CHAPTER

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Correlative two-photon and serial block face scanning electron microscopy in neuronal tissue using 3D near-infrared branding maps

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CHAPTER OUTLINE

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Abstract

Linking cellular structure and function has always been a key goal of microscopy, but obtaining high resolution spatial and temporal information from the same specimen is a fundamental challenge. Two-photon (2P) microscopy allows imaging deep inside intact tissue, bringing great insight into the structural and functional dynamics of cells in their physiological environment. At the nanoscale, the complex ultrastructure of a cell's environment in tissue can be reconstructed in three dimensions (3D) using serial block face scanning electron microscopy (SBF-SEM). This provides a snapshot of high resolution structural information pertaining to the shape, organization, and localization of multiple subcellular structures at the same time. The pairing of these two imaging modalities in the same specimen provides key information to relate cellular dynamics to the ultrastructural environment. Until recently, approaches to relocate a region of interest (ROI) in tissue from 2P microscopy for SBF-SEM have been inefficient or unreliable. However, near-infrared branding (NIRB) overcomes this by using the laser from a multiphoton microscope to create fiducial markers for accurate correlation of 2P and electron microscopy (EM) imaging volumes. The process is quick and can be user defined for each sample. Here, to increase the efficiency of ROI relocation, multiple NIRB marks are used in 3D to target ultramicrotomy. A workflow is described and discussed to obtain a data set for 3D correlated light and electron microscopy, using three different preparations of brain tissue as examples.

INTRODUCTION

Light microscopy (LM) is invaluable to cell biologists as a tool for obtaining dynamic information about cellular function and structure, especially with the aid of fluorescent reporters or dyes. Multiphoton (MP) fluorescence microscopy allows the visualization of these cellular dynamics inside living tissue at greater depth than is possible with conventional fluorescence microscopy (e.g., confocal microscopy) (Zipfel, Williams, & Webb, 2003). This is achieved by employing lower energy, longer wavelength light in the near-infrared part of the spectrum ($\sim$700–1400 nm). To excite a fluorophore, near simultaneous absorption of multiple, lower energy photons are required instead of a single, higher-energy photon in single photon fluorescence microscopy. The probability of MP excitation is extremely low in comparison with single photon excitation. To overcome this, the laser is repeatedly pulsed in ultra-fast (100 fs) bursts to increase the photon density. Because photon density falls away with distance from the focal plane, the chance of obtaining MP excitation is effectively zero outside that focal plane, conferring inherently high three-dimensional (3D) resolution (Zipfel et al., 2003). MP microscopy is advantageous to a cell biologist because the near-infrared light is refracted less as it passes through the tissue. With a sample that refracts very little light, it is possible to routinely achieve