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EDITORIAL

In this special issue

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This special issue of Histochem Cell Biol is devoted to basic aspects of single-molecule super-resolution microscopy. Super-resolution techniques have opened new avenues for the study of cellular structures and molecular events in 2D and 3D at very high resolution in defined parts of cells. The topic is surveyed in three Review Articles, and novel aspects of reagents and image analysis as well as advancement of hardware are presented in four Original Articles.

Markus Sauer and coworkers (Klein et al. 2014) review the history, basic principles and different localization microscopy techniques such as Pointillism, PALM, STORM FPALM, PALMIRA, SPDM, GSDIM and Blink microscopy with emphasis on *d*STORM. This review provides an in-depth overview of the state of the art of two- and three-dimensional single-molecule localization microscopy for live-cell studies and for super-resolution imaging of tissues and organisms. Steven F. Lee and colleagues (Horrocks et al. 2014) focus in their review on pointillism-based super-resolution imaging and its application to biological imaging in both 2D and 3D. This technique permits imaging of single molecules or molecular assemblies with high spatial precision and through time and is open to quantification. Jean-Baptiste Sibarita (Sibarita 2014) reviews the principle of high-density single-particle tracking, aspects of fluorescent probes used, and ways of data acquisition and data analysis. Examples are provided to illustrate the power of high-density single-particle tracking to localize and track single membrane molecules at the nanometer scale and with millisecond temporal resolution.

Holger Erfle and colleagues (Gunkel et al. 2014) report on their development of a method combining two microscope types for initial automatic screening and subsequent super-resolution image analysis of cells of interest. Initially, low-resolution widefield images were screened by automatic image processing including feature space analysis to identify specific cellular phenotypes. The samples were then transferred from the screening microscope to a second microscope equipped for coordinate referencing and *d*STORM imaging. As the authors conclude, this straightforward workflow to transfer samples between different microscopes connects fast high-throughput imaging of a large sample and slow super-resolution imaging to be able to gain directed near-molecule information of selected cells. They also point out the potential of the technique for initial low-resolution screening of live cells followed by super-resolution imaging of the fixed cell samples.

Dylan M. Owen, Katharina Gaus and colleagues (Rossy et al. 2014) describe a method for co-cluster analysis in multichannel single-molecule localization data. They applied a combined univariate and bivariate Getis and Franklin's local point pattern analysis method to investigate the co-clustering of membrane proteins. Through this method, they were able to assess the degree of clustering of each protein to its own species and relative to second species. The method was validated using PALM and *d*STORM of two proteins of the T cell immunological synapse.

Ullrich Köthe and colleagues (Köthe et al. (2014) dealt with the difficulty of correct adjustment of a large number of parameters in reconstruction algorithms applied for localization microscopy. They designed SimpleSTORM, a

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fast, self-calibrating reconstruction algorithm. They could overcome the problems associated with user made parameter tuning by an initial self-calibration phase preceding the actual reconstruction phase. As a result, minimal user input could be combined with good reconstructions. Nonetheless, as stated by the authors, optional configuration by users as may be required in non-standard cases remains possible.

Ulrike Endesfelder, Mike Heilemann and colleagues (Endesfelder et al. 2014) developed a simple method to estimate the average localization precision of a single-molecule localization microscopy experiment based on nearest neighbor analysis. They demonstrate that the method can be used for 2D and 3D single- molecule localization microscopy experiments in a very general context and includes parameters like time dependence, alignment registration and structural labeling factors.

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