

Introduction to Special Issue on Electron Microscopy of Specimens in Liquid

Since the invention of the electron microscope in the 1930s (Ruska, 1986) it has been a wish to image specimens in liquid just like a light microscope does. However, the need for vacuum in the electron microscope initially caused unbridgeable complications. E. Ruska partly circumvented this problem in 1942 by introducing an environmental chamber in the electron microscope (Ruska, 1942). The specimen region was maintained at a low vacuum by placing pump-limiting apertures on the pole shoes of the objective lens and applying a differentially pumped vacuum system. The same principle is still used for today's state-of-the-art environmental transmission electron microscopy. Various systems were developed throughout the years (Parsons et al., 1974) including systems in which a liquid was enclosed between thin membranes (Abrams & McBain, 1944). In the 1970s and 1980s environmental scanning electron microscopy (ESEM) technology was developed for imaging specimens at vapor pressures sufficient to observe liquid water (Danilatos, 1988). ESEM technology also employs a pump-limiting aperture, and, in addition, gas in the specimen chamber is used to enhance detection via a chain of ionization events. In the past decade electron microscopy in liquid has experienced an upsurge in activity triggered by the availability of new types of practically applicable thin membranes to separate a liquid specimen from the vacuum in the electron microscope (Williamson et al., 2003; Thiberge et al., 2004), and the achievement of nanometer resolution in liquid for biological samples (de Jonge et al., 2009) and for nanoparticles (Zheng et al., 2009). It is now routinely possible to conduct experiments in which specimens in liquid are studied with transmission electron microscopy (TEM) or scanning transmission electron microscopy (STEM) for a variety of research fields including biology, materials science, and chemistry (de Jonge & Ross, 2011).

This special issue contains 18 papers about electron microscopy in liquid. Several papers cover instrumentation aspects, including the design of new types of liquid enclosures constructed from dedicated microchips, the combination of X-ray energy dispersive spectroscopy with electron microscopy in liquid, and the introduction of a system to record three-dimensional images of wet samples. New methods have been developed to obtain useful data with electron microscopy in liquid considering, in particular, the influence of the electron beam on the specimen. Examples include methods to study electrochemical processes in liquid, and the loading and unloading of battery materials in electrolyte. It is readily possible to study the formation and self-assembly of nanoparticles using either ESEM or liquid-cell TEM. Other examples of observations in liquid involve the formation of nanodroplets, corrosion effects, and behavior of the liquid – ice interface. Nanoscale patterns can even be written in liquid. The capability to image in wet state also opens the possibility to study polymeric coatings for drug release. Last but not least, labeled proteins can be studied on whole cells both with scanning electron microscopy and STEM, and it now even seems feasible to image live cells.

While methods are being refined and dose-induced effects are separated from relevant data or are used in some cases to induce processes, it can be expected that the technology to record electron microscopy images of specimens in liquid will develop into a mature nanoscale analysis method with broad usage. With the capability to image in liquid it is readily possible to observe processes albeit under careful consideration of electron beam effects, thereby adding the time domain to the two- or three-dimensional structural information. A decade ago, electron microscopy research focused toward the highest possible resolution (Nellist et al., 2004). Since then an increasing number of research groups have pointed their attention to the study of samples in a realistic or close to realistic environment, and for many samples it is important to explore what happens in liquid. *In situ* electron microscopy, including imaging in liquid, will be broadly

represented at major conferences such as the Spring MRS in San Francisco, the Microscopy and Microanalysis (M&M) 2014 meeting in Hartford, CT, the 2014 International Microscopy Conference in Prague, Czech Republic, and the MRS meeting in Boston. The topic area is also represented at various smaller conferences, such as the Conference for In-Situ and Correlative Microscopy, Saarbrücken, Germany, October 14–15, 2014.

The set of papers in this special issue provides a comprehensive overview of the available technology, exemplifies the broad range of subjects that can be covered with electron microscopy of liquid specimens, and includes a critical discussion of the existing experimental challenges. We hope these papers encourage others to utilize these techniques and help to expand the field.

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