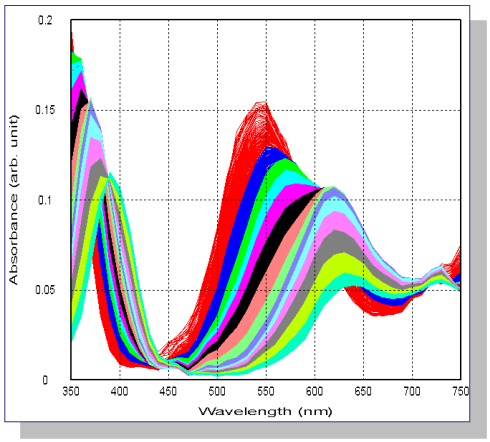
SX20 Stopped-Flow Spectrometer

CONTENTS

SX20 Standard Features

- Optimised for both absorbance and fluorescence detection without the need for reconfiguration. Dedicated detectors are provided for each technique.

- Unsurpassed sensitivity: ultra stable Xe light source and high photometric accuracy.

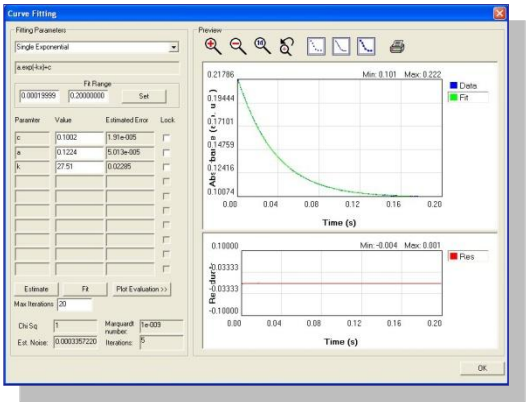


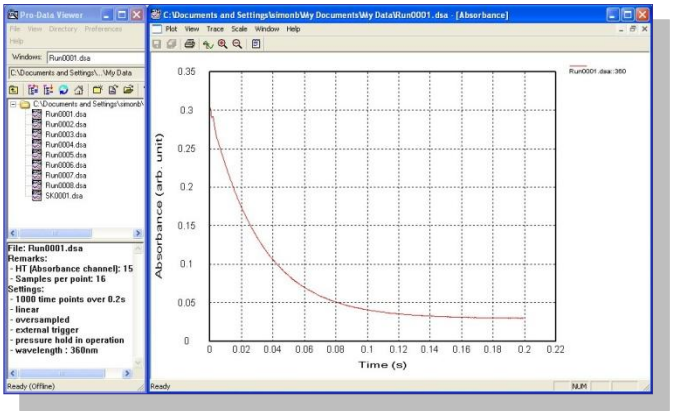
- Programmable monochromator enabling acquisition of time-resolved absorbance spectra and steady-state spectral acquisition.

- Removable cell cartridge with a 20µL volume, 1.1ms dead-time cell (other cells can be fitted). The cell is optimised for both absorbance and fluorescence detection with optical pathlengths of 10mm and 2mm, and both long and short optical pathlengths for fluorescence detection. A short pathlength is essential for many fluorescence applications in order to avoid self-absorption (inner filtering).

- Minimum sample volume requirement of 40µL per syringe.

- **Pro-Data** control software running on Windows™ 7 with comprehensive acquisition, display and analysis tools. Standard features include: wavelength scanning, repeat drives for signal averaging, acquisition of time-resolved spectra, live signal display, linear- logarithmic- and split-timebase, digital oversampling, temperature-dependent scanning and kinetics capability.





- Flow circuit materials suitable for anaerobic experiments and resistant to aggressive reagents.

- Very wide temperature range (+60°C to -20°C).

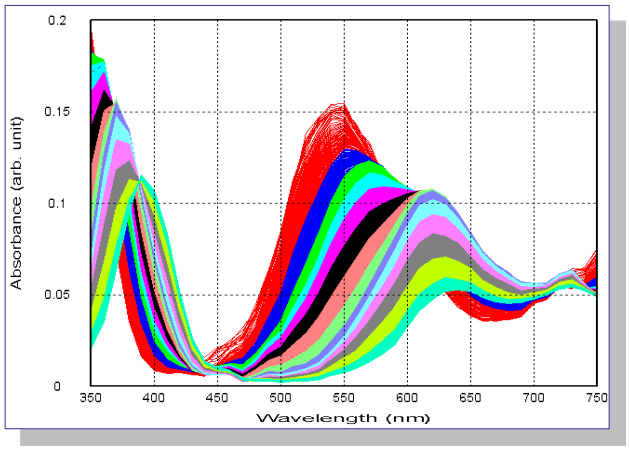
- Flat-screen monitor and network ready PC.

A comprehensive range of upgrade accessories are described on the following pages.

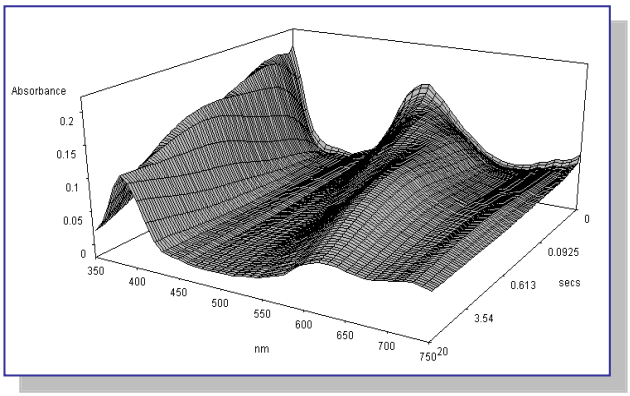
Upgrade Options for new and existing systems.

SX/ProK Global Analysis and Data Simulation Software

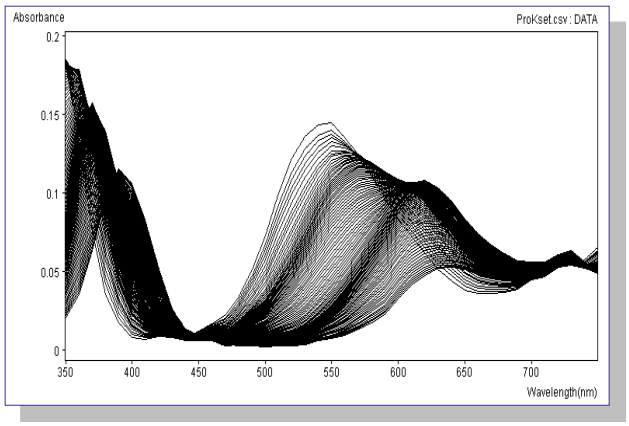
The capability to acquire multi-wavelength absorbance data (time-resolved spectra) is a standard feature of the SX20 system. For the data shown (right), the SX20 was set to automatically acquire a series of kinetic traces at 10nm intervals across the wavelength range 350-750nm. Less than 2mL of each reagent was required for this experiment (including priming the flow lines with sample). Alternatively, one can set the instrument to record kinetics at two or more discrete wavelengths as selected by the user.



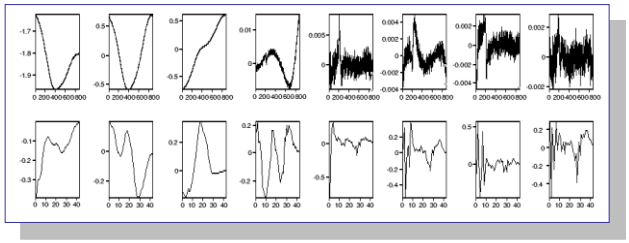
SX/ProK enables global fitting to multi-wavelength kinetic datasets; simultaneously fitting each kinetic trace to the reaction model. The kinetic parameters obtained must satisfy all the wavelength data at each time point therefore provides a more robust fit than single wavelength analysis and enables the study of more complex reactions.



SX/ProK includes a wealth of tools for viewing data and rapidly assessing the quality of fitted data. The figures (right) show 3-D and 2-D representations of the same dataset following transfer to **SX/ProK**.

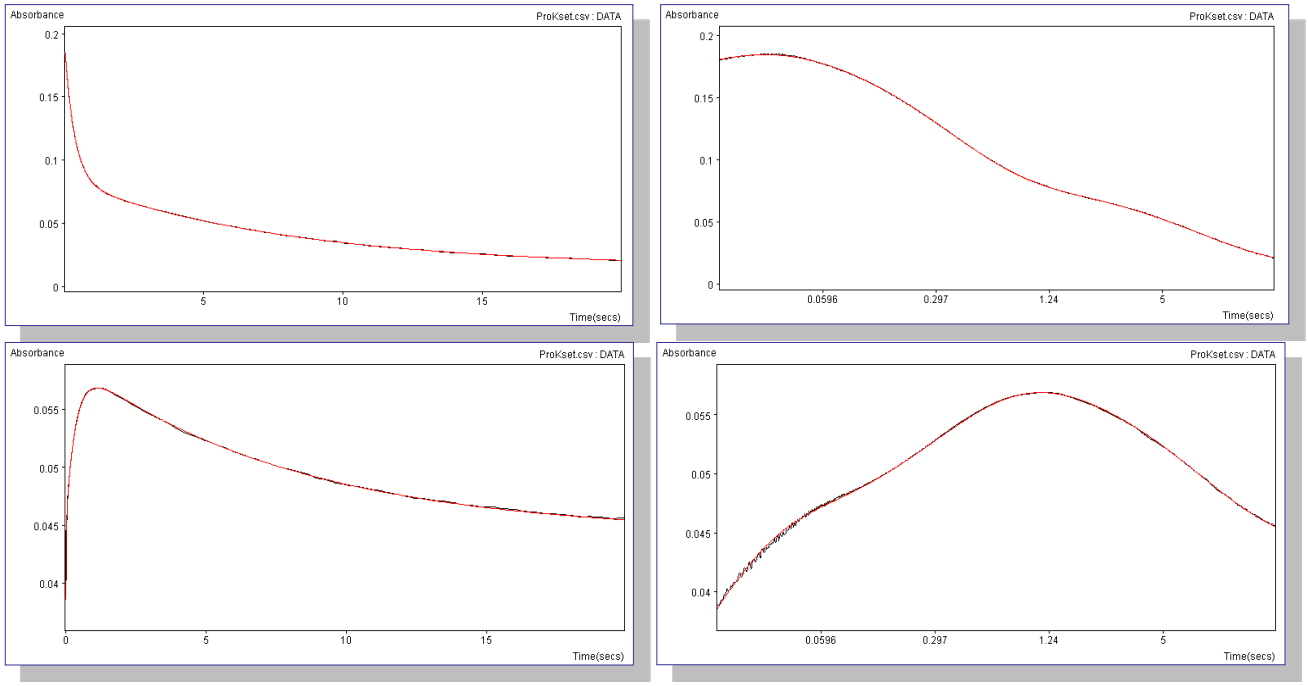


SX/ProK also enables Singular Value Decomposition (SVD) of the dataset. This single-mouse click operation provides a model-free assessment of the number of spectrally distinct reaction components present in the reaction and, by isolating those components that contribute only random noise to the data, enables the user to remove noise elements and improve data quality. In more detail, SVD produces a set of basis spectra, time-dependent amplitudes and singular values. For the data shown here, SVD analysis indicated the presence of 4 distinct species - suggesting a possible 3-step reaction mechanism.



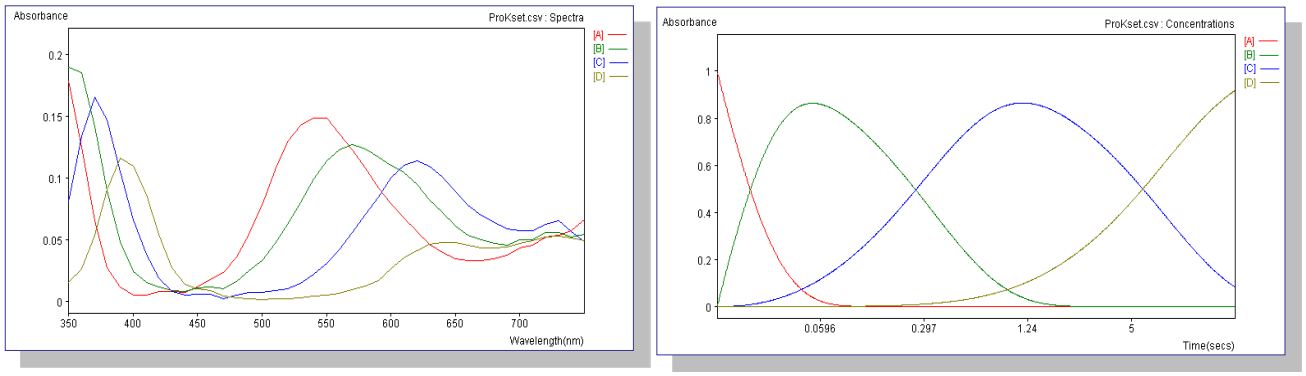
For global analysis of data sets, **SX/ProK** uses an innovative reaction scheme editor in combination with numerical integration techniques to enable fitting. Reaction models are entered in the form of simple steps e.g. A + B > C, C <> D, D > E and the use of numerical integration places almost no limit on the level of complexity of the reaction model.

For the data set shown above, global analysis to the 3-step (pseudo first order) reaction model **A→B→C→D** (entered as A > B, B > C, C > D) yielded calculated rate constants of 62.9s^{-1}, 2.8s^{-1} and 0.13s^{-1}. Close agreement between the fitted curves and all the kinetic data was found - examples of fitted kinetic curves are shown below.



Examples of fitted kinetic curves (overlaid in red on the data) following global analysis of the entire dataset to a 3-step reaction model. The curves show are at 350nm (top) and 690nm (below) and are shown here on both a linear (left) and a logarithmic (right) timescale.

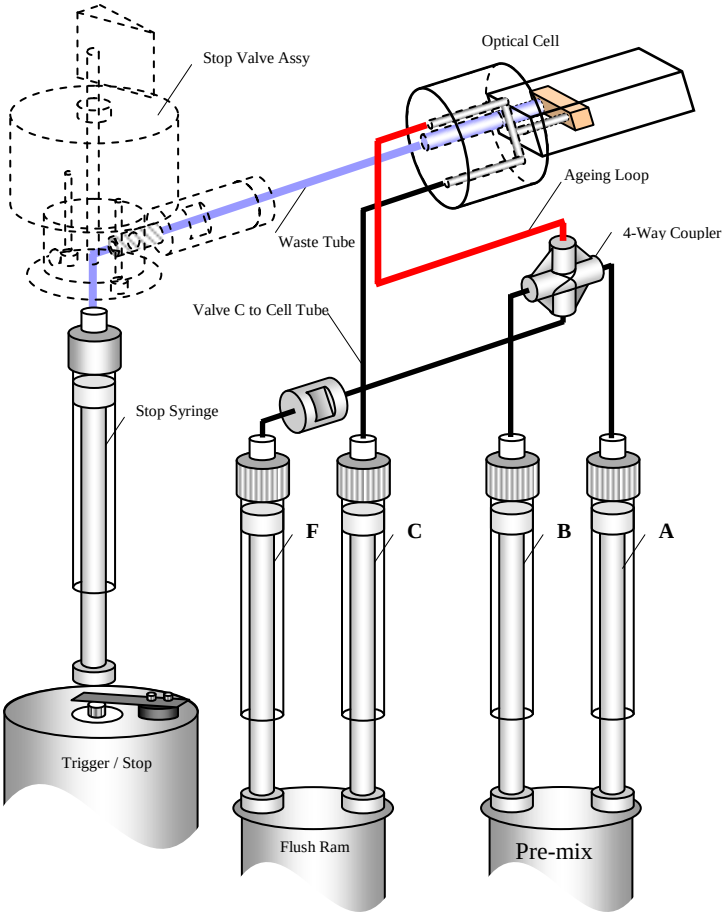
In addition to the optimised rates and the fitted kinetic curves and spectra, **SX/ProK** also generates concentration profiles and best-fit spectra for each reaction component providing clear evidence and support for the presence of short lived reaction intermediates.



Calculated best-fit component spectra (left) and concentration profiles (right - on a logarithmic timescale) following global analysis of the data set to a 3-exponential model.

SX/SQ Sequential-Mixing Accessory

The SX/SQ sequential- (or double-) mixing accessory is specifically designed to study the reactivity of intermediate and transient species. Asymmetric double mixing experiments are also fully supported (e.g. for protein folding/unfolding reactions). This accessory equips the sample handling unit with two drive rams (and 4 syringes). The first drive mixes two reagents (A and B) into an aging loop and, after a user defined aging period, a second drive mixes the aged solution with a third reagent (C) in the stopped-flow cell.



No hardware reconfiguration is required when switching between short and long aging times; the required aging time is simply entered by the user and this can be anything in the range 14ms to 1000s.

Full drive information is provided with each experiment including, drive profiles, calculated age time, drive volume per syringe, and a measurement of the dead-time. Aging times are reproducible to within 1ms.

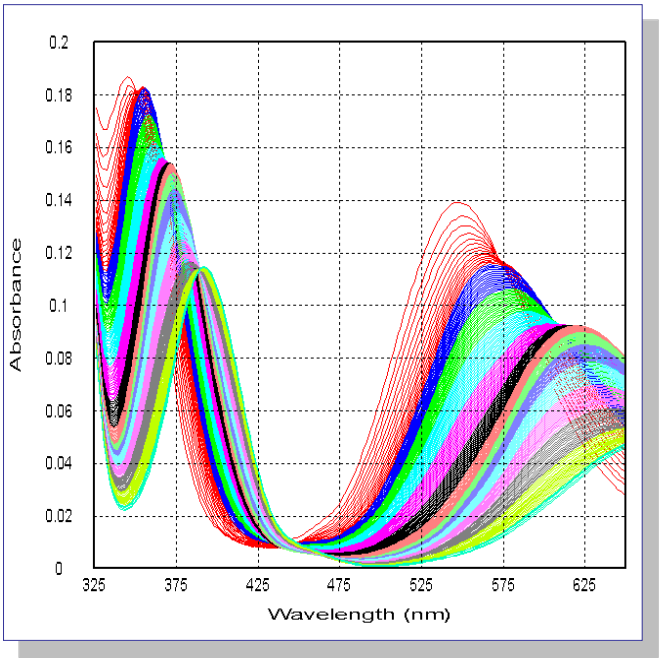
SX/PDA Photodiode Array Detector and SX/ProK Global Analysis

The **SX/PDA** photodiode array option enables sets of time-resolved spectra to be acquired from a single stopped-flow drive. The accessory is a self-contained spectrograph which can be configured in a few seconds without the need to realign or recalibrate the instrument.

The figure (right) shows photodiode array data acquired on the SX20 with option **SX/PDA** and displayed here on the SX20's Pro-Data control software.

Pro-Data also allows data to be edited in both the wavelength and time domains, or displayed as multiple or single kinetic traces.

The data shows time-resolved spectral changes covering the time range from a few ms to 20s that occur during the acid hydrolysis of the complex cation $[Ni(en)_3]^{2+}$ The data was acquired over a logarithmic time scale and the spectra shown here have been edited in the wavelength domain.



Global analysis of the multi-wavelength data sets using either the Applied Photophysics **SX/ProK** (see pages 3 & 4) or **SX/ProK II** (page 13) analysis packages yields more robust kinetic parameters and allows fitting to more complex reaction mechanisms than for single wavelength data.

Features of SX/PDA Photodiode Array

- 256 array that acquires up to 1500 spectra per second.
- Two wavelength ranges are available: PDA-UV:285-725nm and PDA-Vis 330-1100nm (the wavelength range of PDA-UV can be extended to 200nm using the boosted deuterium light source, option SX/UV – see page 7)
- Can be configured in a few seconds and requires no calibration.
- Linear, logarithmic or split time bases may be selected as appropriate to the reaction.
- User selectable digital oversampling.
- The minimum 0.67ms integration time may be increased to improve sensitivity.
- Sequential-mixing experiments are supported.
- User-friendly ProData software controls all experimental aspects with straightforward data transfer to SX/ProK (or ProKII) for data analysis.
- Any spectral region may be zoomed and re-zoomed for closer examination and data over discrete wavelength ranges can be selected for export or analysis.
- PC Pro-K and ProKII global analysis packages enable straightforward and fast access to robustly fitted kinetic parameters, concentration profiles and reaction component spectra.

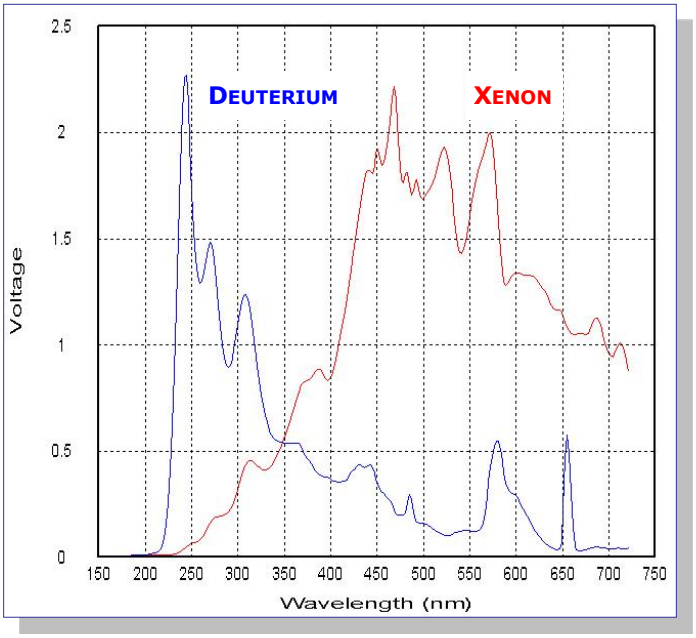
SX/UV Boosted Deuterium Light Source

Designed to compliment the UV photodiode array detector option **SX/PDA**, the boosted deuterium light source allows collection of time-dependent spectra in the far-UV wavelength region. Option **SX/UV** is designed for SX/PDA measurements in the range 200-400nm compared with 285-725nm using the standard xenon light source.

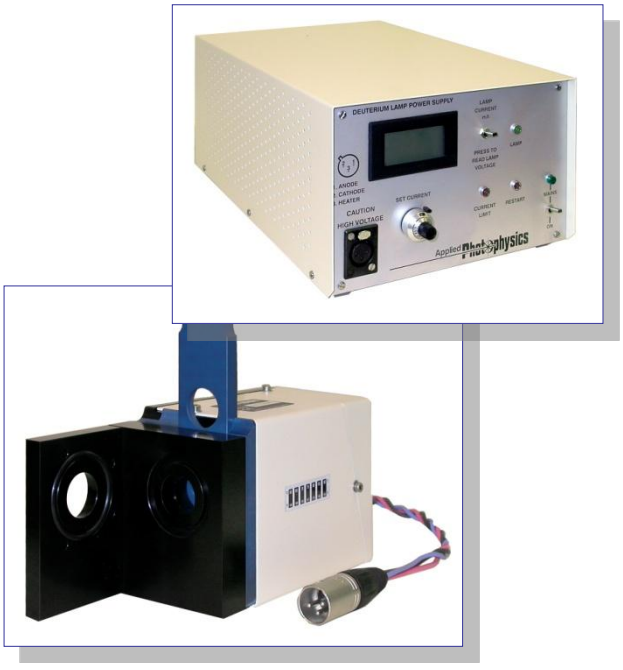
Enhanced UV Region Performance

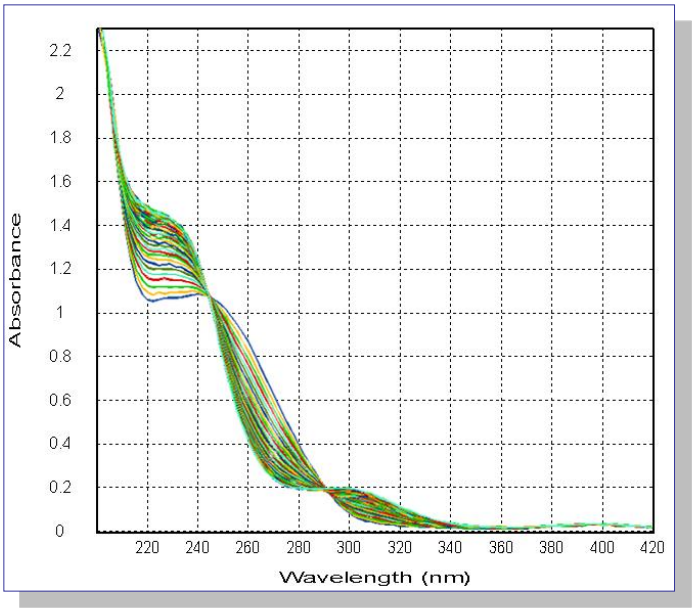
The figure (right) shows the deuterium and xenon lamp spectra. As can be seen, the SX/UV source has intense emission in the UV region and it is essential for SX/PDA measurements below 290nm. Other features include:

- Simple mounting to the SHU cell block
- Simple and rapid reconfiguration between xenon and deuterium light sources
- Adjustable current setting on power supply for signal-to-noise optimisation
- Pre-aligned lamps
- Ozone-free operation



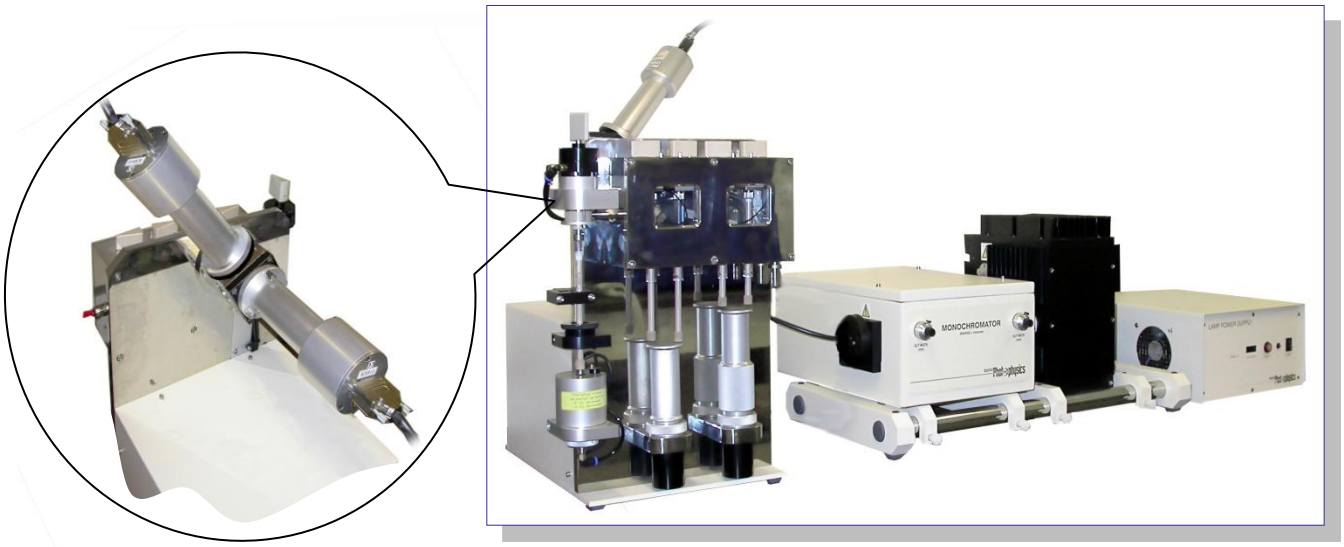
The time-resolved spectra shown (below right) relate to the ring transformation of dimethyl isothiouronium (0.01mM Dimethyl isothiourea was mixed in a 1:1 ratio with 10mM NaOH). The reaction was monitored in the wavelength range 200-420nm, by collecting 2000 spectra over the first 5 seconds.

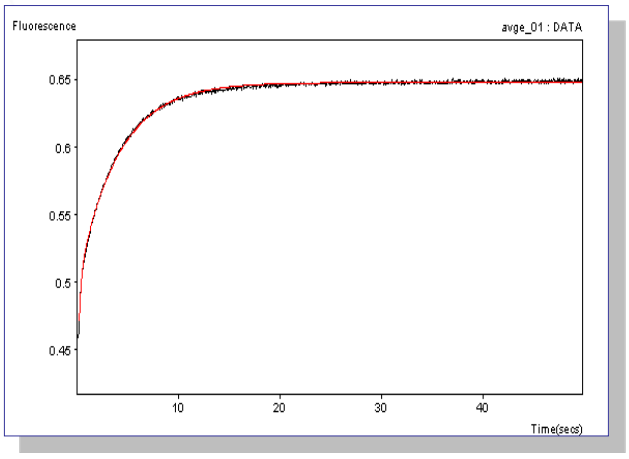


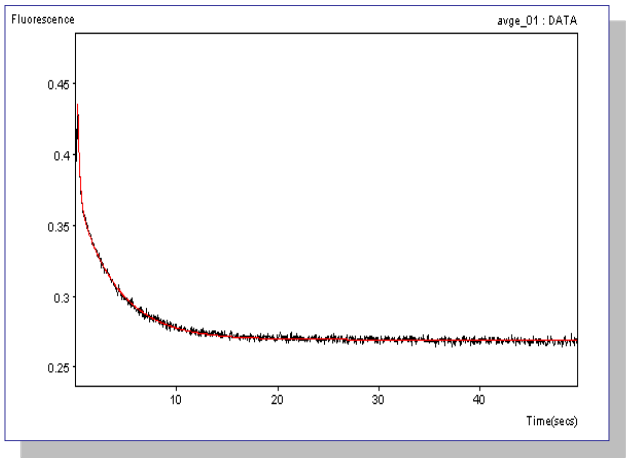


SX/DF Dual Channel Fluorescence Detection

The **SX/DF** option comprises an additional detection channel and fluorescence detector to enable simultaneous fluorescence detection at two emission wavelengths. Both detectors are mounted directly onto the cell-block (as shown below). In general, a cut-off filter is used with one detector to isolate emission at the higher wavelength, and an interference filter is used with the second detector to isolate emission at the lower wavelength. The **SX/DF** accessory finds common application in the study of FRET reactions. Data examples are shown below:







*The kinetic traces above were collected simultaneously using the **SX/DF** accessory. The fitted curves (in red) are overlaid following **SX/ProK** global analysis to a 2-step reaction model.*

The additional detection channel supplied with option **SX/DF** also enables simultaneous absorbance and fluorescence detection (option **SX/DD**) - in effect, this option is included with **SX/DF**.

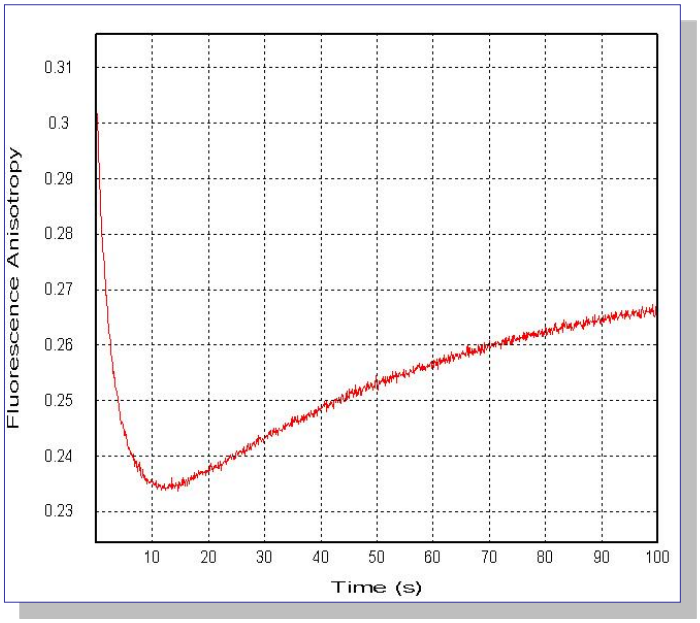
An alternative dual-fluorescence configuration is to mount one of the fluorescence detectors on a second (emission) monochromator (option **SX/SM** – see page 10). This allows the emission wavelength to be selected directly from the control software and hence without the requirement of an interference filter.

SX/FP Fluorescence Polarisation/Anisotropy

Excitation of a fluorophore with plane polarised light results in the preferential excitation of molecules with their absorption moments orientated parallel to the plane of polarisation. Fluorescence polarisation/anisotropy can provide information about changes in the mobility and environment of a fluorophore when it interacts with other molecules.

The **SX/FP** fluorescence polarisation/anisotropy accessory is an easy to fit, dual channel T-format fluorescence polarimeter with a movable calcite input polariser. G-factor determination is controlled from the software and both kinetics and spectra may be acquired in polarisation, anisotropy, total emission and raw data modes, and with full post-acquisition conversion between data modes as required.

Note: option **SX/FP** includes options **SX/DF** and **SX/DD** described above.

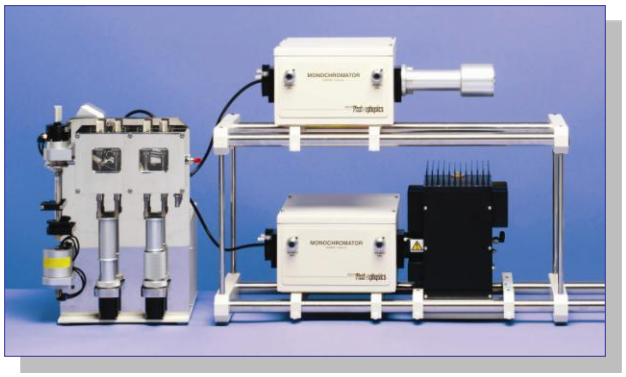


Key Features of SX/FP

- T-format dual channel detector
- Calcite excitation polariser
- DPUV sheet collection polarisers
- Filter holders built into collection pieces allowing reduction of scattered light
- Filter holder built into excitation assembly, for additional flexibility with respect to wavelength selection and rejection
- Straightforward instrument set-up with no optimisation required.
- Robust construction ensures consistent polariser alignment
- Global data analysis using Pro-FP software

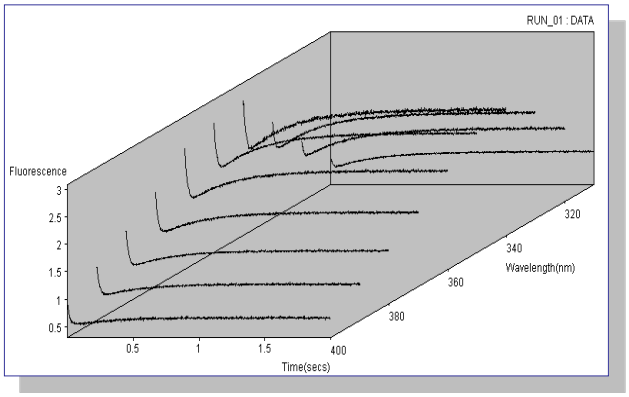
SX/SM Scanning Emission Monochromator

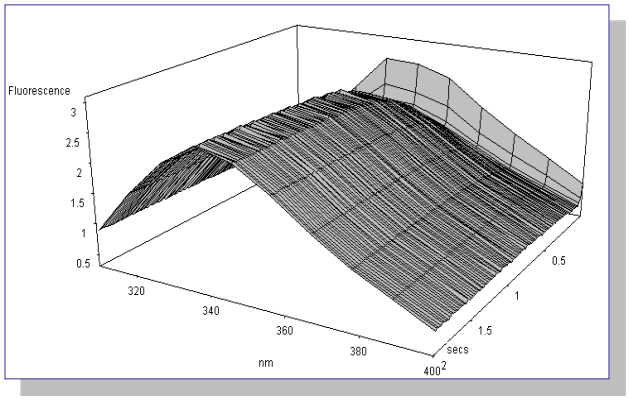
The standard fluorescence configuration, with the fluorescence detector mounted directly onto the cell-block, has exceptional sensitivity. This configuration employs a cut-off filter to block scattered light of the excitation wavelength such that only the fluorescence emission signal is detected. These experimental capabilities are extended with option **SX/SM** which comprises a second programmable monochromator and a light guide to connect the cell-block to the second monochromator and detector as pictured (right). In this configuration the detected emission wavelength can be selected by setting the second monochromator directly from the SX20's control software. This also enables:



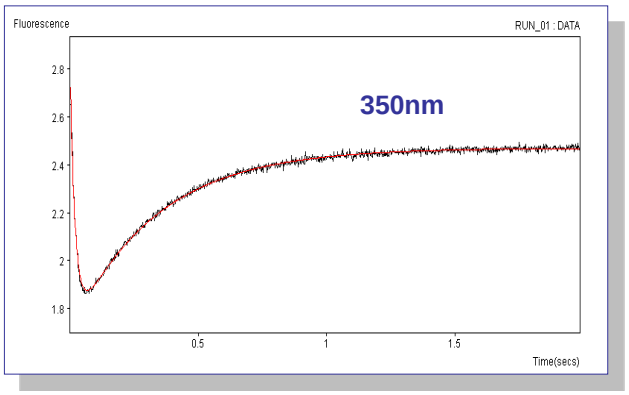
- Automated acquisition of time-resolved emission spectra
- Acquisition of steady-state emission spectra

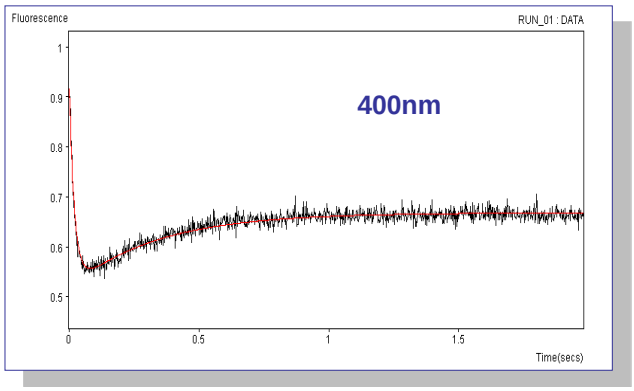
The multi-wavelength dataset below (showing refolding of lysozyme) was acquired from an automated series of stopped-flow drives (between 310nm and 400nm) using option **SX/SM**.





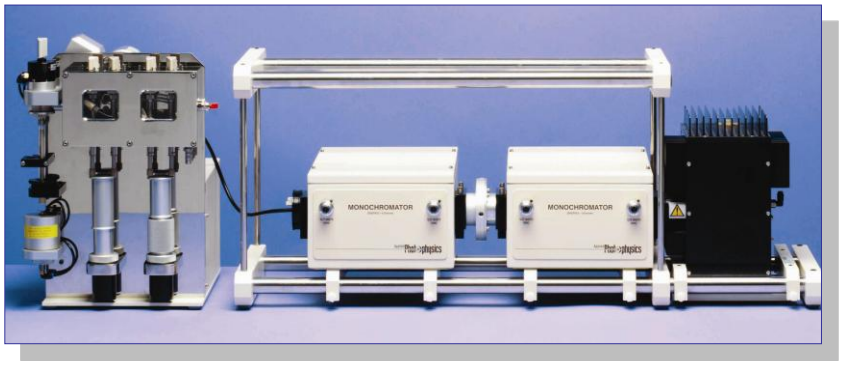
As with multi-wavelength data acquired in absorbance mode (pages 3 and 4), the entire data set can be fitted simultaneously using the **SX/ProK** global analysis software. Examples of fitted data are shown below.





Examples of fitted kinetic traces from the dataset above following global analysis of the whole dataset to a 2-step reaction model (A > B > C). The fitted curves are overlaid in red.

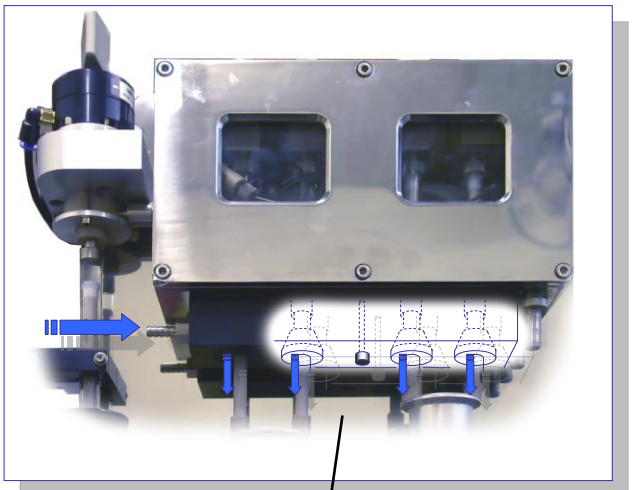
SX/AM Scanning Monochromator (far-UV) and Photometric Accuracy

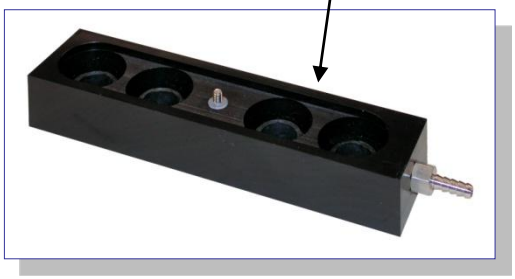


Option **SX/AM** consists of a second programmable monochromator and a coupling unit such that the two monochromators can be connected in series as shown in the figure. The double-monochromator configuration removes stray light error when recording absorbance kinetics in the far-UV wavelength region (see page 15). With the **SX/AM** accessory absorbance kinetics can be recorded over the entire range of the detector (200nm to 850nm) and without any reconfiguration of the instrument. The second monochromator is identical to that used with option **SX/SM** (see previous page) and so purchasing either of these options can, with the addition of only minor components, provide the functionality of the other.

SX/AN Anaerobic Accessory

The anaerobic accessory (SX/AN) equips the SX20 with a high performance bench-top anaerobic capability. The SX20 is designed to enable anaerobic conditions to be achieved with ease – most commonly by purging the flow circuit with a diothionite solution. The flow circuit material is of PEEK(polyetheretherketone), which eases the rapid removal of oxygen, and PTFE (a material from which it is more difficult to purge of oxygen) is present only in the tips of the syringes.





Anaerobic conditions are maintained using the purging manifold that forms the main part of the SX/AN option. This unit mounts over the lower section of the drive syringes and is purged with a steady stream of nitrogen to maintain a oxygen-free environment in the region between the syringe-pistons and the syringe-barrels. This prevents oxygen diffusion across the syringe-tips and contamination of the sample. 3-way valves are also provided to enable anaerobic samples to be introduced to the sample handling unit without coming into contact with the outside environment (air). A full protocol for anaerobic sample introduction is provided.

SX/RC5 5µL volume low dead-time cell

The SX20 features a removable cell cartridge allowing the use of different optical cells to be fitted according to experimental requirements. In addition to the standard 20µL volume cell, a 5µL cell is available with a dead-time of around 0.5ms (see - page 18) and which allows reliable measurement of rates in excess of $3500s^{-1}$.

Each cell is mounted in a pre-aligned cartridge for rapid interchange between cells. The 5µL cell has optical pathlengths of 1mm and 5mm for absorbance, and both observation ports are also suitable for fluorescence detection.

The procedure for changing the cell is straightforward: it takes less than 5 minutes and once fitted, no further optimisation and alignment is necessary.

The SX20 sample handling unit features a novel cell cartridge system that facilitates cartridge mounted optical cells that can be interchanged with a minimum of work. The figure shows a typical cell cartridge.

The Quench-Flow Adapter (see below) is fitted in the same way.

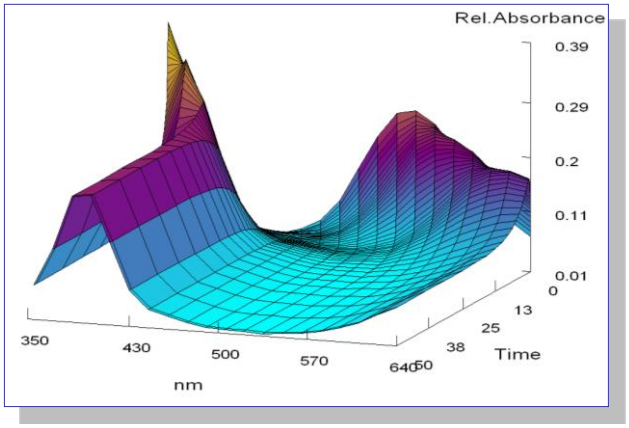
SX/QFA Quench-Flow Adapter

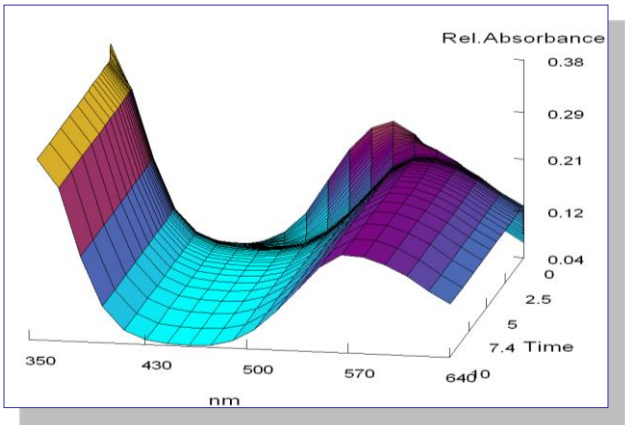
The Quench-Flow adapter can be fitted in a few minutes in place of the standard stopped-flow cell/cartridge. It includes a milli-second dead-time mixer connected to a detatchable flow line (for sample recovery). In combination with the sequential-mixing capability (option **SX/SQ**), this accessory enables quench-flow operation – i.e.

- rapid-mixing of reagents
- incubation for a (user selected) period in the range 15ms to 1000s …
- … followed by rapid-quenching of the reaction and sample recovery.

SX/ProK II - 2nd Order Global Analysis Software

SX/ProK II has all of the functionality of **SX/ProK** (see pages 3 & 4) and in addition allows the simultaneous analysis of *multiple* datasets gathered at different sets of initial conditions (usually concentration). This capability is unique and greatly increases the ability to resolve rates and intermediate spectra of more complex reaction mechanisms directly from stopped-flow data. The simplest example is the 2nd order reaction: A+B > C. Standard global analysis packages (including SX/ProK) cannot directly determine the spectra of A and B from analysis of a multi-wavelength data set as they are linearly dependant; it is necessary to fix the spectrum of one of the reactants. However, by performing the reaction at two (or more) starting concentrations of A and B and submitting all of the datasets to **SX/ProK II**, the spectra and the rate can be directly obtained. A more complex example is the reaction: A+B <> C <> D, where all spectra and all forward and reverse rates can be determined using **SX/ProK II**.





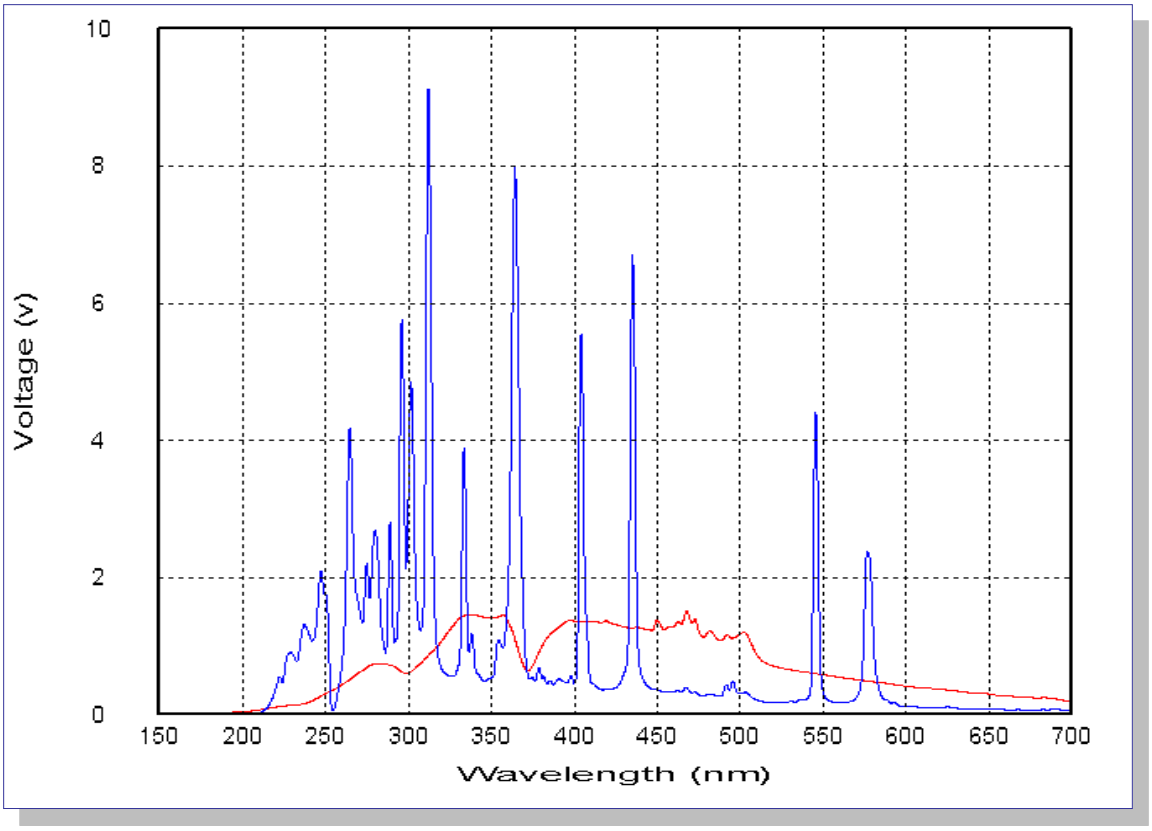
Other features of **SX/ProK II** include:

- Singular value decomposition for spectral component prediction and random noise reduction.
- The use of numerical integration which places virtually no limit on the complexity of the reaction mechanism used for fitting
- A powerful data simulator allowing the user to investigate reaction models, rates and spectral parameters. Random data noise can also be simulated enabling the user to explore the analysis (fitting) capabilities of typical 'idealised' data with the reaction model. The simulation tool can also be used to gain familiarity with the package.
- Mixed data types: simultaneous analysis of, for example, fluorescence and absorbance data to further aid the elucidation of reaction rates and mechanisms
- New support for rapid equilibrium and protonation steps in the reaction model
- Comprehensive visualisation tools for inspecting results

Performance Information

Light Sources

Three types of lamp are available for the SX20 lamp housing: a 150W xenon arc (ozone-free), a 150W xenon arc with spectrosil envelope (ozone-producing) and a 150W mercury-xenon (ozone-producing). All lamps provide outstanding stability and durability conforming to Applied Photophysics's tight specifications. The ozone-free xenon lamp is supplied as standard and is suitable for most applications. The ozone-producing xenon lamp has a much higher light intensity below 240nm. The mercury-xenon lamp has strong Hg emission lines over the xenon spectrum at wavelengths that can be of interest for specific applications in fluorescence and circular dichroism. The figure below shows the output profiles for a xenon lamp (red) and a mercury-xenon lamp (blue).

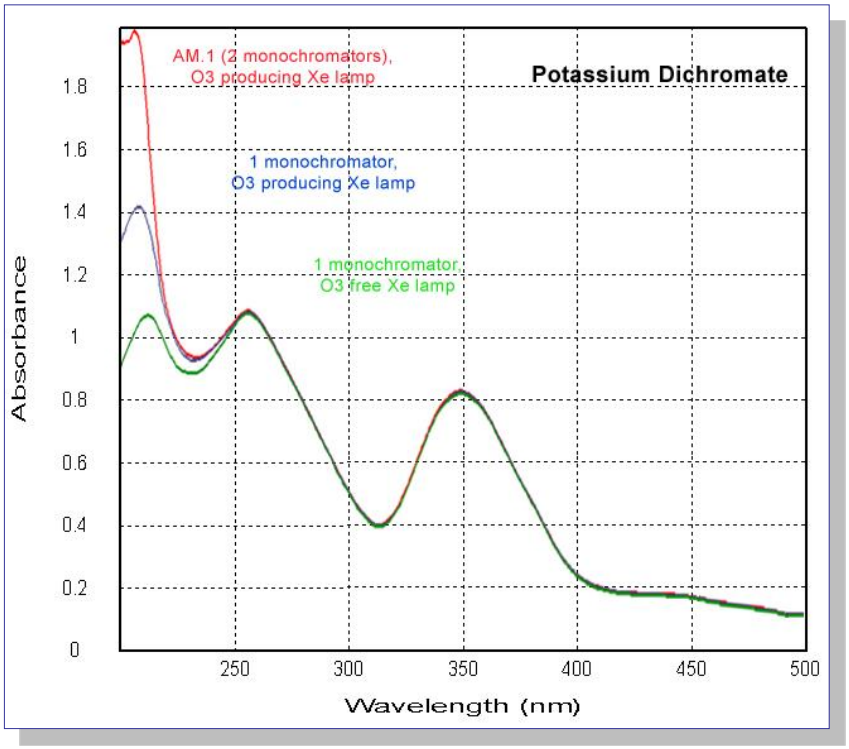


The lamp housing includes a nitrogen purge facility (for use with the ozone producing lamp) and allows external lamp adjustment. Lamps can be readily interchanged but if interchanging the lamp (e.g. between Xe and Xe-Hg) is likely to be regularly required, we recommend purchasing a second back-plate for the lamp housing – this will enable lamp to be changed in a few minutes without the requirement to handle the lamp itself or re-align the lamp when it is fitted.

The lamp power supply unit (PSU) is optimised to provide a stable lamp output (see page 17). Lamp ignition uses a 'SafeStart' igniter system which will not interfere with sensitive electronic equipment.

Photometric Accuracy

The use of spectrophotometry as a tool to follow concentration changes requires that certain criteria are met. With respect to absorption measurements, there must be conformance to the Beer/Lambert law so that absorbance is directly proportional to concentration (i.e. A = e.c.l where e = extinction coefficient, c = concentration and l = optical path). Accurate absorbance measurements in the UV region require that adequate account be taken of spectrometer stray light error. Potassium dichromate is a useful reference material for assessing spectrometer performance. The spectral plots shown in the figure below indicate that the standard SX20 stopped-flow spectrometer has good photometric accuracy down to ~240nm (green and blue traces) whereas using the double monochromator configuration of option **SX/AM** (red trace) there is excellent photometric accuracy to 2AU at 200nm.



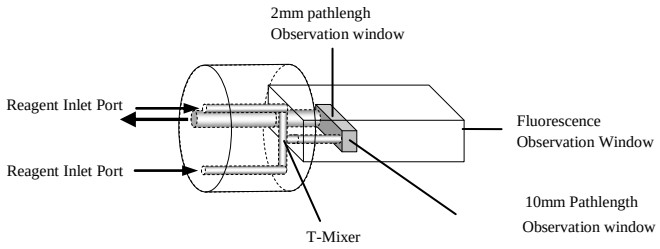
The ozone-free lamp has no emission below 230nm and so the apparent absorbance spectrum in this region with a single monochromator (green trace) is entirely due to stray light error. The ozone-producing lamp does emit below 230nm and so the measured spectrum in this region with a single monochromator (blue trace) is a combination of real sample absorbance and stray light error. When the ozone-producing lamp is used in combination with the double-monochromator configuration (option **SX/AM** – see page 11) there is effectively no stray light error and the true absorbance spectrum of potassium dichromate can be recorded down to 200nm. Comparison of these spectrum with those recorded with a single monochromator indicate that good photometric accuracy can be achieved to at least 240nm using a single monochromator.

Stray light error can also be removed by using a **solar blind detector** in place of the standard detector: by limiting the detectors range (e.g. to cover the region 200nm to 320nm) almost all of the stray light will go undetected and so absorbance measurements will be photometrically accurate.

Cell Design

Absorbance measurements

The standard 20uL volume quartz cell has dimensions 10mm x 2mm x 1mm, and provides optical pathlengths of 10mm and 2mm. Black quartz is used to mask the optical windows. To switch optical pathlengths, the user simply relocates the detector and light guide – as task of about 1 minute.



Fluorescence Measurements and Inner Filtering

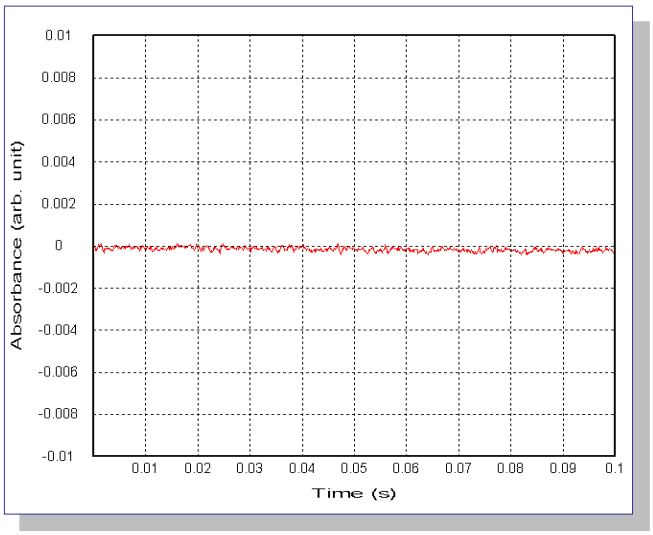
The SX20 cell is uniquely optimised for fluorescence detection because the 'fifth' side of the cell is dedicated to this purpose. This means that:

- The cell is able to incorporate a light pipe specifically designed to maximise collection of fluorescence emission thereby increasing sensitivity.
- The Inner filtering effect (see below) can be low without having to compromise sensitivity by reducing the cell volume.
- No reconfiguration is required when switching between absorbance and fluorescence detection.

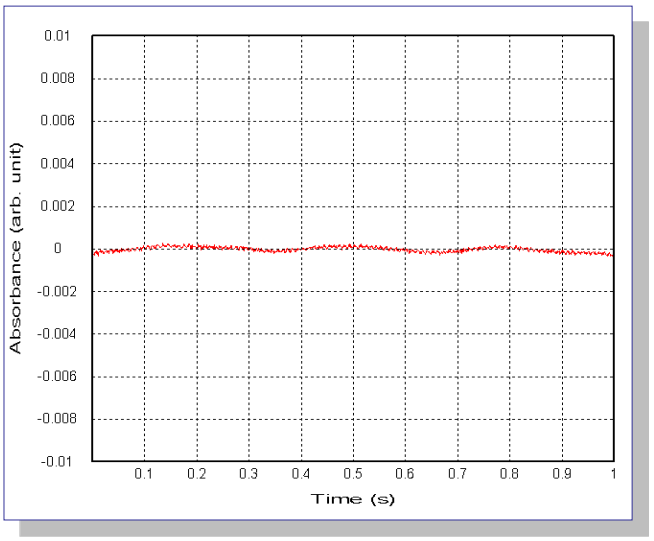
With respect to fluorescence measurements, the inner filter (or self-absorbance) effect must be considered before assuming that the measured signal is directly proportional to the concentration of a chemical species. The inner filter effect is caused by progressive absorption of the fluorescence excitation light as it penetrates the solution being studied, thereby producing progressively less fluorescence signal. Hence a change in the total absorbance during the reaction can produce non-exponential fluorescence kinetics. The effect is minimised by using low sample concentrations and/or low optical pathlength cells. With the SX20 20ul cell, sample excitation via the 10x1mm window (2mm port) gives a low optical pathlength for fluorescence measurements (1.5mm – assuming scattering from the centre of the cell chamber). Excitation via the 2x1mm window gives a higher value (5.5mm). In both cases the entire sample volume can be irradiated. Compare this with a similar stopped-flow cell where the fifth side of the cell cannot be used: here the optical pathlength will be 6mm irrespective of which port is used for sample excitation. This higher value limits the range of sample concentrations that can be used.

Lamp Stability

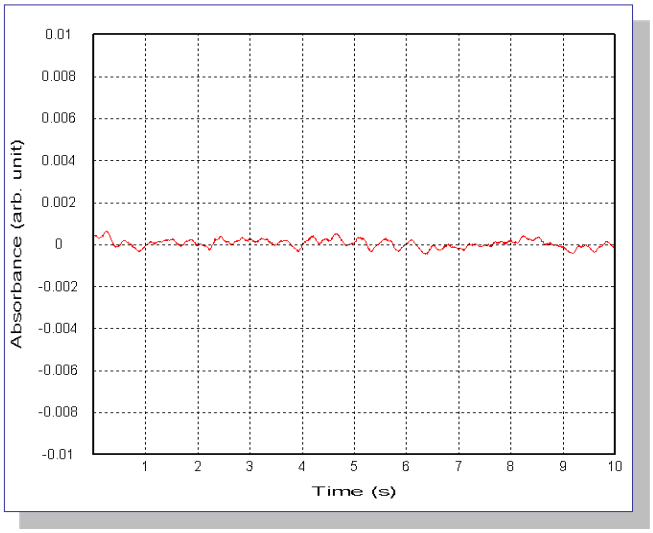
Light source stability is a critical requirement to ensure that small signal changes can be detected and that kinetic traces are reproducible. Lamp stability should be examined for quality in several time domains. Around 100ms will show up ripple originating from the lamp power supply with a frequency related to that of the line supply voltage. The 100ms time period will also show photo-multiplier shot noise due to insufficient light flux. Different types of lamp instability (i.e. plasma instability and arc wander) are revealed between 1 and 10 seconds with long term drift showing over periods of 1000 seconds or more. The traces below illustrate the performance of a typical SX20 instrument with an ozone free xenon arc lamp. The instrument had been allowed to fully stabilise prior to recording these measurements. An absorbance change of 0.001AU represents a signal voltage change of 0.25%.



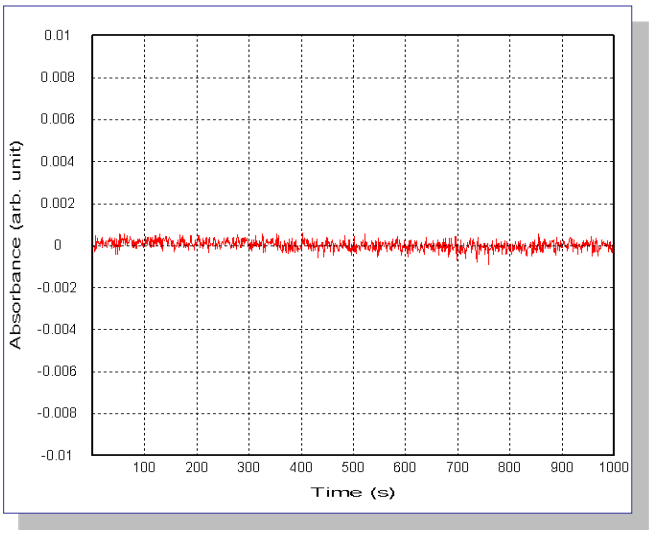
Over 0.1 seconds.



Over 1.0 seconds.



Over 10 seconds.



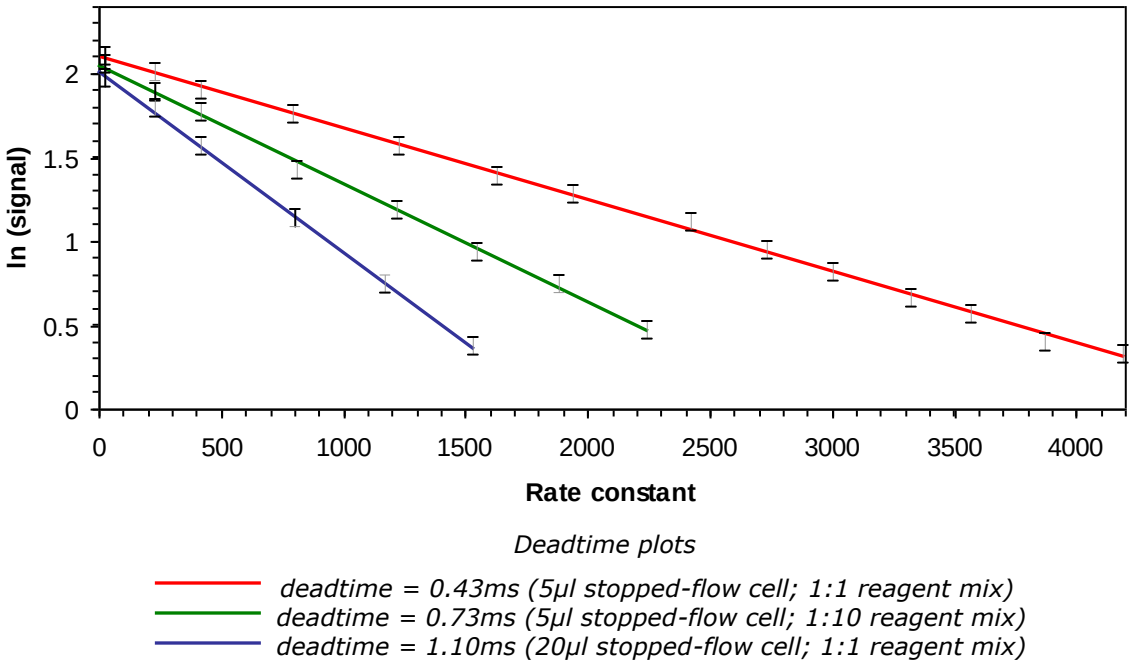
Over 1000 seconds.

Dead-Time Determination

The instrument dead-time can be defined as the earliest time at which valid measurements of the reaction can be made. A short dead-time is an important requirement for measurement of very fast reactions and its value is therefore an indicator of the both instrument's kinetic performance and the overall design quality of the sample handling unit.

The fluorescence quenching reaction between N-acetyltryptophanamide (NAT) and Nbromosuccinimide (NBS) provides a useful tool for measuring the dead-time[1]. To obtain the data shown here, NAT was mixed with a range of NBS concentrations in a 1:1 and 1:10 drive ratio. The reagent concentrations (after mixing) were 10^{-5}M (NAT) and $5x10^{-5}$ to $5x10^{-3}$M (NBS). Excitation was set at 280nm and the fluorescence signal was isolated using a 305nm cut-off filter.

The dead-time can be calculated as the slope of a linear plot of *ln(initial signal) vs. rate constant.* The figure below displays the plots and the corresponding dead-times for 1:1 and 10:1 mixing experiments. It can be seen that using the 5µL cell (option **SX/RC5**) provides a significant improvement in the dead-time allowing measurement of rates in excess of $3500s^{-1}$.

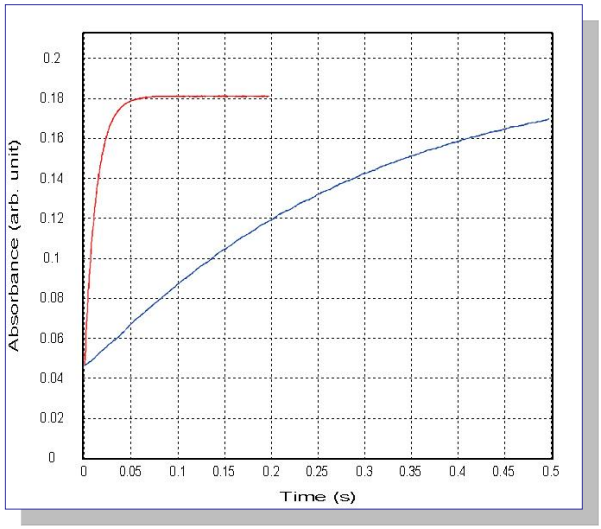


Software Controlled Dead-Time Determination. With option **SX/SQ**, the dead-time can also be measured directly from the instrument control panel simply by doing a stopped-flow drive; the time course of the drive together with the total volume dispensed, the final velocity and the calculated dead-time are recorded. This is a useful feature for checking the instrument's performance and for assessing the effects of parameters such as drive pressure, reagent volume and reagent viscosity on the dead-time without recourse to more lengthy chemical methods as shown above.

[1] Peterman, *Anal. Biochem.*, 1979, **93**, 442.

Low Temperature Kinetics

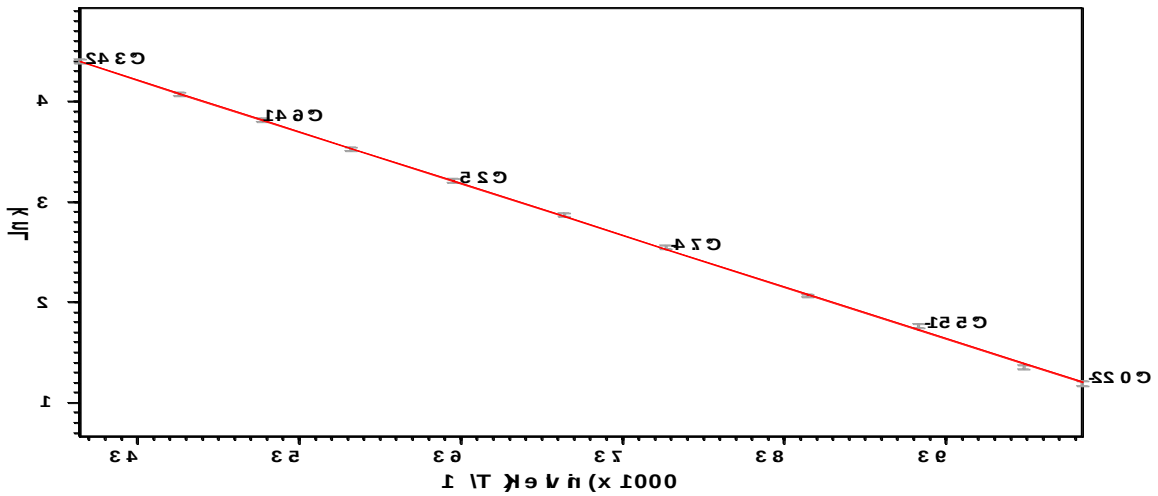
The standard SX20 instrument operates over the temperature range +60°C to -20°C with no requirement for additional accessories. No instrument reconfiguration is required when operating at low temperatures and the sequential mixing facility (option **SX/SQ**) can also be utilised over this temperature range. The SX20 control software can also be linked to a **programmable circulator bath** to enable acquisition of an automated series of kinetic measurements of a range of (user specified) temperatures.



Alkaline hydrolysis of 2,4-DNPA.
Kinetics at 24.3°C (red) and –22.0°C (blue).

Measurements of absorbance changes in the alkaline hydrolysis of 2,4-DNPA (2,4-Dinitrophenylacetate) in methanol are shown here to demonstrate the SX20's performance over the temperature range -22°C to+24°C. 100mM 2,4-DNPA in MeOH was hydrolysed in a 1:1 symmetric mix with 0.3M sodium methoxide (NaOMe) in MeOH. The stopped-flow kinetics were recorded at 360 nm. A Neslab RTE-200 circulator with water:ethylene glycol (50:50) circulating coolant was used for thermostatting.

The figure (above right) shows the kinetic traces acquired at the upper and lower limits of the temperature range. The recorded traces at each temperature were analysed to create an Arrhenius plot (below) from the measured individual rates. The linearity of the data points demonstrates that the SX20 retains its excellent performance even at these low temperatures.



Specifications

Description

The SX20 is purpose designed for high performance stopped-flow research. The standard system provides single mixing, absorbance and fluorescence (or light scattering) detection, scanning capability in steady-state and kinetic modes, all of outstanding sensitivity. It features a biocompatible and fully thermostated flow circuit and supports large ratio asymmetric mixing. Data acquisition, display and analysis is provided by our **Pro-Data** software running under Windows™ 7. **Pro-Data** provides an integrated and easy to use environment with flexible and powerful functionality for a wide range of stopped-flow kinetics applications. A comprehensive range of upgrade options are also available.

General information

- Measurement modes: Absorbance, Fluorescence, Fluorescence Polarisation/Anisotropy, Dual Fluorescence, Sequential Mixing, Quench-Flow. Optimised for both absorbance and fluorescence detection without the need for reconfiguration.
- Ultra stable Xenon lamp power supply with built-in 'SafeStart' igniter.
- 150W Air cooled xenon lamp housing fitted with either a low noise ozone-free Xenon lamp or ozone-producing Xenon lamp.
- Programmable monochromator enabling acquisition of equilibrium absorbance spectra and multi-wavelength kinetic data sets.
- Sample handling unit fitted with removable cell cartridges. The standard 20µL volume cell has a dead-time of 1.1ms, optical pathlengths of 10mm and 2mm, and is suitable for absorbance and fluorescence work. A shorter dead-time 5µL cell can also be fitted.
- Flow circuit materials are suitable for anaerobic experiments and resistant to aggressive reagents.
- Very wide temperature range (+60°C to -20°C).
- Minimum sample volume requirement of 40µL per syringe.
- Nine-stage side window absorption photomultiplier (Range 140 – 850nm).
- Eleven-stage end window emission photomultiplier (Range 280 – 650nm). Alternative tubes are available if extended wavelength ranges are required.
- **Pro-Data** Windows™ control software with comprehensive acquisition, display and analysis tools. Standard features include: wavelength scanning, repeat drives for signal averaging, acquisition of time-resolved spectra (SpectraKinetic technique), linear, logarithmic and split timebase and digital oversampling capability.
- Flat-screen monitor and network ready PC.
- The SX20 stopped-flow spectrometer requires the following environmental conditions:

- Operating temperature: +5°C to +35°C
- Operating Humidity: 20% to 80% non condensing
- Storage temperature: -20°C to +50°C
- Storage Humidity: 5% to 80% non condensing

Instrument Overview

Light Source

Type	150W Xe arc
Ignition	"Safe-Start" – lower RFI
Stability – short term (up to 200ms)	<0.001AU (peak-to-peak)
Stability – medium term (1s to 10s)	<0.001AU (peak-to-peak)
Stability – long term (10s to 1000s)	~0.001AU (peak-to-peak)
Light source warmup - %max. intensity	95% after 0.5hr; 98% after 1.0hr
30min drift (after 1 hour operation	<1%

The light source comprises a high stability, power controlled lamp supply which is used to drive a 150W short arc lamp mounted in convection cooled housing. The combination of this high specification power supply and short arc lamp provides excellent stability over all data acquisition periods. These performance characteristics allow a single light source to be used for UV/Vis absorbance studies as well as for fluorescence excitation.

The standard instrument is supplied with a xenon "ozone-free" lamp which is a useful general purpose lamp. For enhanced operation alternative lamps can be fitted: a 150W xenon with spectrosil envelope (ozone-producing) will enable emission in the far-UV wavelength region (below 240nm) and a 150W mercury-xenon offers strong emission lines at specific wavelengths that can be of interest for specific fluorescence excitation applications.

Lamp ignition is via a novel "SafeStart" built-in igniter which minimizes RFI during lamp ignition and thereby ensuring that there is no possibility of damage to sensitive electronic equipment in the vicinity of the lamp.

Monochromator

Optical layout	Symmetrical Czerny-Turner
Dispersive medium	Diffraction grating
Slits	Bilateral – infinitely variable up to 5mm
Dispersion	4.65nm/mm
Minimum wavelength step	0.1nm
Rate of change when setting to new wavelength	1500nm/min
Standard wavelength range (detector limited)	200 to 850nm

The programmable f/3.4 grating monochromator (Czerny-Turner optical configuration) has continuously variable bi-lateral slits and is supplied with a 1200 l/mm holographic grating (alternative gratings can be fitted). The monochromator is fitted with a stepper motor drive and wavelength selection is controlled from the workstation.
Light output from the monochromator is coupled to the cell cartridge of the stopped-flow sample handling unit via a high quality spectrosil light guide.

Sample Handling Unit

Number of view ports	Two transmission/emission; One dedicated emission
Cell volume (standard cell)	20μL
Optical path (transmission)	2mm and 10mm
Cell volume (optional cell)	5μL
Optical path (transmission)	1mm and 5mm
Dead time (standard cell)	1.1ms (1:1 mix) 1.6ms (10:1 mix)
Maximum final reagent flow rate	18.5μL/ms (1:1 mix)
Typical reagent volume per shot	50μL of each reagent (1:1 mix)
Drive ratios using appropriate syringes	1:1; 2.5:1; 5:1; 10:1; 25:1
Drive mechanism	Pneumatic drive for fast response with automatic sample empty (multiple shots)
Sequential mixing operations	
Age time range	15ms to > 1000s (continuously variable)
Age time selection	Directly from control software (no hardware reconfiguration required)
Calculated dead time	Recorded with each drive
Calculated age time	Recorded with each drive
Drive profiles	Recorded with each drive
Drive volumes	Recorded with each drive

The drive syringes, flow lines and optical cell are surrounded by a thermostat bath. The flow circuit is chemically inert and free of stainless steel and rubber. It is constructed of glass (syringe barrels), silica (optical detection cell), PEEK (sample flow circuit tubing and drive valves) and Teflon (syringe piston seals). A supply of compressed gas, usually nitrogen or air (8 bar pressure), is required to operate the rams.

Electronics & Software

Data acquisition resolution	16 bit
Data input range	+ 10V to – 10V
Maximum digitization range	100kHz
Acquisition period – timebase 1	From 5ms to 360000s
Acquisition period – timebase 2	From 1.25ms to 10000s
Logarithmic acquisition period	From 1ms to 10000s
Data oversampling	100kHz data sampling on linear timebases, variable on log timebase
Auto-range	Automatic optimisation of range settings

The Electronics Unit provides automated control of the sample handling unit and monochromator wavelength setting plus signal acquisition from the photomultiplier tube detectors and data processing. The modular design of the SX20 electronics means only required features need be installed and in the unlikely event of a fault, these are localised, readily identified and if necessary can be quickly and easily repaired by substitution of the appropriate module.

PC Workstation

Model	HP DC5100 EC955ET#ABU
Processor	Intel Pentium 4 630 3.0GHz
Memory	512 Mb DDR2 - PC4200 RAM
Hard Disc Drive	80GB
Network	10/100/1000 Base-T
CD/DVD/Floppy Drives	CD RW/DVD Combo
OS	Windows 7

The SX20 features a Hewlett Packard PC fitted with a fiber-optic interface card that is linked to the Electronics Unit of the SX20 to provide computer control of the instrument via the SX20 Pro-Data Control Software.