SINGLE-MOLECULE LOCALIZATION MICROSCOPY. WHERE NEXT?

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Single-molecule sensitive super-resolution microscopy techniques such as *d*STORM and PALM provide microscopic images with subdiffraction spatial resolution. This enables new insights into how proteins are organized in a cellular context, with a spatial resolution approaching virtually the molecular level [1]. Because of their intrinsic single-molecule sensitivity they allow quantitative access to cellular structures, for example, how proteins are distributed and how they interact with other biomolecules. Ultimately, it is even possible to determine proteins numbers in cells and the number of subunits in a protein complex. Thus, they can pave the way toward a better understanding of how cellular function is encoded at the molecular level. Here, we demonstrate how single-molecule localization microscopy can be used advantageously for subdiffraction-resolution fluorescence imaging, discuss current limitations and point out future prospects.

For example, super-resolution imaging of protein distributions in larger intact tissue volumes with preserved fine structure remains so far challenging. We demonstrate that 3D-*d*STORM in the red spectral range with a high NA water immersion lens and optimized staining procedures can be used to map protein distributions with ~ $20x20x60$ nm^3 resolution in cryosections with a thickness of 25 μm. We recorded thousands of neuronal subcompartments aberration-free in volumes of up to $28x30x14$ μm^3 in 90 min. Using highly specific antibodies we measured protein distributions and clusters with distinct size, number and density in different brain regions. In addition, we developed a new multicolor localization microscopy method that enables quantitative multidimensional *d*STORM. We show how single-molecule sensitive super-resolution microscopy methods can be used successfully in clinical day-to-day diagnostics of cancer diseases to improve next generation personalized immunotherapies.

[1] Sauer, M, Heilemann, M (2017) Single-molecule localization microscopy in eukaryotes. *Chem Rev*, 117:7478-7509.