

# **Imaging of Chromosomes at Nanometer-Scale Resolution, Using Soft X-Ray Microscope**

**K. Takemoto, A. Yamamoto<sup>1</sup>, I. Komura<sup>2</sup>, K. Nakanishi<sup>3</sup>, H. Namba<sup>2</sup>  
and H. Kihara**

## **Abstract**

In order to clarify the process of condensation and distribution of a chromosome with 70nm resolution, process of cell division was observed by soft X-ray microscopy. During prometaphase of mouse fibroblast cell line NIH3T3, each chromosome is clearly visualized at high contrast. Furthermore, thickness of a chromosome is not uniform but varied from 150nm to 750nm. This is considered as a condensing step of chromatin fiber occurring in the prometaphase. During metaphase and anaphase, each chromosome was also observed. The chromosomes were thicker than those in prometaphase. This result implies that during metaphase and anaphase the chromosomes condense and become more compact.

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*Department of Physics, Kansai Medical University, 18-89Uyama-higashi, Hirakata, Osaka, 573-1136, Japan*

<sup>1</sup>*Nagahama Institute of Bio-Science and Technology, 1266, Tamura-cho, Nagahama, Shiga, 526-0829, Japan*

<sup>2</sup>*Department of Physical Science, Ritsumeikan University, 1-1-1, Noji-Higashi Kusatsu, Shiga, 525-8577, Japan*

<sup>3</sup>*SR Center, Ritsumeikan University, 1-1-1, Noji-Higashi, Kusatsu, Shiga, 525-8577, Japan*

## **1. Introduction**

The chromosomes contain DNA which carries genetic information essential to the cell. The chromosomes, which have replicated during the S phase, become compact transportable forms. They are separated and moved to opposite ends of the cell. Each daughter cell exactly inherits one set of the chromosomes. The mechanisms of chromosome condensation and distribution are the fundamentals of a cell cycle. Knowledge of them are consequently of great importance for an understanding of the continuity of lives. The fundamental structural unit of chromatin is an assemblage, called the nucleosome [1]. Although chromosome is organized into the chromatin fiber consisting of nucleosome arrays, the packaging arrangement and structure of the higher levels of chromatin fiber organization is not well understood.

Soft X-ray microscope BL12 is one of the most powerful tools to observe bio-specimens at high resolution and to study function of subcellular structure such as organelles or cytoskeleton [2, 3]. In this report, we describe results of soft X-ray microscopic observation of chromosomes. The structure of chromatin varies considerably as the cell progresses through the cell cycle. In each phase, microstructure of condensed chromatin fibers and chromosomes were observed by light and X-ray microscopy.

## **2. Materials and Methods**

### ***2.1 Sample preparation***

Fibroblast cell line, NIH3T3 cells were cultured in DMEM medium in 5% CO<sub>2</sub> at 37 °C. Cells were attached to polyimide membrane and cultured for 24 hours. The attached cells were fixed in 2% paraformaldehyde in 0.1M Na-phosphate buffer (pH 7.4) (PB) for 5min and washed three times in PB, and permeabilized with 0.3% Triton X-100 for 30min. In order to observe chromosomes by light microscopy, the cells were stained with acetic orcein. After air-dried, cells in mitotic stage were selected under a light microscope, and the same cells were observed under an x-ray microscope.

### **2.2 Soft X-ray microscopy**

X-ray microscopic observation was performed at beamline BL12 [9-10]. The optical element of the X-ray microscope is zone plates used for imaging or focusing. The outermost zone width and aspect ratio define the achievable spatial resolution and diffraction efficiency, respectively. The achieved resolution is c.a. 70nm judging from a knife-edge estimation (20 % -80 %). The expected energy resolution ( $E/\Delta E$ ) is about 160. Size of view field is about  $10 \times 10 \mu\text{m}^2$ . For image acquisition, a sample is scanned in a plane perpendicular to the optical axis. The motion is provided by compact stepping motor actuators (Sigma Koki Co. LTD.) driven linear translation stage. They are located outside the vacuum chamber. A minimum step size is 500nm and working range is several mm. The magnified image is recorded on a

Peltier-cooled, back-illuminated X-ray CCD camera (C4880-21-24WD: Hamamatsu Photonics) with  $512 \times 512$  pixels and a pixel size is  $24 \times 24\mu\text{m}^2$ .

### 3. Results and Discussion

Fig. 1 shows a paraformaldehyde-fixed and air-dried 3T3 cell at prometaphase in mitosis. By staining with acetic orcein, each chromosome is imaged under a light microscope (in Fig. 1a and b). Orcein dye ( $\text{C}_{28}\text{H}_{24}\text{N}_2\text{O}_7$ ) doesn't work as an active dye for X-ray microscopy. In soft X-ray region, DNA and protein absorb strongly X-ray, and the chromosomes are also visible as X-ray dense structure. The chromosomes were clearly imaged under an X-ray microscope as shown in Fig. 1c.

Fig. 2 shows a light and X-ray microscopic images of the same 3T3 cell as Fig. 1 with higher magnification. Due to the limitation of the conventional light microscope, it is difficult to get clear images at high magnification. On the other hand, in Fig. 2c, it is easy for us to distinguish each chromosome. The thickness of a chromosome is not uniform but varied from 150nm to 750nm. Chromatin fiber is the unit of the chromosome. The chromosome with 750nm in thickness is considered to be almost at the final level of packaging of chromosome. The thinner fiber is considered to be a condensing chromatin fiber in the low level compaction.

Figs. 3 and 4 show paraformaldehyde-fixed and air-dried 3T3 cell at metaphase and at anaphase respectively. Each chromosome is also visible as X-ray dense structure (Figs. 3c and 4c). However the chromosome is not clearly imaged under the X-ray microscope, probably by multiple overlapping of the chromosomes.

The thickness of chromatin is estimated to be 870nm (Fig. 3c) in metaphase, and 900nm (Fig. 4c) in anaphase respectively. During metaphase, they condense into the fibers about 900nm in width. It indicates that metaphase and anaphase chromosomes condense and become more compact ones.

### 4. Summary

In order to reveal the packaging arrangement and structure of the highest levels of chromatin fiber organization, X-ray microscopic observations on the cell division of NIH3T3 cells were performed. In prometaphase, each chromosome is clearly visualized with high contrast. Thickness of the chromosome varied from 150nm to 750nm. This is considered as a condensing process of chromatin fiber. In metaphase and anaphase, each chromosome could be also observed under the X-ray microscope. The thickness of the chromosomes was above 900nm. This result implies that during metaphase and anaphase the chromosomes condense and become more compact.

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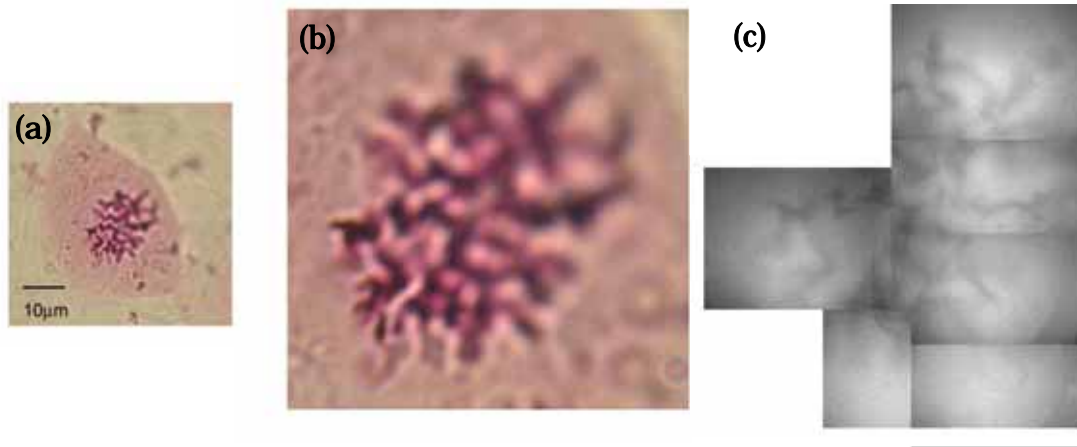


Fig.1 Light and X-ray microscopic images of a NIH3T3 cell at prometaphase in mitosis. (a) light microscopic image, (b) digitally enlarged image of (a), (c) X-ray microscopic image corresponding to (b) at 2.3nm. Exposure time was 120s. Scale bar: 10µm.

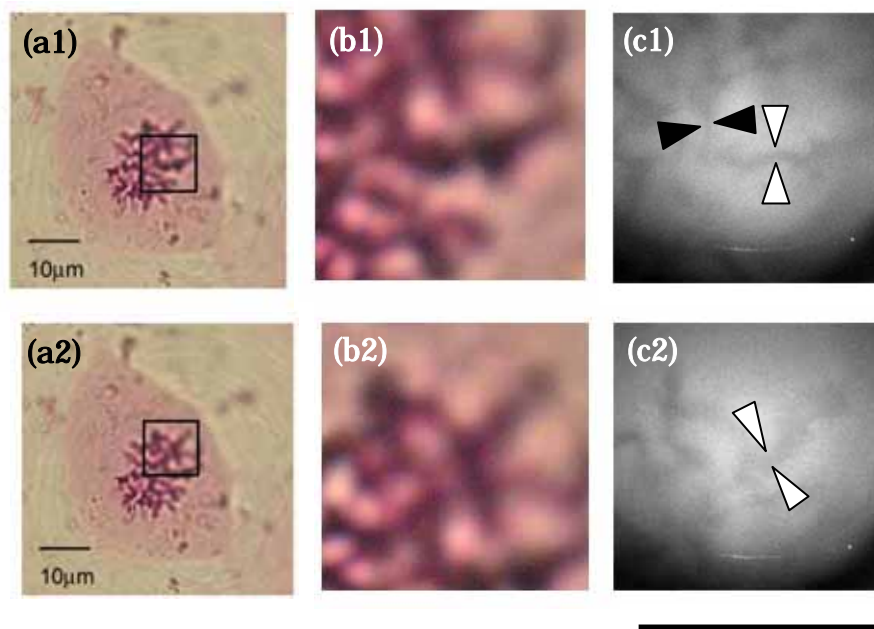


Fig.2 Light and X-ray microscopic images of a NIH3T3 cell at prometaphase in mitosis. It is the same cell with Fig. 1. (a) light microscopic images (b) digitally enlarged images of (a), (c) X-ray microscopic images corresponding to (b) at 2.3nm. Arrowheads indicate chromosome thickness. (c1) Black arrowhead: 150nm, white arrowhead: 470nm, (c2) arrowheads: 750nm. Exposure time was 120s. Scale bar: 10µm.

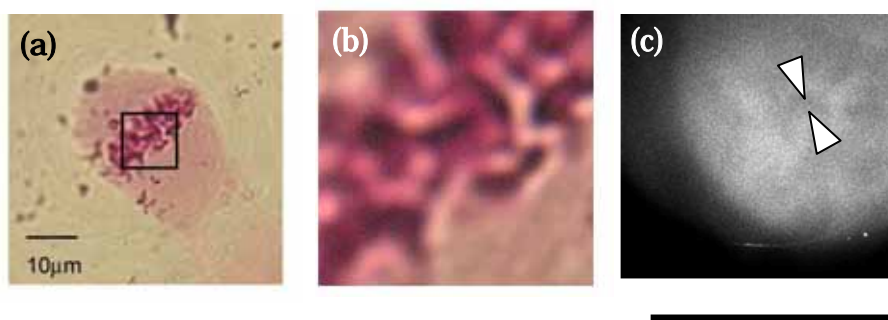


Fig.3 Light and X-ray microscopic images of a NIH3T3 cell at metaphase in mitosis. (a) light microscopic image, (b) digitally enlarged image of (a), (c) X-ray microscopic image corresponding to (b) at 2.3nm. An arrowhead indicates chromosome of 870nm in thickness. Exposure time was 120s. Scale bar: 10µm.

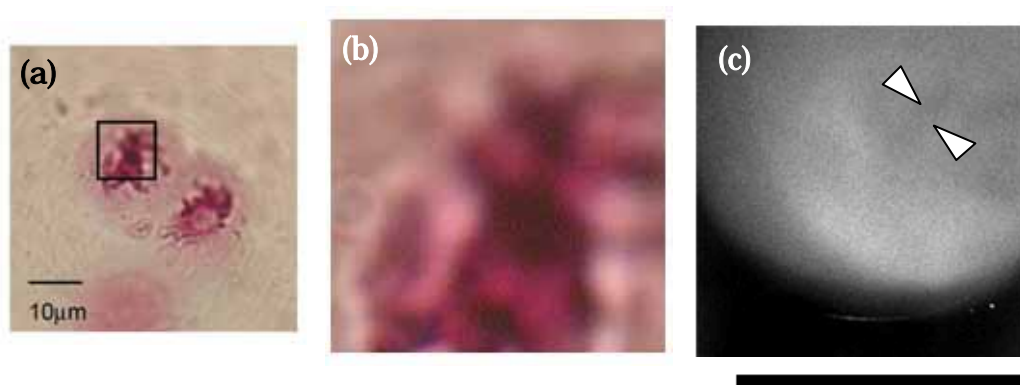


Fig.4 Light and X-ray microscopic images of a NIH3T3 cell in anaphase. (a) light microscopic image, (b) digitally-enlarged image of (a), (c) X-ray microscopic image corresponding to (b) at 2.3nm. An arrowhead indicates chromosome of 900nm in thickness. Exposure time was 120s. Scale bar: 5µm.

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