

Long Term 3D Imaging by FIBSEM for Neurons and Cell Biology and Correlation to Cryo Fluorescence Microscopy

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3D Electron microscopy volume data can be acquired by a variety of approaches. Focused Ion beam – scanning electron microscopy, FIBSEM, offers no limitation on section thickness, so that isotropic voxels with 8 nm or less sampling in x,y,z dimensions can be acquired. The FIBSEM, which is normally limited to a couple days of continuous operation, was refined to enable year-long reliable data acquisition needed for the large volumes of neural imaging and the connectome. Concurrently, this capability opens a new regime where entire cells can be imaged with 4 nm voxel sampling, thereby surpassing partial cell or section limitations to complete cell data. The heavy metal staining for EM contrast gives spatially detailed but generic black and white rendering of protein and membrane defined structures. On the other hand, fluorescence microscopy is highly protein specific, by labeling only a tiny subset (1-3 %) of the thousands of constituent proteins of the cell. Most 99.9% of the cell remains dark. Correlated cryogenic fluorescence microscopy offers a way to combine both without compromising the quality of either EM or fluorescence image. Fluorescent properties at low temperatures (down to 10K) include new regimes of stable fluorescence with highly reduced bleaching, new blinking regimes, good contrast ratios useable for PALM, nonlinearity to excitation power, and photo-reactivation. Multicolor 3D structured illumination SIM images can be acquired on such samples and 2 color, 3D PALM images offer even higher resolution. Examples of such correlative Cryo SIM/PALM and FIBSEM images will be presented on cultured cells.