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Ronald Myers, PhD
JoVE, Editorial Office
1 Alewife Center
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Göttingen, September 27, 2018

Dear Dr. Myers,

Please find enclosed our manuscript “Preparation of biological samples through high-pressure freezing, microwave-assisted processing and minimal resin embedding for FIB-SEM” prepared as invited submission to JoVE.

Since life science has seen many different advances in the realm of acquiring and analyzing samples in 3D the methods of sample preparation have become very important. Depending on the scientific question and the structure of interest different methods can be applied to prepare samples for electron microscopy.

For best sample preservation and best contrast we have combined different important techniques: High-pressure freezing is providing structural preservation close to the native state, while chemical fixation and contrast enhancement by a series of heavy metal impregnations is the best method to obtain excellent contrast for 3D imaging in the scanning electron microscope. By combining cryo-immobilization, freeze-substitution, followed by microwave-assisted post-staining for contrast enhancement, we manage to combine the best of both techniques. In the last preparation sample step the use of minimal resin embedding allows direct and faster targeting inside the FIB-SEM and reduces the time spend on exposing the region of interest.

In our manuscript we describe in detail the necessary preparative steps to provide a helpful resource for researchers working with 3D electron microscopy (FIB-SEM, SBEM and array tomography).

Thank you for handling our manuscript and I am looking forward to your response.

Sincerely,

(Dr. Wiebke Möbius)