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Liquid and Three-Dimensional Scanning Transmission Electron Microscopy for Biological Specimen

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Eukaryotic cells contain a complex organization of proteins and compartments that serve numerous different biological functions. Understanding how these structures are organized is a central challenge for cell biologists [1]. However, state-of-the-art imaging techniques are limited in their capability to image individual proteins inside whole cells in liquid environment and in three dimensions (3D). We have developed a new technique for imaging whole cells in liquid that offers nanometer-scale spatial resolution of individually labeled proteins (Figure 1) [2]. The cells in liquid are placed in a micro-fluidic flow cell with a liquid layer thickness < 10 μm contained between two ultra-thin electron-transparent silicon nitride windows. This flow cell is placed in the vacuum of a scanning transmission electron microscope (STEM) using a dedicated fluid specimen holder. Since the mean-free-path-length for scattering in water is much larger than for materials of a higher atomic number, it is possible to detect nanoscale labels inside a relatively thick layer of water, i.e., samples containing water and gold nanoparticles. The relation between the obtained image contrast and the water thickness was measured and compared using a theoretical model. We have also imaged flowing gold nanoparticles with the STEM using a pixel dwell time as short as 5 μs. The liquid STEM technique was then applied to the imaging of biological specimen. Images of single gold-tagged epidermal growth factor (EGF) molecules bound to EGF receptors on fibroblast cells were recorded. The resolution of 4 nm achieved through a thick liquid layer (~5-7 μm) surpasses alternative approaches previously used, such as imaging of cooled cells in water vapor using an environmental TEM and SEM. For fixed samples, liquid STEM presents an alternative to conventional fluorescence microscopy with a 50X higher resolution.

The primary method to study the 3D organization of cellular structures is tilt-series electron microscopy (EM), with a resolution in the range of 2–20 nm. We have developed an alternative 3D EM technique for cell- and structural biology [3]. Aberration-corrected 3D STEM allows for high-resolution 3D imaging without a tilt stage. In a manner similar to confocal light microscopy, the sample is scanned layer-by-layer by changing the objective lens focus so that a depth-dependent focal series is recorded [4]. In the absence of a pinhole aperture, as used for confocal STEM [5], 3D STEM is comparable to 3D wide-field optical microscopy, in which the 3D image is reconstructed by deconvolution with a point spread function (PSF). The reconstructed 3D STEM images of biological samples show a resolution which is already comparable to that obtained with state-of-the-art tilt-series EM, however, since there is no mechanical tilting of the sample, 3D STEM has several advantages. A 3D STEM dataset can be recorded in a matter of several minutes. The size of the

images is not limited on account of the tilt. In principle, it is possible to record very large datasets containing, for example, several adjacent cells, by a combination of precise stage-movement and scanning large areas. Finally, the 3D spatial distribution of proteins tagged with labels of a high atomic number (such as Au) can be mapped within thick samples and also in liquid.

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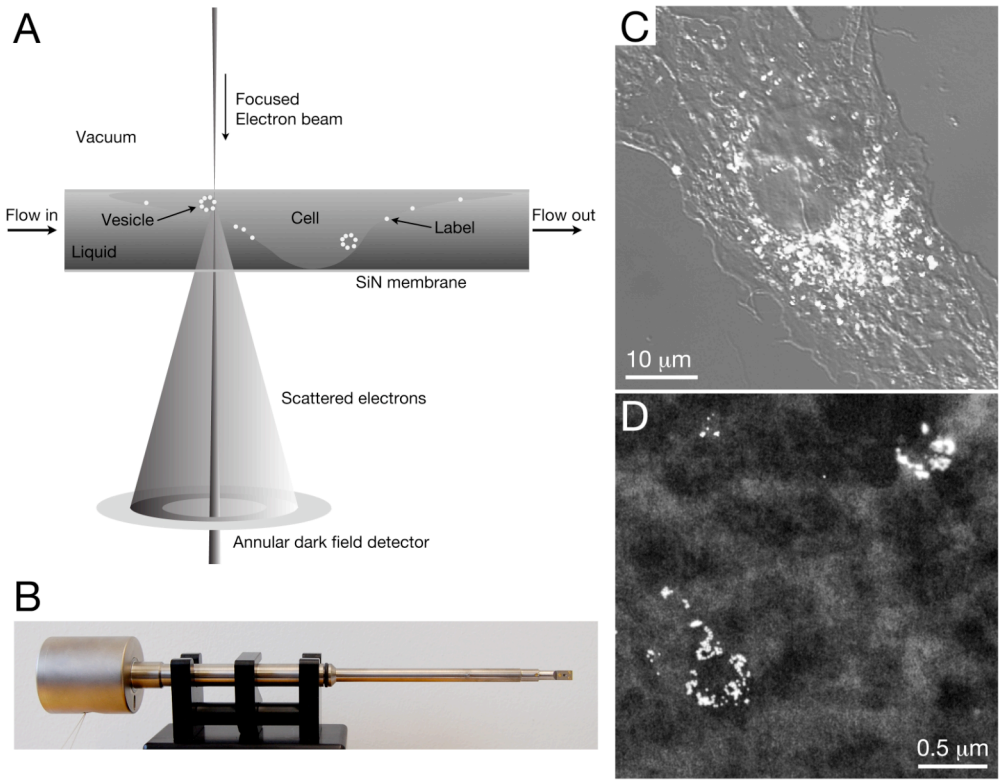

FIG. 1. (A) In liquid STEM a whole cell immersed in liquid is enclosed between two electron-transparent windows. Images are obtained by scanning a focused electron beam over the cell and detecting the scattered electrons with an annular dark field detector.
(B) The fluid holder is used to place the flow cell in the vacuum of the microscope.
(C) Confocal laser scanning microscope image of a fibroblast cell incubated for 25 minutes with quantumdot labeled epidermal growth factor (EGF), overlay of fluorescent and DIC signals. (D) Liquid STEM image of a fibroblast cell in liquid incubated for 25 minutes with EGF-gold. The small spots are individual 10 nm gold labels grouped in a circular cluster, presumably an endosome. The resolution is 50x better than the confocal laser scanning microscope. Image A, B and D from [2].

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