From Electron Microscopy to 3D Printing

Serial Block-Face Scanning Electron Microscopy of Spores

The exhibition “Fungi – Food, Poison and Mythology” in the Museum Wiesbaden, Germany shows more than 1300 fungi models. As a special highlight macroscopic 3D reconstructions of spores are presented which have their origin in serial block-face scanning electron microscopy. This method was applied to embedded spores with diameters of some microns. Computer based 3D reconstructions, 3D printing and the production of hollow casts lead to 126 impressive objects featuring a magnification of about 20,000x.

Fungi – Food, Poison and Mythology

More than 1300 detailed fungi models are currently presented in the exhibition “Fungi – Food, Poison and Mythology”, covering more than 1,100 square metres in four halls of the Museum Wiesbaden. Visitors may find answers to several questions, like: “What are fungi and where can they be found? How can they benefit or harm us humans? In which way did early civilisations benefit from them?” [1]. Many of the presented models were manufactured by Klaus Wechsler, one of Germany’s most renowned taxidermists.

Mycology Meets Electron Microscopy

Figure 1 shows one of the exhibition rooms. Beside several ensembles of biology exhibits (biotopes, ecological niches), dozens of 3D models of spores are attached to the ceiling. Two years before the exhibition opened, it was the aim of the preparation expert Wechsler to get 3D reconstructions of spores. Since these “units of reproduction” are very small he was looking for an appropriate imaging method. By an internet research he found the Austrian Centre for Electron Microscopy and Nanoanalysis (FELMI-ZFE) in Graz, Austria, and visited the institute. At first he was only interested in imaging spores via scanning electron microscopy (SEM) and got to know the low vacuum mode of an environmental scanning electron microscope (ESEM) which enables the direct imaging of samples without an additional electrically conducting layer.
Back home he collected potential specimens of interest and sent them to Graz. Soon he got the results (i.e. micrographs of spores glued on an SEM stub with a carbon tape) and was impressed by the high resolution of the images.

Now he should have been choosing the species for further fabrication, but instead of continuing with conventional steps for preparation and modelling he came up with another idea. Remembering the presentation at the FELMI-ZFE about “serial block-face scanning electron microscopy” he thought: What about 3D reconstructions of spores by electron microscopy and transferring the data to a 3D printer?

**Serial Block-Face Scanning Electron Microscopy (SBEM)**

The technique SBEM, which was developed and firstly published in 2004 [2], enables the reconstruction of internal structures of specimens over hundreds of microns providing 3D information with an imaging resolution typical for SEM. Commonly, this method is performed via an in situ ultramicrotome in a variable-pressure SEM (VPSEM) or an ESEM at a low vacuum mode. It was firstly dedicated to investigations in neuroscience, where it was soon established as a method bridging the gap between high-resolution tomography in a transmission electron microscope (TEM) and light microscopy.

**A New Application for SBEM**

The Austrian Centre for Electron Microscopy was the first institute, which applied this method in the field of materials science and was the first to combine it with energy dispersive X-ray spectroscopy (EDX) [3]. Although there is a focus on projects in materials science, the institute has also several collaborations in the field of biology [4, 5]. However, the aim of producing the data for a 3D print of spores was still new.

Conventionally, samples for SBEM have to be embedded in resin before cutting.
Imaging is done using backscattered electrons (BSE) which deliver compositional contrast. It can be realised either by intrinsic contrast of a sample or especially in the field of biology by staining with heavy metals. A first series of images of pre-cuts of embedded spores already delivered enough contrast without additional staining. Hence, it was possible to reconstruct the spores immediately.

**From a Single Spore to the 3D Model**

Although staining could be omitted, the preparation steps were manifold: embedding the spores in resin, drying of the resin, pre-cutting of the block using a conventional microtome, mounting the specimens in the in situ ultramicrotome (3View of Gatan, Pleasanton, CA, USA), alignment of the electron microscope (ESEM Quanta 600 FEG, FEI, Eindhoven, the Netherlands) and “slice and view” of the samples, getting a stack of images, 3D reconstruction of the spores, exporting the results in a suitable data format for 3D printers, production of masters with a laser sintering method (Materialise, Leuven Belgium), producing of casting mould with the help of the real 3D prints and finally producing several 3D models by filling in polymer into the mould (rotational hollow casting). As a result, Klaus Wechsler produced 126 3D models of spores with diameters between 10 cm and 36 cm. Since the spores’ diameters are in the range of some microns (e.g. 5 µm) and the models have a diameter in the range of decimeters this equals a magnification of about 20,000x. Figure 2 shows five different 3D models of spores in a vitrine. On the bottom of the vitrine micrographs of the spores are presented. Figure 3 shows a table with two photographs of 3D models, one micrograph of the block-face of a specimen with several embedded spores and a 3D reconstruction performed with Avizo. With these impressive models visitors get a better insight into details of these biological objects.

**The Exhibition is Open Until August 5th 2018**

The exhibition opened its doors on June 11th 2017 and lasts until August 5th 2018. Beside the special aspect described in this article, several experiences and impressions can be gained. Numerous experts, lenders and organisations contributed, as listed in [1]. Visitors can explore and use a variety of media and enjoy the great diversity of themes and the quality of the exhibits. So far, this exhibition is the most extensive and far-reaching in the department of Natural History of Museum Wiesbaden. It was preceded by several years of scientific groundwork and research [1].

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