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Introduction

Quantitative fluorescence microscopy

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In 2008, the first international Theodor Förster lecture series took place at the University of Cambridge http://laser.ceb.cam.ac.uk/foerster. Throughout the year, leading researchers were invited from all over the world to hold talks and engage with the life science community at Cambridge. The focus was on developments in quantitative optical microscopy techniques, which are revolutionizing research in the biological sciences today.

The name of the series commemorates the brilliant contributions of Theodor Förster, who, 60 years ago, published his seminal paper on the quantitative theory of electronic energy transfer (Förster 1948). The process, now known as Förster resonance energy transfer (FRET), takes place frequently in nature and refers to the non-radiative transport of energy from a donor to an acceptor molecule. Förster recognized that through a sequence of such interactions, energy can 'migrate' over distances much larger than the molecular scale and in one sweep he had resolved a long-standing puzzle in biology, relating to the extraordinary photoconversion efficiency observed during photosynthesis. Förster's discoveries in the field of excited-state photochemistry had an enormous impact in chemistry, physics and biology already during his lifetime. However, little could Förster have foreseen how his theory on energy transfer would continue to influence science to this day. The literature abounds with thousands of references to FRET. In the life sciences in particular, we have seen a surge of activities around FRET over the past 10 years, where the technique has become an essential tool to study the association and interactions between biological macromolecules. The true power of the technique has only just begun to be unleashed through advances in optical technologies and molecular biology tools for labelling molecules *in situ* in living cells. In 2008, the Nobel Prize in Chemistry was awarded for the discovery and development of fluorescent proteins. Their capability to act as efficient FRET reporters is among their most important properties and Förster's name will continue to be associated with one of the key tools in biophysical research.

The Förster lecture series is a celebration of the ingenious advances that have been made in the optical and biological sciences; at their interface lies one of the richest and most dynamic research fields of current time. This issue contains contributions from many of the speakers at the series and others, and presents recent developments in microscopy with applications at the life science interface.

A fascinating range of topics are covered. The study of the localization, movement, association and conformation of macromolecular complexes within living cells is key to the understanding of all biological processes. Hellriegel & Gratton (2009) describe a novel method for tracking individual particles, for example protein aggregates, at high speed in living cells. The technique locates and tracks particles at nanometre resolution in observation volumes measuring several micrometres in all dimensions. Not only position but also spectral information can be recovered, providing data on the particles' environment. The review by Petrášek & Schwille (2009) explores the origins of fluctuations in fluorescence signals. Usually associated with noise in signals, a careful analysis provides valuable information on the dynamics of the system under investigation, relating to the motion of molecules, their interactions as well as their conformational dynamics. Goedhart & Gadella (2009) show methods of microscopy that quantify the localization, conformation and activity of key signalling proteins in living cells. The use of FRET and genetically encoded FRET biosensors features prominently in the review.

Fluorescence signals are usually indirect measures of the interactions of actual interest. Increasing the number of measurement parameters from fluorescence experiments maximizes one's ability to interpret the underlying molecular processes. The paper by Blum *et al.* (2009) describes a microscope system that extracts spectral and lifetime information from every image pixel, providing sensitive information on fluorescent molecules and their environment.

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Moving from the molecular (nano-) scale to the microscale and even macroscale, Kwon & So describe a quantitative morphometric technique, which identifies rare cells in whole tissue specimens, several cubic millimetres in size (Kwon *et al.* 2009). Using a combination of advanced image post-processing routines and hardware automation, the resolution is adaptively controlled so that rare cells are first identified at low resolution before being measured in detail at high resolution so that morphological data can be retrieved.

The availability of rigorous analysis routines for microscopic imaging data is crucial for their correct interpretation. Elder *et al.* (2009) review a quantitative protocol for retrieving fractions of interacting molecules from FRET-sensitized emission data. The method separates interacting from non-interacting molecules and is applied in the study of dynamically changing interactions between cell-cycle proteins. Hanley's (2009) paper describes a method for the analysis and presentation of spectral frequency-domain FLIM methods, which, among other things, aid in the quantitative interpretation of FRET data. Barber *et al.* (2009) review global analysis routines for the interpretation of time-domain lifetime data for FRET verification, again with a view to separate interacting from non-interacting fractions.

The first international Theodor Förster lecture series was generously sponsored by a number of companies—without their help its organization would have been impossible. Sponsors are listed on the website for the series where podcasts of several of the excellent talks presented are also available.

Particular thanks are due to Dr A. Esposito who played a key role in the organization of the series. Many thanks are also due to Dr N. Becker of the University archive in Stuttgart and Martha Förster for providing pictures and valuable information on Theodor Förster.

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