POINT-SCANNING BASED SUPER-RESOLUTION MICROSCOPY

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The resolution of conventional optical microscopes is limited by diffraction of light to approximately 250 nm. In the last decennium, several techniques have been invented to break this limit, based on photophysical properties of fluorescent molecules, structured illumination or single molecule localization. These techniques are promising and offer an impressive improvement of the resolution but are sometimes hard to apply for real biological experiments due to long acquisition times, limited choice of fluorophores and embedding buffers, phototoxic effects or complex reconstruction algorithms.

The here proposed new microscopy technology is a combination between widefield and image scanning microscopy (ISM) [1]. It is a compromise between improvement of resolution and applicability in biology. Biologists are often interested in improving the resolution just in a part of the cell: for the rest of it, the classical resolution is sufficient.

The complete sample is imaged with widefield illumination, allowing a large field of view with good resolution. Then, the researcher defines the structure of interest which is subsequently imaged with a point-scanning illumination. For this second image in scanning mode the emission light is collected with a fast and sensitive CCD camera. A new reconstruction algorithm calculates the final image of the region of interest leading to improved resolution. With this method, we improve resolution up to a factor of two (down to 120 nm) in a limited region of interest with limited restriction to speed, phototoxicity and staining methods.

We have used this new technique in a neurobiology application where we studied the morphology of dendritic spines. These structures are involved in information transmission and storage in the brain. The dimension of these spines is just beyond the resolution of a wide-field microscope. We efficiently studied the correlation between their morphology and function.