

Preface

Time-correlated single photon counting (TCSPC) was born 54 years ago, in 1960, when Bollinger and Thomas published a paper on the ‘Measurement of Time Dependence of Scintillation Intensity by a Delayed Coincidence Method’. Desmond O’Oonnor and David Phillips published their book, *Time-Correlated Single Photon Counting*, 31 years ago. *Advanced Time-Correlated Single Photon Counting Techniques* appeared 10 years ago.

Can a measurement technique survive for more than half a century? Yes, if it is constantly developing. And there has been a lot of development in TCSPC: The electronics have become orders of magnitude faster, and high-repetition-rate lasers and reversed start-stop have resulted in dramatically shorter acquisition times. Faster detectors are now available and fast excitation sources are more affordable.

A big step forward was the introduction of multidimensional TCSPC in 1993. Optical signals could now be observed not only as a function of the time after an excitation pulse but simultaneously as multidimensional functions of wavelength, polarization, experiment time, spatial parameters, or any other physical quantity describing the momentary state of the measurement object. This paved the way for another breakthrough, the combination of TCSPC with microscopy. Fluorescence lifetime measurements could now be performed in biological tissue, in single cells, and even in single biomolecules. The fluorescence lifetime, formerly considered simply the time a molecule stays in the excited state and possibly undergoes photo-induced reactions, became an indicator of the molecular environment of the molecule.

Techniques based on FRET and other conformation-dependent effects have been developed to investigate the function of biological systems. The combination of spatial resolution on the microscopic and submicroscopic scale with molecular information is one of the big advances of the past several decades, recognized by the award of the 2014 Nobel prize to Eric Betzig, Stefan W. Hell, and William E. Moerner.

Microscopy has created another level of multidimensionality. Histograms of fluorescence parameters can be built up over a large number of pixels in a fluorescence lifetime image or over a large number of molecules that have diffused

through a femtoliter observation volume. These data reveal subpopulations of biomolecules, subpopulations of molecules of different conformations, and transitions between different conformations.

High sensitivity, high time resolution, and the ability of TCSPC to obtain multiple biomedically relevant parameters from a single measurement have led to a variety of clinical applications. These range from fluorescence lifetime measurements on the spatial scale of single cells, to imaging of millimeter- and centimeter-size areas, to diffuse optical imaging techniques that reveal biochemical information from deep inside living tissue.

The complexity of these applications has, however, also created a problem: Understanding advanced TCSPC requires complex, almost magical, thinking, and a solid understanding not only of the TCSPC technique but also of modern microscopy techniques and other optical methods. There is a similar situation in J.K. Rowling's Harry Potter stories (I recommend them as supplementary literature): There are people who can do magic (witches and wizards) and normal people (Muggles) who cannot understand or even see magic when it happens. But there is hope: Hermione Granger was Muggle-born and became the best of her year at Hogwarts.

This book should be considered a continuation of W. Becker, *Advanced Time-Correlated Single Photon Counting Techniques*, Springer (2005). It is an attempt to spread the ideas of advanced time-resolved optical techniques more widely in the scientific community. It contains both chapters about the basics of multidimensional TCSPC and about applications in biology and medicine. The chapters are written by an originator of the technique and by successful users. Our hope is that this combination helps potential users better understand the technique, its various implementations, and encourages its adoption in their own research.

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