Simultaneous Correlative 3D imaging of nanoscale targets and entire cells by Focused Ion Beam Scanning Electron Microscopy

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Ultrastructural imaging across large volume scales is a significant challenge in biology, as traditional methods force a trade-off between image resolution and size. Using novel automated procedures in Focused Ion Beam Scanning Electron Microscopy (FIB-SEM), we simultaneously image large resin embedded biological samples rapidly at intermediate resolutions, and selected sub-volumes at high (up to 3x3x5 nm) resolutions. Here, using a combination of fluorescently labeled HIV core proteins, TRIM5a (a cellular protein that binds HIV) and cell membranes, we first produce a "target map" of an HIV infected cell by fluorescence microscopy. We then generate a correlated high-quality 3D EM volume of the entire cell, and simultaneously, targeted high-resolution 3D images of cytoplasmic TRIM5a, endocytosed HIV virions, and individual HIV cores. This represents a size differential of 10^9-fold in a single dataset collected in a one automated overnight run. These results represent significant progress toward "point-and-click" imaging where one can efficiently visualize the nanoscale local structure as well as mesoscale global context of biological objects of interest.