Development of an Auto-focusing Imaging System in the Soft X-Ray Microscope Beamline of the SR Center in Ritsumeikan University

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Abstract. An X-ray microscope beamline (BL-12) was installed in the SR center, Ritsumeikan University, in 1996 and has been operated for 12 years. After XRM2005, an automated CZP positioning system has been used for daily observation. Using this system, several unique biological samples were observed. In 2007, we started a new project to develop an auto-focusing imaging system in the beamline. The system can be controlled by a single finger and makes possible to perform the multi-wavelength imaging in a wide wavelength region from 1.73 to 4.73 nm. With this system, an edge absorption imaging can be easily applied to many elements.

1. Introduction
At Ritsumeikan University (Kusatsu, Japan), the world smallest electron storage ring with one-body superconducting magnet, ‘AURORA’ has been operated since 1996 (the electron energy of 575 MeV and the ring current of 300 mA). An X-ray microscope beamline (BL-12) was installed also in 1996 and has been operated for 12 years. The present optical configuration of the X-ray microscope is the same with the previous one [1-2]. The optical system consists of two zone plates. In conjunction with a pinhole a condenser zone plate (CZP) acts as a dispersive and focusing element. A CZP is a Göttingen KZP 7 type (diameter: 9 mm, outermost zone width: 53.7 nm, number of the zones: 41,890) [3]. Pinhole diameter is 20 µm. An objective zone plate (OZP) was fabricated at IBM/LBNL (diameter: 50 µm, outermost zone width: 45 nm, number of the zones: 277) [4]. The images are detected by a back-illuminated CCD (C4880-21-24WD, Hamamatsu K.K.). In ray tracing calculation, the wavelength resolution was estimated to be 200 at full-width at half maximum when a beam size was 0.28×2.6 µm² (2σ) and distance between the source and the CZP of 7.1m. Its resolution was estimated to be about 70 nm (20-80%) from the intensity gradient of the knife edge of the mesh [1, 2]. We reported several improvements in the previous paper [5]. In 2004, an automated CZP positioning system was designed and manufactured. This system covers 1.6 - 3.3 nm wavelength range and a number of specimens have been observed since X-ray microscopy conference in Himeji 2005 (XRM2005). Energy calibration was performed by using nitrogen and oxygen absorption edges. Although multi-wavelength observation was possible, it was necessary to adjust the system to an
appropriate energy at each time. Fully automated wavelength scanning system was highly demanded. In 2007, we started a new project to develop a full auto-focusing imaging system in our beamline. This system can be controlled by a single finger. It makes possible to perform the multi-wavelength imaging in a wide wavelength region from 1.73 to 4.73 nm. With this system, an edge absorption imaging is easily applied to many elements. In this paper, details of this project and some recent results after XRM2005 are presented.

2. Vanadium Binding in Dictyostelium discoideum
Vanadium is particularly attractive as a contrast enhancement reagent in soft X-ray microscopy because it has a strong absorption L-edge (2.43 nm) in the water window. In order to evaluate the capability as a labelling agent of vanadium, a labelling operation using vanadium was performed. Dictyostelium discoideum Ax-2 amoebae, cultured in axenic medium (HL5), were collected into centrifuge tubes and washed with 0.01M phosphate buffer. After 1 % glutaraldehyde fixation, the cells were stained with 0.2M VOSO$_4$ dissolved in distilled water for 16 h.

Figure 1 is an image of Dictyostelium cell stained with vanadium, 0.2 M. Optical microscopic observation indicates that the cell size is approximately 7.5 μm in diameter, and there is a core of about 4 μm. Figure 1b and c show X-ray micrographs taken at 2.3 and 2.5 nm, which correspond to the energies above and below vanadium L-edge respectively. Figure 1d shows the vanadium signal which was obtained by subtracting figure 1c from figure 1b. Figure 1d obviously shows that there is a high vanadium dense core, whose size is 4 μm in diameter. Surrounding the core, filamentous structure is observed in the cytoplasm, which may correspond to the cytoskeleton. This result indicates that the nucleus and cytoskeleton are a kind of vanadyl binding sites.

3. Improvement of Soft X-ray Microscope
The previous system was a half-automatic observation system [5]. Since the CZP moves together with the CZP chamber by a stepping motor, the usable wavelength range is restricted by a working stroke of a vacuum bellows which connected to the CZP chamber [1, 2, 5].

In the new system, a CZP chamber is newly manufactured. A particular wavelength is selected by moving the CZP with a new stepping motor along the optical axis of the synchrotron beam in it (see Figure 2). Figure 3 shows schematics and photographs of the mounting assembly for the CZP. The CZP is mounted on piezo positioner stages PP-30 for vacuum (MICOS, Germany). A central stop is also mounted on a 2-axis positioning stage with a tiny SQL Series SQUIGGLE motor SQ-100V for vacuum (New Scale Technologies, Inc, USA). The central stop stage is attached to a CZP holder plate and dependent moving to the CZP is possible. The new CZP chamber covers 1.73 - 4.73 nm wavelength range. Linear actuators are newly attached to the OZP and sample stages. As the results, every stage moving is computer-controlled by b using a LabVIEW (National Instruments (NI)) system. With this system, user can control the system by a single finger and perform the multi-wavelength imaging in a wide wavelength region from 1.73 to 4.73 nm.
4. Summary
After XRM2005, an automated CZP positioning system has been used for daily observation and shows good performance. Using the soft X-ray microscope at BL12, we have evaluated the effect of the contrast enhancement based on vanadium labelling for Dictyostelium discoideum. Using 0.2M VOSO$_4$ staining solution, a cell nucleus and cytoplasm were visible and especially details of cytoskeleton were clearly seen.

In order to offer more user-friendly equipment, a full automatic observation system has been designed and manufactured. Pulse motor is attached to all moving stages and every movement is controlled by pulse. In addition, a new motor control system is developed using LabVIEW. Using this system, user can control the system by a single finger and perform the multi-wavelength imaging in a wide wavelength region from 1.73 to 4.73 nm. During the 2008 fiscal year, we look forward to observing bio-specimens with this new user-friendly X-ray microscope.

![Figure 2](image1.jpg)

**Figure 2.** Schematic of new CZP chamber with the CZP stage.

![Figure 3](image2.jpg)

**Figure 3.** Schematic and photograph of mounting assembly for the CZP.

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