.

6.2 General Advice for Stopped-Flow Experiments

6.2.1 Achievement of fastest dead times

The dead time of a stopped-flow experiment is defined as the time before which observation of the mixture is impossible. Ideally, the dead-time depends only on the flow rate of the mixture exiting the last mixer and the volume between the last mixer and the cuvette.

Nevertheless, an effective stopped-flow experiment depends on a number of other inter-related factors, such as an adequate signal, complete washing of the cuvette, and prevention of cavitations and prudent use of valuable reagents. The relationships between these factors require careful consideration and experimentation. Compromises are often necessary to achieve successful stopped-flow experiments. Some of the most common actions that can be taken to achieve the fastest dead times, and their consequences, are shown in Table3

Table3 – Common Actions to Achieve Fastest Dead Times

IN ORDER TO...	ACTION	BUT THE RISK FOR VERY HIGH FLOW RATE IS...
Lower Dead Times	Increase Flow Rate to high values	cavitations
		overuse of reagent
		inadequate washing
	Decrease Cuvette volume	loss of signal

6.2.2 Washing

As mentioned in section 6.1, it is necessary to completely wash the flow path between the last mixer and cuvette and the cuvette itself during the shot. This ensures that the signal observed after the shot is only of the recently mixed samples. To accomplish this, a sufficient volume of the mixed samples needs to pass through the cuvette during the shot. This volume varies with flow rate, viscosity, and composition of the sample. Using µSFM we recommend to push a minimum of 24µl (total volume) to ensure a good washing of the system.

Cavitation occurs when turbulence creates regions of low enough pressure in a liquid that a "cavity" is formed. This cavity fills with the liquid's vapor. These cavities collapse incompletely, leaving behind small bubbles of vapor 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 for a given flow rate. De-gassing of solutions decreases the probability of cavitation by eliminating gas and lowering the total vapor pressure available to fill the cavities.

6.2.3 Signal amplitude

Signal amplitude is generally proportional to the path length of the cuvette and the concentration of the signal-generating reagent. An increase in signal can then be accomplished by an increase in cuvette path length or an increase in the concentration of the reagent. However, the researcher may be limited by practical concerns such as value of sample, viscosity of sample, dead-times, inherent limitation of the signal (such as inner-filter effect) and sample precipitation. As with the achievement of the fastest dead times, compromises may be necessary to achieve a successful stopped-flow experiment. Table4 shows some of the most common actions that can be taken to improve signal amplitude and their consequences.

Table4 – Common Actions to Improve Signal Amplitude

IN ORDER TO...	ACTION	BUT THE CONSEQUENCE COULD BE...
Increase Signal	Increase Cuvette Path Length	overuse of reagent
		increased dead time
		Inadequate washing
		Inner-filter effect (fluorescence)
	Increase Reagent Concentration	overuse of reagent
		Increased viscosity causing cavitation
		Increase viscosity causing inadequate washing

7 TEST REACTIONS IN STOPPED-FLOW MODE

7.1 Reduction of 2,6-Dichlorophenolindophenol by Ascorbic Acid

A complete description of the reduction of 2,6-dichlorophenolindophenol (DCIP) by ascorbic acid (AA) 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 discoloration. 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. The fast reaction at acid pH can be used to measure the dead time of the µSFM instrument. The slow reaction at basic pH is used to check the quality of the stop, evaluate the washing of the observation cell, and test the variable ratio mixing capabilities. The following sections describe the use of this reaction for testing and exploring its capabilities.

7.2 Evaluation of the Dead Time

The dead time of the SFM can be measured using both the fast and slow reduction reactions of DCIP. An example dead time evaluation is shown in this section. As discussed in section 6.2.1, the dead time of a stopped-flow experiment depends on many factors besides simply the flow rate and cuvette volume. The technique presented here may be adapted to evaluate the dead time under many experimental conditions.

Experimental Conditions:

Slow reaction:

Syringe 1 (10mL)	10 mM sodium ascorbate pH 9
Syringe 2 (10 ml):	1 mM DCIP
Wavelength:	524 nm
Cuvette:	µFC-08
Detection method:	Absorbance
Total Flow Rate (ml/s):	1.2 ml/s

Fast reaction:

Syringe 1 (10mL)	10mM ascorbic acid pH 2
Syringe 2 (10 ml):	1 mM DCIP
Wavelength:	524 nm
Cuvette:	µFC-08
Detection method:	Absorbance
Total Flow Rate (ml/s):	1.2ml/s

The dead time of the experiment is the age of the solution at the observation point. In other words, it is the time for the mixed solution to go from the centre of the last mixer to the observation point. The dead time depends on many factors besides simply the total flow rate and the cuvette volume. But because the hydrodynamics phenomenon is difficult to be taken into account for software calculations, a slight difference between the estimated dead time given by Bio-Kine and the real dead time may be observed.

In basic pH conditions, the reaction is considered as a slow reaction. Therefore, the amplitude of the signal at the stop can be assimilated to the total amplitude of the reaction. In other words the change in absorbance between the mixing point and the observation point is negligible.

In acidic conditions the reaction is much faster, and the change in absorbance between the mixing point and the observation point cannot be neglected. The amplitude of the signal at the stop corresponds to the age of the solution. So knowing the amplitude of the signal and its rate constant in addition to the total amplitude measured with the slow reaction results, it is possible to determine the real dead time of the experiment.

A simplified drawing of the method used for the dead time calculation is given in Figure 22.

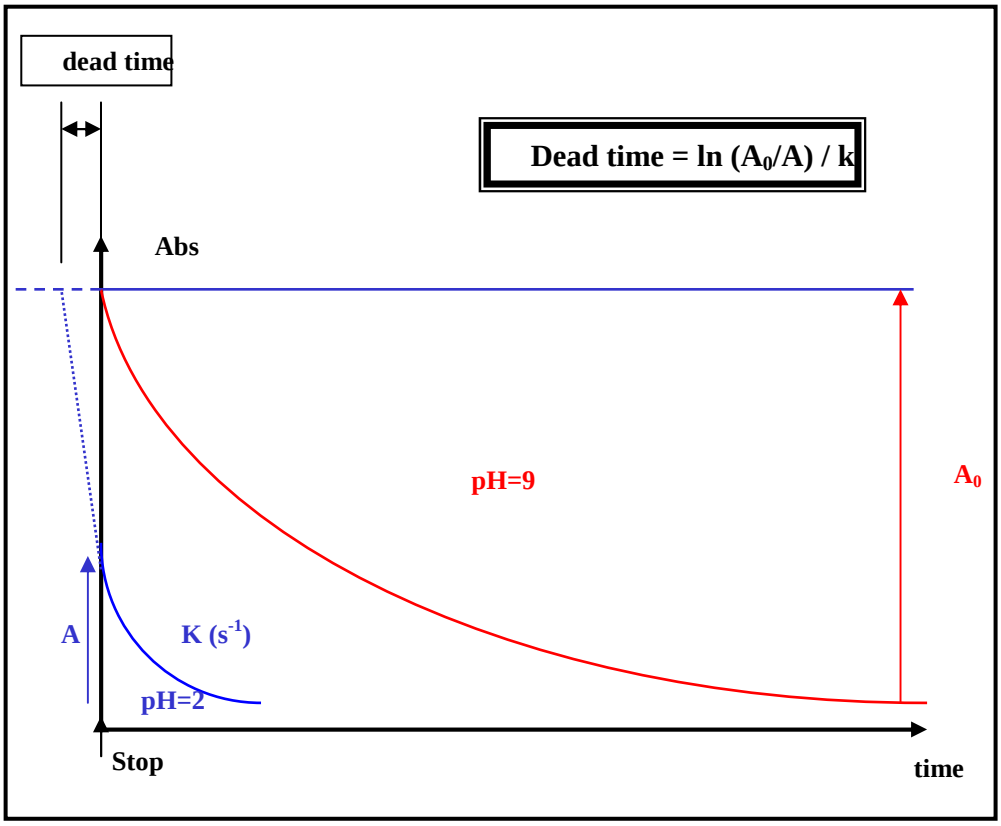


Figure 22: dead time evaluation

Configuration of Bio-Kine:

- Load Bio-Kine.
- Enter the **Install, device** menu and select the stopped-flow device and its USB port.
- Configure the stopped-flow device and the syringe sizes.
- Initialize the syringes, and then fill the syringes with water.
- Push some water into the cuvette and do the absorbance reference.

Slow reaction at pH=9

- Click on the Mixing Sequence button, then on the SFM Options button: select a 2 ms lead time. Validate by clicking the OK button.
- Edit the driving sequence shown in Figure 23 .
- In your acquisition software choose to perform one measurement every 1 ms during 4 seconds.
- Run the sequence.
- The data obtained is shown in Figure 24.

Fast reaction at pH=2

- Click on the Mixing Sequence button.
- Edit the driving sequence shown in Figure 23.
- In your acquisition software choose to perform one measurement every 10 µs during 0.08s.
- Run the sequence.
- The data obtained is shown in Figure 25.

Dead time calculation
From the kinetics at pH =9 we get A_0 = 0.077 A.U.
From the kinetics at pH =2 we get A = 0.063 A.U and k = 207 s^{-1}.
Therefore, **the real dead time is 1 ms.** The estimated dead time given by Biokine software was 1.6 ms.

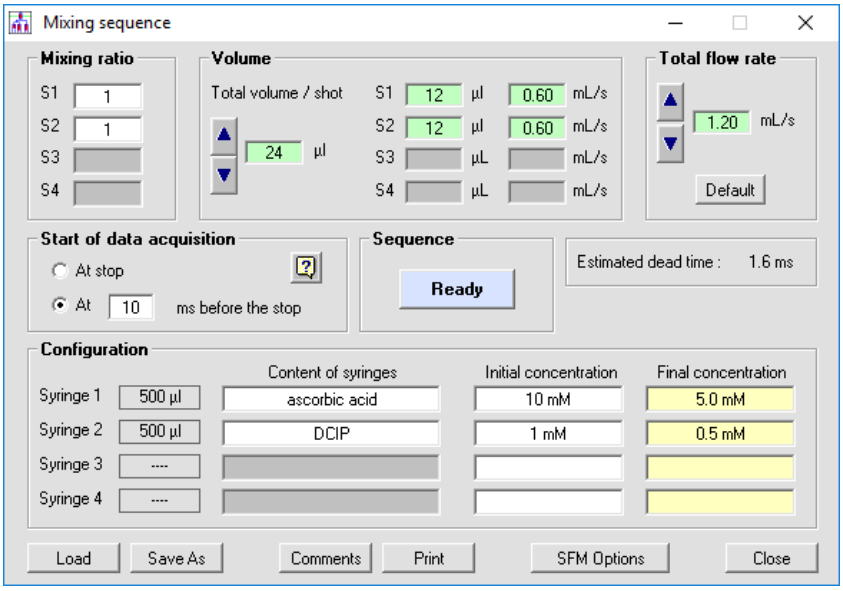


Figure 23: driving sequence for the reduction of DCIP.

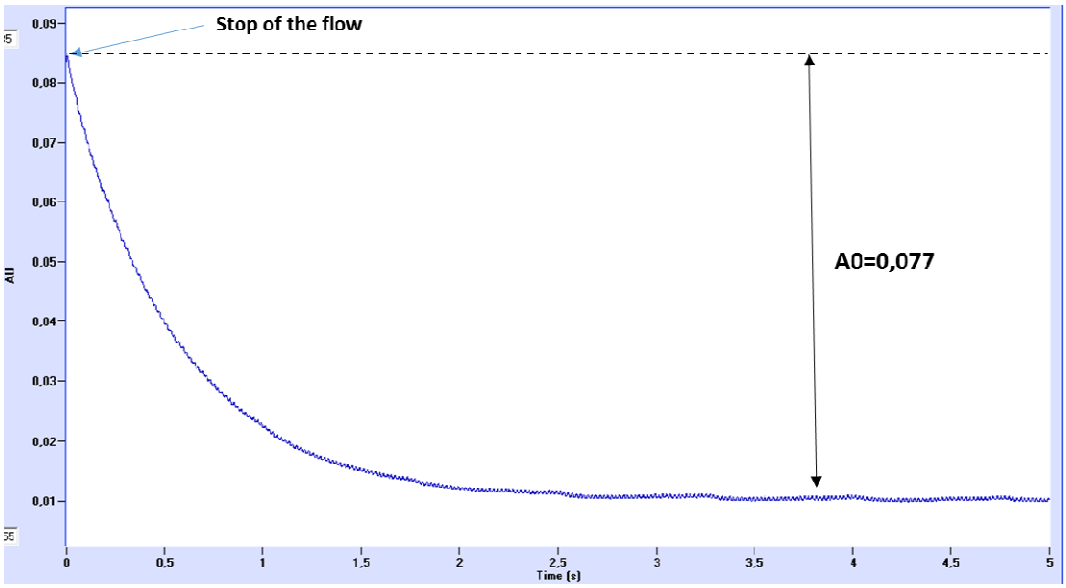


Figure 24: reduction at pH=9.

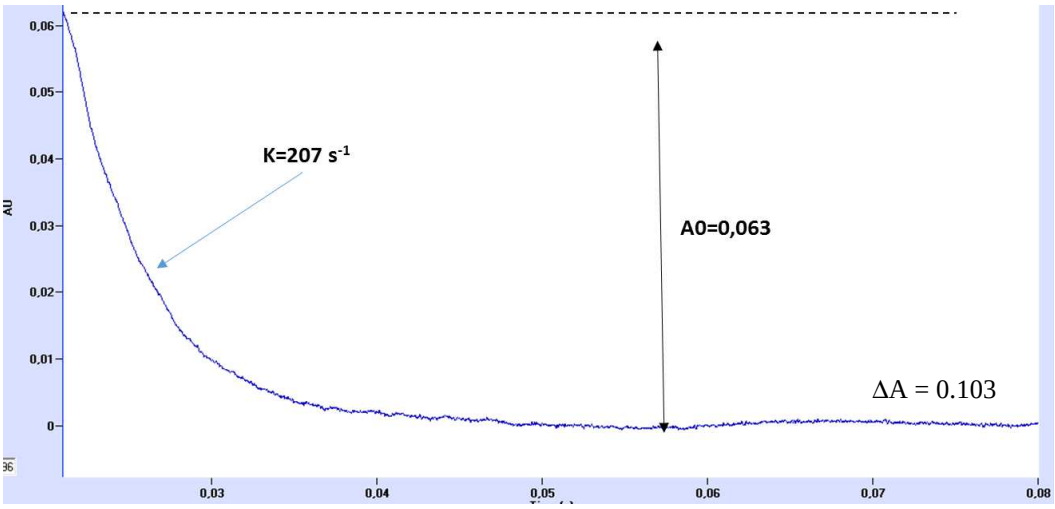


Figure 25: reduction at pH=2.

7.3 Example of experiments using a 200 µl stock solution

The reaction described in section 7.1 is used to illustrate this section. Ascorbic acid 2.5mM is considered as the limited stock and we only have 200µl to do a maximum number of shots. The MPS-70/2 and the detection system are supposed to be connected and configured correctly in the software. As a minimum of 24µl is recommended per shot we will use 12µl of each sample doing a 1:1 mixing ratio.

The sequence shown in Figure 26 will be used for the series of shots.

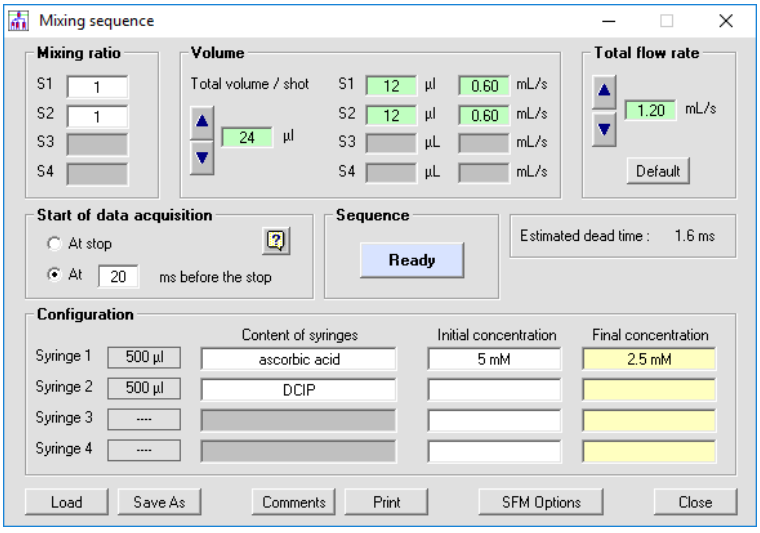


Figure 26 : Example of sequence using 12 µl of each sample

Step 1: Preparation of the µSFM

- For both syringes click on the Load position buttons so the pushing blocks go to the loading position.
- Open the temperature jacket windows and install the empty glass syringes in the instrument

- Move the pushing blocks up from the MPS-70/2 or using Bio-Kine to reach uppermost position using Manual speed n°1 or 2 until where you get a contact between pushing block and the piston of the syringe and you hear a mechanical vibration (higher speed can be used during the long course of the pushing block but be sure you reduce speed just before reaching the piston to limit the stress applied to the glass syringe when it reaches the top)
- Click on Reset buttons so 0 is indicated as amount of solutions in each syringe.

Step 2: Absorbance reference

- For both syringes click on the Load position buttons so the pushing blocks go to the loading position.
- Remove the glass syringes and fill them with 400-500 µl of water using the loading adapters.
- Install the glass syringes in the µSFM
- Move the pushing block up so they get in contact with pistons and empty the syringes half way so you can see water going out of exit tube of the hard-stop. At this point the cuvette is supposed to be filled of water
- Adjust PMT voltage and do the absorbance reference in the software.

Step 3: Loading solutions

- For both syringes click on the Load position buttons so the pushing blocks go to the loading position.
- Close the hard-stop using the Open/Close button.
- Remove the glass syringes.
- Install the loading adapter on the syringe 1 and pump the 200µl from the stock vial making sure not to load any bubble. Remove the adapter.
- The same way load DCIP in syringe 2. As this is not the limiting solution you only need to make sure you pump at least 200 µl. For this example we load 300µl.
- Install the syringes in the instrument.
- Open the hard-stop using the Open/Close button.
- Select Manual speed n°5. In syringe one we have 200 µl so enter 225 µl in the GO TO box so the pushing block moves rapidly up. Select Manual speed n°1 and select 200µl in the GO TO box so the pushing blocks moves to the exact position. Repeat the same procedure for syringe 2 using 325 µl and 300 µl for the two steps.
- At this stage the software must displayed 200 µl and 300 µl as amount of solutions in the µSFM.

Step 4: Mixing sequence

- Edit the stopped-flow sequence shown in Figure 26.
- Click on the READY button. Software indicates that a maximum of 16 shots can be done (corresponding to 12µlx16=192 µl, 8 µl would remain in the machine at the end of the series in this example).
- Activate the Auto-Save button and select the repertory where you want to save all data
- Select a 50 µs sampling period during 0.5 ms.

Step 5: Data collection

- Run the 16 shots automatically.
- Load the traces in the analysis menu.

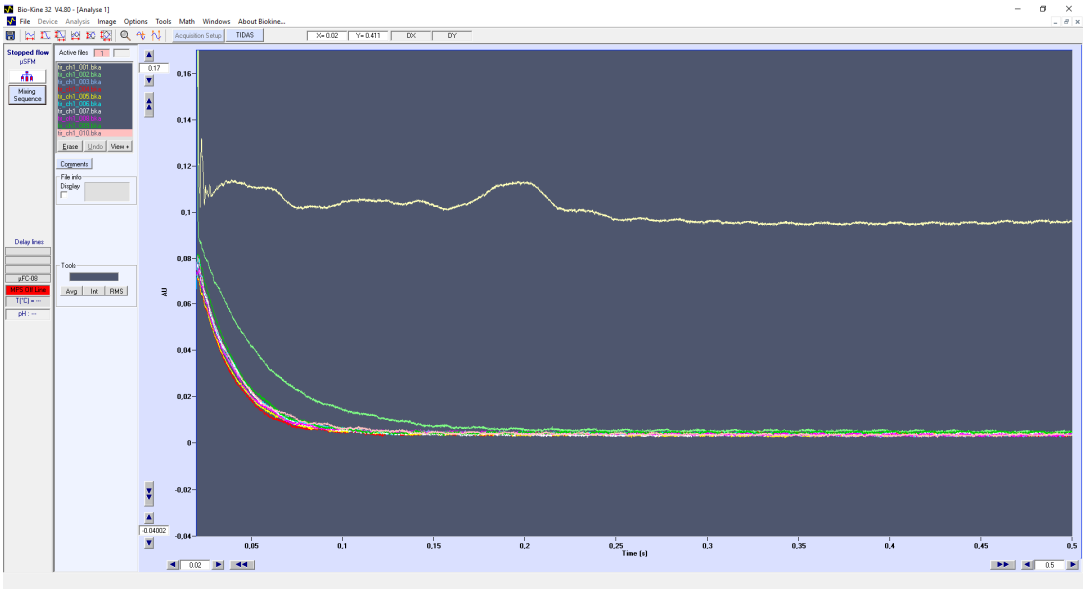


Figure 27 : the 10 first shots recorded

Step 6: data analysis

- The first two shots are clearly not good (traces yellow and green above). They correspond to the 2x12µl (so 24µl) required to fill the priming line. In case you were using 8µl of sample per shot then the 3 first shot would be discarded (corresponding to 3x8=24µl)
- All other traces (from 3 to 15) can be fitted using a single exponential: rate constant and amplitude are reported below

Shot	k (s-1)	amplitude		k (s-1)	amp
1	bad		9	48	0,069
2	bad		10	48	0,073
3	48,4	0,071	11	49	0,071
4	53	0,069	12	52	0,068
5	50	0,068	13	50	0,073
6	49	0,074	14	47	0,074
7	47	0,073	15	46	0,071
8	51	0,07	16	47	0,074
average	48,9571429				
std deviation	2,04439733				

It shows that 14 shots can be done from a 200 µl stock in this condition.

7.4 Refolding experiment using a 50 µl stock solution

Only 50µl of 0.5 mg/ml lysozyme in GndHCl 6M is available for this experiment. The refolding experiment can be initiated by a 10 times dilution of the unfolded protein so final concentration of protein is 0.05 mg/ml in GndHCl 0.6 M. The MPS-70/2 and the detection system are supposed to be connected and configured correctly in the software. We will use 31 µl or solutions (> to the recommended 24 µl) so only 3µl of protein is used at each shot.

Please note that the real injected volume is 3.2 µl (=32/10) of protein and 28.8 µl of water (but software displays 3 and 29 as decimals are not shown)

The sequence shown in Figure 28 will be used for the series of shots.

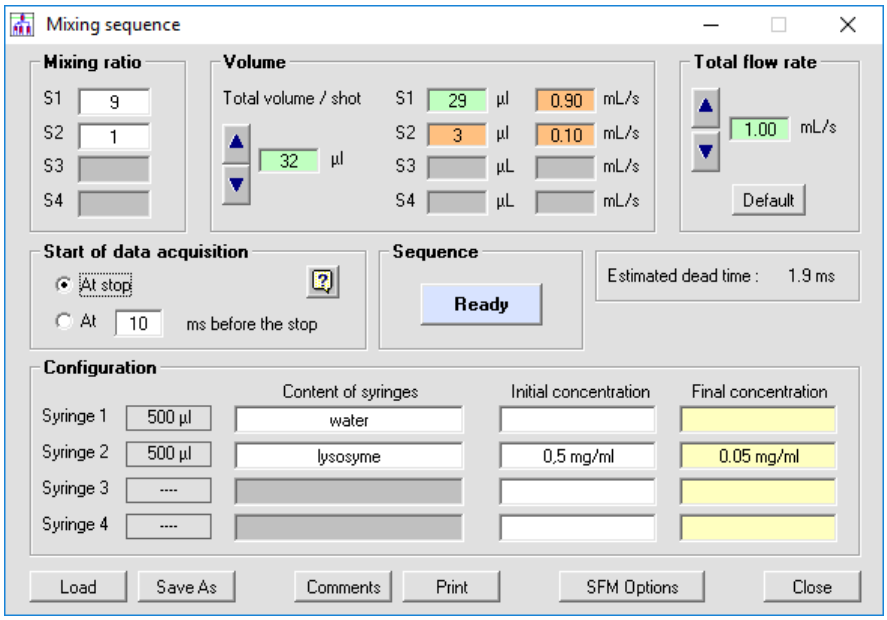


Figure 28: Example of sequence using 3 µl of protein

Step 1: Preparation of the µSFM

- For both syringes click on the Load position buttons so the pushing blocks go to the loading position.
- Open the temperature jacket windows and install the empty glass syringes in the instrument
- Move the pushing blocks up from the MPS-70/2 or using Bio-Kine to reach uppermost position using Manual speed n°1 or 2 until where you get a contact between pushing block and the piston of the syringe and you hear a mechanical vibration (higher speed can be used during the long course of the pushing block but be sure you reduce speed just before reaching the piston to limit the stress applied to the glass syringe when it reaches the top)
- Click on Reset buttons so 0 is indicated as amount of solutions in each syringe.

Step 2: priming with water

- For both syringes click on the Load position buttons so the pushing blocks go to the loading position.
- Remove the glass syringes and fill them with 400-500 µl of water using the loading adapters.
- Install the glass syringes in the µSFM
- Move the pushing block up so they get in contact with pistons and empty the syringes half way so you can see water going out of exit tube of the hard-stop. At this point the cuvette is supposed to be filled of water

Step 3: Loading solutions

- For both syringes click on the Load position buttons so the pushing blocks go to the loading position.
- Close the hard-stop using the Open/Close button.
- Remove the glass syringes.
- Install the loading adapter on the syringe 2 and pump the 50µl from the stock vial making sure not to load any bubble. Remove the adapter.
- The same way load 500µl of water in syringe 1. (as we will do 1:9 mixing ratio we need to load enough so it does not limit the number of shots). Install the syringes in the instrument.
- Open the hard-stop using the Open/Close button.
- Select Manual speed n°5. In syringe one we have 50 µl so enter 75 µl in the GO TO box so the pushing block moves rapidly up. Select Manual speed n°1 and select 50µl in the GO TO box so the pushing blocks moves to the exact position. Repeat the same procedure for syringe 2 using 525 µl and 500 µl for the two steps.
- At this stage the software must displayed 50 µl and 500 µl as amount of solutions in the µSFM.

Step 4: Mixing sequence

- Edit the stopped-flow sequence shown in **Figure 28**.
- Click on the READY button. Software indicates that a maximum of 15 shots can be done (corresponding to 3.2µlx15=48 µl, 2 µl would remain in the machine at the end of the series in this example).
- Activate the Auto-Save button and select the repertory where you want to save all data
- Select a 500 µs sampling period during 2 s.
- Adjust the sensitivity of the detector.9

Step 5: Data collection

- Run the 15 shots automatically.
- Load the traces in the analysis menu.

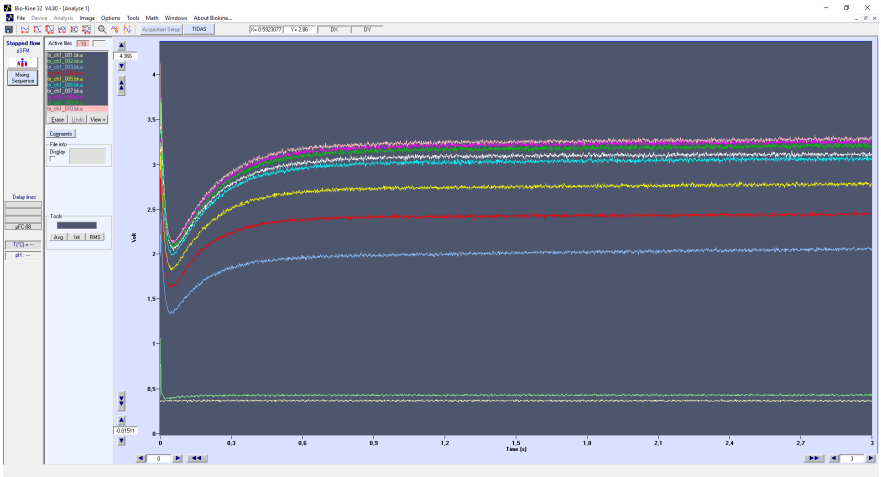


Figure 29 : the 10 first shots recorded

Step 6: data analysis

- The first two shots are clearly not good (traces yellow and green above). The shots 3 to 6 can be fitted with a double exponential but results are not stable. From Shot n°7 the fits are stable. So it means 6 shots in total cannot be used so corresponding to an effective dead volume of 3.2x6=19 µl.
- All other traces (from 3 to 15) can be fitted using a double exponential: rate constant and amplitude are reported below

Shot	k1 (s-1)	k2 (s-1)
1	bad	bad
2	bad	bad
3	bad	bad
4	bad	bad
5	60,9	5,8
6	67	5,4
7	58,2	5,6
8	55,6	5,2
9	54	5,1
10	59,5	5,6
11	56	5,7
12	57,3	5
13	59,2	5,1
14	58,3	5,2
15	53,3	5,1
	56,82222	**5,288889**
-15)	2,226981	0,266667

It shows that 9 shots can be done from a 50 µl stock in this condition !

8 TECHNICAL SECTION

8.1 Solvent compatibility

Any solution used in the SFM system will be in contact with the following materials:

- **PEEK** (flow lines, mixer)
- **Teflon** (piston, cuvette holder)
- **Viton** (o-rings)
- **Quartz** (cuvette)

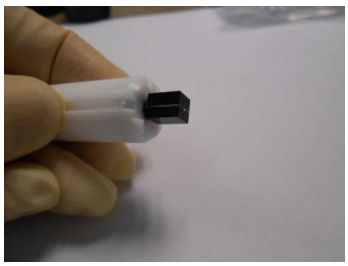
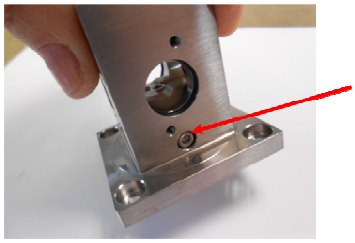
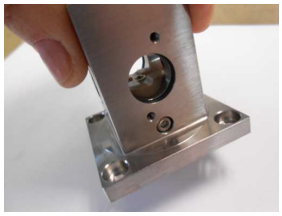
PEEK has excellent chemical resistance to organic and inorganic liquids.
Only concentrated acids like sulfuric and nitric can attack it.
Methylene chloride, DMSO, and THF has some swelling effect, should be used under control.
Maximum operating temperature: 100°C.
Teflon is chemically inert
Viton parts – these parts are most vulnerable chemically
Other materials are available upon request (EPDM, Nitrile, Isolast…). Please contact our commercial service for enquiries.

We highly recommend Kalrez o'rings with an organic solvent.

Please refer to: http://www.superseal.hu/al/catalogs/busak+shamban/isocatal.pdf **for chemical compatibility guide.**

Using a solvent with a non appropriate O-ring material will be not considered under warranty by Bio-Logic.

8.2 Mixer Removal, Examination and Replacement

Step 1 : Remove the observation head from the µSFM body	
Step 2 : Unscrew the cap of the observation head and remove the µFC-08	
Step 3: Remove the two screws on the side of the observation head	
Step 4 : Push out the mixer holder from the observation head from the top	 
Step 5 : Insert the new mixer holder in the empty observation head and secure the position by putting back the two screws removed at step 3. Install back the cuvette and the cap on the observation head	

When changing the mixer we recommend running the µSFM first with water to make sure everything is mounted properly and no leakage is observed.

8.3 Lubrication

The drive screws should be lubricated periodically (once per year) with mineral oil. Access to the drives screws is obtained by removing the cover in front of stepping motors.