Pushing the limits of quantitative SMLM toward deeper, more colours and more throughput imaging

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Abstract:
The recent advent of optical super-resolution techniques represents a new and fundamental step toward understanding biological mechanisms at the molecular level in single cells. Single molecule-based approaches offer the capabilities to count, locate and track the movement of bio-molecules in their cellular environment. However, conventional illumination schemes used with these approaches limit the super-resolution observation to the first micron above the coverslip, preventing imaging live dynamical processes in whole 3D cells or tissues. In addition, monitoring multiple fluorescent species is often achieved at the expense of a loss in spatial and temporal resolutions, conventional experimental setups being typically limited to two simultaneous wavelengths. Finally, despite its high spatial resolution and unique quantitative information, SMLM remains a low throughput technique incompatible with HCS standards.

I will present our projects aiming at overcoming some of these limits, pushing SMLM toward deeper, more colours and more throughput. We will first present our last progress combining single objective Selective Plane Microscopy (soSPIM), adaptive optics (AO) and SMLM to achieve super-resolution imaging of cellular structures up to few tens of micrometers away from the coverslip. I will then show how, by employing a dual-objective imaging configuration compatible with live cell imaging, we can achieve simultaneous 3D single particle tracking of multiple distinct proteins without compromising the spatio-temporal resolution. Finally, I will describe a fully automated quantitative single-molecule-based super-resolution methodology operating in 96-well plates and using HCS based analysis and data mining software in a single workflow.

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