

Towards quantitative high-throughput 3D localization microscopy

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Single-molecule localization microscopy (SMLM) has become a widely used technique to observe cellular structures at the scale of tens of nanometers. However, major bottlenecks are still faced and limit the scope of applications. Here we present new technologies to overcome three of the major limitations of SMLM in throughput, quantitative counting of proteins and 3D resolution. Indeed, SMLM is an intrinsically slow method with acquisition and data analysis times in the minute to hour range. This severely limits the possibility to acquire a sufficient number of experiments in quantitative studies. To nevertheless achieve high throughput in SMLM, we automated the entire workflow of data acquisition and analysis. This enabled us to continuously acquire new experiments over the course of days and analyze them in real time. Another limitation concerns the inhomogeneous photo-dynamics of molecules across the field of view, which is a consequence of the Gaussian intensity profile in microscopes using lasers for excitation. Such inhomogeneity impedes accurate protein counting. To tackle this issue, we developed a homogeneous illumination scheme with the help of a multimode fiber and a speckle reducer [1]. This simple illumination achieves a very high uniformity and results in homogeneous measured localization precisions across the field of view, removing statistical bias from quantitative studies. Finally, we present the use of adaptive optics to improve the 3D resolution of supercritical angle localization microscopy (SALM [2]), with the aim to reach isotropic localization precision in the first few hundreds of nanometers above the coverslip. Altogether, those improvements open the way to better sampled and more precise structural studies in superresolution microscopy.

[1] J. Deschamps, A. Rowald, and J. Ries, "Efficient homogeneous illumination and optical sectioning for quantitative single-molecule localization microscopy," *Optics Express*, **24**, 28080-28090 (2016).

[2] J. Deschamps, M. Mund, and J. Ries, "3D superresolution microscopy by supercritical angle detection," *Optics Express*, **22**, 29081-29091 (2014).