QUANTITATIVE SINGLE-MOLECULE LOCALIZATION MICROSCOPY

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Over the last decade, single-molecule localization microscopy (SMLM) has making it possible to monitor molecular organization and dynamics at the nanoscale, improving our understanding of biological mechanisms at the molecular level. SMLM relies on the cumulative spatial localization of sparsely distributed fluorescent markers and requires dedicated acquisition equipment as well as sophisticated detection algorithms to precisely locate bio-molecules.

Image analysis plays an essential role in microscopy, more especially in SMLM, allowing deciphering mechanisms underlying biological processes in a statistical and nonbiased manner. However, while quantitative image analysis has been thoroughly used on pixel-based images, quantifying SMLM data from molecular coordinates remains challenging.

This presentation will cover the methods and challenges to achieve quantitative SMLM, from the acquisition to the data analysis. We will discuss how the analysis of spatio-temporal localization patterns allows gathering quantitative information on the organization, dynamics and stoichiometry of bio-molecules in their cellular environment. Dedicated quantification techniques will be presented with their advantages and limits, and potential artefacts inherent to SMLM data will be discussed.