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3D X-ray Microscopy: High-resolution Stereo-imaging with the Göttingen X-ray Microscope at BESSY

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A multiple-angle viewing stage has been built at the Forschungseinrichtung (FE) Röntgenphysik (University of Göttingen) and was implemented at the Göttingen x-ray microscope at BESSY (Schmahl et al. 1993). First TXM (transmission x-ray microscope) stereo images have been taken of sheaths of mineral accumulating bacteria *Leptothrix Ochracea* and diatoms. It results that the three-dimensional structure of these specimens can be revealed at high resolution. For the studies a microzoneplate with an outermost zonewidth of about 40 *nm* was used at a wavelength of λ=2.4 *nm*. Structures of 40 *nm* size can clearly be seen.

INTRODUCTION

The aim of x-ray microscopy is to study specimens in a natural environment at resolutions far beyond those of visible light microscopes. Image-formation is based on the natural contrast of the specimen-structures against the surrounding media. Currently this technique is applied to various fields of interest including biology, medical research and soil science. The investigated specimens typically have a thickness of up to 10µm, which is similar to the size of the image-field of the Göttingen TXM. It is therefore obvious that the three-dimensional structure of the objects must be considered. As reflection at interfaces is neglegibly small in the soft x-ray domain, it is possible to look into thick specimens that are opaque for visible light. The TXM is therefore an ideal tool for the study of three-dimensional specimens. When using a TXM in the traditional way, the information about one dimension is lost. Since the depth of focus of the TXM is of comparable size as the thickness of the objects, an appropriate approach to recover this information is to apply the technique of stereoscopy to the TXM. First stereoscopy-experiments with resolutions beyond those of visible light methods have been performed by the Lawrence Berkeley Laboratory (Loo et al. 1992), using the scanning transmission x-ray microscope (STXM) at the National Synchrotron Light Source, Brookhaven National Laboratory.

EXPERIMENTAL

The optimum tilt angle for stereoscopic images can be estimated from the classical parallax equation, which relates the parallax ΔY to the angle of tilt, the magnification M and the vertical separation Δh of two points in the object at the angle midway between the two tilts.

$$\Delta Y = 2\Delta h M \sin(\Theta/2) \quad (1)$$

For a good three-dimensional impression, the parallax ΔY should be in the range between 3 and 5 *mm* (Hudson and Makin, 1970), when stereo-pairs are viewed at a distance of 25 *cm* from the eyes. Assuming a total magnification of *M*=4000 and an object thickness of 5 µm, optimum tilt angles Θ between 5 and 15 degrees can be calculated. At the Göttingen TXM the maximum tilt angle is too small for stereo-views, if a conventional specimen holder with large dimensions in the direction perpendicular to the rotation axis is used. To solve this problem without major changes to the microscope, a multiple-angle viewing stage with a specimen-support of 0,5 *mm* width was constructed. Currently, tilt angles of about 40 degrees can be achieved with this modification.

RESULTS

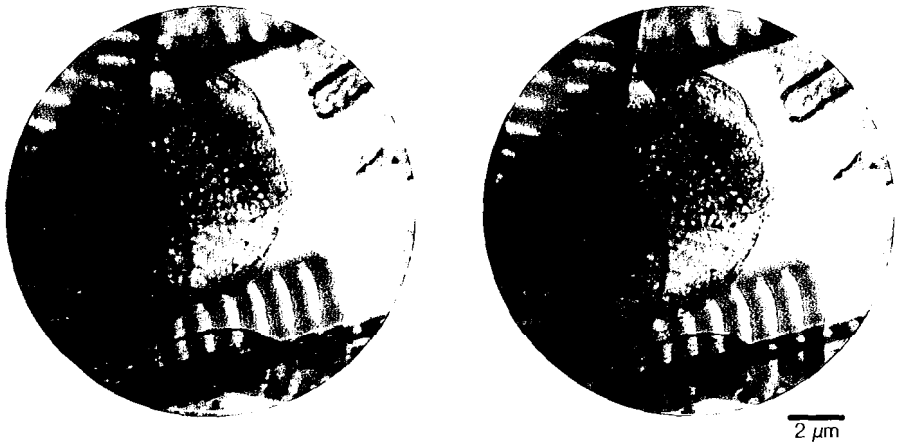

Fig. 1. Stereo-pair images of sheaths of the mineral accumulating bacteria *Lepothrix Ochracea*. The stereo angle is 12.5 degrees.

Fig. 2. Stereo-pair images of diatoms at a stereo angle of 5 degrees.

In a first series of experiments images of sheaths of mineal accumulating bacteria *Leptothrix Ochracea* (Fig. 1) and diatoms (Fig. 2) were taken from various viewing angles. The exposure times were around 2 seconds. From measurements of the object-thickness it can be seen that the range of depth that contributes to the three-dimensional image is much bigger than the depth of focus. In Fig. 1 for example, the obect-thickness is 9 μm, whereas the depth of focus is about 1.5μm. It is therefore very likely that stereo-images can be taken at even higher resolution, even if the depth of focus is less than 0.5 μm, when a microzoneplate with an outermost zonewidth of 20 *nm* is used. Stereoscopy combined with x-ray microscopy proofs to be a powerful technique for studying the three-dimensional structure of specimens from biology and soil science, as the results show. For future applications of this technique it will be advantageous to combine it with the cryo-preparation method, which is also under development at the FE Rontgenphysik (Schneider and Niemann 1994). The range of specimens, which can be examinated will be extended to objects in aqueous medium and further applications in biology and medical research will be enabled.

REFERENCES

Schmahl G, D Rudoph et al. 1993. X-Ray Microscopy Studies Optik **93:** pp. 95-102.

Loo jr, BW, S Williams, S Meizel, XS Rothman. 1992. X-Ray stereomicroscopy: High resolution 3-D imaging of human spermatozoa in aqueous suspension with natural contrast. Journal of Microscopy, Vol. **166:** Pt 2, May pp. PR5-PR6.

Hudson B, MJ Makin. 1970. The optimum tilt angle for electron stereo-microscopy Journal of Physics E: Scientific Instruments Volume **3:** p 311.

Schneider G, B Niemann. 1994. Cryo-X-Ray Microscopy: First Images of Specimens at Low Temperatures X-Ray-Science (Newsletter of the Kings College, London), Issue 2, Summer.