Mapping of intracellular light elements including Ca, P and S in mammalian cells by soft X-ray contact microscopy

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Soft X-ray microscopy is a powerful tool for high-resolution mapping of light elements in thin specimens such as a biological cell with a few-micron thickness. We have explored the method to obtain intracellular distribution of light elements particularly of minor light elements such as Ca, P, and S. Since abundant major light elements, C, O and N are constituent elements of most biomolecules, the distributions of those elements are similar to a cell image formed by X-ray absorption. On the other hand minor light elements are contained in specific biomolecules; for example, nucleic acids and phospholipids contain phosphorus, while proteins do not. In addition in some cases the distribution of those elements is known to reflect physiological response.

For the elemental mapping we have used contact microscopy with an electronic zooming tube that has sensitivity over the wide wavelength range covering the absorption K or L edges of constituent elements in a cell. From the absorption spectrum of one pixel in a cell image we have developed a computer program to obtain the quantities of minor elements whose absorption edges are hidden by large absorption of major light elements. In our previous algorithm, elemental content of C, O and N that is calculated from the absorption jump across the K absorption edge is subtracted from the absorption spectrum, resulting in the appearance of the absorption jump at the absorption edges of minor elements. The elemental content of these elements is obtained by the same procedure as major elements [1].

In the present study, we have improved the above method by introducing iteration procedure until absorption jumps at all the detected absorption edges become zero. In every iteration process we can find new elements in a specimen by the recognition of absorption jump at the absorption edge and improve the accuracy of elemental content. The content of all the elements whose absorption edges become evident is calculated by solving a simultaneous equation in which absorbance is expressed as a linear combination of mass thicknesses of constituent elements. The algorithm was checked for the images of dried KNO₃ and 2'deoxyguanosine ($C_{10}H_{13}N_5O_4$) with known elemental composition. With these standard specimens we had a good agreement with the expected values by choosing appropriate wavelengths for imaging at the both sides of the absorption edges of these elements. Then the program was applied to the image of dried human HeLa cells. Distribution of phosphorus was more preferential in the nuclear region than those of C, O or N, while sulfur seems to distribute uniformly in a cell. Interestingly calcium was mainly accumulated in the nuclear region. A simplified method based on the similar iteration algorithm is now being developed.

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STXM investigations of biological tissues

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In contrast to the conventional use of scanning transmission x-ray microscopy as an analysis tool for materials, especially extending to "soft materials", we have investigated some biological tissues to ascertain an alternative and promising avenue of the application of zoneplate based scanning x-ray microspectroscopy. As the number of x-ray microscopes worldwide increases, the technique should be able to augment the arsenal of techniques that could be of use to the biological community under suitable experimental conditions.

We have investigated several biological samples taking advantage of the polymer STXM at the Advanced Light Source. A set of experiments focused on the investigation of biological fibers, in particular the spectroscopic properties of spider webs. Preliminary energy dependent line scans across the fibers yielded no distinct dichroism mechanism and thus no direct evidence for a preferential orientation of the monomers. Another interesting biological area is the analysis of human hair. Although the lateral resolution in STXM (<35 nm at BL5.3.2) is currently about an order of magnitude higher than transmission electron microscopy (TEM), the resolution achieved was sufficient in recognizing the internal structure and morphology of human hair. However, no specific spectral signatures were distinguished which would have allowed identification of distinct chemical species.

	Previous imaging with
	hv=320 eV
	hv=531.6 eV
	hv=400.3 eV
1233	hv=390 eV
-	hv=288.3 eV
	hv=284.6 eV

A 20 x 20 μ m² STXM image of a microtomed xanthopan eye recorded at the ALS 5.3.2 STXM (hv = 288.3 eV). The smaller rectangular areas were pre-recorded images which subsequently indicate different contrast enhancement that become amplified due to x-ray imaging on the same area of the sample.

Radiation damage or in-situ chemical changes due to the high photon flux density are often a disadvantage in x-ray microscopy. However, in some cases it may be advantageous as we have demonstrated for insect eyes. We have investigated microtomed samples from the rear part of the compound eye (taken from the moth xanthopan morgani praedicta). Scanning smaller areas with different photon energies (on-resonance, off-resonance) has yielded different contrast when scanning the sample on an absorption edge. Although the mechanism which causes the damage is not clearly understood and in particular not predictable, it should become a useful tool in x-ray microscopy in the foreseeable future.

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Micro-CT of Pseudocneorhinus bifasciatus by projection x-ray micosopy

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The projection X-ray microscope utilized a very small X-ray source from a thin (0.1-3 μ m) target metal film that is excited by the focused electron beam of a scanning electron microscope (SEM). If an object is placed just below the target metal film, the diverging X-rays enlarge the shadow of the object. Because no X-ray optics such as zone-plate is used, focal depth is infinite in principle. Taking this advantage, we have applied projection X-ray microscope for three-dimensional (3-D) structure analysis by means of cone-beam computed tomography (CT). The projection images of a small arthropod (*Pseudocneorhinus bifasciatus*, 5 mm in length), was recorded in every 3 degree for whole direction (360 degree) with a stepping motor controlled sample rotator. The 3-D image was reconstructed from corn-beam projections using filtered back-projection algorithm.

Three dimensional reconstructed image was calculated as $256 \times 256 \times 256$ boxel data. In Fig.A-C, outermost surface of the reconstructed image is shown using three-dimensional visualization software (AVS) and it reproduces the original object precisely. Some part of slice images vertical to the rotation axes and two directions along the rotation axes were shown in Fig. D, Fig.E, and Fig. F, respectively. The reconstructed 3-D image shows detailed internal structures of an opaque object.



Quality Improvement of Soft X-Ray Image in FCSXRM ETL Mark III

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The table-top Flash Contact Soft X-ray Microscope (FCSXRM) ETL Mark III is the soft x-ray microscope system for convenient use in laboratory [1-2]. Laser-produced plasma is suitable for a flash x-ray source. An x-ray image of a specimen is recorded in terms of contact mode on a photo resist PMMA (polymethyl methacrylate) membrane supported on a silicon chip of 5-mm square. After chemical development of the PMMA chip, the x-ray image is enlarged with an atomic force microscope (AFM). The principle of soft x-ray imaging for a living cell is carbon imaging, which provides a carbon density map of the specimen in water. In the soft x-ray wavelength range, carbon and oxygen have absorption edges at 4.4nm and 2.3nm respectively. The mass absorption coefficient of carbon is about 10 times greater than that of oxygen within the wavelength range between these absorption edges. Using this wavelength range (so called "water window"), one can obtain the soft x-ray image of the living cell in water.

Quality of the x-ray image obtained by FCSXRM ETL Mark III depends on the uniformity of the thickness of water surrounding the specimen. In this system, an x-ray window (0.5-mm square) made of silicon nitride membrane separates a vacuum chamber for plasma production from a sample holder kept under atmospheric conditions. Flexure of the x-ray window due to pressure difference between the vacuum chamber and the sample holder causes the non-uniformity of water thickness under the x-ray window, and then it produces crucial unevenness of contrast in the x-ray image. In case of a large specimen with several hundreds micrometers in size, the unevenness of contrast is critical at the central part of the x-ray window. To reduce the flexure of the x-ray window during x-ray exposure, the vacuum chamber was filled with helium gas and/or a spacer was located between the PMMA membrane and the x-ray window. These improvements gave successful x-ray images of large cells cultured on the PMMA chip. Using this technique, extra cellular matrix of 3Y1 cells were examined. A possible role of soft x-ray imaging in biological research will be discussed.

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Observation of thick biological specimens in their natural aqueous environment has been one of the major subjects for X-ray microscopy (XRM). Among those specimens, eukaryotic chromosome is one of the most attractive ones for XRM for the following reasons: a) It has characteristic hierarchical structures from DNA to a chromosome with the sizes ranging from nanometer to micrometer level; b) some of the intermediate structures have actually been still in controversy even by using current high resolution imaging methods such as electron microscopy and atomic force microscopy; and c) XRM is an imaging method to obtain transmitted images of specimens at the resolution-range compatible with the most size levels of hierarchical structures in a chromosome. Thus, X-ray microscopy is expected to have potential to give some new information on the architecture of a chromosome. Numerous attempts to visualize both dry and hydrated chromosomes from various organisms have been made using various types of XRM. However, most of them have not been necessarily attempted to clarify the organizing processes from nucleosomes to a chromosome. As a result, there have been few discoveries to add to the knowledge of the chromosome structures obtained mainly by electron microscopy for understanding the architecture of a chromosome despite that the resolution of XRM has already been accomplished to the level of several ten nanometers. Based on the review of the past studies on chromosomes using XRM including our works, we will discuss the problems of X-ray microscopy for the study of chromosome structures and to propose some approaches to solve the problems.

The 18ID BioCAT microprobe for X-ray imaging, microXAFS and microdiffraction studies on biological samples

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Microfocus capabilities have been recently incorporated at BioCAT that allow x-ray imaging, microXAFS and microdiffraction studies on biological samples. The microprobe optics comprises a pair of platinum coated silicon KB mirrors, 4x0.5x0.2 inches. The energy range of the BioCAT microprobe is 3.5 keV up to 17 keV. Sample holder is mounted in a XYZ high precision positioner (0.1 micron). A set of fluorescent detectors: Ketek Si drift detector with polymer window for light elements detection, Fe and Zn Bent Laue analyzers and a Ge detector. A high sensitivity CCD detector designed specifically for synchrotron time–resolved applications is used for microdiffraction experiments. The combination of the x-ray microbeam and the existing fast scanning capabilities of the BioCAT beamline allow performing x-ray imaging and microXAFS measurements. The system allows measuring samples with different spatial resolutions. 100 microns, 20 microns and 3.5 microns beam sizes are commonly used.

This paper reports the commissioning results of the KB mirrors and the first results of x-ray fluorescence mapping, microXAFS and microdiffraction at the BioCAT 18ID undulator beamline. A 3.5 x 3.5 micron^2 beam at 10 keV was measured by means of a knife edge Ni thin film. Comparison of standard 2D step scans and fast continuous scans will be presented. Examples of x-ray fluorescence mapping of neurodegenerative brain tissue and microEXAFS at the Zn K edge in brain tissue will be shown. Micro x-ray diffraction experiments of fibrous collagen specimens has been performed. The setup allows to scan the sample for regions of greater crystalline areas and reduce spatial spread of reflections originating from fibrous crystallites of small dimensions (~15-50 nm) and therefore obtain a better signal to noise ratio.

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X-ray CT and DEI images of the biological soft-tissue using synchrotron X-rays

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Biological soft-tissue embedded in marine invetebrates are imaged using 20, 30 and 40 keV synchrotron X-rays utilizing X-ray CT and diffraction-enhanced imaging technique. The choice of energy is chosen based on the quality of the image. Visualized the embedded features at different regions within the shell. The choice of optimum energy allowed us clear visibility of the soft-tissue internal structure with void space. The sensitivity of X-ray imaging to soft-tissues must be improved with monochromacy, for better contrast, in particular, utilizing the new X-ray modalities, such as, diffraction-enhanced imaging. Recently, refraction properties of X-rays turn to be more attractive advantages for imaging over the absorption properties. Refraction is orders of magnitude more sensitive, particularly for biological or low materials. Diffraction-enhanced imaging (DEI) technique exploits these refraction for differentiating the biological soft-tissue with high collimated synchrotron X-rays. Compared the images obtaine using X-ray CT and DEI.

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Images of the rat bone and lumber vertebra using diffraction-enhanced imaging technique

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Different age groups of the rat bone, lumber vertebra and phantoms of different composition are imaged using 20, 30 and 40 keV synchrotron X-rays. The choice of optimum energy is chosen based on the quality of the image for better visualization and analysis. Visualized the embedded spongiosa within the bone, in order to know the embedded features in a more visible way with DEI. Diffraction-enhanced imaging technique provided, considerable improvement in contrast compared to conventional radiography. The scattered radiation is reduced considerably with the use of this novel technique, which provided considerable new information with medical and biological samples. Performed the rocking curve analysis at all the studied energies.

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Nickel Induced Lateral Crystallization of Amorphous Silicon Studied by SPESM

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The crystallization of amorphous silicon has been a subject of research because of its scientific interest and technological importance. Since Low temperature polysilicon (LTPS) technology is the novel technology specific for Display application. Recently, metal induced lateral crystallization (MILC) of amorphous silicon (a-Si) has been studied intensively. It is proven that MILC is a technique for lowering the crystallization temperature of a-Si. The application of MILC can be used in fabricating Silicon Thin Film Transistor (TFT). The studies show that MILC enables reduced-temperature solid-phase crystallization, which leads to the realization of TFTs at a significantly reduced process temperature with improved characteristics. However, the undesirable contamination of metals to the poly-Si thin film is a concern for practical applications. Thus, it is interesting to study the interaction of metal patterned film with a-Si film. In our report, the reaction of Ni metal with a-Si thin film has been studied with SPESM (Scanning Photoelectron SepctroMicroscopy). Lateral variations in the local chemistry of the MILC of a-Si were directly imaged. The information on the lateral distribution of the different chemically-shifted phases as function of the local chemical environment for each phase was also obtained.

Scanning Transmission X-ray Microscopy (STXM) of Onion-type Polymer Microspheres

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Polymer microspheres consisting of concentric layers with different chemical compositions and physical properties may be useful as 3D-photonic bandgap devices and as catalyst supports. They are prepared here for the first time by precipitation polymerization of crosslinking monomers under conditions where thermal or chemical perturbations of the polymerization conditions are used to write concentric patterns into the growing microspheres. STXM at ALS beamline 5.3.2 is then used to map the compositions of the resulting onion-type microspheres with high spatial and chemical resolution. For these samples we use the C1s $\rightarrow \pi^*$ transitions for divinylbenzene (DVB) at 285.2 eV and for the phenylmaleimide carbonyl at 288.4eV. The microsphere formation involves polymerizing crosslinking monomers including DVB in marginal solvents such as acetonitrile, such that the forming polymers aggregate in controlled

fashion to form mono-disperse microspheres of about 3 micron diameter. Under certain conditions, this aggregation becomes sensitive to thermal fluctuations of the polymerization, permitting rings of lower crosslink density to be inscribed into the growing microspheres by imposing a thermal profile on the polymerization. Fig. 1 shows one possible thermal profile imposed during a 20 hour polymerization, the corresponding internal ring structure as seen by TEM, the quantitative DVB map of one quadrant of a sphere, and a radial line profile. This line profile can be used to calculate the refractive index profile of these microspheres, and hence evaluate potential use as photonic band gap materials. In analogy, doping with carefully

selected comonomers such as phenylmaleimide at, for example, 4 and 8 hours into a similar precipitation polymerization gives rise to the



Fig. 1 Thermally generated polymer onions. (left) T(reaction) vs. time (hr) and TEM. (right) STXM-derived DVB map, and radial profile.



Fig. 2 TEM image, STXM-derived PMI map of DVB-PMI onion; PMI structure.

corresponding poly(DVB-*co*-PMI) layers. **Fig. 2** compares a TEM image (mapping electron density) with a C 1s STXM derived quantitative map of the PMI component. Use of chemically reactive comonomers such as itaconic anhydride (IA) instead of PMI permits subsequent layer-specific chemical modification of the onion microspheres. Experiments are in progress to trap organometallic catalysts at specific layers for subsequent catalytic reactions.

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Atomic Image around Mn Atoms in Diluted Magnetic Semiconductor Zn_{0.4}Mn_{0.6}Te Obtained by X-ray Fluorescence Holography

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Recently, diluted magnetic semiconductor $Zn_{1-x}Mn_xTe$ has attracted much attention as a spintronic material. From an X-ray diffraction [1], the lattice constant of $Zn_{1-x}Mn_xTe$ linearly changes with varying *x* (Vegard's law), while an XAFS results [2] showed almost unchanged Mn-Te and Zn-Te bond lengths (Pauling's rule). This discrepancy led to a question of how the large MnTe₄ tetrahedra can be squeezed into the small ZnTe₄ lattice.

X-ray fluorescence holography (XFH) is a new technique that allows one to investigate a three-dimensional local image around a specific element. The sample was irradiated by intense X-rays of certain energies beyond the Mn-K absorption edge at BL37XU/SPring-8 in order to obtain the Mn-K fluorescence hologram [3]. A three-dimensional atomic image around the Mn central atoms was derived from the holograms using Barton's algorithm, as shown in Fig.1. The nearest- and third-nearestneighbor Te atoms are clearly visualized. However, the second-nearest-neighbor Zn or Mn atoms are barely visible in this image due probably to a highly distorted cation Zn(Mn) sub-lattice.

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Fig.1 Schematic view of $Zn_{1-x}Mn_xTe$ crystal (a), atomic image around Mn atom on the (110) plane obtained from the present XFH experiments (b). Large and small circles indicate the anions (Te) and the cations (Zn or Mn).

X-ray Microdiffraction for Polymer Materials at BL24XU of SPirng-8

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In SPring-8, hard X-ray microbeam, which was obtained by phase zone plate made of tantalum, is in operation at the Hyogo-BL (BL24XU hutch C1). We have investigated the skin/ core structure of polymer single fiber using an X-ray microbeam. In this study, as the further application of hard X-ray microbeam, localized microstructures of polymer/polymer interface was measured by X-ray microdiffraction method.

Laminated polymer films were obtained by the melt-pressed linear low-density polyethylene (PE) and isotactic polypropylene (PP). A position and a tilt of the film were precisely adjusted by monitoring the intensity of Thomson scattering from the sample as shown in figure 1. X-ray beam was aligned parallel to surface of the laminated film. By changing the sample position, diffraction patterns can be obtained from PE/PP laminated films with 0.9 micron (vertical) \times 1.7 micron (horizontal) beam at the 15keV in a five minutes per pattern by imaging plate.

Figure 2 shows the X-ray diffraction profiles of the laminated films. Changing the sample position relative to the microbeam across the interfacial region. Approaching to PP side form PE side, the diffraction intensity of PP gradually increased. According to these results, it was indicated that the laminated film possesses an interphase region with a few micrometers thickness.



Fig.1 Schematic representation of the X-ray microdiffraction method for film sample.



Fig.2 X-ray diffraction profiles of PE/PP laminated film.

Observation of Polymer Blends by X-Ray Phase Tomography

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Blending polymers is of importance because properties of plastic materials can be tailored for specific applications. Phase separation is observed in polymer blends and plays a role determining properties. Its structural analysis is therefore needed especially three dimensionally. X-ray phase tomography is a candidate for that purpose since its high sensitivity enables us to depict phase-separation structures without treatments, such as labeling with high-Z elements or selective etching, for contrast enhancement.

We demonstrate phase separation in blends of polystyrene (PS) and poly(methyl methacrylate) (PMMA) with phase tomography using a crystal X-ray interferometer. Mixtures of PS and PMMA with 1:1 volume ratio were kept at 180 °C for phase separation in cylindrical holes 2 mm in diameter made on a copper plate sandwiched in a melt-press machine. A sample 2 mm in diameter was thus prepared and put in a cell filled with water

placed in a beam path of the X-ray interferometer. Phase measurements were performed with 17.7-keV X-rays at 400 angular positions during 180° sample rotation, and a three-dimensional image mapping the refractive index was reconstructed. A CCD-based X-ray image detector of a 3.14-µm effective pixel size was employed.

As shown in Fig. 1, bicontinuous feature of PS-rich (dark) and PMMA-rich (bright) regions was depicted clearly. The density of each region evaluated is consistent from the image with spinodal decomposition. Thus, phase tomography is a powerful unique technique for three-dimensional and observation of a polymer blend quantitatively.



Fig. 1 Three-dimensional image of a PS/PMMA blond obtained by phase tomography.

Surface Engineering with SPEM

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The goal of the project is the development of X-ray lithography with monomolecular resists and zone-plate-focused X-ray beam provided by the scanning photoelectron microscope (SPEM) station [1-3]. As such resists, we used various thiol-derived aliphatic and aromatic self-assembled monolayers (SAMs) on Au and Ag substrates. The fabricated patterns were imaged and characterized by the same SPEM setup used for their fabrication. The major advantage of this approach is its flexibility, so that any pattern can be generated by the exact settings of exposure time per specific area. Further, the patterns can be used as templates for the selective attachment of various substances by in situ exposure to the respective compounds in the gas phase. In parallel, every step of the pattern fabrication and modification can be monitored and analyzed by SPEM.

In this paper, we will present the current status of the project with emphasis on the fabrication of multi-exposure and gradient patterns, and the tailored modification of functional groups. We will address the most important parameters of the patterning process such as selectivity, lateral resolution, and optimal dosage, as well as discuss potential drawbacks of the approach.



C 1s SPEM images of microbeam patterned $CF_3(CF_2)_9(CH_2)_2S/Ag$ at the position of hydrocarbon and fluorocarbon emissions. The written lines are 10 micrometer long; they consist of individual points placed 100 nm away from each other. The dwell time per point is 150, 120, 60, 90, and 30 ms, going from the topmost line to the bottom one.

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Characterization of soft matters and trace element analysis by X-ray microprobe at the Photon Factory

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The X-ray microprobe has become indispensable tool for the material characterization. A Kirkpatrick-Baez (KB) X-ray microbeam system of BL4A at the Photon Factory has been used for the elemental mapping of biological and mineralogical samples. X-ray diffraction experiments, such as micro-grazing exit diffraction from thin films and meso-porous silica films and the intensive study of the dynamical behavior of liquid crystals and polymers, have been also performed. The routine analytical system both for X-ray fluorescence analysis and X-ray diffraction/scattering experiments is working successfully, though the beam size is around 5 micron due to the relatively large source size of the PF ring. For the X-ray fluorescence analysis and elemental mapping, a double multilayer monochromator is used to enhance the incident intensity, while a double crystal monochromator is used for the micro-XAFS and micro-diffraction experiment. The double multilayer monochromator is also effective for small angle scattering. The KB system uses a pair of elliptically shaped Rh coated X-ray mirrors with a glancing angle of 2.8 mrad which covers the X-ray energy up to 20 keV. For spectroscopic applications, a silicon drift detector has recently introduced for the higher counting rate experiment in addition to the conventional Si(Li) detector. Two dimensional detectors, CCD X-ray cameras with an image intensifier or a tapered optical fiber, become indispensable for X-ray diffraction and scattering experiments.

In this presentation, the time-resolved micro-diffraction experiments for the characterization of the dynamic and static behavior of smectic liquid crystals are described. With an appropriate combination of the monochromator and the detection system, the time resolution of a few micro-sec and the spatial resolution of the a few microns were established. The dynamic local layer response of chiral smectic liquid crystals to the external electric field was revealed for the first time.

Characterization of Polyelectrolyte Hollow Microcapsules in water by X-Ray Microscopy

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Polyelectrolyte hollow microcapsules have received great interest recently due to their unique properties and the fine-tuning of their shell thickness in the nanometric scale. They are prepared by the alternative deposition of oppositely charged polyélectrolytes (Layer-by-Layer technique) on colloidal particles and subsequent dissolution of the sacrificial template [1]:



These capsules are well characterized in dry state by atomic force microscopy or electronic microscopies, giving sub-micron and nanometric resolution. However in wet state, the resolution is much worse, due to the use of optical microscopes. Here we present the results obtained in water using a X-Ray microscope, allowing a resolution up to 20nm. This powerful technique was used to measure *in situ*, in water suspension, the tickness of capsules made of sodium poly(styrene sulfonate) (PSS) and poly(diallyldimethyl ammonium) chloride (PDADMAC), whose size and shell thickness were tuned by changing the temperature.

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Study of Surface Orientation of Carbon Alignment Layers by Polarized PEEM

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Today's flat panel displays are largely based on liquid crystal (LC) molecules that can modulate light transmission of the display through changes in orientation. The alignment of LC molecules on carbon alignment layers is hence not only an interesting scientific but also an important technological problem. Noncontact method such as ion beam irradiation without suffering from electrostatic discharging and dust contamination has been suggested to replace the current rubbing alignment process [1-2]. However, the anchoring energy of LC molecules on an ion beam treated surface is not stable. Here we used photoemission electron microscopy (X-PEEM), polarization-dependent near-edge X-ray absorption fine structure (NEXAFS) and polarized attenuated total reflection infrared spectroscopy (ATR-IR) measurements to investigate the orientation order of the carbon alignment surface. The alignment surface such as polyimide (PI) and diamond-like carbon (DLC) are treated with a hydrogen ion beam for a configuration alignment of surface bonding and passivation. With simultaneously sputtering and passivation, the results reveal a preferential asymmetric in-plane alignment of DLC layer at the substrate. In-plane orientation order of rubbed PI molecules with modifying the main or side chain structure are explored as well.

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Modulating the X-ray Polarization at a bending magnet for magnetic fullfield soft X-ray transmission microscopy

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Full field soft X-ray transmission microscopy is a powerful tool to image at high spatial resolution and with element specific magnetic contrast magnetic microstructures in low dimensional magnetic systems that are currently discussed both for fundamental and applied studies. Circularly polarized x-rays are required for magnetic X-ray microscopy since x-ray magnetic circular dichroism(XMCD), i.e., the dependence of the photoabsorption cross section at element-specific binding energies on the relative orientation between photon spin and magnetic moments serves as the magnetic contrast mechanism [1, 2]

To control the circular degree of helicity at the full-field soft X-ray microscopy beamline XM-1 (BL 6.1.2) at the Advanced Light Source in Berkeley CA we have integrated a vertical adjustable slit aperture with variable width. Thus we are able to modulate either the right or left elliptically polarized X-rays emitted from the bending magnet. We demonstrated that the magnetic contrast of the magnetic domain structure recorded at the Fe L₃ edge in a 60nm amorphous Gd₂₅Fe₇₅ thin film can be modulated by the X-ray helicity modulation and it scales with the degree of circular helicity in agreement with theoretical calculations. This technique enables us to significantly reduce non-magnetic background contributions and to enhance magnetic contrast by comparing two images taken with opposite spin of the x-rays. It also opens a new avenue for lock-in recording schemes that might be advantegous for imaging fast magnetization dynamics with high resolution magnetic soft X-ray transmission microscopy.

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Domain structures of patterned Co thin films deposited on low-index Cu surfaces

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The different domain structures between continuous and patterned Co thin film elements are studied by the X-ray Photoemission Electron Microscope (PEEM). Using an *in-situ* mask, we are able to prepare micron-sized elements on two low-index copper surfaces; Cu(100) and Cu(110). The impact of Cu crystal orientation on continuous Co film is significant; a layer-by-layer growth mode on Co/Cu(100) with the in-plane easy axis lying along <011> direction[1], and a thickness-dependent growth mode for Co deposition on Cu(110) whose in-plane easy axis exhibits a 90° switching upon the subsequent carbon monoxide adsorption[2]. For these two cases, substrate induced uniaxial anisotropy plays a dominant role in determining the magnetic properties of Co layer. For the patterned thin film element on single crystal surface, the geometry constrain introduces additional shape anisotropy. As a result, it is expected that the domain structures of Co elements to be different from those continuous films (see Fig. 1). In this study, full-field PEEM images with magnetic contrast were constructed with polarized photons irradiating the sample. The X-ray images are then used to examine the different domain structures between continuous and patterned films.



<u>Figure 1</u>: Domain structures of 8 Å thick, as-deposited continuous and patterned Co films on Cu(100).

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Diffraction-Enhanced X-ray Imaging of Hydride in Alpha-Titanium and Titanium Alloy

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Although titanium and its alloys are promising materials for preserving hydrogen, until now it has been impossible to visualize hydrides in titanium using non-destructive methods,, that is, conventional radiography that utilizes absorption of X-rays. Therefore, the present work was planned and performed with the aim of confirming the visualization of titanium hydride in alpha-titanium by using the diffraction-enhanced X-ray imaging method.

Titanium crystals (99.99 at. %, 1 x 5 x 20 mm) were annealed at 560 in hydrogen gas at 1 atm for 66 min. resulting in the formation of titanium hydride in the specimen. To observe the specimen cross-section, another specimen was prepared with hydride deposited on the surface using electrolytic charging.

The present observations were performed at a vertical-wiggler beamline, BL-14B (precision X-ray optical station), at the Photon Factory in the High Energy Accelerator Research Organization (KEK) in Tsukuba, Japan. The collimator and the analyzer were adjusted at the asymmetric and symmetric 220-diffraction condition, respectively. Absorption images of the specimen were also recorded without an analyzer crystal. The exposure time for all refraction images was 2 - 3 min for 30 keV X-ray.

Diffraction-enhanced image of hydrogen annealed titanium show weak contrast images as white weathers. The images were not observed in the absorption contrast condition and in the specimen before annealing in hydrogen gas. We founded that the white contrast image originated by refraction of hydride. To confirm that the image does indeed show hydrides in titanium, we also examined a cross-section of the specimen covered with surface hydride. The cross-sectional image of the electrolytic charged titanium specimen shows white and black contrast images at the circumference of the specimen. And the contrast of the image is reversed between low-angle and high-angle side image of the rocking curve. In contrast with titanium, refraction-contrast images of hydride were not observed in titanium-aluminum alloy. Aluminum atoms prevent the hydride formation.

We were able to obtain a high-contrast projection image of a hydride in titanium using refraction-contrast radiography. The study shows that this is a promising new technique for non-destructive inspection of bulk material systems with only small differences between refraction indexes of the components.

Evaluation of Hydrogen Diffusion in Alpha-Titanium by Diffraction-Enhanced X-ray Imaging Technique

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There have been a number of investigations of the hydrogen diffusion in titanium in order to develop hydrogen-storage materials and so on. However, the majority of investigations deal with isotope experiments or indirect detection techniques such as internal frictions of hydrogen atoms. Therefore, we applied the diffraction-enhanced X-ray imaging technique to visualize hydride, TiH_2 , in alpha-titanium polycrystals and evaluated the hydrogen diffusion.

The alpha-titanium specimens $(1 \times 5 \times 15 \text{ mm})$ were prepared with hydride deposited on the surface using electrolytic charging at room temperature and cut into a 1 mm thick slice for cross-sectional observation. Electrolytic charging time and current density were 18 or 48 hours and 5 mA/mm², respectively. The observation was carried out in BL-14B at the Photon Factory in KEK, Japan. The collimator and the analyzer were adjusted at the asymmetric and symmetric 220-diffraction condition, respectively. Low-angle and high-angle diffraction images were recorded on X-ray films with nearly half maximum of the rocking curve from the analyzer. The X-ray energy was 30 keV and the exposure time was about 3 min.

Hydride layer was visualized by thick black or white line parallel to the surface. Intensity profile of the refraction image of hydride was obtained from the low-angle and high-angle images by subtraction. In the titanium-hydrogen system, heat of solution and formation are negative. So, the hydride formation was subject to diffusion process of hydrogen atoms. Hydrogen diffusion from the surface was calculated using a solution of one-dimensional diffusion equation. Deviation angle of X-ray by refraction was calculated from hydride density distribution using the Snell's low. Finally, X-ray intensity profile obtained from the deviation angle using the rocking curve was compared with the intensity profile of refraction images of the hydride. The obtained diffusion coefficient of hydrogen in alpha-titanium, $D = 3 \times 10^{-15} \text{ m/s}^2$, is slightly larger than the widely accepted value at room temperature. The result is explained by the assumption that the specimen temperature rose during the electrolytic charging by reactions and Joule heating.

Patterned magnetic multilayer studied by X-ray PEEM

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In advance material research, the exchange coupled magnetic thin films is a subject under intensive study. From material engineering point of view, a system with tunable properties through adjusting its macroscopic parameters such as film thickness and lateral dimensions is attractive for its quick turn around time. To gain a fundamental understanding on such correlation, however, it requires additional insight other than the measurement of averaged response. Indeed, recent studies on the coupled magnetic thin film layers have observed clear variation in the domain size as well as their magnetization orientations due to the competition between in-plan and out-of-plan magnetization when the film thickness is changed [1-2]. Similarly, the domain structures in coupled sandwich structure, NiFe/Rh/NiFe, were found to be in different dimensions depending one whether the coupling is ferromagnetic or antiferromagnetic [3]. In this study, we use X-ray photoemission electron microscope to study the domain structures of exchange coupled Co/Cu/Co/Cu(100) system that is patterned into micron sizes. The Co layers in this sandwich structure is known to exhibit oscillatory interlayer coupling as a function of Cu spacer layer thickness [4], and the patterned Co/Cu(100) has different domain structure comparing with the continuous film. By varying the geometrical dimensions and individual layer thickness, we study the domain structures of patterned multilayer system.

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