doi:10.1017/S1431927611010579
Microsc. Microanal. 17 (Suppl 2), 2011

Biological Applications of Fluorescence Photoactivation Localization Microscopy

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Since the advent of super-resolution imaging methods, great advances have been made in our capability to understand biological systems. While diffraction limits the smallest structures that can be imaged in a conventional light microscope to approximately 200-250 nanometers, many biological processes occur on molecular length scales. Fluorescence photoactivation localization microscopy [1,2,3] (FPALM) can image biological samples with resolution of 20-30 nm. This recently developed method offers a means by which previously inaccessible biological questions can now be addressed.

In conventional fluorescence microscopy, the majority of labeled molecules are visible at the same time, resulting in diffraction-dependent blurring of structures on length scales less than 200-250 nm. In contrast, localization microscopy gathers image information by measuring the positions of (localizing) many small subsets of fluorescently labeled molecules within a sample. By imaging these molecules separately and stochastically, at a low enough density where each visible molecule is spatially distinct from the others, fluorescent molecules imaged with FPALM can be localized with a precision of just a few nanometers, and with an overall density of >10000 per um^2 within the focal plane. Images are then rendered by plotting the positions of all localized molecules.

Localization microscopy has rapidly evolved to image live cells [1], multiple molecular species [4,5], three-dimensional samples [6,7] and molecular orientations [8]. FPALM has been applied to a variety of biological imaging applications, including membrane, cytoskeletal, nuclear, and cytoplasmic proteins in fixed and living cells.

This tutorial describes how to successfully obtain and analyze FPALM images. Additionally, examples of successful biological applications and potential new applications of the technique will be described. The major topics to be covered are: i) Concepts of FPALM; ii) Instrumental setup (Fig. 1) and iii) Applications.

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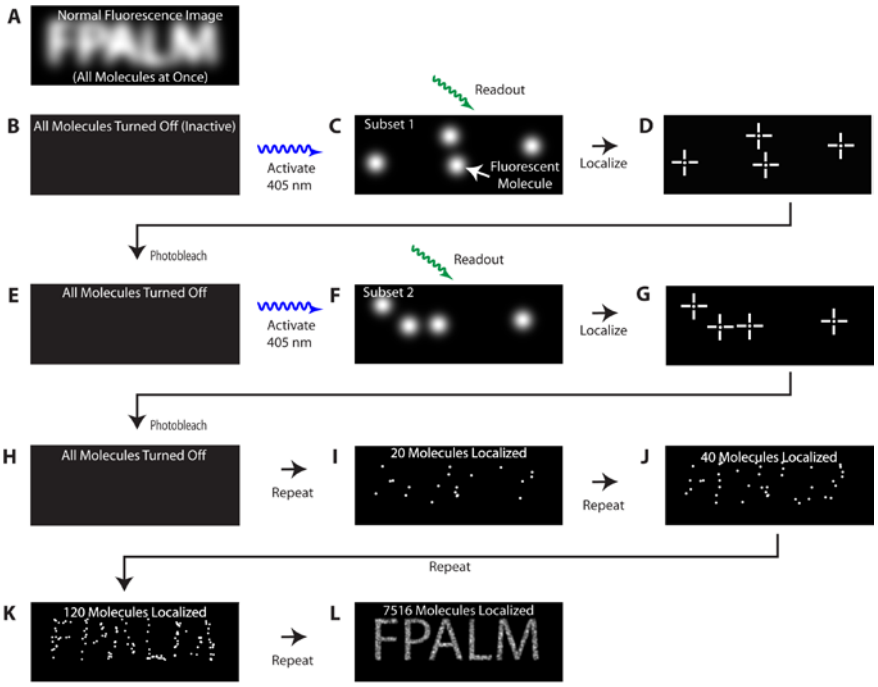

Figure 1. The molecules in the sample are initially inactive (B, E, H) until an activation laser is used to active a subset of molecules in the sample (C, F). The activation laser is turned off and the fluorescence from the labeled proteins that were photoactivated is collected using a readout laser. Concurrently with the readout laser illumination, a high-sensitivity camera records movie frames of the fluorescence of the photoactivated molecules. These movie frames are analyzed to identify and localize activated molecules (D, G, I-K) for the duration of their fluorescence. Molecules will spontaneously photobleach under the high-intensity illumination of the readout laser, which reduces the number of visible molecules to a low enough density that each visible molecule is distinct from others. The process of activation, imaging, localization, and photobleaching is repeated for many cycles to generate data containing tens or hundreds of thousands of molecules. Final images are rendered by plotting the positions of all localized molecules.

https://doi.org/10.1017/S1431927611010579 Published online by Cambridge University Press