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Introduction

Light microscopy on the move

Light microscopy is coming up with novel functional imaging schemes, nonlinear scattering imaging modes, and improved resolution in live cells. This current movement is reflected in this special issue.

Enabling quantitative functional imaging of biological processes at ever-increasing resolution and sensitivity, light microscopy has taken a prominent place in the life sciences. This success is based on the recent advancements in (photo)physics, chemistry, laser physics, and molecular biology, as well as on their cross-fertilization.

The field took a giant leap after the discovery of genetically encoded fluorescent proteins. They not only provided spatial information on the subcellular scale, but also allowed observation of the dynamic behavior of the proteins in action. The design and exploitation of minimally perturbing endogenous optical sensors opened up a new window of opportunity for biology: the observation of (bio)chemistry at work.

The advent of compact and tunable lasers, as well as of ultrasensitive detectors, paved the way for exploiting the innate photophysical properties of the fluorescent markers and for realizing innovative optical instruments. All in all, this has led to imaging approaches that map the temporal, spectral, and polarisation properties of fluorescence to subsequently reveal the function of proteins in the cell.

Fluorescence is not the only read-out mode of cellular information, because many cellular constituents are also able to alter the wavelength of the light after intense scattering. Catalyzed by advancements in pulsed laser physics, the almost forgotten field of higher harmonics imaging is taking another leap. In fact, this nonlinear microscopy mode is rapidly rolling to maturation. During the past years, optical imaging has also witnessed the breaking of the diffraction barrier in fluorescence microscopy, which changed the perception about the resolving power of light microscopy.

Featuring eight original contributions and a minireview, this special issue is a lively account of some of the latest advances in biological microscopy. Three groups report on functional imaging of protein dynamics in cells with fluorescent protein constructs. Another three articles concentrate on higher harmonics optical imaging. Whereas a further contribution deals with resolution improvement, the minireview highlights the power of combining confocal microscopy and fluorescence correlation spectroscopy to study lipid ''raft'' organization.

To examine diffusion of proteins in living cells, Fukano et al. present a novel set-up equipped with a digital micromirror device for free control of the illumination area. Atsushi Miyawaki's group shows by analysis of fluorescence recovery after photobleaching that diffusion into all processes of hippocampal neurons is at equivalent speeds. This finding argues against a postulated specific diffusion barrier at the axon–soma boundary.

Farla et al. use photobleaching approaches analyzed by computer models to study the interaction between a nuclear receptor and DNA. Thus, Adriaan Houtsmuller's group shows that ligand binding to the ligand binding domain of the androgen receptor stabilizes the androgen receptor–DNA complexes. Interestingly, a mutant androgen receptor lacking the ligand binding domain displayed substantially shorter immobilization with higher transcriptional activity.

The group of Yasushi Hiraoka combines several photochemical approaches to study the dynamic properties of a nuclear protein BAF that is involved in nuclear assembly, chromatin architecture, and gene regulation. On the basis of their combined analysis of fluorescence recovery after photobleaching and acceptor photobleaching resonance energy transfer experiments on a GFP fusion of BAF, Shimi et al. propose a novel touch-and-go model where BAF binds frequently yet transiently with emerin at the inner nuclear membrane.

4Pi-microscopy improves the axial resolution of focusing fluorescence microscopy through the coherent use of two opposing lenses. Whereas the gain in axial resolution has been extensively demonstrated for fixed cells and live baker's yeast, 4Pi-imaging of live mammalian cells is still considered challenging. In this special issue, Egner et al. show that these challenges can be met. Featuring a 3D resolution of 100–120 nm, the images of the mammalian Golgi apparatus reportedly are the sharpest images obtained so far in this organelle in the living state.

During the past 2 years, the recording of the third harmonics signal turned out to be more powerful than estimated in the past. The current progress in higher harmonic imaging is reflected in the articles by the groups of Yaron Silberberg, Chi-Kuang Sun, and Vlad Yakovlev. For example, Oron et al. show that third harmonic generation (THG) microscopy is a robust method for obtaining structural information on a variety of biological specimens, also allowing

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depth-resolved imaging of inhomogeneities. Virtually no background is observed from the surrounding homogeneous media. These authors further show that THG is particularly suitable for imaging of biogenic crystals, enabling extraction of the crystal orientation.

After giving a general outline of second harmonic generation (SHG) and THG under microscopy conditions, Sun et al. demonstrate the suitability of this nonlinear scattering contrast mode for developmental biology. Noninvasive optical penetration is illustrated, as well as prolonged imaging. The generation of higher harmonics in microscopy has the potential to offer new insights into the studies of embryonic morphological changes and complex developmental processes. Nonlinear optical scattering may capture subtle developmental information occurring deep inside live embryos and larvae.

Shcheslavskiy et al. characterize the third harmonics signal when focusing into different solutions and the sensitivity of THG to detect collagen stuctures. In particular, a transformation of collagen was observed with THG in solution. It was found that structures as small as 50 nm can be detected inside living cells using the current level of technology.

Squire and colleagues examine red-edge anisotropy microscopy that enables dynamic imaging of fluorescence resonance energy transfer (FRET) between identical fluorophores (homo-FRET) in cells. An experimental set-up for steady-state fluorescent anisotropy microscopy is described and can be used to acquire anisotropy images in live cells at main-band and red-edge excitation of enhanced green fluorescent protein (EGFP). Homo-FRET suppression of protein fusion constructs that consist of two and three EGFP molecules connected by short linkers represents a novel approach to the dynamic measurement of homo-FRET in live cells that will be of importance in the biological sciences for detecting oligomerization and concentration-dependent interactions between identically labeled molecules.

Finally, Kahya et al. review the lipid domain formation and dynamics in model membranes, such as giant unilamellar vesicles (GUVs). Lipids in eukaryotic cell membranes have been proposed to cluster in ''rafts'' with different lipid/protein compositions and molecular packing. GUVs may serve as a model system for elucidating the physical mechanisms of raft assembly, how lipids organize and move in rafts, and how they modulate membrane fluidity. The group of Petra Schwille combines confocal fluorescence microscopy and fluorescence correlation spectroscopy to investigate lipid dynamics and organization in GUV-rafts prepared from various lipid mixtures.

Enjoy reading!

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