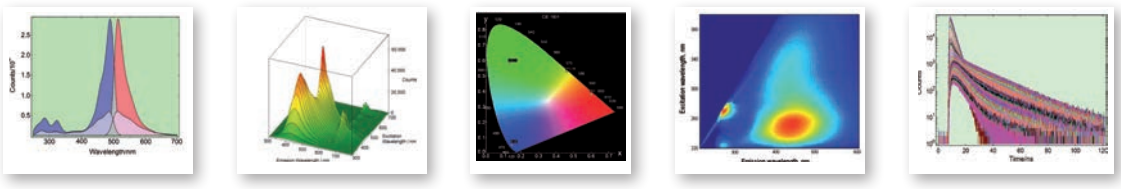
EDINBURGH
INSTRUMENTS

FS5

Spectrofluorometer

from Single Photons
to a Multitude of Measurements



FS5

An unprecedented, modern spectrofluorometer, developed and manufactured by Edinburgh Instruments in the UK

Edinburgh Instruments' fully integrated, purpose built spectrofluorometer; the *FS5*. The instrument is designed and engineered to the highest standards of sensitivity, acquisition speed, ease of use and sampling flexibility.

The *FS5* provides everything users have come to expect from Edinburgh Instruments, setting new standards for a mid-price range fluorometer, from the company with over 40 years of experience in fluorescence spectroscopy. The *FS5* is directly suited for global analytical and research markets, and provides straightforward options of measurement upgrades using interchangeable and 'intelligent' sample measurement modules for a broad range of samples and their applications.

- Ultra High Sensitivity – Single Photon Counting Technique
- High Dynamic Range and Fast Acquisition Speed
- Unrivalled Software, Tailored for Fluorescence Spectroscopy
- Unparalleled Range of Measurement Modules
- Comprehensive Measurement Upgrade Routes, including:
  - NIR – Extension of Spectral Coverage up to 1650 nm
  - POL – For Measurements of Polarisation and Anisotropy
  - MCS – Lifetime Measurements in Microsecond to Second Range
  - TCSPC – Lifetime Measurements in Picosecond to Microsecond Range

APPLICATIONS

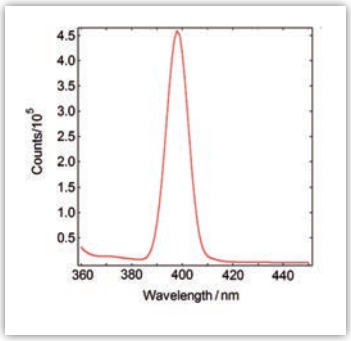
- Photochemistry
- Biochemistry
- Material Research
- Cell Biology
- Pharmaceutical Industry
- Analytical Chemistry
- Medicine
- Environmental Science
- Manufacturing
- Food Science and Agriculture

FS5

FS5 – Exceptional Instrument Sensitivity

The *FS5* is designed to meet the highest measurement specifications in the research and analytical markets. To achieve optimal sensitivity, resolution, and acquisition speed, we engineered an air-sealed excitation path to enhance the excitation power in the UV, select only the optimal performance photomultipliers in temperature stabilised housings, utilise an optical design that includes plane, spherical, toroidal, ellipsoidal mirrors and optimised mirror coatings; the FS5 is truly unrivalled in its spectral performance and sensitivity.

Not only does this superior design guarantee the highest sensitivity and broadest wavelength coverage, but it has also been optimised for a small bright focus at the sample position. This is important for measuring small sample volumes and benefits many sample holder attachments like plate reader, fibre launch optics, titrators and sample positioners.



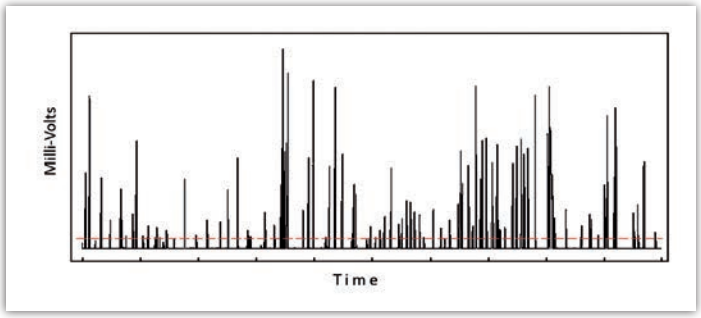
The Raman spectrum of water, exited at 350 nm, with a spectral bandwidth of 5 nm in excitation and emission, measured with an integration time of 1 s, is generally used as a means to specify sensitivity.

The FS5 guarantees a signal-to-noise ratio of >6000:1 for these measurement conditions, calculated from the signals taken at the peak at 397 nm (b) and the background at 450 nm (a) using SNR_{SQRT} *= (b-a)/SQRT(a).*

FS5 – Photon Counting

Single Photon Counting is light detection at the quantum limit. This detection method is intrinsically digital, and therefore less susceptible to noise interference than other methods. As a true counting technique, each measured count-value (photon) comes with a known statistical accuracy. As such, the technique is not only the most sensitive, it also offers unrivalled analysis of your data.

In the *FS5*, Single Photon Counting is employed for all measurement modes, not only for standard spectral scanning and kinetic measurements, but also for the optional fluorescence and phosphorescence lifetime measurements.



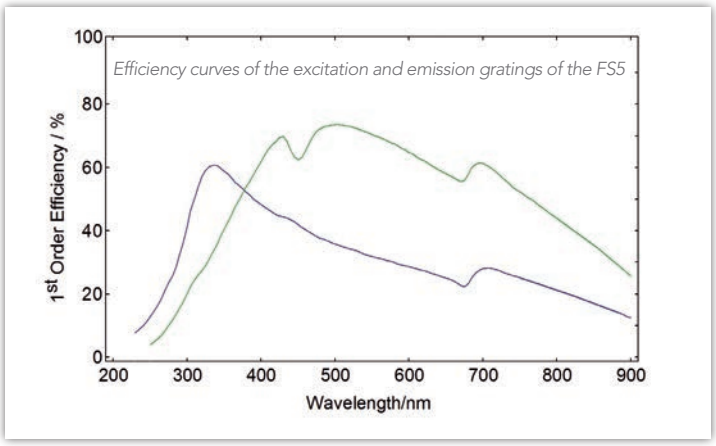
This figure shows typical pulses from a single photon counting photomultiplier.

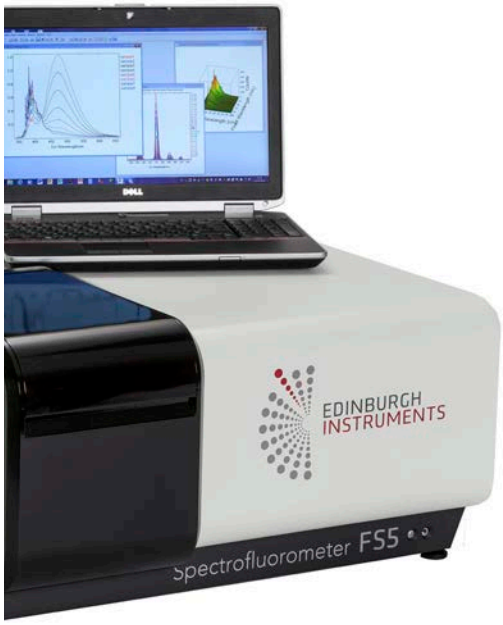
Only photon pulses above a certain threshold value are counted; this eliminates unstable background and associated noise. The amplitude of each accepted pulse is not relevant, a feature of digital data processing.

FS5 – Unmatched Optical Design

The *FS5* has an optical design that is superior to all known instruments of this class utilising optical components that are specially selected to optimise performance.

The embedded monochromators are the largest in its class with a 225 mm focal length. This ensures better imaging quality and higher optical throughput, increasing resolution and enhancing sensitivity. Furthermore, the instrument uses a minimum of optical reflections; all optical coatings are of highest reflectivity and lowest scatter. We use gratings with the best reputation (Richardson Gratings) and have selected those with highest efficiency and best wavelength coverage.



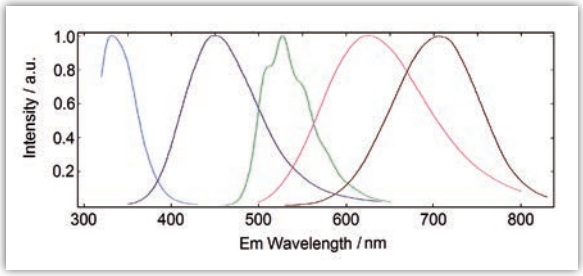


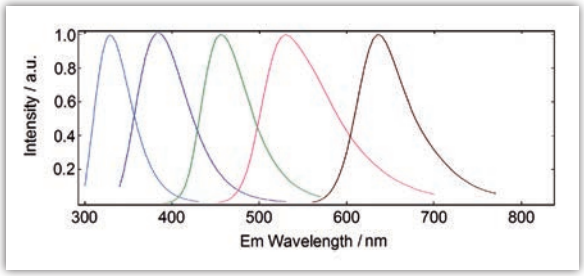
FS5 – Supreme Reliability and Accuracy

Thorough calibration procedures are used to guarantee correct data representation. Spectral calibration is used to ensure wavelength accuracy and repeatability and photometric calibration is used to ensure presentation of true spectra without distortion of instrumental throughput functions.

Traceable standards from NIST and BAM are used for calibration throughout the full wavelength coverage to verify calibration, in photons per unit bandwidth, of recorded spectra.

The *FS5 delivers data you can trust and utilise for high impact results in the research and analytical markets.*





NIST standards (top) and BAM standards (bottom), as measured with the FS5 under recommended measurement conditions

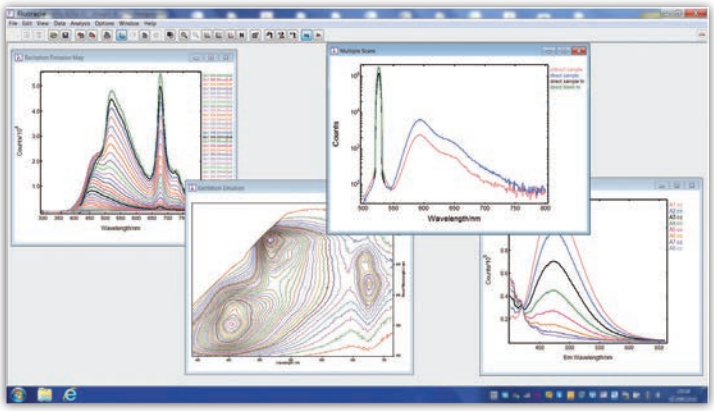
FS5 – Unrivalled Software: Fluoracle®

The operating software for the *FS5* Spectrofluorometer is named Fluoracle. An exceptional software package specifically written to handle data and instrumentation in fluorescence spectroscopy.

Fluoracle controls all *FS5* steady state and time-resolved spectrometer features with a straightforward design concept: focus on all modern photoluminescence spectroscopy applications, while at the same time provide a user friendly interface with 'ready to publish' outputs.

Whether you select a basic spectral scanning version of the *FS5*, or you go for an advanced version that includes TCSPC lifetime measurements, or integrating sphere measurements, the software will provide all instrument options, from data acquisition, to analysis and presentation.

Load / Save Measurement Settings ✓
Batch Measurements ✓
Detailed Measurement Properties ✓
Higher Order Removal ✓
ASCII Input / Output ✓
Recognition of Sample Holders / Accessories ✓
Chromaticity Analysis ✓
Quantum Yield Analysis ✓
Reflection / Absorption ✓

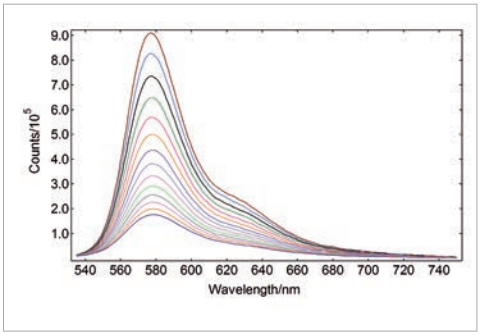


Measurement Examples

Emission Scans - with temperature dependence

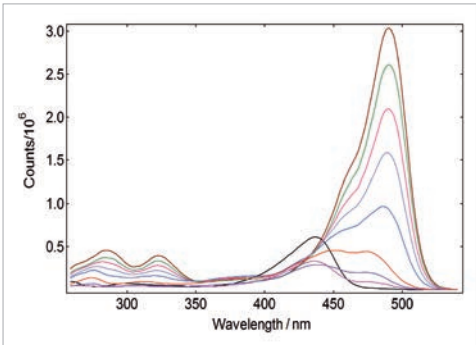
Rhodamine B, unlike other Rhodamine derivatives, has a chemical structure that is not entirely rigid. The diethylamino groups are interacting with the solvent and their mobility is therefore coupled to the solvent temperature.

This causes the fluorescence intensity to have a strong dependence on the sample temperature. The example shows this effect, measured with the TE cooled sample holder.



Rhodamine B in water, OD_{525} = 0.1
Spectral bandwidth: 2.5 nm, dwell time: 0.1 s
Temp Accuracy Band: 0.5°C, Temp Stabilisation Time: 10 min

Excitation Scans - with pH dependence



Fluorescence excitation spectra are more selective than absorption spectra, as they reveal – by virtue of the selected emission wavelength – the absorption of a particular emitting species.

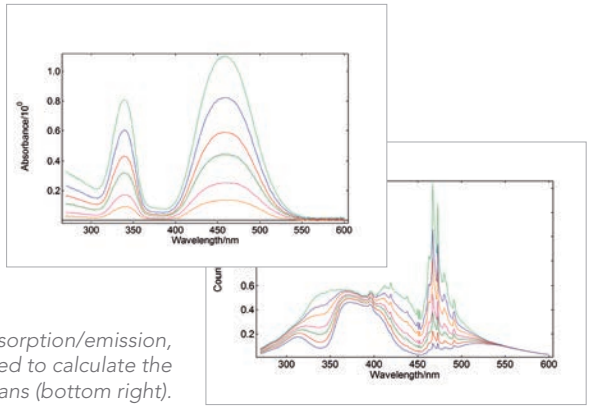
Accurate excitation spectra require a sensitive instrument, as the concentration of the sample must be kept low to avoid inner filter effects, and require reliable spectral correction, as the xenon lamp spectrum has narrow features at certain wavelengths.

Fluorescein in water, with pH adjusted between pH2 and pH7
Spectral bandwidth: 1.5 nm, dwell time: 0.1 s
pH adjusted between pH2 (blue shifted spectrum) and pH7
(spectrum of maximum intensity)

Synchronous Scans - with concentration dependence

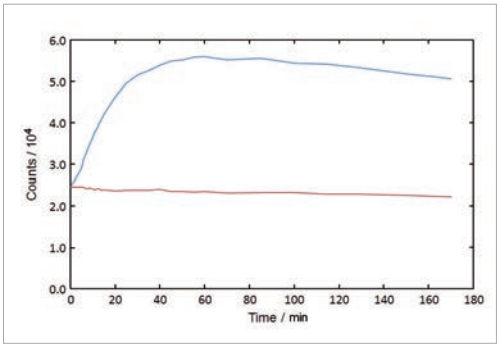
In synchronous spectral scans the excitation and emission monochromators scan at the same time with a fixed wavelength offset. For dilute mixtures this type of scan is used to identify species with a strong overlap between absorption and emission.

Synchronous scans, together with the integrating sphere attachment, can also be used to measure the absorption spectra of strongly scattering powders.



YAG: Ce powder, diluted with $BaSO_4$ to study the effect of re-absorption/emission, concentration change from 100 % down to 20 %. The software wizard is used to calculate the absorbance (top left) from the raw data of synchronous scans (bottom right).

Absorption Scans / Kinetic Scans

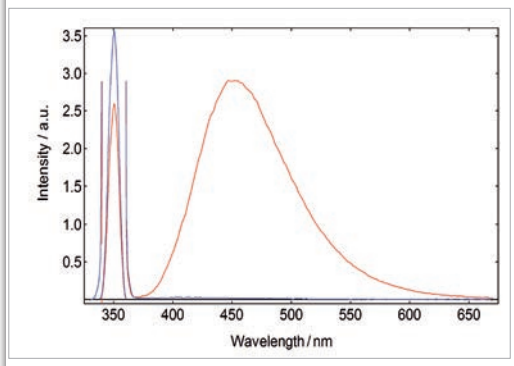


The *FS5* can record the time course of a fluorescence signal, and – at the same time – record the signal transmitted through the sample. This enables experiments to be performed with chemically or biologically unstable samples, or with samples where very small changes are to be measured very accurately.

The transmission detector comes as standard in the *FS5*.

Caspase Assay, fluorescence time course recorded for a 100 % enzyme addition (blue) and a 0 % enzyme control (red). Peptide cleavage is recorded by an organic dye excited at 400 nm, emitting at 460 nm.

Measurements of absolute fluorescence quantum yield



Fluorescence quantum yields can be measured by using the optional integrating sphere. The absolute method requires two measurements; the number of absorbed photons and the number of the emitted photons. The number of absorbed photons of a sample is determined by the reduction of the light scatter compared to a blank measurement.

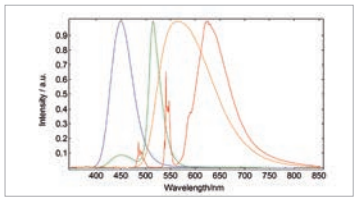
The quantum yield calculation is made using a wizard within the operating software.

Quinine bisulphate in perchloric acid.
The red curve shows the scan over the excitation scatter at 350 nm and the emission of the sample, the blue curve shows the scatter of the blank measurement. The scatter region has been scaled by a factor 1/100 for better demonstration.

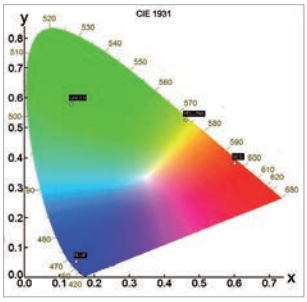
Chromaticity and Colour Co-ordinates

The lighting industry requires precise determination of the colour co-ordinates of fluorescent powders.

The *FS5* provides Chromaticity analysis tools for the determination of colour co-ordinates and luminocity values using CIE 1931 and CIE 1976. The example shows four commercial powders with blue, green, yellow and red emission.



Show/ID	Name	X	Y	I
1	BLUE	0.14888	0.05283	2.435E-005
2	GREEN	0.13487	0.57890	1.036E-004
3	RED	0.60068	0.38197	7.201E-005
4	YELLOW	0.46109	0.52544	3.103E-004



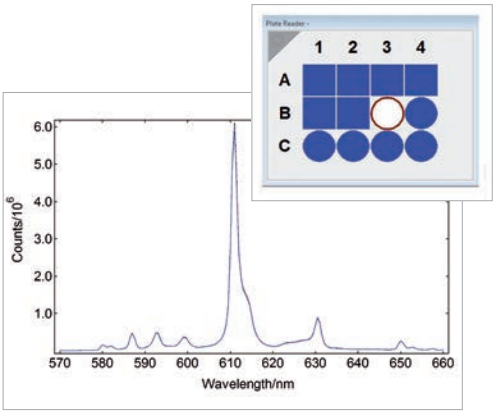


Plate Reader

Multiple sample measurements can be made using the plate reader sample holder accessory. This can be used for liquid samples with commercial plates of up to 96 wells, but is also suitable for routine quality assessment of fluorescent powders. As with all the sample holder accessories this is also compatible with the *FS5* upgrade options, including the lifetime upgrades.

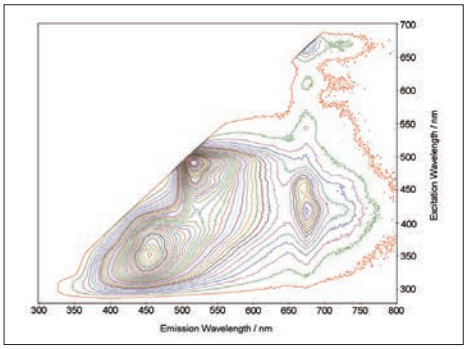
Quality control measurement of Y_2O_3 Eu $^{3+}$ powder samples, in a 12 well powder tray.
The graph shows 12 superimposed identical measurements. The inset is an example of the measurement progress display.

Excitation-Emission Maps

Excitation-Emission Maps (EEMs) provide a 'Finger Print' of complex mixtures of substances. These maps can be measured either by a series of emission scans with stepwise increase, or decrease of the excitation wavelength or by a series of synchronous scans and stepwise increase of the excitation-emission offset.

A map measurement over a wide range of excitation and emission wavelengths, as shown here, can only be performed properly if higher order scatter is automatically removed during the measurement.

Chinese Green Tea (Wuyi region).
Spectral Band width: 5 nm, step size: 2 nm, dwell time: 0.1 s



Upgrade Options

The upgrade options are modifications or additions to the main body of the *FS5*. All upgrades are best installed at the time of manufacture, but they can also be retro-fitted by a qualified service engineer during an on-site visit.

All the standard features of the *FS5* are retained when an upgrade option has been added. Many of the options can be combined.

This offers outstanding flexibility for an instrument of this class.

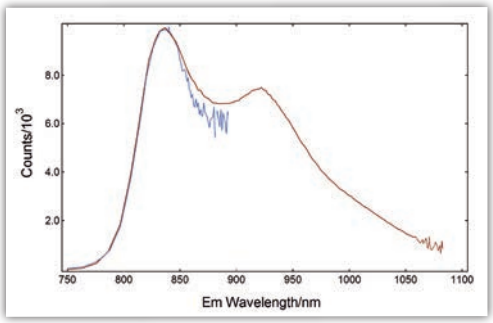
FS5 – NIR Extension of the Spectral Coverage into the Near Infrared

The *FS5-NIR* has a SECOND detector fitted to expand the operating spectral range without sacrificing the performance of the standard instrument.

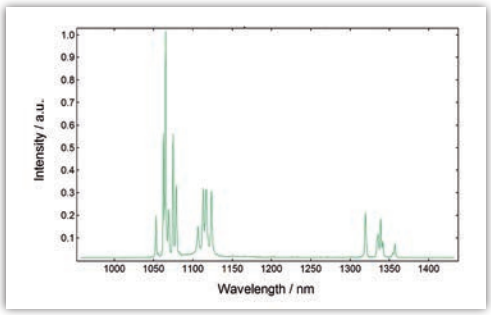
There are three NIR versions available: *FS5-NIR* is fitted with a cooled side window PMT for sensitivity up to 1010 nm; the *FS5-NIR+* is fitted with a TE-cooled NIR-PMT for sensitivity up to 1650 nm; and the *FS5-NIRA+* is fitted with an InGaAs analogue detector for spectral measurements up to 1650 nm. All three options are supplied with suitable NIR gratings.

The *NIR* and *NIR+* options are based on single photon counting for maximum sensitivity and are compatible with any of the lifetime options, should they be added.

For the *NIR+* and *NIRA+* options we recommend to replace the standard PMT-900 with the extended range PMT which provides high sensitivity from 200 nm up to 980 nm.



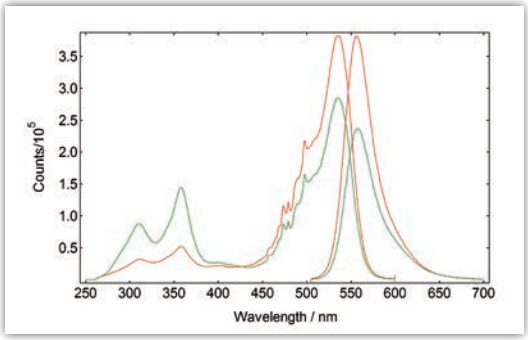
Measurement of the emission spectrum from an infrared laser dye with standard FS5 photomultiplier (blue) and with the FS5-NIR (brown)



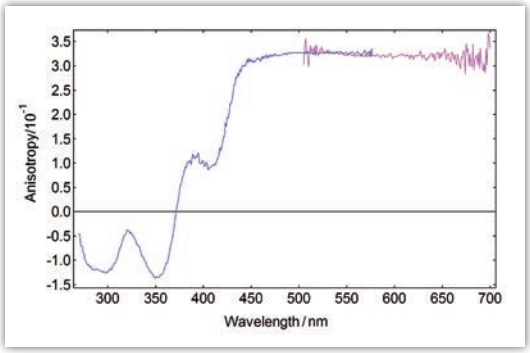
The emission spectrum of Nd:YAG, measured with the FS5-NIR+

Fluorescence Anisotropy measurement of Rhodamin 6G in glycerol at room temperature.

Raw data for vertical (red) and horizontal (green) emission polarisation plane.



Steady state anisotropy of excitation (blue) and emission (magenta)

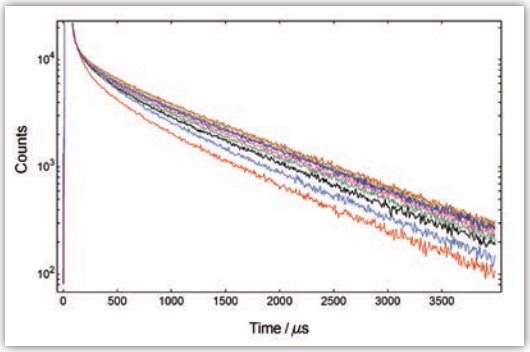


FS5 – POL Measurements of Fluorescence Polarisation and Anisotropy

This upgrade comprises the standard *FS5* with fully computerised polarisers in both excitation and emission arms. The polarisers enable polarised fluorescence and fluorescence anisotropy studies.

Automated measurements and generation of anisotropy curves, both raw and G-factor corrected, are supported by the software. If combined with the *TCSPC* option, time-resolved fluorescence anisotropy measurements and analysis will also be possible.

FS5-POL uses calcite polarising prisms with an operational range of 240 nm - 2300 nm for both excitation and emission. This ensures compatibility with the standard detector of the *FS5* and with the *NIR* options *FS5-NIR, FS5-NIR+ and FS5-NIRA+*.



MCS measurements in the microsecond and millisecond time scale on an example immune-assay.

In this assay, the FRET rate of the donor-acceptor pair (Europium cryptate-APC) used to label the antibodies depends on the concentration of the antigen. Very low antigen concentrations can be measured due to the discrimination by lifetimes.

FS5 – MCS Measurement of Lifetimes in Microsecond to Second Range

The instrument has all the features of the standard *FS5*, plus the capability of long lifetime (>5 µs) measurements for the time-resolved measurement of strong phosphors and rare earth emissions.

The changeover between the standard continuous light source and the pulsed xenon flash lamp is software controlled and the acquisition mode automatically changes from standard photon counting to time-resolved photon counting. The software incorporates fitting and reconvolution analysis for lifetime evaluation.

For longer working sessions in lifetime mode, the continuous lamp can be switched off via the spectrometer software as it is not required. This saves energy and increases the lifespan of this lamp.

The instrumental response width of this instrument is 2 µs. Lifetimes from below 5 µs to above 10 s can be accurately measured.

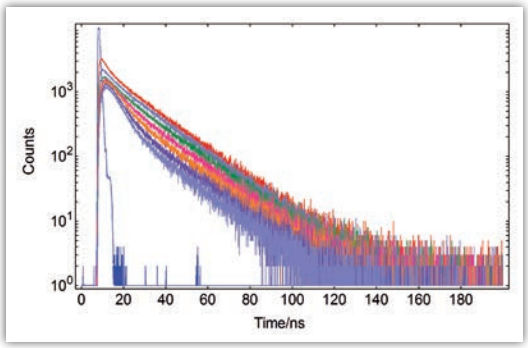
FS5 – TCSPC Measurement of Lifetimes in Picosecond to Microsecond Range

The instrument has all the features of the standard *FS5*, plus the capability of fluorescence lifetime measurements in the picosecond, nanosecond and the lower microsecond (<10 µs) time range. The *FS5-TCSPC* version requires picosecond pulsed diode lasers and LEDs for excitation, which are simply attached to the special *FS5-TCSPC* housing and are compatible to all sample holder options.

There is no stand-alone laser driver or data acquisition module. The software is fully compatible with all measurement options and offers numerical reconvolution and curve fitting.

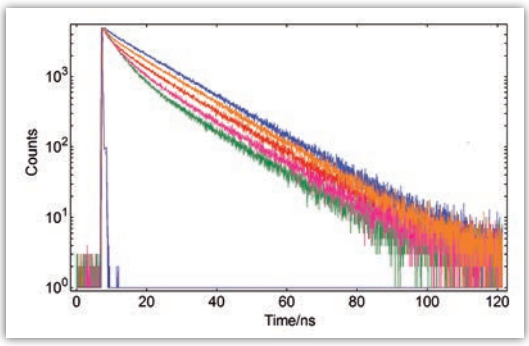
Note that the picosecond diode lasers (EPL series) and the picosecond pulsed LEDs (EPLED series) come with different output wavelengths. More than one of these picosecond pulsed light sources may be required to cover your range of applications.

TCSPC lifetime measurements with the standard *FS5* detector will have an instrumental response width of ~800 ps (*FS5-TCSPC*). The exact value depends on which EPL or EPLED model is used. For challenging applications the instrumental response width can be improved by fitting a SECOND, faster detector (*FS5-TCSPC+*). Using an EPL as an excitation source in this configuration will result in an instrumental response width of ~250 ps.



Measurement example for the FS5-TCSPC upgrade, using an EPLED 280 and the standard detector. (Sample: Norharmane in ethanol, at different emission wavelengths.)

The FS5-TCSPC, with EPL / EPLED sources.



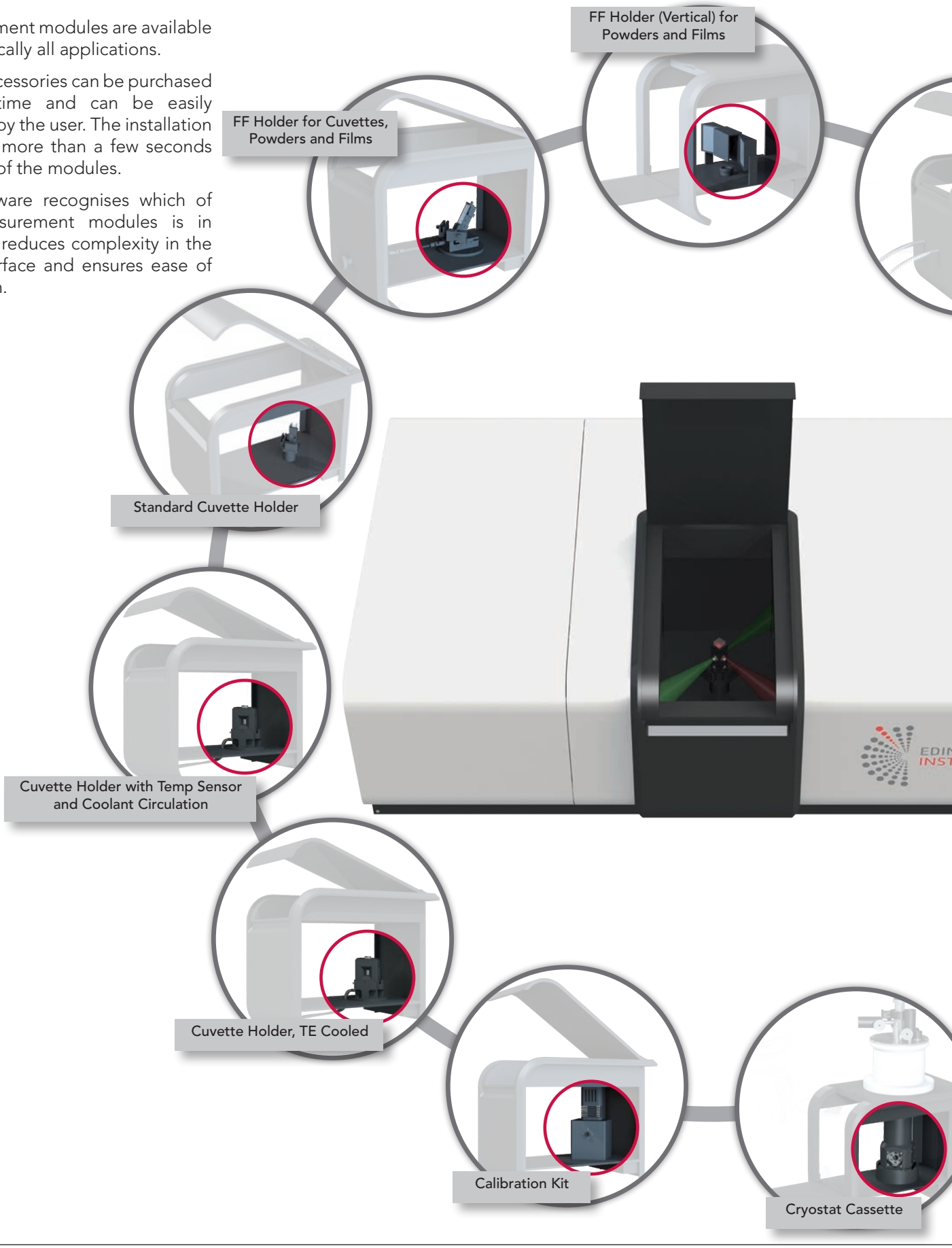
Measurement example for the FS5-TCSPC+ upgrade, with EPL 405 and faster detector. (Sample: Hematoporphyrine in PBS, at different emission wavelength.)

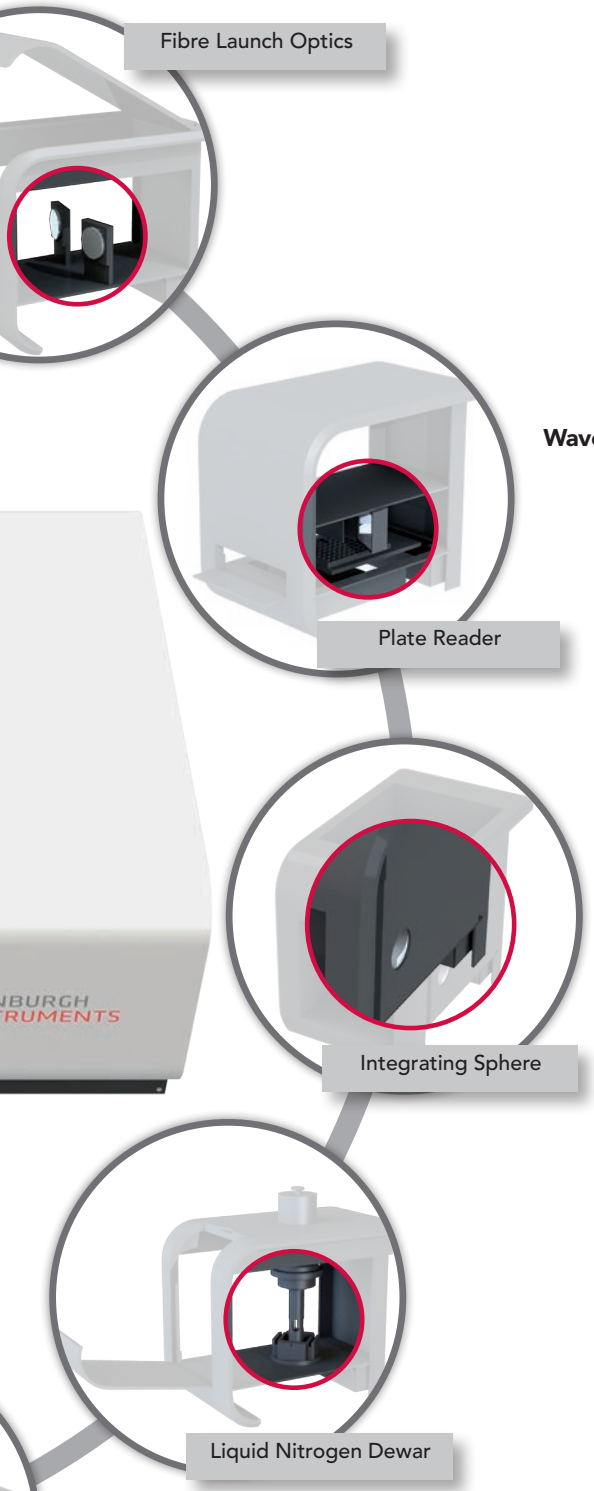
Measurement Modules

Measurement modules are available for practically all applications.

These accessories can be purchased at any time and can be easily installed by the user. The installation takes no more than a few seconds for most of the modules.

The software recognises which of the measurement modules is in use. This reduces complexity in the user interface and ensures ease of operation.





Specifications

Optics:	All-reflective for a wavelength independent focus with high brightness (small focus) at the sample
Source:	150 W CW Ozone-free Xenon arc lamp
Monochromators:	Czerny-Turner design with plane gratings for accurate focus at all wavelengths and minimum stray light
Spectral Coverage – Excitation:	230 nm – 1000 nm
Spectral Coverage – Emission:	200 nm – 870 nm
Filter Wheels:	Fully automated; included in both the excitation and emission monochromators
Bandpass – Excitation/Emission:	0* to 30 nm, continuously adjustable
Wavelength Accuracy – Excitation/Emission:	± 0.5 nm
Scan Speed – Excitation/Emission:	100 nm/s
Integration Time:	1 ms – 200 s
Emission Detector:	PMT-900, spectral coverage 185 nm – 900 nm, cooled and stabilised (Extended PMT, 185 nm - 980 nm available)
Reference Detector:	UV enhanced silicon photodiode
Transmission Detector:	UV enhanced silicon photodiode
Water Raman Signal:	≥400,000 cps at 397 nm emission, excitation 350 nm, 5 nm bandpass, 1 s integration time
Signal-Noise Ratio of Water Raman Signal:	SNR_{SQRT} > 6000:1
Dimensions:	104 cm (w) x 59 cm (d) x 32 cm (h)
Weight:	55 kg

*resolution limit of 0.3 nm

Upgrade Specifications

FS5 - MCS

Source:	5 W microsecond xenon flashlamp
Lifetime Range:	<5 µs to >10 s

FS5 - TCSPC

Sources:	Picosecond diode lasers (EPL series) Picosecond pulsed LEDs (EPLED series)
Lifetime Range:	<150 ps to >10 µs
Lifetime Range of TCSPC+ version:	<25 ps to >10 µs (EPL excitation)

FS5 - NIR

Spectral coverage of NIR:	200 nm - 870 nm plus 600 nm - 1010 nm
Spectral coverage of NIR+:	200 nm - 870 nm plus 950 nm - 1650 nm
Spectral coverage of NIRA+:	200 nm - 870 nm plus 870 nm - 1650 nm

Note: NIRA+ for spectral measurements only, Extended PMT, 185 nm - 980nm recommended with NIR+ and NIRA+

FS5 - POL

Computer Control:	In/Out of beam, polarisation angle 0°- 90°
Spectral Coverage:	240 nm - 2300 nm (excitation and emission)

EDINBURGH INSTRUMENTS

FS5

Spectrofluorometer

For more information contact:

Edinburgh Instruments Ltd
2 Bain Square
Kirkton Campus
Livingston
EH54 7DQ
United Kingdom

+44 (0)1506 425 300
+1-800-323-6115 (US Office)
sales@edinst.com
ussales@edinst.com (US Office)
www.edinst.com

Registered Office: Lake House, Market Hill, Royston, Herts SG8 9JN. UK Reg No: 962331 England VAT No GB 271 7379 37

May 2017