Rapid Kinetics and Spectroscopy instruments

Page also available in **PDF** format

Stopped-Flow/Quench-Flow

Instruments on the move with your experiments

Since 1983, Bio-Logic develops and manufactures Rapid-Kinetics instrumentation, associating quality with innovation to produce instruments a step above the rest.

The results of our efforts produced the world's first rapid filtration system and commercial stepping-motor driven stopped-flow.

Over 300 Bio-Logic rapid kinetics **instruments** have been **installed worldwide**. The reason for our success lies in the quality of our instruments.

We understand that investing in instrumentation is not a trivial matter. What arrives in your lab today is going to be part of your research program for years to come. Our instruments are designed to last and grow with your research program. We are dedicated to supporting our instruments, be it the initial or the most recent instrument to come from our factory. Our first instruments are still in use today !

Over the years our instruments have grown and changed, fueled by customer / manufacturer interaction.
The Bio-Logic development team is dedicated to listening to client feedback and seeing which direction research is moving. Our instruments evolve with client needs and in anticipation of them.

This tradition continues on today with our current line of rapid mixing instruments.

The Bio-Logic SFM

The Bio-Logic Stopped-Flow Modules (SFM) are a fusion of technology, design and innovative control. Each element of the fusion compliments the others making the Bio-Logic SFM most powerful and flexible rapid mixing instruments existing today.

Principle of operation

All Bio-Logic SFM instruments are based on our innovative use of stepping-motors to drive the syringes.
With modern microprocessor techniques, stepping-motors offer control and power unmatched by any other fast driving system.

Each stepping-motor is controlled by one microprocessor enabling micro-positioning of the motor's rotor with an accuracy equivalent to 6400 steps per motor revolution. This corresponds to a volume precision of 30 to 200 nanoliter per micro-step (depending on syringe installed). The damping

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

The high performance of each SFM and the high speed of the stepping-motors is achieved because of the quality of the power-supply.

Each Mixer Power-Supply (MPS) unit contains independent power-supplies for each syringe, each driven by their own microprocessor. The sequence of impulses sent to the stepping-motors is stored in the memory of each motor board. One main microprocessor board synchronizes all the power supplies, and communicates with the computer via a serial interface.

Stepping motors advantages
The use of stepping-motors and a high quality power supply, gives each SFM the following advantages.

- **Completely Variable Mixing Ratios:**
  Because the flow rate and volume delivered by each syringe is independent of the others, mixing ratios are not fixed by syringes sizes. Mixing ratios are defined by the user, on-line.
- **Precise Flow Rate and Volume Control:**
  The flow rate and volume delivered by each syringe is chosen by the user, not defined by the instrument.
- **Reproducibility and Regularity:**
  The reproducibility and regularity of the linear translation of the syringes and the absence of pressure artifact allow optical recording during liquid flow.
  This capability greatly facilitates the determination of the initial phase of the reaction being monitored during stopped-flow experiments and makes the equipment suitable for accurate, continuous flow experiments.
- **No Overpressure Artifacts:**
  Syringe movements are completely controlled by microprocessor and can be halted in a fraction of a millisecond. This abolishes the need for a stop syringe during stopped-flow experiments and eliminates the stop artifact present in most conventional stopped-flow systems.
- **Mixing of viscous solutions:**
  Syringe velocity is programmed and thus is independent of the solution viscosity. Very asymmetric mixing such as pure glycerol against water can therefore be achieved.

Mixer technology
The mixer is the heart of a stopped-flow instrument. Although being often referred as "T-mixer" a straight T-connection between 3 tubes is capable of mixing two solutions only in limited cases (aqueous solutions, symmetrical 1:1 mixing, limited range of flow rate). The mixer used in the Bio-Logic Stopped-Flow are all based on the "ball mixer" technology which is a proven design for creating turbulence in the most stringent conditions. Because of it the Bio-Logic Stopped-Flow can operate in a wide range of fluidic conditions such as : high dilution ratio (up to 1:100), high range of flow rate (from 1 to 20 mL/s), mixing of highly viscous solutions against water (viscosity up to 2000 centipoises or 2 pascal.second have been achieved).

Applications

The application of the Bio-Logic SFM family of instruments is large and evolving. We have made our instruments as versatile as possible so that they are not the limiting factor in your research.

Here is a sampling of how our instruments have been used:

	Measured by:
Stopped-Flow Experiments	• UV/VIS Absorbance • Light Scattering • Fluorescence
• Protein Folding • Conformational Changes	

- Substrate Binding
- Enzyme Kinetics
- Substrate Transport in Vesicles

- Circular Dichroism
- Fluorescence Anisotropy
- FTIR
- X-ray scattering
- Conductivity
- Mass Spectrometry

Quench-Flow Experiments

Measured by:

- Substrate Binding
- Enzyme Kinetics
- Protein Folding Kinetics
- Conformational Changes
- Second Messenger Studies

- Radioactive labeling
- Hydrogen/Deuterium Exchange followed by NMR or Mass Spectrometry

Freeze Quench Methods

Measured by:

- Trapping of kinetic intermediates by ultra-fast freezing

- Solid-State NMR
- EPR
- X-ray scattering
- EXASF

The range of application of our instruments extends beyond the several examples listed here and is growing every day !

If you have questions about your specific application and would like to know how it can be supported by a Bio-Logic rapid-kinetics instrument, **contact us** !. We would be happy to discuss your application with you.

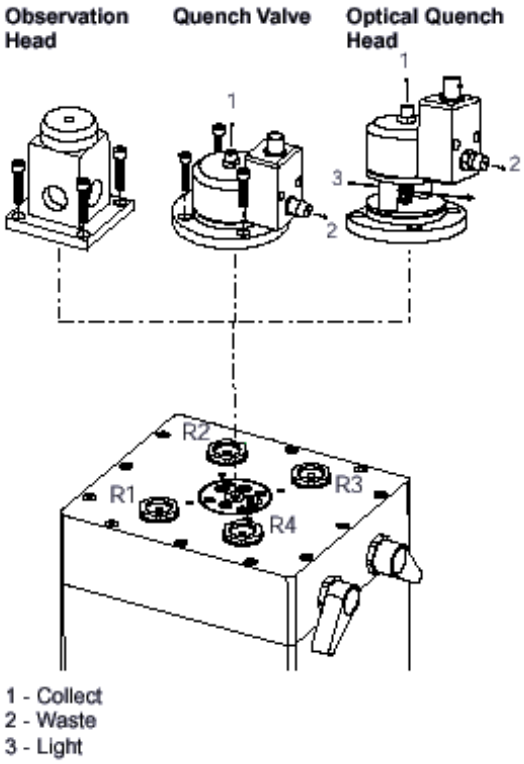
Mechanical Design

A Characteristic Design

Each SFM is designed to be a versatile rapid-mixing instrument. It is not confined to a single functionality, but adaptable and expandable to a variety of rapid-mixing experiments.

This is due to the "open" mechanical design of an SFM. The syringes and motors are housed in a body that accepts attachments created for different types of experiments. Every SFM can be configured for Stopped-Flow, Quench-Flow, Optical-Quench and many other experimental conditions.

Switching from one mode of operation to another is just a matter of replacing one attachment with another. This operation requires only a few minutes and no specialized tools.

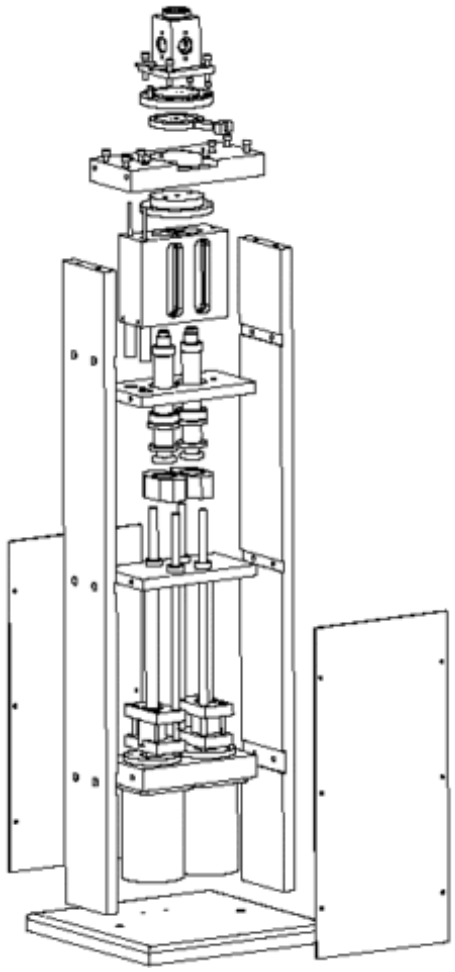


Design Features

Vertical mounting of the SFM syringes facilitates purging of bubbles which might interfere with experiments. Bubbles can be evacuated from the instrument with a few pushes of each drive syringe.
The syringes, valves, and observation chamber can be very uniformly thermoregulated through the use of a circulating temperature bath connected to the SFM. The coolant flows through two internal circuits: one around the injection and reservoir syringe ports and the other through the valve block and observation head. This thermoregulation prevents the occurrence of temperature artifacts on a very wide temperature range, and permits rapid-kinetics studies even at low temperatures.

Quality Construction

Every SFM is carefully constructed of high quality materials. The parts in contact with the sample and the buffers are all machined out of materials selected for their inert characteristics



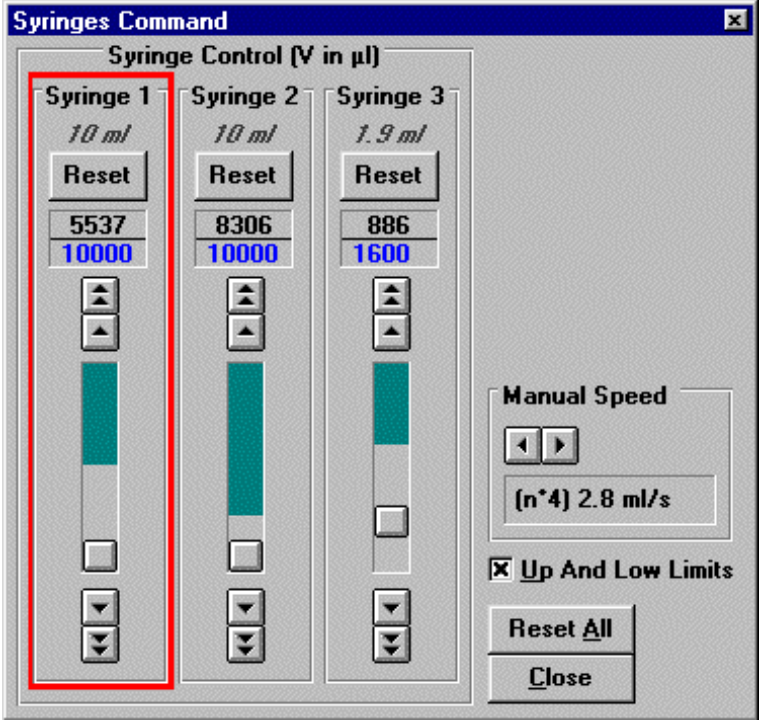
Instrument control

The Mixer Power Supply (MPS) that controls each Stopped-Flow Module (SFM) is programmed by computer using the MPS32 software common to all Bio-Logic SFM instruments.

The MPS32 software operates under the latest version of Windows environment (Win95, Win98, WinME, WinNT, Win2000, WinXP)

The software is easily configured for any of our SFM instruments. Through the use of various menus and windows, the MPS32 software allows the user to:

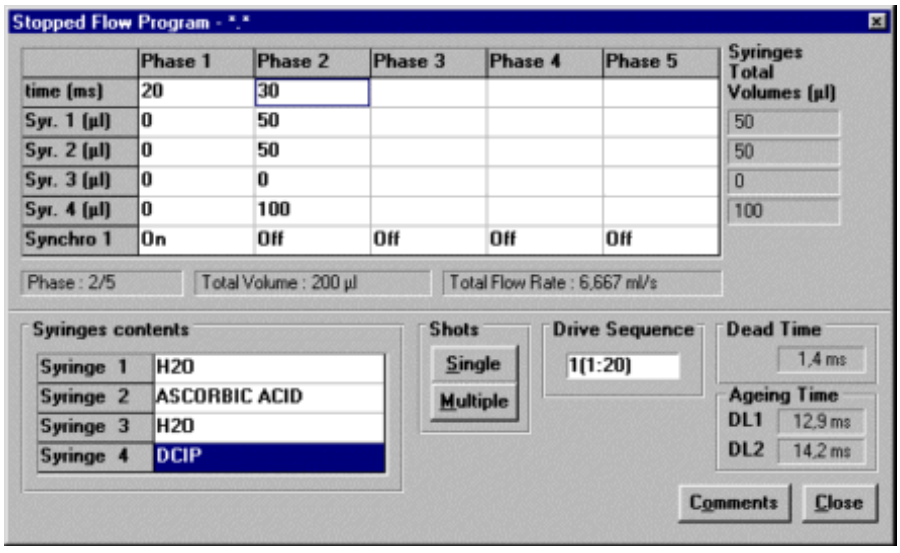
- know the volume of the solution in each syringe
- perform manual or automatic movement of the syringes
- create a driving sequence with complete control of the volume delivered and flow rate of the syringes
- save or recall driving sequences
- start or stop experiments
- program the synchronization pulse used to trigger the acquisition system
- load the spectrometer data acquisition software: Bio-Kine



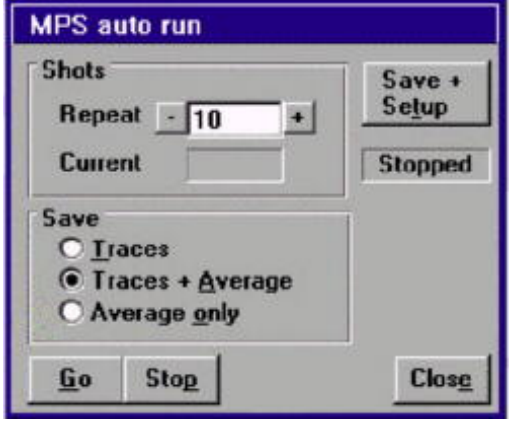
The MPS32 software makes very accurate estimations of expected dead and ageing times based on the current driving sequence, cuvette and delay lines used.

Dead and ageing time estimations are updated anytime the driving sequence or configuration parameters are changed.
These estimations are only possible because the user has complete control over syringe flow rate and volume delivered.

Up to 20 phases can be included in a driving sequence, allowing experiments involving complicated steps and washings to be executed with ease.



Automation of experiments is possible through the MPS32 software.
The MPS32 software can be used in conjunction with the spectrometer Bio-Kine acquisition and analysis software to automate experiments and acquisitions. Multiple shot averages can be made with a single button push.



SFM-20

SFM-20 with its MPS-20 power supply. **Click** to enlarge

The SFM-20 is Bio-Logic's entry level rapid mixing instrument. It is the most efficient instrument on the market in terms of sample economy.

The SFM-20 has been designed to fulfill the requirements of users that need only a basic 2-syringe instrument and are not willing to compromise on priming volume and sample economy. Experiments using in total only a few hundreds of microliter of reactants are within the capability of the SFM-20.

Even though it is our "basic" mixing instrument, its performance is on par with our 3 and 4-syringe instruments.

High precision drive mechanics allows dilution ratios exceeding 1/100.

The standard **optical head** is associated to a large choice of flow **cuvettes**. Time course of the reaction is followed by optical methods, see the **Spectrometer** pages for description of our dedicated fast kinetics UV/Vis spectrometers.

SFM-20 detail. **Click** to enlarge

Animated SFM-20 click **here** (316 kb)

Number of syringes	2
Driving mechanism	One stepping-motor per syringe
Number of mixer	1
Minimum injection volume per syringe	2-15 µl depending on installed syringe
Observation cuvette	from 0.8 to 10 mm light path (click **here** for details)
Priming Volume	50µl/syringe
Minimal dead time	1.5 ms (with FC-08 cuvette) - 0.4 ms with the **microcuvette**
Flow-rate range per syringe	0.003 - 5.8 ml/s depending on installed syringe
Variable ratio range	Continuously variable from 1/1 to 1/200
Trigger	Programmable trigger for data acquisition and synchronization of accessories
Material	PEEK & glass
Syringe volume	1 - 10 ml
Dimensions	200 x 140 x 802 cm
Weight	11 kg

SFM-300/400

SFM-400 with its MPS-60 power supply. **Click** to enlarge

The SFM-300/400 instruments are the latest evolution in our 3 and 4-syringe instruments. They offer the capability to carry out complex, multi-mixing experiments with the lowest ageing and deadtimes.

They are still the first and only multi-mixing instruments that can be used as both optical stopped-flow and quench-flow instruments.

High precision drive mechanics allows dilution ratios exceeding 1/100.

Stopped-Flow configuration is achieved by installing the **optical head** associated to a large choice of flow **cuvettes**. Time course of the reaction is followed by optical methods, see the **Spectrometer** pages for description of our dedicated fast kinetics UV/Vis spectrometers.

SFM-400 in SF mode. **Click** to enlarge

Quench-Flow mode is obtained by installing ageing loops between the mixers and the programmable Quench-Flow valve at the exit of the instrument.

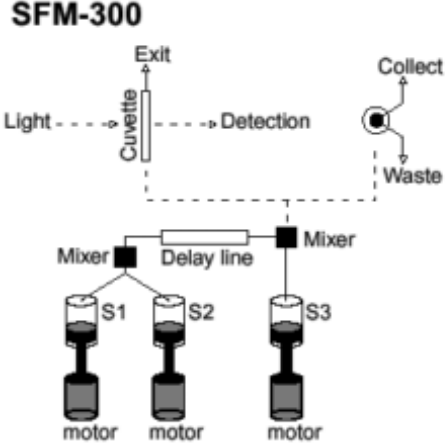
SFM-400 in QF mode. **Click** to enlarge

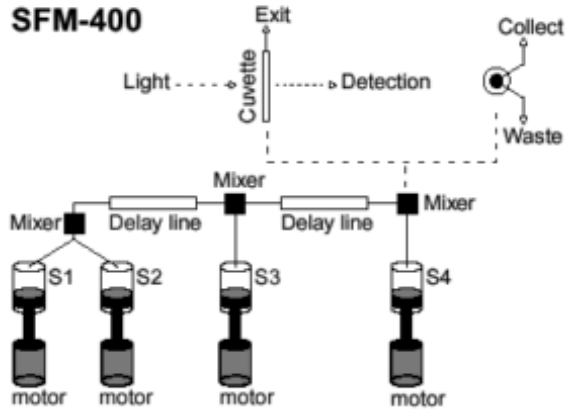
SFM-400 in titration mode. **Click** to enlarge

Number of syringes	3 (SFM-300) or 4 (SFM-400)
Driving mechanism	One stepping motor per syringe
Number of mixers	2 (SFM-300) or 3 (SFM-400)
Ageing volume between two mixers	adjustable from 25 to 1000 µl
Minimal dead time	0.6 ms with FC-08 cuvette - 0.25 ms with the **microcuvette**
Minimum injection volume per syringe	10 – 30 µl depending on installed cuvette
Observation cuvette	from 0.8 to 10 mm light path (click **here** for details)
Flow-rate range per syringe	0.010 – 10 ml/s depending on installed syringe
Variable ratio range	Continuously programmable from 1/1 to more than 1/100
Minimal ageing time in Quench-Flow mode	< 2 ms
Trigger	Programmable trigger for data acquisition and synchronization of accessories
Material	PEEK
Syringe volume	1.9 – 10 ml
Dimensions	200 x 197 x 522 cm
Weight	13 kg (SFM-300) or 14 kg (SFM-400)

SFM-400 in SF mode using an umbilical connection. **Click** to enlarge

SFM-300 and Mix & freeze accessory. **Click** to enlarge





SFM-300 and SFM-400 diagrams for Stopped-Flow and Quenched-Flow mode

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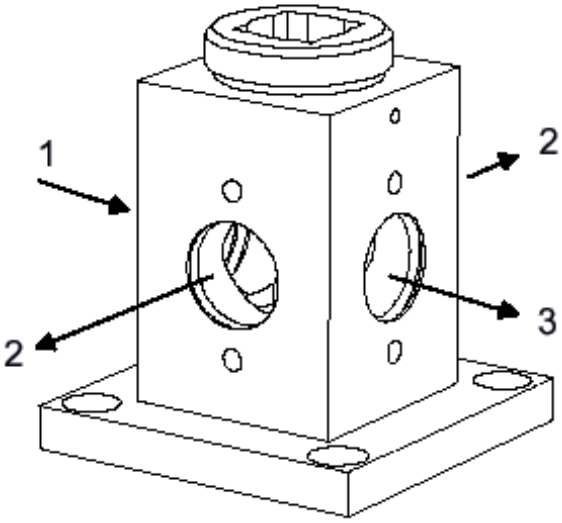
Stopped-Flow optics

The Observation Head

The observation head is the interface between the Stopped-Flow modules SFM-20/300&400 and the recording spectrometers for optical Stopped-Flow experiments. The observation head houses the flow cell (cuvette). A large variety of **flow cells** have been developed to optimize the optical conditions specific to each mode of observation. Installation and exchange of observation cell is an easy operation that requires a couple of minutes without any optical readjustment.

It is also a unique feature of the SFM instrument, that the observation head allows insertion of standard 1 cm x 1 cm cuvette for steady-state measurements and calibration of the optical system.

The observation head has four optical ports for connections: one for illumination, two at 90° to the light source for fluorescence, light scattering and fluorescence anisotropy measurements, and one at 180° to the light source for absorbance, transmittance and circular dichroism measurements.



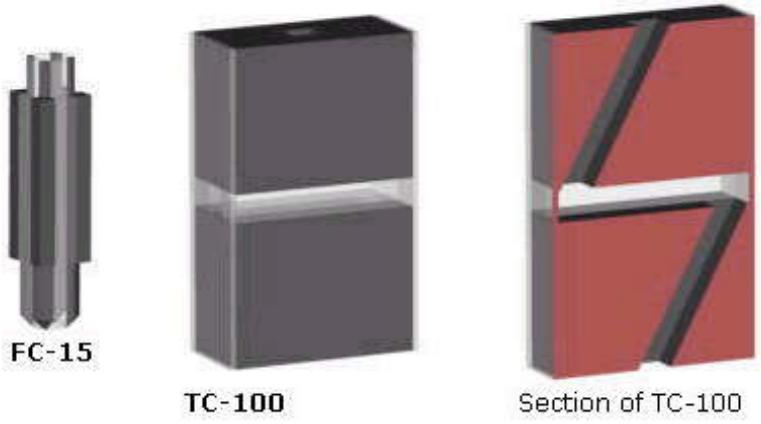
1 - Light Source
2 - Fluorescence, Light Scattering, Anisotropy
3 - Absorbance, Transmittance, Circular Dichroism

The optical ports used for detection can be equipped with lens or fiber optics to direct the transmitted or emitted light wherever it is needed.

In most standard configurations, the optical head is set directly on the top of the Stopped-Flow instrument. This ensures mechanical and optical stability and also the lowest volume between the drive syringes and the last mixers. However, for some applications it is necessary to have a physical separation between these two parts of the instrument. This is achieved by the **Umbilical link**.

Stopped-Flow observation cuvettes

The observation cuvette is one of the most critical parts of all Stopped-Flow instruments. Indeed, it is extremely important to adapt the cuvette to the parameter being observed. For example, it would be inappropriate to use the same cuvette for measuring a small absorbance change and for measuring a fluorescence change of a compound having a high absorbance and producing strong inner filter effects



If our standard cuvettes do not satisfy your specific experimental requirements, we invite you to **contact us** about custom-made cuvettes.

The observation head has been designed so that the observation cuvettes can be exchanged within a few minutes. This is often recognized by our users as one of the many advantages of the SFM systems.

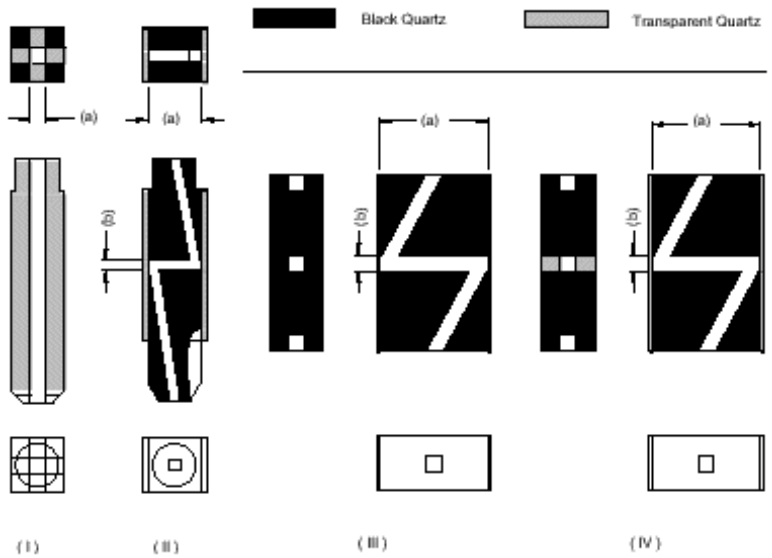
The table **below** shows the cuvettes presently available and their specifications. There are two general styles of cuvettes:

FC (fluorescence cuvette)

FC type cuvettes have blackened edges to reduce light scattering in fluorescence configuration. The best dead time performance are obtained with the FC-08 cuvette while the FC-15 and FC-20 cuvettes are the best choices for CD experiments in the far UV where their large aperture facilitates low noise recording at these wavelengths.

TC (transmittance cuvette)

TC type cuvettes have been primarily designed for absorbance and transmittance experiments, however in the TC-xx/yyF models, both sides along the light path are transparent. These models of cuvettes can also be used for fluorescence experiments using dilute samples and excitation with a laser or any other low divergence light source. Cuvettes of the TC.xx/10 type have a 1x1 mm² cross section and cuvettes of the TC.xx/15 type have a 1.5x1.5 mm² cross section.



Observation cuvettes - geometry

Observation cuvettes - optical specs. and applications						
Ref.	**Design. (1)**	**Drawing N°**	**Light path (mm) (a)**	**Aperture (mm) (b)**	**Volume (µL) (2)**	**Main applications**
054-08	FC-08	I	0.8	n.a.	11	Fluorescence, light scattering
						Fluorescence,

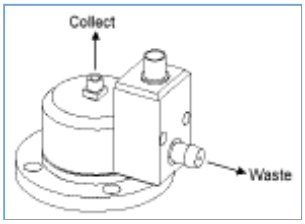
054-15	FC-15	I	1.5	n.a.	30	light scattering, high absorbance
054-20	FC-20	I	2.0	n.a.	50	CD, fluorescence
054-51	TC-50/10	II	5	1.0	20	Absorbance, CD, fluorescence
054-55	TC-50/15	II	5	1.5	40	Absorbance, CD, fluorescence
054-70	TC-100/10T	III	10	1.0	30	Absorbance, CD
054-60	TC-100/10F	IV	10, 1.0 (3)	1.0	30	Absorbance, CD, fluorescence
054-71	TC-100/15T	III	10	1.5	45	Absorbance, CD
054-61	TC-100/15F	IV	10, 1.5 (3)	1.5	45	Absorbance, CD, fluorescence

Notes:
(1) All cuvettes are in Suprasil (transparent from 185 to 2500 nm)
(2) Volume from a last mixer to center of observation area. Dead time is proportional to this volume and to the inverse of the total flow rate. The driving software issues an estimated dead time that takes into account all these parameters
(3) Light path when the cuvette is turned by 90°

Quench-Flow mode

In this configuration, the Stopped-Flow module SFM-300 and SFM-400 are converted into full feature Quench-Flow instruments.

SFM-300/Q features a three-syringe Quench-Flow with one delay line, two mixers and a diverting valve for waste and collect. This allows mixing between two reagents (in syringe 1 & 2 respectively), followed by ageing in delay line and subsequent quenching by mixing the aged mixture with the solution contained in the third syringe.



Quench-Flow exit valve.
Click to enlarge

SFM-400/Q features a four-syringe Quench-Flow with two delay lines, three mixers and a diverting valve for waste and collect. This instrument allows more sophisticated mixing sequences such as mixing of two reagents (in syringe 1 & 2), first ageing in the first delay line followed by mixing with a third reagent (in syringe 3) and a second ageing phase in the second delay line before final quenching with the contents of the fourth syringe.

Various values of ageing time in the delay lines are obtained by modifying either the solution flow rate and/or the delay line volume (continuous-flow mode) or alternatively by including pauses in the sequence timing (interrupted-flow mode). The Windows based software allows an efficient control of the operational modes and of the solution flow rates as well as of washing and collect periods.


Click to enlarge

The **/Q** versions are delivered with a full set of delay lines allowing reaction ageing from a few millisecond to several seconds.

Flash quenching with a photoreactive reagent is also a mode that can be easily implemented with the SFM. (for details click **here**). Many other configurations are possible, and you are invited to **inquire** about their feasibility.

The commercial reference SFM-x00/QS has all the components for the Stopped-Flow (apart from Spectrometer) and Quench-Flow applications.

An SFM-x00/S or an SFM-x00/Q can easily be updated to an SFM-x00/QS.

Accessories

A wide variety of accessories are available to adapt and expand the functionality of an SFM.

These include:

The **cryo-stopped-flow** assembly extends the range of operation of the Bio-Logic stopped-flow instruments to far sub-zero temperatures. It allows **rapid kinetics** experiments to be performed **down to -90°C** - click **here** for more details.

New **submillisecond cuvette** and mixer: This microcuvette accessory is a specially designed observation head that allows observation of very fast kinetics. Its provides the record performance of **0.25 ms dead time** in stopped-flow mode (for SFM-300 & SFM-400, 0.4 ms for SFM-20) - click **here** for more details.

High Density Mixer - Used for minimizing convection artifacts when mixing solutions of widely differing viscosities. Highly recommended for protein folding-type experiments.

Delay Lines - Delay lines can be inserted between mixers to adjust sample ageing times for all types of experiments.

Titrator and Peltier element controller: Because of its extreme precision of volume delivery, the Bio-Logic stopped-flow instruments feature the most accurate titrator of the market. By a simple software choice and an easy hardware installation, any SFM-300/400 or SFM-20 can be converted in a **high precision** two syringe **titrator**. The titrator device also comes in a version that includes a Peltier Cell temperature cycler. This Peltier/titrator system benefit from a full integration with Spectrometer control software (Bio-Kine32). As such it can easily be used in absorbance, fluorescence, fluorescence anisotropy and circular dichoism - click **here** for more details.

Mix & Freeze - click **here**

Umbilical Connection - click **here**

X-ray head - click **here**

Optical Quench Accessories - click **here**

Adaptation for **EPR** experiment - **contact us**

Bio-Logic also specializes in building custom accessories and adapting our instruments to new experimental methods. Please do not hesitate to **contact us** if you need a special development.

TOP

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