Improved Methods for Conjugate Immunofluorescence and Electron Microscopic Array Tomography.

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Array tomography (AT) is a high-resolution, high-content, volumetric imaging method based on acquiring images of planar arrays of serial ultrathin sections followed by computational volume reconstruction and analysis. Arrays can be imaged either by high-resolution, high-dimensional immunofluorescence (IF/AT) or by field emission scanning electron microscopy (FESEM/AT). IF/AT provides for high-dimensional measurement of tissue antigens, while FESEM/AT provides the classic electron microscopic view of membrane ultrastructure. Until now, however, conjugate imaging of individual specimens by IF/AT and FESEM/AT in sequence has been limited by conflicting requirements for antigenicity and ultrastructure at early stages in tissue processing. These conflicts have enforced severe compromises image quality.

Here we demonstrate greatly improved conjugate IF/AT – FESEM/AT imaging based on new freeze-substitution methods of tissue preparation. In pilot studies of mouse cortical synapses, we show that this new methodology permits both high-quality IF imaging of at least six proteins (and probably many more) and excellent FESEM visualization of synapse ultrastructure, including synaptic vesicles, the synaptic cleft and the postsynaptic density. In addition, neuronal membranes and cytoskeletal elements such as microtubules are well defined, thus allowing tracing of the neuronal connections. Information obtained by this conjugate proteometric and ultrastructural analysis is critically important for providing molecular context to neural circuit mapping, such as identifying the neurotransmitters and receptors present at each synapse. The techniques to be described also may be useful for research on other, non-neuronal tissues.