

Multi-parameter fluorescence nanoscopy through single molecule switching

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Fluorescence far-field optical microscopy is one of the most important tools in life science. Highly specific tagging with fluorescent markers allows functional imaging of intact and even living samples using visible light with minimal impact on the system under consideration. By tagging different molecular species with different types of markers, their spatial and temporal correlation can be explored. Importantly, the markers themselves can function as ultra-small chemical or physical sensors reporting on their micro-environment by changing their spectroscopic properties. Depending on for example, pH, temperature or binding state, the markers' emission can be used to classify their immediate vicinity. One has long argued that a severe drawback of using far-field optical microscopy is its limited resolution which impedes the separation of similar objects which are closer together than about half of the wavelength used (~250nm). In dense samples the resolution limit thus also limits the detection of spectroscopic properties of the markers to bulk recording because unlike in single molecule spectroscopy of dilute solutions, fluorescence is simultaneously detected for several of the labels.

We show how sequentially switching single, isolated emitters to a fluorescent state and recording their fluorescence allows not only precise determination of their position in 3D and thus the assembly of images with nanoscale resolution but also the application of techniques from single molecule spectroscopy. This allows us to efficiently distinguish between different molecular species.

References:

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