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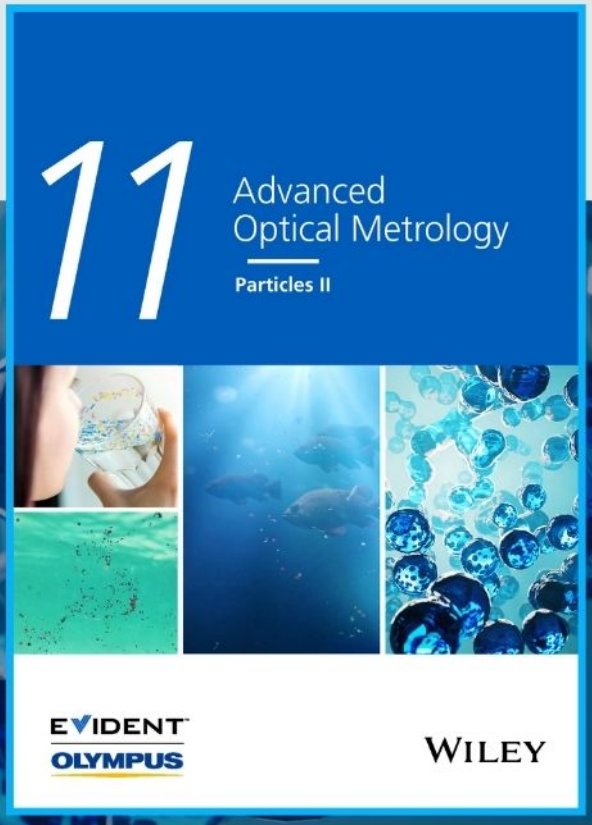

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EDITORIAL

Cryo-focussed ion beam in Life Sciences (and beyond)

It was in this journal, in 2006, that Mike Marko and coworkers from Albany (US) published a paper in which they demonstrated the possibility of using a focussed ion beam (FIB) to prepare thin sections (i.e. lamellae) from frozen hydrated Life Science samples. The so-called cryo-lamellae were subsequently transferred to a cryo-transmission electron microscope (cryo-TEM) to record images of cells embedded in vitreous ice.[1] Their work sparked a lot of activities around the globe, with multiple groups developing their own 'cryo-TEM prep' methods. A small but steadily growing community of cryo-FIB-users started organising small workshops across Europe (Colerain, UK, 2008; Utrecht, The Netherlands, 2009; Vlaardingen, The Netherlands, 2012; Lausanne, Switzerland, 2014; Nottingham, UK, 2017). The discussions between the groups clearly helped and inspired the development of various approaches towards cryo-TEM preparation to where we are today. Today's status can be seen in the literature, as what is being published is not only describing further innovative cryo-TEM preparation methodologies, but also describing scientific contributions to various fields in Life Sciences based on established cryo-TEM preparation methods. Cryo-TEM preparation is becoming a high-end standard technique for high-end scientific questions. Hence, this special issue of *Journal of Microscopy* clearly reflects that cryo-TEM preparation is the main application for cryo-FIB instruments.

For this special issue, we have brought back together colleagues from those early days of cryo-TEM preparation developments to both reflect on their techniques and to look ahead of where to go next. Hence, primarily discussed in this special issue are the three routes towards cryo-TEM observations of cryo-FIB made lamellae: Thinning cells on a grid,[2] lift-out techniques[2,3] and thinning of high-pressure frozen samples.[4]

The cryo-TEM preparation application is the result of about three decades of developing cryo-FIB techniques. The FIB milling technique had become popular in semiconductor research in the 1980s. However, it was recognised that the FIB introduces defects and other types of damage in the samples. Meanwhile, preliminary work in the late 1980s resulted in successful observations of frozen hydrated Life Science samples in a cryo-scanning electron microscope (SEM) in the very early 1990s.[5] Soon, FIB milling was performed under cryo-conditions to mitigate the damaging effect of the ion beam.[6] Around that time, the combined FIB-SEM instruments became increasingly popular. With ongoing improvements of FIB-SEM instruments, the first feasibility studies of cryo-FIB-SEM work on frozen hydrated Life Science samples were published by Hans Mulders[7] and Ingo Gestman *et al*.[8] The potential of the technique was quickly recognised and resulted in where we are today.

Thus, the work of cryo-TEM preparation is based upon three decades of developing cryo-FIB-SEM techniques. This is even longer if we include various ways of preparing Life Science samples. Therefore, the extensive basics of cryo-FIB operations, including a detailed description of the preparation and transfer options are discussed in the paper by Mike Hayles and Matthijs de Winter.[9] Their aim is to inspire and help forward newcomers to the field and to serve as a general resource for more experienced cryo-FIB-SEM operators.

One could say that cryo-FIB in Life Sciences has matured in less than 20 years, but that does not mean there is nothing left to do. Jakub Kuda and coworkers[2] discuss cryo-FIB-SEM tomography of unstained biological samples, a technique first introduced by Andreas Schertel in 2013.[10] The FIB is used to mill away thin consecutive slices and the resulting cross sections are imaged by the SEM. The series of SEM images is used for a digital reconstruction of the analysed volume. The results of cryo-FIB-SEM tomography are very promising, but the image formation mechanism is not yet fully understood. The image is formed by depositing charge at or just behind the cross section which affects the secondary electron generation of subsequent electron beam scans. Needless to say, the SEM scan strategy requires precise tuning. The kind of cellular features that can be observed and to what detail is still a question to be researched.

Another development which has been researched recently is the potential all-in-one instrument,[4,11] when not only the making of cryo-TEM lamella is done in the FIB-SEM instrument, but also the observations are made in the FIB-SEM instrument. The latter is done by placing a solid-state detector underneath the cryo-TEM lamella and performing transmission imaging with the SEM. This would be a complimentary technique to, for example, the

extraordinary high resolving power single particle analysis by TEM. However, many applications may not require such high resolving powers, making the cryo-FIB-SEM a powerful stand-alone instrument.

Still there is more, as other observed phenomena are interesting for scientific exploration. For example, unpublished work by the first author indicates that gallium from the ion beam may form small (tens of nanometre) droplets on the surface during milling. Gallium droplet formation has been observed before[12] and it is a relevant finding, as the density difference between gallium and cellular material induces curtaining, that is, a rough finish of the FIB milled cross section. Another intriguing observation is the effect of a cross section being charged up by the electron beam and becoming unsusceptible to water vapour deposition from a close proximity warm surface. When considering the dipole moment of water molecules, one could be tempted to think that an electrical field could repel water vapour. Although currently somewhat speculative, if that works with reasonable electrical field strengths, it will offer potential sample protection against gaining layers of ice over time.[13]

As you can see, there are still a lot of interesting developments to be studied within the field of cryo-FIB, and not just in the field of Life Sciences. Cryo-FIB-SEM work is done in *Material Sciences*[14,15] and *Earth Sciences*[16] too. For examples, some polymers are very sensitive when irradiated by ions and electrons. Cooling down those samples may help to mitigate some of the damaging effects of the beams, just as with the semiconductor materials that started off cryo-FIB work back in the 1990s.

To conclude, we hope that this special issue about cryo-FIB applications serves as a guide and a general resource to both newcomers and experienced researchers in the field. But first and foremost, as generations come and go, we hope this special issue of *Journal of Microscopy* encourages new researchers to join and continue building the wonderful discipline of cryo-FIB work.

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