Abstract. Deinococcus radiodurans has been known to withstand radiation levels up to 1,000 times than that would kill normal human cells. To cope radiation damage during soft X-ray observation of living cells, D. radiodurans incubated with tellurium oxyanions was used as the X-ray microscopy sample. The first observation was successfully performed. In combination of antifreeze solution and subzero temperature, along with carbon window, the cell observation will be more closely to the living condition.

1. Introduction
Soft X-ray microscope is expected to be one of the promising tools for observing living cells and tissues with nm order resolution. However, in order to observe living cells, there are several problems to be solved. The most serious problem is radiation damage. Doses of about $10^4$ Gy exhibit immediate morphological changes. Based on Sayer’s approach [1, 2], the dose necessary to image the 50 nm protein structure in a 10 µm water layer is $10^5$ Gy.

Deinococcus radiodurans has been known to withstand radiation levels up to 1,000 times than that would kill normal human cells. Exponential-phase cultures of D. radiodurans survive 5,000 Gy of $\gamma$ radiation. D. radiodurans belongs to a unique family of eubacteria characterized by an exceptional capacity to withstand the usually lethal effects of DNA-damaging agents including ionizing radiation, UV light and desiccation [3]. D. radiodurans also exhibits high resistance to tellurium oxyanions [4]. Oxyanions of tellurium, like tellurite ($\text{TeO}_3^{2-}$) and tellurate ($\text{TeO}_4^{2-}$), are highly toxic for most bacteria. However, tellurite-resistant bacteria do exist in nature and they often reduce tellurite to its elemental and less toxic form $\text{Te}_x$ that is accumulated as black deposits inside the cell. Tellurite toxicity results from its ability to act as a strong oxidizing agent to generate reactive oxygen species (ROS) in cell [5]. D. radiodurans genome possesses a ter operon (terZ-ABDEF) which confers tellurite resistance [4]. It has been shown that Rhodobacter capsulatus cells incubated with potassium tellurite exhibit increased superoxide dismutase (SOD) activity and increased resistance to tellurite when exposed to the $\text{O}_2^-$ generator paraquat [6]. On the other hand, it has been shown that loss of $\text{sod}$ gene renders D. radiodurans sensitive to ionizing radiation [7]. These facts lead us to use D. radiodurans cells incubated with tellurium oxyanions for soft X-ray examination in order to make living cells observation successful by reducing radiation damage.

2. Materials and methods
2.1. Sample Preparation

*Deinococcus radiodurans* strain R1 (ATCC13939) was grown at 30 ºC on TGY agar (0.5 % Bacto tryptone, 0.3 % Bacto yeast extract, 0.1 % glucose and 1.5 % Bacto agar) supplemented with 250 µg/ml of potassium tellurate. One loop of colony was resuspended in 1 ml of MilliQ water, and 1 µl of the cell suspension was placed on a grid with poly-vinyl-formale (PVF) membrane and air-dried.

2.2. X-ray Microscopy

X-ray microscopic observation was performed at beamline BL12 [8, 9]. Optical elements of the X-ray microscope used for imaging and focusing are zone plates. In conjunction with a pinhole (20 µm in diameter), a condenser zone plate (CZP, diameter: 9 mm, outermost zone width: 53.7 nm, number of the zones: 41,890) acts as a dispersive and focusing element. The expected energy resolution (E/ΔE) is about 160. Outermost zone width of ZP defines the achievable spatial resolution. The objective zone plate (OZP) had an outermost zone width of 45 nm. The achieved resolution is c.a. 70 nm judging from the knife-edge estimation (20 % - 80 %). The magnified image was recorded on a peltier-cooled, back-illuminated X-ray CCD camera.

3. Results and Discussion

The most serious problem during the observation of living cells using X-ray microscopy is radiation damage of cellular components including DNA, protein and membrane lipid. In this study, *D. radiodurans* that possesses highly efficient mechanisms to protect oxidative damage was selected as the sample of X-ray microscopy observation. Figure 1 shows a transmission electron microscopic image of *D. radiodurans* in which four cells gather together, forming a tetrad with septa. ROS such as O₂⁻ are generated during the radiolysis of water, an indirect effect of ionizing radiation. Cells become vulnerable to damage, a condition referred to as oxidative stress, when ROS exceed the capacity of endogenous scavengers to eliminate them. As a defense against ROS, organisms contain two major antioxidant enzymes; SOD and catalase [10]. Therefore, one way to reduce radiation damage of cells is to enhance activity of radical scavengers prior to irradiation. In this study, *D. radiodurans* grown in medium containing tellurate was used for X-ray microscopy observation to enhance intracellular SOD activity. Figure 2 shows a typical X-ray microscopic image of *D. radiodurans* cells. Observation wavelength was 2.3 nm and exposure time was 2 min. Electron microscopy and electron spectroscopy imaging have shown that tellurite resistant bacteria contain intracellular crystals of black metallic tellurium [4]. In Figure 2, each cell with spherical-shaped structure had a dense body that assumed to be derived from accumulated tellurium in cytoplasm.

In order to actually observe a cell in the living state, the observation method needs to be optimized. Cryogenic technique promises reduction of radiation damage of bio-specimens. In the case of *D. radiodurans*, the observation in cryogenic temperature is unnecessary for reducing radiation damage. Using antifreeze solution instead of water, it is possible to decrease the temperature of biological specimens to subzero temperature without freezing. In the above case, the spectral range “4.4 nm < λ < 5 nm” that follows immediately after the K-absorption edges of carbon seems to be more attractive than the water window. In the wavelength range so-called “carbon window”, organic materials show strong penetration of X-ray, while carbon is relatively non-absorbing. Combined with metal labeling, metal binding site is clearly visualized like fluorescence of optical microscopy observation as shown in figure 3. In addition, because the antifreeze solution is a major free-radical scavenger, it is possible for specimens to obtain higher radiotolerance. Using these ideas, the cell observation will be more closely to the living condition.

4. Conclusion

Soft X-ray microscopy was applied to the visualization of *D. radiodurans*. The first X-ray microscopic observation of air-dried cells at room temperature was successfully performed. In combination of antifreeze solution and subzero temperature, along with carbon window, the cell observation will be more closely to the living condition.
Figure 1. Transmission electron microscopic image of *D. radiodurans*.

Figure 2. X-ray microscopic image of *D. radiodurans*. Observation wavelength is 2.3 nm and exposure time is 2 min.

Figure 3. Amplitude contrast and phase contrast of 50 nm protein structure with composition C_{94}H_{139}N_{24}O_{31}S and density 1.35 g/cm$^3$ in ethylene glycol (EGOH). In labeled protein, 50 nm gold structure is used as a labeling element. Calculation is performed assuming an ideal imaging with a nickel phase plate in back focal plane [11].

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