

Validation of Fluorescence Lifetime Apparatus

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Abstract

During the past 20 years there has been a rapid growth in the use of fluorescence spectroscopy. Key applications include medical imaging, healthcare and more recently nanotechnology. Whilst steady-state (time-integrated) fluorescence measurements have been used to characterise diverse samples in terms of emission strength, peak wavelength and spectral shape, limitations to their effectiveness include reduced discrimination of spectrally overlapping fluorophores due to their relatively broad emission bands.

Time-dependent measurements provide additional information about the underlying fluorescence dynamics and have the advantages of enhanced discrimination among fluorophores, especially for those with overlapping emission spectra. Fluorescence lifetime measurements are also sensitive to various environmental parameters such as pH, temperature and solvation. Another major advantage of lifetime-based sensing is the fact that fluorescence lifetime is independent of signal strength.

Recently instrumentation costs have decreased dramatically. This, combined with the size and complexity of operation, has opened fluorescence lifetime measurements to a wider range of applications and expertise.

The well-established time-domain technique of time-correlated single-photon counting (TCSPC) can resolve picosecond lifetime with high sensitivity and dynamic range. In the work reported here a commercial instrument is characterised and calibrated. The performance of the apparatus was then validated using measurements of fluorescence lifetime standards and the data analysis tested with simulated data. Further tests were performed on important molecular probes, including quantum dots and Enhanced Green Fluorescent Proteins (EGFP).

Non-linearity of the time measurement is the most important source of errors in TCSPC measurements. Traditionally timing accuracy, stability and linearity is tested using pulse generators or electrically calibrated delays. Here we develop a novel method based on the optical delay of a laser pulse. The linearity of the TCSPC system and PMT detector can also affect the measurement of the fluorescence lifetime and these were investigated using superposed laser pulses.

Using this system it will then be possible to make traceable measurements of candidate fluorescence lifetime standards, which in turn can be used to validate measurement systems in diverse fields such as clinical sensing and diagnostics and fluorescence lifetime imaging.

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