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Development of a soft X-ray microprobe for single cell radiobiology

CHEN Liang YAN Jingwen JIANG Shiping* YU Yang

National Synchrotron Radiation Laboratory, University of Science & Technology of China, Hefei 230029, China

Abstract An X-ray microprobe for radiobiological studies was developed which deliver precise doses of radiation to the selected individual cells. The facility used synchrotron radiation as soft X-ray source. A zone plate combining with a pinhole produced a fine probe from bending magnet for single cell irradiating with defined doses. The diameter of microprobe at the target position was about 2 μm by scanning a knife-edge with an AXUV photo diode. The fluxes of soft X-rays at 516.7 eV (2.4 nm) were about 5.4×10^4 photons/s.100mA measured with the photo diode. The absorbed dose rate for typical yeast cells was about 11.34 Gy/s with the storage current of 100 mA. A preliminary experiment for yeast cells irradiation has shown that the microprobe had a definite biological effect for radiobiological investigations. The soft X-ray microprobe at "water window" region has provided a useful tool for single cell irradiating damage and a capability of individually irradiating a certain numbers of cells each time.

Key words Synchrotron radiation, Soft X-rays, Microprobe, Radiation biology

1 Introduction

Radiation damage caused by soft X-rays has attracted research interests ever since X-ray was discovered. Different energies of X-rays have been used for radiobiology[1,2]. X-rays can cause DNA strand break[3] and chromosome aberrations[4], induce gene mutation and cell inactivation[5]. However, soft X-rays are more effective than conventional X-rays as the radiation effectiveness increases with decreasing X-ray energy[6]. When radiation energy is at the inner-shell absorption edges of the composing elements (such as K, L-shell absorption edges), the radiation effect becomes stronger[7].

There has been considerable interest in producing a soft X-ray microprobe for single cell radiobiology, and a few facilities have been used for different types of radiation effect studies[8,9]. Focused beams of X-rays, as a probe in controllable precision in micrometers, can deliver well defined doses to specific cells or sub-cellular components, and effects of non-targeted responses, such as bystander effects, can be systematically studied. Soft X-rays interact with materials *via* almost entirely photoelectric effect and therefore subject to very little scattering. having moderate LET and large numbers of photoelectrons with well defined energies, they produce in a cell nanometer-scale tracks, which can be used for studies on radiation damage to a single cell.

Soft X-rays of energies near the K-shell edges of O (0.54 keV), N (0.39 keV) and C (0.28 keV) are desirable for studies on radiation damage[10,11]. Lying in the *water window* region, where organic materials show strong absorption, while water is relatively non-absorbing[12], soft X-rays produce large numbers of photoelectrons and Auger electrons[13], and may cause unique characters in radiobiology[10]. When biological samples are irradiated by X-rays at the *K*-shell edges of O, N and C, the main elements of biological bodies, the X-rays are absorbed greatly and large fall-off in dose is deposited, hence an increase of probabilities for DNA or chromosome damage.

The soft X-ray microprobe project at NSRL, after many years of difficulties in focusing the X-rays, has been in progresses in recent years, thanks to the development of X-ray optical elements, such as zone plates, which is capable of providing focused probe sizes of 50 nm or less at energies of a few hundred eV[14]. In this paper, we report development of the

* Corresponding author. *E-mail address:* spjiang@ustc.edu.cn

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NSRL soft X-ray microprobe, with working wavelengths of 2.2–4.8 nm, which cover the entire water window region. With the intense quasi-monochromatic soft X-rays, the microprobe is good for radiobiological studies of soft X-rays near the K-shell edges of O, N and C.

2 Materials and methods

The soft X-ray microprobe was based on an 800 MeV storage ring at NSRL. It uses a linear monochromator to select soft X-rays in the water window for radiobiological studies. Fig. 1 is the schematic layout of the beamline and microprobe. Synchrotron radiations from a bending magnet are reflected by a plane mirror at a grazing angle of 2.5°, so as to cut off high energy radiations. The plane mirror, made of SiO_2 and coated with 30 nm Ni, is mounted on a stage to perform linear and tilting adjustments.

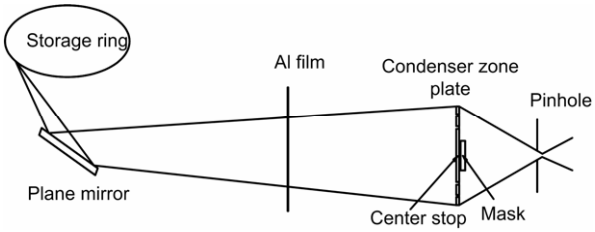

Fig.1 Schematic layout of the beamline and microbeam system.

A 2-μm Al foil is used to reduce the intensity of radiations on samples and to reduce heat accumulation on the optical elements (such as CZP) caused by low energy radiations (<200 eV).

The microprobe for single-cell irradiation can be divided in two parts: the monochromator and X-ray focusing assembly, and the target-finding and -aligning assembly. Lights from a bending magnet have a board spectrum of radiations (so called white light), and a monochromator is needed to select desired soft X-rays. We use a condense zone plate (CZP) combining with a pinhole to constitute the linear monochromator. The CZP is a circular diffraction grating with radially increasing line densities, such that different energies of X-rays are diffracted to an axial focus with different focal lengths. The higher the X-ray energy, the longer the focal length is. The pinhole was just before the first order focus of selected X-rays. Adjusting the CZP-pinhole distance along the light axis, one obtains the needed X-rays, with minimized contribution of the unfocused and unwanted energies. The Φ 9 mm CZP has an outmost zone width of 50 nm, and a Φ4 mm central stop, where there is a central mask to block off the zero order X-rays completely. To increase the monoenergetic X-rays and minimize contributions of X-rays above and below the selected wavelength, a small pinhole of Φ5 μm is used. The cells to be irradiated are placed on a mylar dish 0.2 mm behind the pinhole and irradiated in air. The CZP and the pinhole are mounted on a stage adjustable in *X*, *Y* and *Z* directions by precision lead screws. The resolution and reproducibility of the stages are all 1 μm.

The target-finding and -aligning assembly is of importance. In a radiobiological experiment a great number of cells are irradiated, procedures for finding and aligning the cells should be fast and automated. The microprobe is installed with a closed loop 3-axis positioning nano-motion stage (Nanomotion, Israel), with a resolution of 200 nm and adjustable ranges of ±5 mm at *X*, *Y* and *Z* directions. A schematic view of finding and aligning cells is shown in Fig 2.

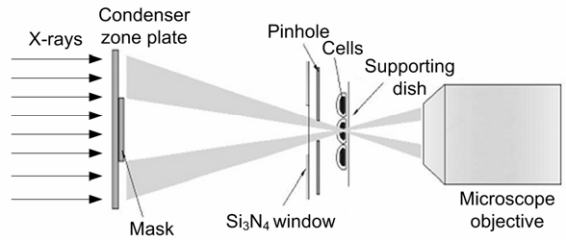

Fig.2 Experimental layout for single-cell irradiation.

Cells were viewed *in situ* using a visible light CCD and an objective lens (Nikon, 20×), which are supported on a two-axis stepper-motorised stage of 1μm resolution and 1μm reproducibility. The stage is on a vertically mounted linear bearing adjustable precisely in vertical direction.

The selected X-rays go out of the vacuum system through a Si_3N_4 window (0.25×0.25 mm). The pinhole, sample stage and target-finding system are held in a PMMA box, in which a UV lamp is used to sterile the sample before irradiation. The CZP, pinhole, sample stage and finding system are all on a marble platform with dimension of 2200×1100×500 mm (as shown in Fig.3), which were all aligned offline by laser before assembled together on the marble platform.

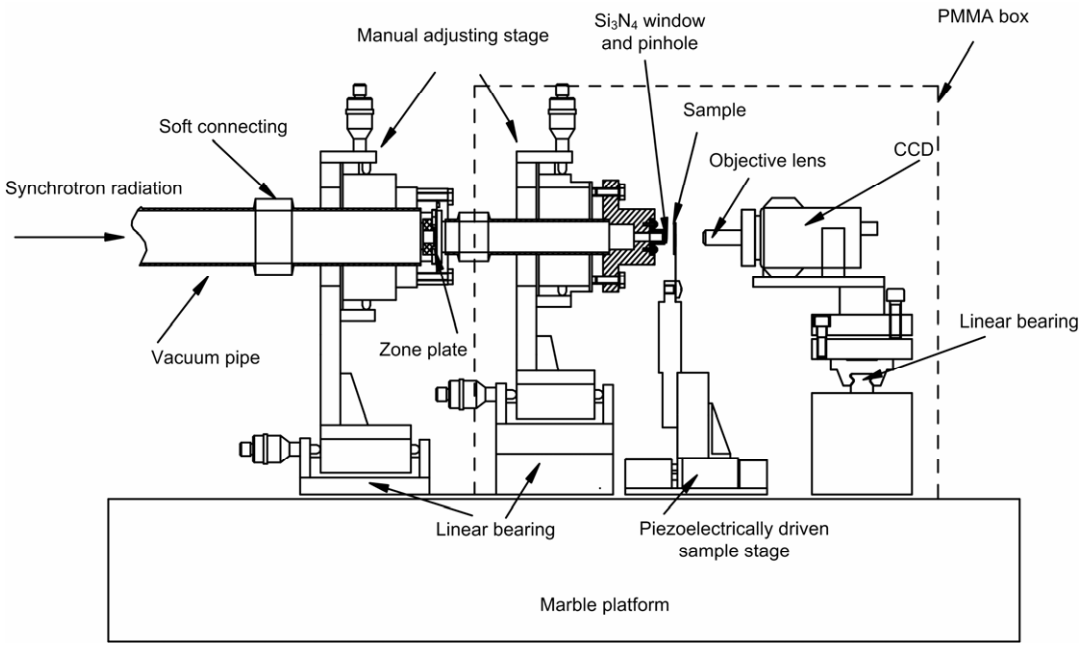

Fig.3 Schematic view of the soft X-ray microprobe for single cell irradiation.

The position of focus is established by precisely scanning a knife-edge of a Si_3N_4 window half plated with 200 nm Au film. The scanning procedure is achieved by using the nano positioning stage. The flux is detected by an AXUV photo diode (AXUV-100G.). When the X-ray focus centers through the pinhole, a sharp increase in the X-ray photon-current can be observed. The diode is also used to characterize the spot size and X-ray intensity at the sample position by

$$\Delta I = N \times \eta \times \xi \times (100/I_A)$$

where ΔI is the current detected by the diode, N is the X-ray flux, η is the detection efficiency of the diode, and ξ is the quantum efficiency for generating electron-hole pairs. The energy for generating a pair of electron-hole is 3.64 eV (provided by the manufacturer). The spot profile at the target position measured with 516.7 eV X-ray is shown in Fig. 4. The spot size is 2 μm at the focal position by the knife-edge. From the photon-current in Fig.4, the flux can be estimated at about 5.4×10^4 photons/s with a storage current of 100 mA.

For cell alignment, the pinhole is adjusted to the first order focus of the CZP with the CCD, and it is labeled on the screen. The pinhole is moved for 200 μm toward the CZP, and this is the right spot to position the cells. The nano-stage is adjusted until the cells can be seen clearly by the CCD while lens remains immobile, and this means that the cells are almost at the focal position of the CZP. The selected cells are maneuvered to the labeled pinhole position for irradiating them one by one with the microprobe. The cell alignment and parameters of the microprobe are shown in Fig.5 and Table 1.

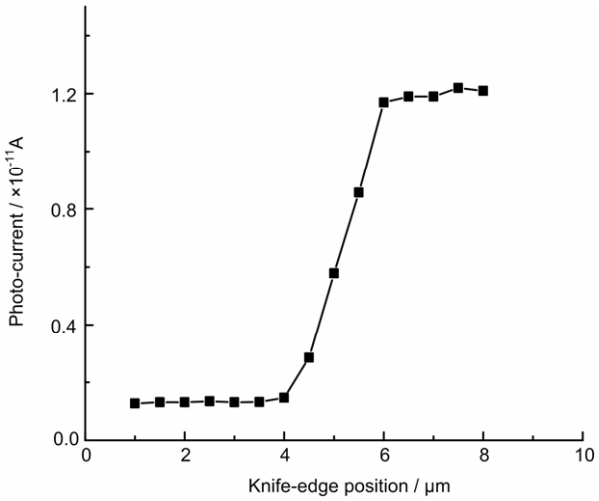

Fig.4 Spot profile scanned by a knife-edge (storage ring current: 100 mA).

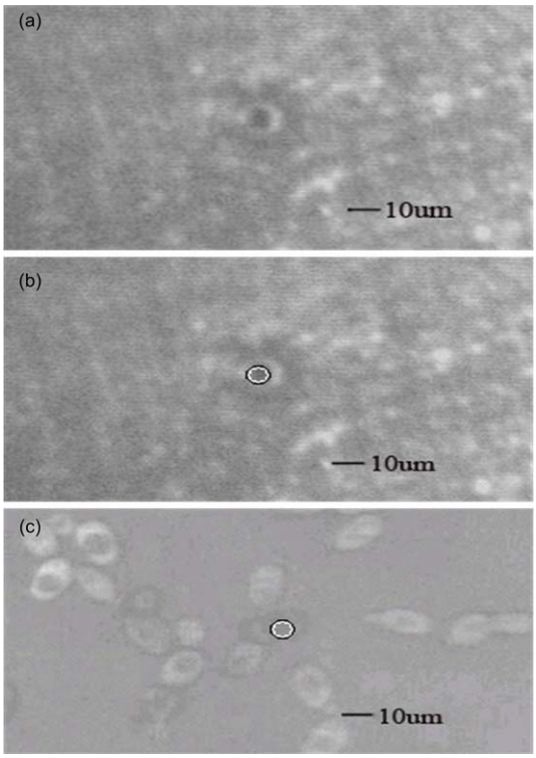

Fig.5 Cells-alignment. Finding the pinhole (a), labeling it on the screen (b) and Maneuvering the cell to the label (c).

Table 1 Parameters of the soft X-ray microprobe

X-ray energy	258-564eV
Spot diameter	2μm
Flux	5.4×10^4 photons/s.100mA@516.7eV
Resolution of sample stage	200nm

3 Results

In this paper, 516.7 eV X-ray (near the K edge of O) was used. The first-order focal length of the CZP at 516.7 eV is 187.5 mm, as can be calculated by $f \approx D\times\delta/\lambda$, where D is the diameter of CZP, δ is the outmost zone width of the CZP and λ is the X-ray wavelength. The CZP-pinhole distance was adjusted to 187.3 mm. The samples to be irradiated were yeast cells (*Saccharomyces cerevisiae*), which had typical diameter of 8 μm, average density of 1.06 g/cm^3 and the element contents of H (10.7%), O(82.3%), C(5.3%), N (1.7%) and S (0.2%)[15]. The irradiation dose rate is about 13.19 Gy·s^{-1}·(100 mA)$^{-1}$, calculated with the number of X-ray photons and 1 J=6.25×10^{18} eV[16]. The mass absorption coefficient of the yeast cell at 516.7 eV (2.4 nm) is about 2157.095 cm^2/g. Calculating with $I=I_0(1-e^{-\mu\rho d})$, where I and I_0 are the absorbed and incident fluxes of X-rays, respectively, ρ and d are the average density and diameter of yeast cell, and $\mu=\sum(\mu_i,\eta_i)$, where μ_i and η_i are the mass absorption coefficient and mass fraction of i^{th} element, respectively, the dose delivered to a cell is about 86% of the irradiating dose, and the absorbed dose rate of a cell is about 11.34 Gy/s.

Before irradiation, the yeast cells were cultured in YPD (1% yeast extract, 2% peptone, 2% glucose) medium for two days and harvested in a stationary phase. The cell concentration was diluted and adjusted approximately to 1×10^5 per mL and a 2 μLsuspension was used for each run of irradiation. The Mylar support kept humid during experiment, which lasted 40 min.

After irradiation, the cells were washed down by 0.5 mL PBS buffer (pH 7.4) and dyed with 0.1 mL, 0.5% trypan blue solution for 3 min. After centrifugating the cells at low speed for three times, the dead cells (interphase death) could be determined with a light microscope. Just the dead cells can be dyed purple by the trypan blue[17]. The irradiated and the control (no irradiation) yeast cells are shown in Fig.6. The survivals to 516.7 eV X-ray and ^{60}Co γ-rays are shown in Fig.7. The 516.7 eV X-ray is more efficient than ^{60}Co γ-rays in inducing cell killing. The irradiation experiment was repeated for three times and the error bars, calculated from results of the three experiments, were mainly from the cell-killing statistics.

a

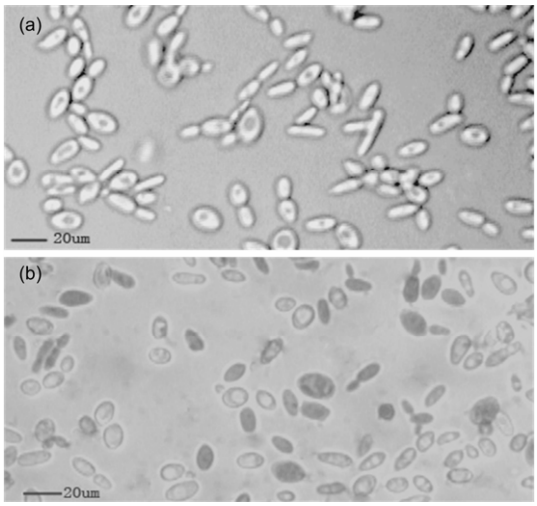

Fig.6 Control (a) and irradiated (b) yeast cells, where the dark ones (dyed with trypan blue) are dead cells.

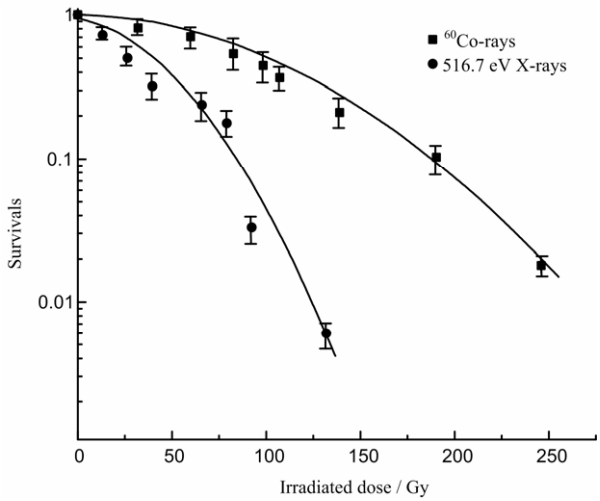

Fig.7 Survivals of yeast cells irradiated with 516.7 eV X-rays (•) and ^{60}Co γ-rays (▪). The curves were obtained by the least-squares fitting method, assuming a linear-quadratic model.

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