DECIPHERING MOLECULAR ORGANIZATION AND DYNAMICS USING LOCALIZATION-BASED SUPER-RESOLUTION MICROSCOPY

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Deciphering molecular organization and activity at the molecular level has become possible thanks to the recent advent of super-resolution microscopy. Techniques based on the sequential stochastic photo-conversion of sparse subsets of single fluorophores, i.e. (f)PALM or (d)STORM, rely on the ability of determining the center of the point spread function (PSF) created by each single point emitter. They have become extremely popular due to their affordability and relatively simple implementation on a conventional microscope. They allow the localization and tracking of a large number of biomolecules with close to molecular accuracy (down to 10 nm in lateral and 40 nm in axial) and millisecond scale temporal resolution.

Nevertheless, a major step relies in the quantitative image analysis, often time-consuming and not easy to handle by non-specialists. This crucial task often involves complex processing techniques adapted to image topology and quality, since few methods take into consideration the pointillist nature of single-molecule data. We will discuss this issue and present a novel general segmentation framework allowing robust and automatic quantification of protein counting and organization at different scales, from the cellular level down to clusters of a few fluorescent markers.

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