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X-Ray Microscopy Resource Center at the Advanced Light Source

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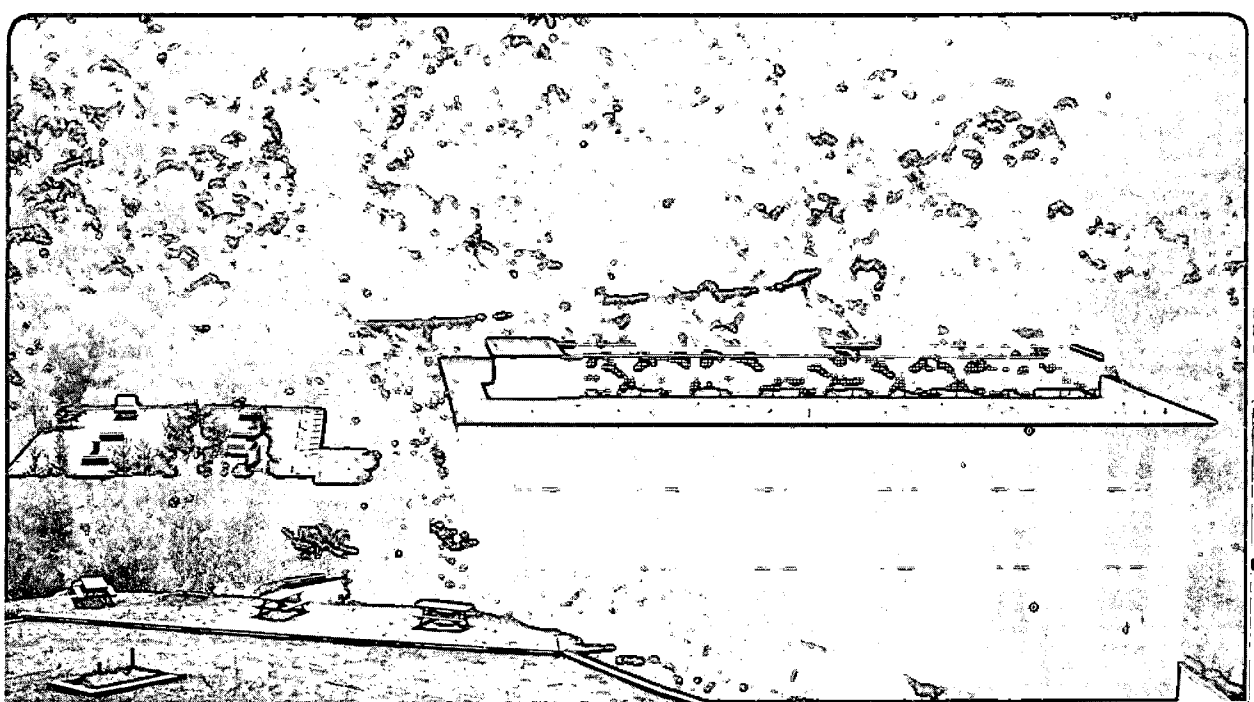

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X-ray Microscopy Resource Center at the Advanced Light Source

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1. Introduction

The high spectral brightness of undulator radiation from the Advanced Light Source (ALS) offers a great scientific opportunity for biological x-ray microscopy. X-ray microscopy extends visible light microscopy to higher resolution and makes use of unique contrast mechanisms. It does not compete with techniques such as electron microscopy in terms of resolution, but rather offers unique advantages, including the opportunity to take images of samples in an aqueous environment. For a considerable range of resolution and sample thickness the radiation dose in x-ray microscopy is lower than in electron microscopy under the same imaging conditions [1,2].

To exploit this opportunity a Biological X-ray Microscopy Resource Center will be built at the ALS. Two types of x-ray microscopes are to be built: an x-ray microscope (XM) and a scanning x-ray microscope (SXM). These two microscopes serve complementary needs. The XM gives high quality images at comparably short exposure times, while the SXM is optimized for low radiation dose. High resolution is accomplished in both microscopes with Fresnel zone plate lenses. The SXM produces a diffraction-limited focus point, which is scanned across the sample; therefore the SXM can use only the spatially coherent portion of the radiation. Accordingly the SXM is best operated on an undulator source with its small phase space. On the other hand, an XM can use the full brightness, including the incoherent fraction of the source. This means it can be operated with either a bending magnet or an undulator source. Although exposure times are shorter with an undulator, the XM can be installed initially at a bending magnet, which can be available at an earlier time, and thus permits the development of diverse biological community at an earlier time. Later this XM can be moved to the undulator, or left at the bending magnet for developmental and less demanding experiments.

The schematic layout of the x-ray microscopes is shown in figure 1. The microscopes make use of undulator radiation from a 3.65 cm period undulator and a bending magnet. The microscopy area will be enclosed in a noise and dust isolated room. Following initial design work which began early 1992, it is planned that construction work will begin early in 1993. It is expected to take about one year to build the first microscope, the XM at a bending magnet. It will be operational at the end of 1993. Design and some long lead time construction work for the SXM will also begin in 1993. The main effort for the SXM will occur in 1994. The undulator is expected to be operational early in 1995, so that the SXM will be ready for operation early in 1995.

The x-ray microscopes at the ALS will be high quality instruments for biological research. They provide high resolution and great flexibility in a reliable and easy accessible system. A high quality visible light microscope is included for sample selection, adjustment, and check. Laboratories for sample preparation and characterization are planned on site. Thus the X-ray Microscopy Resource Center at the ALS allows a broad range of biological research. A diverse community of biologists will evolve as soon as the first x-ray microscope becomes available. Because the XM at the bending magnet can be available earlier, we plan to build it first, with construction of the SXM to follow. To continuously maintain at least one operational microscope, moving the XM to the undulator will be delayed until the SXM is fully operational. It may be possible to keep the XM at the bending magnet for less demanding experiments.

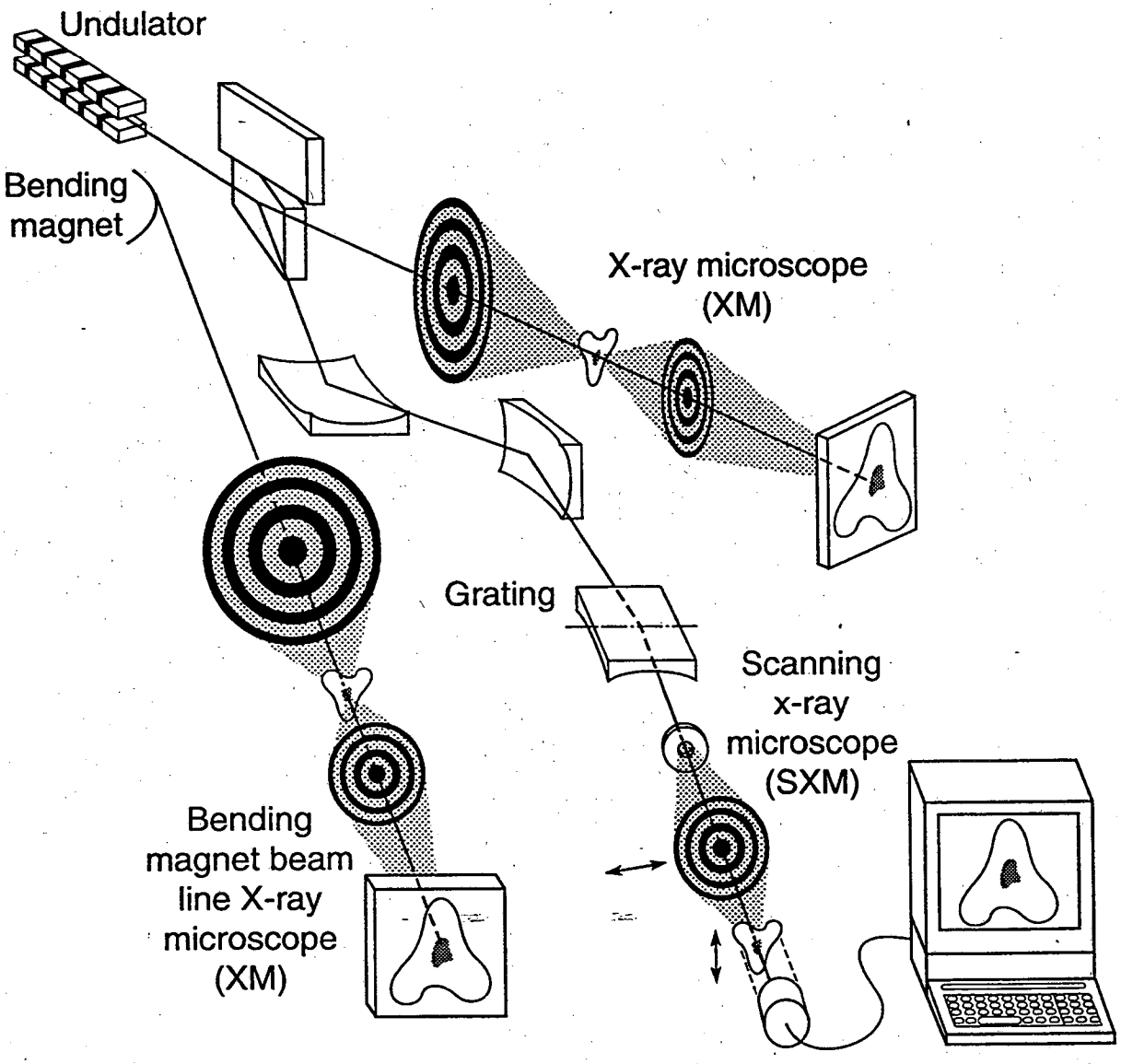

Fig. 1: Schematic layout of the Biological X-ray Microscopy Resource Center at the ALS.

2. Specifications of the X-ray Microscopes

The spatial resolution of the x-ray microscopes is determined by the resolution of microscope objective lenses used. Highest resolution is currently achieved by zone plate lenses. As for visible light microscopy, the diffraction limited resolution δ is expressed as a function of the wavelength λ and the numerical aperture NA of the lens:

$$\delta = 0.61\frac{\lambda}{NA}.$$

For zone plate lenses, where the numerical aperture is a function of wavelength, this formula can be written as:

$$\delta = 1.22\Delta$$

with Δ being the width of the outermost zone. Zone plates with an outermost zone width of 300Å are presently available [3]. In early experiments we expect to use zone plates with an outermost zone width of 250Å [4], which could achieve a diffraction limited resolution of 300Å. An example of an x-ray micro graph is shown in figure 2. The sample, a dried human sperm, was chosen to illustrate the imaging capabilities of x-ray microscopy. The image was taken in a collaboration with the Göttingen x-ray microscopy group with their XM at BESSY [5] at a wavelength of 24Å. The micro zone plate with an outermost zone width Δ=350Å was fabricated by Erik Anderson in a collaboration of the Lawrence Berkeley Laboratory/Center for X-ray Optics with IBM. The image shows detailed structures, especially in the head and tail of the sperm. Artifacts from the drying process, probably salt crystals, are concentrated in the aft region of the specimen.

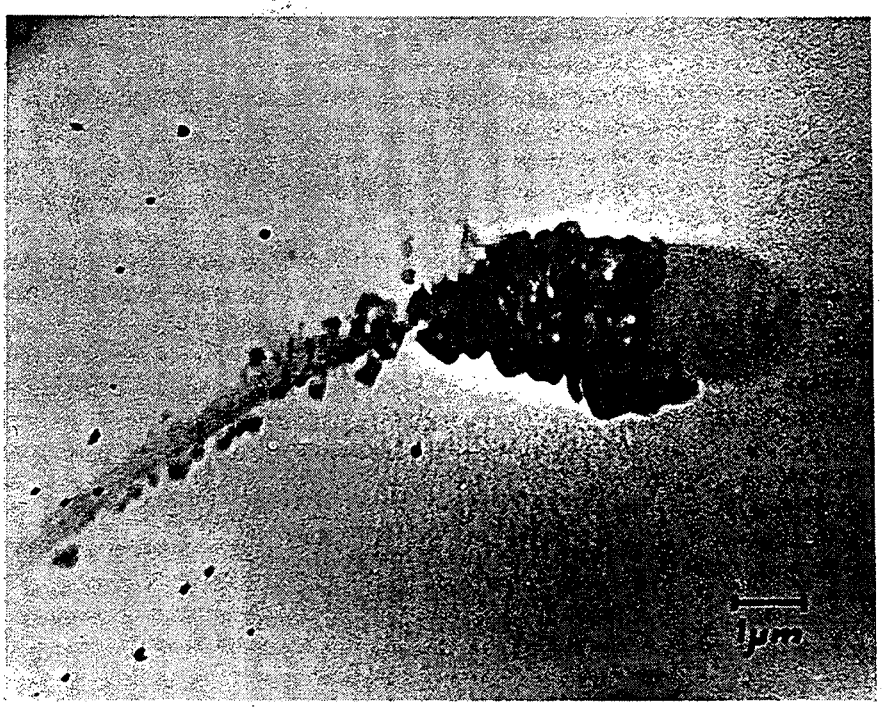

Figure 2: XM image of a dried human sperm [6] taken with the Göttingen XM at BESSY using a micro zone plate with Δ=350Å, x-ray magnification of 300x. The image shows detailed structures, especially in the head and the tail of the sperm. Artifacts from the drying process, probably salt crystals, are concentrated in the aft region of the specimen.

The diffraction limited resolution is independent of the type of microscope (XM or SXM) used. Both microscopes can achieve a resolution approaching the diffraction limit. Fortunately the zone plates available are of a very high quality, so that they do not significantly reduce the resolution of the system. Limiting factors are photon noise, imperfect illumination in the XM, non-spatial coherence in the SXM, and scanning errors. In all present x-ray microscopes, the

resolution of the system is better than twice the diffraction limit. It is our goal to have a system resolution at the ALS, which is closer to the diffraction limit than existing designs.

The field of view will be 30μm with the SXM and at least 15μm with the XM. The exposure times depend on the image quality. Higher quality images require more photons, causing longer exposure times, and increasing the radiation dose applied to the sample. According to Rose [7] a signal to noise ratio (S/N) of 5 is needed to detect a feature. Assuming a Poisson distribution of the counts in the detector, it needs 25 counts to reach S/N=5. For more gray levels in the image, more counts are needed. For purposes of comparison we consider an image with 1000 counts per pixel (this gives a S/N=32), a 1000x1000 pixel array each of 300 Å size. Exposure times for this type of images at the ALS will be 0.02 sec with the XM at the undulator, 5 sec with the SXM on the undulator, and 3 sec with the XM at a bending magnet (see table 1).

The wavelengths used for x-ray microscopy are designed to take advantage of the natural contrast in the so called water-window, between 24Å and 45Å, the K-absorption edges of oxygen and carbon. Both x-ray microscopes, the XM and SXM, are compatible with phase contrast [8,9] methods. The XM design allows different illumination schemes to insure optimized performance for phase contrast and amplitude contrast. The SXM can be equipped with an area sensitive detector to allow phase sensitive experiments [10]. The tuning range of the undulator source (see table 2 and figure 4) is much wider than the water-window. For samples in water the penetration of x-rays through water must be high enough to allow whole cell imaging. Outside the water window this is near a wavelength of 10 Å (see figure 3). These wavelengths also allow low dosages for phase contrast x-ray microscopy [2,9]. As these wavelengths are within the tuning range of our undulator, we might operate even over 2keV photon energy (6Å) in the future.

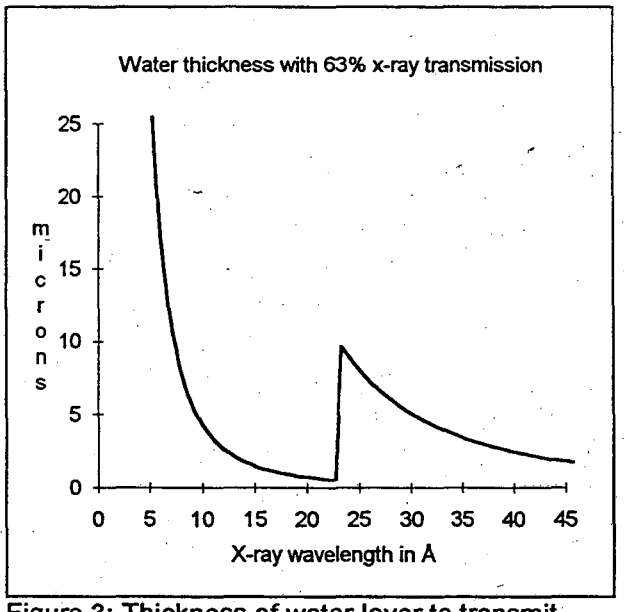

Figure 3: Thickness of water layer to transmit 63% of x-rays as a function of the x-ray wavelength.

The x-ray microscopes at the ALS allow several operating modes. A versatile design will even enable us to use operating modes that will become important in the future. Any flexibility that does not limit high quality and high resolution imaging will be preserved. These include phase contrast, 3D imaging, and fluorescence. The microscopes can also operate at multiple wavelengths. This allows contrast sensitive to elemental compositions and to some extent to chemical bonding [11].

It will be possible to rotate the sample around an axis to record multiple view images and gather 3D information. Uncomplicated means of doing so provide stereo images and focal series, but more sophisticated methods like micro-tomography are also feasible, within the constraints of the total acceptable radiation dose.

Table 1. Exposure times preliminary estimate:

300 Å Resolution	X-ray microscope (XM)	Scanning x-ray microscope (SXM)
1000 Counts 1000x1000 pixels 30 μm field	3 sec (B) 20 msec (U)	5 sec

Times for the XM are for a bending magnet (B) or for an undulator (U).

A very interesting operation of the SXM will be fluorescence microscopy with x-ray excitation and x-ray or visible light detection. Especially fluorescence microscopy with x-ray excitation and visible light detection will be very useful, when appropriate fluorescence dyes become available[12].

Operation modes, that might be used in more distant future include: Dark field imaging, Schlieren method, x-ray interferometry, and confocal microscopy.

3. Undulator for X-ray Microscopy

High spectral brightness, as needed for x-ray microscopy, will be provided by an undulator. It is anticipated that the majority of experiments will be performed at x-ray wavelengths in the water-window, especially near 24 Å. Therefore the design of the undulator was established by the following criterion:

- First, the spectral brightness inside the water-window, especially near 24 Å, should be as high as possible.
- Second, there should be no gap in the tuning curve between the 1st and 3rd harmonic, and a certain step in brightness can be tolerated.

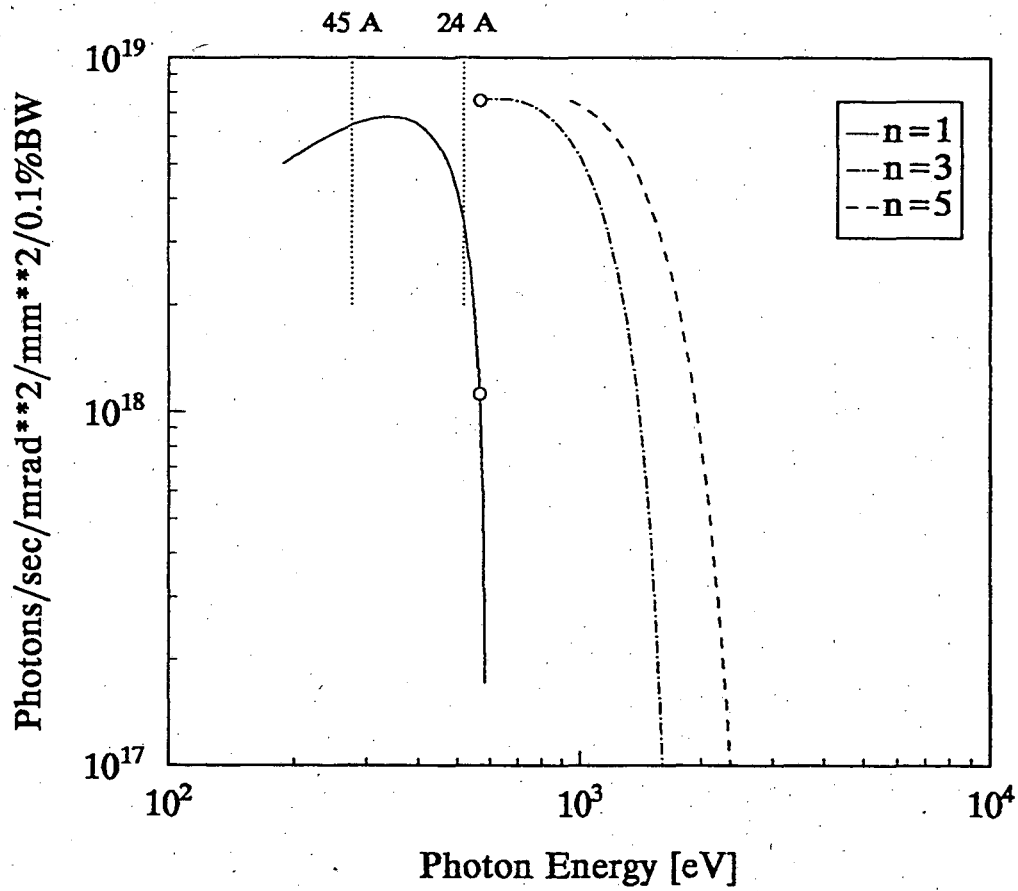

Figure 4: Spectral brightness of the 3.65 cm period undulator for x-ray microscopy at the ALS. The water-window from 24 Å to 45 Å is indicated by vertical marks.

This will be accomplished with a period length of 3.65 cm. The anticipated working conditions of the undulator at the ALS are summarized in table 2. The spectral brightness as a function of photon energy is shown in figure 4. The information has been compiled based on electron beam data from the ALS handbook [13], with new estimates of the maximum magnetic field, which are slightly higher than reported in the ALS handbook.

Table 2	
Electron beam energy	1.5 GeV
Beam current	400 mA
Undulator period	3.65 cm
Magnet periods, length of undulator	123 periods, 4.5 m
Spectral brightness at 24Å	$3 \cdot 10^{18}$ photons/(sec·mm^2·mrad^2·0.1%BW)
Tuning range	Deflection parameter K: $0.2 \leq K \leq 2.05$
1st harmonic	21.3-65.5 Å (189-583 eV)
3rd harmonic	7.1-21.8 Å (567-1750 eV)
5th harmonic	4.26-13.1 Å (945-2920 eV)
Beam size σ rms	63 µm (V), 330 µm (H)
Beam divergence σ' rms	16 µrad (V), 30 µrad (H)

4. Beam Lines for X-ray Microscopy

The x-ray microscopes at the ALS will be operated on two beam lines, an undulator beam line and a bending magnet beam line. The beam lines will be adjacent and reside in a noise and dust isolated room, as shown in figure 5. Besides the microscopes there is space reserved for additional branch lines: A White Light Station and a Coherent Optics Station. The XM at the bending magnet uses its own beam line and can be used full time. At the undulator different branch lines must time-share radiation from the undulator. As exposure times are on the order of seconds, and setup times to install and adjust the samples are much longer, the branch lines are served on a time sharing basis by use of a so-called pop-up mirror. This pop-up mirror directs the full undulator radiation to one branch line at a time. Thus if a branch gets service, it also gets uncompromised performance.

4.1. XM Branch Lines

The x-ray microscope (XM) will first be installed at an ALS bending magnet. The location of this beam line is next to the undulator as shown in figure 5. It is an uncomplicated beam line. There is no monochromator preceding the end station. The necessary monochromticity will be provided by the condenser system of the XM in the end station. To reduce the amount of unwanted higher energy photons, the radiation from the bending magnet will be low-pass filtered by use of a plane mirror.

After completion of the SXM the XM may be upgraded based on the experience then and installed at the undulator too. The XM branch line at the undulator will be similar to the bending magnet beam line, except that the first mirror will be the pop-up mirror discussed in the SXM beam line section. Therefore the XM at the undulator will be operated in time sharing with the other branch lines.

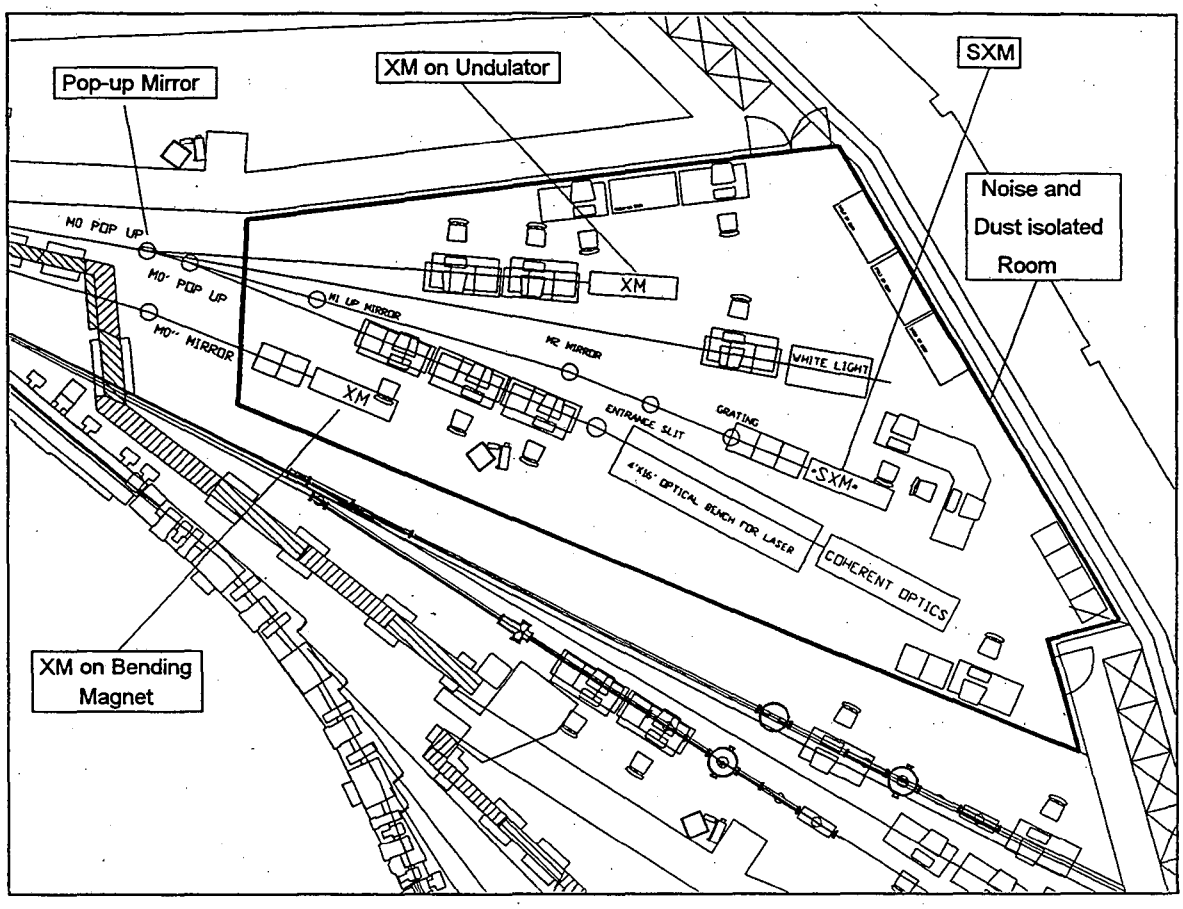

Fig. 5: Beam line layout of the Biological X-ray Microscopy Resource Center at the ALS.

4.2. XM End Station

The x-ray microscope end station is designed to make the advantages of x-ray microscopy available to the user. By providing interchangeable visible and x-ray microscopy the concept is: switch from the visible light microscope to the higher resolving x-ray microscope. Therefore a state of the art visible light microscope is an integral part of the XM. This concept has been proven valuable with the Göttingen x-ray microscope[5] and their laboratory microscope[14]. Our dominant design goal is to maximize the capabilities for state of the art visible light microscopy, so that the biologist can choose from a large variety of contrast mechanisms available in visible light microscopy to examine his sample, switch to the XM, take the XM image, and then check the sample again with visible light.

The versatile use of visible light microscopy will be possible, because the sample holder within its environmental chamber will be precisely transported between two positions -- the XM and the visible light microscope. The precision of this movement will be sufficient to preview and completely align the sample with the visible light microscope, thus avoiding unnecessary x-ray sample damage, and unnecessary use of time-shared undulator radiation.

The optical elements of the XM are a condenser zone plate and a micro zone plate as shown in figure 6. The condenser zone plate (KZP) will be from the University of Göttingen, which is to be provided as part of a collaboration. Together with a pinhole of 15 µm diameter, this KZP will operate as a linear monochromator with $\lambda/\Delta\lambda$=300. The sample specimen resides in proximity to this pinhole. As the image of the source, that is produced by the KZP to illuminate the sample, is

smaller than the monochromator pinhole, the KZP will be scanned to provide a homogeneous illumination.

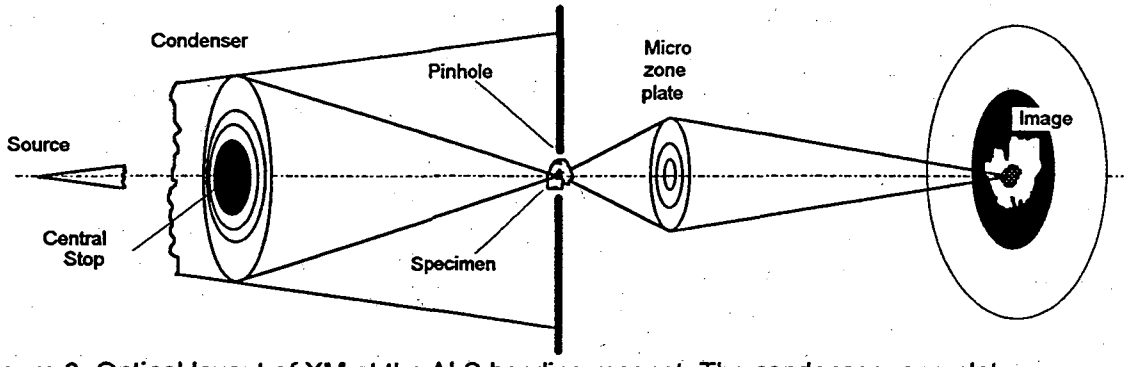

Figure 6: Optical layout of XM at the ALS bending magnet. The condenser zone plate illuminates the sample. In combination with the pinhole this setup also forms a linear monochromator. A homogeneous illumination of the sample will be provided by scanning the condenser zone plate.

The micro zone plate forms an enlarged image of the sample on a detector. Micro zone plates with varying focal length from below 500µm up to several millimeters can be used in the XM. As described in a previous paragraph, we expect to use 250Å outer zone width lenses in early experiments, which permit a diffraction limited resolution of 300Å.

Several detectors are available with the XM. High resolution images will be recorded on a soft x-ray CCD camera, where the best detective quantum efficiency is expected. For adjustment purposes a detector system with an x-ray sensitive micro channel plate (MCP) will be used. This allows on-line control of x-ray positioning and alignment as needed. An optional camera using high resolution photographic emulsions is also planned.

4.3. SXM Branch Line

The SXM branch line is part of the undulator beam line. It includes a specialized monochromator. Several possible beam line optics and monochromator designs have been analyzed. A possible design is shown in figure 7. A plane pop-up mirror directs the undulator radiation into different undulator branch lines for the XM and the SXM. A Kirkpatrick-Baez (KB) system focuses the beam for the SXM vertically to an entrance slit of a grating monochromator and horizontally onto a pinhole, which also acts as the exit slit of the monochromator. The monochromator, having a constant deviation of 174°, uses a 38-m, 300-grooves/mm spherical grating with varied line space. A resolving power of 500 to 1000 is expected in a wavelength range from 20Å to 50Å

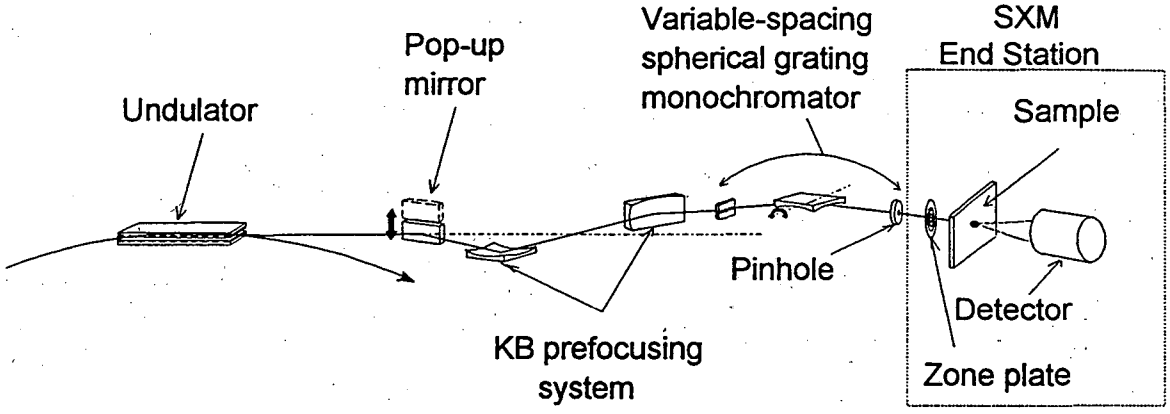

Figure 7: SXM beam line optics consisting of a pop-up mirror, a Kirkpatrick-Baez (KB) prefocusing system, a variable spacing spherical grating monochromator. The SXM end station is shown in more detail in figure 8.

The radiation delivered to the SXM end station by the monochromator should be stable in wavelength and intensity. Changes in wavelength cause a modest loss in resolution due to defocus, while unmonitored changes in intensity result in false contrast. Wavelength changes can be reduced below acceptable limits, but variations in the source position and direction will cause intensity variations that are difficult to correct. This cannot be avoided if the beam line optics include focussing onto intermediate apertures like a slit or pinhole. That is why we investigated other designs, that have no intermediate apertures.

In an alternate design the source is directly demagnified to a diffraction limited point by use of a zone plate. The spatial coherence is chosen by the acceptance angle of the micro zone plate. The temporal coherence is provided by a varied line space grating. This also is a less complex system. The difficulty with this design is that one particular varied line space grating only allows a very limited range of wavelength tuning. Therefore the monochromator for the SXM will be a trade off between stability, tuning range, and complexity.

4.4. SXM End Station

The scanning x-ray microscopy end station follows the same concepts as the XM end station described earlier. The sample stage and environmental chamber are compatible, so that any sample prepared for x-ray microscopy can either be imaged in the XM or in the SXM. The incorporation of the visible light microscope into the SXM is similar to that in the XM.

The schematic layout of the SXM end station is shown in figure 8. A high resolution micro zone plate is used to produce a diffraction limited scanning spot on the sample. In the case of the monochromator with the Kirkpatrick-Baez prefocusing system as shown in figure 7, the source is represented by the pinhole at the end of the monochromator. In the alternate design without intermediate apertures, the source lies in the undulator. The end station can accommodate both cases by using a micro zone plate with proper focal length for the correct demagnification of the source.

The SXM end station consists of three important parts: micro zone plate, scanning stage, and detector system. The micro zone plate has the same resolution as in the XM. Because of the high photon flux from the ALS undulator, the scanning stage must provide high speed and high precision scanning. We expect exposure times of 5 µsec per pixel and 5 msec per line for a typical image. These high scanning speeds require accelerations not suitable for the sample. Therefore the high speed scanning will be done by horizontally scanning the micro zone plate, and the low speed scanning will be done by moving the sample vertically line by line.

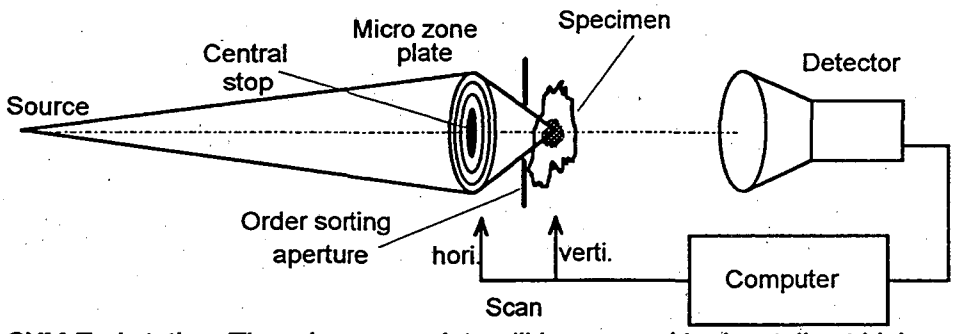

Figure 8: SXM End station. The micro zone plate will be scanned horizontally at high speed and the sample will be scanned vertically.

A great advantage of the SXM is that there are fewer radiation losses following the sample. This is because every photon that penetrates the sample reaches the detector. Consequently there is less radiation dose necessary to achieve a certain signal to noise in the image with an SXM compared to an XM. If the intensity of the source is unstable, the image contrast will be degraded and more radiation is necessary to get the same image quality. This effect depends on counting statistics and the desired image quality. Images taken with a low photon count are limited by photon noise. High quality images with a large number of photons are more sensitive to intensity variations of the source. In this situation the radiation dose necessary for a certain image quality can be lower with an XM than with a SXM. These limitations of the SXM are from technical imperfections and not from physical limits. Therefore they can be reduced. This is done by choosing a stable beam line optics and monochromator design and by measuring the intensity of the source for every pixel. Therefore we can correct the images and maintain the advantages of the SXM over a greater range of image qualities.

7. Acknowledgments

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