Single-Molecule Localization Super-Resolution Microscopy of Synaptic Proteins

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Abstract

Recent years have witnessed huge progress in the field of light microscopy with the development and implementation of new approaches leading to dramatic improvements in the spatial and temporal resolution of this form of imaging, most particularly in its biological applications. The limitations in spatial resolution imposed by the diffraction of light have been circumvented by resorting to different strategies, which are briefly outlined in the Introduction. These protocols are intended to provide practical guidelines for the imaging of synaptic proteins using one such strategy, namely, single-molecule stochastic localization super-resolution microscopy.

The protocols use neuronal cells from the hippocampus of rodent embryos as the experimental paradigm and outline the steps for obtaining dissociated neurons and establishing primary cultures for in vitro studies. The techniques can be adapted to the culture of neurons from other brain regions. Procedures for handling fixed and live specimens are described, as well as the use of extrinsic fluorescent probes and fluorescent proteins, mounting media, examples of hardware configurations, software for image analysis, and some hints for the implementation of minimalist approaches to single-molecule localization nanoscopy.

Keywords Nanoscopy, Neuronal cell culture, Sample preparation, Single-molecule imaging, Staining, Super-resolution microscopy

1 Introduction

Optical ("light," "wide-field," "far-field") microscopy is undoubtedly the most popular technique for imaging cells and tissues, and the successful combinations of appropriate probes (organic dyes, fluorescent proteins, and inorganic nanoparticles, e.g., quantum dots), new light sources (solid-state lasers, light-emitting diodes), and improved detectors (CCD cameras, avalanche photodiodes) have made fluorescence microscopy the method of choice, essentially because of the unparalleled selectivity and sensitivity achieved in biological applications, pervading practically all realms of biology. Moreover, recent years have witnessed a revolution in fluorescence microscopy: using conventional lenses and visible light it has been possible to circumvent the century-old limit dictated by the

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