Prospects for imaging "live" biological structures with high spatial and temporal resolution in the DTEM

James E. Evans[1,2], Katherine L. Jungjohann[3], Peony C. K. Wong[2], Po-Lin Chiu[2], Gavin H. Dutrow[2], Ilke Arslan[3,4], and Nigel D. Browning[1,2,3,4]

1. Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, USA
2. Dept. of Molecular and Cellular Biology, University of California, Davis, Davis CA, USA
3. Dept. of Chemical Engineering and Materials Science, University of California Davis, Davis, CA, USA
4. Fundamental Computational Sciences Directorate, Pacific Northwest National Laboratory, Richland, WA, USA

Nigel.browning@pnnl.gov
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The goal of the Dynamic Transmission Electron Microscope (DTEM) is to be able to directly observe transient phenomena in materials/biological systems with both high spatial (~1nm or better) and high temporal (~1μs or faster) resolution [1]. The approach differs from many other experimental methods aimed at high temporal resolution analysis by obtaining a direct real-space image of the transient phenomenon being studied – thereby removing the phase retrieval problem that exists for diffraction experiments. Another issue that directs the design of experiments in the DTEM is that for many of the key scientific challenges involving structure-property relationships in materials science and structure-function relationships in biology, the transient processes involve significant re-arrangement of the atoms/molecules in the structure at specific locations making dynamic observations essentially irreversible in nature. This establishes the requirement for "single shot" imaging in the DTEM that effectively constrains the temporal resolution that can be obtained, and defines the types of reactions that can be studied in the microscope.

High temporal resolution is achieved in the DTEM by using a short pulse laser to create a pulse of electrons through photo-emission. This pulse of electrons is propagated down the microscope column in the same way as in a conventional high-resolution Transmission Electron Microscope (TEM). The only difference is that the spatial resolution is limited by the electron-electron interactions in the pulse (a typical 10ns pulse contains ~10^9 electrons). To synchronize this pulse of electrons with a particular dynamic event, a second laser is used to "drive" the sample a defined time interval prior to the arrival of the laser pulse.

A key first step in the use of DTEM to study transient processes in materials science, chemistry and biology is to control the environment around the sample. Here the commercial TEM platform that serves as the base for the DTEM allows the environmental controls for the DTEM to build on the many years of in-situ TEM developments – from in-situ straining and biasing through to gas and liquid stages. In this presentation, we will discuss the development and implementation of an in-situ liquid stage developed in collaboration with Hummingbird Scientific that allows atomic scale images and electron energy loss spectra to be obtained from samples suspended in solution [2,3]. The functionality of this stage has a wide range of applications from studying corrosion in materials science, imaging battery technologies all the way to imaging "live" biological systems.

The development of the in-situ liquid cell has intriguing possibilities for studies in biology of live and hydrated specimens. *Live* biological processes have previously only been observed by light microscopy at low spatial resolution (>20 nm) where the cellular components are localized through fluorescent labels. Although TEM has routinely allowed high-resolution (~1 nm) ultrastructural imaging of biology, this has only been for samples in the arrested (fixed or frozen/cryogenic) state. The ability to achieve both high spatial and high temporal resolution with the DTEM means that its combination with in situ liquid imaging may avoid the negative spatial resolution effects of Brownian motion for particles in suspension. Figure 1 shows the first direct images of purified macromolecular protein complexes using the *in situ* liquid cell in an aberration corrected scanning transmission electron microscope (STEM). These results establish the viability of using the liquid cell technique to visualize the interface between biology and nanotechnology with high fidelity while also probing the interactions of biomolecules within solution [4]. This method represents an important advancement

towards direct high-resolution observation of biological processes and conformational dynamics in real-time using the DTEM described above. These results along with the development path for future DTEM capabilities for imaging biological structures will be discussed in detail in this presentation [5].

References:

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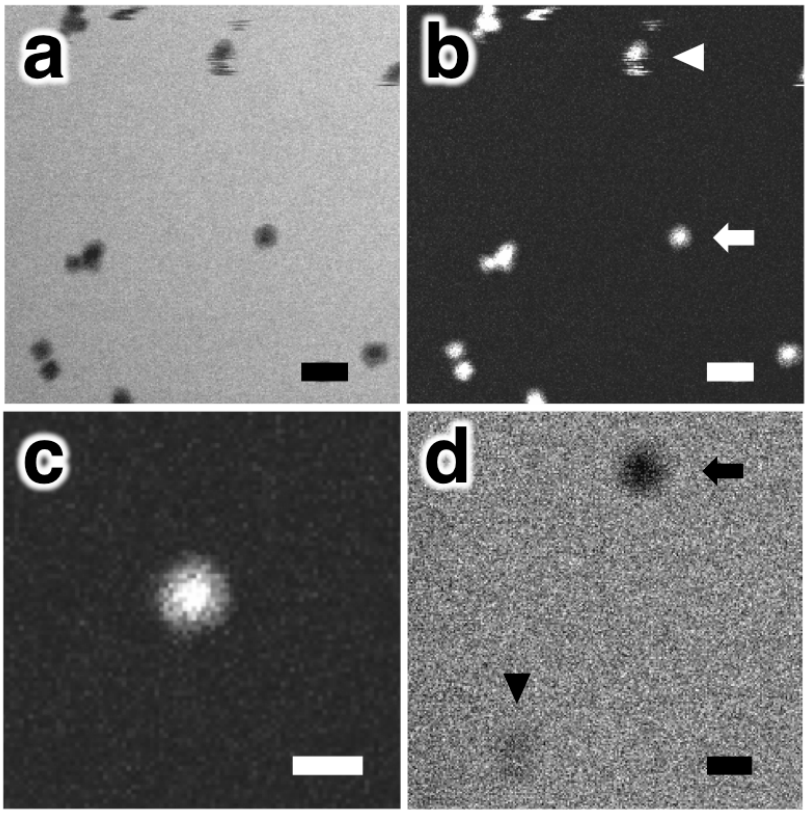

Figure 1 (*a&b) In situ bright field and dark field STEM images of a suspension of ferritin molecules in a buffered saline solution. The white arrowhead points to a ferritin molecule that moved during acquisition. c) Magnified view of ferritin molecule indicated by white arrow in (b) depicting both the outer protein shell and more dense (brighter) inner iron oxide nanoparticle. d) In situ bright field STEM image of ferritin and apoferritin. Scale bars are 10 nm. Adapted from [4].*