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Radiation Damage of Biological Specimen in Environmental Electron Microscopy

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Proteins labeled with nanoparticles can be studied in their native context of the plasma membrane in intact cells in liquid using scanning transmission electron microscopy (STEM) [1,2]. One approach is the usage of a microfluidic chamber enclosing the cell entirely in liquid and imaging at 200 keV beam energy [1]. An alternative approach is to study the cell maintained under a thin liquid layer using environmental scanning electron microscopy (ESEM) at 30 keV [2]. An important question is to which extent the effect of electron beam induced radiation damage changes the structure of cells in liquid.

In order to examine what type of radiation damage is of influence, one should first look at the specific question that the microscopic study aims to address. We are interested in the dimer formation of the epidermal growth factor (EGF) receptor (EGFR), a transmembrane receptor playing a critical role in the pathogenesis and progression of many different types of cancer. In Comparison to super-resolution fluorescence microscopy, the enhanced resolution of STEM (with a spatial resolution of a few nanometres) enables us to distinguish between monomers, dimers and clusters of labelled proteins while the cell remains intact and in its natural liquid environment. However, due to its invasive nature, electron microscopy entails several complications like interactions of the electron beam and/or radiolysis reactions with the solvent. Hence, the main limiting factors for the imaging of biological samples are changes in the structural integrity of a cell caused by radiation damage. What matters in our experiment is that the locations of proteins are determined with sufficient resolution to examine the compositions of protein complexes and as close as possible to the native situation. Possibly, some radiation damage to the biological structure can be tolerated as long as the protein positions remain the same. In our case, we specifically label certain protein species with nanoparticles. Tolerance for radiation damage can thus be evaluated based on the shift of nanoparticles. According to literature the limit of radiation damage for biological molecules in a water vapor environment [3] equals ~1 e-/Å², which we consider as the lower limit for unfixed cells in saline buffer solution. In a previous study, we demonstrated the ability to detect gold labeled EGFR proteins with a resolution of 4 nm in fixed cells for 200 keV Liquid STEM. The electron dose applied was in the range of 7×10^2 e-/Å² for which no radiation damage was observed [1, 2]. However, the effect of electron beam irradiation on cells in liquid has remained mostly unexplored.

We examined the electron dose tolerance of COS7 cells in liquid. Gold nanoparticles of 10 nm diameter were adhered to EGFRs via a streptavidin-biotin bond. The cells were fixed with glutaraldehyde and kept in pure water during electron microscopy. Two experimental approaches were used. 1) Cells fully enclosed in liquid in a microfluidic device at 200 keV. 2) Cells maintained in an open wet environment using ESEM at 30 keV (Fig. 1). Several regions on different cells were repeatedly imaged in order to assess the effect of increasing electron dose. The electron dose limit for radiation damage was defined from the onset of shifts of the labels > 2 nm. It was found in our ESEM experiments that the label positions remained mostly unchanged between two images recorded with a total electron dose of 430 e^-/Å² (Fig. 2A). Yet, most of the nanoparticles at the edges of the image had shifted while recording a series of images with a total dose of 8815 e^-/Å² (Fig. 2B). In conclusion, fixed COS7 cells can be studied in liquid with electron microscopy, while the positions of the labeled EGFRs do not significantly change for an electron dose sufficient to detect the labels.

References:

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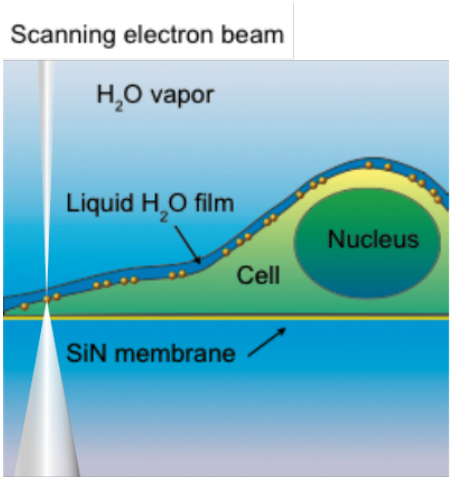

Figure 1. Experiment to test radiation damage of ESEM of a eukaryotic cell in hydrated state. Schematic of the setup. Live eukaryotic cells are grown on a supporting silicon nitride membrane and incubated with specific protein labels consisting of gold nanoparticles (AuNPs). Imaging is done by scanning a focused electron beam over the cell. Transmitted electrons are recorded with the STEM detector located beneath the sample. The cell is maintained in a saturated water vapor atmosphere (740 Pa, 3 °C), while a thin layer of water covers the cell.

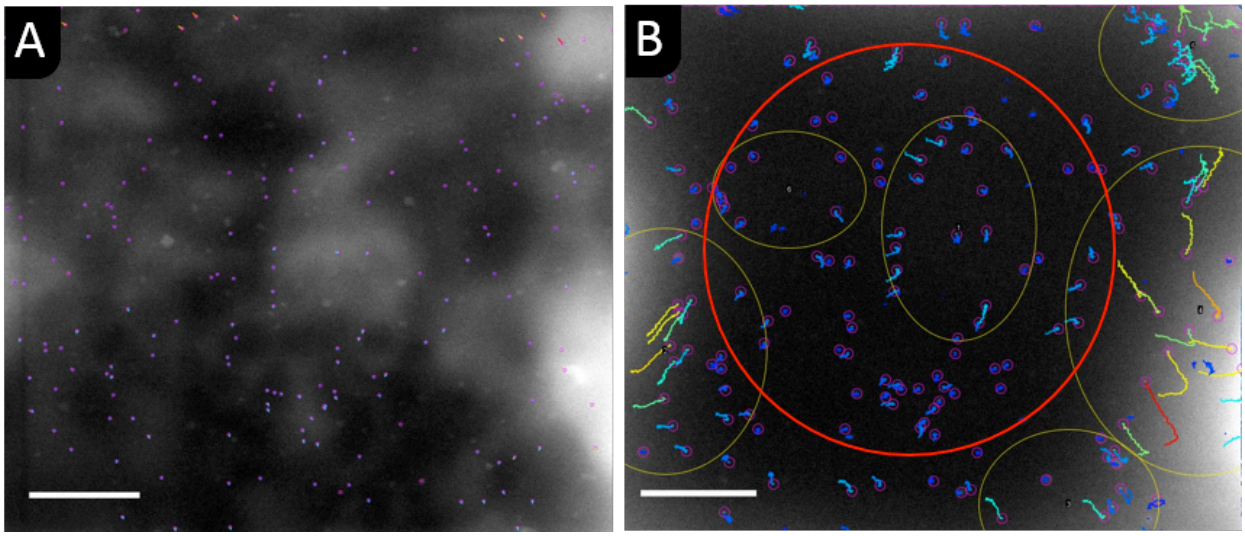

Figure 2. Micrographs of the same region on a COS7 cell with gold nanoparticles conjugated to epidermal growth factor receptors in the plasma membrane. (A) ESEM-STEM image showing the Au NP label as bright spots on the background shapes in grey of the biological material. The locations of Au NPs detected in a second image at the same position are shown in blue color. The two images were recorded with a total electron dose of 2x215 $e^-/Å^2$. Most Au NP positions overlapped between the two images, only a few streaks are visible at the top of the image indicating shifts. (B) Image showing the shifts of Au NPs after the recording of a series of images with a total dose of 8815 $e^-/Å^2$. The largest shifts were observed at the edges of the image. The mean grey value of the imaged area decreases with increasing dose due to the destruction of the eukaryotic cell. Scale bar: 500 nm.

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