C6-O-02

doi:10.1093/jmicro/dfv202

Mapping Synapses by Conjugate Light-Electron Array Tomography

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Synapses of the mammalian CNS are diverse in size, structure, molecular composition, and function. Synapses in their myriad variations are fundamental to neural circuit development, homeostasis, plasticity, and memory storage. Unfortunately, quantitative analysis and mapping of the brain's heterogeneous synapse populations has been limited by the lack of adequate single-synapse measurement methods. Electron microscopy (EM) is the definitive means to recognize and measure individual synaptic contacts, but EM has only limited abilities to measure the molecular composition of synapses. This report describes conjugate array tomography (AT), a volumetric imaging method that integrates immunofluorescence and EM imaging modalities in voxel-conjugate fashion. We will illustrate the use of conjugate AT to advance the proteometric measurement of EM-validated single-synapse analysis in a study of mouse cortex.

C6-O-03

doi:10.1093/jmicro/dfv203

Quantifying adult brain ultrastructure using focussed ion beam scanning electron microscopy

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Electron microscopy is an essential technique for visualising the structure and connectivity of the nervous system. In the past, this was achieved with the slow and error-prone method of serial section transmission electron microscopy. However, more recently, scanning electron microscopy has provided automated methods for imaging significant volumes of tissue. In this talk I will show how focussed ion beam scanning electron microscopy (FIBSEM) can acquire image stacks with near isotropic voxels with which computer algorithms can help speed up the reconstruction and analysis process. With the high precision of this method we can target axons and dendrites that were previously imaged, in vivo, giving insights into the plasticity of adult neural connectivity; as well as revealing morphological characteristics of excitatory, inhibitory, and neuromodulatory axons. Dense reconstructions of all neurites contained within volumes of neuropil allow us to use a 3D approach for making a complete structural analysis. With this data we can quantitatively describe the ultrastructure of a brain region, as well as compare animals at different ages. These experiments show that FIBSEM is a reliable imaging tool, capable of producing a quality of serial images that enable rapid morphological analysis.