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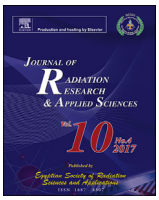

Single floating cell irradiation technique with an X-ray microbeam

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ABSTRACT

A single floating cell irradiation technique with X-ray microbeam has been developed for the microscopic research of radiation effects on floating cells in a liquid medium. This technique is a combination of X-ray microbeam and laser technologies. A preliminary experiment on the survival of budding cells of the yeast *Saccharomyces cerevisiae* (RAD52) was performed with the X-ray microbeam system. Micro-pits were fabricated on the bottom of a culture dish in a liquid medium, using green laser beams. The yeast cells were put into the micro-pits one by one by using focused infrared laser beams. A 50-kV X-ray microbeam 12 μm (FWHM) in diameter was propagated into the targeted yeast cell. The maximum dose rate on the targeted cell was estimated to be 0.09 Gy/s from the results of beam profile measurements and photon-electron transport calculations. The X-ray irradiation effect on the cell lethality was clearly observed for the cell exposed to X-rays of 100 Gy.

1. Introduction

Microbeams are important tools in radiobiological research (Bartzsch, Cummings, Eismann, & Oelfke, 2016; Folkard et al., 2001; Narita et al., 2015; Patrono et al., 2015). Such techniques have provided opportunities to deliver precise doses to pre-selected individual cells and have been useful for investigation of the effects of spatial interactions of ionizing radiation around targeted cells. Further, with regard to various biological endpoints, there are many reports on the radiation effects such as cellular inactivation, radiation mutagenesis, and oncogenic cell transformation. In the beginning of the use of microbeams, many researchers studied the bystander effect (effects in unirradiated cells receiving signals from irradiated cells) (Liu et al., 2015; Lobachevsky et al., 2015; Mancuso et al., 2012; Rastogi, Coates, Lorimore, & Wright, 2012). Recently, microbeam technology with sophisticated molecular biological techniques has also been used to understand molecular processes in radiobiological research (Drexler et al., 2015; Suzuki, Yamauchi, Oka, Suzuki, & Yamashita, 2011). Non-targeted effects, including bystander, are known to occur following both low and high doses of radiation and other stressors. Radiation-induced bystander signals may originate with diffusible mediators (Azzam, de Toledo, Spitz, & Little, 2002; Matsumoto et al., 2001; Shao, Furusawa, Aoki, Matsumoto, & Ando, 2002). Most in vivo data involving shielding part of an animal (Buonanno et al., 2015; Fernandez-Palomo et al., 2015) are complicated by systemic factors such as blood and endocrine factors, making it difficult to resolve the role of the immune system in the process (Blyth & Sykes, 2011; Munro, 2009; Tomita & Maeda, 2015).

A further improvement in the microbeam technique is desired for the progress of the microscopic study of radiation biology. For example, the normal microbeam irradiation technique needs the process of attaching cells and is not enough to target cells suspended in liquid medium such as blood. Therefore, we developed an X-ray irradiation technique involving a combination of X-ray microbeam and laser technologies for single floating cells. In this research, the laser technology included laser processing and optical tweezers. The optical tweezers are powerful micromanipulation tools for biological cells (Hu, Chen, Chen, Xu, & Sun, 2017). The optical tweezers technique was based on the force of the radiation pressure acting on a dielectric particle (a targeted cell) (Ashkin, Dziedzic, & Yamane, 1987; Greulich, 2017). The restoring force was generated when the dielectric particle was removed from the equilibrium position. In brief, the manipulation of the dielectric particle was performed with the positioning of the laser focal spot. Compared with other biological manipulation tools, such as

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micropipette aspiration, atomic force microscope, and electrokinetics, the optical tweezers have no special requirement for the material of the particle being manipulated and can provide a powerful noncontact force on ~10 μm-scale cells. Thus, the applications of optical tweezers can be used in drug delivery (Drews, 2000), cell fusion (Steubling, Cheng, & Berns, 1991), and mechanical study of DNA (Rusu et al., 2001).

In a preliminary experiment, a micro-pit chamber was fabricated on the bottom of a culture dish by using laser processing. The micro-pit chamber is one of cell-patterning technologies to use for arranging individual cells at designated positions (Tanaka et al., 2016). Floating yeast cells in a liquid medium were moved and stored using the optical tweezers. The targeted cell subsequently was irradiated with an X-ray microbeam. The morphological changes of the cells were observed after the X-ray microbeam irradiation.

2. Experimental

Fig. 1 shows a schematic view of the X-ray microbeam system. The detail of the X-ray microbeam system was reported in a previous paper (Sato et al., 2008). The X-ray microbeam system was set on a vibration-isolated optical table. The major components of the microbeam system were a microfocus X-ray tube, a glass capillary, a fluorescent X-ray detector, a vacuum chamber, a sample chamber for a culture dish, *XYZ* stepper-motorized stages, an optical microscope, and 2 lasers.

2.1. X-ray microbeam system

The microfocus X-ray tube and the glass capillary were made by Horiba, Ltd. (Kyoto, Japan) and were parts of the XGT-5000 commercial X-ray microbeam system (Ohzawa, Komatani, & Obori, 2004). The focal spot size at the rhodium target of the X-ray tube was about 30 μm in diameter. The maximum voltage and current of the tube were 50 kV and 1 mA, respectively. The glass capillary, 100 mm in length, had an effective function of X-ray focusing for a polychromatic X-ray source. X-rays that entered the capillary with a small incident angle were repeatedly reflected on the surface of the inner wall of the glass capillary. The X-rays were collected at a spot through the glass capillary. The irradiation of the X-ray microbeams was thus performed. The entrance of the capillary was set at 30 mm from the focal spot of the rhodium target. The glass capillary and the fluorescent X-ray detector were housed in the vacuum chamber at a pressure below 100 Pa. Polypropylene films of 4 μm thickness were used as a vacuum window.

The sample chamber was set on the stepper-motorized sample stage (SGSP20-35(XY), Sigma Koki Co., Ltd., Saitama, Japan). The images of targeted cells in the sample chamber were obtained with the objective (UPLSAPO60XW, Olympus Co., Tokyo, Japan), the CCD camera (DP30BW, Olympus), and a 150 W halogen lamp.

2.2. Characterization of the X-ray microbeam

To obtain an X-ray energy spectrum, an X-ray silicon detector (XR-100, Amptek, Inc., Bedford, MA, USA) was set at the sample stage. Pulses from the energy amplifier of the detector were analyzed with a multi-channel pulse height analyzer (MCA8000,

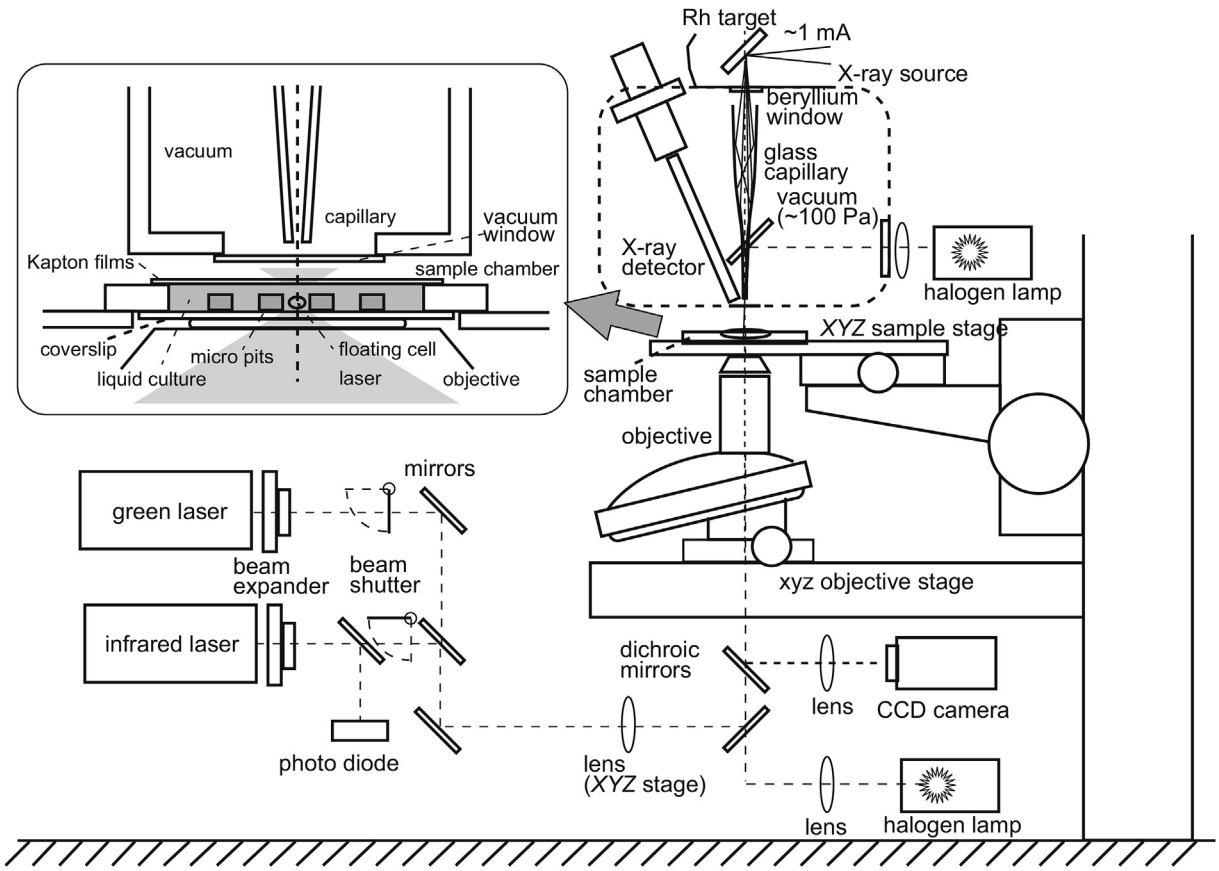

Fig. 1. A schematic drawing of the X-ray microbeam system with optical tweezers.

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