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HIGH RESOLUTION X-RAY MICROSCOPY WITH ZONE PLATE MICROSCOPES

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Résumé - Des expériences de microscopie par rayons X ont été réalisées à l'aide d'un microscope à rayons X installé sur l'anneau de stockage BESSY à Berlin.

Abstract - X-ray microscopy experiments are described which have been performed with an x-ray microscope at the electron storage ring BESSY in Berlin.

I - INTRODUCTION

X-ray microscopy can be used for investigations in the fields of biology, medicine, physics, especially materials science, and probably other fields, as polymer science, ceramics, geology, and thin film technology.

For the examination of biological specimens there is a gap between light and electron microscopy. Such specimens are normally hydrated and are composed of low atomic number atoms thus having low contrast for visible light and electrons. For the examination in light- and especially electron microscopes the biological samples have to be treated chemically and/or physically which may alter the elemental composition or ultrastructure of the sample.

In the soft x-ray wavelength region between 2.3 to 4.4 nm the absorption coefficients of water and e.g. protein differ by about one order of magnitude. As already pointed out by Wolter /1/ this difference provides a natural contrast mechanism for the investigation of wet biological samples.

Fig. 1 - X-ray microscope at the BESSY storage ring in Berlin.
Microscopy with soft x-rays requires intense x-ray sources as well as high resolution x-ray lenses. Intense x-radiation is provided by the synchrotron radiation of electron storage rings. Additionally, x-ray plasma sources, possibly suited as laboratory sources for x-ray microscopy, are under development. Suited optical elements for x-ray microscopy work are zone plates.

The state of the art of x-ray microscopy - including x-ray sources, x-ray optical elements, x-ray microscopes and applications of x-ray microscopy - is described in the volume "X-RAY MICROSCOPY"/2/.

Fig. 2 - Schematic arrangement of the x-ray microscope.

Fig. 3 - Arrangement of the x-ray optical elements.
II - X-RAY MICROSCOPE

The x-ray microscope consists of a condenser zone plate, a diaphragm in the object plane, a micro zone plate, a camera and a channelplate to convert the x-ray image to a visible image (Fig. 1 and 2).

The condenser zone plate is illuminated by polychromatic x-radiation of the x-ray source located at a distance of 15m and concentrates the radiation into the object plane. In addition the condenser acts together with the diaphragm as a linear monochromator to provide the quasimonochromatic radiation with $\lambda/\Delta\lambda=n/2$ necessary for imaging with the micro zone plate having n zones. The spectral resolution is given by $\lambda/\Delta\lambda=D/2d$ with D = diameter of the condenser and d = diameter of the diaphragm. The micro zone plate generates an enlarged image of the object placed on the diaphragm. This image can either be viewed directly using the channelplate or be photographed.

To avoid reduction of the contrast in the enlarged x-ray image by zero order radiation the condenser was apodized as shown in Fig. 3. The apodized region of the condenser firstly avoids zero order radiation of the condenser reaching the object and second ensures that in the image field the central region is free from zero order radiation and radiation from all negative orders of the micro zone plate. In addition, a stop shades second and higher order radiation.

The zone plates are made by an interferographic method as described by Schmahl et al /3/. The parameters of the micro zone plates (MZP) and condenser zone plates (KZP) used up to now in our x-ray microscopy experiments are given in Table 1 ( $r_1$ = radius of the first zone, $r_n$ = radius of the outermost zone, $d_r$ = width of the outermost zone, $n$ = zone number, $f$ = focal length ).

Fig. 4 - Diatom, imaged with $\lambda = 4.5\text{nm}$, x-ray magnification 500 x.

Fig. 5 - Part of a giant chromosome of the salivary glands of larvae of Chironomus thummi. $\lambda = 4.5\text{nm}$, x-ray magnification 250 x.
At the BESSY storage ring, x-ray microscopy experiments have been made using the condenser zone plate KZP 3 and the micro zone plate MZP 3 and the wavelength $\lambda = 4.5\,\text{nm}$. With a source diameter of about 1 mm the monochromatic image of the source in the object plane has a diameter of $d = 20\,\mu\text{m}$ resulting in $\frac{\lambda}{\Delta \lambda} = 225$. This meets the requirements of MZP 3.

With the storage ring current of 100 mA, a condenser zone plate efficiency of 4% and absorption losses in the membranes of the entrance window and object chamber of about 50% the photon density in the object plane is about $4 \times 10^4$ photons/$\mu\text{m}^2\,\text{s}$.

X-ray photographs have been made with an x-ray magnification between 250 x and 500 x with exposure times ranging from 2 to 30 seconds. Examples are shown in Fig. 4 and Fig. 5 with a resolution of about 0.05 $\mu$ m.

### III - SCANNING X-RAY MICROSCOPE

The necessity to build a scanning x-ray microscope results from the advantageously reduced radiation dose of such a system compared with an imaging x-ray microscope.

In an imaging x-ray microscope the x-rays first enter the object and then pass the magnifying micro zone plate, which has usually a diffraction efficiency of less than ten percent. Thus, more radiation passes the object than is diffracted into the image. In an x-ray scanning microscope the x-rays first pass the micro zone plate. Only the x-rays which are diffracted into the scan spot enter the object and they all contribute to the image. Also the detective quantum efficiency of the counter in the scanning microscope is much higher than of photographic plates used up to now in the imaging microscope. But one has to bear in mind that the dosage advantage of a scanning x-ray microscope decreases with the development of efficient area detectors and zone plates with high diffraction efficiencies (phase zone plates). It should also be mentioned that the time necessary to scan an image is always longer than the time necessary in the imaging mode.
Fig. 6 shows a schematic of the scanning system which has been built at the Göttingen University and will be tested at the electron storage ring BESSY. The small x-ray image spot which is necessary for a scanning microscope is achieved with two zone plates. The first zone plate is several mm in diameter and by adjusting a pinhole of some \( \mu \)m in diameter to the focus of a selected wavelength, it acts also as a linear monochromator. The following micro zone plate, now illuminated with quasimonochromatic radiation from the pinhole, produces the scan spot of some ten nanometer in diameter; the spot is the demagnified image of the pinhole. The transmitted radiation will be measured by a high efficient x-ray detector and a picture will be built up on a monitor. As an x-ray detector gas proportional or gas scintillation counters can be used with 50 \% or more detective quantum efficiency. This high quantum efficiency in combination with the special optical arrangement leads to a radiation dose in the object about two orders of magnitude less than in the imaging x-ray microscope used up to now. The scanning stage is performed mechanically and must be able to produce step sizes of 5 nm in order to achieve 10 nm resolution. The steps will be done with arms of lever which rest at torsion-joints free of backlash. The amplitude will be measured at the free end of the lever. The motions and the image formation is controlled by a computer.

References

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