

composed of hundreds of proteins, selectively controlling all traffic between the nucleus and the cytoplasm. The architecture of the NPC is central to understanding nuclear transport. However, due to its sheer size, its local environment and its dynamic nature, determining its structure at molecular resolution remains a challenge for conventional techniques. Combining FIB milling, cryo-ET, and image processing enables the study of the NPC in its native environment, free of the distortions caused by purification. This approach has not only revealed the NPC architecture at unparalleled resolution, but also captured different conformational states in action. Other uses of cryo-FIB/ET to study diverse cellular environments at molecular detail will be presented, including actin networks, the architecture of cell division, and the distribution of macromolecular complexes within organelles such as mitochondria.

#### 1813-Pos Board B705

##### Development for Dynamic Live Cell Imaging by Cryo-Electron Tomography and Stem

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Cryo-EM tomography of intact cells is an emerging technology that complements crystallography, NMR and single molecule imaging techniques. Its strength is in that it reveals the spatial arrangements of key proteins and complexes during intracellular signaling and mechanical events like motility and division. In this meeting, we describe dynamical Live Cell imaging by Cryo-EM tomography using the Titan Krios microscope with 300 kV electron column and HAADF detector. Observed living cells were grown directly on Holey Carbon Support Film. In order to make sure that the cells are suitable for observation we analyzed the some parameters of cell movement and cell division on Quantifoil using Fiji and compared these properties with those of cells grown on a normal cell culture plastic plate. After phosphate-buffered saline washing, the cells were done rapid freeze fixation (vitrification) by dropping in liquid ethane using Vitrobot<sup>TM</sup> Mark IV instead of the usual chemical fixation and were transferred onto the microscope immediately while keeping the environment under liquid N<sub>2</sub>. Cells were imaged over an angular range from -70 degrees to 70 degrees at 2 degrees x cos  $\theta$  tilt increments automatically and analyzed with Inspect 3D and Amira software to provide 3D images and Volume rendering respectively.

We observed some unique architectures of a part of Lamellipodium in GFP-Myosin X expressed COS 7 cell and them from near the nuclear membrane to plasma membrane in COS 7 and several eukaryotic cells (HeLa, NIH3T3... etc.) by Cryo-EM tomography using STEM using intact cells and vitreous cell sections.

At future, our system can provide any new information about many kinds of cells and organelles during important events.

#### 1814-Pos Board B706

##### New Method for Applying Multiple Samples to a TEM Specimen Grid

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Transmission electron microscopy (TEM) enables visualization and structural determination of biological macromolecules. Examination of several samples by TEM is greatly limited by the time that it takes to insert each sample on the microscope, and the deteriorating vacuum on the TEM after this process is repeated several times. Nevertheless, when observing a specimen by TEM, in almost all cases the examined area of the grid represents a small fraction of the available area. Thus, in theory, a TEM grid has space for more than one sample. Here we have exploited the microarray technology to apply multiple samples to a single TEM specimen grid. Microarrays can deposit small quantities of sample using a printer-like machine that transfers the liquid from the reservoir to the assay surface. We demonstrate that microarray technology can be used to accurately position picoliter quantities of sample within micrometric distances in the reduced space of the TEM grid with negligible cross-contamination. This technique permitted the deposition of samples into arrays of 2 to 36 discrete spots on a single TEM grid. The TEM grid containing a sample microarray can be negatively stained. After introducing the microarray grid in the microscope every spot can be inspected and images can be recorded digitally or on film. In conclusion, the microarray method can dramatically decrease the time necessary for TEM grid prep-

aration and examination at least by one order of magnitude, while preserving the vacuum of the electron microscope. This new method is very suitable for screening and data collection in experiments that generate a multiplicity of samples.

#### 1815-Pos Board B707

##### Direct Visualization of HIV-1 with Correlative Live-Cell Microscopy and Cryo-Electron Tomography

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Cryo-electron tomography (cryoET) allows 3D visualization of cellular structures at molecular resolution in a close-to-physiological state. However, due to the poor signal, low contrast, and radiation sensitive nature of unstained frozen-hydrated specimens, acquisition of tomographic projection series is not generally selective in choice of imaging targets. Therefore, the full potential of cryoET for 3D cellular imaging is realized, especially for cellular processes that are rare or dynamic. In order to overcome this limitation in cryoET analysis, approaches for correlating fluorescent light microscopy and cryoET are highly desirable, not only to complement the structural information obtained from cryoET with the dynamic functional data from fluorescent labeling, but also to guide sampling in cryoET. Such tools are particularly valuable for investigating the early events of HIV-1 infection in cells, which are infrequent and difficult to catch. Here, we report on a methodology that combines high speed 3D live-cell imaging with cryoET tools. We applied this technology to visualize the process viral entry into HeLa cells, following of the same particles. Through direct 3D visualization we identified HIV-1 particles that are smaller than the diffraction limit of light microscopy (~ 100 nm). They were found attached to plasma membrane and in MVBs after cell entry. We also showed, for the first time under near-native conditions, that intact hyperstable mutant HIV-1 cores are released into the cytoplasm of host-cells. We anticipate that the methodology established here will not only constitute a useful tool for studying virus-host cell interactions at various stages during infection, but will also open new ways to investigate cell signaling events and many other cellular processes in general.

#### 1816-Pos Board B708

##### Structural Plasticity within the Postsynaptic Density

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The postsynaptic density (PSD) is a large protein complex that clusters neurotransmitter receptors at the synapse and organizes the intracellular signaling molecules responsible for altering the efficiency of synaptic transmission – termed synaptic plasticity. We propose that synapses from different parts of the brain place unique demands on the process of synaptic transmission and that the structure and composition of the PSD play a role in providing these distinctive properties. To begin to address this question, PSDs were isolated from adult rat cerebella, hippocampi and cortices, three brain areas amenable to straightforward isolation that contain unique distributions of neuronal cell types. Electron-tomography (ET) was used to visualize the fine morphology of the isolated PSDs and calculate total protein occupancy within the PSD structure. Immunogold labeling was utilized to quantify protein composition and distribution of key signaling and scaffold molecules. Although the mean surface area did not significantly differ between PSD types, the PSD thickness, as measured from Cryo ET reconstructions, differed significantly between PSD types. Labeling densities for PSD-95 and  $\alpha$ CaMKII were found to differ dramatically among the PSD types, while all regions had moderate to high labeling for  $\beta$ CaMKII, illustrating the importance of  $\beta$ CaMKII to the PSD structure. PSD-95, a scaffold protein, was absent from a fraction of cerebellar PSDs, unlike hippocampal and cortical PSDs, showing that protein composition varies between PSD types. Ripley's K function analysis of immunogold labeled PSDs showed that PSD-95 was clustered in cerebellar PSDs, unlike other PSD types, suggesting a different function for PSD-95 in cerebellar PSDs. In contrast,  $\beta$ CaMKII was found to have similar non-random organization in all PSD types. These results support the idea that the composition and structure of the PSD are modified to achieve the specific synaptic functions required of each brain region.