Development of soft X-ray contact microscope for in-situ identification of organelles

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Abstract. A contact microscope was developed with the use of a scintillator plate which shows high quantum efficiency and linearity in the soft X-ray (SX) wavelength region. With the use of the scintillator plate, a SX image can be observed instantly by a visible (VI) optical microscope. Transmittance spectra of rat hepatic cells are shown to demonstrate as the performance of the contact microscope.

1. Introduction

Normal incident optics coating with a reflection multilayer show high spatial resolution in extreme ultraviolet (EUV) wavelength region ($\lambda=2 \sim 50$ nm), because the wavelength in EUV region is one or two magnitude shorter than that in VI region. In addition, normal incident mirror optics have convex and concave mirrors, therefore the aberration can be corrected by combination of the mirrors. As the result of the correction, normal incident multilayer optics have wide field of view [1]. When the EUV multilayer optics are applied to an observation tool for bio-cells, it is possible to observe a small organelle less than several tens of nm in a large tissue more than several hundreds of $\mu$m.

An example of the observation sample that needs both of the high spatial resolution and the wide field of view is neuron connections in brain. The functions of neurons as a key component of brain were thoroughly investigated and many of the functions are well known [2]. Nevertheless, the expression mechanism of brain functions is still unknown. For the time being it is widely believed that the form and number of neuron connections lead to expression of the brain functions. To study the neuron connections in brain, all connection points of neurons will be clarified through the observation of the whole brain and the diagram of the connections will be created by the clarified connection points [3]. The connection points are synapses which size is several tens of nm in diameter and connections of neurons integrated form a brain in size of from several mm to several cm in diameter.

As an observation tool for the sample that needs both of the high spatial resolution and the wide field of view, transmission-type EUV microscope, TXM$^3$, with normal incident multilayer optics was implemented in a working 13.5 nm wavelength [4]. The light of the 13.5 nm wavelength is absorbed well by water, then wet bio-cells are hard to observe. When water in bio-cells can be decreased and make fixed and dry sections which are used in TEM observation, bio-cells will be possible to be observed in the 13.5 nm wavelength. On the other hand, the plan of the neuron-connection-diagram will be carried by fixed brains [3], therefore we investigate fixation and stain methods for 13.5 nm wavelength imaging [5] and fabricate a conventional evaluation tool for fixation and stain methods.

The tool fabricated for evaluating the preparation methods is a contact microscopy with the use of scintillator plate and developed on the basis of an image detector for EUV imaging [6]. The merit of
the contact microscope developed here is the flexibility of observation wavelength because there is no
objective lens in the system, the sensitivity of the scintillator material for wide wavelength range from
EUV to HX region, and the coexistence with VI microscope that is used for readout optics of
conversion images. The details of the Soft x-ray COn tact Microscope (SCOM) will be presented in
this paper.

2. Evaluation of SCOM
The fabricated microscope SCOM is composed of a scintillator plate converting a SX image into a
VI one, a visible microscope to read and magnify the VI image (Fig. 1). The scintillator plate has a
wide detection wavelength range in SX region ($\lambda=0.2 \sim 50$ nm) and is transparent in VI region ($\lambda=380
\sim 820$ nm). The VI microscope is composed of an infinity optical system: an objective lens (UV20X,
Union Optical Co. Ltd.), an optical window for vacuum isolation, a tube lens (UV tube lens, Union
Optical Co. Ltd.), and a CCD camera (C4742-98, Hamamatsu Photonics). As the results of these
features, SX images can be directly compared with VI ones changing the irradiation light.

2.1. Quantum efficiency of the scintillator
Scintillator material is a key component in the system, since quality of SX images depends on the
specifications of the scintillator plate. The specifications that relate to the one of SCOM are quantum
efficiency $Q(\lambda_{SX})$ for a wavelength $\lambda_{SX}$ in SX region, and linearity of emission intensity to incident
light intensity.

Using quantum efficiency $q_{scin}(\lambda_{SX})$ of the scintillator plate, numerical aperture of VI objective
lens $N_A$, transmittance of VI microscope $T_{VI}(\lambda_{scin})$ at the emission wavelength $\lambda_{scin}$ of the scintillator
material, and quantum efficiency $q_{CCD}(\lambda_{scin})$ of the CCD image sensor, total quantum efficiency
$Q(\lambda_{SX})$ of SCOM is represented as

$$Q(\lambda_{SX}) \propto q_{scin}(\lambda_{SX}) T_{VI}(\lambda_{scin}) q_{CCD}(\lambda_{scin}) \left( 1 - \sqrt{1 - N_A^2} \right). \quad (1)$$

Except for quantum efficiency $q_{scin}(\lambda_{SX})$ of the scintillator, all parameters are known: transmittance of
the VI microscope is more than 0.8 and dispersion of transmittance of the VI microscope is flat [7],
quantum efficiency of the CCD image sensor is 0.63 at the emission wavelength [8]. Total quantum
efficiency $Q(\lambda_{SX})$ can be obtained from measurement results.

We chose Ce:LYSO (Oxide Co.) as a material of the scintillator plate because its quantum
efficiency is high in SX region. Quantum efficiency of Ce:LYSO was measured with the use of
SCOM and determined by Eq. (1). All measurements were carried out at beamline BL11D in Photon
Factory, KEK, Japan under the conditions: wavelength resolution, $\lambda \Delta \lambda$, 500, exposure time, $5 \sim 30$ sec,
wavelength of incident light, $5 \sim 20$ nm.

Relative quantum efficiency of Ce:LYSO measured is presented in Fig. 2. The results obtained coincide with the

![Figure 1. Schematic representation of SCOM.](image1)

![Figure 2. Wavelength dependence of relative quantum efficiency.](image2)
past results [6]. The values showed the maximum at around 7 ∼ 8 nm and decreased slowly as the increase of the wavelength to 20 nm.

2.2. Linearity of the scintillator

To evaluate the linearity of Ce:LYSO, integrated emission intensity was measured changing the incident photon intensity at the 13.5 nm wavelength by changing a width of the exit slit in the beamline. In advance of the linearity measurement, conversion coefficients from the drain current of the Au mesh into the number of incident photons were obtained at each wavelength. Incident photon number was estimated from the photo-diode (AXUV100, IRD Inc.) current of which quantum efficiency is known. To obtain the production efficiency of the electrons in the CCD pixels, the background image was subtracted from the measured CCD image at first. At second, numbers of all the pixels were integrated, and finally the integration value was normalized by the number of the CCD pixels. Produced number of electrons for 1 pixel of the CCD camera is plotted in Fig. 3. A linear relationship exits between the incident photons and produced electrons. The quantum efficiency of the electrons is 7.4×10⁻³ electrons/photon obtained from the interpolation of the plotted data.

2.3. MTF of SCOM

In order to evaluate the modulation transfer function (MTF) of the system, slanted edge response of Si wafer was measured [9]. A sample used for the edge response measurement was a 100 nm thick Si₃N₄ membrane, which boundary between the Si wafer and the Si₃N₄ membrane was used as the edge. The edge image was taken at a wavelength 12.7 nm, exposure time of the image was 0.1 sec, and MTF of the system was obtained numerically from the edge image. The MTF obtained is represented in Fig. 4. The MTF increases with the increase of the spatial frequency, and the MTF value is less than 0.1 at the spatial frequency region more than 300 LP/mm. The dip structures observed at values of spatial frequency 150, 350, and 700 LP/mm, are alias from the pixel size of the CCD camera.

The optimal cutoff frequency of the system is 1760 (LP/mm) using a 450 nm scintillator emission wavelength, and a 0.4 numerical aperture for the objective lens. The comparison of the measured MTF curve with the optimal cut-off frequency, shows that the MTF values of the system are relatively low in the high frequency region. This is because the intensity of the stray light in and around the scintillator plate is high.

3. Transmittances of organelles using SCOM

Transmission spectra of rat hepatic cells obtained from transmission images are demonstrated using SCOM. The preparation of the sections for measurements is as follows: a block of rat hepatic cells was embedded by epoxy resin at first and stained by uranyl acetate at second. The block embedded and stained was sliced into 400 nm thick sections by an ultramicrotome. Each section was placed on a TEM grid.

Before hepatic cells observation, light source image was acquired at each observation wavelength.

![Figure 3. Linearity of the scintillator plate at wavelength 13.5 nm.](image1)

![Figure 4. MTF of SCOM](image2)
And this, one of the sections was placed tightly on the scintillator plate, and observed changing the wavelength. Light source and hepatic cell images were corrected by subtraction of background images acquired after each observation, and normalized by the exposure time. Finally the hepatic cell images were normalized by light source images on the assumption that there is no interference in the sections.

The transmittance image at 13.5 nm wavelength using our TXM³ is presented in Fig. 5. Transmittances of some organelles obtained from each image are plotted at each wavelength in Fig. 6. All transmittance spectra decrease monotonically as the increase of the wavelength. At 11 ~ 13 nm wavelength region, two dip structures are observed in the nucleus and the cytoplasm. Those are originated from O₄.₅ absorption edges of uranium atoms which are contained in uranyl acetate solution used in staining. Because the uranyl acetate binds to a specific protein containing in organelles and the blood vessels have no organelles in its tube structure, no dip structures are observed [10].

4. Summary

Usually organelles are observed with VI microscopes, and their shape is recognized from those images. In SX images, the shapes of organelles are different from the VI images therefore identification of SX images needs careful comparison of a SX image with a VI image. The contact microscope fabricated here can take both SX and VI images at the same position of an observation sample by the change of the illumination light. The use of this microscope makes SX/VI comparisons feasible and relatively easy.

Figure 5. SX image of rat hepatic cell staining with uranyl acetate. N, nucleus, W, sinusoidal vessel, R, red blood cell, BV, blood vessel, X, unknown.

Figure 6. Transmittances of organelles in rat hepatic cell.

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