

Stopped-flow accessories



- Automatic titration
- Cryogenic applications
- Chemical quench-flow
- Freeze quench
- EPR stopped-flow
- Optical quench
- Optically delayed studies
- Temperature jump
- Conductimetric applications
- FT-IR stopped-flow
- Umbilical link
- Neutron head
- X-Ray/beam line studies



Configure the SFM to your needs

The SFM family of stopped-flow instruments are designed for versatility.

The instruments are easily adaptable and expandable to a wide variety of rapid mixing techniques and applications.

Any SFM can be switched from optical stopped-flow, to chemical quench-flow, to freeze quench, to automatic titration capabilities, to cryo operation, to a beam-line, all in minutes, using off the shelf options.

All of these accessories are user changeable, letting you adapt the SFM-2000/3000/4000 to your labs needs without buying a completely new system.

Use the SFM series for:

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Titration accessory

The programmable titrator takes advantage of high precision stepping motors for accurate micro-volume delivery.

Volumes as low as 2 μl can be injected using 1.9 ml syringe.

The titrator accessory is designed to accept standard 1 cm x 1 cm cells, and includes a micro magnetic stirrer. A 5.5 mm diameter port is available for external devices (pH or nitric oxide electrodes, temperature probes)

The titration head is connected to the SFM circulating bath circuit, but an optional Peltier element is available for independent control of cuvette temperature.

Titration sequence design

The titrator is fully automated from Bio-Kine software with user-defined concentration steps. Automatic increment functions are also available, including a variable function to change the increment during titration.

Bio-Kine automatically tracks solution volumes, and alerts the user to out of range conditions.

When acquisition is made using a Bio-Logic spectrometer, a series of titration sequences is done automatically. External detection devices can be synchronized by adjusting the steps and acquisition duration.

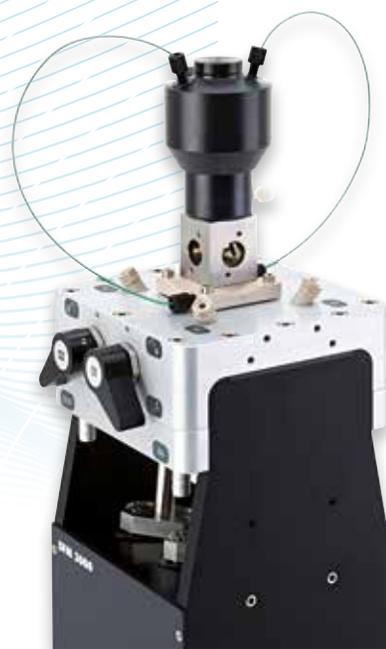
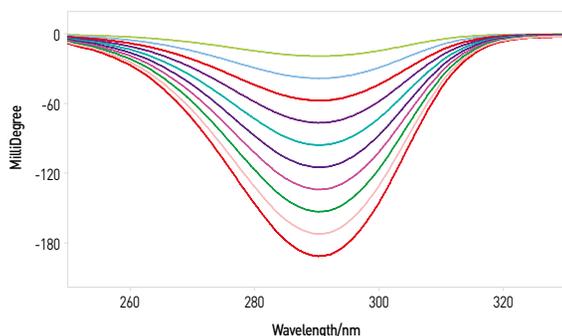
Data collection

The user selects between single wavelength titration and spectral titrations.

When the titration experiment is done at a single wavelength using a Bio-Logic spectrometer, Bio-Kine software automatically builds the titration curve at each step by plotting signal versus concentration. Depending on the spectrometer model spectra can also be recorded at each titration step and data displayed and analyzed as a 3D file.

Applications include:

- denaturation studies,
- binding reaction,
- pH denaturation,
- dissociation constants.



Specifications

Minimum injection volume	2 μl (1.9 ml syringe) 10 μl (10 ml syringe)
Injection ports	3
Detection windows	3
External probes port	1 (\varnothing 5.5 mm)
Temperature control	yes (-20°C to +85°C)

Compatible with

all SFM models

Titration accessory

Catalog n°

045-01

CS-90°C, cryo-stopped-flow

Liquid N₂ cryo-stopped-flow

The cryo option extends the range of Bio-Logic stopped-flow instruments to -90°C. It allows transient state kinetics experiments to be performed in single mixing mode.

Temperature control

CS -90°C includes a cryo-bath and heater. The cooling is done by circulating liquid N₂ in a coil immersed in cryo-solvent. Temperature dependence studies can be done from -90°C to +20°C without any reconfiguration. A temperature probe in contact with the cuvette gives a precise temperature reading of the reaction.

Mixer and observation cell assembly

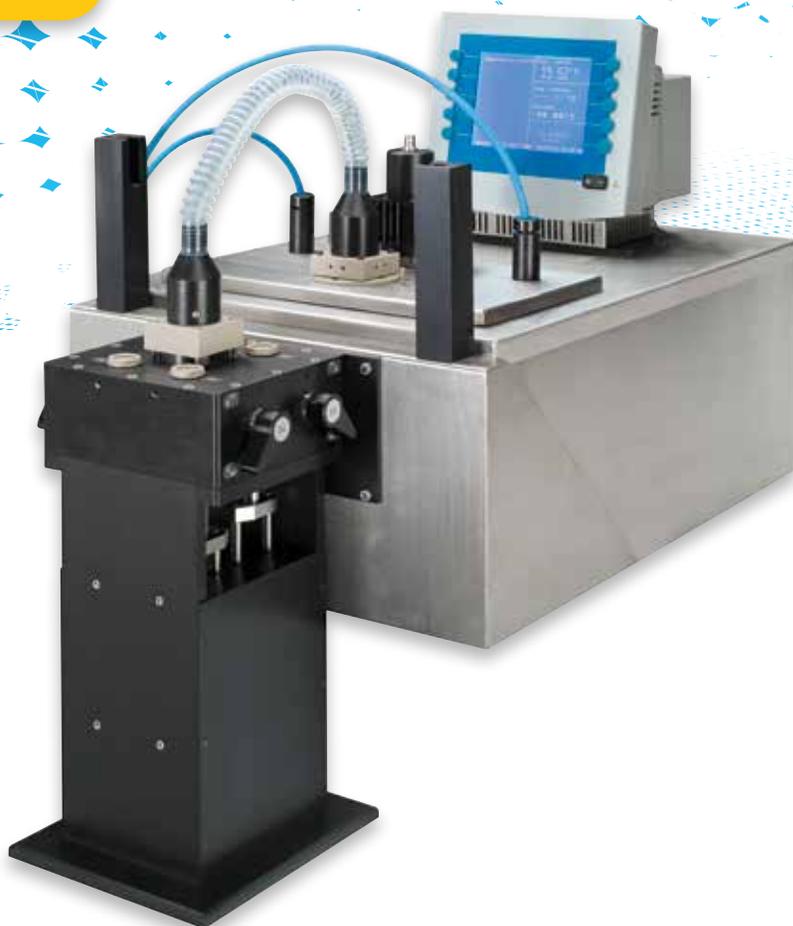
The umbilical connector connects the SFM to the mixing chamber. The chamber, 200 µl reactant transfer lines, and the last mixing stage are immersed in the cryo-solvent. CS-90°C is equipped with FFKM O-rings for full solvent compatibility. Anaerobic operation is standard. Detection is made using fiber optic cables, and the CS-90°C is compatible with all MOS models. A diode array (MOS-DA) is the most popular spectrometer for the CS-90°C. Absorbance spectra can be collected at the fastest rate available (400 µs per spectrum) with the diode array to trap the structure of reaction intermediates invisible at ambient temperature.

CS -90°C includes:

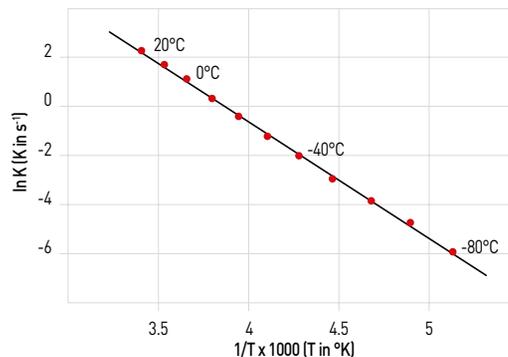
- stopped-flow adaptor, umbilical link,
- mixing compartment including 1 cm light path cuvette,
- 2 fiber optics (2 m long) with SMA connectors,
- FFKM O-rings for full solvent compatibility,
- temperature probe,
- heater for temperature regulation,
- 22 liters cuve and coil for liquid N₂ circulation.

Site requirement:

- cryo-solvent (oil, isopropanol, ethanol, etc.),
- pressurized liquid nitrogen tank,
- tubing to connect cryo-stopped-flow to N₂ tank.



Arrhenius plot



Dinitrophenylacetate reaction with sodium methoxide

Specifications

Temperature range	from -90°C to +20°C
Dead time	2 ms
Mixing ratio	1:1 to 1:5
T control of umbilical link	yes
Single mixing applications	

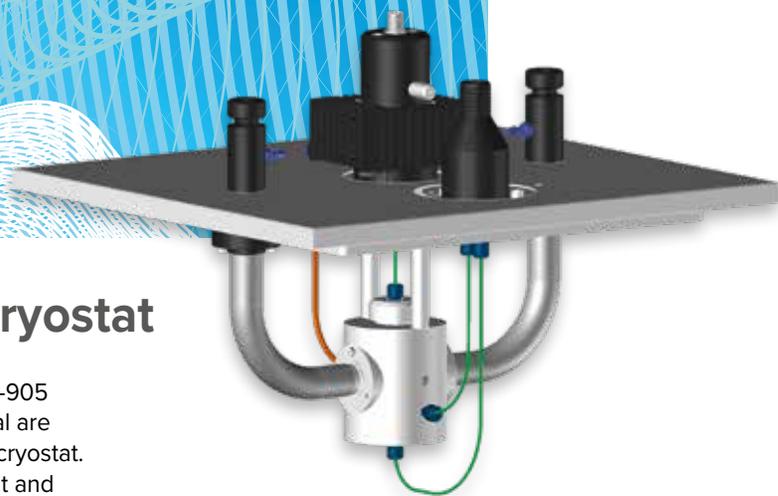
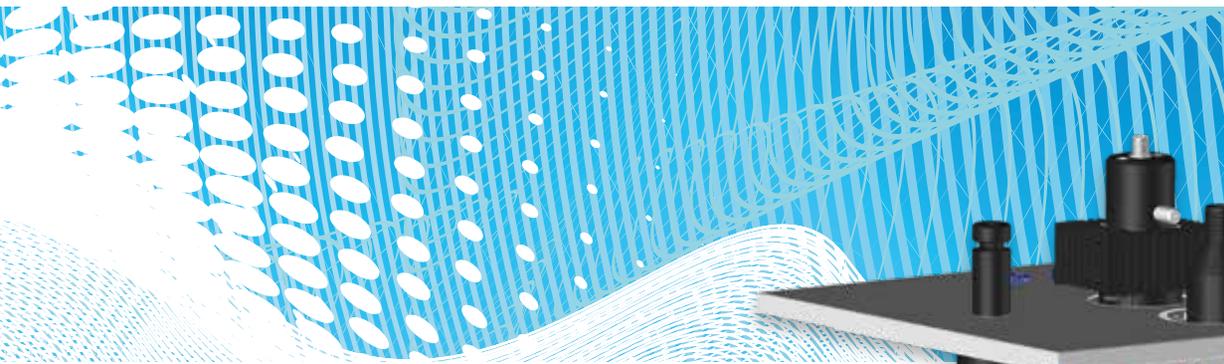
Compatible with

all SFM models

CS-90°C, cryo stopped-flow

Catalog n°

053-11/29



Cryo-accessory for external cryostat

Bio-Logic also offers a cryo-accessory compatible with the CC-905 cryostat from Huber GmbH. The mixing assembly and umbilical are similar to CS-90°C. A stopped-flow is attached directly on the cryostat. The temperature of the bath is set from the manual control unit and is stable to within a 0.1°C.

Customization of cryo-accessory is also possible for coupling with other cryostat models (minimum bath aperture should be 270 x 150 mm and minimal depth 150 mm), contact Bio-Logic for customized solutions.

Cryo-accessory includes:

- stopped-flow adaptor,
- umbilical link,
- mixing compartment including 1 cm light path cuvette,
- 2 fiber optics (2 m long) with SMA connectors,
- FFKM O-rings for full solvent compatibility,
- temperature probe.

Specifications

Temperature range	from -90°C to +20°C
Dead time	2 ms
Mixing ratio	1:1 to 1:5
T control of umbilical link	Yes
Single mixing applications	

Compatible with

all SFM models

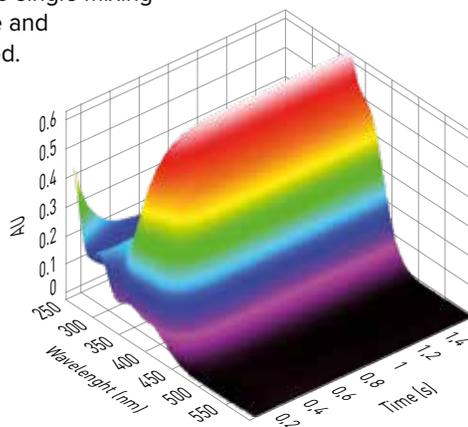
Catalog n°

Cryo-accessory for external cryostat

053-11/31

Double mixing cryo-stopped-flow

Double mixing cryo-stopped-flow is possible with the SFM-4000. Solutions are brought to cryo-bath using HPLC tubing. Two mixers are immersed and a 100 µl delay line is built-in. User can customize the delay line volume if required. Ageing time as low as 15 ms can be reached before proceeding to second mixing. Double mixing is available with liquid N₂ and cryostat cooling methods. The SFM-4000 can also be used for single mixing applications. The delivered items are similar to single mixing set-up except that one delay line and second built-in mixer are included.



Specifications

Temperature range	from -90°C to +20°C
Dead time	3 ms
Mixing ratio	1:1 to 1:5
T control of umbilical link	no
Single and double mixing applications	

Compatible with

SFM-4000/400

Catalog n°

Double mixing cryo-stopped-flow

Double mixing CS-90°C

053-11/29D

Double mixing cryo-accessory for external cryostat

053-11/31D

Quench-flow upgrade

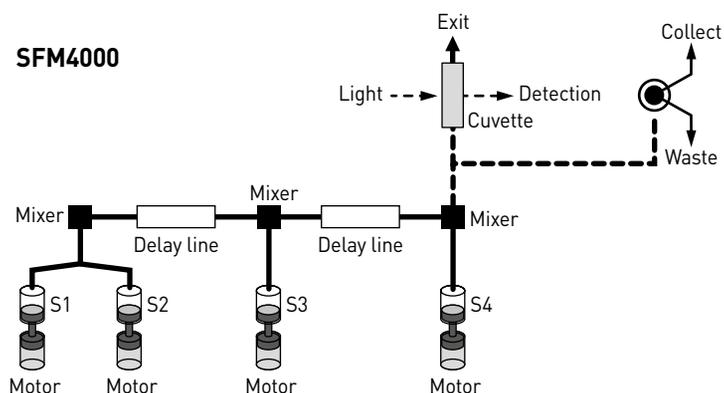
SFM-3000 and SFM-4000 systems can be converted quickly to top performance quench-flow systems by exchanging the stopped-flow observation head for the quench-flow accessory.

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.

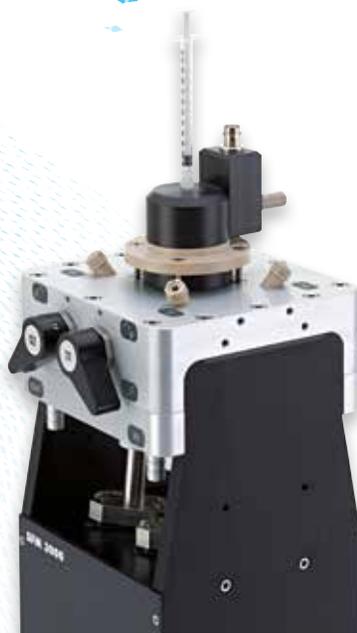
The independent stepping motors remove all limitations of a single drive mechanism, and mixing ratios can be changed freely just as in stopped-flow observation mode while only consuming 50 µl of reagent.



The SFM-4000/Q is the only system with 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 "MIX - AGE - MIX - AGE - MIX" experiments can be done rapidly.

A long list of papers using our instruments in this application field is available on Bio-Logic website.



Specifications

Minimum ageing time	2 ms
Ageing method	set of delay lines
Temperature range	0°C to +85°C
Sample consumption (depending on mixing ratio and delay line used)	50 µl

Compatible with

**SFM-3000/4000,
SFM-300/400**

Quench Flow upgrade

QF upgrade for SFM-3000

QF upgrade for SFM-4000

Catalog n°

054-11/41

054-11/42

Freeze quench

The freeze quench technique uses extreme cold to stop the reaction. Freeze quench is used to study full kinetics or to trap reaction intermediates whose presence has been determined by stopped-flow experiments.

Freeze quench techniques are extensively used to study metallic reaction centers in **metalloproteins** and **metalloenzymes**.

The reactants are mixed and aged for a user-defined time inside the calibrated ageing loops before ejection for freezing.

A built-in ejection nozzle at the exit of each ageing loop sprays the aged solution. Mixing is fully controlled from Biokine software.

Freezing is done by ejection into a -130°C isopentane bath cooled down by liquid N_2 circulation. The mixing part is independent from the freezing part so coupling to cold surface freezer is possible.

Sample consumption depends largely on the amount of material necessary for off-line analysis. For example, to get about 1 cm of crystals in an EPR tube, the user needs around 200 μl of solution delivered to the ejection nozzle.

Ageing times, including flying time and freezing time, range from 9 ms to several minutes.

To limit the number of steps during the packing process and avoid the risk of warming up the frozen solution, aged solution is ejected directly into a PTFE funnel. The sample holder is attached to the funnel so the sample can be packed easily with the included packing rod. Holders are available to collect samples for spectroscopic analysis by EPR/NMR, Mossbauer, and XAFS.

Freeze quench accessory includes:

- umbilical connector,
- built-in Berger Ball mixer,
- set of ageing loops with built-in ejection nozzle,
- dewar and one collection kit,
- packing rods.

Site requirement:

- liquid nitrogen and circulation system,
- isopentane,
- magnetic rod and magnetic stirrer plate,
- sample holder (EPR tube, NMR tube, etc.),
- packing rods.



XAFS



Mossbauer



EPR
NMR

Specifications

Priming volume of umbilical	200 μl
Ageing method	set of ageing loops
Flying time	< 1 ms
Minimum ageing time in ageing loop	3 ms
Freezing time (using isopentane)	4-5 ms
Minimum ageing time (total)	9 ms

Compatible with

all SFM models

Freeze quench accessory

Catalog n°

053-11/10

EPR stopped-flow

Electron Paramagnetic Resonance (EPR) detection with freeze quench and stopped flow has been used for many years to follow kinetics.

The modular design of the Bio-Logic SFM series now allows easy coupling of a stopped-flow mixer with a variety of EPR flow resonators.

The time dependent change of amplitude of an EPR signal in a distinct field value can be followed.

This makes stopped-flow a useful technique for measurements of rate constant for formation, decay or conversion of paramagnetic species.

The EPR stopped-flow accessory consists of an umbilical connector specially engineered such that a Berger Ball mixer can be fitted on one end to minimize the dead volume of the set-up.

A flow resonator is attached directly at the exit of the mixer using an adaptor ring, or optionally with HPLC type tubing.

The temperature is controlled from the driving syringe to the mixer.

The dead time of the EPR stopped-flow set-up depends on the design of the flow resonator. The geometry chosen will limit the flow rate and determines the dead volume.

Data showing 330 μ s dead time using two dielectric rings type resonator* have been published.

EPR stopped-flow accessory includes:

- umbilical connector,
- built-in Berger Ball mixer with open exit,
- adaptor for resonator,
- Viton® O-rings.

Not included:

- EPR flow resonator.

* G. Lassmann, P.P. Schmidt, W. Lubitz,

Journal of Magnetic Resonance, 172 (2005) 312-323



Specifications

Umbilical volume	200 μ l
Built-in mixer	Berger Ball
Material	peek
Dead time (depending on resonator geometry)	0.33 ms

Compatible with

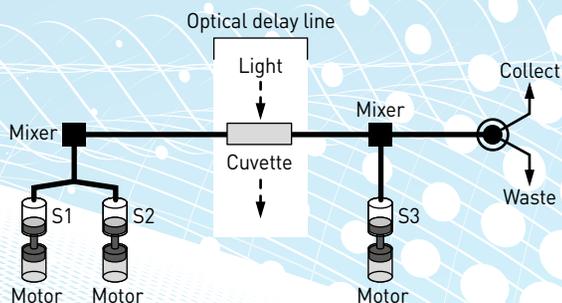
all SFM models

EPR stopped-flow adaptor

Catalog n°

053-11/33

Optical delay line



Specifications

Cuvette	FC-15
Light path	1.5 mm
Intermixer volume	89 μ l
Dead time (from center of mixer to center of cuvette)	31 μ l

Compatible with

all SFM models

Optical delay line accessory

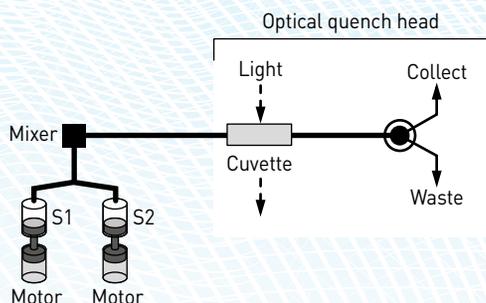
Catalog n°
053-20

The optical delay line is used for applications where the light is used to generate a reactant or to trigger a sample change before chemical quenching.

The sample flows through the cuvette under constant illumination or the flow is stopped and the sample illuminated at a preset time before mixing with a final reactant/quencher. The cuvette access ports allow beams as large as 5 mm in diameter to be used for illumination.

The optical delay line is compatible with SFM-3000 and SFM-4000 only. A FC-15 cuvette is delivered in standard but the optical delay line is compatible with all FC type cuvettes.

Optical quench head



Specifications

Cuvette	FC-15
Light path	1.5 mm
Illuminated volume	37 μ l

Compatible with

all SFM models

Optical quench accessory

Catalog n°
053-11/11

The optical quench head is used for applications where intense light (laser, flash lamp, etc.) is used to quench a reaction.

A sample can flow through the cuvette under constant illumination, or the flow can be stopped and the sample illuminated at a preset time. Cuvette exposure is optimized for maximum quenching.

The open air design of the head allows quick dissipation of heat without compromising the amount of sample exposed to the light source.

The optical quench head is compatible with all SFM models. A FC-15 cuvette is delivered in standard but all FC type cuvettes are compatible with the accessory.

mT-Jump, rapid mixing temperature jump

The mT-Jump accessory achieves temperature changes by mixing two solutions of different initial temperatures T1 and T2. The final temperature of the mixture (T3) is calculated from the initial temperatures T1 and T2 and the mixing ratio of the two solutions.

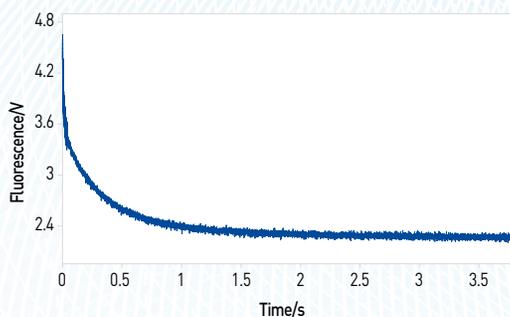
Three thermoelectric Peltier elements are used to control the initial temperatures of the two solutions and that of the observation cell after mixing.

The user defines the mixing ratio for the reaction and the amplitude of the temperature jump. The temperature of each Peltier element is then automatically adjusted. The mT-Jump system can accommodate all FC-type cuvettes and is compatible with all MOS models.

The amplitude and direction of the jump is entirely controlled by the user (up to $\pm 40^\circ\text{C}$ jump), offering new investigation domains, for example, protein refolding studies.

Main applications with millisecond resolution include:

- folding/refolding without chemical denaturant,
- polymerization.



Refolding of horse heart cytochrome c by cold jump from 85°C to 60°C .



Specifications

Mixing ratio	from 1:1 to 1:4
Storage lines	150 μl
Cuvette compability	all FC cuvettes
Dead time	1.5 ms
Communication	USB
Temperature range	from $+5^\circ\text{C}$ to $+90^\circ\text{C}$
Maximum temperature jump	$\pm 40^\circ\text{C}$
Precision of T Jump	$\pm 0.1^\circ\text{C}$
T stability (cuvette)	variation $< 1\%$ in 30 s
T stability (storage line)	$\pm 0.2^\circ\text{C}$ at 80°C

Compatible with

all SFM models

mT-Jump accessory

Catalog n°

045-11/4

MCS-200, conductimetric detector

Conductivity detection can be an alternative to standard optical detection technique. Changes in the solution's conductance in the course of a chemical reaction can be induced either by a change in the concentration of positively or negatively charged ions or molecules or by a change of their mobility inside the solution.

MCS-200 is based on Fast-Fourier-Transform (FFT) Impedance Spectroscopy.

The instrument allows recording steady state **impedance spectra** of the sample in a frequency range of 10 Hz to 200 kHz, and measuring rapid kinetics with a time resolution of **200 μ s per data point**. Resistance, conductance and specific conductance are measured in real time with a 3 ms dead time.

The conductimetric cuvette is a customized FC-20 cuvette with 1.6 mm diameter gold electrodes (glassy carbon electrodes available on request) built in to the cuvette walls. Simultaneous absorbance measurements are possible.

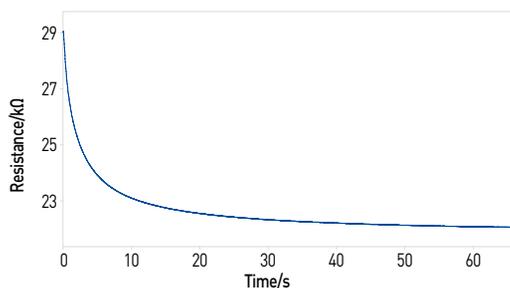
Cyclic voltammetry technique is available for efficient electrode cleaning.

MCS-200 includes:

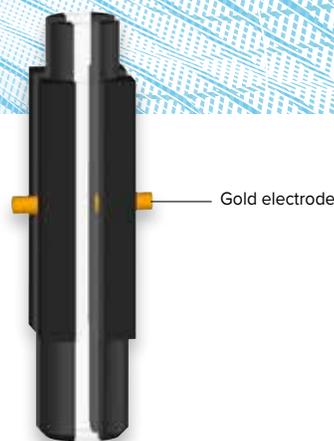
- dedicated observation head with ground connection,
- conductivity cell ,
- control unit equipped with impedance board and low current capabilities,
- hard-stop,
- special Bio-Kine software version
(for impedance measurements, Nyquist representation included).

Main applications include:

- (in)organic reduction/oxidation,
- proton exchange,
- metal ligand complexation,
- micelles formation from surfactants,
- ion exchange.



Fe II oxydation by H_2O_2



Specifications

Conductivity range	1 to 500 μ S.cm ⁻¹
Accuracy	1%
Electrical cell constant	6.4 cm ⁻¹
Light path	2 mm
Fastest acquisition time	200 μ s
Minimum dead time	3 ms
Electrode material	gold (\varnothing 1.6 mm)

Compatible with

all SFM models

MCS-200 detector

Catalog n°

091-64

FT-IR stopped-flow

Improvement in Fourier Transform Infra-Red (FT-IR) technology and use of step scan acquisition have made coupling the FT-IR with stopped-flow instruments a valuable tool for evaluating reaction rates in Infra-Red region.

FT-IR spectroscopy provides structural information in terms of bonds and mobility. It can be a very useful accessory to study infrared kinetics in the amide I region of a protein's vibrational spectrum.

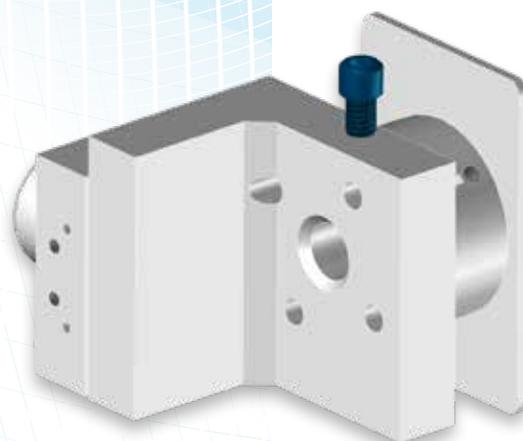
The FT-IR accessory is usually attached to stopped-flow using an umbilical connector so only the observation cuvette is fitted into FT-IR sample compartment. The FT-IR flow cell is mounted in a holder compatible with most FT-IR instruments.

The flow cell uses CaF_2 windows and user can freely select light path from 15 μm to 500 μm by changing a spacer between windows.

Depending on the light path the total flow rate may be limited to 1ml/s. The dead time under these conditions is determined by the acquisition speed of the detector.

FT-IR accessory includes:

- FT-IR flow cell holder with built-in Berger Ball mixer,
- set of CaF_2 windows,
- set of spacers,
- direct exit tube.



Specifications

Windows material	CaF_2
Light path	15 μm , 25 μm , 50 μm , 100 μm , 200 μm , 500 μm
Dead time (with 100 μm spacer and 3 ml/s flow rate)	6 ms

Compatible with

all SFM models

FT-IR accessory

Catalog n°

053-17

Umbilical link

Some stopped-flow applications require special mounting options. This is often the case on a beam line where there is no room to mount the observation head directly on top of the mixer body. The umbilical connection allows the stopped-flow mixer body and the observation head, (with last mixing stage), to be separated by up to 45 cm. With the umbilical connection you can use Bio-Logic's advanced mixing technology with more devices, and in more applications. The umbilical connection is compatible with all Bio-Logic stopped-flow instruments.

For external coupling or difficult access application

Applications where use of umbilical may be necessary:

- installation on a synchrotron beamline or a neutron scattering line,
- cryo-stopped-flow experiments at sub-zero temperatures,
- high temperature stopped-flow ($T \geq 80^{\circ}\text{C}$),
- cooling of the observation head and flow lines while the rest of the instrument is maintained at a different temperature (when stock solutions are not very stable at experimental temperature),
- positioning of the observation head in a spectrometer magnet (for this application all parts will be metal free),
- coupling to FT-IR or EPR spectrometer.



Specifications

Volume of flow line ⁽¹⁾	200 μl
Number of flow lines	2
Length of umbilical link ⁽¹⁾	45 cm
Temperature control	yes

(1): different length available (multiple of 45 cm)

Compatible with

all SFM models

Umbilical link accessory

Catalog n°

047-62

Neutron head

Combining stopped-flow technology with small-angle neutron scattering gives the user valuable information about the early stages of reactions occurring during growth and formation of micelles or vesicles.

It can also be applied to other soft condensed matter studies such as growth of inorganic particles in an organic matrix or growth of mesoporous structures.

Small-Angle Neutron Scattering (SANS) is a technique that measures the deviation at small angles (from less than one degree to several degrees), of a neutron beam due to small particles in the sample. These structures could be clusters in alloys, polymers, or biological macromolecules and their dimensions are usually in the range of 10 to 100 nanometers.

The neutron scattering observation head is designed with a 44° opening for a large solid angle so the detector can get the maximum scattering information. A standard Berger Ball mixer is built into the neutron head. Reactions takes place in a 1 mm light path quartz cuvette.

The stopped-flow instrument can be fitted onto the neutron beamline using the umbilical link to mount the neutron head if needed.

While the maximum flow rate is usually limited to 2 ml/s, with the neutron cell, the overall dead time is determined by the acquisition speed of the detector.

Neutron scattering accessory includes:

- observation head with large opening window,
- FFKM O-rings (stopped-flow must also be equipped with FFKM for full solvent compatibility),
- quartz cuvette,
- Berger Ball mixer (hard-stop not included).

Typical applications include:

- soft condensed matter,
- micelles and vesicles,
- protein/protein interactions,
- change of quaternary structure.



Specifications

Solid angle aperture	44°C
Light path	1 mm
Temperature control	yes
Spare cuvette part number	OPT3100Q

Compatible with

all SFM models

[Neutron head accessory](#)

Catalog n°

053-11/34

X-Ray accessory

Time Resolved Small Angle X-Ray Scattering (TR-SAXS) has become a popular technique to characterize nanostructures.

The X-Ray observation head is engineered with a 60° opening to maximize the solid angle so both Small Angle (SAXS) and Wide Angle (WAXS) scattering applications can be covered. A standard Berger Ball mixer is built-in in the X-Ray head.

The X-Ray head is available with three sizes of capillary holder (OD 1.1, 1.5 and 2 mm). All holders can be fitted in the same observation head. A special capillary holder with 90° windows is also available for simultaneous optical and X-Ray measurements. Depending on capillary geometry a flow rate up to 7-8 ml/s can be used.

The SFM can be fitted onto the synchrotron beamline or bench-top X-Ray station. If space is not available around the beam line the X-Ray head can be combined the umbilical link.

X-Ray head includes:

- observation head with large opening window,
- capillary holder,
- Berger Ball mixer,
- hard-stop (capillaries not supplied).

For applications where the user needs to simultaneously measure XAFS and absorbance along a longer light path, a customized TC-50 cuvette (5 mm light path) with thinner walls is available. Contact Bio-Logic for details.



Specifications

Solid angle aperture	60°
Maximum flow rate	8 ml/s
Temperature control	yes

Compatible with

all SFM models

X-Ray accessory

		Catalog n°
X-Ray upgrade	for small capillaries, OD 1.1 mm	053-11/18
	for medium capillaries, OD 1.5 mm	053-11/25
	for large capillaries, OD 2 mm	053-11/36

Miscellaneous

Cuvette

The SFM observation head was designed so the cuvette can be changed in seconds without draining the temperature control circuit. The choice of a cuvette is made depending on the detection technique, the signal level needed, and the rate of the reaction to follow. The dead time is inversely proportional to flow rate and therefore cuvette volume. The signal level is proportional to path length. The fastest dead time (0.2 ms) is achievable with microcuvette accessory (μ FC-08).

Longer light paths are usually more adapted for absorbance measurements and shorter light paths for fluorescence.

Cuvette	light path	Catalog n°
FC-08	0,8 mm	054-08
FC-15/7.5	0,75 mm and 1,5 mm	054-15/7.5
FC-15	1,5 mm	054-15
FC-20/10	1 mm and 2 mm	054-20/10
FC-20	2 mm	054-20
TC-100/10F	1 mm and 1 cm	054-60
TC-50/10F	1 mm and 5 mm	054-53
TC-50/15T	1,5 mm and 5 mm	054-55
μ FC-08	0,8 mm	053-05
μ TC-100/10	1 mm and 1 cm	053-05/3
TC-50 cuvette holder		054-62
TC-100 cuvette holder		054-63
FC cuvette holder		054-64

Syringes

All SFM instruments are delivered with 10 ml reservoir syringes as standard. Smaller syringes are available for large mixing ratio applications or titrations. Plungers are also available as spares. Syringes are generally user changeable.

Complete syringes (barrel + plunger)	Catalog n°
1.9 ml syringe	950-27/1
6.8 ml syringe	950-27/2
10 ml syringe	950-27/3
3.6 ml syringe	950-27/4

Plungers only

1.9 ml syringe plunger for SFM-X000	950-27/6
6.8 ml syringe plunger for SFM-X000	950-27/7
10 ml syringe plunger for SFM-X000	950-27/8
3.6 ml syringe plunger for SFM-X000	950-27/9

O-rings

All SFM instruments are delivered with Viton® O-rings as standard. These are suitable for any biochemical applications with aqueous samples. For applications requiring the use of organic solvents, the SFM must be equipped with Perfluoroelastomer (FFKM) O-rings. These can be installed at the time of manufacture, or added later.

Standard kit	Catalog n°
Standard Viton® O-ring kit	950-00/20
For full solvent compatibility	
O-ring kit FFKM for SFM-2000/S	950-00/30
O-ring kit FFKM for SFM-3000/S	950-00/31
O-ring kit FFKM for SFM-4000/S	950-00/32
O-ring kit FFKM for /Q upgrade SFM-3000	950-00/33
O-ring kit FFKM for /Q upgrade SFM-4000	950-00/34

Viton® is a trademark of DuPont Performance Elastomers L.L.C

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