

# Technical Report

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## Fluorescence Lifetime Plate Reader for Life Science and Biotechnological Applications based on the Technique of Time Correlated Single Photon Counting

### 1 The New NanoTaurus Plate Reader

Edinburgh Instruments has launched a new product for Life Science and Biotechnological industry – a Fluorescence Lifetime Plate Reader called the NanoTaurus. The instrument of relatively small size has very high sensitivity, versatility and performs with the possibility to conduct multi-parametric experiments in homogeneous biological sample formats under computer control. These properties make this instrument a promising universal bench-top fluorescence laboratory for biotechnological and scientific applications.



## 2 Why Use Fluorescence in Life Science and Biotechnology?

One of the most powerful tools for molecular and cellular biology as well as for drug discovery is the use of receptor-ligand affinity screening. Early work involved the use of radioactive ligands to identify a binding event; however, there are numerous limitations involved in the use of radioactivity for high throughput screening. These limitations have led to the creation of highly sensitive, non-radioactive alternatives. For the investigation of receptor-ligand interactions, fluorescence detection is the most powerful one. In many fields it has successfully replaced radioactive isotope labelling. Fluorescent dyes have an "environmental advantage": they have a longer shelf life, are inexpensive to discard and safer to handle.

There are two major advantages of fluorescence. One is the potential to conduct parallel measurements by using different colored dyes or monitor different fluorescence characteristics, and the other, the potential to perform measurements in homogeneous biological sample format allowing time resolved continuous data acquisition.

Fluorescence is characterized by the absorption and emission spectra. The absorption and emission peaks are shifted by 15 to 100 nm (Stokes-Shift). The efficiency of this process is described by the quantum yield. A "good" fluorophore is characterized by strong absorption (a high extinction coefficient), high quantum yield and a large Stokes-Shift. In contrast to spontaneous emission of radioisotopes, fluorescence detection requires employment of excitation light sources. The light source has to harmonize with the fluorophores used. It should radiate near the absorption peak. Since the emitted fluorescence has a certain bandwidth, the different fluorophores should be separated by 15-50 nm to be detectable concomitantly. Depending on the instrument configuration two to four fluorophores can be detected simultaneously.

An important fluorescence property is the lifetime. This parameter represents a rate of fluorescence emission decay after short pulse excitation. It depends on the molecular structure and on the molecular environment, and together with the absorption and emission spectra can be used as molecular "signature". Within certain limitations the lifetime is independent of sample conditions such as concentration, volume, excitation and emission wavelengths, and can therefore be considered as a "self referenced" characteristic. The differing fluorescent lifetimes of diverse dyes with similar wavelengths could be used to analyze various probes simultaneously.

Emission of some fluorophores is influenced by solvent polarity, temperature, pH, viscosity or neighboring molecules, particularly by other fluorophores. Neighboring fluorophores may transfer energy to each other, through a phenomenon known as Fluorescence-Resonance-Energy-Transfer (FRET). By means of this feature, molecular sensors can be developed, which provide information on the presence or configuration of molecules. This allows continuous observation and analysis of a reaction avoiding tedious washing-off or separation of reaction components, as in all other conventional assays that rely on binding. Examples for this type of "conventional" method are: Northern- and Southern-Blot, reverse blot techniques, PCR-ELISA on micro titer plates or membranes, cDNA arrays or DNA-chips and their detection with radioactive labeled markers or haptens and enzyme-conjugated antibodies.

### 3 What is the Advantage of Time-Resolved Fluorescence Measurements?

Any of the above mentioned fluorescence parameters can be employed for the design of fluorescence-based assays. However, due to the variety of processes affecting fluorescence probe emission, it is sometimes difficult to attribute observable changes to specific mechanisms. For example, changes in steady-state anisotropy, a fluorescence characteristic that is often used in fluorescence-based applications, could result from changes in the fluorescence lifetime or due to changes in the solvent viscosity. In another FRET-based method, used for study ligand – receptor interactions, changes in the acceptor's fluorescence intensity could be either due to a shortening of the fluorescence lifetime (dynamic quenching) which indicates proximity of the donor and acceptor molecule or a static quenching of its emission. By combining lifetime and steady-state measurements one can unambiguously interpret experimental results and accurately evaluate experimental parameters. In addition, time-resolved measurements can significantly increase the number of fluorescence-based applications and assays, and discriminate unwanted fluorescence or scattering emissions always present in biological experiments.

### 4 How Does the New Plate Reader Work?

Short pulse laser excitation employed in the plate reader allows monitoring of fluorescence emission decay in the pico- or nanosecond time domain by using the Time Correlated Single Photon Counting (TCSPC) method. Due to the sensitivity of TCSPC and the high repetition frequency of the laser pulses, an acquisition time required for readout of one well is as short as 0.5 s for 100 pM fluorescein solution in PBS. The lifetime evaluation software processes experimental data in real time. The lifetime resolution allows efficient discrimination of background fluorescence and scattering. It also allows studying fluorescence probes rotational diffusion or lifetime resolving of photochemical processes initiated by the laser excitation. Importantly, due to using different excitation and emission wavelengths and the fluorescence lifetime analysis one can simultaneously measure several emission parameters of several fluorophores as e.g. fluorescence intensities, lifetimes and anisotropies. In addition, an original design of the plate reader provides high spatial selectivity such that fluorescence of cells lying on bottoms of the multi-well plate can be distinguished from the bulk fluorescence. Because of that the plate reader allows performing fluorescence assays in homogeneous format when experimental protocol does not require additional separating steps such as washing or centrifugations.

Simultaneous monitoring of different experimental parameters, the spatial selectivity and the homogeneous format provide a unique possibility to obtain comprehensive experimental data from one experiment. Such a multi-parametric experimental design is necessary when experimental samples are available in limited amounts or experiments cannot be repeated several times. In addition, monitoring of different parameters in a course of the same experiment increases the confidence and reliability of the experimental data and significantly reduces time required for studies. All these features of the plate reader provide significantly advanced experimental facilities for scientific and biotechnological applications.