



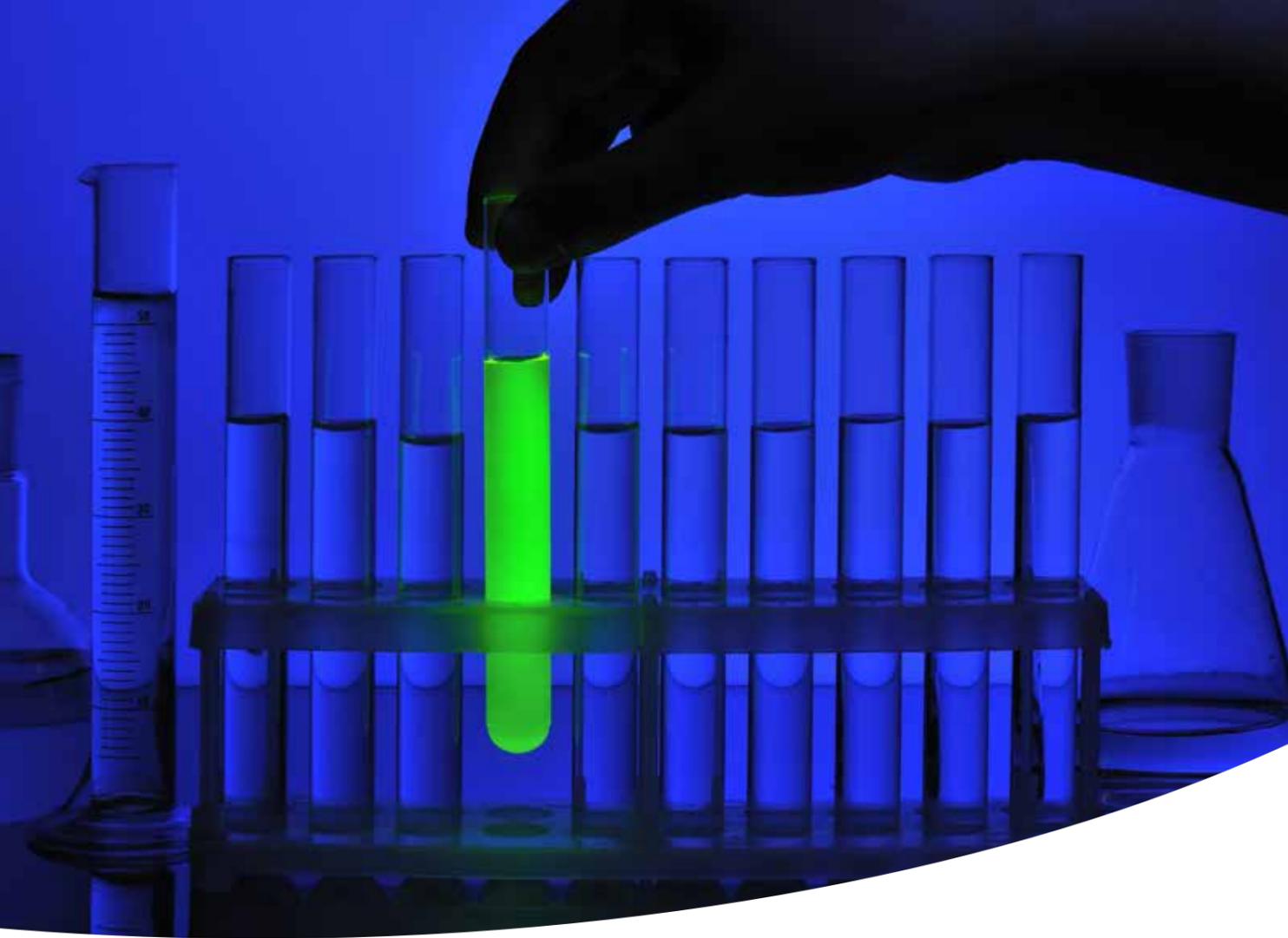
EDINBURGH  
INSTRUMENTS

Pride in Precision

# FLS980

Photoluminescence  
Spectrometer





# FLS980

## Flexibility at your fingertips

The Edinburgh Instruments FLS980 series of spectrometers continues to set new standards in both steady state and time resolved fluorescence spectroscopy. Based on single photon counting techniques, they will surpass your expectations for technical performance, reliability and

ease of use. The modular construction enables systems to be flexibly configured to meet your individual needs. The FLS980 spectrometer will enable you to push the boundaries of your scientific research.

Edinburgh Instruments has been at the forefront of research, development and manufacture of state of the art luminescence based products for over 35 years. During this time a worldwide reputation for quality and innovation has been established. The use of fluorescence measurement techniques is expanding rapidly, particularly within life sciences, biotechnology and materials research. Edinburgh Instruments meets this challenge through product development of affordable, reliable and high quality instrumentation that can be tailored and upgraded to evolve with the science.



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# FLS980 The World's Most Sensitive Spectrofluorimeter

The FLS980 is a computer controlled, modular spectrofluorimeter for measuring steady state luminescence spectra in the ultraviolet to near infrared spectral range with single photon counting sensitivity. It combines ultimate sensitivity with high spectral resolution and excellent stray light rejection.

The performance of the FLS980 makes it ideally suited for demanding applications in the broad areas of photophysics, photochemistry, biophysics and materials research.

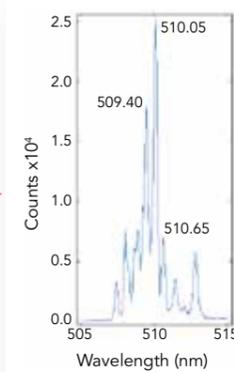
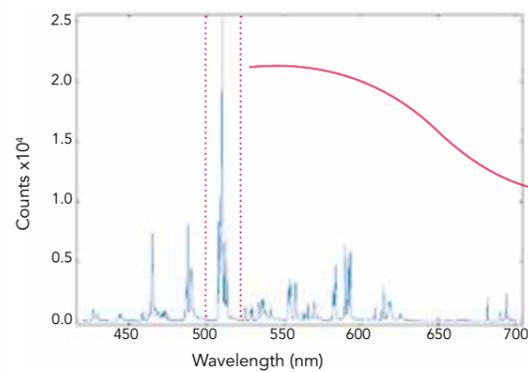
## Spectral Range

Research trends show that it is increasingly important to study luminescence characteristics over a broad spectral range from the ultraviolet to the infrared.

The FLS980 is supplied with computer controlled, triple grating turret monochromators, with up to three different gratings permanently fitted. This allows a large spectral range to be covered and provides maximum flexibility and ease of use. Each grating is individually optimised for both spectral range and linear dispersion. Grating selection,

wavelength tuning and slits are controlled via the F980 software. A computer controlled beam steering mirror allows the rapid selection of a detector. Two detectors, with independent exit slits, can be mounted on the single emission monochromator, (three on the double emission monochromator). Single photon counting photomultipliers are available covering the wavelength range from 200 nm - 1700 nm, while analogue detectors extend the wavelength range to beyond 5000 nm.

## Resolution



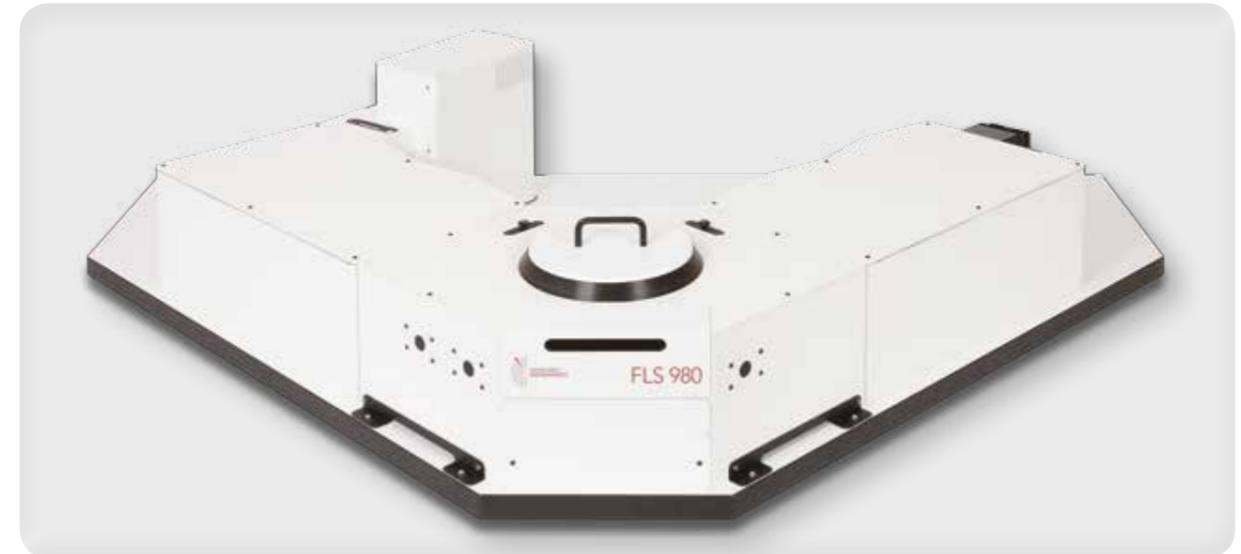
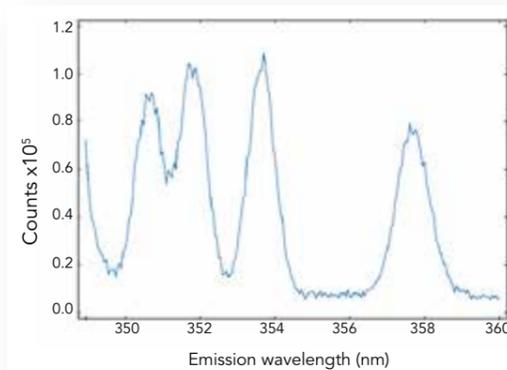
The FLS980 uses Czerny-Turner monochromators with high quality diffraction gratings for high dispersion and excellent imaging quality. Wavelength tuning is by micro-stepper motor driven with a minimum step size of 0.05 nm. Spectral details as close as 0.1 nm can be resolved over the spectral range from UV to NIR.

**Sample:** Europium  
**Measurement Conditions:**  $\lambda_{ex} = 395$  nm,  $\Delta\lambda_{ex} = 5$  nm,  $\Delta\lambda_{em} = 0.05$  nm, step size = 0.05 nm, integration time = 1 s

## Stray Light

Stray light suppression is vital for samples that exhibit a low quantum yield or a high level of scattering. The FLS980 exhibits high stray light suppression. This reduces the possibility of stray or scattered light swamping the fluorescence signal. Single or double grating monochromators are available with stray light rejection of 1:10<sup>5</sup> and 1:10<sup>10</sup>, respectively.

**Sample:** Raman Spectrum of CCl<sub>4</sub>  
**Measurement Conditions:**  $\lambda_{ex} = 348$  nm,  $\Delta\lambda_{ex} = 0.5$  nm,  $\Delta\lambda_{em} = 0.7$  nm, step size = 0.05 nm, integration time = 5 s

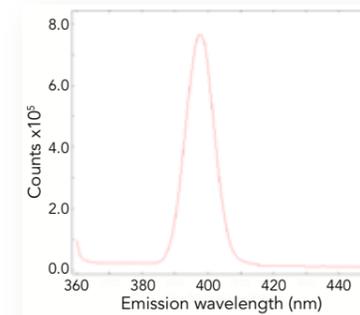


FLS980 Spectrofluorimeter with red-cooled PMT and double monochromators

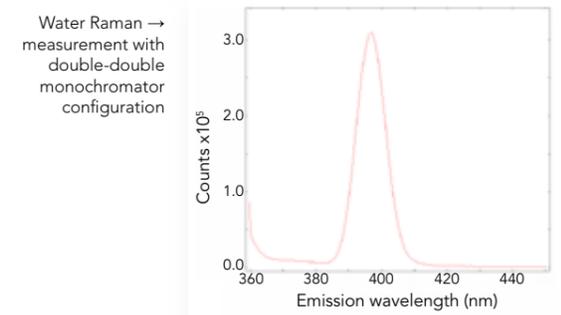
## Sensitivity

Single photon counting is an unparalleled method for the measurement of low level optical radiation. Edinburgh Instruments have optimised this technique in the FLS980. A signal to noise ratio of >25,000:1 for a measurement

of a water Raman spectrum under standard measurement conditions is guaranteed. This sensitivity allows the spectra of weak dye solutions, as low as 100 fM, to be routinely measured.



← Water Raman measurement with single-single monochromator configuration



Water Raman → measurement with double-double monochromator configuration

**Sample:** Raman Spectrum of Distilled Water  
**Measurement Conditions:**  $\lambda_{ex} = 350$  nm,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 5$  nm, step size = 1 nm, integration time = 1 s  
 Signal to noise ratio > 25,000:1 for water Raman signal,  $\lambda_{peak} = 397$  nm, noise measured at 450 nm

The water Raman signal has a strong wavelength dependence, which results from the spectral output of the xenon excitation source, the throughput of both excitation and emission monochromators and from the spectral responsivity of the detector.

It is often beneficial to look beyond the ultimate water Raman specification under "standard conditions" which

is in the UV spectral range. If the major focus of your research is in the visible and near infrared that is where the instrument should be optimised.

Edinburgh Instruments can configure your system with additional grating sets in both excitation and emission monochromators to provide you with sensitivity in the range where you need it.

## Spectral Correction

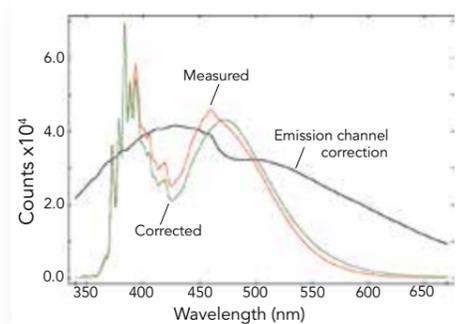
Spectral correction is necessary to obtain the true excitation and emission spectra of the sample, free from any instrumental effects. Comprehensive spectral correction, using factory measured correction files, is standard practice when using the FLS980.

Uncorrected excitation spectra are affected by the spectral output of the light source and the throughput of the monochromator. The correction file is obtained by using the built-in calibrated reference detector that monitors a fraction of the excitation light.

Similarly, raw emission spectra are affected by the monochromator efficiency and the spectral response of the detector. Unique correction files for each spectrometer and grating/detector combination are obtained during calibration at the Edinburgh Instruments factory using calibrated light sources. With a simple mouse click, the FLS980 produces corrected spectra you can trust.

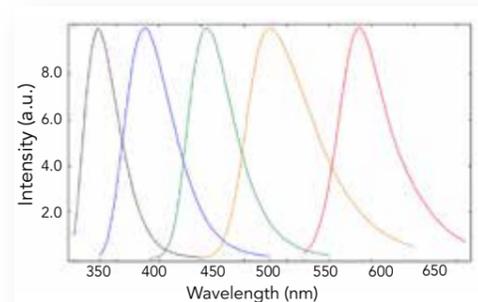
Edinburgh Instruments provides correction files in photon irradiance. This means that all photoluminescence spectra present the number of photons per unit band width. If required, spectral irradiance (optical power per unit band-width) can be recalculated.

G-factor correction in anisotropy studies is available.



The effect of spectral correction shown on a typical emission spectrum.

**Sample:** Pyrene in cyclohexane ( $2 \times 10^{-3}$  M)  
**Measurement Conditions:** Xenon Lamp (Xe1), Detector (R928P) with  $\lambda_{ex} = 338$  nm,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 0.5$  nm, step size = 0.5 nm, integration time = 1 s



Customers can verify the spectral correction using commercial standards. The graph shows the BAM standards F001-F005.

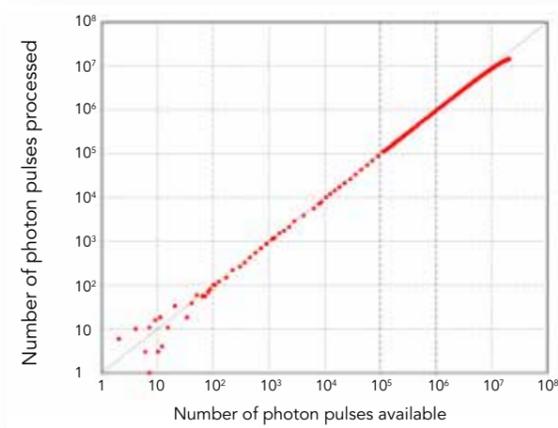
**Sample:** BAM standards in ethanol, prepared to manufacturers instructions.  
**Measurement Conditions:** Xe1, R928P, with  $\lambda_{ex} = 280$  nm (F001), 315 nm (F002), 380 nm (F003), 420 nm (F004), 550 nm (F005),  $\Delta\lambda_{ex} = 0.5$  nm, integration time 1 s, step size 1 nm.  $\Delta\lambda_{em}$  was adjusted to optimise fluorescence signal intensity

## Photon Counting and Signal Processing

Single photon counting is the most sensitive measurement technique in the field of fluorescence spectroscopy. It is fast and has a high dynamic range. Furthermore, the technique is digital, making it insensitive to background noise from detectors and electronics. The FLS980 spectrometer incorporates cutting edge single photon counting technology with multiple 100 MHz counters working in parallel.

The dynamic range is limited by the pulse width of the photon detector and by photon statistics.

When analogue infrared detectors are used, their output current is processed by 4 MHz current-frequency converters. The resulting dynamic range is similar to photon counting detectors.



Dynamic range of the standard R928P photomultiplier in standard photon counting mode.

Most detectors are linear over more than 6 orders of magnitude.

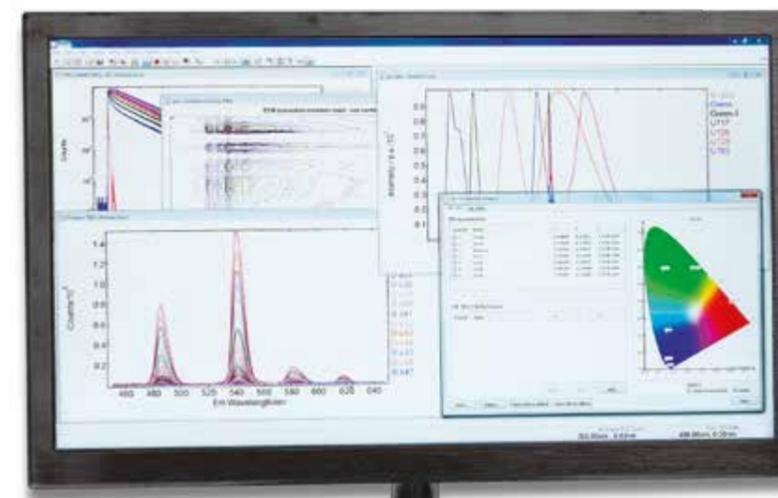
## Software Interface

The F980 spectrometer operating software is Windows™ 7 and 8 compatible and is based on a data centred design that enables you, the user, to focus on your measurement. This guarantees ease-of-use in the operation of a modular and potentially complex spectrometer.

Measurement set-up and data acquisition is made through an intuitive menu system. Key spectroscopic parameters are easily accessed through functional groupings, while common measurement routines can be saved as method files to allow previous experiments to be easily repeated. Tabbed dialogue boxes and particular scan parameters are always visible during set-up. The current status of the instrument is also continuously displayed.

A unique feature of the F980 software is that all modes of data acquisition, including spectral scanning and lifetime acquisition in both MCS and TCSPC modes, are controlled from within one software package. Modern light sources, detectors, complex sample holders (plate reader, XY sample stages, titrator) and cooler options (thermostated sample holders and cryostats) are supported and fully software controlled.

Comprehensive data import and export facilities are provided to ensure compatibility with many other popular analysis programs. Graphics can be exported to a standard Windows metafile or directly cut and pasted into word processing, graphics and desktop publishing programs.



## Software Functionality for Steady State Spectroscopy

### Measurement Modes

- Signal rates
- Excitation spectra
- Emission spectra
- Anisotropy spectra
- Kinetic measurements
- Synchronous spectra
- Corrected spectra
- Temperature resolved spectra map
- Synchronous spectra map
- Excitation-emission map
- Sample temperature monitoring
- Reflection measurements
- Absorption measurements
- Absolute quantum yield measurements
- Multiple sample position and well-plate measurements

### Control Features

- Wavelength selection ( $\lambda_{ex}$  &  $\lambda_{em}$ )
- Grating selection
- Spectral band widths
- Integration time per data point
- Sample selection (multi-position sample wells)
- Programmed excitation shutter
- Programmed attenuator
- Source and detector selection
- Online spectral data correction
- Post acquisition spectral correction
- Polariser selection and orientation
- X-Y Sample stage control
- Sample temperature control
- Cryostat control
- Microscope stage control
- Plate reader control
- Titrator control

### Data Manipulation & Display

- Arithmetic (+, -, ×, /, append)
- Scaling / multiplication factor
- Normalise
- Baseline subtraction
- Crop range
- Smooth
- 2D, 3D, Contour and text
- Grid ON / OFF
- Differentiation / Integration
- Peak search
- Correction
- Anisotropy (G factor corrected)
- Logarithmic / linear scales
- Cursor locations
- Join, split and extract frames
- Spectral correction wizard
- Absolute quantum yield wizard
- Chromaticity calculation and display wizard

Optional hardware may be required for some measurement modes and control features.

# FLS980 - The World's Most Advanced Fluorescence and Phosphorescence Lifetime Spectrometer

The FLS980 is a modular, computer controlled spectrometer for measuring photoluminescence lifetimes spanning a vast time range of more than 12 orders of magnitude from picoseconds to seconds. Like spectral measurements, lifetime measurements are made by single photon counting. Therefore the FLS980 stands for unmatched accuracy and sensitivity in the acquisition of time resolved data.

Two different single photon counting techniques are required to cover the large time range: Time Correlated Single Photon Counting (TCSPC) and Multi-Channel

Scaling (MCS). Edinburgh Instruments has pioneered TCSPC, the now widely accepted technique of choice for accurate fluorescence lifetime data acquisition.

The FLS980 is the only product worldwide that combines all three counting techniques, spectral scanning, TCSPC and MCS in a unique package of hardware and software components. The system is modular-configurable and at the same time both user friendly and task oriented. It is the cutting-edge tool for extending the boundaries in photo-physics, photochemistry, biophysics and material research.

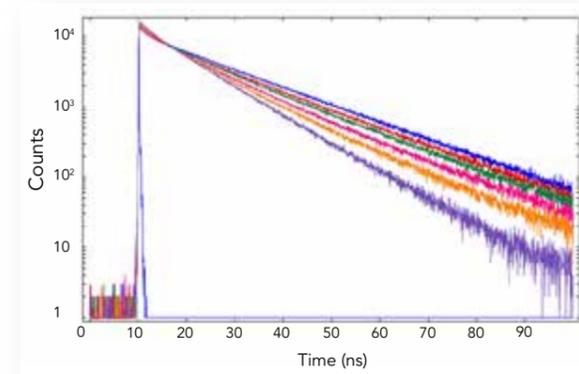
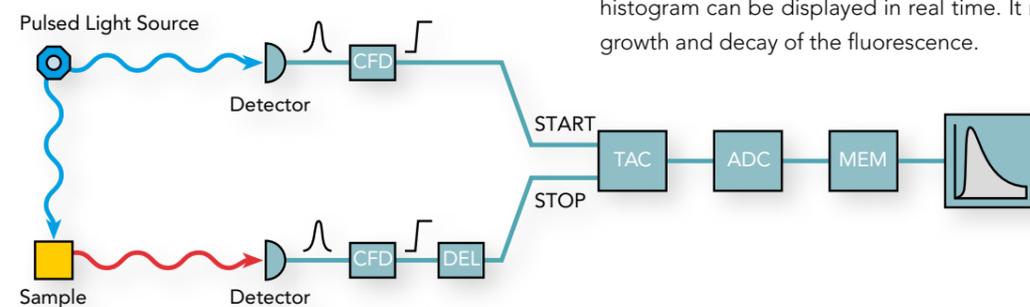
## Time Correlated Single Photon Counting - Fluorescence Lifetime Measurements

Time Correlated Single Photon Counting (TCSPC) is a technique for the acquisition of single photons. It has a time resolution of picoseconds to nanoseconds. The technique is a digital counting technique, counting photons that are time correlated in relation to a short excitation light pulse.

In TCSPC the sample is repetitively excited using a pulsed light source with a high repetition rate. During the measurement a probability histogram builds, which relates the time between an excitation pulse (START) and the observation of the first fluorescence photon (STOP).

The fact that the time at which a fluorescence photon is incident on the detector can be defined with picosecond resolution is critical to the operation and precision of TCSPC. The output pulses from a photomultiplier, corresponding to individual photon detection, have a significant spread in pulse height. This implies that timing based on an amplitude threshold would be subject to considerable jitter. A Constant Fraction Discriminator (CFD) is used to extract precise timing information from the detector pulse output using a method that is largely independent of the amplitude of the pulse.

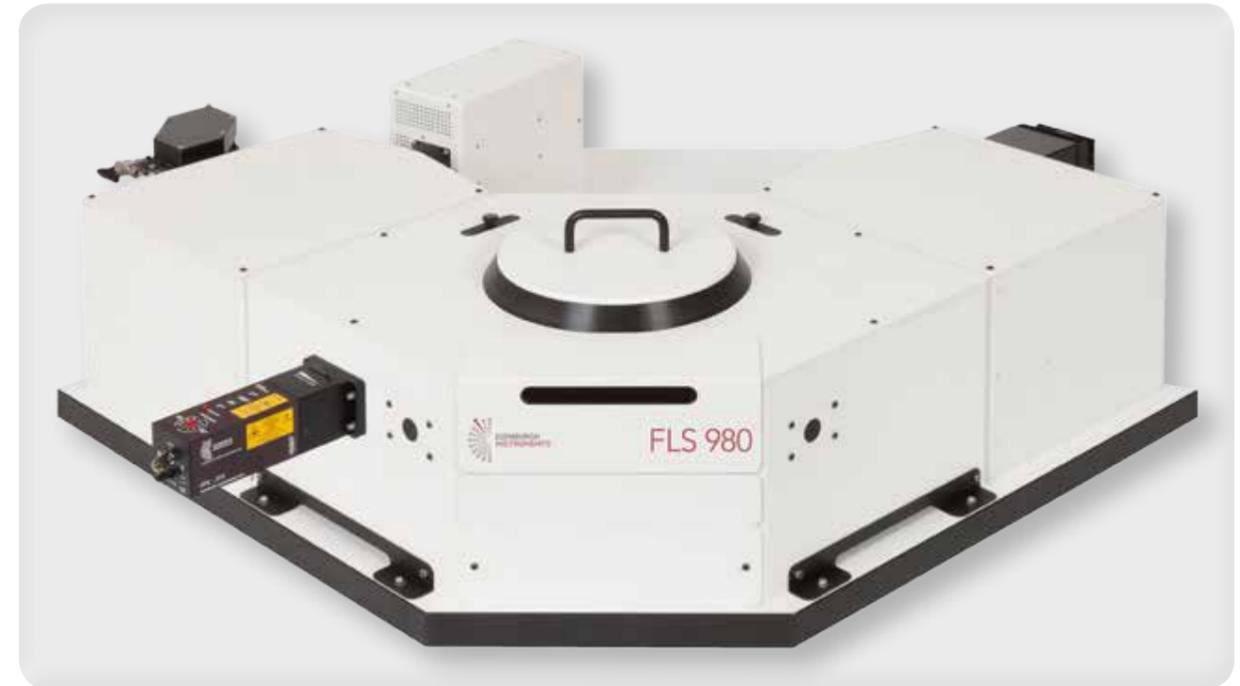
The START signal triggers a linear voltage ramp of the



**Sample:** Organic dye used in caspase assay for monitoring peptide cleavage by enzymes. Fluorescent dyes for six different enzyme concentrations are shown. Assay products and substrates can clearly be identified by fluorescence lifetimes.

**Measurement Conditions:** EPL-405, MCP-PMT  
 $\lambda_{ex} = 405 \text{ nm}$ ,  $\Delta\lambda_{ex} = 2 \text{ nm}$  (laser line width),  $\lambda_{em} = 440 \text{ nm}$ ,  $\Delta\lambda_{em} = 10 \text{ nm}$   
 timing bin resolution: 48.83 ps, number of bins for full time range: 2000  
**Acquisition time:** 120 s for each curve

Time to Amplitude Converter (TAC). This ramp is stopped when the first fluorescence photon is detected. The TAC produces a voltage output, which is proportional to the time between the START and STOP signals. This voltage is read by an analogue to digital converter (ADC) and the value is stored in the memory (MEM). Summing over many START-STOP cycles, the evolution of the probability histogram can be displayed in real time. It represents the growth and decay of the fluorescence.



FLS980 Fluorescence and Phosphorescence Lifetime Spectrometer with nanosecond flashlamp and picosecond pulsed diode laser for TCSPC measurements and microsecond flashlamp for MCS measurements.

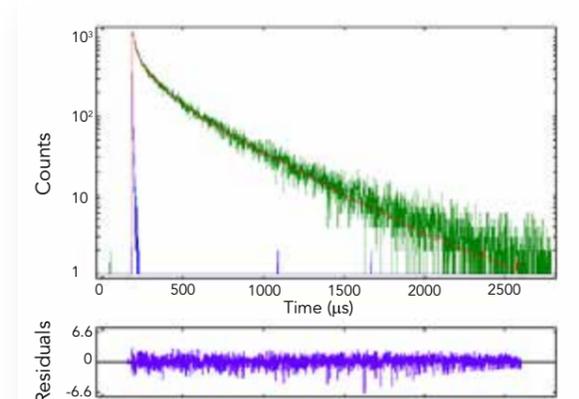
## Multi-Channel Scaling - Phosphorescence Lifetime Measurements

Multi-Channel Scaling is a technique for the acquisition of single photons with a time resolution of tens of nanoseconds to seconds.

Photons are counted in a time window, which sweeps across the full time range following each excitation pulse, creating a histogram of counts versus time. The data quality of the resulting histogram is improved by adding the data of repeated sweeps.

The minimum time window of the FLS980 MCS data acquisition electronics is 10 ns. Lifetimes from about 10 ns can be realistically measured with suitable narrow pulsed excitation sources.

The sample is excited, and data are collected, repetitively with a low to medium repetition rate source, such as the standard microsecond flashlamp, or lasers operating in the kHz regime.

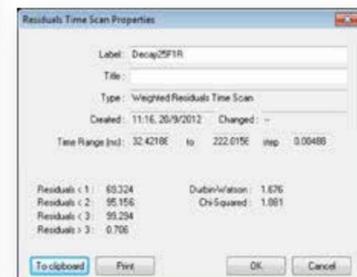
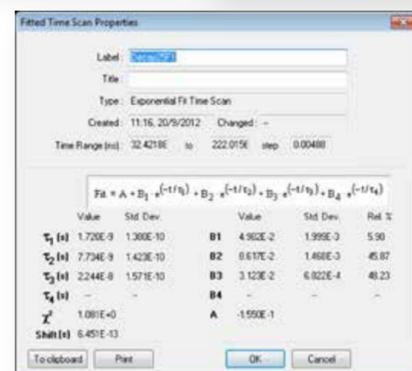
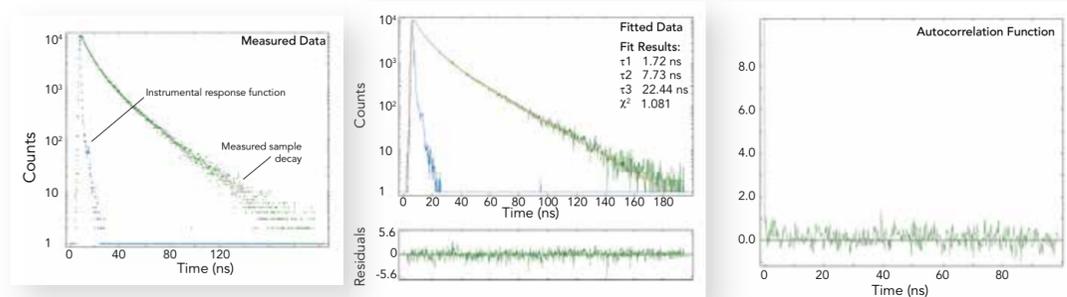


**Sample:** Rare earth doped glass  
**Measurement Conditions:**  $\mu\text{F2}$  and R928P  
 $\lambda_{ex} = 340 \text{ nm}$ ,  $\lambda_{em} = 430 \text{ nm}$ ,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 2.2 \text{ nm}$ ,  
**Fit Result:** reconvolution fit with three exponential terms  
 $\tau_1 = 14.5 \mu\text{s}$ ,  $\tau_2 = 114 \mu\text{s}$ ,  $\tau_3 = 400 \mu\text{s}$

## Standard Fluorescence Lifetime Data Analysis

The F980 software provides analysis tools for standard decay fitting (tail fits) and numerical reconvolution. With numerical reconvolution short lifetime components can be extracted from the raw decay data which would otherwise be distorted or masked by the instrumental profile.

The analysis routine provided is based on the Marquardt-Levenberg algorithm. Up to four exponential decay components can be fitted, with shift and offset fitting as standard. The algorithm is robust, delivers results in a blink of an eye, and is presented in a user friendly interface.



The example shows the analysis of data measured by TCSPC. A random residual bar (centred around Zero) and a chi-squared value close to unity are indications of a good fit and an appropriate model (in the example a

3-exponential decay). Additional fit quality parameters are available for quality assessment, such as autocorrelation functions, the Durbin-Watson parameter and standard deviations.

## Advanced Fluorescence Lifetime Data Analysis (FAST)

For the advanced analysis of fluorescence and phosphorescence decay kinetics Edinburgh Instruments offers the FAST Software package. This software package sets new standards in precision, robustness and speed of fluorescence lifetime data recovery. FAST provides unsurpassed accuracy and fits are 100% convergent.

FAST contains a library of advanced data reconvolution and curve fitting routines based on proprietary data processing algorithms, which in both speed and reliability surpass the conventional Marquardt-Levenberg algorithm.

The user can have complete confidence in the quality and reproducibility of the analysed data as it has been comprehensively tested with real and simulated data for validation.

Despite the sophisticated and challenging analysis models, FAST is easy to operate, with an intuitive and

user friendly interface. A wide range of data input, on screen visualisation, hardcopy and clipboard facilities are available.

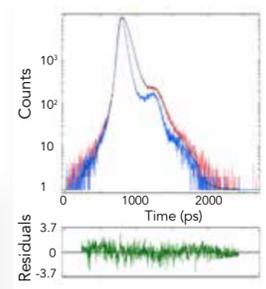
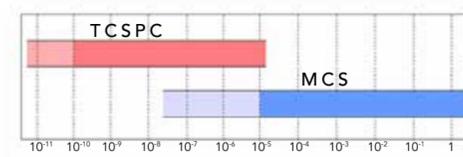
Experimental data can be analysed one at a time or in batch mode. The latter is particular useful for larger data sets such as in assay development and screening.

List of advanced analysis tools:

- Lifetime distribution analysis
- Exponential components analysis
- Support plane analysis for the calculation of lifetime confidence intervals
- Global exponential components analysis
- Stretched exponential components analysis
- Förster kinetics analysis
- Micellar quenching kinetics analysis
- Analysis of time resolved fluorescence anisotropy kinetics

## Lifetime Range

Edinburgh Instruments have unique data acquisition electronics that combine both TCSPC and MCS (TCC1 card). This enables a lifetime range that extends over 12 orders of magnitude, from a few picoseconds to seconds. Therefore, the range of lifetimes that can be measured is limited only by the light sources and detectors available.



Sample: DASPI in ethanol.  
Measurement Conditions: EPL375 and MCP-PMT, emission polariser set to magic angle.  
 $\lambda_{ex}$  = 375 nm,  $\Delta\lambda_{ex}$  = 1.5 nm (laser line width),  $\lambda_{em}$  = 585 nm,  $\Delta\lambda_{em}$  = 20 nm

Timing bin resolution: 2.441 ps, number of bins for full time range: 2096

Acquisition time: 80 s;

Width of IRF: 95 ps, extracted decay time:  $(68 \pm 5)$  ps (confidence interval verified by FAST support plane analysis).

The graph illustrates the range of lifetimes for both data acquisition techniques. The darker shaded ranges are covered with the FLS980 standard sources, nanosecond flashlamp for TCSPC and microsecond flashlamp for MCS. The lifetime range for TCSPC can be extended by use of short pulsed sources and high speed detectors. The MCS time range can be extended by nanosecond pulsed low-to-medium repetition rate lasers.

The shortest lifetimes that can be reliably extracted from raw measurements will require reconvolution analysis to be performed. Overall, the value of the lifetime resolution is influenced by many factors, such as width of the IRF, stability of the system, accuracy of the data for decay and IRF, experimental conditions and consistency for both decay and IRF measurement, single or multiple exponential functions.

## Software Interface

While measurements of photoluminescence lifetimes may have been cumbersome in the past, with the FLS980 they are just as simple as spectral measurements.

All setup and control functions for the state of the art data acquisition electronics are embedded in the F980 spectrometer control software. Changing from spectral measurements to lifetime measurements is practically a mouse-click away. Complex hardware configurations are

factory set, your focus is solely on the measurement of your sample and the interpretation of your data.

The F980 comprises full reconvolution analysis routines. Advanced analysis software (FAST) is available as an option.

Data display and output is straight forward and compatible to modern computer hardware and software technology.

## Software Functionality for Time Resolved Photoluminescence Measurements

### Measurement Modes

- Signal rates
- Manual lifetime measurement
- Multiple lifetime measurement
- Time resolved excitation spectra
- Time resolved emission spectra
- Fluorescence anisotropy
- Temperature controlled lifetime measurements
- Sample temperature monitoring
- Multiple sample position and well-plate measurements

### Control Features

- Wavelength selection for excitation and emission monochromators
- Sample selection (multi-position sample holders)
- Detector selection (up to 3)
- nF920 flashlamp voltage, frequency control and gas pressure monitor
- Programmable iris attenuator
- Multiple sources
- Polariser selection and orientation
- Cryostat control
- Measurement to peak counts or preset time
- Forward or Reverse mode
- CFD settings on START and STOP: threshold, zero crossing and divider
- Channel Selection:
  - 512 - 8192 channels (TCSPC)
  - 500 - 8000 channels (MCS)
- Time Range Selection:
  - 2.5 ns - 50  $\mu$ s (TCSPC)
  - 5  $\mu$ s - 1000 s (MCS)

### Analysis Features

- Full data reconvolution using a non-linear least square fitting routine:
- Exponential reconvolution or simple tail fit
- 1-4 independent exponential decay times, fixed or as free fit parameters
- Shift parameters, fixed or as a free fit parameter
- Background fit, fixed or as a free fit parameter
- Chi-squared goodness-of-fit test
- Weighted residuals, Durbin-Watson parameter
- Autocorrelation function
- Anisotropy calculation
- Time resolved spectra

# Reveal the Secrets of



## Temperature Controlled Sample Holders



**TE Cooled Sample Holder**  
Cuvette holder with temperature control between -10°C to 105°C (an extended version is available). The holder is fully controlled by the F980 software allowing both set temperature and temperature map measurements to be conducted. A four sample position version is also available.

## Dewar for Sample Measurements at 77 K



This low cost option for measurements at 77 K comprises a quartz Dewar in a robust mount with clear access to the sample from all four directions. The sample is contained in a quartz sample rod that is immersed into liquid nitrogen. The assembly comes with a top hat lid for the sample chamber.

## Cryostat Systems



Oxford Instruments liquid nitrogen or helium cryostats with ITC controllers are used when low temperature measurements are required. The F980 software communicates with the cryostat controller allowing families of temperature dependent steady state and lifetime data to be acquired under computer control. The cryostat is supplied with an adapter to fit to the sample chamber.

## Microscope Connections

An upright or inverted microscope (manufactured by Nikon) can be connected to the FLS980 spectrometer by fibre attachments for widefield excitation. Steady state spectral and/or lifetime measurements can be acquired.



## Excitation Sources

### Xe1 Xenon Arc Lamp

The Xe1 is a 450 W ozone free xenon arc lamp that emits continuous radiation from 230 nm - 2600 nm (after optics the excitation range is typically 230 nm - 1000 nm). The lamp has an integrated power supply with hour and power display. The light from the xenon arc is refocused into the monochromator by means of a high quality off-axis ellipsoidal mirror.

### µF2 Microsecond Flashlamp

The µF2 is a pulsed xenon microsecond flashlamp producing short, typically a few µs, high irradiance optical pulses at repetition rates up to 100 Hz. As a result, this is an ideal source for phosphorescence decay measurements in the range from microseconds to seconds.

### nF920 Nanosecond Flashlamp

The nF920 is a thyatron triggered, all metal, pulsed flashlamp. It operates with a hydrogen or nitrogen gas fill to provide sub-nanosecond optical pulses over the VUV to VIS spectral range, 115 nm - 400 nm depending on optics and gas fill (typically 200 nm - 400 nm), at repetition rates of up to 100 kHz.

### Additional Sources for TCSPC Measurements

#### • EPLs – Diode lasers

EPL-Series lasers produce picosecond duration pulses (typically <100 ps) at repetition rates up to 20 MHz and are therefore ideal for applications for TCSPC measurements. These lasers are compact and require only a power adapter for operation. EPL lasers are available with laser wavelengths of 375 nm, 405 nm, 445 nm, 470 nm, 485 nm, 515 nm and discrete wavelengths above 630 nm. Other picosecond pulsed semiconductor lasers may also be used.

#### • EPLEDs - Light Emitting Diodes

Pulsed light emitting diodes of the EPLED series produce sub-nanosecond (typically <750 ps) optical pulses at repetition rates up to 10 MHz and are therefore ideally suited to TCSPC measurements. EPLEDs are available with emission covering the UV-Visible spectrum, starting from 250 nm. Other picosecond pulsed light emitting diodes may also be used.

### Alternative Sources for TCSPC Measurements

#### • Ti:Sapphire lasers

#### • Supercontinuum fibre lasers

**Note:** Laser safety precautions must be followed when using a spectrometer with a laser.

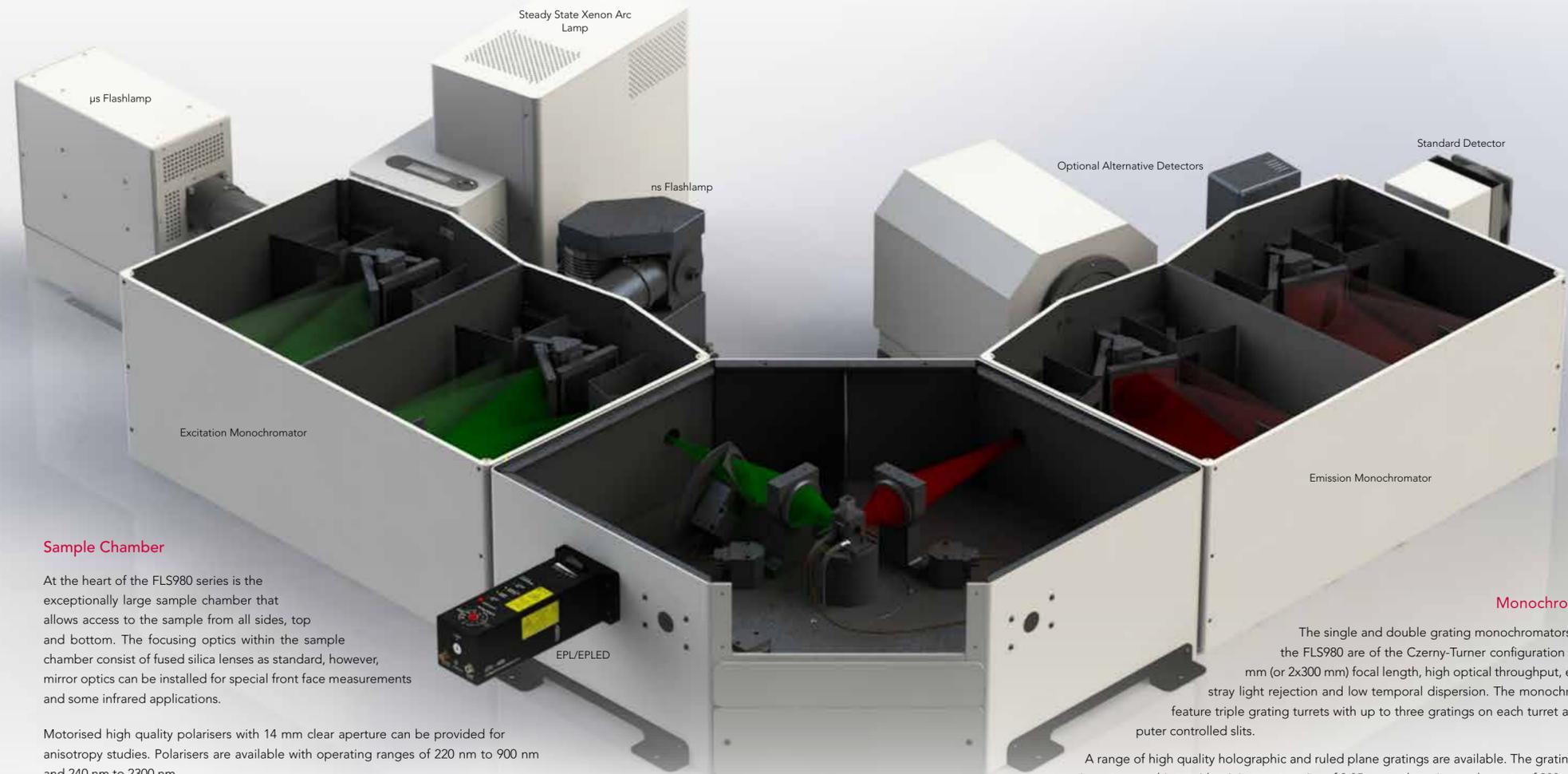
### Alternative Sources for MCS measurements

#### • µF1 5 W microsecond flashlamp

#### • Harmonics of Q-switched solid state lasers (e.g. Nd:YAG)

#### • Optically pumped parametric oscillators (OPOs) or dye lasers

# FLS980 Combined Time Resolved & Steady State Photoluminescence Spectrometer



## Sample Chamber

At the heart of the FLS980 series is the exceptionally large sample chamber that allows access to the sample from all sides, top and bottom. The focusing optics within the sample chamber consist of fused silica lenses as standard, however, mirror optics can be installed for special front face measurements and some infrared applications.

Motorised high quality polarisers with 14 mm clear aperture can be provided for anisotropy studies. Polarisers are available with operating ranges of 220 nm to 900 nm and 240 nm to 2300 nm.

An additional laser input port on the side of the sample chamber (in the picture fitted with an EPL picosecond pulsed diode laser) provides access for external lasers. The beam is steered along the standard excitation path which ensures flexibility and consistency for sample holder options such as the front face sample holder or integrating sphere.

The bottom access to the sample chamber ensures compatibility with top loading cryostats and simplifies both access and alignment of other sample holders.

## Monochromators

The single and double grating monochromators used in the FLS980 are of the Czerny-Turner configuration with 300 mm (or 2x300 mm) focal length, high optical throughput, excellent stray light rejection and low temporal dispersion. The monochromators feature triple grating turrets with up to three gratings on each turret and computer controlled slits.

A range of high quality holographic and ruled plane gratings are available. The grating turrets are micro-stepper driven with minimum step size of 0.05 nm and maximum slew rate of 200 nm/s.

Excitation monochromators have an integrated computer controlled excitation shutter for controlling the light exposure to the sample, emission monochromators have an integrated shutter for detector protection.

All monochromators are available with computer controlled swing mirrors for port selection and with a computer controlled filter wheel for higher order removal and further stray light control.

## Detectors

A full range of detector options are available to enhance the range of spectral coverage and to reduce the instrumental response width.

### R928P in cooled housing (standard)

This PMT has a wavelength coverage of 200 nm to 870 nm and a dark count rate of <50 cps (at -20°C). The detector is operated in single photon counting mode throughout all time ranges. When operated in TCSPC mode the instrumental response width is ~600 ps.

### High speed photomultiplier in cooled housing (optional)

This detector has a slightly reduced overall sensitivity, but with a narrower, 200 ps, detection response width, it benefits TCSPC applications. The spectral coverage of this detector is 230 nm to 870 nm, with a dark count rate of <100 cps (at 0°C).

### MCP-PMT in cooled housing (optional)

This is the best detector for fast TCSPC measurements. The MCP-PMT has a detector response width of <25 ps. Integrated into the FLS980 Edinburgh Instruments guarantees an instrument response width of <50 ps. The detector assembly features a dark count rate of <50 cps (at -20°C), the spectral coverage is 200 nm to 850 nm.

### R2658P in cooled housing (optional)

This side window photomultiplier has an extended near infrared (NIR) sensitivity, the spectral range of this detector is 200 nm -1010 nm and a dark count rate of 100 cps (at -20°C). The detector response width is 600 ps.

### NIR-photomultiplier (optional)

NIR-PMTs enable photon counting operation up to a spectral limit of ~1700 nm. These detectors are therefore the ultimate choice in this extended spectral range, which was formerly only accessible with analogue detectors. Fully automated liquid nitrogen cooled versions and thermoelectrically cooled versions are available, the latter with a reduced spectral coverage on the short wavelength side of ~950 nm.

### Analogue Detectors (optional)

Three versions of InGaAs detectors with spectral coverage up to ~1.65 µm, 2.05 µm and 2.55 µm are available, all are thermoelectrically cooled. The responsivity of these detectors reduces with increasing wavelength coverage. Typically the detectors are used in steady state mode, but with pulsed lasers and transient recording lifetime applications are possible.

InAs and InSb detectors covering up to 5.5 µm are available. These detectors come with liquid nitrogen cooling.

## Sample Holders

### Front Face Sample Holder

The front face sample holder has external adjustment for accurate sample positioning. The accessory comes with inserts for demountable cuvette (as shown) film/slide clamp and holder for microspheres.



### 3-Position Cuvette Turret

The three-position cuvette turret has coolant circulation and temperature probe. Filter holders are provided for all three individual sample positions.



### Integrating Sphere

The integrating sphere allows absolute quantum yield, reflectance and chromaticity measurements to be taken. It comes with sample holders for directly and indirectly excited liquids, powders and films.



### Plate Reader

The unit is coupled to the FLS980 with a bifurcated quartz fibre bundle. Spectral scanning, lifetime measurements, as well as conventional intensity readings can be made on 12 to 384 well plates. Liquids or powder samples can be scanned.



### Other Sample Holder Options

- Front face sample holders on rotational stages
- Front face sample clamps (film slides)
- Front face sample holder on XY stage
- Computer controlled XY sample stage
- Clamp for EL sample (on slides)

### Fibre Launch Options

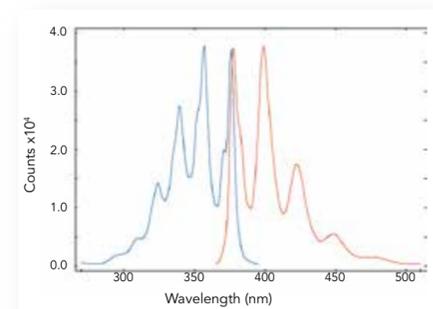
- Launcher for bifurcated fibre bundle (plate reader, remote sensing)
- Launcher for single fibre bundle (microscope sample emission)
- Launcher for FC terminated fibres
- Launcher for liquid light guides (microscope illumination and sample emission)



# Steady State Measurement Examples and Applications

## Excitation and Emission Scans

Excitation and emission spectra are standard measurements in fluorescence spectroscopy. The figure demonstrates a measurement of a well documented standard test solution of anthracene in degassed cyclohexane.

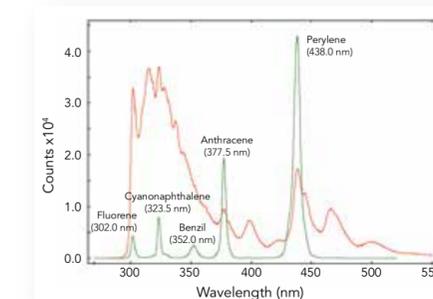


**Sample:** Anthracene in Cyclohexane ( $10^{-6}$ M)  
**Measurement Conditions:**  $\lambda_{ex} = 358$  nm for emission scan,  $\lambda_{em} = 400$  nm for corrected excitation scan,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 0.4$  nm, step size = 1 nm, integration time = 1 s

## Synchronous Scans

In synchronous scans, both excitation and emission monochromators are scanned synchronously with a preset offset.

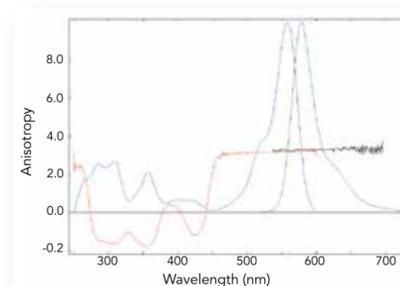
The figure demonstrates a sample of five different aromatic hydrocarbons dissolved in cyclohexane, measured with a conventional emission scan (red) and a synchronous scan with zero offset (green). The five hydrocarbons are resolved by the synchronous scan.



**Sample:** Five aromatic hydrocarbons dissolved in cyclohexane  
**Measurement Conditions:**  $\lambda_{ex} = 280$  nm for emission scan,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 0.5$  nm, step size = 0.5 nm, integration time = 1 s, offset = 0 nm

## Steady State Fluorescence Anisotropy

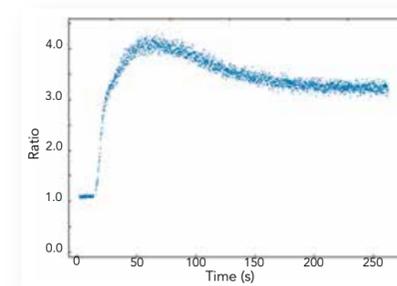
The steady state fluorescence anisotropy is obtained from polarised excitation and emission spectra. Fluorescence Excitation Anisotropy spectra are often the more important of the two due to the direct relationship of the excitation anisotropy to the orientation between the excitation and emission dipoles. Temperature and solvent dependent analysis of such anisotropy is a powerful tool for directly studying the molecular rotation of the fluorophore or the rotation of molecules labelled with fluorophores.



**Sample:** Rhodamine B in glycerol ( $10^{-6}$ M)  
**Measurement Conditions:**  $\lambda_{ex} = 515$  nm for emission scan,  $\lambda_{em} = 610$  nm for excitation scan,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 1$  nm, step size = 1 nm, integration time = 1 s, sample temperature =  $+10^{\circ}\text{C}$   
 G-factor corrected steady state fluorescence anisotropy (red, black curve), anisotropy free excitation scan and emission scan for comparison (blue and green curve, respectively)

## Kinetic Measurements

Kinetic scans reveal temporal changes of the sample fluorescence at fixed excitation and emission wavelengths. Luminescence emission in the milliseconds to seconds range, such as long phosphorescence, chemical reactions or chemical migration in cells, can be studied. As an example, using the FLS980 in T-geometry for dual wavelength detection, simultaneous measurements of the  $\text{Ca}^{2+}$  active fluorophore Indo-1 can be made with both emission arms set to different wavelengths.

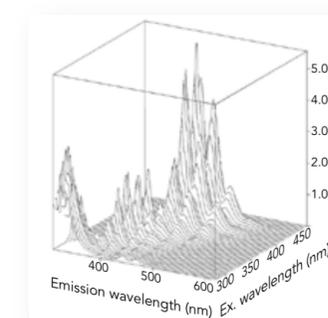


**Sample:** Human platelet cells loaded with Indo-1 in 1mM  $\text{Ca}^{2+}$   
**Measurement Conditions:**  $\lambda_{ex} = 340$  nm,  $\lambda_{em1} = 485$  nm,  $\lambda_{em2} = 410$  nm,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 1$  nm, integration time = 0.5 s

## Excitation - Emission Maps

The variety of measurement, display and analysis options of the FLS980 spectrometer allows easy and fast investigation of unknown luminescent samples or samples which contain different fluorophores.

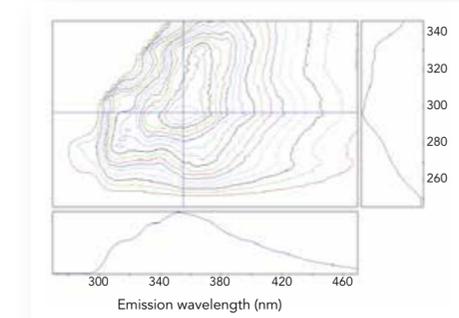
One method is to measure a series of emission scans within a selected range of excitation. The result is then demonstrated either in a 3D plot or in a contour plot.



**Sample:** Three organic dyes in solution: naphthalene, anthracene, perylene  
**Measurement Conditions:** Xe1, R928P  
 $280$  nm  $\leq \lambda_{ex} \leq 460$  nm,  $310$  nm  $\leq \lambda_{em} \leq 620$  nm,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 2$  nm, integration time = 0.5 s, repeats per scan: 1.

## Contour Plot

Excitation, emission and synchronous maps can be conveniently demonstrated as contour graphics with a single mouse click on the tool bar. These maps provide a fingerprint of the sample. The cursor allows the user to directly select and compare excitation and emission characteristics.

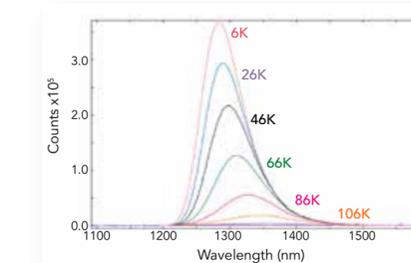


**Sample:** Crude oil. Excitation - Emission Map  
**Measurement Conditions:**  $245$  nm  $\leq \lambda_{ex} \leq 345$  nm,  $275$  nm  $\leq \lambda_{em} \leq 465$  nm,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 1.0$  nm, step size = 1.0 nm, integration time = 0.1 s

## Temperature Maps

The F980 spectrometer control software can communicate with Oxford Instruments Optistat DN (liquid nitrogen) and Optistat CF (liquid helium) cryostats (along with TE controlled sample holders).

Temperature maps can be made by acquiring a series of emission, excitation or synchronous scans for a predefined temperature range. The individual measurements are automatically started when the target temperatures are reached.



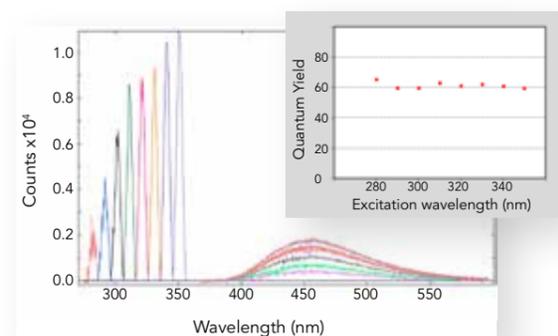
**Sample:**  $\text{CuInSe}_2$  (a material used for photovoltaic cells)  
**Measurement Conditions:** F980 controlled Optistat<sup>CF</sup>, Xe1, NIR-PMT,  $\lambda_{ex} = 694$  nm,  $\Delta\lambda_{ex} = 10$  nm,  $\Delta\lambda_{em} = 5$  nm, step size: 1 nm, integration time: 0.2 s,  
**Temperature Range:** 6K-106K, step 20K



## Absolute Quantum Yield Measurements

The absolute method for fluorescence quantum yield measurements is becoming more widely used than the relative method, as it does not require a quantum yield standard, is readily applicable to liquids, films and powders and can be extended into the near infrared spectral range.

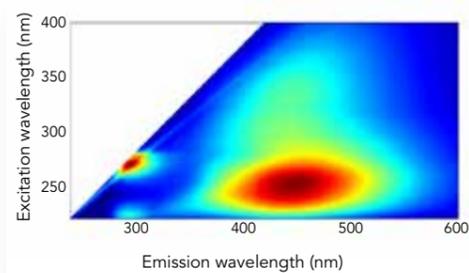
The picture shows the independence of the fluorescence quantum yield from the wavelength of excitation for a standard organic dye. The graph shows the area of absorption for eight different excitation wavelengths on the left, while on the right it shows the corresponding emission spectra, scaled by a factor of 5. The inset shows the calculated quantum yields.



**Sample:** Quinine bisulphate in perchloric acid  
**Measurement Conditions:** integrating sphere,  $\Delta\lambda_{ex}=5.0$  nm,  $\Delta\lambda_{em}=0.5$  nm, 0.3 s integration time

## Water Quality Assessments

Excitation-Emission Maps of water samples are successfully used to assess the quality of the water. Water from rivers and lakes contains specific levels of dissolved organic matter, which emits in the UV spectral range. A contour plot of an excitation-emission map as shown in the picture provides a unique finger print of the organic matter content of the water sample.

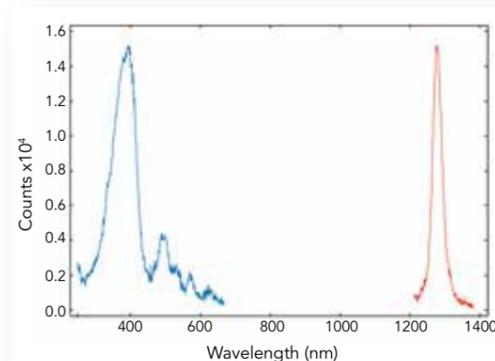


**Sample:** Water from the River Almond near Edinburgh Photonics  
**Measurement Conditions:** Xe1 and R928P;  $\Delta\lambda_{ex}=2$  nm,  $\Delta\lambda_{em}=2$  nm, step size: 2 nm, integration time: 0.1 s

## Steady State Singlet Oxygen Emission

The emission of singlet oxygen is known to be very weak and, historically, powerful laser excitation has been used to monitor this.

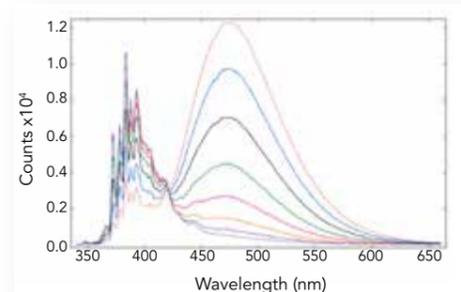
However, both excitation and emission spectra of singlet oxygen can be measured using the FLS980 with a broadband xenon lamp. The figure demonstrates a measurement of singlet oxygen luminescence generated from hematoporphyrin in ethanol. In a mixture of photosensitizers, the excitation spectrum may be used to identify the singlet oxygen generator.



**Sample:** Singlet Oxygen Luminescence generated from  $10^{-5}$  M hematoporphyrin in ethanol  
**Measurement Conditions:**  $\lambda_{ex}=380$  nm for emission scan,  $\lambda_{em}=1270$  nm for excitation scan,  $\Delta\lambda_{ex}=\Delta\lambda_{em}=2.0$  nm, step size = 1.0 nm, integration time = 3 s

## Pyrene Monomer – Excimer Equilibrium

Measurements on samples with a high extinction coefficient are often affected by the inner filter effect. This can lead to false spectra as the incident radiation is absorbed near the input face of the cuvette. The emission spectrum of pyrene in cyclohexane is shown as a function of pyrene concentration. The measurements were made in a triangular cuvette to avoid the inner filter effect. At the lowest concentrations only the monomer lines in the wavelength range 370 nm - 400 nm are observed, while at the highest concentrations the excimer peak at ~ 480 nm dominates.

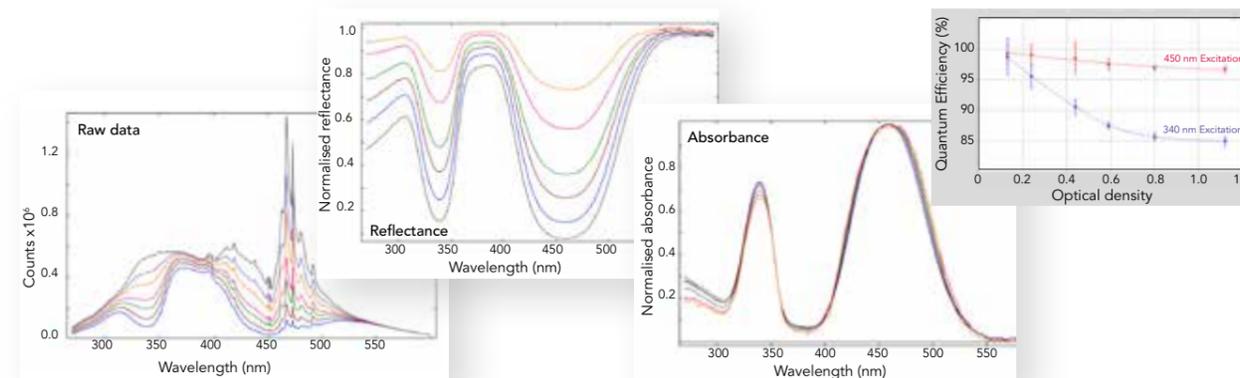


**Sample:** Pyrene in cyclohexane  
**Measurement Conditions:**  $\lambda_{ex}=335$  nm  $\Delta\lambda_{ex}=\Delta\lambda_{em}=0.5$  nm, step size 1.0 nm, integration time 1.0 s

## Reflection, Absorption and Quantum Yield Measurements of Phosphor Powders

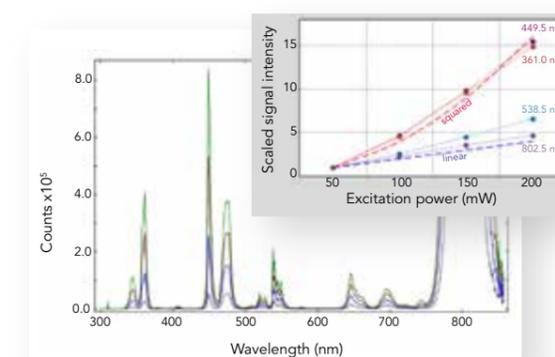
The properties of phosphor powders for the lighting and display industries can be measured using the integrating sphere accessory.

Spectral dependent reflectance and absorbance are measured by means of synchronous scans. Absolute photoluminescence quantum yields are measured with emission scans that are further processed using the quantum yield wizard provided by the F980 software.



**Sample:** YAG:Ce powder  
**Measurement Conditions:** Xe1, R928P, integrating sphere  $\Delta\lambda_{ex}=5$  nm,  $\Delta\lambda_{em}=0.2$  nm, synchronous scan, integration time 1 s  
Synchronous scans for reflectance/absorbance, emission scans for quantum yield.

## Fluorescence Upconversion



**Samples:**  $Er^{3+}/Yb^{3+}$  doped  $TiO_2$   
**Measurement conditions:** 1 W diode laser with power adjustment, standard PMT, front face sample holder  
 $\Delta\lambda_{ex}=980$  nm,  $\Delta\lambda_{em}=0.25$  nm, integration time 0.5 s

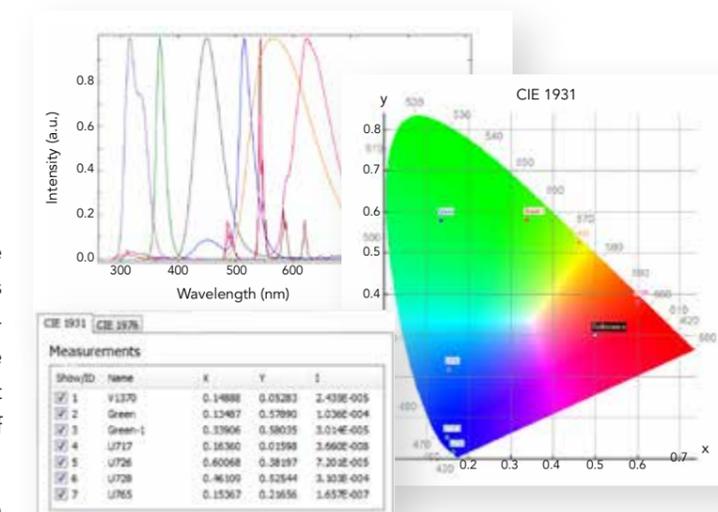
Fluorescence upconversion materials absorb light in the near infrared and produce emission of shorter wavelengths in the visible spectral range. These upconversion materials are currently the focus of research for their use in dye sensitized solar cells. Efficient upconversion is important for the efficiency of solar panels in the near infrared part of the sun spectrum.

The picture above shows the upconversion emission of an erbium-ytterbium doped  $TiO_2$  powder, for four different levels of excitation power at 980 nm. As shown in the insert, some of the upconversion lines scale close to linear with excitation power, whereas others scale approximately to the square of the excitation power.

## Chromaticity

For fluorescent powders that are used in the lighting industry, the specification of the colour of light and its luminous intensity are important.

The graphs below show an example of the measurement and analysis of 7 different powders. The chromaticity option of the F980 calculates and displays colour co-ordinates for CIE1931 and CIE1976, and also produces luminance values.



**Samples:** Luminescent powders  
**Measurement Conditions:** Xe1, R928P integrating sphere with powder accessory.  $\Delta\lambda_{ex}=5.0$  nm,  $\Delta\lambda_{em}<1.0$  nm, step size: 2 nm, integration time: 1 s

# Time-Resolved Measurement

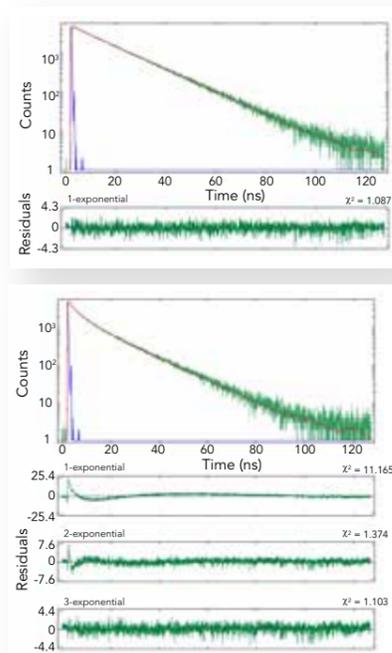
## Single and Multiple Exponential Decays

Fluorescence lifetime is an excellent parameter for analysing the interaction of fluorophores with their micro-environment, such as solvents, neighbouring fluorophores or non-fluorescing molecules. These "environmental" effects will reduce the natural decay process (characterised by the natural lifetime), to shorter and often more complex decay kinetics.

Most fluorescence decay kinetics are analysed by single or multiple exponential models. The user fits the raw data to a specific model. The quality of the fit will determine whether or not the selected model was appropriate, and – if it was – the result will provide the fit parameter such as lifetimes and pre-exponential factors.

The example shows two measurement results of the same homogeneous solution, taken at two different emission wavelengths. The decay at the shorter wavelength is clearly a single exponential, the decay at the longer wavelength is best characterised by three exponential components.

**Sample:** Hematoporphyrin IX in phosphate buffer (pH7.2)  
**Measurement Conditions:** EPL405, MCP-PMT  
 $\lambda_{ex} = 398 \text{ nm}$ ,  $\Delta\lambda_{em} = 1 \text{ nm}$ , rep. rate = 1MHz,  $\lambda_{em} = 620 \text{ nm}$  (top graph),  
 $\lambda_{em} = 620 \text{ nm}$  (bottom graph),



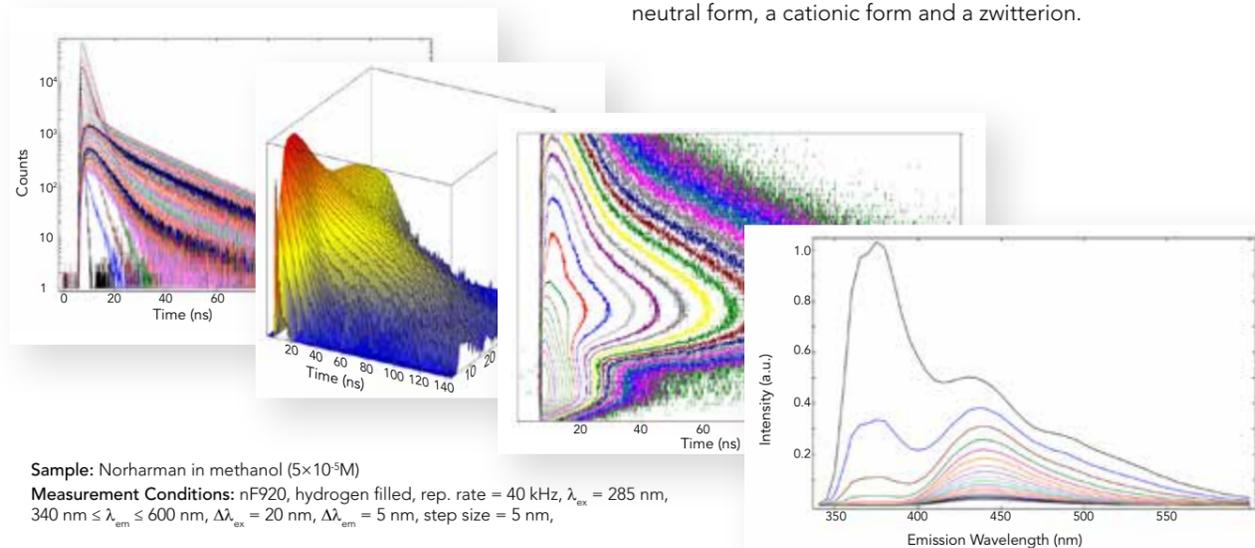
**Data Analysis:** multi-exponential reconvolution (F980). Confidence intervals verified by support plane analysis (FAST).  
 $\tau_1 = (15.02 \pm 0.03) \text{ ns}$  (top graph)  
 $\tau_1 = (14.80 \pm 0.20) \text{ ns}$ ,  $\tau_2 = (4.62 \pm 0.55) \text{ ns}$ ,  $\tau_3 = (0.81 \pm 0.20) \text{ ns}$  (bottom)

## Time Resolved Emission Spectroscopy (TRES)

TRES is a powerful tool in fluorescence lifetime studies. A family of decay curves is automatically measured as a function of a pre-set experimental variable, for example excitation or emission wavelength.

This measurement example shows complex fluorescence decay dynamics for a single fluorophore, Norharman, dissolved in a protic solvent.

Global Analysis (FAST) reveals three emitting species: a neutral form, a cationic form and a zwitterion.



**Sample:** Norharman in methanol ( $5 \times 10^{-5} \text{ M}$ )  
**Measurement Conditions:** nF920, hydrogen filled, rep. rate = 40 kHz,  $\lambda_{ex} = 285 \text{ nm}$ ,  $340 \text{ nm} \leq \lambda_{em} \leq 600 \text{ nm}$ ,  $\Delta\lambda_{ex} = 20 \text{ nm}$ ,  $\Delta\lambda_{em} = 5 \text{ nm}$ , step size = 5 nm,

# Examples and Applications - TCSPC

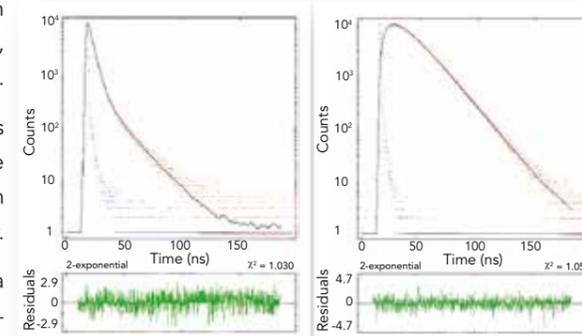
## Monomer-Excimer Kinetics

While many fluorescence lifetime measurements can be described by single or multiple exponential decays, often formation (or growth) kinetics can be observed too.

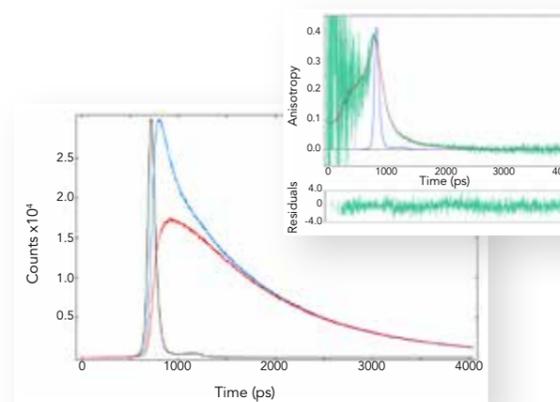
The example shows monomer-excimer kinetics of pyrene, with the monomer showing a double exponential decay, while the excimer displays an exponential rise, followed by an exponential decay.

Rise and decay times are often linked. Lifetime data analysis with linked parameters (Global Analysis) is possible by using the advanced data analysis package FAST.

**Sample:** Pyrene in cyclohexane ( $10^{-2} \text{ M}$ )  
**Measurement Conditions:** nF920, hydrogen filled, rep. rate = 40 kHz,  $\lambda_{ex} = 335 \text{ nm}$ ,  $\lambda_{em} = 395 \text{ nm}$  for monomer emission,  $\lambda_{em} = 465 \text{ nm}$  for excimer emission,  $\Delta\lambda_{ex} = \Delta\lambda_{em} = 5 \text{ nm}$ , measured in front face due to high sample concentration.  
**Fit Result:** Double exponential global fit:  $\tau_M = 9.3 \text{ ns}$ ,  $\tau_E = 15.4 \text{ ns}$



## Time Resolved Fluorescence Anisotropy



By exciting the sample with vertically polarised light and recording the emission in both the vertical and horizontal plane, one can calculate the fluorescence anisotropy of a homogeneous sample. The fluorescence anisotropy reveals the average rotational diffusion time of the molecules.

The measurement example shows that rotational diffusion in the picosecond time scale can be accurately measured. Most samples show rotational diffusion. To avoid this effect when precise fluorescence lifetime measurements are required, the emission polariser must be set to magic angle conditions (and vertically polarised excitation used).

**Sample:** POPOP in cyclohexane (IRF, decays with parallel and crossed polariser – left plot), Fluorescence Anisotropy (raw data and fit - right plot).  
**Measurement Conditions:** EPL375, MCP-PMT.  $\lambda_{ex} = 375 \text{ nm}$ ,  $\Delta\lambda_{ex} = 2 \text{ nm}$ ,  $\lambda_{em} = 390 \text{ nm}$ ,  $\Delta\lambda_{em} = 2 \text{ nm}$   
**Data Analysis:** Full anisotropy reconvolution (FAST) with ellipsoidal rotor model. The rotational diffusion times are 110 ps, 150 ps and 620 ps, respectively. (A spherical rotor model results in a fit with significantly increased chi-square. POPOP is a rod-like molecule.)

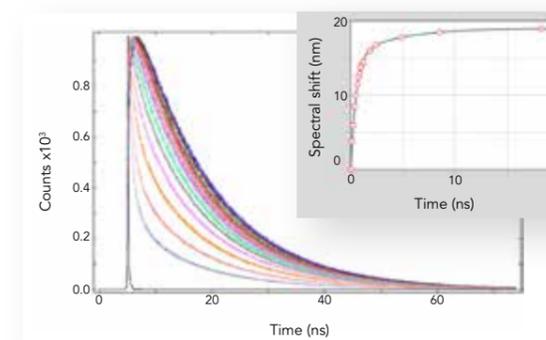
## Solvent Relaxation Dynamics

Fluorescence kinetics can be complex for homogeneous dye solutions in polar solvents, even if rotational diffusion is eliminated, i.e. measurements with magic angle conditions.

The measurement shows 16 different fluorescence decays in a polar-viscous solvent (glycerol) containing one fluorophore species, with multi-exponential decays at shorter wavelengths and rise-decay kinetics at longer wavelengths.

Normalised emission spectrum spectral peak positions can be determined, by using global exponential decay analysis (FAST) and comparing the global pre-exponential factors, and plotted as a function of time.

The insert shows that spectral peak position shifts in a double exponential manner with time.



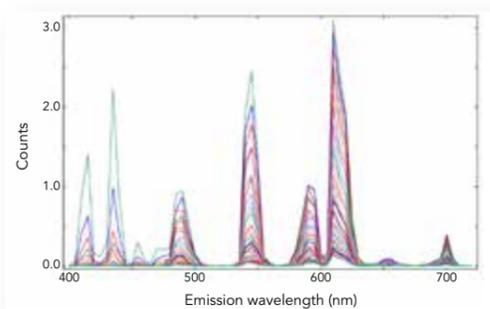
**Sample:** 3-amino-N-methylphthalimide in glycerol ( $5 \mu\text{M}$ )  
**Measurement Conditions:** EPL-375,  $\Delta\lambda_{em} = 5 \text{ nm}$ ,  $450 \text{ nm} \leq \lambda_{em} < 600 \text{ nm}$ , 10 nm steps, 8000 channels per decay.  
**Analysis:** Global Analysis with 4 lifetimes linked (FAST).

# Time-Resolved Measurement Examples and Applications - MCS

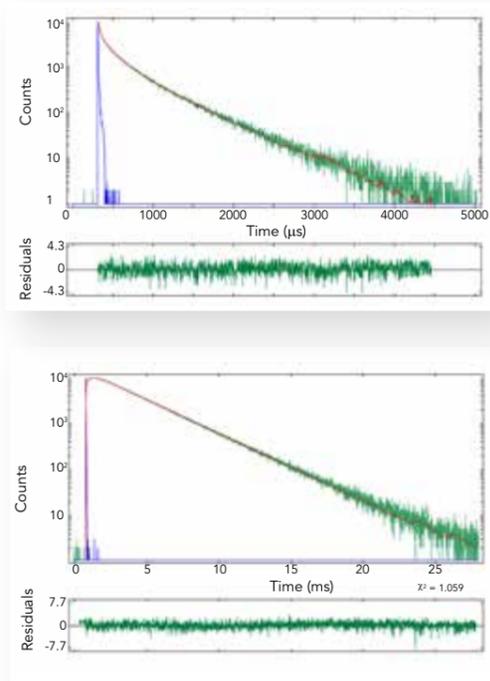
## Time Resolved Measurements of Lanthanides

The photoluminescence emission lifetime of lanthanides extends over a large time range from nanoseconds to seconds where the method of choice for time resolved measurements is the MCS technique. Due to the high dynamic range and the accuracy resulting from counting statistics, complex decay analysis can be performed.

The pictures show time resolved measurements from a lanthanide doped glass sample at two different emission wavelengths. At the shorter wavelength the decay is best fitted with a three exponential terms, while at the longer emission wavelength the initial rise is followed by a millisecond decay.



**Sample:** rare earth doped glass (same as for lifetime measurements)  
**Measurement Conditions:**  $\mu$ F2,  $\lambda_{ex}$ =370 nm,  $\Delta\lambda_{ex}=\Delta\lambda_{em}$ =2.5 nm, rep. rate: 100 Hz, step 10 nm. Spectra produced for every 50  $\mu$ s.



**Sample:** rare earth doped glass  
**Measurement Conditions:**  $\mu$ F2,  $\lambda_{ex}$ =370 nm,  $\Delta\lambda_{ex}$ =2.5 nm,  $\lambda_{em}$ =430 nm,  $\Delta\lambda_{em}$ =2.5 nm, rep. rate: 100 Hz, measurement time: 2min (top graph)  
 $\lambda_{ex}$ =370 nm,  $\Delta\lambda_{ex}$ =1.7 nm,  $\lambda_{em}$ =612 nm,  $\Delta\lambda_{em}$ =1.7 nm, rep. rate: 20 Hz, measurement time: 8 min (bottom graph)

**Data Analysis:** multi-exponential reconvolution.  
Good fit results were achieved with four exponential decay model (top graph) and model comprising two exponential rise and one decay functions (bottom graph)

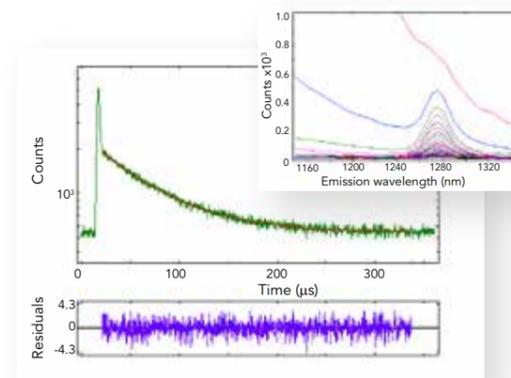


## Time Resolved Singlet Oxygen Measurements

### Time Resolved Singlet Oxygen Measurements

Time resolved singlet oxygen measurements are challenging as the emission at 1270 nm is very weak. The emission lifetime is solvent dependent and becomes comparatively short in aqueous solutions.

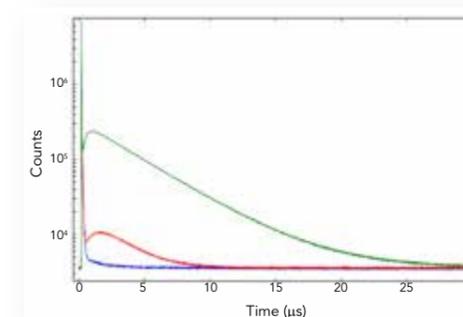
The figures below shows that time resolved singlet



**Sample:** Singlet oxygen generated by  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  in  $\text{D}_2\text{O}$ . The sample is oxygen saturated.  
**Measurement Conditions:**  $\mu$ F2, rep. rate 100 Hz, NIR-PMT  $\lambda_{ex}$  = 450 nm;  $\Delta\lambda_{ex}$  = 20 nm,  $\lambda_{em}$  = 1270 nm,  $\Delta\lambda_{em}$  = 12 nm  
**Data Analysis:** Tail fit ignoring contribution of sensitizer emission:  $\tau$ =58.5  $\mu$ s  
**Inset:** Time resolved emission spectra of  $[\text{Ru}(\text{bpy})_3]\text{Cl}_2$  in  $\text{D}_2\text{O}$ . Spectral slices of 10  $\mu$ s width allow the singlet oxygen emission to be discriminated from the background.

oxygen measurements are possible with the standard microsecond flashlamp as excitation source. By measuring time resolved spectra the singlet oxygen emission can be discriminated from the background.

The figure on the right shows a time resolved singlet oxygen emission measurement with exceptional high temporal resolution. A nanosecond pulsed laser source is required for this type of measurement.



**Sample:** Singlet oxygen generated by chlorine e6 in water/ethanol (green) and pure water (red), instrumental response function (blue)  
**Measurement Conditions:** pulsed laser, rep. rate 1 kHz, NIR-PMT  $\lambda_{ex}$  = 355 nm,  $\Delta\lambda_{em}$  = 1 nm,  $\lambda_{em}$  = 1270 nm  
**Data Analysis:** Data Analysis by FAST:  
in water/ethanol rise time: 280 ns, decay time: 4.0  $\mu$ s  
in pure water: rise time 1.1  $\mu$ s, decay time 1.9  $\mu$ s

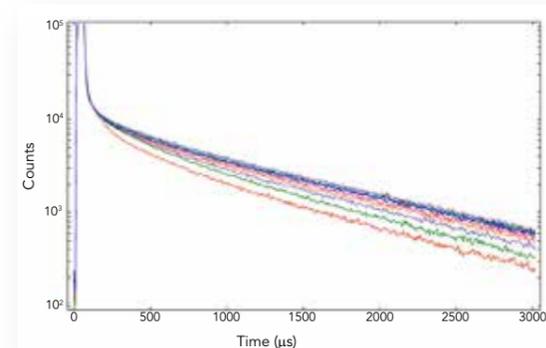
### Time Resolved FRET Measurements

Time resolved measurements of FRET in the millisecond time scale can provide more accurate data than other FRET techniques as background emission from the assay in nanosecond regime can be removed by detector gating and other residual background in the microsecond time scale is eliminated during data analysis.

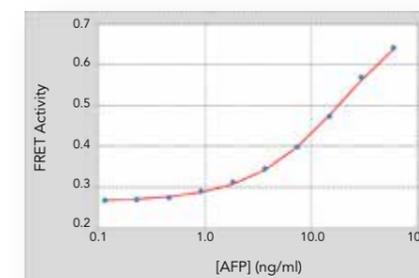
The example shows the change in the fluorescence decay kinetics of the donor Europium cryptate (EuK), quenched

by a nanosecond emitting acceptor. The fraction of FRET quenched donors is proportional to the concentration of the antigen concentration, in this case Human Alfa Fetoprotein (AFP).

The high sensitivity of this time resolved FRET measurement in this homogeneous immunoassay allows for antigen concentrations that are significantly lower than the cancer marker threshold.



**Sample:** EuK / acceptor labelled antibodies, AFP antigen, incubated for 1 hour at 22°C.  
**Measurement Conditions:**  $\mu$ F2 and (65  $\mu$ s off -) gated PMT.  $\lambda_{ex}$  = 320 nm,  $\Delta\lambda_{ex}$  = 20 nm  $\lambda_{em}$  = 612 nm,  $\Delta\lambda_{em}$  = 5 nm, rep rate 100 Hz



**Data Analysis:** 4-exponential fit, the two short lifetime components account for the microsecond background, the third and fourth components represent the donor's FRET-quenched and non-quenched emission, respectively. The relative amplitude of the FRET-quenched component,  $b_3/(b_3+b_4)$ , is directly proportional to concentration of the antigen.  
Data Analysis was performed using FAST.

## Special Spectrometer Configurations



FLS980 tailored for steady state and time resolved (ps -  $\mu$ s - TCSPC mode) spectroscopy in the spectral range up to 1700 nm. Additional sources for time resolved measurements are EPLs/EPLEDs, connected to the sample chamber laser port and a super-continuum source, connected to the double grating excitation monochromator.

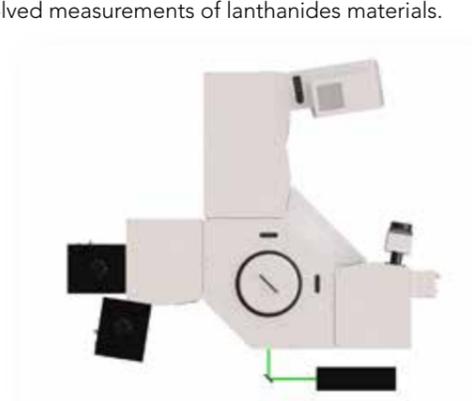


FLS980 tailored for steady state and time resolved spectroscopy ( $\mu$ s - seconds - MCS mode) in the spectral range up to 870 nm (R928P detector) or 1010 nm (R2658P detector) with added laser excitation (low rep rate OPO) for tuneable narrow pulsed excitation.

This system was configured for steady state and time resolved measurements of lanthanides materials.



FLS980 in T-geometry with CCD camera for spectral measurements on one emission arm and NIR-PMT for spectral and lifetime measurements in the NIR on the other emission arm. The source for the spectral measurements is the Xe1 xenon lamp, the source for the lifetime measurements in microsecond to second range is the  $\mu$ F2 pulsed xenon lamp.



FLS980 in T-geometry configured for steady state spectroscopy in the visible and near infrared spectral range. The right emission arm supports a photomultiplier and an InGaAs detector. The left emission arm has an InAs and an InSb detector fitted.

The infrared detectors are less sensitive, therefore a powerful continuous laser source is required for sample excitation.

FLS980 adapted for steady state and time resolved (ns- $\mu$ s TCSPC mode) VUV excited spectroscopy. The instrument comprises a VUV monochromator with deuterium lamp for continuous measurements and a nF920 nanosecond flashlamp for pulsed excitation.



Some spectrometer configurations include class 3R, 3B or class 4 laser products. Laser safety procedures must be followed.

## FLS980 - Technical Specifications

### System

Optical Configuration	Right angle geometry (standard) Additional geometries are available for non-standard applications
Optics	Lens optics (standard) and/or mirror optics (optional)

### Sensitivity (steady state)

Signal to Noise Ratio of water Raman peak	>25,000:1 Excitation wavelength = 350 nm, Spectral bandwidth = 5 nm, integration time = 1 s.
-------------------------------------------	-------------------------------------------------------------------------------------------------

### Lifetime Range (time resolved)

TCSPC	TCSPC	MCS	MCS
with nF920	with fs-laser/MCP	with $\mu$ F2	with pulsed laser
100 ps up to 50 $\mu$ s	5 ps up to 50 $\mu$ s	1 $\mu$ s up to 10 s	10 ns up to 10 s

### Monochromators

Type	Czerny-Turner
Focal length	300 mm (single) 2 x 300 mm (double)
Gratings	Mounted to triple grating turret, fully computer controlled
Slits	<25 $\mu$ m to 10 mm, fully computer controlled
Stray Light rejection	10 <sup>5</sup> (single) or 10 <sup>10</sup> (double)

### Gratings

Type	Plane holographic or ruled grating	Double grating monochromator
Standard	Single grating monochromator 1800 grooves/mm 250 nm / 500 nm blaze (ex, em respectively)	Double grating monochromator 1200 grooves/mm 300 nm / 500 nm blaze (ex, em respectively)
Dispersion	1.8 nm/mm	0.9 nm/mm
Resolution	0.05 nm - 18 nm	0.05 nm - 9 nm
Wavelength Accuracy	$\pm$ 0.2 nm	$\pm$ 0.2 nm
Wavelength Repeatability	$\pm$ 0.1 nm	$\pm$ 0.1 nm
Minimum Step Size	0.05 nm	0.05 nm
Options	Gratings with 300 - 2400 grooves/mm, optimised from VUV to NIR are available.	

### Excitation Sources

Type	450 W ozone free Xenon Arc Lamp	Microsecond flashlamp	Nanosecond flashlamp
Mode of operation	steady state	time-resolved (MCS)	time-resolved (TCSPC)
Spectral Range	230 nm - 1000 nm	200 nm - 1000 nm	200 nm - 400 nm
Pulse Width	n/a	1 $\mu$ s - 2 $\mu$ s	<1 ns
Option	Ozone generating lamp with range 200 nm - 1000 nm	Low to medium repetition rate pulsed lasers	Picosecond pulsed diode lasers (<100 ps) and LEDs (<950 ps)

### Detectors

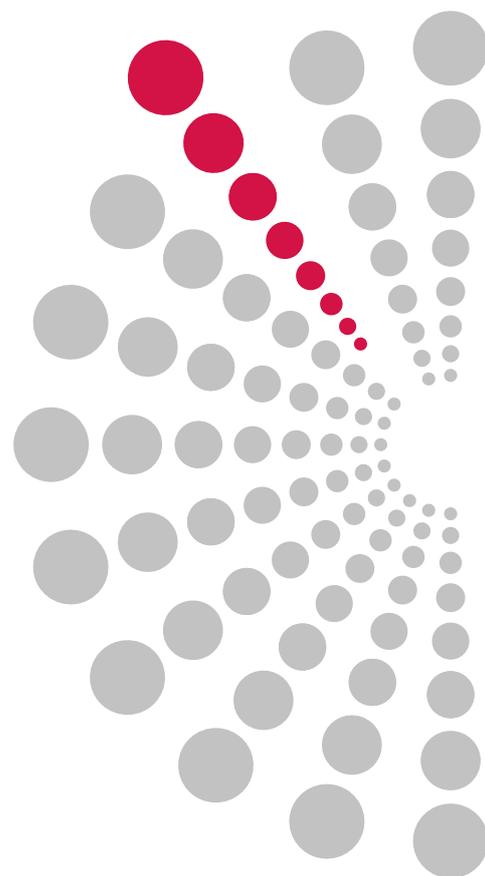
Photomultiplier	R928P	NIR-PMT	R2658P	high speed	MCP-PMT
Spectral Range	200 nm - 870 nm	300 nm - 1700 nm	200 nm - 1010 nm	230 nm - 870 nm	200 nm - 850 nm
Dark Count Rate	< 50 cps (-20°C)	<200 kcps (-80°C)	< 100 cps (-20°C)	< 100 cps (0°C)	< 50 cps (-20°C)
Instrument Response Function	600 ps	800 ps	600 ps	200 ps	<50 ps
Options	A variety of other photomultipliers and analogue detectors are also available.				

### Data Acquisition

Model	CB acquisition module	TCC acquisition module
Modes of operation	Counting, MCS	Counting, MCS, TCSPC
Number of detector channels	3	3
Max. number of time bins	8000	8000 (MCS), 8192 (TCSPC)
Min. width of time bins	10 ns	<610 fs
Time range selection	5 $\mu$ s - 1000s	5 $\mu$ s - 1000 s (MCS), 2.5 ns - 50 $\mu$ s (TCSPC)
Computer Interface	USB	USB

### Software

Operating System	Windows™ 7 or 8
Data Manipulation	Mathematical, Smoothing, Integration, Differentiation, 2-D and 3-D graphics, Contour plots, Chromaticity, Quantum Yields, Multi-exponential Deconvolution Lifetime Analysis.
Option	FAST - Advanced Fluorescence Lifetime Analysis Software (Lifetime distributions, batch analysis, global analysis, advanced anisotropy analysis, FRET analysis, stretched exponential analysis, micellar quenching and Förster quenching)



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