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Editorial overview: Molecular imaging

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Optical microscopy is a very popular technique for investigating the living cell. Especially, far-field illumination in combination with fluorescence detection of tagged molecules such as proteins offers minimal invasiveness yet large specificity and sensitivity. Unfortunately, its full applicability is still limited due to several reasons. For example, introduction of a fluorescent label may still disturb the system or its information content may be biased by limited signal due to, for example, photobleaching. In the context of the latter case, one has to keep in mind that the illuminating light might introduce phototoxic effects on the system under study. Therefore, great efforts are undertaken to improve the labeling characteristics and minimize illumination stress. Yet, the largest limitation of far-field microscopy is probably its limited spatial resolution. Owing to diffraction, light cannot be focused to an infinitely small spot, which for visible light amounts to a lower limit of about 200 nm. As a consequence of this limitation, similar objects that are closer together than 200 nm cannot be discerned and details of, for example, cellular protein distributions can be visualized only to a certain extent. Fortunately, recent years has seen the development of super-resolution far-field fluorescence microscopy or nanoscopy techniques such as STED, GSD, RESOLFT, (f)PALM, (d)STORM, GSDIM, etc. (see e.g., [1–9]). All fluorescence nanoscopy techniques in one way or the other apply the reversible transition between states of different fluorescence emission characteristics (e.g., between a dark and a bright state), thereby ensuring that the measured signal only stems from molecules within a region of the sample that is much smaller than 200 nm, thus reaching molecular-scale imaging of cellular components. Efforts in correlatively combining optical nanoscopy with other microscopy tools such as electron microscopy will thereby significantly increase the information content. The enclosed range of articles gives an update on recent advances in labeling technology, reduction of phototoxicity, applicability of optical nanoscopy as well as combination with electron microscopy.

The first review by [Cieslinski and Ries](#) highlights optical microscopy methods that helped to gain structural insights into the yeast kinetochore. The toolbox of methods includes two-color fluorescence microscopy, Total-Internal-Reflection-Fluorescence (TIRF) microscopy and quantitative super-resolution microscopy. The questions addressed are the inner and outer kinetochore in budding and fission yeast.

How important and useful super-resolution fluorescence microscopy is for cellular biology is also addressed by [Jakobs and Wurm](#), who report on using super-resolution STED microscopy to investigate protein distributions in mitochondria with high detail. The authors further discuss open questions in mitochondria research and specific challenges. Similarly, [Blom and](#)

Widengren highlight several examples how STED microscopy has and potentially will advance cell-biology related research. Specifically, the authors highlight how advances in microscope setup, laser technology and fluorescent labels have improved the applicability of STED.

Neurobiology is a field that has exceptionally profited from super-resolution microscopy in the last decade, as reviewed by **Willig and Barrantes**. Specific structural features in neuronal cells could be visualized using STED microscopy, such as the regular arrangement of cytoskeletal motifs or the shape and size of protein clusters. Single-molecule tracking in addition provided dynamic information, and revealed nanodomains of neuronal receptors. Related, **Hosy, Butler and Sibarita** outline how super-resolution (f)PALM/(d)STORM nanoscopy has advanced the modern vision of synaptic function, particularly deciphering the organization of post-synaptic proteins, and offering new insight into the mechanism of synaptic transmission.

Next to resolving structural features of sub-diffraction size, single-molecule based (f)PALM/(d)STORM nanoscopy methods provide straight-forward access to quantitative information such as protein numbers. This is realized by light-stimulated activation of single fluorophores, yet requiring a careful analysis in order to extract reliable numbers. **Durisc, Cuervo and Lakadamyali** review pitfalls in quantitative super-resolution imaging, and discuss strategies for robust and reliable image analysis. **Cognet, Leduc and Lounis** describe how the single-molecule information can be used to advance the (f)PALM/(d)STORM technology for studying molecular mobilities and cellular dynamics by combining it with tracking and time-resolved imaging. How this technology at the end can bring up new perspectives on gene expression regulation and thus the geometry of the nucleus is described by **Woringer, Darzacq, and Izeddin**.

A key question in cell biology is the dynamics of proteins on cell membranes. In particular, the activation of membrane receptors, protein-protein interactions and transport processes are initiated at membranes. In the last years, imaging fluorescence correlation spectroscopy (imaging FCS) has emerged as a quantitative bioimaging tool to investigate such cellular processes in live cells, as reviewed by **Singh and Wohland**. Multiplexing and spatio-temporal correlations are accessible, as well as a high spatial and temporal resolution. In combination with sophisticated illumination schemes such as single-plane illumination, a selective excitation and read-out inside live cells becomes possible. How proteins rule the organization of the cell membrane by controlling the formation of lipid domains, and how advanced imaging methods such as optical nanoscopy can help to reveal this mystery is reviewed by **Rossy, Ma and Gaus**.

The development of molecular bioimaging is closely connected to the development of reporters for fluorescence. **Correa Jr** summarizes tag-mediated protein labeling for live cells, which are promising tools to monitor protein dynamics and interactions. Here, proteins are typically co-expressing a second protein that serves as a tag. This tag protein specifically processes a substrate that itself is conjugated to a synthetic fluorophore, which allows for stoichiometric labeling. Various fluorophore substrates are available, designed for photoactivation, for super-resolution imaging or single-molecule tracking. Another class of fluorescent reporters which are indispensable in modern bioimaging are fluorescent proteins. **Shcherbakova and Verkhusha** review a particular class of fluorescent proteins which can be modulated by light. The authors discuss the basic principles and photochemical reactions that underlie photoactivation or photoconversion, as well as light-induced chromophore transformations. They further outline which of these fluorescent proteins are particularly suited for different super-resolution microscopy techniques.

The importance of advancing probe and label technology for molecular imaging is further highlighted by other reviews. **Adam, Berardozzi, Byrdin, and Bourgeois** introduce the reader to the usefulness of photoswitchable proteins and their versatile use in several directions of advanced microscopy, but they also bring up current limitations and pathways for optimization. Photostability and brightness are two important parameters in advanced optical microscopy. This is pictured by **Juette, Terry, Wasserman, Zhou, Altman, Zheng, and Blanchard** by the design of intramolecularly stabilized, 'self-healing' fluorophores, which then significantly improve the performance of, for example, single-molecule FRET experiments. The applicability of the superior luminescence properties of nitrogen-vacancy (NV) color centers in diamond for advanced molecular imaging is outlined by **Balasubramanian, Lazariiev, Arumugam, and Duan**. Especially, the authors depict the sensitivity of these probes for nanoscale biosensing when exploiting the dependency of the luminescence on external magnetic fields.

Processes in living cells are often occurring at speeds that are beyond the scope of bioimaging techniques. **Winter and Shroff** review recent advances in high-speed fluorescence microscopy. They discuss approaches for both point-scanning and parallel detection, and cover diffraction-limited and super-resolution microscopy. To-date, a temporal resolution of 1 kHz in diffraction-limited imaging is possible. The authors in addition discuss how high-speed imaging depends on experimental parameters, and how it affects the spatial resolution or the sample penetration, and phototoxic effects by the illuminating light.

Finally, Kaufmann, Hagen, and Gruenewald introduce the reader to the prospects and challenges of studying biological structures with fine details using fluorescence cryo-microscopy. This approach has the potential to combine the optical read-out with electron microscopy, thereby combining the powers of both techniques for molecular imaging.

References

1. Hell SW: **Far-field optical nanoscopy**. *Science* 2007, **316**: 1153-1158.
2. Bates M, Huang B, Zhuang XW: **Super-resolution microscopy by nanoscale localization of photo-switchable fluorescent probes**. *Curr Opin Chem Biol* 2008, **12**:505-514.
3. Hell SW: **Microscopy and its focal switch**. *Nat Methods* 2009, **6**:24-32.
4. Hell S: **Far-field optical nanoscopy**. In *Single Molecule Spectroscopy in Chemistry*. Edited by Gräslund A, Rigler R, Widengren J. Berlin: Springer; 2009:365-398.
5. Lippincott-Schwartz J, Manley S: **Putting super-resolution fluorescence microscopy to work**. *Nat Methods* 2009, **6**:21-23.
6. Heilemann M, Dedecker P, Hofkens J, Sauer M: **Photoswitches: key molecules for subdiffraction-resolution fluorescence imaging and molecular quantification**. *Laser Photon Rev* 2009, **3**:180-202.
7. Heintzmann R, Gustafsson MGL: **Subdiffraction resolution in continuous samples**. *Nat Photon* 2009, **3**:362-364.
8. Patterson G, Davidson M, Manley S, Lippincott-Schwartz J: **Superresolution imaging using single-molecule localization**. *Annu Rev Phys Chem* 2010, **61**:345-367.
9. Eggeling C, Willig KI, Barrantes FJ: **STED microscopy of living cells — new frontiers in membrane and neurobiology**. *J Neurochem* 2013, **126**:203-212.