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Spectroscopic analysis beyond the diffraction limit

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Abstract

The recent surge in spectroscopic Single-Molecule Localization Microscopy (sSMLM) offers exciting new capabilities for combining single molecule imaging and spectroscopic analysis. Through the synergistic integration of super-resolution optical microscopy and single-molecule spectroscopy, sSMLM offers combined strengths from both fields. By capturing the full spectra of single molecule fluorescent emissions, sSMLM can distinguish minute spectroscopic variations from individual fluorescent molecules while preserving nanoscopic spatial localization precision. It can significantly extend the coding space for multi-molecule super-resolution imaging. Furthermore, it has the potential to detect spectroscopic variations in fluorescence emission associated with molecular interactions, which further enables probing local chemical and biochemical inhomogeneities of the nano-environments. In this review, we seek to explain the working principle of sSMLM technologies and the status of sSMLM techniques towards new super-resolution imaging applications.

Keywords: Super-resolution microscopy; Fluorescence spectral imaging; Single-molecule spectroscopy

1. Introduction

In the past decade, a wide range of super-resolution imaging modalities, including structured illumination microscopy (SIM), stimulated emission depletion microscopy (STED), and single-molecule localization microscopy (SMLM), have been developed to improve the optical imaging resolutions far beyond the diffraction limit of light (Hell, 2007; Huang et al., 2010; Liu et al., 2015; Moerner, 2015). These techniques have offered tremendous opportunities for researchers to “see” cellular architectures at single-nanometer scale, which was previously considered unresolvable by optical microscopy. However, despite the successes in precisely capturing the spatial locations of individual fluorescence molecules, the fluorescent emission information were only detected by rather limited color channels, leaving the rich molecular spectroscopic signatures largely un-utilized.

To address the above-mentioned difficulties, multiple research groups recently reported a new class of imaging technology based on the working principle of SMLM that simultaneously recorded the spatial

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