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Cryo-FIB Machining: An Alternative to TEM Cryo-Sections Cut with Diamonds?

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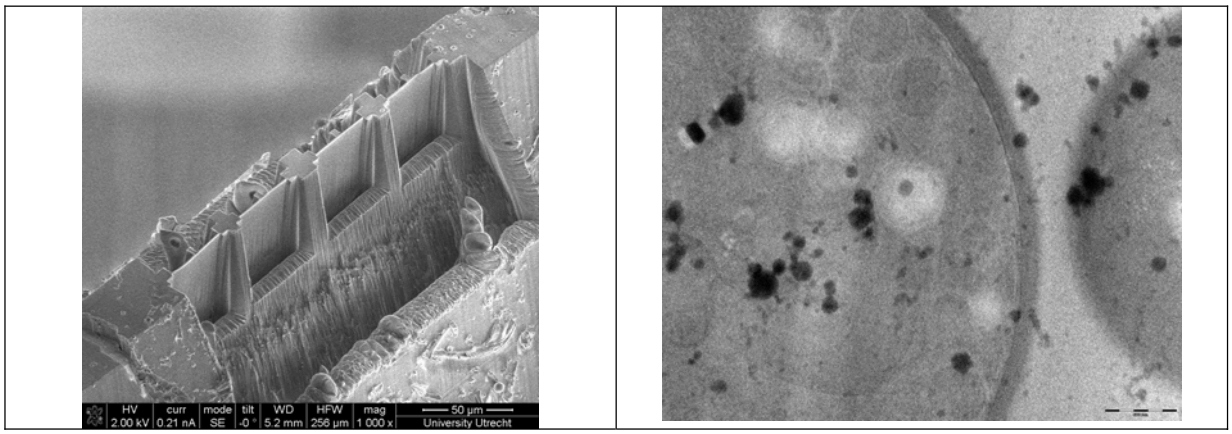
Since the beginning of electron microscopy, sample preparation has been a matter of debate. In an electron micrograph every single detail down to about a few nanometers is visible; therefore the electron microscopists are constantly concerned to preserve biological matter as close as possible to nature. Today's consent is that a physical arrest by cryo-fixation is the only method to preserve the living in its actual physiological state. Furthermore also the observation should be done in ice, as water is the natural solvent of life. During dehydration macromolecules may collapse [1] and the heavy metal stains tend to bind better to certain molecular sites, depicting more their distribution than the actual structure of the macromolecules. In structural biology this concept has become a standard since a long time [2]. For cell or tissue biology the implementation is more difficult because the sample has to be thin in order for the electron beam to pass through. Further, small objects like proteins can easily be vitrified in a thin film of water but cells and pieces of tissue require more advanced techniques. Therefore high-pressure freezing has become the method of choice [3, 4]. Depending on the natural antifreeze within the tissue, slices of 100 µm up to a 500 µm can be preserved. Subsequently, sections have to be prepared that allow TEM imaging. The group of Jacques Dubochet [5] has improved cryo-sectioning of vitrified biological samples and coined the term CEMOVIS, cryo-electron microscopy of vitreous sections. *Every advantage has a disadvantage* (after the famous Dutch soccer player Johan Cruijff): CEMOVIS allows the observation of cells vitrified in their physiological surrounding without the influence of chemicals, but the sectioning process induces physical damage like knife marks, compression and crevasses [6] that become worse with increasing thickness of the sections. For high resolution *in situ* analysis of molecular complexes the method is very valuable [7]. For cellular electron tomography, however, thicker cryo sections, up to 500 nm, are desired. To overcome the limitations of sectioning, Mike Marko and his group introduced a thinning technique using a focused ion beam (FIB). Cells were frozen on an electron microscopy grid, then the ice was thinned in the ion beam to allow cryo-electron tomography [8, 9]. A new approach was described by Rigort et al. [10]. They have established a correlative chain from imaging fluorescent cells grown on EM-grids, followed by coarse thinning in a cryo-ultramicrotome and final polishing with the FIB. We set out to make connectivity from samples cryo-fixed by high-pressure freezing until imaging in a cryo-TEM [11]. The heart is a newly developed cryo-stage for the FIB-SEM (Nova Nanolab 600, FEI Company), a cryo-nanolathe called CryoNanoBench. Samples are cryo-fixed in a copper tube by a high-pressure freezer (EMPact, Leica Microsystems), then the copper is trimmed off in a cryo-ultramicrotome. Thereafter, the sample is mounted in a specially designed ferrule, cryo-transferred into the CryoNanoBench. In the FIB-SEM a thin lamella is cut at the tip of the sample. Back through the newly developed transfer chain, including the Quorum cryo-transfer system (Quorum Technologies) the sample is loaded into a modified cryo-holder (Gatan, Inc) and transferred into the cryo-TEM for observation. In this presentation we will explain the setup in detail.



Yeast cells were cryo-fixed in a copper tube by high-pressure freezing and prepared according to the description above. The SE image on the left hand side depicts are row of thinned lamella. On the right hand side a TEM image of the frozen-hydrated yeast cells is shown.

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