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EDITORIAL OPEN ACCESS

Cell biology needs super-resolution

Oliver von Bohlen und Halbach

The understanding of cellular functions requires detailed knowledge of all factors and their cellular distribution. Conventional microscopy has played (and still plays) a key role in cell biology. Microscopy combined with specific fluorescent labeling techniques has allowed to analyze the spatial distribution and/or the dynamics of subcellular structures or protein of interest in detail. However, conventional microscopy is limited in resolution and therefore, structures smaller than 200 nm can hardly be visualized. The problem is that the maximum resolution of lens-based microscopy is constrained by the diffraction limit of light. During the transmission through an objective lens, diffraction limits the minimum size of a focal point and results in a three-dimensional point spread function (PSF) of the light. Thus, if two structures are too close together, their individual PSFs cannot be distinguished. Thus, the two points cannot be resolved. Roughly spoken, even under best conditions, the optical resolution of a light microscope is limited to approximately half of the wavelength of the light used. Therefore, only structures that were at least 200 to 350 nm apart (depending on the wave-length of the light) could be resolved by light microscopy [1]. Recently, several new technologies, termed "super-resolution microscopy" or "nanoscopy", have been developed that circumvent the diffraction limit and shift the optical resolution down to 20–100 nm. Among these technologies are three different far-field methods, termed STED, SIM, and PALM/STORM, that have become popular.

Stimulated emission depletion microscopy (STED) relies on the depletion of the fluorophore's excited state to reduce the detection PSF. To obtain diffraction-unlimited images, STED assembles an image by scanning pixel by pixel with a laser and removes the out-of focus light using a pinhole in the optical path [2]. The resolution of a STED system in the X-Y axis is about 20–100 nm [1, 3].

Structured illumination microscopy (SIM) is based on conventional wide field fluorescence microscopy. An advantage of the SIM technique is that it does not require a special fluorescent probe for the resolution improvement [4]. SIM illuminates the entire field with a striped pattern of light. The excitation pattern mixes with the spatial pattern of the sample and produces an interference pattern (called "moiré fringe"). The excitation pattern is translated and rotated to generate a series of image with different moiré fringes. Since the illumination pattern is known, it can be mathematically removed from the moiré to gain access to the normally irresolvable higher resolution information in the sample [5]. The resolution of a SIM system in the X-Y axis is about 100 nm [1, 5]. Interestingly, the SIM technique can also be used in the tree-dimensional space. 3D-SIM allows to obtain 3D-images with ~100 nm lateral and ~300 nm axial resolution [4, 5].

Photoactivation localization microscopy (PALM), and stochastic optical reconstruction microscopy (STORM) represent specific single-molecule localization techniques. These techniques rely on the localization of single fluorophores based on their intensity data. The main principle of these techniques is based on the possibility to turn a subgroup of sparsely-distributed fluorescent molecules in subsequent imaging cycles on and off with the result of obtaining few sparse emitting molecules per cycle and captured image [2]. Series of a few hundred or thousand images can be processed into high-resolution images reaching a resolution in the range of about 30 nm [1].

Currently, the development of super-resolution microscopy just has started and will continue to evolve. Super-resolution microscopy has impressively improved

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the resolution in the X-Y axis. However, it should be kept in mind that biological structures are three-dimensional. Therefore, there is a need to extend super-resolution microscopy to 3D. In addition, image resolution often comes at the price of acquisition-speed and living cells may transport molecules faster than the super-resolution systems may capture images. Super-resolution microscopy in general will evolve as well as specialized system will be developed that will focus on higher resolution, high sampling speed or specific acquisition within thick tissues.

Super-resolution technologies offer the opportunity to localize proteins within the cell very precisely without the need for electron microscopy. Super-resolution microscopy will also allow to study small subcellular structures that have a length or width near and/or below the diffraction limit of the light, as e.g., the dendritic spines of a neuron. Thus, super-resolution microscopy has been successfully used to determine the nanoscopic spine localization of a mGluR5 accessory protein, called Norbin [6]. Moreover, by using PALM in combination with quantum dot tracking, morphological dynamic changes in spine morphology have been analyzed [7].

Although these technologies are currently not widely used for biological research, current super-resolution approaches allow fascinating new insights in cellular structures. The future developments in the field of super-resolution microscopy will have the power to revolutionize biological research.

Keywords: Cell biology, Nanoscopy, Photoactivation localization microscopy, Structured illumination microscopy (SIM), Stimulated emission depletion microscopy (STED), Super-resolution microscopy

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Conflict of Interest

Authors declare no conflict of interest.

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