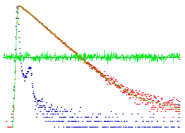


Configuring a time-resolved system

The configuration of a fluorescence lifetime system is, in the main, determined by the lifetime and wavelength of the sample to be measured. This strongly influences the choice of the pulsed excitation source and the detector. The choice of timing electronics is also important, as it affects the lifetime resolution and the measurement time duration. The dead time of the electronics is particularly influential for efficient data acquisition. Finally the selection of optical platform depends on if the sample is cuvette based or requires spatial resolution using a microscope. Time-resolved capability can even be added to steady state spectrometers to form hybrid systems. However, this note will only concentrate on dedicated time-resolved systems.

System components

The main constituents of a time-resolved system are illustrated in Fig. 1. These will be briefly considered.

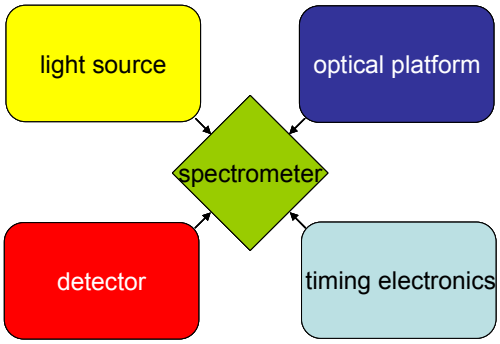


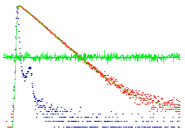
Fig. 1. Component parts of a time-resolved spectrometer

- **Light sources**

The principal excitation sources at present are solid state lasers and LEDS. These can range in wavelength from the UV (~250nm) to the NIR (~1310nm). Generally lasers have higher peak powers and produce narrower optical pulses. These sources emit at a single wavelength with a narrow spectral width. Thus, a complete spectral coverage requires the use of several sources. Broadband coverage is possible by the use of a coaxial flashlamp or a supercontinuum laser. The former is limited in repetition rate (tens of kHz), while the latter runs at a high repetition rate (80MHz), which is reduced using a pulse picker. It is also possible to use Ti-sapphire lasers, which are required for two-photon excitation measurements, but use of a pulse picker is advisable, and frequency doubling or trebling may be required. It is the optical pulse width (FWHM) that determines the shortest lifetime that can be measured, with the shortest coming from a femtosecond laser. Long pulsed LEDs are available for longer (phosphorescence) timescales. For a majority of fluorescence applications solid state lasers and LEDs are more than sufficient.

- **Detectors**

The transit time spread (TTS) of a detector affects the shortest lifetime that can be measured. Detectors may require separate amplifiers and discriminators or these can be built into one package. The state of the art TCSPC detector is a microchannel plate photomultiplier (MCP), which is recommended for the measurement of very short-lived fluorescence (ps range). Although these relatively expensive detectors are very sensitive, they are limited in the count rate that can be applied to them. Also, their count rate response is not linear and they should not be considered general purpose detectors. They are usually not recommended for steady state or phosphorescence measurements, where there is a likelihood of exposure to high light levels exists or a linear response is needed. Some types of photodiodes (SPADs) also exhibit a low TTS, but their small active area limits their practical application and their response can be strongly wavelength dependent. Picosecond detection modules, combining a fast response detector along with HV supply, amplifier and discriminator, give a good TTS (typically 150ps) and are robust enough for most applications. Some side window photomultipliers can also be used; they are robust and provide a lower cost option, but generally exhibit higher dark noise and a broader TTS. The latter limits the shortest lifetime that can be measured.



Standard detectors can have a wavelength sensitivity up to ~900nm. It is possible to measure time-resolved decays up to 1700nm by the use of more specialised detectors, however, this wavelength sensitivity comes at the cost of high dark count rates. This can be problematic as these counts contribute to the "stop signal", which affects the range of lifetimes that can be measured.

- **Timing electronics**

These can either be "stand alone" modules or cards inserted into a computer. It should be noted that as a computer is an electronically noisy environment, it is advisable to process any electronic signal prior to arrival to any inserted card. The rapid evolution of computers can threaten to make some card formats obsolete. Factors to consider with timing electronics are; time-resolution per point, overall time range and dead time. To measure short-lived species a small time per point is necessary in order to have sufficient points to fit the data. A low dead time enables efficient conversion of photons from the sample to data points in the acquired histogram. This is important when higher count rates are used. The overall time range should be considered if measurements are needed on both fluorescence and phosphorescence timescales. Some modules will cover a large range while others may require additional cards.

- **Optical platform**

The first aspect to consider is if spatial information requiring the use of a microscope needs to be obtained. If a sample chamber is selected, then thought needs to be given to the means of wavelength selection, see Fig. 2. If a broadband excitation source is used then an excitation monochromator may be required. Typically solid state sources do not require any additional wavelength selectivity so are usually attached directly to the excitation arm of the sample chamber. The emission wavelength can either be selected by filters or a monochromator.

If lasers or anisotropy measurements are envisaged then the addition of polarisers on both excitation and emission is required.

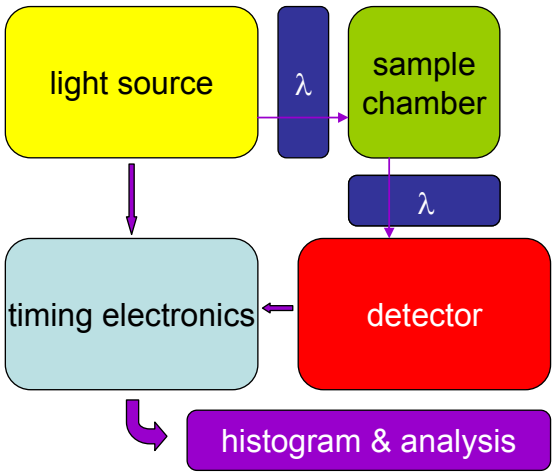


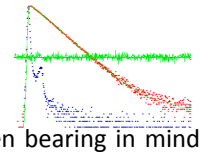
Fig. 2. Block diagram of a typical time-resolved system

System performance

The overall performance of a system is limited by its weakest component. Having a very "fast" detector with a "slow" light source or vice-versa will not allow for the measurement of short-lived species. The performance of each part should be considered and matched in relation to the range of samples to be measured. Generally, the shortest lifetime that can be determined via reconvolution analysis is 10 % of the instrumental response. This can be estimated using the following equation,

$$\Delta t_m \approx \left[\Delta t_{exc}^2 + \Delta t_{\det}^2 + \Delta t_{elect}^2 + \sum_i \Delta t_i^2 \right]^{1/2}$$

Where, Δt_m is the measured response, the subscripts *exc*, *det* and *elect* refer to the excitation source, detector and timing electronics respectively. Other factors (subscript *i*) can also be present, but generally uncertainty in the measured response is dominated by that of the source and detector.



Choice of *HORIBA Scientific* system

When configuring a system the two following questions should be kept in mind;

- *What lifetimes do I wish to measure?*
- *What wavelength range do I need?*

Following are some general guidelines to help with the initial step, concentrating on the choice of light source and detector. Fig. 3 charts currently available component blocks that comprise a system.

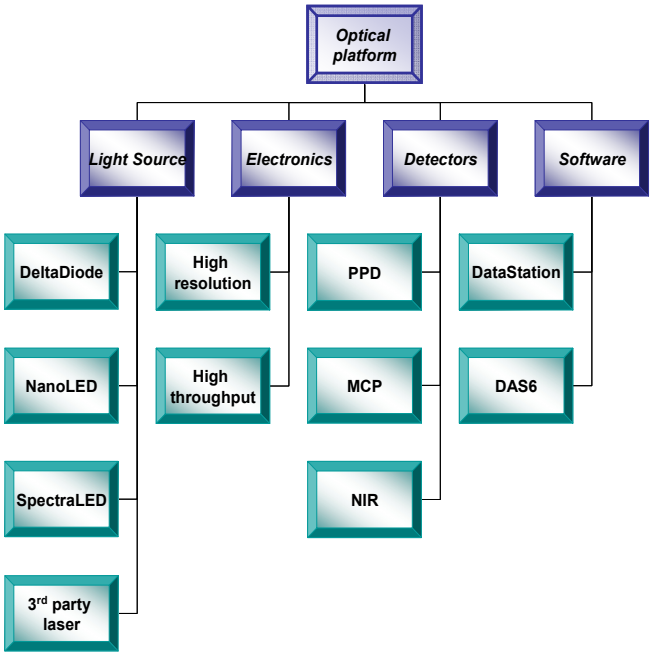


Fig. 3. Chart of TCSPC system components

Concerning the individual components and system range, updated specifications can be found on the website at;

www.horiba.com/scientific/products/fluorescence-spectroscopy/lifetime/

www.deltatcspc.com

www.picocomponents.com

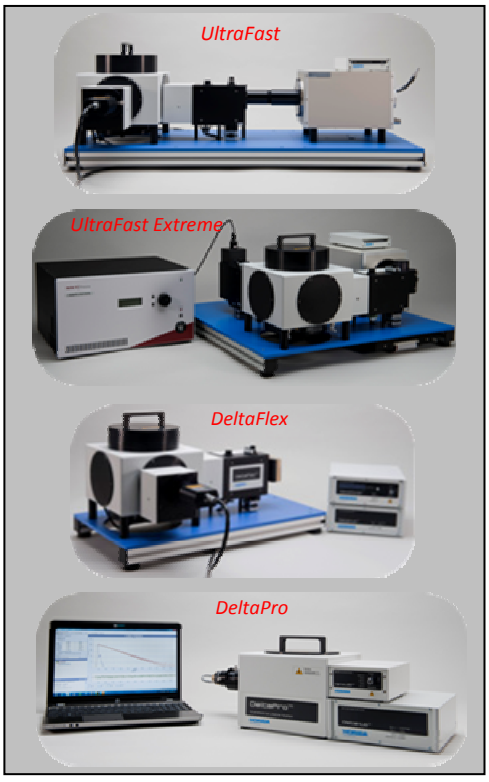
Considering a non microscope system, then bearing in mind the two fundamental questions, the choice of system can be guided by the chart on the following page. This uses minimum lifetime and maximum wavelength as the guiding principles. However, this should be just treated as a rough guide, as the versatility and flexibility of these systems means that other combinations of components are possible and it should be pointed out that systems and components are continuously being developed.

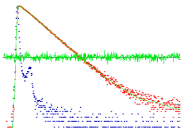
Also the minimum lifetime that can be measured will to some degree depend on the sample. Criteria can change depending on the complexity of the decay kinetics and the quantum yield.

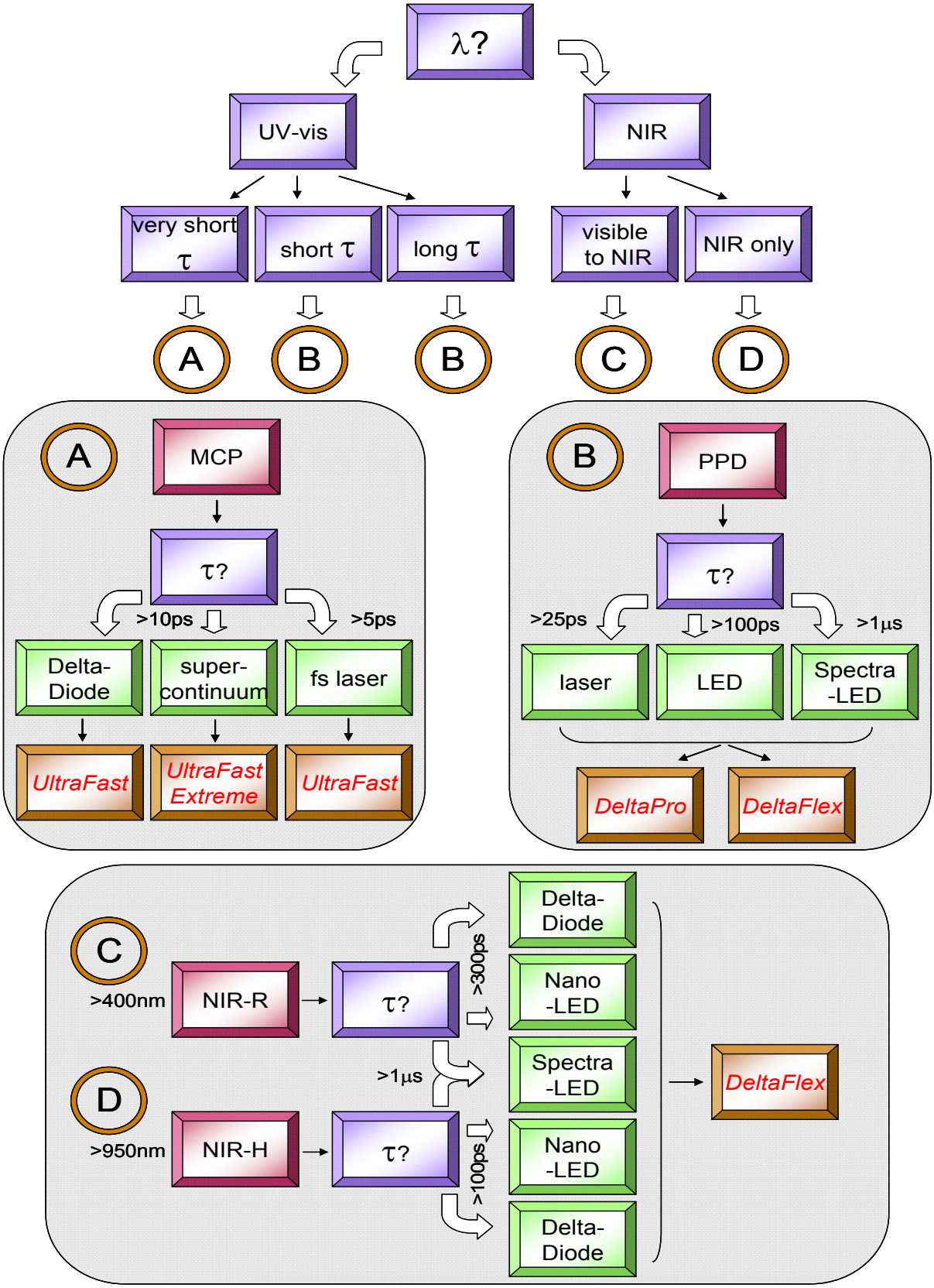
For many applications requiring the ultimate in time-resolution the *FluoroCube UltraFast* system is recommended. However for a majority of uses either *DeltaPro* or *DeltaFlex* system can be employed, which are advantageous for phosphorescence and high throughput measurements involving *DeltaDiode*s.

The lifetime values given are for rough guidance only and the lifetime is not the only determining factor in the choice of system.

All specifications and appearances are liable to change without warning, please contact your agent for details.







JOBIN YVON Technology

HORIBA Scientific

info.sci@horiba.com www.horiba.com/scientific

USA: +1 732 494 8660
UK: +44 (0)20 8204 8142
China: +86 (0)10 8567 9966
France: +33 (0)1 64 54 13 00
Italy: +39 2 5760 3050
Brazil: +55 (0)11 5545 1514
Germany: +49 (0)89 4623 17-0
Japan: +81 (0)3 6206 4717
Other: +33 (0)1 64 54 13 00