Synergy of SEM and Ultramicrotomy

SBEM Spreading from Life Sciences to Materials Science

Serial block-face scanning electron microscopy (SBFSEM, SBEM) is a well established method in life sciences. The combination of ultramicrotomy and scanning electron microscopy enables imaging with high resolution and the reconstruction of big volumes compared to other methods. Recent publications, where computer simulations were performed additionally to volumetric information about different materials, show that this method will contribute to material sciences, technology and industry services.

Serial Block Face Scanning Electron Microscopy, SBEM

In 2004 a groundbreaking paper was published by Denk and Horstmann [1] introducing a new technique called serial block-face scanning electron microscopy, abbreviated SBFSEM or SBEM. Here an ultramicrotome is located in the specimen chamber of a scanning electron microscope that enables the investigation of electrically non-conductive samples, either a variable-pressure scanning electron microscope (VPSEM) or an environmental scanning electron microscope (ESEM). Automated slicing and imaging of the block-face of a specimen is performed using a diamond knife for cutting. This serial imaging leads to a stack of micrographs which can be used for the 3D-reconstruction of specimens. The advantage of the use of a diamond knife in comparison to e.g. an ion beam which would be available in a focused ion beam microscope (FIB) is the fact that no implantation of ions into the material takes place and additionally a comparatively bigger sample volume can be investigated in an adequate time [2].

A Great Impact on Life Sciences

The first decade of SBEM was primarily characterized by publications in the field of life sciences. Especially in neuroscience two main advantages of the method can be combined: imaging with SEM resolution and investigating volumes of interest with macroscopic dimensions. This is possible since cuboids of about 0.6 x 0.6 x 0.6 mm³ can be cut by the microtome. As an example a 3D-reconstruction of a bundle of neurons of the visual system of a grasshopper is shown in figure 1. Here different colors indicate different single neurons.
Even synaptic connections can be identified which is vital for the understanding of the neurons’ functionality. In neuroscience, not only the authentic 3D-reconstruction of several neurons is relevant but also their precise connectivity. For this purpose special computer tools were developed in the community in order to shorten the time of investigation [3]. Below the bundle of neurons a typical block-face of the specimen can be seen, where the different biological structures, the cell membranes and the cell organelles were made visible by using heavy metal staining. This pretreatment increases not only the compositional contrast delivered by backscattered electrons (BSE), but hardens also the material in order to make it sliceable at room temperature. Figure 1 correlates to a paper where the connectivity within the grasshopper’s visual system was described [4]. There is high demand for this method, not only in neuroscience but generally in life sciences. Concerning the preparation techniques and the number of publications SBEM can be regarded as well established in life sciences.

**SBEM of Soft Matter Materials**

In 2009 first results in material sciences were presented in [5], describing applications on different soft matter materials. Again compositional contrast was used either due to different phases in the material or due to specimen preparation.
As an example figure 2 shows several steps in the 3D-analysis of a polymer blend (isotactic polypropylene/ethylene propylene rubber particles) after a tensile test. Here a staining protocol similar to life sciences had to be applied using ruthenium tetroxide, which enables the differentiation between the polypropylene matrix and the rubber particles. Figure 2a shows the slices of the block faces with particles and cracks, figure 2b the binarized images after applying a segmentation technique. The resulting 3D-reconstruction in figure 2c shows the cracks developing in the polymer blend as well as the crack surfaces and the position of the cracks related to the distribution of the particles. Additionally, further analyses like the distance map of the particles can easily be calculated (fig. 2d).

In [2] and [5] investigations on other types of soft matter materials are listed such as embedded paper, embedded membranes and materials including particles like talcum filled polypropylene (compositional contrast due to different phases) or foam-like structures like polymer monoliths (heavy metal staining).

**SBEM of Metals and Alloys**

In a recent paper [6] it was outlined that George E. Thompson from the University of Manchester and co-workers “have been at the vanguard of developing workflows to investigate the corrosion behavior of aluminum and magnesium, the influence of organic coatings on corrosion pathways, the distribution of pigments in the paint, and the distribution of intermetallics during casting or forming” (literally from [6]). In this paper several examples are described and special aspects of cutting metals with a diamond knife are mentioned. Although limits of the method are also discussed in [6] it is concluded that SBEM is capable of generating 3D-images of different engineering materials at nanoscale resolution over large volumes.

As an example, figure 3a shows the block face of an aluminum specimen. The compositional contrast due to regions of different chemistry enables a 3D-reconstruction (fig. 3b) of a sample volume showing the distribution of fine and coarse precipitates. In figure 3c the setup of the SBEM system is shown. While imaging is typically done with the BSE detector, the combination of SBEM and energy dispersive x-ray spectroscopy (EDS) was first published as 3D-elemental mapping in [2] and [7] (see also the online film with the title “3D elemental mapping in the ESEM”). Here an aluminum-copper alloy specimen of the type EN AW 2024 T351 was investigated and different precipitates (e.g. Mg₂Si, Al₂CuMg) were reconstructed from 3D-elemental maps of the elements Al, Cu, Mg, Mn, and Si.

**An Established Method with a Promising Future**
More than a decade after the publication of [1] SBEM can be regarded as well established in life sciences. However, recent publications show that this method is even promising in the field of materials science. It is not only useful to get 3D-reconstructions of volumes of interest but also to apply mathematical models [8] and furthermore computer simulations [9, 10]. Additionally the combination with other methods like EDS or Electron backscatter diffraction (EBSD) will contribute to science, technology and industry services.

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References


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