Serial block face scanning EM for 3D analysis in materials science

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The method

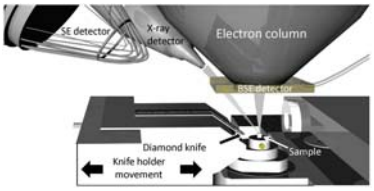


Fig. 1. Schematic of the setup: an ultramicrotome is attached to the door of an environmental scanning electron microscope enabling serial sectioning and imaging with different signals using typically backscattered elctrons, but also secondary electrons and X-rays.

Serial block face scanning electron microscopy (SBFSEM, nowadays called SBEM for serial block-face electron microscopy) combines the slicing technique of an ultramicrotome and the imaging capabilities of an environmental scanning electron microscope (ESEM). Automated slicing and viewing of the specimen´s blockface can be performed typically using backscattered electrons (BSE) for imaging.

The results presented here were carried out using an ESEM Quanta 600 FEG (FEI, Eindhoven, the Netherlands) and the serial block face sectioning and imaging tool 3View™ of Gatan, Inc. (Pleasanton, CA, USA). The 3View™ system is controlled by the software Digital Micrograph™.

Preparation

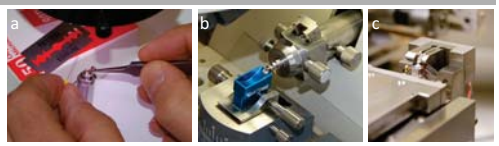


Fig. 2. Steps of preparation: fixation of the specimen on a rivet (a), precutting with a conventional microtome (b), alignment of sample holder and diamond knife (c).

Specimens of biological or soft material are typically fixed in a resin and glued on a special rivet which is screwed in a specimen holder. The samples usually have to be stained, in order to get sufficient compositional contrast. Then precutting with a conventional microtome has to be performed. After mounting of the specimen holder at the stage of the microtome the holder and the diamond knife have to be aligned. After these steps the automated slice and view process delivers stacks of images for 3D reconstruction.

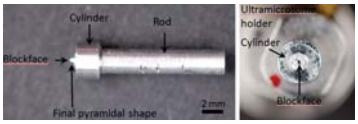


Fig. 3. The aluminium-copper alloy sample was manufactured by standard milling in order to get a rod and subsequent trimming with a glass knife delivers a pyramidal shape at the top for *in situ* ultramicrotomy.

Heterogenous polymeric materials

This technique was originally developed for neuroscience and is, up to now, mostly used in life sciences [1]. However, first results in the field of materials science were published in [2] and [3].

SBFSEM is operated in the low vacuum mode of the ESEM, enabling the investigation of electrically nonconductive materials without the necessity of a conductive coating. It gives three dimensional information about the structure of materials like paper samples, polymer blends, particle filled polymers and interfaces. Paper samples and talcum filled polypropylene [4] have sufficient intrinsic compositional contrast due to the different chemical composition of the phases. In the case of polymer blends staining with e.g. ruthenium tetroxide is required for imaging.

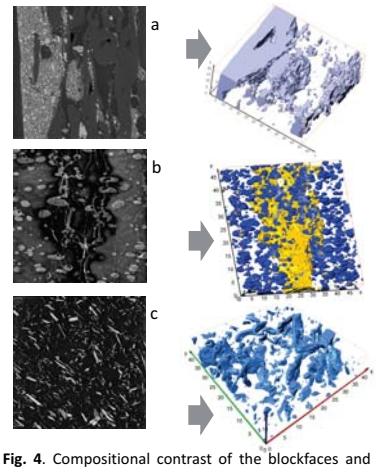


Fig. 4. Compositional contrast of the blockfaces and correlated 3D models: a: paper, b: iPP/EPR blend; c: talcum filled PP (image widths correlated to the dimensions of the 3D model, unit: microns).

Polymeric membranes

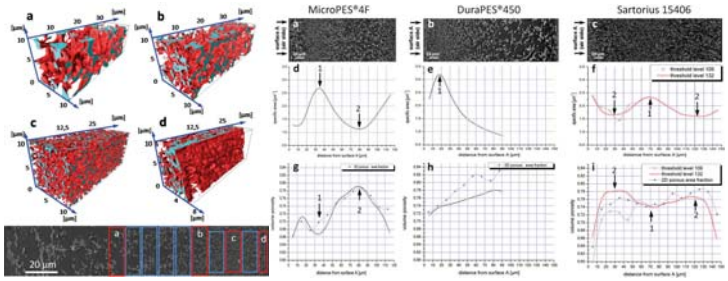


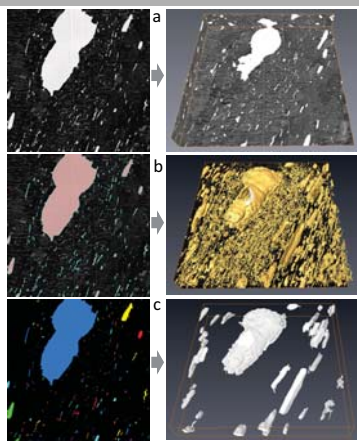
Fig. 5. Left: 3D reconstructions of different layers of the DuraPES®450 membrane: a: support layer; b: intermediate layer; c: separation layer; d: air side of the membrane. Each of these 3D reconstructions gives one data point for the respective parameter profiles as shown right: specific surface area (d-f) and volume porosity (g-i) as a function of the distance from surface A for the respective membranes.

Aside from three dimensional representations of special volumes, software programs like Avizo® deliver additional data, which are fundamental for further simulations. The 3D reconstruction of polyethersulfone membranes [3] delivered quantitative values for the characterisation of membranes´ properties. Before performing *in situ* ultramicrotomy the membranes had only to be fixed with resin. No staining had to be performed, since the sulfur containing material delivered enough compositional contrast.

Aluminium - morphological information

Fig. 6. Contrast and thresholding: a: compositional contrast and the correlated ortho-slices; b: thresholding and furhter isosurface rendering elucidates morphology and orientation of the precipitates; c: by labeling of the inhomogeneities different geometrical properties (e.g. by a volume threshold) can be filtered.

The aluminium-copper alloy of the type EN AW 2024 T351 (by AMAG, Ranshofen, Austria) delivers compositonal contrast due to different chemical elements of the precipitates in the material. Additionally it is sliceable with a diamond knife. By different levels of thresholding morphological information and statistical values concerning different geometrical properties can be gained. Due to its electrical conductivity *in situ* ultramicrotomy can be performed in the high vacuum mode of the ESEM.



Aluminium - 3D elemental mapping

Additionally to the three dimensional (3D) structural information it is desirable to get 3D chemical information by energy dispersive X-ray spectrometry (EDS) [5]. The recording of the respective elemental maps was carried out with an X-Max® Silicon Drift Detector (Oxford Instruments Analytical Ltd., UK) with an 80 mm² active detection area. This system facilitated the investigation of the distribution of the chemical phases in the aluminum-copper alloy.

A total of 200 slices with a thickness of 100 nm, corresponding to a volume of (26 × 22 × 20) µm³, was cut. After each cut an image and a spectrum map were recorded (total measurement time: about 22 hours). Subsequently the spectrum maps were quantified with the INCA™ software.

Fig. 7 shows the resulting 3D reconstructions of the distribution of the different phases. In (a) the reconstruction of the major S-phase (Al_2CuMg) can be seen, in (b) further minor phases like $Al_{20}Cu_2Mn_3$ (T-phase, violet), Al_2Cu (blue) Al_7Cu_2Fe (grey), $Al_{12}(Mn,Fe)_3Si$ (green) and Mg_2Si (red) are marked by different colors.

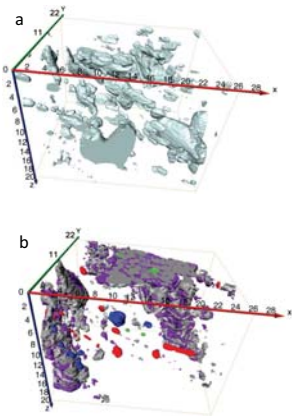


Fig. 7. 3D distribution (unit: microns) of different phases in the aluminium-copper alloy (see text).

References

[1] W. Denk & H. Horstmann PLoS Biol 2 (2004): e329.

[2] A. Zankel, B. Kraus, P. Poelt, M. Schaffer, E. Ingolic, J. Microsc. 233 (2009) p140.

[3] H. Reingruber, A. Zankel, C. Mayrhofer, P. Poelt, J. Membr. Sci. 372 (2011) p66.

[4] T. Koch, D. Salaberger, A. Zankel, H. Reingruber, A. Steiger-Thirsfeld, Y. Voronko, S. Seidler, Macromol. Symp. 315 (2012) p122.

[5] A. Zankel, R. Reingruber and H. Schroettner, Imaging and Microscopy 2 (2011) 35.

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