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Aims and Scope

Fluorescence spectroscopy, fluorescence imaging and fluorescent probes are indispensible tools in numerous fields of modern medicine and science, including molecular biology, biophysics, biochemistry, clinical diagnosis and analytical and environmental chemistry. Applications stretch from spectroscopy and sensor technology to microscopy and imaging, to single molecule detection, to the development of novel fluorescent probes, and to proteomics and genomics. The *Springer Series on Fluorescence* aims at publishing state-of-the-art articles that can serve as invaluable tools for both practitioners and researchers being active in this highly interdisciplinary field. The carefully edited collection of papers in each volume will give continuous inspiration for new research and will point to exciting new trends.

Preface

Get her excited red or green;
let it depend on what she's keen.
Quite soon quite radiant she will be seen;
in colours true of her she'll gleam,
but mind the nanoseconds in between.
For they might tell which way to lean:
perhaps she's other than you had foreseen.
Although, be patient when you try this scheme;
a single flash that lights the scene
is not enough to get her theme.
In fact, her moods are so extreme
that truly random she might seem.
And yet, if patiently you sift her stream
a pattern of true beauty can be seen.
So sum your count and be serene.

In 1926 the physicist Frithiof Wolfers and the chemist Gilbert N. Lewis coined the name photon for the quantum of light discovered about 20 years earlier. Even if it may look a little superficial at first glance, let us note the involvement of chemists here and elsewhere in the evolution of quantum physics. Indeed, the overwhelming success of quantum mechanics as a modern scientific theory is rooted not so much in pure physics but in its inescapably convincing explanatory power for virtually all aspects of physical chemistry and material science. Modelling atoms and molecules as quantum mechanical systems undergoing transitions between quantum states, some of them involving photon absorption and emission, was the key to understanding and eventually even exploiting virtually all previously mysterious spectroscopic effects. In this sense it is not a surprise that the methods addressed in this volume are now used more often in chemistry and related fields than in pure physics. In fact, spectroscopic methods have become indispensible in biochemistry because light as a probe, suitably applied, can be used in living cells without any damage to the specimen and without unduly spoiling the functions or processes

under investigation. Even though classic spectroscopy does not require working with single photons, it turns out very useful that it *can* be done so. This is the case when there are only very few molecules involved in the processes of interest, in particular in the vital processes explored by molecular biology. Even more interesting than classic spectroscopy is the scenario where yet another quantum mechanical property is used: the lifetime of the excited state. It turns out that the average time a molecule spends in this state is so specific to that molecule and its environment that it can be used as a fingerprint of the molecule in addition to its spectrum and/or as a probe for certain environment parameters. Even though the lifetime of an individual excited state is completely unpredictable according to quantum mechanics, the average lifetime is both measurable and meaningful. In practice it can be observed as the duration of luminescence from an ensemble of molecules excited by a short flash of light. Going by the observed phenomenon one speaks of fluorescence or phosphorescence lifetime measurements. In that case the necessary averaging of the individual excited state lifetimes is achieved implicitly because of the simultaneous observation of photons from the entire ensemble. The other interesting scenario is that of averaging the excited state lifetime across multiple cycles of excitation and photon emission observed on just one molecule. Indeed, by virtue of ergodicity, this kind of measurement gives the same results as the ensemble measurement. In this case time-correlated single photon counting (TCSPC) is the method of choice. It allows luminescence lifetime measurements on single molecules and other isolated quantum systems. Combining spectral information and, e.g., fluorescence lifetime one can use this refined "finger print" of the molecules of interest to identify them even in the presence of significant background. It was largely this idea, combined with confocal detection, which finally led to the incredible achievements in single molecule detection, single molecule spectroscopy, and even microscopy by means of TCSPC. Powerful methods such as the exploitation of Förster resonant energy transfer (FRET) as a molecular ruler became routine tools for the investigation of protein folding and interaction when they were made accessible for single molecules along this way.

However, TCSPC is not only useful with single molecules. As we will show in the first chapters of the present volume, it also helps to achieve better time resolution with typical detectors, even in ensemble measurements. These technology related chapters will cover the state of the art of current hardware, hopefully also convincing the reader that despite the inherent statistical nature of the measurement, modern users can rely on incredibly fast instruments and must not be as patient as our little old-fashioned opening poem might suggest. Indeed it is mostly the memory of "oldfashioned" instrumentation that left the impression of slowness attached to TCSPC. This may be illustrated by personal memories held probably not only by the editors: "I recall with nostalgia the long nights spent alone in a dimmed basement lab waiting for collection of at least 1000 counts in the peak by our beautiful TCSPC monster powered by a 45 kHz flashlamp." This is the past. Meanwhile things have changed dramatically, not only because of faster TCSPC electronics but very much also because of faster (and easier to use) lasers, to which a chapter in its own will be dedicated. The remainder of the chapters are covering a

rich eclectic mixture of application topics as well as methodology in experiment and data analysis. Despite of the importance of life science applications we tried to embrace a much wider scope, including, e.g., defect centers in diamond as single photon sources and quantum sensors, as well as optical tomography and super resolution microscopy. Similarly, on the methodology and instrumentation side, we aimed to show the interesting new options arising from the combination of apparently distinct methods such as classic TCSPC and fluorescence lifetime with methods based on intensity fluctuation. Together with the authors, to whom we express our gratitude here, we hope to provide a volume of both an immediate value as a current overview of the field and some longer term value as a collection of reference texts.

Prague, Czech Republic — Peter Kapusta
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Contents

Modern TCSPC Electronics: Principles and Acquisition Modes

Michael Wahl

Abstract Time-correlated single-photon counting (TCSPC) is an extraordinarily versatile and sensitive technique. While it was initially used almost only to measure excited state lifetimes, it can today be used much more flexibly, embracing and combining experimental methods that in the past required separate instrumentation. This has become possible by time-tagged event recording and modern time measurement circuitry. This chapter shows how such technologies operate with regard to electronics, data processing, and applications. Some implementation details will be exemplified by state-of-the-art TCSPC instruments and a recent software package for TCSPC data acquisition and analysis.

Keywords Coincidence correlation · Picosecond timing · Single-photon counting · TCSPC · TDC · Time tagging

Contents

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