920 Series

Steady State and Time Resolved Fluorescence Spectrometers
Edinburgh Instruments has been at the forefront of the 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. With the use of fluorescence measurement techniques expanding rapidly, particularly within the life science and biotechnology sectors, Edinburgh Instruments continue to lead the market in product development of affordable, reliable and high quality instrumentation.
The Edinburgh Instruments 920 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 920 series of spectrometers will enable you to push the boundaries of scientific research.

**FS920**

**STEADY STATE FLUORIMETER**

Based on the single photon counting technique, the FS920 is the world’s most sensitive steady state fluorimeter. In addition, it can be easily configured to measure phosphorescence decay kinetics from microseconds to seconds.

**FL920**

**FLUORESCENCE LIFETIME SPECTROMETER**

Based on time correlated single photon counting (TCSPC) the FL920 is the system of choice for measuring complex fluorescence decay kinetics in the picosecond to microsecond range.

**FLSP920**

**COMBINED STEADY STATE AND LIFETIME SPECTROMETER**

The FLSP920 is a complete fluorescence laboratory in a single instrument. It combines all the features of the FS920 and the FL920, together with phosphorescence spectra and lifetime capabilities.
FS920 - The World’s Most Sensitive Spectrofluorimeter

Steady State Spectrofluorimeter

The FS920 is a modular, computer controlled spectrofluorimeter for measuring steady state luminescence spectra in the ultraviolet - near infrared spectral range with single photon counting sensitivity. It combines ultimate sensitivity with high spectral resolution and excellent stray light rejection. The performance specifications of the FS920 make it ideally suited for demanding applications in the broad areas of photophysics, photochemistry, biophysics and semiconductor study.

Sensitivity

Single photon counting is an unparalleled method for the measurement of low level optical radiation. Edinburgh Instruments have optimised this technique in the FS920. A peak count rate of >750,000 cps and a signal to noise ratio of >6000:1 for a measurement of a water Raman spectrum under standard measurement conditions are guaranteed. This sensitivity allows the spectra of weak dye solutions, as low as 100fM, to be routinely measured.

Sample: Raman Spectrum of Distilled Water
Measurement Conditions: $\lambda_{\text{ex}} = 350\text{nm}$, $\Delta\lambda_{\text{ex}} = \Delta\lambda_{\text{em}} = 5\text{nm}$, step size = 1nm, integration time = 1s
Signal to noise ratio > 6000:1 for water Raman signal, $\lambda_{\text{peak}} = 397\text{nm}$, RMS noise measured at 450nm

Sample: 100 fM fluorescein in 0.1N $\text{H}_2\text{SO}_4$
Measurement Conditions: $\lambda_{\text{ex}} = 485\text{nm}$, $\lambda_{\text{em}} = 525\text{nm}$, $\Delta\lambda_{\text{ex}} = \Delta\lambda_{\text{em}} = 4\text{nm}$, step size = 1nm, integration time = 1s (curves are background subtracted)
Resolution

The FS920 uses Czerny-Turner monochromators with high quality diffraction gratings for high dispersion and excellent imaging quality. Wavelength tuning is micro-stepper motor driven with a minimum step size of 0.05nm. Spectral details as close as 0.1nm can be resolved over the spectral range from UV to NIR.

Stray Light

Stray light suppression is vital for samples that exhibit a low quantum yield or a high level of scattering. The 920 series of spectrometers 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 1:10^5 and 1:10^10, respectively.

Sample: Europium
Measurement Conditions: λ_ex = 395nm, Δλ_ex = 5nm, Δλ_em = 0.05nm
step size = 0.05nm, integration time = 1s

Sample: Raman Spectrum of CCl_4
Measurement Conditions: λ_ex = 348nm, Δλ_ex = 0.5nm, Δλ_em = 0.7nm, step size = 0.05nm, integration time = 5s
Spectral Range

The trend in research is that it is increasingly important to study luminescence characteristics over a broad spectral range from the ultra-violet to the infrared.

The FS920 is supplied with triple grating turret monochromators, with up to three different gratings permanently fitted. This allows a large spectral range to be covered, providing maximum flexibility and ease of use. Each grating is individually optimised for both spectral range and linear dispersion. Grating selection and wavelength tuning is micro-stepping drive controlled via the F900 software. A computer controlled beam steering mirror allows the rapid selection of two detectors mounted on independent exit slits of the emission monochromator. Single photon counting photomultipliers are available covering the wavelength range from 185 -1700nm, while analogue detectors extend the wavelength range to 5000nm.

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 previously measured correction files, is standard practice when using the FS920.

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 are obtained during calibration at the Edinburgh Instruments factory using calibrated light sources. With a simple mouse click, the FS920 produces corrected spectra you can trust.

The figure shows the effect of spectral correction on a typical emission spectrum.

G-factor correction in anisotropy studies is also available.

Fast Photon Counting

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 FS920 spectrometer incorporates state of the art single photon counting technology in the form of the PCS900 computer plug-in card. This is a fast pulse counter scalar card that is capable of operating with count rates of up to 100MHz. The PCS900 card can operate in two single photon counting modes for both steady state measurements and phosphorescence decay measurements.

In the later, the card operates in Multi Channel Scaling (MCS) mode. Here, 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. Measurements are repeated many times and added to improve the signal to noise ratio. The minimum time window for the PCS900 card is 200ns, allowing phosphorescence lifetimes from 400ns to 10s to be measured. Optional dedicated MCS cards are available with minimum time window down to 1ns.
Software Interface

The F900 advanced software package is based on a data centred design that enables the user to focus on the measurement. Such an approach offers significant benefits in system ease-of-use. Extensive on-line help guides the user through measurement sequences. Measurement set-up and data acquisition is made through a transparent menu system. Key spectroscopic parameters are easily accessed through functional grouping. Tabbed dialogue boxes and particular scan parameters are always visible during set-up. The current instrument status is also continuously displayed.

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

In keeping with the modularity of the FS920 spectrofluorimeter, the F900 advanced software is a user friendly and flexible package. Irrespective of the system configuration and accessories, the F900 software provides the user with complete control. Measurement modes within the software reflect such system configurations by means of a simple operating environment.

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Measurement Examples

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 $10^{-5}$M anthracene in degassed cyclohexane. The sample is also used as a standard for time resolved measurements.

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 dependant 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.

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 FS920 in T-geometry for dual wavelength detection, simultaneous measurements of the Ca$^{2+}$ active fluorophore Indo-1 can be made with both emission arms set to different wavelengths.

The figure demonstrates the “ratio curve” of the two simultaneous kinetic scans for Indo-1 loaded human platelet cells in a $10^{-3}$M Ca$^{2+}$ environment. 10 seconds after the beginning of the scan the cell membranes were activated with 250nM ionomycin.

Sample: Anthracene in Cyclohexane ($10^{-5}$M)
Measurement Conditions: $\lambda_{ex} = 358$nm for corrected emission scan, $\lambda_{em} = 400$nm for corrected excitation scan, $\Delta \lambda_{ex} = \Delta \lambda_{em} = 0.4$nm, step size = 1nm, integration time = 1s

Sample: Rhodamine B in glycerol ($10^{-5}$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 = 1nm, integration time = 1s, sample temperature = +10°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)

Sample: Human platelet cells loaded with Indo-1 in 1mM 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 = 0.5s
Synchronous Scans
In synchronous scans, both excitation and emission monochromators are scanned synchronously with a preset offset. These scans can be used in order to separate and identify fluorescence components in mixtures. For low concentrations, each fraction of the molecules has a peak at a characteristic wavelength of the synchronous spectrum.

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).

Excitation - Emission Maps
The variety of measurement, display and analysis options of the FS920 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.

Temperature Maps
The F900 spectrometer control software can communicate with Oxford Instruments Optistat DN (liquid nitrogen) and Optistat CF (liquid helium) cryostats. Temperature maps can be made by acquiring a series of emission scans, excitation, or synchronous scans for a predefined temperature range. The individual measurements are automatically started when the actual target temperature is reached.

Sample: Crude oil from an Asian oil field. Excitation - Emission Map
Measurement Conditions: 245nm ≤ λ_ex ≤ 345nm, 275nm ≤ λ_em ≤ 465nm, Δλ_ex = Δλ_em = 1.0nm, step size = 1.0nm, integration time = 0.1s

Sample: Polymer sample with two aromatic molecules embedded: Naphthalene and Anthracene
Measurement Conditions: 270nm ≤ λ_ex ≤ 390nm, 300nm ≤ λ_em ≤ 500nm, Δλ_ex = Δλ_em = 0.4nm, step size = 2.0nm, integration time = 0.2s

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: Triphenyldiamine
Measurement Conditions: Δλ_ex = 380nm, temperature range 90K ≤ T ≤ 107K in 1K steps, stabilisation time = 10s. Δλ_em = Δλ_em = 1.0nm, step size = 1.0nm, integration time = 0.2s

Sample: Five aromatic hydrocarbons dissolved in cyclohexane
Measurement Conditions: λ_ex = 280nm for corrected emission scan Δλ_ex = Δλ_em = 0.5nm, step size = 0.5nm, integration time = 1s, offset = 0nm for synchronous scan

Sample: Five aromatic hydrocarbons dissolved in cyclohexane
Measurement Conditions: λ_ex = 280nm for corrected emission scan Δλ_ex = Δλ_em = 0.5nm, step size = 0.5nm, integration time = 1s, offset = 0nm for synchronous scan
Emission in Near Infrared Spectral Range

Studies in the near infrared spectral range are becoming increasingly important among spectroscopy scientists. Long wavelength optimised gratings and special detectors are required to cover this range.

A large variety of infrared detectors can be integrated into FS920 spectrometers, including NIR photomultipliers, Germanium, InGaAs, PbS, InAs, InSb and MCT detectors, both Peltier and liquid nitrogen cooled.

The strong and structured emission spectrum of a laser rod of Nd:YAG is shown for the wavelength range from 850-1700nm measured with a NIR-PMT.

Sample: Nd:YAG
Measurement Conditions: $\lambda_{ex} = 357\, \text{nm}, \Delta \lambda_{ex} = 10\, \text{nm}, \Delta \lambda_{em} = 0.1\, \text{nm}$, step size = 0.1nm, integration time = 1.0s

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 FS920 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 $10^{-4}\, \text{M}$ hematoporphyrin in ethanol
Measurement Conditions: $\lambda_{ex} = 380\, \text{nm}$ for emission scan, $\lambda_{em} = 1270\, \text{nm}$ for excitation scan, $\Delta \lambda_{ex} = \Delta \lambda_{em} = 2.0\, \text{nm}$, step size = 1.0nm, integration time = 3s

Semiconductor Emission

A number of applications for the FS920 require alternative excitation sources, detection devices or special sample handling facilities. Emission spectra analysis of a GaInAs quantum well requires upgraded features in all three areas of the system.

The figure demonstrates emission spectra of such a sample at three different temperatures measured using an argon ion laser for excitation, a photomultiplier with a GaAs photocathode as a detector and a helium cryostat for temperature control.

Sample: GaInAs quantum wells at three different temperatures
Measurement Conditions: $\lambda_{ex} = 514.5\, \text{nm}$ (all curves are spectrally corrected), $\Delta \lambda_{ex} = \Delta \lambda_{em} = 0.5\, \text{nm}$, step size = 0.5nm, integration time = 1.0s

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 low concentrations ($1.6\times10^{-4}\, \text{M}$ – pink curve) only the monomer lines in the wavelength range 370-400nm are observed, while at high concentrations ($2.0\times10^{-2}\, \text{M}$ – blue curve) the excimer peak at ca 480nm dominates.

Sample: Pyrene in cyclohexane
Measurement Conditions: $\lambda_{ex} = 335\, \text{nm}$ (all curves are spectrally corrected), $\Delta \lambda_{ex} = \Delta \lambda_{em} = 0.5\, \text{nm}$, step size 1.0nm, integration time 1.0s
The FS920 can be easily upgraded from steady state mode to facilitate time resolved phosphorescence spectra and phosphorescence decays.

Phosphorescence is generally weak and characterized by a long lifetime, typically in the range from microseconds to seconds. At room temperature, phosphorescence lifetimes can be shortened and either the scattered light from the sample or the sample fluorescence can swamp the signal. It is often not possible to separate these effects solely by spectral means, and time resolved measurements are the only solution.

Phosphorescence measurements require a pulsed light source. Typically a microsecond pulsed xenon flashlamp for sample excitation is used. Time resolved single photon counting detection is made using the Multi-Channel Scaling mode of the PCS900 card. Here, individual photon pulses are counted within a narrow time window that is scanned over the complete time range. This process is repeated over many flashes to improve the signal to noise ratio. As an additional option, the photomultiplier can be gated to avoid saturation due to strong scattered light or sample fluorescence.

Time resolved phosphorescence spectra are obtained by a two-fold process. A series of decay measurements at different wavelengths are recorded and then the data is sliced at different time windows after the excitation pulse. This technique separates phosphorescence from fluorescence and reveals unambiguous sample kinetics.

The figure below shows a family of decay curves at different emission wavelengths for a Nd:YAG laser rod measured under computer control using single photon counting in the NIR spectral range. Time resolved spectra are shown on the right by slicing this data every 30µs.

![Decay curves](image1)

Sample: Nd:YAG laser rod
Measurement Conditions: µF900, rep. rate = 100Hz, NIR-PMT detector, $\lambda_{ex} = 357nm$, $\Delta\lambda_{ex} = 10nm$, $\Delta\lambda_{em} = 3nm$, step size 2nm.

An example of a typical case, where the sample exhibits strong fluorescence and weak phosphorescence that is typically 3-4 orders of magnitude less at room temperature, is shown right. The three curves show time resolved spectra at the long wavelength tail of the emission of a derivative of the aromatic molecule anthracene in toluene at 0, 3µs and 12µs after the excitation pulse. The phosphorescence band around 770nm is only fully resolved at longer time delays.

This data was measured using a µF900H high power Xenon flashlamp. For some phosphorescence applications laser sources with higher pulse energy can also be integrated.

![Decay curves](image2)

Sample: 9,10-dichloro-2,6-bis(bromomethyl)anthracene in iodosluene
Measurement Conditions: µF900H, rep. rate = 100Hz, $\lambda_{ex} = 400nm$, $\Delta\lambda_{ex} = 20nm$, $\Delta\lambda_{em} = 10nm$, step size = 10nm.
## FS920 - Technical Specifications

### System

| Optical Configuration | Right angle geometry (standard) Additional geometries are available for non-standard applications |

### Sensitivity

| Water Raman Spectrum | Excitation wavelength = 350nm Spectral bandwidth 5nm, integration time 1s |
| Peak Counts | > 750,000cps @ 397nm |
| RMS Noise | < 125cps @ 450nm |
| Signal to Noise Ratio | > 6000:1 |

### Excitation Source

| Type | Continuous Xenon Arc Lamp |
| Lamp | 450W, ozone free |
| Spectral Range | 230 – 2600nm |
| Adjustment | XYZ, focussing, rear mirror |
| Depth of Focus | 150mm to ∞ |
| Option | ozone generating lamp with spectral range 200-2600nm |

### Monochromator

| Type | Czerny-Turner |
| Focal Length | 300mm |
| Gratings | mounted to triple grating turret, fully computer controlled |
| Slits | <10µm to 10mm, fully computer controlled |
| Stray Light Rejection | 1:10⁴ (1:10¹⁰ for double monochromators) |
| Option | Double Grating Monochromator |

### Grating

| Type | Plane holographic or ruled grating |
| Standard | 250/500nm blaze, 1800 grooves/mm |
| Dispersion | 1.8 nm/mm |
| Resolution | 0.05 – 18 nm |
| Wavelength Accuracy | ± 0.2 nm |
| Wavelength Repeatability | ± 0.1 nm |
| Minimum Step Size | 0.05 nm |
| Options | A variety of gratings with 300-2400grooves/mm, optimised from VUV to NIR are available |

### Detector

| Photomultiplier | Blue Sensitive | Red Sensitive |
| Spectral Range | 185 – 680 nm | 185 – 870 nm |
| Window Material | UV Glass | UV Glass |
| Dark Count Rate (+24°C) | < 100 cps | < 2000 cps |
| Dark Count Rate (-25°C) | < 5 cps | < 50 cps |
| Options | To allow NIR measurements, photomultipliers and analogue detectors are available |

### Data Acquisition

| Photon Counting Scalar Card | Model PCS900 Plug-in PC Card |
| Mode of Operation | Single photon counting / multi-channel scaling |
| Maximum Count Rate | 100MHz |
| Computer Interface | PCI |

### Software

| Operating System | Windows® |
| Data Manipulation | Mathematical, Smoothing, Integration, Differentiation, 2-D and 3-D graphics, Contour Plots |

Edinburgh Instruments Ltd. has a policy of continuous product development and reserve the right to amend specifications without prior notice (Apr 07)
Excitation Sources

Xe900 Xenon Arc Lamp
The Xe900 is a 450W ozone free xenon arc lamp that emits continuous radiation from 230nm to 2600nm. The lamp is fully adjustable in two orthogonal planes for alignment optimisation during operation. It also has an adjustable rear reflector and an f/1 spectrosil B condensing lens for optimum imaging.

nF900 Nanosecond Flashlamp
The nF900 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 NIR spectral range, namely 110-850nm depending on optics and gas fill, at repetition rates to 100kHz.

µF900 Microsecond Flashlamp
The µF900 is a pulsed xenon microsecond flashlamp producing short, typically a few µs, high irradiance optical pulses at repetition rates up to 100Hz. As a result, this is an ideal source for phosphorescence decay measurements in the range from microseconds to seconds. The µF900 comes in a 5W version or in a standard 60W version.

Alternative Light Sources
The 920 spectrometers have convenient access for coupling alternative light sources to the sample chamber.

Sources for TCSPC Measurements (repetition rates from 10kHz to 100MHz)
• EPLs – Picosecond pulsed semiconductor diode lasers
EPL-Series lasers produce picosecond duration pulses (typically <100ps) at repetition rates up to 20MHz and are therefore ideal for applications in TCSPC measurements. These lasers are very compact and require only a power adapter for operation. EPL lasers are available with laser wavelengths of 375nm, 405nm, 445nm, 475nm, and discrete emissions above 630nm. Other picosecond pulsed semiconductor lasers may also be used.
• EPDs - Light Emitting Diodes
Pulsed light emitting diodes of the EPD series produce sub-nanosecond (typically <750ps) optical pulses at repetition rates up to 20MHz and are therefore ideally suited to TCSPC measurements. EPDs are available with emission covering the UV-Visible spectrum, starting from 265nm. Other picosecond pulsed light emitting diodes may also be used.
• Ti:Sapphire lasers
• Supercontinuum fibre lasers

Sources for MCS measurements (repetition rates up to 1000 Hz)
• Harmonics of Q-switched solid state lasers (eg Nd:YAG)
• Optically pumped parametric oscillators or dye lasers

Sample Chamber
At the heart of the 920 series is the universal sample chamber with integrated spectrometer hardware controller, eliminating open interconnecting cables. It can accommodate an array of optional accessories including lens or mirror focussing and collecting optics. Motorised high quality Glan Thompson prism polarisers with a 14mm clear aperture allowing transmission from 220nm to over 2000nm can be provided for anisotropy studies. Wavelength filters can also be conveniently added. In addition, the sample chamber can accept variable temperature liquid nitrogen or helium cryostats to cover the range 4-500K, closed cycle coolers and EPR Dewars.

The chamber can accept a wide range of single or multiple temperature controlled sample stages for liquids, solids or powders. In addition, it can also accept magnetic stirrers, a stopped flow accessory, optical filters and fibre optic coupling.
The monochromators used in the 920 series spectrometers are of the Czerny-Turner configuration with 300mm focal length, high optical throughput (f/4.2), excellent stray light rejection (1:10^5) and low temporal dispersion. The monochromators feature a triple grating turret with up to three gratings, fully computer controlled from within the spectrometer operating software. A range of high quality holographic and ruled gratings are available. The grating turret is micro-stepper driven with a minimum step size of 0.05nm and a maximum slew rate of 200nm/s.

The monochromators have computer controlled slits and the spectrometer software displays the band with settings in nanometre units, taking automatically into account the opto-mechanical properties of the grating.

All excitation monochromators are equipped with dual entrance slits, selected by a computer controlled flip mirror. Consequently, up to two permanently aligned excitation sources can be selected. As an option an external swing mirror can be fitted to allow the selection of up to three different light sources.

All emission monochromators are equipped with dual emission slits that can be selected by software. This allows mounting of two permanently fixed detectors on each emission arm.

Double grating monochromators with three computer controlled entrance slits (excitation) and three computer controlled exit slits (emission) are available for increased performance in stray light rejection. The double monochromators also benefit from an increase of the linear dispersion compared to the standard single grating monochromators.

**Detectors**

**Photomultiplier**

Single photon counting photomultipliers are now available spanning the range from 185nm to 1700nm. Edinburgh Instruments selects detectors for optimum performance including spectral response, high amplification, low dark count and low transit time spread. Optional Peltier cooled housings are available to reduce the dark noise, particularly for red sensitive photomultipliers. NIR devices are supplied with a cooling system to operate at approximately -80°C.

The Instrument Response Function (IRF) of the photomultipliers, measured with short pulse laser excitation, varies from 200ps in the visible range to 800ps in the NIR.

**Micro - Channel Plate Photomultiplier (MCP-PMT)**

An MCP-PMT with an IRF of <50ps is recommended for applications demanding the highest time resolution.

**Analogue Detectors**

Thermoelectrically or liquid nitrogen cooled infrared detectors with responsivities from 900-5000nm are used for steady state or time resolved emission measurements.
Sample Holder Options

**Single Sample Holder**
The standard sample holder for all 920 series systems consists of a single cuvette holder with filter retainer and a liquid cooling option, complete with continuous temperature monitoring.

**3 Position Sample Turret**
For multiple sample measurements at a regulated temperature, a computer controlled sample holder incorporating up to three samples on a computer controlled turret is available. The liquid coolant may be circulated through all three sample holders; a temperature probe is mounted to one of the three holders. A magnetic stirrer can be mounted underneath each sample position for sample agitation. Filter retainers for each individual sample are fitted as standard.

**Front Face Sample Holder**
The single sample holder for front face excitation is mounted on a translational platform for sample position optimisation. Filter holders are fitted as standard to allow ND and band pass filters to be used.

**Front Face With Rotation**
Designed specifically for samples such as thin films, powders, microscope slides and fibres, this single sample holder is mounted on a rotation stage allowing the angle to be adjusted external to the sample chamber.

**Stopped Flow**
Rapid kinetic accessory for either manual or computer controlled multi-mixing capabilities. Comprises sample handling unit fitted with two 2.5ml drive syringes, 600mm long umbilical, operating drive, square mixing/observation cuvette with standard dimensions (10mm path way)

**Plate Reader**
Multi-well plater reader attachment for plate formats with up to 384 wells. The plate reader is coupled to the spectrometer by optical fibre bundles. The plate reader is fully controlled from within the spectrometer operating software and comes with temperature monitoring and safety shutter interlock options.

**Integrating Sphere**
150mm diameter integrating sphere for quantum yield measurements in the spectral range 200-2500nm. The sphere is easily mounted in place of the standard sample holder and comes with holders for cuvettes and powders.
Accessories and Sample Cooling Options

**T-Geometry**

The system can be configured as a T-Geometry with two emission arms allowing two emission monochromators and up to four detectors to be permanently fitted to the system.

**Mirror Geometry**

The sample chamber can be configured in mirror geometry for improved front face measurements or measurements in the infrared beyond 3000nm. Once installed, the change-over between lens optics and mirror optics is simple and takes only seconds. A special mirror assembly is also available for powder measurements on a tray.

**TE Cooled Sample Holder**

Thermoelectrically cooled 4-window cuvette holder with stand alone controller that enables stable temperature control of samples from -10°C to 105°C. The temperature can be held constant with +/- 0.02°C precision and can be rapidly changed.

**EPR Dewar**

Provides temperature stability at 77K when low sample temperature analysis is required.

**Cryostat Systems**

Oxford Instruments liquid nitrogen or helium cryostats with ITC controllers are used when low temperature measurements are required.

The F900 software communicates fully 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.
# FL920 - Technical Specifications

## System

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</table>

## Mode of Operation

**With nF900, Pulsed Laser or LED**
- Time Correlated Single Photon Counting

**With µF900 or Pulsed Laser**
- Time Resolved Single Photon Counting (Multi Channel Scaling)

## Lifetime Range

<table>
<thead>
<tr>
<th>TCSPC with nF900</th>
<th>100ps - 50µs</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCSPC with fs-laser / MCP-PMT</td>
<td>5ps - 50µs</td>
</tr>
<tr>
<td>MCS with µF900</td>
<td>400µs to 10s</td>
</tr>
<tr>
<td>MCS with pulsed laser</td>
<td>400ns to 10s, optional 1ns to 2.5ms</td>
</tr>
</tbody>
</table>

## Excitation Source

<table>
<thead>
<tr>
<th>Type</th>
<th>Nanosecond Flashlamp, hydrogen or nitrogen filled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse Width</td>
<td>1 ns (typical)</td>
</tr>
<tr>
<td>Pulse Repetition Rate</td>
<td>up to 100kHz (40kHz typical)</td>
</tr>
<tr>
<td>Spectral Range</td>
<td>110 – 850 nm (depending on gas fill)</td>
</tr>
<tr>
<td>Options</td>
<td>microsecond flashlamp, EPL picosecond diode lasers, EPD picosecond pulsed light emitting diodes</td>
</tr>
</tbody>
</table>

## Monochromator

see FS920 specifications

## Detector

<table>
<thead>
<tr>
<th>Type</th>
<th>(for standard detectors, see FS920 specifications)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Options</td>
<td>MCP-PMT</td>
</tr>
<tr>
<td>Spectral Range</td>
<td>160-850 nm</td>
</tr>
<tr>
<td>Dark Count Rate</td>
<td>&lt; 50 cps @ -25ºC</td>
</tr>
<tr>
<td>Instrument Response Function</td>
<td>&lt; 50ps</td>
</tr>
</tbody>
</table>

## Data Acquisition

<table>
<thead>
<tr>
<th>TCSPC Card</th>
<th>Model TCC900 Plug-in PC Card</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Count Rate</td>
<td>3 MHz</td>
</tr>
<tr>
<td>Time Channels per Curve</td>
<td>up to 4096</td>
</tr>
<tr>
<td>Minimum Time per Channel</td>
<td>610fs</td>
</tr>
<tr>
<td>Timing Jitter</td>
<td>&lt; 25ps</td>
</tr>
<tr>
<td>Computer Interface</td>
<td>PCI</td>
</tr>
</tbody>
</table>

## Software

<table>
<thead>
<tr>
<th>Operating System</th>
<th>Windows®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Manipulation</td>
<td>Marquardt-Levenberg algorithm, up to 4 exponential decay, time resolved spectra, anisotropy</td>
</tr>
<tr>
<td>Option</td>
<td>FAST - advanced fluorescence lifetime analysis software (lifetime distributions, batch analysis, global analysis, advanced anisotropy analysis, FRET analysis, quenching models)</td>
</tr>
</tbody>
</table>
The FL920 is a modular, computer controlled spectrometer for measuring luminescence lifetimes spanning the range from picoseconds to seconds. Based on the measurement principle of Time Correlated Single Photon Counting (TCSPC), the FL920 offers the highest dynamic range and temporal resolution available. It has proved itself as the system of choice for high quality fluorescence research. The FL920 can be easily upgraded to measure phosphorescence lifetimes using the Multi-Channel Scaling technique.

**Fluorescence Lifetime Measurements**

Luminescence decay kinetics give a complete picture of the fluorophore and its interactions within the microenvironment. From this, the researcher can gain a deep understanding of the reaction mechanisms and electronic structure of the excited states of organic and inorganic molecules. In addition, it provides the industrialist with a powerful tool for quality control and testing.

An example of fluorescence decays measured using TCSPC is shown in the adjacent figure. This illustrates how detailed information on complex intermolecular interactions can be revealed by lifetime measurements made across an emission spectrum which has little structure.

The scope for applications of fluorescence lifetime measurements is large and growing. From single molecule detection to lanthanide tracing; from singlet oxygen monitoring in photodynamic therapy to the study of new C-60 “fullerenes”; from Tryptophan protein studies to the characterisation of rare earth doped glasses; the kinetic information given in fluorescence lifetime measurements opens the door to a greater level of understanding.

The biomedical researcher who employs fluorescent probes in the study of enzymes, dynamics and structure of nucleic acids, protein folding and DNA sequencing, can use a-priori fluorescence lifetime knowledge of the fluorescent probe to characterise the system. The materials physicist studying semiconductors and novel structures such as quantum wells and quantum dots or the quality control technician in a wafer foundry can monitor photoluminescence decays to characterise the doping or impurity level present. The research assistant monitoring drug interactions in the pharmaceutical sector can study energy transfer mechanisms using fluorescence lifetimes as the indicator.

**Sample:** Hematoporphyrin IX in water at pH 7.2.

**Measurement Conditions:** Picosecond Diode Laser, rep.rate = 1MHz, MCP-PMT Detector, $\lambda_{ex} = 400$nm

Emission changes from single lifetime of 14.8ns at 620nm to a triple exponential decay of 0.8ns, 4.4ns and 14.8ns at 750nm

FL920 Fluorescence Lifetime Spectrometer
Time Correlated Single Photon Counting (TCSPC) is a digital counting technique, counting photons that are time correlated in relation to an excitation light pulse.

In TCSPC the sample is repetitively excited using a pulsed light source and the measurement builds a probability histogram relating 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 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.

**The Benefits of TCSPC**

- **Single Photon Sensitivity** - Time resolved measurements at the quantum limit
- **Time Domain Form** - Direct measurement and observation in real-time
- **Digital Counting** - Reducing background noise associated with detectors and signal processing electronics
- **Enhanced Temporal Resolution** - The short transit time spread of the detector determines the temporal resolution, in contrast to the overall detector pulse response width
- **Safe and Robust** - Insensitive against source intensity fluctuations and detector amplification noise
- **Noise Statistics** - The Poissonian characteristics of the signal noise gives rise to significant benefits for data deconvolution and fit evaluation. This is in contrast to the Gaussian noise associated with analogue techniques.
- **High Dynamic Range** - Due to the Poissonian nature of the noise, long and short lifetimes with large and small amplitudes can be reliably extracted from a single measurement.
Historically, cumbersome Nuclear Instrumentation Modules (NIM) rack mounted electronic units were the norm for TCSPC systems. With the consequent complex mass of interconnections and settings, measurements demanded a high level of user expertise and were often prone to RF interference.

State of the art plug and play PCI card technology has recently revolutionised the approach to TCSPC. All Edinburgh Instruments fluorescence lifetime spectrometers incorporate the TCC900 card. Here, only the optical trigger from the light source and the raw detector output need to be connected to the START and STOP inputs on the TCC900.

This card incorporates all the necessary electronic modules required for TCSPC, including:

- Constant Fraction Discriminator on both START and STOP inputs
- Variable nanosecond delay
- Time to Amplitude Converter with time ranges from 2.5ns to 50μs
- Up to 4096 time channels
- Minimum channel width 610fs
- Low dead time allowing measurements to be converted at MHz rates
- Differential non-linearity <1% peak to peak
- Timing jitter <25ps

Data Acquisition Speed

With the nF900 nanosecond flashlamp, operating under standard conditions, a typical high quality TCSPC measurement, with 10⁴ counts in the peak, can be acquired in under 10 minutes.

However, with the advent of mode locked and high repetition rate lasers, and the vastly improved data harvesting of the TCC900 card, high quality TCSPC data can be routinely and reliably collected in a matter of seconds.

Lifetime data can still be extracted from a relatively small number of counts collected in a matter of milli-seconds. Consequently, the method lends itself to single molecule studies, where the molecule is present only for a short time, or in routine multi-well plate assays.

In a TCSPC measurement, it is important to ensure that no more than one fluorescence photon is detected for each excitation pulse. The detection of more than one photon per pulse will affect the histogram statistics, skewing the distribution to shorter times, leading to erroneous measurement results. This is known as “the pulse pile-up problem”. In order to ensure that only one photon per light flash is detected, the photon STOP rate is kept low (usually 5% or lower) in comparison to the repetition rate of the excitation source.

With mode locked or high repetition rate lasers at repetition rates up to 100MHz, the upper limit of timing events becomes set at a few Mcps. Until recently electronics were not available to cope with this rate. The short dead time of the TCC900 card ensures that this high count rate can be fully utilised.

As a rule of thumb, the interval between successive excitation pulses should be more than 10 times the lifetime to be measured. Hence, the maximum lifetime which should be measured with a laser operating at 100MHz repetition rate is typically 1.0ns. To measure longer lifetimes, all that is required is that the repetition rate of the laser is reduced.
The range of lifetimes that can be measured by TCSPC extends from a few ps using short pulse, high repetition rate laser sources and micro-channel plate photomultiplier (MCP-PMT) detectors to 50µs, the practical lifetime limit for TCSPC measurements. The lifetime range of the FL920 can easily be extended to cover 12 orders of magnitude from picoseconds to seconds with the Multi-Channel Scaling upgrade.

Sample: Cryptocyanine in acetone (10⁻⁴M)
Measurement Conditions: Ti: Sapphire laser, mode locked, pulse picked, MCP-PMT detector, λ<sub>ex</sub> = 700nm, λ<sub>em</sub> = 730nm, Δλ<sub>em</sub> = 4nm
Fit Result: Single exponential: τ = 53.8ps

The nF900 nanosecond flashlamp, the standard excitation source for the FL920, has a broad spectral output from the VUV to 850nm, dependent on filler gas.
Picosecond semiconductor diode lasers, short pulse light emitting diodes (LEDs) and picosecond and femtosecond laser systems are becoming increasingly used for excitation in the visible and near infrared range. All can be easily integrated into the FL920 system.
A variety of single photon counting photomultipliers are available covering emission from the UV to infra-red, 185nm-1700nm. Technical details can be found on the specification pages.

Sample: Erythrosin B in water (5x10⁻⁵M)
Measurement Conditions: nF900, hydrogen filled, rep. rate = 40kHz, λ<sub>ex</sub> = 470nm, λ<sub>em</sub> = 570nm, Δλ<sub>ex</sub> = Δλ<sub>em</sub> = 18nm
Fit Result: Single exponential: τ = 81ps

Sample: [Cr(bpy)<sub>3</sub>(ClO<sub>4</sub>)<sub>3</sub>] in 0.1M sulphuric acid
Measurement Conditions: nF900, hydrogen filled, rep. rate=5kHz, λ<sub>ex</sub> = 360nm, λ<sub>em</sub> = 728nm, Δλ<sub>ex</sub> = Δλ<sub>em</sub> = 18nm
Fit Result: Single exponential: τ = 45.7µs

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Spectral Range

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Software Functionality

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

Control Features
- Wavelength selection for excitation and emission monochromators
- Sample selection (3 position)
- nF900 flashlamp voltage, frequency control and gas pressure monitor
- Programmed iris attenuator
- multiple sources
- Polariser selection and orientation
- Cryostat control
- Measurement to peak counts or preset time
- Forward or Reverse mode
- Selection of 512, 1024, 2048, 4096 channels for the full TAC range
- CFD settings on START and STOP: threshold, zero crossing and divider
- Time settings – TAC range from 2.5ns – 50µs, delay, offset

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
Decay curve analysis, the extraction of meaningful physical properties of the sample from the raw data, is of crucial importance for time-resolved luminescence measurements. The Edinburgh Instruments F900 reconvolution software package is reliable and well proven, setting the benchmark for precision and ease of use. Gone are the days of data analysis being a slow and laborious process. Using the F900 reconvolution software, standard lifetime analysis is complete in the blink of an eye. In the simplest case of a single fluorescence decay process, the fluorescence intensity, \( f(t) \), will follow an exponential dependence on time:

\[
f(t) = f(0) \exp\left(-\frac{t}{\tau}\right)
\]

where \( \tau \) is the characteristic fluorescence lifetime and \( f(0) \) is the fluorescence intensity at time zero.

More complex fluorescence decays can be represented by a sum of up to four exponential terms, and by a constant background, \( (A) \):

\[
f(t) = A + \sum_{i}^{d} B_i \exp\left(-\frac{t}{\tau_i}\right)
\]

In practice, instrumental processes affect the measured fluorescence decay. The theoretical fluorescence decay is superimposed by noise (Poisson noise), and the initial part of the decay can be broadened by the finite pulse width of the excitation source, the transit time spread of the detector, and other potential optical and electronic contributions. Taking the above effects into account, the actual measured time dependent fluorescence intensity becomes a convolution of the instrumental response function \( P(t) \) and the theoretical sample decay:

\[
F(t) = \int_{0}^{t} P(t - t') f(t') \, dt'
\]

The aim of the numerical analysis is to fit the function \( F(t) \) to the measured sample decay, thus extracting the theoretical fluorescence decay model \( f(t) \) by eliminating the effects of the instrumental response function and the effects of the noise. Typically, the shortest lifetimes, which can be recovered from the raw data using the reconvolution analysis, are between 0.1 – 0.2 times the width of the instrumental response function. The F900 software uses well-established, non-linear least squares reconvolution procedures based on the Marquardt-Levenberg algorithm. Up to 4 lifetimes can be fitted from up to 10000 data channels with iterative shift fit, background fit and parameter fixing options. The fitted decay curve, together with lifetime and amplitude values, weighted residuals, \( \chi^2 \) goodness of fit, autocorrelation function, Durban Watson parameter, etc. are all calculated, to allow an assessment of the quality of the fit.

The correct fit is found for three exponential decays with a \( \chi^2 \) value of 1.081, a clean autocorrelation function, more than 99% of all data points falling within 3 standard deviations and a Durban Watson parameter of 1.676.
Advanced Fluorescence Lifetime Data Analysis

For the advanced analysis of fluorescence and phosphorescence decay kinetics Edinburgh Instruments offer FAST (Fluorescence Analysis Software Technology). This software package sets new standards in precision, robustness and speed of fluorescence lifetime data recovery. FAST provides unsurpassed accuracy and all fits are literally 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.

Analysis Tools

- Individual multi-exponential fluorescence decay analysis
- Batch analysis of sets of time courses
- Global analysis of sets of decay data with wavelength dependence (TRES data), temporal dependence, concentration dependence, multi-titre plates, etc.

The user can have complete confidence in the quality and reproducibility of the analysed data as hundreds of real and simulated data have been used 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.

Global fluorescence lifetime analysis of a porphyrin in aqueous solution. The analysis was made with three linked lifetimes yielding to three distinct lifetime related spectra shown in the inset, total emission also shown in the inset for comparison – black)

Global Fluorescence Lifetime Data Analysis

Global Analysis is a powerful tool for the simultaneous analysis of a set of multi-exponential decay curves. During the simultaneous analysis one or more fit parameters may be linked, i.e. they remain free floating, but they are identical in all individual decay curves. The FAST Global Analysis is exceptionally fast, even with large datasets.

Fluorescence lifetime distribution analysis of three different quantum dot samples. The lifetime distributions of the three samples are distinctively different, although the raw lifetime data only show marginal differences.

Distribution of Lifetime Analysis

The easy-to-use Lifetime Distribution Analysis calculates lifetime distribution functions on up to 200 logarithmically spaced lifetime-intervals with the possibility of simultaneous evaluation of the shift and background parameters. The resulting lifetime distribution is free of any model, i.e. it is not pre-shaped by Gaussian or Lorentzian distributions.
**Measurement Examples**

### Single Exponential Decay Kinetics

Anthracene in cyclohexane is one of the most documented standards for fluorescence lifetime spectroscopy and is used as a standard test sample in the FL920 spectrometers. The decay is a single exponential with a lifetime of 5.1 ns.

Sample: Anthracene in cyclohexane (5 x 10^{-5} M), degassed  
Measurement Conditions: nF900, hydrogen filled,  
rep. rate = 40kHz, \( \lambda_{ex} = 358\text{nm}, \lambda_{em} = 400\text{nm}, \Delta \lambda_{ex} = \Delta \lambda_{em} = 16\text{nm} \)  
Fit Result: Single exponential: \( \tau = 5.1 \pm 0.1 \text{ ns} \)

### Multiple Exponential Decay Kinetics

The fluorescence decay of Hematoporphyrin IX in a phosphate buffer solution is a good example for a complex decay kinetic. The measured data at 720 nm shows poor quality fitting for a single or double exponential decay but is perfectly represented by a triple exponential decay.

Sample: Hematoporphyrin IX in a phosphate buffer (pH 7.2)  
Measurement Conditions: picosecond diode laser, \( \lambda_{ex} = 398\text{nm} \), rep. rate = 1 MHz, MCP-PMT detector, \( \lambda_{em} = 720\text{ nm} \)  
Fit Result: (relative fluorescence contribution in brackets)  
\( \tau_1 = 14.80 \pm 0.06 \text{ ns (69.69\%)} \)  
\( \tau_2 = 4.62 \pm 0.09 \text{ ns (27.11\%)} \)  
\( \tau_3 = 0.81 \pm 0.05 \text{ ns (3.20\%)} \)

### Monomer-Excimer Kinetics

Multi-exponential fluorescence kinetic studies, including both growth and decay of the fluorescence emission, are standard measurements with the FL920 spectrometer. The monomer-excimer kinetics of pyrene clearly illustrates these different forms. The monomer emission decay displays two exponential terms whereas the excimer emission is characterised by an exponential growth followed by a single exponential decay.

The intrinsic lifetimes of the two measurements are not independent and Global Analysis with linked lifetimes is the preferred method of analysis for this type of model.

Sample: Pyrene in cyclohexane (10^{-2} M)  
Measurement Conditions: nF900, hydrogen filled,  
rep. rate = 40kHz, \( \lambda_{ex} = 335\text{nm}, \lambda_{em} = 395\text{nm} \) for monomer emission, \( \lambda_{ex} = 465\text{nm} \) 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: \( \tau_1 = 9.3\text{ns}, \tau_2 = 15.4\text{ns} \)
Time Resolved Single Photon Counting in the NIR Spectral Range

Measurements on an InGaAs/InP quantum well structure illustrate high temporal resolution when the technique of time correlated single photon counting is used in the near infra-red spectral range. The quantum well emission (red) is characterised by a simpler two exponential decay compared to the substrate (green).

**Sample:** InGaAs/InP quantum well  
**Measurement Conditions:** picosecond diode laser, $\lambda_{ex} = 760\text{nm}$ (blue curve), rep. rate = 1.0MHz, NIR-PMT detector, $\lambda_{em1} = 920\text{nm}$ (green curve), $\lambda_{em2} = 1330\text{nm}$ (red curve), $\Delta \lambda_{ex} = \Delta \lambda_{em} = 5\text{nm}$.

Time Resolved Fluorescence Anisotropy Measurements

Time resolved polarisation measurements reveal the rotation rates of the emitting molecules and have many applications in structure determination, membrane fluidity, polymer dynamics and protein engineering. The example shows a study on a water soluble polymer, labelled with dansyl chromophores located at a given distance from the polymer backbone that is defined by the intervening spacer unit and the environmental pH. The figure below left demonstrates a polarised fluorescence decay experiment with the instrument profile and the G-factor corrected measurement with parallel and crossed polarisers.

**Sample:** Water soluble polymer, labelled with dansyl chromophores, in aqueous solution  
**Measurement Conditions:** nF900, hydrogen filled, rep. rate = 40kHz, $\lambda_{ex} = 330\text{nm}$, $\lambda_{em} = 540\text{nm}$, $\Delta \lambda_{ex} = \Delta \lambda_{em} = 15\text{nm}$, G-factor = 1.413

The figures in the centre and on the right show the raw data and fitted curves for four different fluorescence anisotropy measurements of a polymer sample at different pH. For low pH the dansyl labels are locked to the polymer backbone and the individual rotation of the chromophore is restricted, resulting in a slow rotational diffusion time. For high pH the dye is quasi-free, resulting in a faster rotation. In all cases a residual anisotropy $r_\infty$ can be observed, caused by the quasi-rigid polymer.

**Sample:** Hematoporphyrin in dry ethanol ($5.3 \times 10^{-5}\text{M}$)  
**Measurement Conditions:** Nd:YAG laser, $\lambda_{ex} = 355\text{nm}$, rep. rate = 10Hz, NIR-PMT detector, multi-channel scaling mode, $1200\text{nm} \leq \lambda_{em} \leq 1350\text{nm}$, $\Delta \lambda_{em} = 2\text{nm}$, step size 2nm

The measurement of the time resolved emission of singlet oxygen at about 1270nm is very attractive as it reveals important knowledge on the activity of the excited oxygen species, its environmental properties, and on the photosensitive molecules which generate the singlet oxygen. The measurement below shows the high accuracy with which the extremely weak singlet oxygen emission can be measured by single photon counting. The single photon counting technique is also opening up the chance to measure time resolved singlet oxygen emission in the nanosecond time scale, which is not accessible with analogue detectors.
**Measurement Examples**

**pH Dependant Fluorescence Lifetime Measurements**

Tryptophan is one of the most important amino-acids in biological fluorescence spectroscopy. The fluorescence is sensitive to the environment, thus allowing the study of the protein structure, dynamics and function in its vicinity.

Lifetime measurements were made on a 50µM solution of tryptophan as a function of pH.

The measured decay curves were analysed using Global Analysis. The overall behaviour is well described by three decay components of varying relative amplitude.

**Sample:** Tryptophan (50µM)

**Measurement Conditions:** nF900, hydrogen filled, rep. rate = 40kHz, 
\(\lambda_{ex} = 290\text{nm}\), \(\lambda_{em} = 330\text{nm}\),

(Above Right) fluorescence decay curves measured as a function of pH, global fit results

(Right) Plot of the relative fluorescence contribution of the individual lifetimes versus pH

**Time Resolved Emission Spectroscopy (TRES)**

TRES is a very powerful tool in fluorescence lifetime studies. A family of decay curves is automatically measured as a function of an experimental variable (for example excitation or emission wavelength).

The TRES measurement shown is of Norharman in methanol. In this protic solvent the fluorescence decay is complex. The fluorescence originates from the neutral form of Norharman at short wavelengths, the cation at intermediate wavelengths, and the zwitterion form in the long spectral range.

**Sample:** Norharman in methanol (5×10^{-5}M)

**Measurement Conditions:** nF900, hydrogen filled, rep. rate = 40kHz,  
\(\lambda_{ex} = 285\text{nm}, \lambda_{em} = 600\text{nm}\), \(\Delta \lambda_{ex} = 20\text{nm}\), \(\Delta \lambda_{em} = 5\text{nm}\), step size = 5nm,

(Above Left) Raw data

(Left) Fit results (global analysis), with three lifetimes of 2.27ns, 5.02ns, and 18.0ns

(Above) Contour plot with cross hair and corresponding emission spectrum and decay profile
The FLSP920 combined steady state and fluorescence lifetime spectrometer can be coupled to a fluorescence microscope by means of optical fibres. With this set-up measurements can be made with spectral, temporal and spatial resolution.

Special Applications

Fluorescence Microscopy

- Inverted microscope (with optional pillar and condenser for trans-illumination)
- Fluorescence microscopy using epi-illumination
- Fluorescence imaging using epi-illumination and CCD camera
- Spectral scanning of the wide-field (using wide-field epi-illumination) or with spatial resolution (using lasers for excitation)
- Fluorescence or phosphorescence time resolved measurements of the wide-field or with spatial resolution

VUV Spectroscopy

When VUV excitation is required (down to 115nm) the FLSP920 spectrometer can be upgraded with a complete VUV excitation arm that includes the VUV light source(s), VUV excitation monochromator and VUV coupling optics.

- steady state deuterium light source for spectral measurements between 115nm and 400nm
- pulsed deuterium light source for lifetime measurements in the microsecond to second time scale using multi-channel scaling
- nanosecond flashlamp for lifetime measurements in the nanosecond time scale using time correlated single photon counting
- VUV monochromator with triple grating turret and up to two entrance slits
- all optical components and flanges in excitation channel evacuated
- Nitrogen purging of the immediate surrounding of the sample

X-Ray Fluorescence Spectroscopy

Edinburgh Instruments can offer an X-ray source as an alternate excitation source for use together with the FLSP920 spectrometer.

The unit comprises the X-ray source and power supply all contained within a safety chamber, featuring sample exchange facility. The X-ray excited light emitted by the sample is focused with fluorescence collection optics to a fibre optic bundle and then delivered to the FLSP920 sample chamber. The FLSP920 spectrometer can measure the X-ray excited spectra by using the standard emission scan option.

- 30kV, 2mA tungsten target X-ray excitation source for the generation of X-ray excited spectra in the FLS290 spectrometers
- safety chamber with sample exchange facility for routine sample exchange
- picosecond pulsed X-ray source available for time-resolved studies
Additional Fluorescence Spectrometer Products

NanoTaurus
Fluorescence Lifetime Plate Reader

mini-Tau
Ultra-compact Fluorescence Lifetime Spectrometer

LifeSpec
Picosecond Lifetime Spectrometers

OB920
Fluorescence and Phosphorescence Lifetime Spectrometer