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Techniques and Applications of X-ray Microscopy

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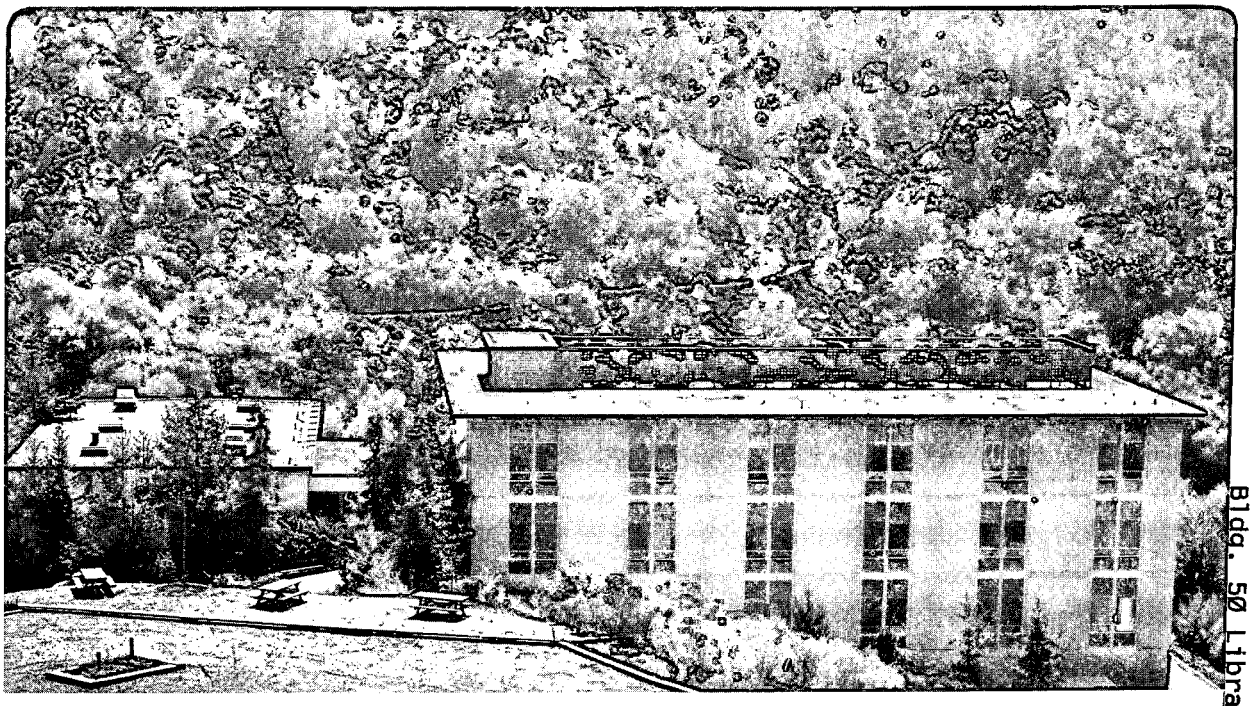

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Techniques and Applications of X-ray Microscopy

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X-ray microscopy has evolved to the point where a five fold improvement in resolution over visible light microscopy is achieved with further improvements expected in the near future. Applications of x-ray methods are being developed for both biological and material sciences and include high resolution imaging, elemental and chemical mapping, and x-ray excited fluorescence techniques. The critical components of an x-ray microscope are the light source, optical elements, detector, and sample holder. A Biological X-ray Microscopy Resource Center is planned at the Advanced Light Source (ALS) in Berkeley. This center will include an x-ray microscope (XM) and a scanning x-ray microscope (SXM). Both microscopes will make use of zone plates as high resolution lenses. Experiments have been performed with the Göttingen x-ray microscope at BESSY (Berlin) to test zone plates, x-ray CCD detectors, and sample preparation techniques. A variety of biological materials including sperm cells, human erythrocytes, and other mammalian cells have been studied using different methods of sample preparation (e.g., fixed and unfixed, different supports, suspension media, etc.). With the x-ray microscope (XM), the use of the CCD detector led to a great decrease in exposure time (approx. 10 fold) compared to photographic emulsion, and eliminated the time needed for photographic processing. We report on these experiments and discuss the implications for the system design of the Biological X-ray Microscopy Resource Center at the ALS.

1. INTRODUCTION

X-ray microscopy is expected to yield improved resolution compared to visible light microscopy without the constraints of maintaining samples in vacuum or resorting to preparing very thin sections. The resolution of x-ray microscopes is presently limited by optical components and the radiation damage to the sample, but not by the wavelength of the radiation. The highest resolution achieved is about five times that of visible light microscopy with further improvements expected from future development of x-ray optics (predominantly zone plates). X-ray microscopes can image samples in aqueous physiological environments and with specimens substantially thicker than in an electron microscope (up to 10 μm). Although a major objective of x-ray microscopy is the imaging of untreated biological material initially in the living state, it is not limited to it.

Fixation and labeling techniques uniquely designed to take advantage of the properties of soft x-rays are presently being developed. Recent progress indicates that fluorescent labeling techniques, which play an important role in visible light microscopy, can be extended to higher resolution by use of x-ray microscopes [1]. Instrument design at the ALS will be specifically developed to exploit these evolving methodologies.

2. TECHNIQUES

X-ray microscopes can be designed analogous to conventional visible light microscopes, or as scanning x-ray microscopes. The best examples of these microscopes are the Göttingen X-ray Microscope at BESSY [2,3] and the Scanning X-ray Transmission Microscope (STXM) at the NSLS [4,5].

2.1. High resolution x-ray imaging

The most basic imaging method of x-ray microscopy depends on natural contrast produced in biological samples in native physiological environments. Natural contrast is provided by element specific x-ray interactions with atoms in the sample. Within the so called x-ray water-window between 2.34 nm and 4.37 nm (the K-absorption edges of oxygen and carbon), x-rays are more strongly absorbed in biological material than in water. This results in amplitude contrast images. Along with x-ray absorption, contrast can also be attained with a phase contrast x-ray microscope [3].

X-ray microscopes of the Göttingen type achieve the highest possible resolution and the shortest exposure times.

2.2. Scanning x-ray microscopy

Scanning x-ray microscopes are more complex and make much less efficient use of the delivered x-ray flux because they can operate only with the spatially coherent portion of the synchrotron radiation; as a consequence an undulator is highly desired for practical scanning x-ray microscopy.

Although imaging times for scanning microscopes are comparatively long, they have significant advantages in certain types of applications. Since scanning microscopy does not require a lens downstream of the specimen, the necessary radiation dose to get a given signal to noise ratio is greatly reduced. This is because the zone plate lenses presently have efficiencies below 11%. In addition, the scanning mode makes it possible to use fluorescent labeling techniques whose resolution is given by the size of the scanning spot and not the wavelength of the emitted radiation [1,6].

2.3. Elemental and chemical mapping

As the atomic scattering factors depend on the wavelength, images taken at different wavelengths can be used to determine the elemental distribution within the sample at very high spatial resolution [7]. This allows elemental maps including those of carbon, nitrogen, chloride, potassium, and calcium. As the fine structure of the absorption edges depends on the chemical state of the element, in some cases even chemical distributions can be obtained [7].

3. X-RAY MICROSCOPES PLANNED AT THE ALS

The Center for X-ray Optics (CXRO), Lawrence Berkeley Laboratory, has begun to design complementary imaging and scanning x-ray microscopes for the Advanced Light Source (ALS) in Berkeley. These microscopes will form a Biological X-ray Microscopy Resource Center [8]. The x-ray microscope (XM) will be installed on a bending magnet first. Its optical design is similar to the Göttingen X-ray Microscope at BESSY and is planned to begin operation in 1994. The scanning x-ray microscope (SXM) will use the radiation from an undulator with 123 poles and a period of 3.65 cm. The high brightness of this undulator at 2.4 nm ($3x10^{18}$ photons sec^{-1} mm^{-2} $mrad^{-2}$ 0.1%BW) will allow images of 1000 by 1000 pixels to be captured within 5 seconds (1000 photons detected per 30 nm pixel). In the case of the imaging microscope, the undulator will permit 1000 by 1000 pixel images to be acquired within 20 msec.

3.1. X-ray microscope (XM) on an ALS bending magnet

The XM will be the first microscope completed at the ALS. It will be installed on beam line 6.12, which is a bending magnet port. The horizontal and vertical beam size are expected to be 67 μm and 140 μm (rms) with a divergence of 0.45 mrad (rms) at 2.4 nm wavelength. To remove hard x-rays, a mirror deflects the radiation horizontally from the source to the microscope with an angle of 6°. The use of this mirror also shields the x-ray microscope from the direct beam of the ALS. The sample is illuminated with a linear monochromator, using a condenser zone plate. An enlarged high resolution image of the sample is formed on a CCD camera by a second zone plate acting as an objective lens. The CCD is a thinned and back-illuminated device [9]. Exposure times for images with 1000 by 1000 pixels using the bending magnet source are expected to be 3 seconds (1000 photons detected per 30 nm pixel).

The mechanical design of the x-ray microscope provides full integration of state of the art visible light microscopes that will permit examination of the sample before and after x-ray imaging. To achieve this, we incorporated two visible light microscopes into the design. The first visible light microscope will be used to view the sample before mounting in the XM (external microscope). Initially, a Zeiss Axioplan equipped with several contrast methods including phase contrast and fluorescence will be used. Subsequently, this can be replaced with any other microscope including a confocal microscope. Using the external microscope, the sample will be precisely mapped and focused for significant features. Their locations will be stored digitally and later used by the XM for positioning and focusing. The sample carrier will use kinematic mounts to assure precise positioning of the specimen in either microscope. As the depth of field of a high resolution visible light microscope is comparable to an x-ray microscope, there will be no fine-focusing necessary with the XM. This will permit a high degree of automation of the image taking process and should provide high experimental throughput.

The second microscope will be mechanically built into the XM (alignment microscope) and will be used to view the sample mounted in the x-ray microscope stage. This microscope will employ Nomarski interference contrast (in reflection) and epi-

fluorescence and will provide the necessary placement accuracy to check the alignment of the sample when mounted in the XM.

Eventually, a cryogenic and tilt stage will be incorporated into the XM providing multiple views for stereo and tomographic reconstructions. The cryogenic feature will also be very useful in preventing radiation damage to the specimens.

4. RECENT EXPERIMENTS AT BESSY

The following presents examples of recently obtained results using the Göttingen x-ray microscope at BESSY.

Several experimental runs were done with zone plates fabricated by CXRO [10]. Short exposure times (a few seconds or less), visible light pre-focusing [2], and the newly installed back-illuminated CCD [9] provided greatly increased specimen turnaround times.

Glutaraldehyde fixed malaria infected human red blood cells were imaged wet in physiological solution. *Plasmodium falciparum* infected human erythrocytes from continuous *in vitro* culture, were fixed in 2% glutaraldehyde in physiological buffered salt (PBS), washed, and then imaged in the XM. Figure 1 shows two cells, one of them infected with a mature trophozoite stage parasite. The adjacent cell is uninfected and shows the characteristic biconcave morphology. In further experiments we plan to investigate the morphological changes characteristic of parasite development during its life cycle. In addition, we expect the parasite infected cells to present an ideal test object for evaluating tomographic reconstruction techniques. It is noteworthy, that the short exposure times of the x-ray microscope makes it practical to investigate a large number of specimens; this is crucial to many biological imaging problems because of their generally large statistical variations, whether it be morphological or structural.

Figure 2 shows an x-ray microscope image of a human metaphase chromosome from a T1 cell. The cells were fixed in methanol-acetic acid, which appeared to be less favorable compared to the glutaraldehyde fixation we used with other samples.

Figure 3 shows the sperm-head of a marsupial mouse with its tail attached. The sample was fixed in 3% glutaraldehyde. The image consists of an assembly of individual exposures of adjacent areas. The tail is unusually large for a sperm, and we show only a very small part of it here.

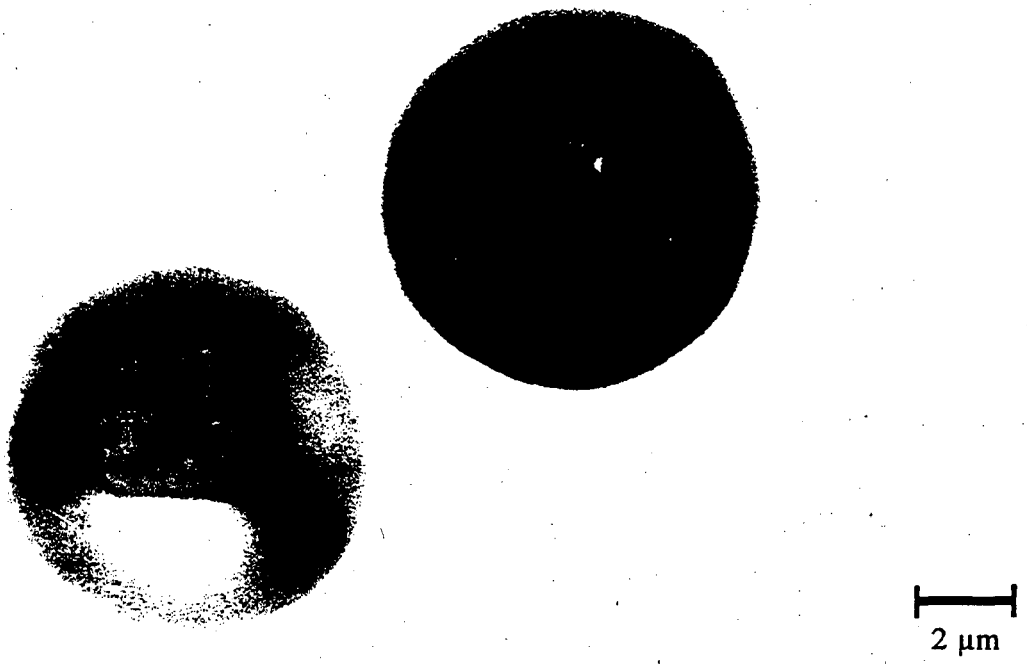

Figure 1. Intact human red blood cells. One is infected with a mature trophozoite stage malaria parasite. The specimen was imaged with the Göttingen X-ray Microscope at BESSY in Berlin using a zone plate with 35 nm outermost zone width.

Figure 2. Metaphase human chromosome from a T1 cell. (Sample prepared by K. Bjornstad, E. Blakely, Lawrence Berkeley Laboratory, USA)

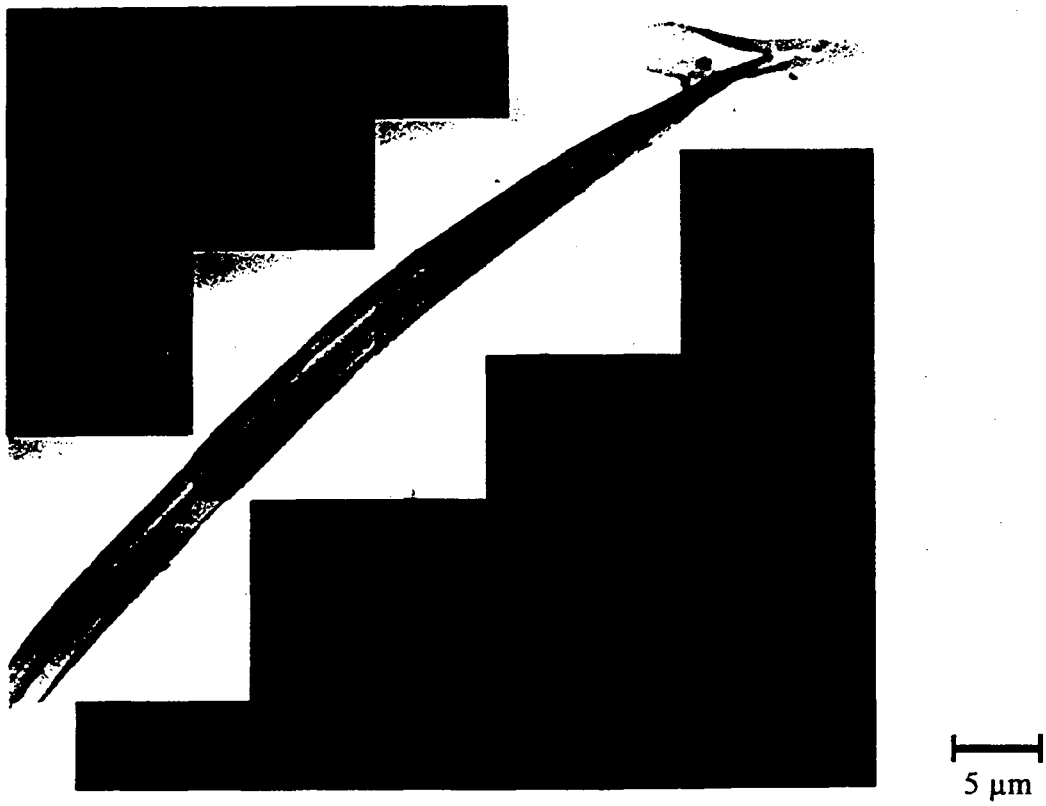

Figure 3. Sperm head of a marsupial mouse. The sample was fixed in 3% glutaraldehyde. The image shown here was constructed from adjacent exposures. As the tail of this sperm is very large, only a very small part of it is shown. (Sample prepared by W. Breed, Univ. Adelaide, Australia and R. Balhorn, Lawrence Livermore National Laboratory, USA)

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