



2, 3 and 4 SYRINGE STOPPED-FLOW
A unique design for single, double
and triple mixing applications

SFM-2000/3000/4000 MARKS A NEW STEP IN THE COMBINATION OF HIGH PERFORMANCE AND MODULARITY

The **SFM-2000/3000/4000** stopped-flow systems are the result of nearly 30 years of innovation and development at Bio-Logic.

The **SFM-3** was introduced in 1987. This was the first three syringe stopped-flow system using independent stepping motor technology, Berger Ball mixers and an electro-valve hard-stop.

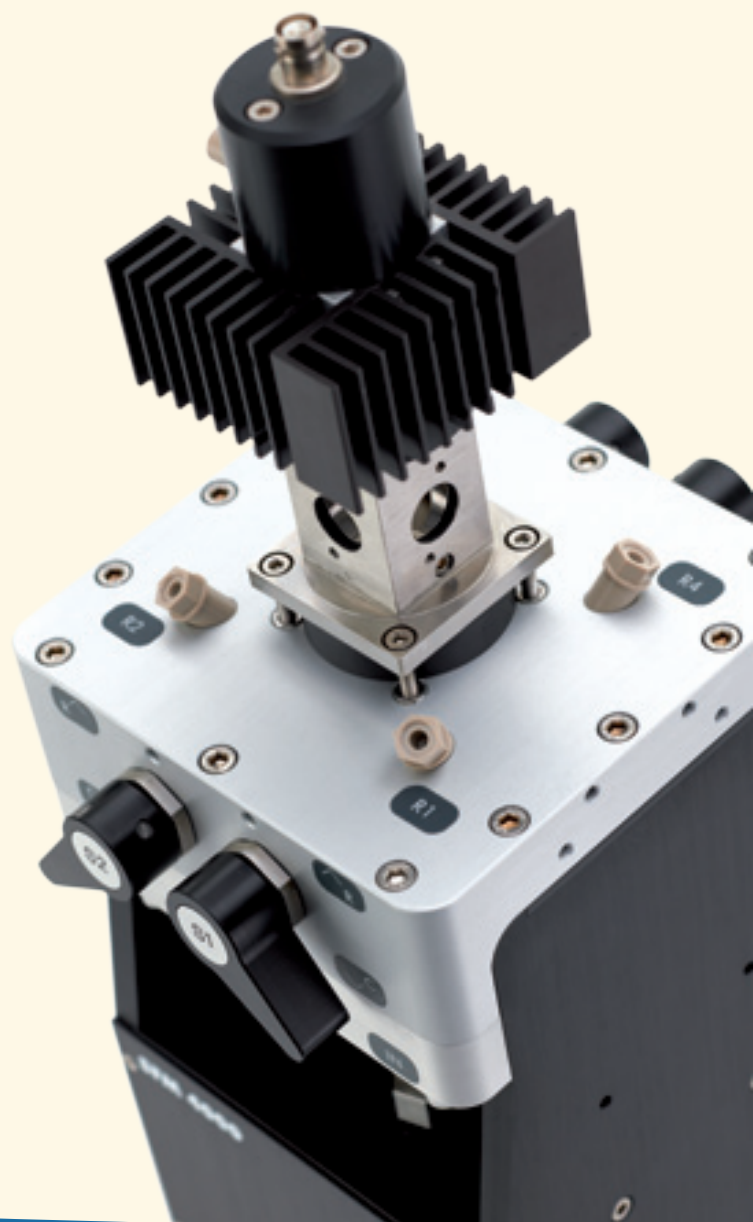
In 1992 the four syringe **SFM-4** brought triple mixing capability to the rapid kinetics field, increasing the range of applications available.

1999 was a milestone year with the introduction of the two syringe **SFM-20**, the **SFM-300** and **SFM-400**. The **SFM-300/400** reduced the size of instrument, dead volume, sample consumption, and dead time by nearly a factor of two. The **SFM-300** also brought dead time down to 200 μ s based on published results, making it the reference system for very rapid reactions.

2011 brings the introduction of the third generation **SFM-2000/3000/4000** systems. With a common drive chassis, this generation offers simpler upgrades to three or four syringes, smaller dead volume, and improved overall performance.

UNIQUE FEATURES

- Independent stepper motor control for each syringe
- Automatic concentration dependent studies
- Mixing ratio is fully programmable from Bio-Kine
- Ratios can be changed without syringe or hardware changes
- μ l precision – No pressure artifacts
- Modularity from SFM-2000 to SFM-3000 and SFM-4000
- Quench-flow capabilities using optional quench valve



Outstanding performance and unsurpassed flexibility make the **SFM-2000/3000/4000** family the benchmark rapid kinetics mixers for today's laboratory.

All Bio-Logic **SFM mixers use independent stepping motor technology**, for full independent control of volume, flow rate and drive power in each syringe. Precise control allows mixing ratios from 1:1 to 1:100 to be achieved quickly and repeatedly. A series of shots at various concentrations can be programmed and run quickly without refilling or changing syringes. Bio-Logic's stepper motor drive has proven itself to be the best for nearly thirty years. Flexible ratio control, precision, accuracy, speed and the ability to handle extreme viscosity ranges are well known features of Bio-Logic system

Advanced Berger-Ball technology mixers are used to provide the best turbulent mixing over the widest range of flow, viscosity and temperature conditions. Berger-Ball technology offers outstanding consistency and repeatability over T-style mixers.

The SFM stopped-flow family is designed for versatility. **The instruments are easily adaptable and expandable to a wide variety of rapid mixing techniques and applications.** A single SFM can be switched from optical stopped-flow, to chemical quench flow, to freeze quench, to automatic titration, to cryo operation, to a beam-line, all in minutes, with off the shelf options. All of these accessories, and more, are user changeable to let you adapt the SFM-2000/3000/4000 to your needs in the lab, without buying a complete new system.

GENERAL SPECIFICATIONS

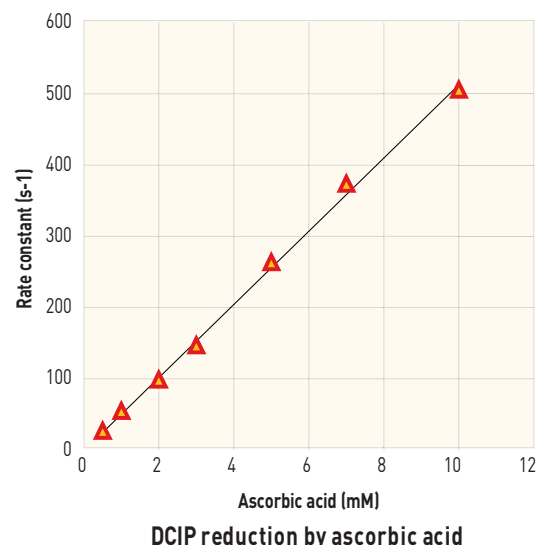
- SFM-2000: single mixing
- SFM-3000: single and double mixing
- SFM-4000: single, double and triple mixing
- Independent syringe control of volume
- Independent control of flow rate
- 200 μ s dead time
- Mixing ratio 1:1 to 1:100
- Low dead volume 88 μ l
- Sample economy
- Automated concentration dependent studies
- Compatible with: MOS-LED, MOS-200/200M, MOS-450/AF-CD, MOS-DA, MCS-200
Many external devices with appropriate options



MIXING PERFORMANCE

Precision of sample delivery with stepping motors is outstanding. The Bio-Logic design delivers μl precision and accuracy at all flow rates, over a wide viscosity and mixing ratio range.

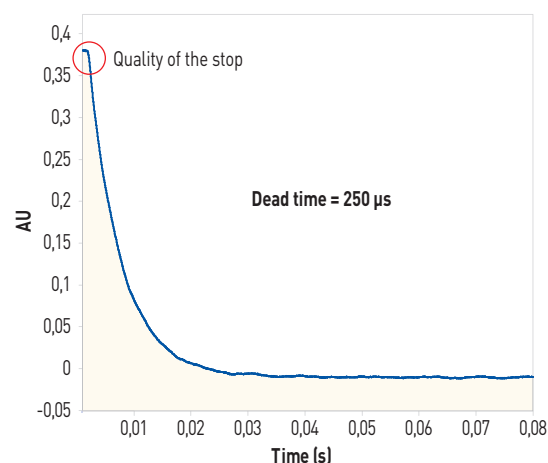
The reduction of DCIP by ascorbic acid illustrates SFM mixing consistency and speed. A standard configuration with three 10 ml syringes is used, with buffer in syringe 1, ascorbic acid in syringe 2, and DCIP in syringe 3. Ascorbic acid concentration is varied by changing the mixing ratio from 19:1:20 to 0:20:20 in Biokine software. Since the syringes do not need to be reloaded between shots, a full series of experiment can be done in less than a minute



DEAD TIME PERFORMANCE AND QUALITY OF THE STOP

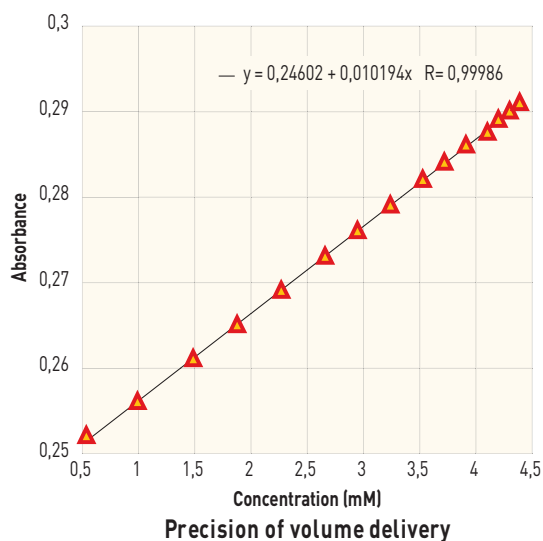
With independent stepping motors driving the syringes, the user has full control of flow velocity. Accuracy of flow rate delivery can easily be checked by measuring faster dead time with increasing flow rates.

Dead times to 200 μs can be obtained with the micro-cuvette option. Measurement of low dead time is enhanced with the responsive hard-stop electro-valve and a pre-trigger. Flow in the cuvette is stopped instantly without the pressure artifacts common observed with systems using stop syringes. A pre-trigger is user selectable to check that the mixing reaches a stationary state before the flow stops.



VOLUME PERFORMANCE

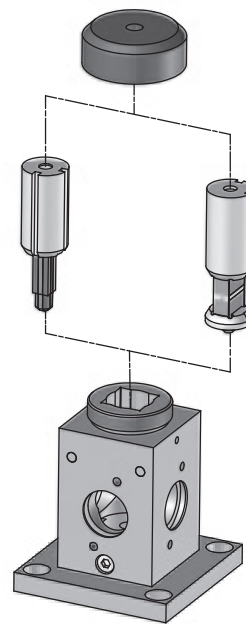
Bio-Logic's newest SFM mixers have dead volumes a factor of two better than the previous. All internal volumes were reduced to minimize priming requirements. Precision of volume delivery is maximum, stepping motors can deliver solution with a sub-microliter precision. The plot illustrates the linear performance of an absorbance check in titration mode by doing consecutive injections of 10 μl , 5 μl , and 2 μl steps (see figure on the right).



CHANGE YOUR CUVETTE IN 30 SECONDS!

It is essential for the user to have a wide choice of cuvettes, and to be able to change them quickly. The SFM-2000/3000/4000 models deliver both. A choice of 10 cuvettes is available with light path from 0.75 mm to 10 mm, and volumes from 3 μ l to 50 μ l (cuvettes with dual light path are available: for example 1 cm and 1 mm). Fluorescence optimized cuvettes with a short light path (0.8 mm) are available to minimize inner filter effects.

The SFM is designed so the user can change the cuvette in seconds, without dismantling the system or draining the temperature circuit. Like the rest of the system, you set it up to match your experiment, rather than design the experiment to match the instrument.



PRECISE TEMPERATURE CONTROL

All driving syringes, delay lines, mixers and mixing chamber can be temperature controlled by connecting a water bath circulator. The same circuit is used for the entire cooling path to prevent a temperature gradient observed when different circuits are used for syringe and cuvette. Options for individual syringe temperature control or individual mixing chamber temperature control are available if multiple control temperatures are needed.

An optional temperature probe is available for installation on the observation cuvette; it provides temperature readout in Biokine software with a 0.1°C precision.

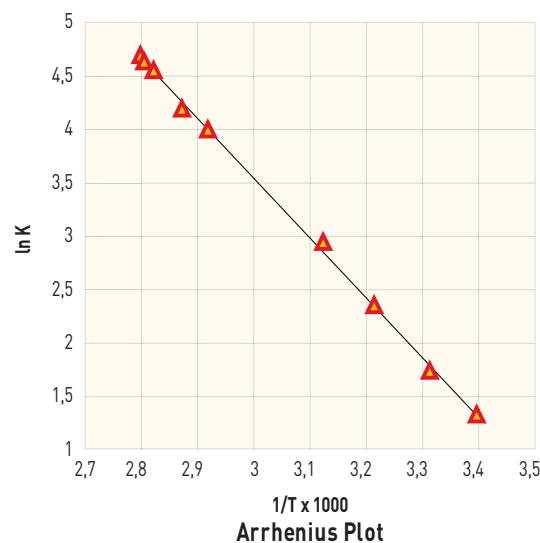
A INVESTMENT FOR YEARS OF TOP PERFORMANCE

Our SFM's are recognized for their long life with full performance. 20 year old instruments are capable of the same performance as when they were new. Routine maintenance can be done easily by users, with inexpensive parts.

Our SFM's were designed to be modular instruments and are the standard reference for non classical applications. Easy access to the mixing head makes external coupling possible, for example: laser excitation, flash photolysis or coupling to a streak camera.

Most of our stopped-flow systems installed 20 years ago are still in working condition. By choosing Bio-logic you choose an instrument which will age well and follow the evolution of your research.

Our R&D team also provides special development services so the instrument can be customized to your needs and applications. Our expertise includes electronic, optical, software and mechanical engineering. Please contact your local representative or Bio-logic offices for details.



BIO-KINE is the reference software for stopped-flow measurements. Intuitive and easy to use, Bio-Kine controls the stopped-flow, the detection device, follows the acquisition, and analyzes data.

SINGLE MIXING APPLICATIONS

Two levels of operation are provided for mixer control. The first level is designed for rapid and easy experiment design.

The user sets the mixing ratio, the size of the cuvette and the volume of samples. Color coded windows display calculated values and alert the user to out of range conditions. The estimated dead time is automatically displayed, and a pre-trigger is also available to be sure the stationary state is reached accurately.

This interface is ideal for routine mixing designs, occasional users, or students.



MULTI MIXING APPLICATIONS

The classic interface allows sophisticated and unrestricted instrument programming. This is ideal for multi-step sequences such as double jump experiments. It can also be used to include a pre-washing phase and for coupling with external devices, (synchrotron lines for example).

In a double mixing application, samples 1 and 2 are mixed in phase 1 before incubation in phase 2. Then it is mixed with a third reactant in phase 3, and the second kinetics is followed optically.

Estimated dead time and ageing time are automatically calculated by the software. This mode is also suitable for chemical quench, freeze quench and optical quench modes. In these applications ageing time is also automatically calculated according to user's parameters.

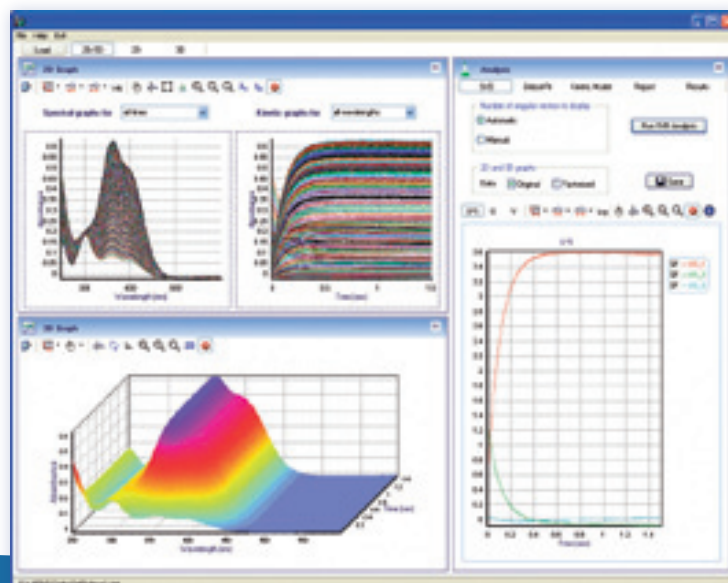


GLOBAL FITTING / SVD ANALYSIS

Bio-Logic now provides SVD (Single Value Decomposition) analysis tools with all new mixers. This feature is ideal to analyze data generated by diode array detectors, and multi-wavelength data generated by **MOS-200/M** and **MOS-450**.

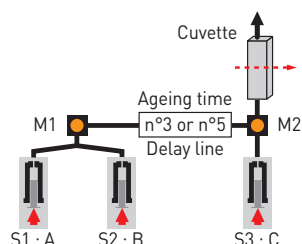
It includes:

- automatic estimation of number of vectors,
- fast SVD analysis,
- global fitting with residual analysis (2D and 3D),
- levenberg-Marquardt and Simplex fit algorithms
- large selection of kinetics models
- import of data from clipboard or text files.



SAVE SAMPLE, TIME and MONEY.
Independent stepping motors offer the best automation possibilities.

AUTOMATIC VARIATION OF AGEING TIME



This protocol was designed to automate double jump experiments, such as folding/unfolding experiments. Samples A and B are mixed and then aged for a user-defined time.

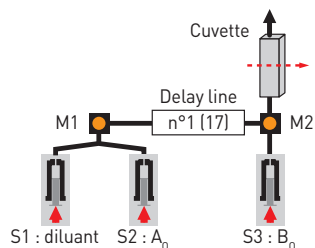
After incubation the aged solution is mixed with sample C so the second kinetics can be observed optically.

The user defines the mixing ratios for the 3 reactants, then enters the ageing times he wants to reach.

Bio-Kine automatically calculates the optimum sequence according to the user parameters, (cuvette, mixer, flow rate), and displays the corresponding dead time. Each ageing step can be repeated for averaging. This mode is also available for external detectors.



AUTOMATIC CONCENTRATION DEPENDENCE STUDIES



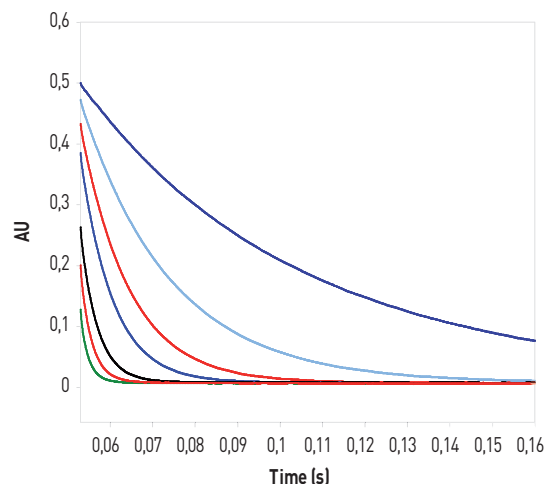
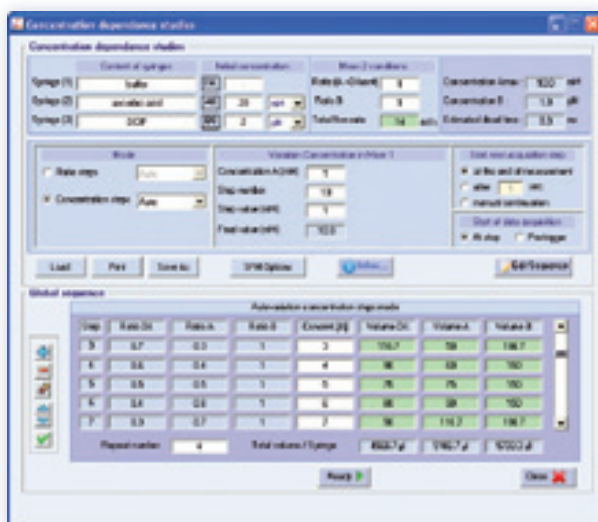
This protocol studies the reaction $A+B$ by varying the concentration of A at each shot while keeping B constant. The first mixer is used to set the concentration of A by mixing A with buffer. Then A is mixed with B in the second mixer and the reaction is followed optically. The dead time is identical for all concentration steps.

The user enters the mixing ratio between A and B at each mixing step, and the final concentration of A required. Bio-Kine automatically calculates the optimum volume to push from each syringe.

Mixing ratios can be varied from 1:1 to 1:20 using the same configuration.

Each concentration step can be repeated for averaging, and concentrations at each step are automatically saved with data to facilitate data analysis.

This mode is also available when using an external detector. The method is ideal for rapidly collecting data for a Chevron plots.



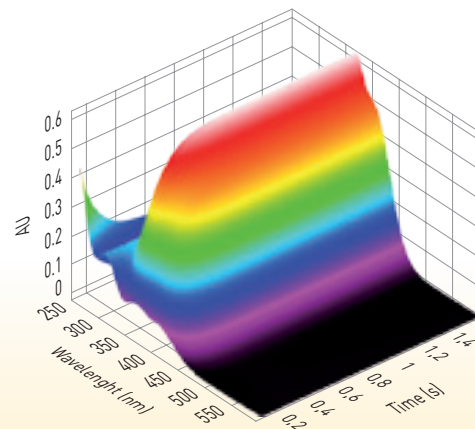
OPTICAL COUPLING



MOS-200/200M

Absorbance, fluorescence, chemiluminescence, and 90° light scattering

The MOS-200/200M are perfect for all basic rapid kinetics studies, with data acquisition down to 10 μ s resolution. The systems include a manual or motorized monochromator, dual light source, and high sensitivity photomultiplier (PMT). Multiple detector and T-format anisotropy options are available. The motorized model offers full automation for multi-wavelength studies.



MOS-DA

Diode array spectrometer

The fastest Diode Array spectrometers on the market for UV, visible and near IR, with speed down to 0.4 ms/spectra. High quality 3D software interface. SVD analysis through Bio-Logic's software package.



MOS-LED

LED spectrometer

MOS-LED is the choice for routine measurements or users who do not need the modularity of MOS-200.

Excitation is done with one of over 60 high intensity LED sources. An optional PMT detector adds fluorescence capability. Data can be sampled at a rate down to 100 μ s.



MOS-450/AF-CD

Absorbance, fluorescence, circular dichroism, LD, fluorescence anisotropy and 90° light scattering

The most advanced kinetics and steady state spectrometer on the market with a double excitation monochromator and double light source. No purging of optics needed in kinetics mode, and no need to handle lamps for operation. Time resolution to 10 μ s. Bio-Kine control software allows full automation and multi-wavelength experiments with automatic spectral reconstruction.



Biochemistry

Protein folding/unfolding

Independent syringes and completely variable mixing ratios make the SFM-2000/3000/4000 the ideal system for studying protein folding. These studies often involve mixing solutions of up to 2000 cp viscosity, with is easily done with our specially designed micro-volume Berger Ball mixers.

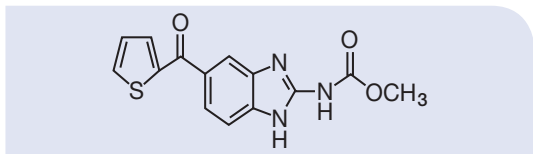
For reactions longer than 10-15 seconds, a unique high density mixer is available to minimize convection artifacts. The flexibility of mixing ratios and differing sample viscosities offered by the SFM give the user unmatched control of experiment design and sample economy.



Water & ions transport in vesicles

A common stopped-flow application is fast tracking of transient kinetics of water or solute transport, across the membranes of small, (10-500 nm scale), pressure sensitive vesicles. With the SFM's precise control of total flow rate, and a synchronized electro-valve to stop sample flow, shear forces and over pressure artifacts are minimized. The combination minimizes changes in vesicle integrity.

The SFM coupled to one of our spectrometers is an ideal system for 90° light scattering and fluorescence which is often used to follow such reactions in blood cells, aquaporins, liposomes, and for water purification applications.



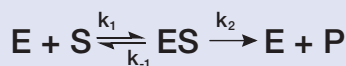
Organic & inorganic chemistry

Structural changes

Understanding reaction mechanisms and characterizing the structure of reaction intermediates are common goals in organic and inorganic chemistry. These reactions often occur in organic solvents, requiring instruments that can maintain design function and performance in an aggressive environment. The SFM-2000/3000/4000 mixer components and optional Kalrez O-ring set easily withstand exposure to solvents such as THF and toluene.

For studies where a reactant is oxygen sensitive, the SFM anaerobic option provides a simple and reliable way to perform an experiment in an oxygen limited environment.

A -90°C cryogenic option is available to capture intermediates with very short life times.

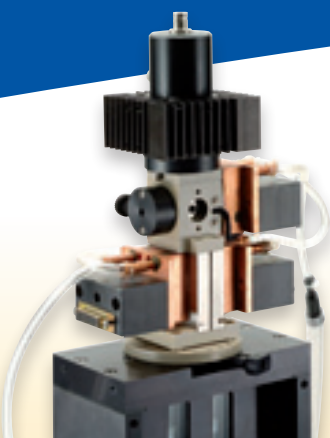


Enzyme kinetics

Ligand binding conformational changes

Single and multi-substrate reactions can be followed with stopped flow techniques to study the catalytic mechanism of an enzyme, and to learn about its role in the cell. Mechanisms can be investigated by changing reactant concentrations, and by adding fluorescent dyes to the protein to enable observation of movements during reactions. With independent stepping motors, users can change mixing ratios after each shot without refilling the system, so a series of concentration studies can be done rapidly. Bio-Kine's analytical tools provide immediate information about the reaction mechanism.

Applications possibilities are unlimited: micelles, second messenger, drug design, polymerization, and more.

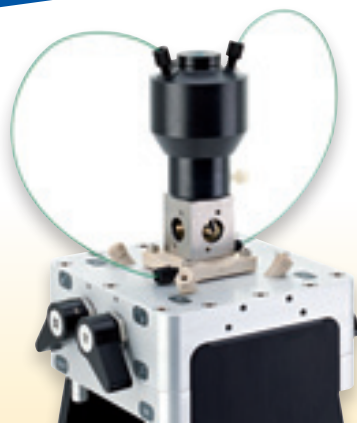


mT-jump

The mT-jump head replaces the standard SFM observation head. mT-jump achieves temperature changes by mixing two solutions of initial temperatures T1 and T2. The final temperature (T3) of the mixture is calculated from the initial temperatures T1 and T2 and the mixing ratio of the two solutions. A T-jump with a dead time of 0.7 ms can be measured.

Reactions can be followed with a ms resolution. A cooling or heating jump of up to 40°C is possible.

It is compatible with all stopped-flow models in all modes of observation such as circular dichroism, fluorescence, absorbance, and fluorescence anisotropy. With this accessory, protein folding and refolding can easily be studied without adding denaturant to the protein!



TITRATION

The titration head replaces the standard SFM observation cell in seconds. It is designed to accept standard 1 x 1 cm cells, and includes a magnetic stirrer for continuous sample mixing. Temperature is controlled through the stopped-flow circulation loop. Three detection windows are provided for measurements, and a 5.5 mm diameter port is available for external devices such as pH electrodes, nitric oxide electrodes, and temperature probes.

The programmable titrator takes advantage of **high precision stepping motors for accurate micro-volume delivery of solutions**. Volumes **as low as 2 ul can be injected** using a 1.9 ml syringe.

Concentration steps are user-defined. Automatic increment functions are available, including a function to change the increment during titration. **Bio-Kine automatically tracks solution volumes, and alerts the user to out of range conditions.** This prevents an experiment from running out of solution during the sequence.



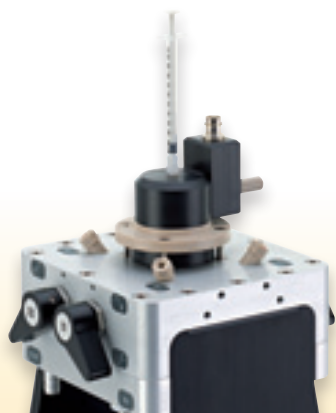
CUSTOMIZATION

The SFM-2000/3000/4000 observation head was designed to allow easy coupling to external devices. Optional heads and adaptors have been designed for neutron scattering, EPR, large and small angle X-ray, multi-angle light scattering, FT-IR, and conductimetry.

Connection options are available for some third party spectrophotometers, fluorimeters, EPR, and other systems. If you have a system and want to connect it to stopped-flow mixer, contact us with the details and we can advise you on what options are available.

EASY INTERFACING

- Fiber optic accessory
- Single and double light links
- Umbilical connection



QUENCH-FLOW

SFM-3000 and SFM-4000 systems can be converted quickly to top performance quench-flow operation by exchanging the stopped-flow observation head for a quench-flow collecting valve.

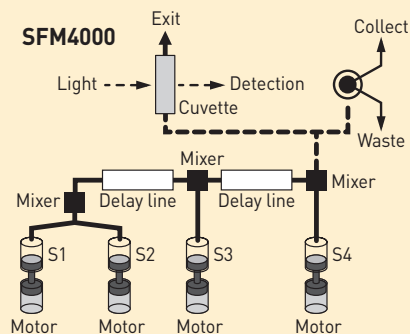
Ageing times are varied by selecting delay lines. Mixing is made in continuous flow mode, interrupted flow mode, or in Bio-Logic's unique pulse mode (single mixing applications only).

Bio-Logic's stepping motor technology controls flow rate precisely from Bio-Kine software. Long calibration procedures to estimate the flow rate are not required. The SFM drive delivers the exact requirements, every time.

The independent stepping motors remove all limitations of a single drive mechanism, and mixing ratios can be changed freely just as in stopped-flow observation mode.

The SFM-4000/Q is the only system in the world having 4 independent syringes and 3 mixers. It is a unique system for triple mixing applications, such as De/H exchange experiments or radioactive labeling. The user controls two ageing times from the software, and series of experiments can be done rapidly.

A long list of papers using our instruments in this application field is available.



FREEZE QUENCH

The freeze quench accessory consists of an umbilical connector, a set of ageing loops, a diverting electrovalve, and a dewar with a sample holder.

Reactants are loaded in the mixer syringes, then the reaction is initiated by mixing 2 or more solutions. The reaction is aged for a user-defined time inside the calibrated ageing loops. A built-in ejection nozzle at the exit of the ageing loop sprays the aged solution towards the dewar.

Freeze quench techniques are extensively used to study of metallic reaction centers in metalloproteins and metalloenzymes. Different sample holder are available for XAFS, EPR, NMR or Mossbauer spectroscopy.

SPECIFICATIONS

Drive mechanism: independent stepping motors

Number of syringes	2 (SFM-2000), 3 (SFM-3000), or 4 (SFM-4000)
Number of stepping motors	1 per syringe
Precision of stepping motors	10.4 nl per microstep
Number of mixers	1 (SFM-2000) 2 (SFM-3000) or 3 (SFM-4000)
Mixer type	Berger-Ball mixer or optional HDS
Stop mechanism	electrovalve

Syringes

Syringe material	PEEK
Syringe size	10 ml (6.8 ml, 3.6 ml and 1.9 ml are optional)
Anaerobicity	anaerobic operation standard

Sample consumption

Priming volume	from 88 µl per syringe
Flow rate range	0.003 to 10 ml/s (per syringe)
Mixing ratio	fully variable from 1:1 to 1:100
Minimum injection volume	10-25 µl depending on syringe size in stopped-flow mode
Minimum injection volume in titration mode	2 µl

Observation head

Number of detection windows	3
Cuvette	quartz; choice of 10 models with light path from 0.75 mm to 1 cm
Optional	capillary for X-ray measurements
Minimum dead time	0.2 ms using microcuvette (0.8 mm light path), < 0.7 ms using smallest standard cuvette
Material	PEEK, Viton full solvent compatibility using Kalrez o' rings (option)

Note

Bio-Kine software and a USB connected hardware interface is included with the SFM-2000/3000/4000. A PC is required in addition for controlling the SFM system, with a Pentium IV, and Windows 2000, XP, Vista or 7.

Temperature control

Temperature range	-20°C to +85°C (standard) down to -90°C using cryo - option up to 160°C using high temperature umbilical and mixing head
Temperature control	water bath circulation (optional)
Temperature probe	optional PT100 allows direct reading of temperature in Bio-Kine

Synchronization with detection

Trigger	fully programmable; 5V TTL trigger in/out available
Optical coupling	fiber optics or direct attachment

Compatible detection devices

MOS-LED	absorbance, fluorescence, dual absorbance
MOS-200/200M	absorbance, fluorescence, 90° light scattering, T-format anisotropy (optional)
MOS-DA	diode array, absorbance
MOS-450/AF-CD	absorbance, fluorescence, 90° light scattering, circular dichroism, anisotropy (EMFA)
MCS-200	conductivity

General

Dimensions	200 x 197 x 522 mm
Weight	12 kg (SFM-2000), 13 kg (SFM-3000), 14 kg (SFM-4000)
Communication	USB
Power	110 V-220 V

Upgrades

Chemical quench-flow
Freeze quench (EPR, Mossbauer, NMR, XAFS)
EPR stopped-flow
Upgrade to SFM-3000 and SFM-4000
XAFS head
Neutron scattering head
Titration
Optical quench

Specifications are subject to change

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