Diffraction enhanced imaging of rat kidney

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Abstract
Objective The purpose of this study was to explore the potential of diffraction enhanced imaging of rat kidney. Methods The dissected kidney of rats with the thickness of 2mm and 120µm were imaged by X-ray diffraction enhanced imaging at the Beijing Synchrotron Radiation Facility (BSRF). Histologic slides of the specimens were made after imaging and compared with the diffraction enhanced imaging of the specimens. Results The straight collecting ducts and papillary tubules that can’t be detected by conventional radiography are visible clearly down to approximately 30µm by DEI in dissected kidney of rats. The artry and vein that can only be detected through are visible clearly by DEI, too. Conclusion The results suggest that DEI with synchrotron X-ray has the potential to be of use in the study of micro lesion of renal medulla and vessel. This technique provides a new way of imaging a property of biological tissues not yet exploited.

Key words X-ray, diffraction enhanced imaging, kidney
Improvement in S/N Ratio of Our Soft X-ray Spectro-Reflectometer with a Laser-Produced Plasma Source

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We constructed a spectro-reflectometer with a laser-produced plasma (LPP) source in the previous work [1]. While the measurement error of 2% was achieved, the performance has been unstable for evaluation of soft X-ray multilayer mirrors, which was mainly caused by intensity fluctuation of the LPP source. For the purpose of signal to noise ratio improvement, we developed a novel intensity monitoring system using a grazing incidence beam splitter. We also improved detection electronics and software.

The optical system of the spectro-reflectometer is shown in Fig. 1. A Nd:YAG laser (Quanta-Ray DCR-2A, 1064 nm, 10 Hz, 800 mJ) is used to produce plasma on a samarium rod target. The monochromator consists of a spherical pre-focusing mirror of a radius of curvature of 5 m at a grazing incidence angle of 6°, a spherical grating (R = 3 m, 600 grooves/mm) and a pair of slits. A rotating debris shutter was designed and added in front of the pre-focusing mirror. A 2.5 nm thick Ru film supported by a Si$_3$N$_4$ membrane (NTT-AT corp.) was set as a beam splitter at the entrance of a sample chamber at a grazing incidence angle of 20°. The transmitted main beam toward the sample is used for reflection or transmission measurements with an EMT (Hamamatsu, R515). The reflected beam is used for intensity monitoring with another EMT. After the improvement of the detection electronics and software, a 1% shot to shot fluctuation of the intensity ratio of transmitted and reflected beams was achieved. The photon flux at a wavelength of 13.5 nm with 0.6 mm wide slits was estimated to be $1.75 \times 10^5$ photons/pulse.

Development of a Quarter-wave Plate near Carbon K-edge by Using Graphite

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We present a quarter-wave plate near photon energy 290 eV. A quarter-wave plate is a polarization element that retards the phase between s- and p-components 90 degrees. It changes polarization state, from linear polarization to right or left circular polarization for example. By using the quarter-wave plate, the circularly polarized light is available without a light source specialized for circular polarization, such as helical undulator in the synchrotron ring.

Crystals with low symmetry were calculated to have large phase retardation near its absorption K-edge [1]. These crystals were predicted to work as a quarter-wave plate. We measured polarization properties of graphite crystal, which has K-edge at 284.1 eV. The evaluation was carried out by using linearly polarized synchrotron radiation at BL27SU of the SPring-8. A versatile apparatus for polarimetry and ellipsometry [2] was attached to the beamline. Rotating analyzer method was performed in the apparatus to obtain the phase retardation and polarizance, normalized difference between the reflectivity of s- and p-components. Figure 1 shows the measured phase retardation and polarizance as a function of glancing angle at 289.1 eV. At the glancing angle of 6.1 degree, the graphite had the phase retardation 90 degrees and worked as a quarter-wave plate.

In the soft x-ray region, a quarter-wave plate is usually designed by transmission-type multilayer. However, a quarter-wave plate of this type for 290eV is not available. The throughput of graphite was as high as 1%, which is much higher than that of a phase retarder designed by multilayer. Lamellar crystals would be new type of polarization elements in the soft x-ray region.

![Figure 1](image-url)  
*Figure 1. The phase retardation (panel a) and polarizance (panel b) of the graphite at 289.1 eV.*

Narrow Band Mo/Si EUV Multilayers with Thick Si Structures

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Narrow band Mo/Si multilayers as a high throughput monochromator are needed in our EUV and soft x-ray interferometer composed of multilayer mirror optics with a laser produced plasma laboratory source [1]. The observable number of interference fringes depends on temporal coherence length \( \lambda^2 / 2\pi \Delta \lambda \). The FWHM of spectral reflectance of standard Mo/Si multilayers at the wavelength 13.5 nm is about 0.5 nm, which is insufficient for our interferometer. This is the reason why narrowing the bandwidth of the multilayer reflection is necessary. There have been narrow bandwidth designs of the Si/B4C [2] and the Si/Si3N4 [3] multilayers. In these multilayers, the pair materials have low contrasts at the optical constants. The basic design idea for narrow band multilayers is to make the total thickness of effective layers in reflection thicker than the temporal coherence length needed, because the reflections at the layer interfaces interfere with coherent component of themselves. We designed Mo/Si multilayers using higher order Bragg reflection with thick Si layers and constant Mo layer thicknesses because Si has a low absorption coefficient and too thin Mo layer causes island structure.

Mo/Si multilayers shown in Table 1 were designed and fabricated by an ion beam sputtering system. The integer \( m \) is the order number of Bragg reflection, which equals to one for the standard first Bragg reflection. With the Mo layer thicknesses \( d_{\text{Mo}} \) fixed at 2.5 nm, the multilayers of the period thicknesses \( m \times 6.9 \) nm were fabricated to 40 periods with \( m = 1, 2, 3, 4 \) and 8. The reflection spectrum in Fig. 1 were measured at PF BL-12A with SR of \( p \)-polarized light set at an angle of incidence of 5°. As shown in the table, the bandwidth and the reflectance of \( m = 3 \) multilayer mirror were 0.17 nm and 32%, respectively, which realized the temporal coherence length of much longer \( 13\lambda \).

Table 1. The Si layer thickness, the \( p \)-reflectance, the reflection bandwidth and the temporal coherence length of each \( m \)-th Bragg multilayer mirrors.

<table>
<thead>
<tr>
<th>( m )</th>
<th>( d_{\text{Si}} ) (nm)</th>
<th>( R ) (%)</th>
<th>( \Delta \lambda ) (nm)</th>
<th>( L / \lambda ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.4</td>
<td>60</td>
<td>0.47</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
<td>11.3</td>
<td>45</td>
<td>0.24</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>18.2</td>
<td>32</td>
<td>0.17</td>
<td>13</td>
</tr>
<tr>
<td>4</td>
<td>25.1</td>
<td>10</td>
<td>0.13</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>52.7</td>
<td>11</td>
<td>0.09</td>
<td>24</td>
</tr>
</tbody>
</table>

Fig. 1. The \( p \)-reflectances of \( m \)-th Bragg multilayer mirrors measured at PF BL-12A.

Distribution analysis of hydrophilic component in polysulfone hollow fiber using scanning transmission x-ray microscope (STXM)

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Polysulfone (PSF) hollow fiber is widely used as a membrane filter in water-treatment systems, medical devices, and so on. When used for these purposes, PSF is often blended with some kinds of hydrophilic polymers in order to gain good wettability or biocompatibility. Therefore, the nanometer scale distribution of such hydrophilic component in a hollow fiber is very important. We have investigated the distribution of poly(N-vinylpyrrolidone) (PVP) in a PSF hollow fiber STXM at Advanced Light Source (ALS) beamline 5.3.2. An ultrathin section of PSF hollow fiber embedded in epoxy resin was prepared for STXM analysis. A series of mapping data (49 mappings) was obtained at the same analysis area by varying the photon energy with 0.2 eV step near the C K-edge NEXAFS region. Singular value decomposition analysis using internal (PSF and epoxy) and external (PVP) standard spectra showed clear distribution of each component. It has been clearly revealed that the PVP-rich thin layer exists on the surface of PSF. The average thickness of this PVP rich layer was measured to be 35 nm. The results will be compared with cross sectional TEM images.

![Image of polysulfone hollow fiber distribution analysis](image-url)
An X-ray microscopy perspective on the effect of glutaraldehyde fixation on cells
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Abstract

X-ray microscopy (XM) is the only microscopy technique that can provide high resolution (30 nm) imaging of biological specimens without the need to fix, stain or section them. We aim to determine the effect, if any, of glutaraldehyde fixation on algae cells from the XM perspective and thus provide beneficial information for both X-ray and electron microscopists on artifacts induced by glutaraldehyde fixation.

Three species of microalgae, *Microcystis aeruginosa*, *Anabaena spiroides* and *Chlorella vulgaris*, were used in this study. XM images were obtained from unfixed and glutaraldehyde-fixed cells and cell diameter and % X-ray absorbency measured. The mean diameter of cells from fixed preparations was smaller than unfixed ones; the mean diameter of *M. aeruginosa* cells was significantly reduced from 3.92 µm in unfixed cells to 3.43 µm in fixed cells (P < 0.05); in *C. vulgaris* the diameter of cells was also significantly reduced from 3.50 µm in unfixed to 2.98 µm in fixed samples (P < 0.05); while there was no significant reduction in the diameter of *A. spiroides* cells (4.04 µm to 3.90 µm). The protein crosslinking mechanism of glutaraldehyde probably generated free water molecules, which play an important role in radiation damage induced by X-rays. This was seen as mass loss and cell shrinkage, which in the present study occurred more frequently in fixed cells than unfixed cells. In addition, we demonstrated that the uptake of glutaraldehyde by cells makes all protein constituents in the cell organise into a closely packed configuration, thus causing a rise in percent X-ray absorbency. In fixed cells, this rise was approximately by a factor of two compared to unfixed samples where protein constituents inside the cell are arranged in their native form.
Development of a High-Angular-Resolution Microdiffraction System for Reciprocal Space Map Measurements

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X-ray reciprocal space mapping is a powerful method for characterizing the strain status of strained thin layers because the variation in orientation of the crystal planes (mosaic structure) can be distinguished from the variation in lattice spacing. This method becomes much powerful with using x-ray microbeam. We therefore developed a new high-anguler-resolution x-ray microdiffraction system.

The new diffractmetor is setup at the BL46XU of the SPring-8 (Fig. 1). A phase zone plate is employed for a focusing device. A narrow slit is placed in front of the phase zone plate in order to partially irradiate the zone plate. This realizes the focused beam with small size and small angular divergence. The beam size and angular divergence are measured to be 1.0 µm × 2.8 µm and 60 µrad, respectively at an x-ray energy of 15 keV. The sample stage consists of high precision stepping motor driven stages including θ-2θ rotation stages and XYZ linear stages.

We demonstrated that the local strain and the crystallinity of SiGe layers can be analyzed using this system. Figure 2 shows two-dimensional reciprocal-space map around the Si 004 and SiGe 004 diffraction spots. The profile of the SiGe 004 peak, broadened along the Qₓ direction, consists of several small peaks. This result indicates that submicrometer-sized crystal domains exist in the SiGe layer.

Figure 1. Top view of the system used for X-ray microdiffraction.

Figure 2. The reciprocal-space maps around the Si 004 and SiGe 004 spots of samples.
Since the last X-ray microscopy conference (Grenoble 2002), we have made considerable progress in all aspects of micro and nano fabrication.

Through systematic and careful control of thin film deposition parameters, we have been able to minimize stress in zone materials, which in turn has enabled us to fabricate high-resolution nanostructures with minimum dimensions below 50 nm, and thickness of up to 1.5 μm. Tungsten is still the material of choice for the fabrication of diffractive optical elements, because it offers exceptional diffraction efficiencies throughout the most used X-ray energy range. It is also a very stable material, due to the close matching of its thermal expansion coefficient with that of Si/Si3N4, which are the most common substrate materials. Examples of our recent work in tungsten will be shown, both diffractive optics and Test Objects (TOs), with aspect ratios of >5 and in some cases near 10.

More recently, we have been involved with the design and fabrication of a new generation of condenser Zone Plates for Imaging microscopy, which should give a uniformly illuminated large (30-50 μm) object field, as well as minimize some unwanted diffraction effects common in modern high coherence beamline designs.
Development of beam splitters for the EUV region

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We are developing beam splitters for the extreme ultraviolet (EUV) region, which have application to interferometric X-ray microscopy, polarization experiments, and so on. The beam splitters were designed for the EUV region such as wavelengths of around 6 - 30 nm, and various incident angles. The fabrication involves the deposition of multilayers on a SiN membrane by magnetron sputtering, and the subsequent removal of the SiN membrane by reactive ion etching. Figure 1 is a photograph of a fabricated transmittance type beam splitters for a wavelength of around 6 nm. The window is 10 mm square. Measurements on Beamline 6.3.2 of the Advanced Light Source revealed the reflectivity of a CoCr/C beam splitter to be 8.7% and the transmittance to be 4.4% at a wavelength of 6.36 nm and an incident angle of 45 degrees. The reflectivity of the Cr/C beam splitter to be 5.8% and the transmittance to be 6.6% at a wavelength of 6.15 nm and an incident angle of 80 degrees. Figure 2 is a measured reflectivity and transmittance of CoCr/C multilayers for an incident angle of 45 degree.

![Fig. 1 Photograph of transmittance type beam splitters.](image)

![Fig. 2. The measured reflectivity (dotted line) and transmittance (solid line) of a CoCr/C beam splitter.](image)
Microprobe EXAFS at the SRS


Microprobe applications are becoming routine on modern 3rd generation synchrotron sources and benefit from the high brightnesses and small source sizes inherent in them. Older, second generation facilities are characterised by larger sources sizes and have lower degrees of photon beam collimation, so are not as competitive at producing very small x-ray focii. We have successfully built and commissioned a Kirkpatrick-Baez pair of elliptically bent mirrors for EXAFS measurements on an existing wiggler beamline on the SRS. This produces a sub-50 micron focal spot with sufficient flux to collect meaningful EXAFS data over the 5 to 15keV energy range. The resulting spatial resolution is well suited to a number of environmental, Earth sciences and archaeological problems and the ability to conduct small beam x-ray spectroscopy represents a new area for UK science.
X-ray refraction imaging of the lung and histological correlations

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Rationale and Objectives;
Authors performed this study to observe microstructures of the lung using synchrotron radiation beam and matched findings with histological observations.

Materials and Methods;
X-ray refraction image from ex-vivo ventilating rat lung was obtained with 8KeV of monochromatic beam. Obtained images were analyzed and compared with conventional light microscopic findings from same sample.

Results;
Pulmonary microstructures including alveolar ducts, alveolar sacs, alveoli, alveolar walls and perialveolar capillary networks were clearly identified and had good correlation with conventional light microscopic findings. The shape of alveoli kept more round than in microscopic image.

Conclusion;
The findings suggest that synchrotron radiation provides a novel research tool for respiratory medicine and possible clinical application in near future.
Analytical designing of two-aspherical-mirror anastigmats permitting practical misalignments for soft-X-ray imaging

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With use of soft X-ray multilayer mirrors of several tens % reflection at normal incidence, practical microscopes with a laboratory source have been expected to be realized as an alternative to the conventional zone-plate microscope, which requires a narrow beam of synchrotron radiation available at limited location and machine-time. Historical Schwarzschild optics has also been demonstrated for imaging microscopes for soft X-rays.\textsuperscript{1-2)} However, the resolution still stays at a few micrometer range.

Although the Schwarzschild optics has good characteristics of small aberration, it has a practical drawback in high alignments accuracy required. As Horikawa\textsuperscript{3)} has pointed out, for imaging by a soft X-ray of 3.98 nm in wavelength, a permissible alignment error of the Schwarzschild mirrors falls within 300 nm for achieving diffraction limit imaging. Such a high sensitivity to misalignments can be the dominant difficulty for implementing the mirror optics of several cm in diameter and several tens cm apart at sufficient stability under various disturbances such as temperature drifts and mechanical vibrations.

The most promising solution to overcome this difficulty would be to seek for low alignment sensitivity configuration allowing larger misalignments by extending the spherical mirrors to aspherical, since such mirrors are now commercially available. For versatile designing to find new solution groups, an analytical method should be much more useful than a standard computer designing based on ray tracing and numerical optimization. Closed-form equations usable for this purpose to search for two-aspherical-mirror anastigmats have been previously treated.\textsuperscript{3)} The equations were found impractical however, because the solution groups were described by one variable indirect to practical design parameters. Therefore, the pupil obstruction needs to be calculated separately, which lacks insight to find practical solutions of high throughput essential in the soft X-ray optics composed of the partial reflection mirrors.

In this presentation we propose a new analytical method of designing based on new practical equations formulating aberrations for searching two-aspherical-mirror anastigmats in terms of the pupil obstruction of the optical systems. Then we introduce additional aberration terms caused by a slight misalignment to discuss the misalignment sensitivity of the solution groups. These formulations are then used for searching the anastigmats groups of soft X-ray microscopes respecting low sensitivity to misalignments and a large field of view.

References
Status of the Cryo X-ray Microscope in Aarhus

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The Institute for Storage Ring Facilities at the University of Aarhus operates an X-ray microscope in the soft X-ray region at a bending magnet source on the ASTRID storage ring. After an upgrading process the possibility of investigating cryogenically fixed samples mounted in a vacuum object chamber is now given at this microscope. In comparison to experiments taken at room temperature in an atmosphere of air and/or helium an enhanced stability against radiation damage is especially observable in biological samples. For mounting an object under cryogenic conditions in the microscope a modified Gatan cryogenic sample holder is used. In this poster the design of the object chamber will be outlined and first results of imaging experiments will be presented.
A scanning microscope using laboratory X-ray sources

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Currently high-resolution static X-ray microscopy is mainly performed with synchrotron radiation [1,2] or laser plasma sources [3,4]. Recently EUV transmission microscopy with high-harmonic radiation was demonstrated with a resolution of better than 400 nm [5]. Here we present a compact scanning transmission microscope developed for EUV and soft X-ray laboratory sources, including high-harmonic sources[6]. With a laser plasma source the resolution of the scanning microscope is determined to 500 nm at $\lambda = 17 \text{ nm}$. This is close to the theoretical resolution of 350 nm. With the new technique of high-harmonic generation the way is paved to examine highly dynamical processes, applying visible-pump and X-ray-probe techniques, time-resolved photoelectron or X-ray fluorescence spectroscopy. We demonstrate first results of the scanning transmission microscope using plasma and high-harmonic radiation for imaging different objects. The 13 nm and 17 nm oxygen lines of a laser plasma source and the 61$^{th}$ harmonic of a laser based high-harmonic radiation source have been used. The high-harmonic source was driven by 25 fs/1 mJ pulses of a Ti:sapphire laser. To ensure a uniform, monochromatic illumination the radiation is spectrally narrowed with filters and/or a Mo/Si multilayer mirror optimised for 13 nm. The set-up of the microscope can easily be modified for imaging in the water-window region between the K absorption edges of oxygen and carbon (2.34 - 4.38 nm). This spectral region is especially adapted for biological and medical investigations and desperately waiting for suitable high resolution laboratory microscopes.

X-Ray Spectromicroscopy Studies of the Effect of Chain Length and Substrate Temperature on the Growth and Morphology of n-Alkane Thin Films

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X-ray microscopy has been used to study the morphology and growth of thin films of linear n-alkanes prepared by vacuum evaporation onto freshly cleaved NaCl(001) surfaces, where molecules can align along the [110] and [-110] directions on the NaCl(001) surface. X-ray microscopy experiments were performed using the Scanning Transmission X-ray Microscopes (STXM) on beamlines 5.3.2 and 11.0.2. at the Advanced Light Source (ALS). NEXAFS microscopy at the Carbon K-edge reveals that the morphology and orientation of these vapor deposited n-alkane thin films changes systematically with the chain length and the substrate temperature during deposition. Figure 1 presents STXM images for hexacontane (HC, C60H122) deposited onto the NaCl(001) surface at different substrate temperatures recorded at 287.6 eV (e.g. C1s → σ*C-H transition). These images show strong contrast, attributed to different molecular orientations of the different domains. The size of the domains depends on the deposition substrate temperature, an effect that can be attributed to the increased molecular mobility and a decreased crystal nucleation density during growth at elevated substrate temperatures. Complementary phenomena are observed when the chain length of the n-alkane molecules is varied.

(a) Room temp (b) 36°C (c) 40°C (d) 45°C

Figure 1. X-ray microscope images of hexacontane (C60H122) deposited onto NaCl(001) surfaces at different substrate temperatures. These images were recorded with a x-ray energy of 287.6 eV corresponding to the C 1s → C-H transition.

Acknowledgements: Research was supported NSERC, CFI, Saskatchewan Synchrotron Institute and the University of Saskatchewan, and performed on the BL 5.3.2 and 11.0.2. STXM microscopes at the Advanced Light Source, supported by the U.S. Department of Energy.
CaPeRS and LOX: Photoemission Electron Spectromicroscopy at the Canadian Light Source

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This presentation will discuss recent research results and future prospects for the Canadian Photoelectron Research Spectromicroscope (CaPeRS) facility. This photoelectron emission microscope (PEEM) was purchased from Elmitec GmbH and was used on multiple beamlines at the Synchrotron Radiation Center (SRC, Stoughton, WI) since April 2002. At the SRC, the CaPeRS microscope was used for surface and micro-scale chemistry of a wide range of heterogeneous materials, including the absorption of proteins on patterned polymer surfaces, chemical characterization of grain boundary precipitates in metal alloys and of carbonaceous matter in geochemical materials.

A corresponding data acquisition software package, LOX, has been developed to integrate the microscope with beamline, shutter and microscope control and for acquiring complementary signals.

The microscope was recently moved to the Canadian Light Source (CLS, Saskatoon, SK), where it is now being recommissioned for use on a several beamlines, including a specialized branch of the spectromicroscopy beamline (Apple II EPU source, entrance slit-less PGM monochromator, 250 – 2000 eV energy range) where the increased photon flux and greater experimental flexibility will be harnessed later in 2005.

Acknowledgements: The CaPeRS X-PEEM microscope was funded by NSERC (Major Installation), the Province of Saskatchewan and the University of Saskatchewan, and has been used at the Synchrotron Radiation Center (supported by the NSF) on facility beamlines and beamlines of the Canadian Synchrotron Radiation Facility (supported by NSERC).
Industrial Applications of Scanning Transmission X-Ray Microscopy at The Dow Chemical Company

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We have been using the scanning transmission x-ray microscope (STXM) at the 5.3.2 beamline at the Advanced Light Source for submicron materials characterization to provide understanding and feedback for new materials design projects at Dow. STXM provides the useful ability to measure molecular composition at the 35 nm spatial scale for determining the chemical structure of polymers and other soft matter. For instance, additive dispersion in polymeric materials can impact polymer properties. An example of the application of STXM to understanding the distribution of additive in a bulk polymer is shown in Figure 1. STXM was able to detect the concentration in the bulk and when the additive levels were high enough, detect phase separation or precipitation of the additive into particles. Taking advantage of the dipole selection rules for near edge x-ray absorption fine structure (NEXAFS) spectroscopy, one can also learn about the molecular orientation of polymers. This poster will share some recent applications of STXM to industrial research and development projects at Dow.

Figure 1. STXM images of PP foam sections containing 8000ppm (left) and 4000ppm (right) Irganox 1010 antioxidant additive.
Mach-Zehnder interference microscopy using X-ray laser

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Recently, an x-ray laser is used as the coherent soft x-ray source for several
applications; such as interferometry of the electron density of laser plasmas and the
study of the dynamics of a surface domain structure of ferroelectric materials. At the
Advanced Photon Research center, we have developed a fully coherent x-ray laser
(XRL) at the wavelength of 13.9 nm. We propose a Mach-Zehnder interference
microscopy with the fully coherent XRL using two transmission gratings as beam
splitters.
2D and 3D Imaging of Breast Cancer and Lung Alveoli

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2D and 3D images due to refraction of breast cancer specimen and lung healthy specimen have been successfully observed. A novel system Dark-field Imaging (DFI) has been applied to visualize human breast cancer and lung alveoli specimen with 5 micrometer spacial resolution and high contrast resolution in 2D form. An example of DFI image of breast cancer is shown in Fig.1.

Fig.1. DFI image of breast cancer specimen. This sample is micro papillary carcinoma, which involved lactis duct. Its view dimension is 2.5 mm in square which is a part of a specimen with size of 26 mm (width) x 22 mm (height) x 2.8 mm (thickness). Obtained image showed very high contrast.

Fig.2. Pathological image of the neighbouring region of the same sample as for the DF image in Fig. 1 with the same scale

The algorithm for 3D reconstruction of X-ray image due to refraction has successfully achieved recently. That was applied to 2 kinds of human tissues, one breast cancer and the other lung alveoli. Both rod shaped specimens have the size of 3.5 mm in diameter and 4 mm in length. Their marvelous results are shown in Fig. 3 (a) and (b).
Contact X-ray microscopy and micro-radiography on lithium fluoride detectors

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The lack of a good detector for Contact X-ray Microscopy (CXRM) has limited up to now the performances of this simple and cheap microscopy technique. In this contribution, a new detector based on Lithium Fluoride (LiF) crystals or films is proposed. Experimental results demonstrating the high spatial resolution (sub 100 nm) and the high dynamic range achievable on a LiF detector will be presented. Also for applications on contact micro-radiography of biological samples, where generally photographic films or CCD cameras are widely used, this novel image detector presents interesting potential applications due to its higher resolution. A comparison between a LiF detector and other image detectors will be discussed. Finally, preliminary experimental results of CXRM of some biological specimen (leptolyngbya, etc.) imaged on LiF will be shown.
Detection of heavy metals in different biological samples

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Different biological samples (typically leafs) have been analyzed by soft X-ray microradiography at different wavelength values in order to detect their heavy metals intake, either as a natural content (for example magnesium) or induced by artificial doping. Qualitative and quantitative measurements of the metal intake have been obtained by microradiography at 1-2 keV photon energy. These results are interesting for phytoremediation applications. The technique has been applied also for the detection of atmospheric pollutant intake into lichens. Preliminary results of all these different topics will be shown.
Development of high energy micro-XRF analysis using Fresnel zone plate optics

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High-energy (over 20 keV) microbeam is an attractive technique for X-ray fluorescence (XRF) analysis. Recently, we have developed sputtered-sliced Fresnel zone plate (ss-FZP) as an X-ray focusing device and successfully obtained an X-ray microbeam at 100 keV [1]. In this study, XRF spectroscopy using a microbeam was applied for the first time to the hyperaccumulator plants of Cd in order to reveal the distribution of such toxic heavy elements in their tissues and cells.

The microbeam optics has been constructed at BL37XU of SPring-8. A single-bounce monochromator with Si 111 reflection of Bragg angle of 1.5 degree provides 75.5 keV photons. A focused microbeam was evaluated by the knife-edge scan method. The beam size was estimated to be 2\(\mu\)m (V) x 5\(\mu\)m (H). The X-ray fluorescence intensities were measured by Ge-SSD. The plant samples cultivated with a medium containing Cd were subjected to the analysis. The samples were prepared as a slice of tissues by microtome for the organ analysis.

Figure 1 a) shows an optical microscope image of the sample. The two-dimensional distributions of the trace elements in the plant tissues were clarified. \(\mu\)-XRF imaging of Cd and Mo are shown in Fig. 1 b) and c), respectively. It was found that Cd and Mo were accumulated in the sieve tissues of the plants. This study has demonstrated that high-energy microbeam is a new effective tool for ultra-sensitive analysis of trace heavy elements.

References

Fig.1 a) optical microscope image of sample, \(\mu\)-XRF imaging of b) Cd, and c) Mo (pixel size: 2\(\mu\)m (V) x 5\(\mu\)m (H), dwell time: 1 sec.).
Initial Development of a sub-micron Angle Resolved Photoemission Microscope

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We have begun initial development of a sub-micron angle resolved photoemission microscope. The current test system consists of an SES-200 detector and a zone plate based focusing system operating at 180eV photon energy. We have measured angle resolved spectra using the SES-200 angle-dispersive collection mode at resolution of ~500nm. We have used this to show orientational contrast on highly oriented pyrolytic graphite (HOPG). The domains on HOPG are on the order of 1-20 microns and are well orientated along the c-axis but show random azimuthal order. We are able to clearly image these domains even though they show no chemical contrast, and can measure the single crystal band structure on disordered polycrystalline sample. We believe this demonstrates the promise of such a system for the measurement of materials which cannot be found in bulk single crystals.