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Minimal Resin Embedding of Multicellular Specimens for Targeted FIB-SEM Imaging

Nicole Schieber[1], Pedro Machado[2], Sebastian Markert[3], Christian Stigloher[3], Yannick Schwab[1,2], Anna Steyer[1]

[1.] Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany
[2.] Electron Microscopy Core Facility, European Molecular Biology Laboratory, Heidelberg, Germany.
[3.] University of Wurzburg, Imaging Core Facility, Biocenter, Germany.

Correlative light and electron microscopy (CLEM) is a powerful tool combining a large field of view, of a potentially living specimen with high-resolution and the full ultrastructural context. The most crucial and often most time consuming step in a CLEM workflow is to find back the region of interest, which is challenging in single cells, but even more difficult in multicellular organisms. Therefore we have progressed on techniques for minimal resin embedding of multicellular specimens for targeted focused ion beam-scanning electron microscope (FIB-SEM) imaging (Kizilyaprak, Bittermann [1], Belu, Schnitker [2]). We have found slightly different approaches for zebrafish, *Platynereis* and *C. elegans* with the same goal to remove as much resin as possible. This enables direct targeting of the structure of interest in the FIB-SEM. The goal of this was two-fold: on one hand, reducing the layer of resin exposes the surface structures for direct visualization by LM and SEM, and on the other hand, the physical properties of the resin are compatible with stable imaging (using the FIB-SEM).

To minimize the resin, there were slight variations of the method between the three model organisms. For all, once the samples were taken through the EM processing they were gently placed with a toothpick on either absorbent paper, filter paper or Aclar, depending on the size of the organism. In each case the samples were gently moved around on their substrate until there was no more resin surrounding them.

For *C. elegans*, they were left on the piece of thick Aclar after being stripped of excess resin using a toothpick. The samples were polymerized in a 60°C oven for 48 h. The worms on top of Aclar were cut out and put on a SEM stub. All were sputter coated with gold for 180 seconds at 30 mA (Quorum, Q150RS). Silver paint was added to the samples to help with sample stability and charging. For the sake of presentation, all three models were mounted together on a SEM stub, but would be treated separately otherwise (Figure 1A).

The areas that were acquired were dictated only by the questions we had for each of the different model organisms, but were not limited by the technique since the whole organism was accessible. This method can be applied across a wide range of multicellular organisms as demonstrated and might as well be used in serial bloc-face SEM. The power of the minimal resin embedding relies on the straightforward targeting of precise regions of interest on multicellular organisms. Leading to an enhanced throughput, we expect this method to reach routine applications for volume EM imaging in a large number of laboratories.

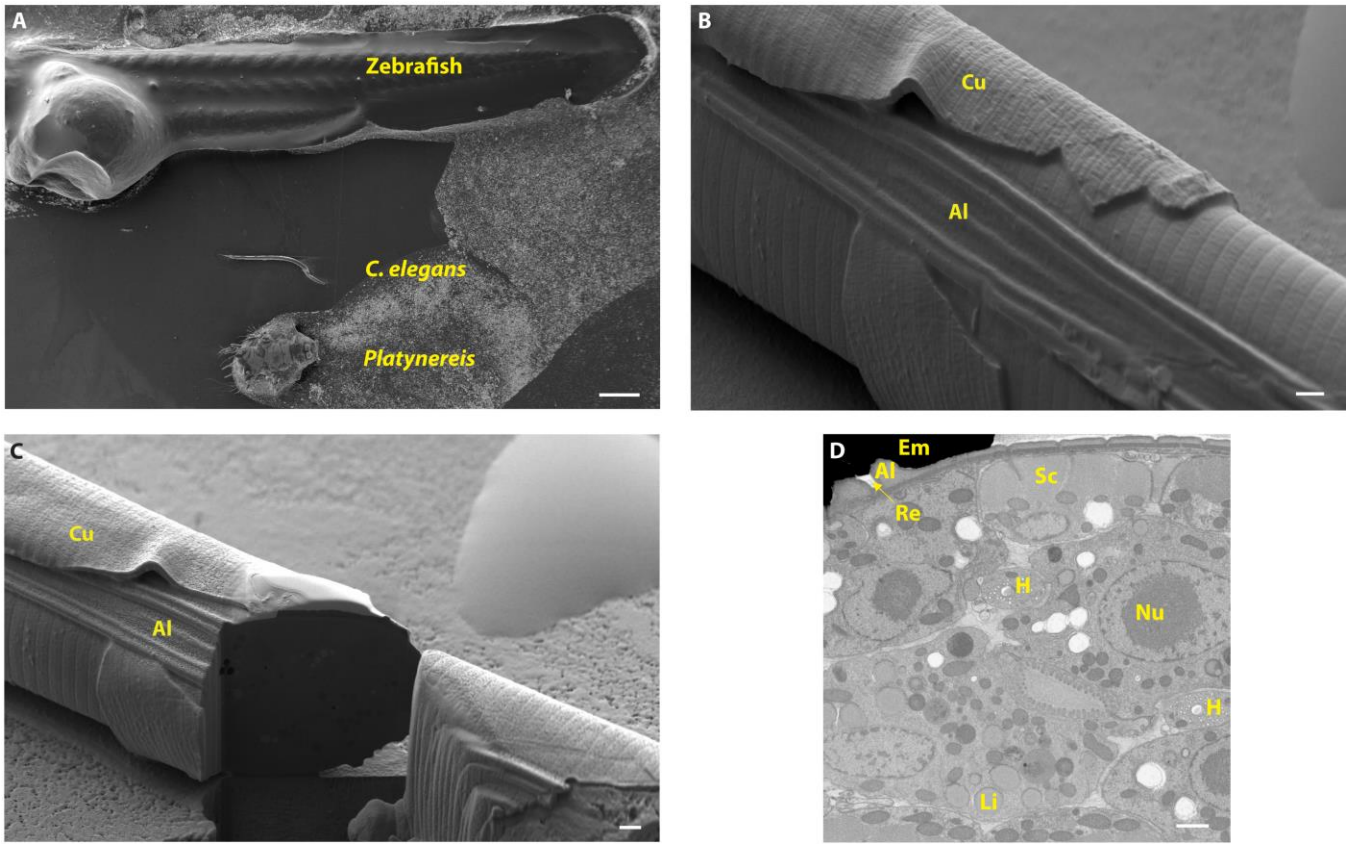

Figure 1. Overview of polymerized samples and FIB-SEM acquisition set up. A) SEM (SESI) image of zebrafish, *C. elegans* and *Platynereis* embedded with minimal resin inside a FIB-SEM, scale bar 200 µm. B) Detail SEM (SESI) image showing anatomical features of the *C. elegans* dauer larva including the cuticle (Cu) and the alae (Al) in their distinct pattern, scale bar 2 µm. C) SEM (EsB) imaging of the exposed cross-section, scale bar 2 µm. D) SEM (EsB) high magnification imaging of the exposed cross-section, scale bar 1 µm. Anatomical features of the *C. elegans* dauer larva including the cuticle (Cu) and the alae (Al) in their distinct pattern are visible in the cross-section as well as: Em = empty space, H = H-cell, Li = lipid droplets, Nu = nucleus, Sc = sarcomers, Re = resin.

References:

[1] Kizilyaprak, C., et al., *FIB-SEM tomography in biology.* Methods Mol Biol, 2014. **1117**: p. 541-58.

[2] Belu, A., et al., *Ultra-thin resin embedding method for scanning electron microscopy of individual cells on high and low aspect ratio 3D nanostructures.* J Microsc, 2016. **263**(1): p. 78-86.

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