

Applications and Instrumentation Advances with the Stony Brook Scanning Transmission X-ray Microscope

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ABSTRACT

Scanning transmission X-ray microscopes (STXM) are well matched to the optics of high resolution monochromators, offer a variety of imaging modes and can minimize radiation damage to the specimen. We describe the Stony Brook STXM at the NSLS. This microscope is used for a variety of studies by many users; we briefly outline its use for studies of hydrated colloidal systems and for dark field microscopy on immunogold labeled specimens as examples. In order to keep pace with developments in zone plate optics, spectroscopy and a variety of imaging modalities, the microscope is being redesigned and its characteristics are discussed. Its primary x-ray detector will be a new multiwire proportional counter with high count rate capability.

Keywords: x-ray microscopy, soft x-ray microscopy, spectromicroscopy, proportional counter, dark field microscopy

1. INTRODUCTION

Scanning transmission x-ray microscopes using Fresnel zone plate optics were first developed at Stony Brook around 1980.^{1,2} The original motivation for the choice of the scanning geometry was to place the 5 - 15 % efficient Fresnel zone plate before rather than after the sample in the beam path, thereby minimizing radiation dose to the sample. This advantage has become somewhat less important as more efficient zone plates have been developed, and with the introduction of cryo microscopy techniques.^{3,4} However, at the same time other beneficial characteristics of scanning microscopy have become clear, including its straightforward coupling to undulator sources and high resolution monochromators and its flexibility for a number of imaging modes, e.g. scanning luminescence x-ray microscopy (SLXM),⁵ which can not be done in a conventional transmission x-ray microscope.

In this paper we briefly describe the room temperature scanning transmission x-ray microscope (STXM) at beamline X-1A at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL), and illustrate its capabilities by two recent applications. New applications like these have placed new demands on the microscope, and furthermore improvements in zone plate optics⁶ have made clear the shortcomings of our existing scanning stage. We have therefore begun a redesign of the microscope, and a new detector has already entered operation.

2. THE SCANNING TRANSMISSION X-RAY MICROSCOPE AT THE NSLS

We present here a brief summary of the features of the current STXM^{7,8} and outline some of the changes it has undergone in recent years. In addition, the X-1A beamline at which it is mounted has undergone considerable change.^{9,10}

The microscope makes use of a 35 period, $\lambda_0 = 8$ cm undulator in the NSLS x-ray ring, which provides high tunable flux from 200 to 800 eV.¹¹ Two branch beamlines are used for scanning x-ray microscopy and other experiments including holography and diffraction; the room temperature microscope which is described here presently operates on the X-1A outboard branch line. Fig. 1 shows the basic components of the X-1A outboard branch beamline and the STXM. Mirrors, which focus and deflect the beam, are not shown in the diagram. A toroidal mirror focuses

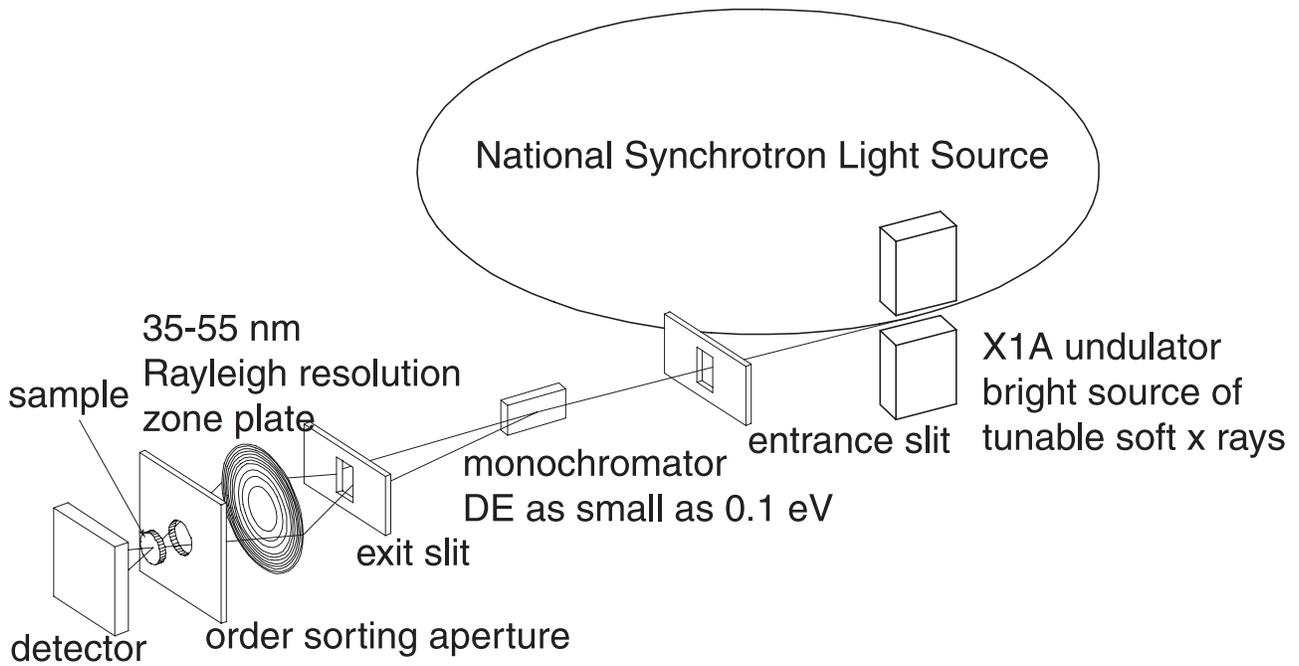


Figure 1. Schematic diagram of the Stony Brook STXM on the X-1A beamline at the NSLS¹²

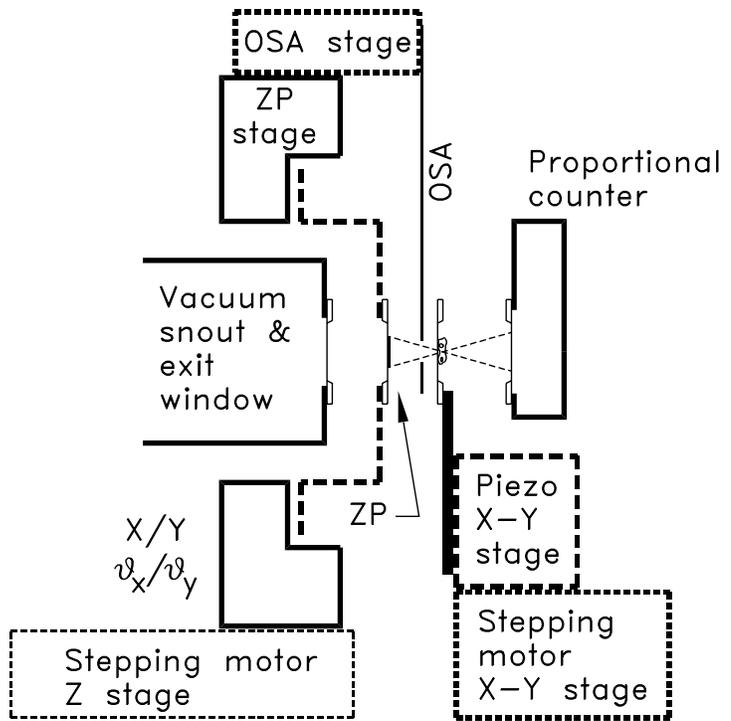
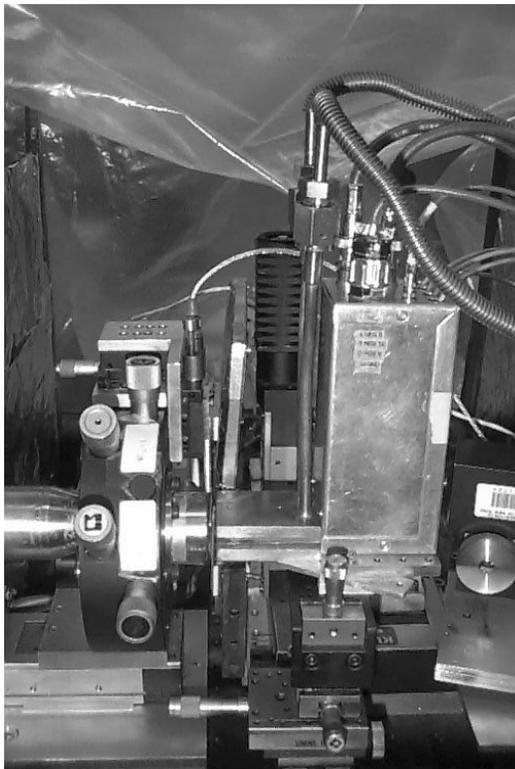


Figure 2. Left: Photograph of the STXM
Right: Schematic diagram showing the STXM Fresnel zone plate (ZP), order sorting aperture (OSA), scanning mechanism and detector

the beam onto the entrance slit of a spherical grating monochromator (SGM) in the horizontal plane and onto the exit slit in the vertical plane. The focal point of the SGM in the horizontal plane coincides with the exit slit also, so that an astigmatic focus is formed, which determines the effective source size for spatial coherence considerations. The variable entrance slit setting of the monochromator determines the energy resolution, which can be as good as $\lambda/(\Delta\lambda)=4000$.

The beam leaves the beamline vacuum through a 100 nm thick silicon nitride window, which is 250 by 250 μm in size, and enters the atmosphere of the STXM chamber. The microscope is isolated from vibrations of the experimental floor by an air table. Due to the fact that the area of uniform illumination of the exit window typically is not much larger than the zone plate itself, the table has to stay at a constant position with respect to the beam. The microprobe is formed by coherently illuminating a zone plate which has a central stop. To isolate the beam diffracted into the first order focus, an order selecting aperture (OSA) is inserted between the zone plate and the focal plane. The aperture is slightly smaller than the central stop, and the location is chosen so that it should transmit all the first order focused radiation, but stop the unwanted orders.

We also require a high degree of spatial coherence to achieve the highest attainable resolution, namely the diffraction limited spot size $1.22 \lambda/\text{NA}$ (NA is the numerical aperture). From the convolution of the spot size with the geometrical image of the source we can calculate the intensity point spread function (PSF) as limited by the source. Fourier transformation of the PSF yields the modulation transfer function (MTF). The MTF is the relative amplitude of the spatial frequencies in the image compared to those in the object and thus is a measure of the attainable resolution. As the product of source size times angle subtended (i.e. phase space volume) by the zone plate is increased, the MTF worsens. One can show that the illumination phase space accepted should be on the order of 1λ to operate at the diffraction limit.¹³ The X-1 undulator fills a phase space of 100λ in the horizontal. As a result, two scanning microscopes can be illuminated simultaneously while a conventional spectroscopy beamline also uses the undulator beam.

Some of the components of the STXM are shown in Fig. 2. There are two ways of moving the sample perpendicular to the beam. Coarse scans with steps on the μm level and translation of the sample are provided by stepping motors. For high resolution scans a flexure stage with piezoelectric actuators is employed, which has capacitance micrometers in a closed loop feedback system.¹⁴ The transmitted x-rays are detected by a constant flow proportional counter. Fig. 2 also shows the recently developed multiwire proportional counter described in section 5. Typical experiments with pixel dwell times of some msec at a rate of 500 kHz favor photon counting statistics compared to a detector operating in current mode, because of the intrinsic electronic noise of these devices. Count rates beyond some MHz are beginning to favor the current or charge integration mode of detectors featuring very low noise electronics. We will be exploring current mode detectors in the future.

The microscope is under the control of a VAX-station 4000/90 computer workstation, which controls the scan and the data acquisition through a CAMAC interface. A graphical user interface is used to perform position and energy scans.

3. RECENT APPLICATIONS

3.1. Spectromicroscopy on Hydrated Colloidal Systems

The favorable capabilities of x-ray microscopy for investigating hydrated samples at atmospheric pressure have been applied to oil/water emulsions stabilized by solids (in collaboration with Sven Abend from the group of Gerhard Lagaly, Institut für Anorganische Chemie, Universität Kiel, Germany). It has been known for a long time that colloidal particles such as clays can act as stabilizers in emulsions.¹⁵ However, the surface of the clay usually has to be modified by organic cations (surfactants) in order to obtain a stable emulsion. The oil-water emulsions examined here have been stabilized using only negatively charged clay (sodium montmorillonite, Wyoming) and positively charged calcium/aluminum layered double hydroxide (LDH) without any additional surface active agents.¹⁶ This new technique of stabilizing emulsions is of interest for a large variety of applications in cosmetics and pharmaceutical products, because from an environmental and toxicological point of view it is desirable to avoid surfactants. Due to the interaction of clay and layered double hydroxides that have opposite charge, heterocoagulates form. These heterocoagulates surround and cage the oil droplets in the emulsion and prevent them from coalescing and forming two separate oil/water phases.

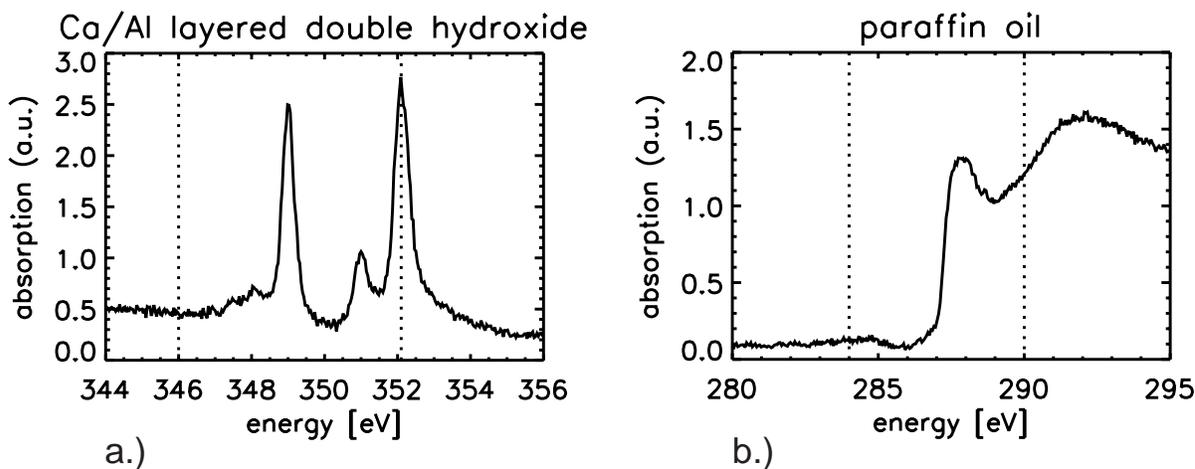


Figure 3. Near edge absorption edge spectra taken in the STXM.

a.) spectrum of Ca/Al layered double hydroxide suspended in water near the calcium *L*-absorption edge

b.) spectrum of paraffin oil (bulk sample) near the carbon *K*-absorption edge

The spectra were obtained from fixed small object regions, limited in extension only by the spot size (70 nm) and zone plate movement caused by re-focusing. The dashed lines refer to photon energies used for imaging in Fig. 4.

X-ray microscopy was used to study the structural properties of these emulsions and to better understand the stabilization process. Taking advantage of x-ray absorption edge contrast, oil could be distinguished from water near the carbon *K*-edge and the Ca/Al layered double hydroxide from clay (which contains no calcium) near the calcium *L*-edge by taking images at photon energies with characteristic absorption properties for carbon and calcium respectively (Fig. 4). The x-ray absorption spectra of paraffin oil near the carbon edge and LDH near the calcium edge are shown in Fig. 3; photon energies used for imaging are marked with dashed lines.

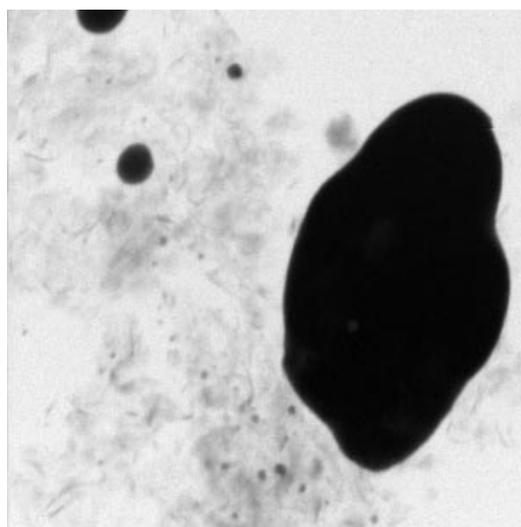
From the experimental results, the existing model of the formation of heterocoagulates could be confirmed. Information about the spatial distribution of clay and LDH as well as details of the particles and their orientation in the oil/water interface could be obtained.

3.2. Dark Field Microscopy for Immunogold Labeled Specimens

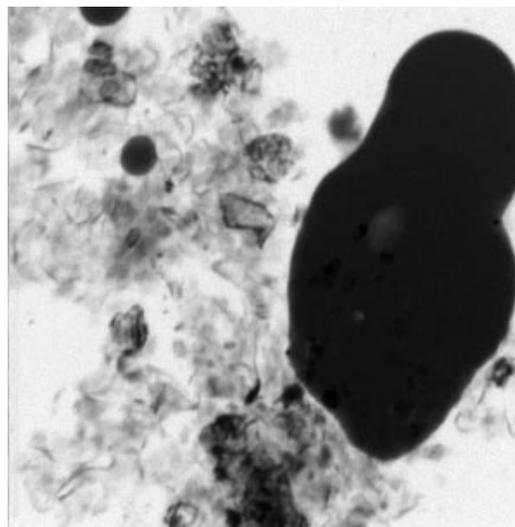
For many studies in biology it is very beneficial to label specific sites of biochemical activity in a cell. While efforts are underway to do this using scanning luminescence microscopy¹⁷, dark field microscopy in a STXM allows one to unambiguously identify gold labels in cells.¹⁸ This is accomplished by using a stop in front of the detector so as to obtain annular dark field imaging conditions. More recently, rod-shape stops have been used with good success and alignment in one dimension only, and labels as small as 20 nm in size have been used to label the nuclear lamina.¹⁹ In order to better understand the relative merits of different x-ray microscope configurations for dark field imaging, Vogt *et al.* have carried out numerical calculations using a partially coherent imaging model. These calculations have shown that the best contrast for closely spaced particles should be obtained when the STXM detector is apertured to 1.5 times the numerical aperture of the zone plate objective, and that it is not beneficial to make the center region (corresponding to the central stop of the zone plate) of the detector sensitive.¹⁹ This has highlighted the need to have rapidly interchangeable, accurately aligned detector apertures in the STXM, adding to the requirements for optimum use of the microscope.

4. REDESIGN OF THE STXM

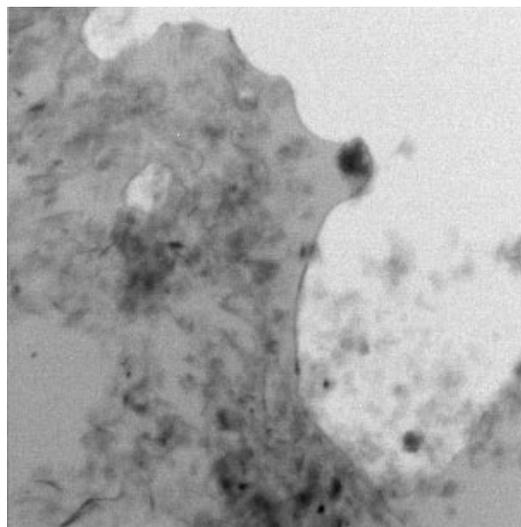
The existing STXM sees heavy use as a productive facility for studies in biology, polymer science, colloid and environmental science, geochemistry and other fields. As the number and variety of applications have developed, a number of new capabilities have become desirable. As zone plates have advanced in resolution, imperfections in the existing scanning stage have become limiting factors in obtaining the highest possible image resolution. In addition, a cryo STXM has been developed⁴ which uses most, but not all, of the available beamtime on the inboard branch



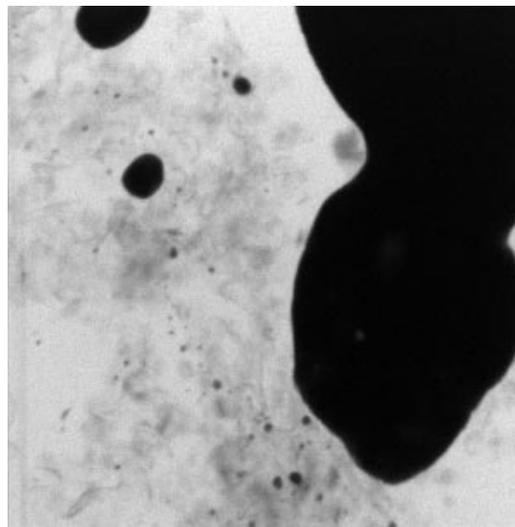
a.) 3.583 nm
346 eV



b.) 3.519 nm
352 eV



c.) 4.365 nm
284 eV



d.) 4.275 nm
290 eV

Figure 4. Images of an oil-water emulsion stabilized by solids (equal amount of clay and LDH) with x-ray absorption contrast, taken with the wet cell in the STXM. The photon energies used for the various images are shown in Fig. 3.

a.) Calcium weakly absorbing, only clay (which contains no calcium) appears dark

b.) Calcium strongly absorbing; LDH structures visible

c.) Carbon weakly absorbing; the paraffin oil droplet appears white

d.) Carbon strongly absorbing

During the experiment, the regions of the interface stabilized by heterocoagulate remain at a fixed position (lower part), while the uncaged oil droplet can disperse over time (upper part).

The images have 300 by 300 pixels with a pixel size of 70 nm. The data acquisition time was 10 (20) min for images taken near the calcium (carbon) edge.

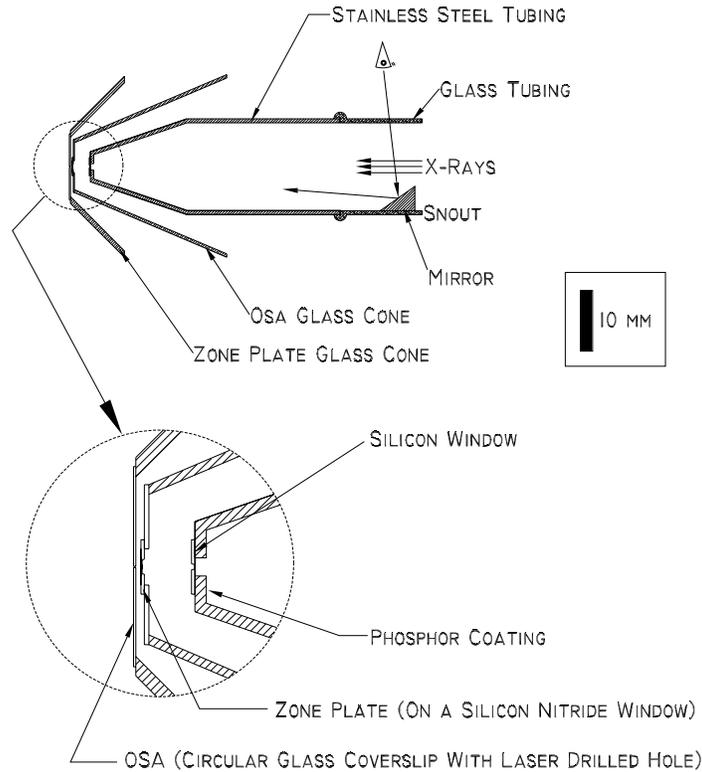


Figure 5. Schematic diagram of the zone plate, OSA and snout region of the new STXM

of the beamline, creating opportunities for operation of a second room temperature STXM. For this reason, we have undertaken to redesign the microscope with a number of new features which we now describe.

4.1. Requirements for the New STXM

It has become common to change zone plates as needed for various experiments. For some studies, the highest resolution zone plates now in routine use (30 nm outermost zone width in 80 μm diameter⁶) are desired, while studies of wet specimens at the carbon *K*-edge often require the longer focal length of 160 μm diameter, 45 nm outermost zone width zone plates.²⁰

Some imaging modes require special x-ray detectors, e.g. a CCD camera²¹ for Wigner phase microscopy²² or a detector with central stop for dark field microscopy.^{18,19} Provisions to mount these instead of the primary detector for bright field imaging have to be made.

With the prospect of higher resolution imaging using zone plates with an outermost zone width as small as 20 nm,⁶ we desire to have a better piezo stage and mounting to minimize vibrations and sensitivity to thermal expansion. Although our current stage design¹⁴ (see also Fig. 2) is wonderfully compact for first generation capacitance-micrometer-coupled piezos, we estimate the position noise to be on the order of 10-15 nm which limits the attainable image resolution.

We want to continue using the STXM at atmospheric pressure, since all components, including the sample, are then easily accessible and do not have to be vacuum compatible. The $1/e$ absorption length of air in the 3.1-4.4 nm wavelength range is 1.6 - 3.6 mm, while in helium it is 20 - 55 mm. Therefore, in the current microscope helium flow outlets are located at several points below the x-ray beam path to reduce absorption. The turbulent helium flow can lead to variations in the transmitted beam intensity, and the remaining and varying concentration of oxygen and nitrogen in the beam path makes quantitative spectroscopy near the oxygen (≈ 530 eV) and nitrogen *K*-edge (≈ 400 eV) difficult. To avoid the problems of a turbulent helium flow, we want for the new microscope a well sealed chamber that can be quickly evacuated to a pressure of about 100 Pa and backfilled with helium

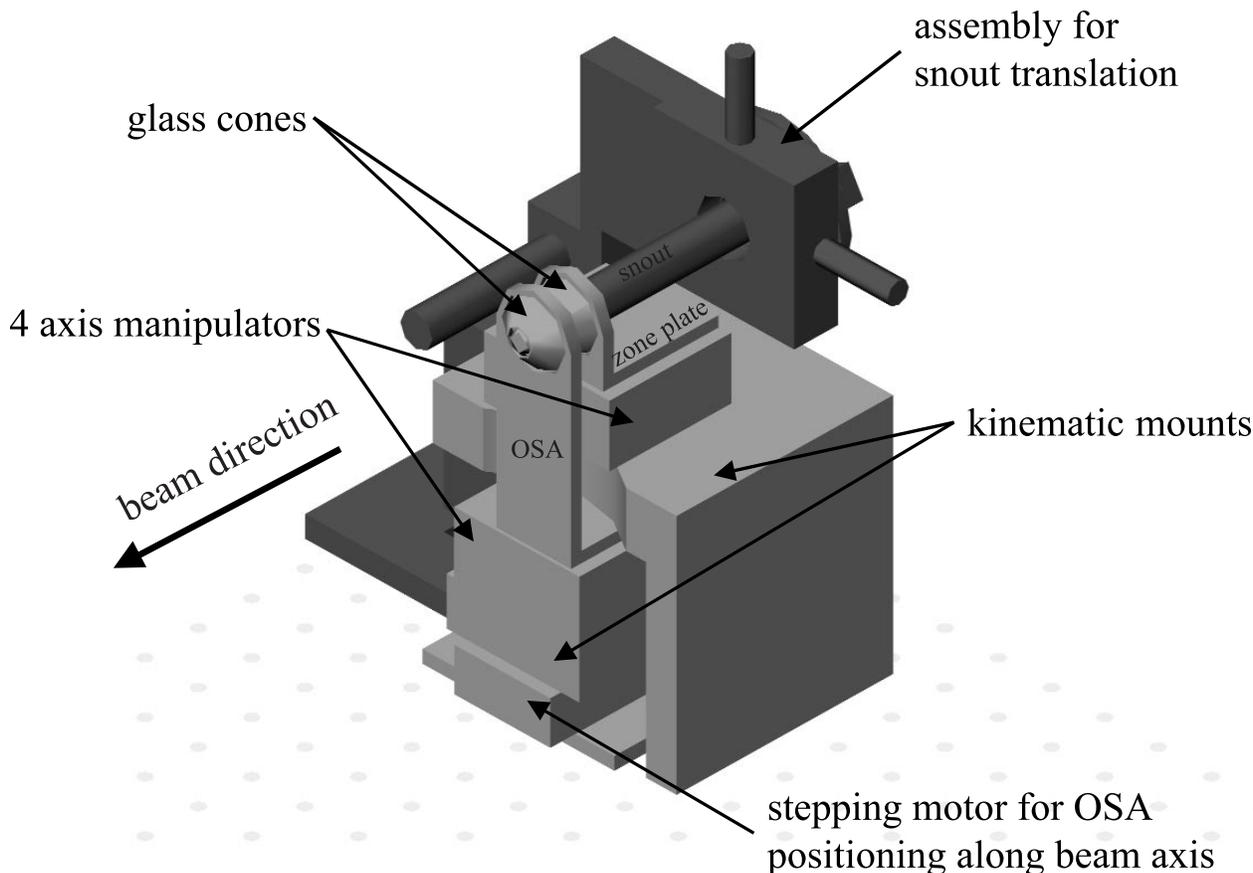


Figure 6. Perspective view of the assembly for the zone plate, the OSA and the snout

to atmospheric pressure. This corresponds to a reduction in partial pressure of air by a factor of 1000.

For suppression of second diffraction order radiation from the monochromator an order sorting mirror assembly has been installed on the beamline, which can be inserted into the beam path. It consists of two parallel polished glass plates, off which the beam is reflected at an adjustable grazing angle (20-70 mrad). Insertion causes the beam height to shift by ≈ 2 mm. We therefore need to be able to translate and tilt the new microscope chamber accurately and reproducibly (our goal is $20 \mu\text{m}$ and 0.1 mrad respectively). Another beneficial aspect of accurate positioning is that we can compensate for beam shifts by moving the whole chamber, rather than separately readjusting the exit window, zone plate and OSA.

We also want to be able to easily pass beam through the STXM chamber and deliver it to downstream experiments like diffraction or the cryo STXM.⁴

For the new STXM we want to be capable of detecting x-ray photon count rates up to several MHz without the loss of linearity or introduction of detector noise. We have had adequate performance with a single wire gas flow proportional counter in the current STXM. For the new microscope we developed a constant flow multi wire proportional counter operating at low pressure, which is described in some detail in section 5.

While improving the microscope hardware, we are also planning on upgrading the electronics and software. The present system runs with a VAX/VMS computer and CAMAC electronics. We are able to collect only one signal at 14 bit precision, and furthermore the software architecture is of a type that is no longer likely to be familiar to new users of the microscope.

4.2. Design of the new STXM

For the design we try to use mostly commercially available parts to keep the cost and engineering effort down. Making the arms connecting the zone plate and the sample as stiff and light as possible, we seek a high resonant

frequency to minimize vibrational amplitudes, which otherwise spoil the resolution.

We are using 4 pneumatic isolators supporting a 90 by 90 cm tabletop for vibration isolation. To get a well sealed microscope chamber we want to use a custom made rectangular vacuum vessel with a removable plexiglass top sealed by an O-ring. By supporting the chamber at three points, we are able to define both position and tilt in the horizontal and vertical plane. Custom made linear manipulators employing low profile linear motion stages provide translation and tilt in the horizontal plane. They offer high accuracy linear travel and can take high loads (550 kg for one manipulator) while minimizing the height. Three machine screw actuators are to be used for adjusting the height and vertical tilt of the chamber.

Most of the electrical and signal lines will be fed through the chamber wall using a PC board, which pairwise links connectors soldered on different sides of the board. The PC boards will be glued onto an aluminum support frame which will bolt onto the chamber using an O-ring seal.

We plan to use glass cones as supports for the zone plate and OSA (Fig. 5) allowing the visual check of the alignment. Compared to materials like plastic, glass offers the advantage of a low coefficient of thermal expansion. We will also try to use #1 circular glass coverslips (120 μm thick) with a laser drilled hole as OSAs. This has the advantage that one can see the zone plate through the OSA during alignment with a visible light microscope. The snout is a combination of stainless steel and glass tubing with a conical stainless steel adapter, that holds the silicon nitride exit window. A mirror in the glass part makes it possible to view the position of the x-ray beam on the vacuum side of the exit window region, which is coated with phosphor.

Compact four axis manipulators allow for position and tilt adjustment of the glass cones (Fig. 6). The zone plate, glass cone, angle bracket and 4 axis manipulator make up a permanently assembled unit, which is kinematically mounted; the OSA is held similarly. Not only does this design allow easy interchange of zone plate and OSA without the loss of alignment, but the alignment in the microscope may be transferred to an offline setup of kinematic bases on an optical rail. With this setup we will be able to prealign matched pairs of zone plates and OSAs with their manipulators offline.

As in the current STXM we use the combination of encoded stepper motors and a piezo actuated flexure stage with capacitance micrometers in closed loop feedback for scanning the sample on the μm and nm scale respectively. We plan to mount the sample kinematically to the piezo stage and to also allow the use of a wet specimen chamber developed by U. Neuhausler.²³

We will have room for accommodating three detectors on a motorized platform. Long range, encoded stepping stages will allow easy and reproducible switching among detectors. We hope to explore new imaging modes, e.g. differential interference phase contrast,²⁴ in which a scanable detector is needed or helpful.

The beam path can be cleared by removing the OSA and zone plate assemblies from their kinematic mounts and moving the long range sample and detector horizontal stages all the way to one side. We want to explore the possibility of directly passing beam to downstream experiments without the installation of a beam pipe.

A visible light microscope with a CCD readout will be used for alignment of the zone plate and OSA and for sample inspection. The multiwire gas proportional counter (see section 5) is planned to be used as primary x-ray detector for bright field imaging.

We will now use a cheaper yet more capable PC running the Linux operating system and using GPIB electronics. With this system, we are able to record up to 8 analog signals and 6 pulse rate signals simultaneously at each pixel with 1 msec dwell times, all at 16 bit precision (with pulse rate division available on the scalers). This is accomplished by using devices that are able to run without computer communication during the course of a scan line; data is then rapidly transferred to the computer using direct memory access (DMA) transfers before a new scan line is begun. The scan software is being developed in C++ using the Qt graphics toolkit from www.troll.no.

5. THE MULTIWIRE GAS PROPORTIONAL COUNTER

For most of the STXM applications only the number of photons transmitted by the sample is of interest. For best contrast, high dynamic range and minimal noise is required. Typical photon count rates range from some kHz to several MHz. The detector should be completely insensitive to visible light, since it is a serious problem to shield the microscope chamber from ambient light. The counter should also have long term stability since an automated spectromicroscopic dataset (images taken at many different wavelengths) can take many hours.

A single wire proportional counter with a continuous flow of gas at atmospheric pressure was employed for many years in the current STXM. It has low intrinsic noise (about 100 Hz) and is completely insensitive to visible light. Drawbacks of this detector, however, are the difficult assembly procedure and limited lifetime due to loss of wire

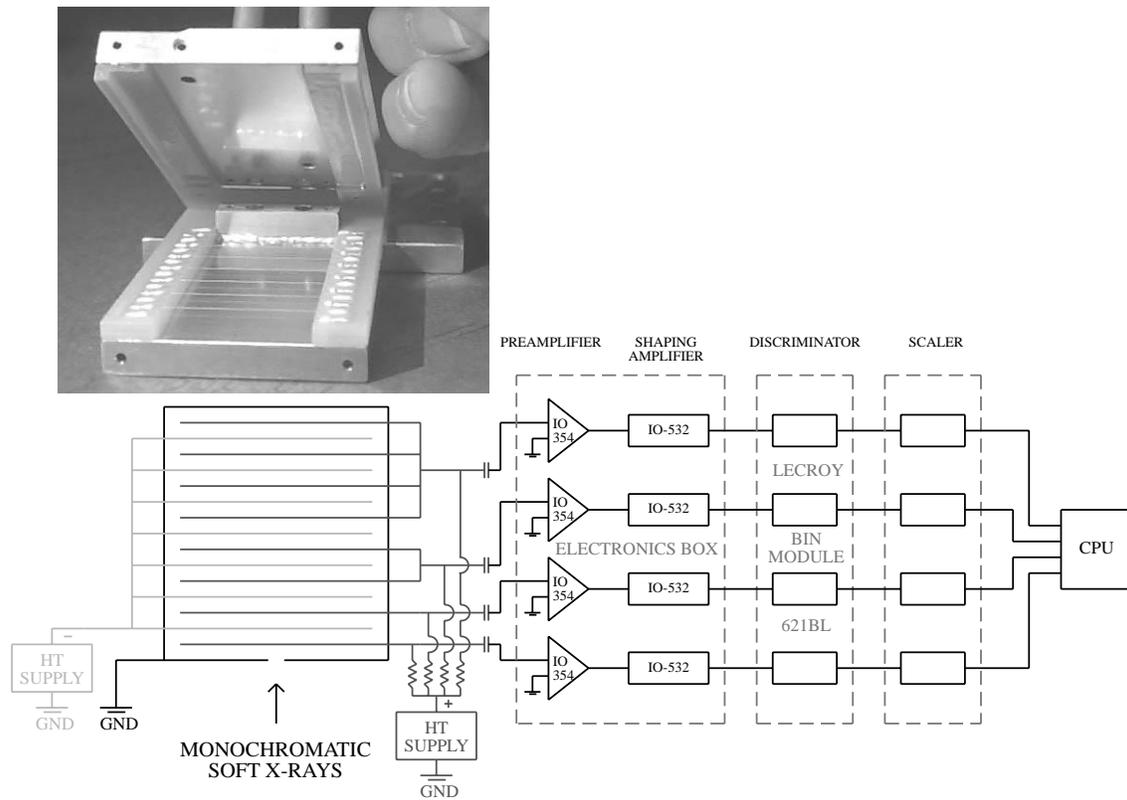


Figure 7. Top: Photograph of the counter body before assembly. The electronics box is not attached. Bottom: Functional diagram of the detector and electronics

tension, as well as saturation problems at high count rates and resulting discharges.

A novel multiwire gas proportional counter with better reliability, lower noise and higher saturation count rate has been developed. A schematic layout of the counter with electronics is shown in Fig. 7. The monochromatic x-ray beam enters the detector through a 100 nm thick, 250 by 250 μm area silicon nitride window with 20 nm Cr coating for conductivity. Depending on the photon energy, the window transmits on the order of 60 % of the x rays. The eight anode wires of the detector are connected in groups (from the front) of 1, 1, 2 and 4. Because of the exponential decrease in x-ray photon intensity in the detector (length 5 cm), comparable count rates can be obtained on all four groups when the composition and pressure of the gas are optimized for a specific photon energy. Low Z gas mixtures such as neon with a CO_2 quench are being tested. Each group of anode wires feeds an electronic channel comprising a high bandwidth preamplifier and shaping amplifier. The system is optimized to minimize electronic noise and provide an output waveform just several nanoseconds wide²⁵; this permits high count rates at modest avalanche sizes, helping to prevent space charge saturation.

The detector was studied in the energy range from 270 eV to 800 eV on the STXM at beamline X-1A at the NSLS. The principle of spreading photons over more than one channel has been demonstrated with the counter response to photon intensity being quite linear. Fig. 8 shows the result of a linearity test for one of the four counter channels.

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We wish to thank Sue Wirick for her continuous help at X-1A. Y. Wang and T. Oversluizen were involved in the early design of the new microscope. We would also like to thank E. Von Achen for his patient work on the new proportional counter.

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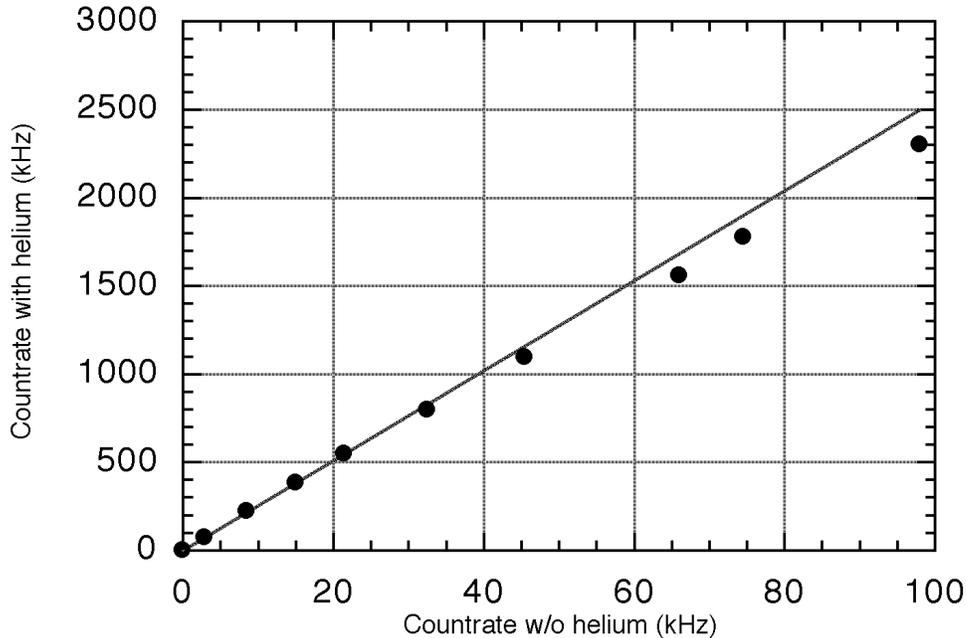


Figure 8. Measurement of the linearity of one detector channel. A point on the curve is obtained by measuring the counterate for a particular photon flux with and without helium in the beam path. One assumes perfect linearity at very low counterates (x-axis). Saturation effects due to the limited bandwidth of the electronics and space charge formation begin to be significant at counterates exceeding 1 MHz for one channel. The linear fit is based on the first four points.

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