Ion irradiation in liquid of μm^3 region for cell surgery

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We present here a cell surgery scheme involving selective inactivation or disruption of cellular structures. Energetic ions are injected into a cell through a tapered glass capillary like a microinjection method. A slight but essential difference from microinjection is that a thin window is prepared at the outlet so that no liquid material can flow in or back through the outlet while still allowing energetic ions to penetrate into the cell. An ~MeV He ion beam from such a capillary having 10 μm outlet diameter inactivated a selected volume ($\sim\mu m^3$) of fluorescent molecules located in a HeLa cell nucleus. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2834695]

When an energetic ion is injected into a cell, chemical bonds of biological molecules are broken via electronic (excitation, ionization) and nuclear recoil processes as well as secondary processes due to various radicals formed in the cell. A typical animal cell is about 50 μm in diameter, composed of complex intracellular organelles and assembled macromolecules, which range in size from more than 1 μm (e.g., chromosomes, mitochondria, and nuclear compartments) down to 100 nm (e.g., centriole and nuclear pore complex) or less. It is then naturally expected that a micrometer/nanometer beam of ions has a high potential to induce space-selective inactivation or disruption of arbitrary cellular structures and would be a unique and potential tool to investigate their functions in living cells. Major advances in cell biology have frequently sprung by introducing different and unexplored viewpoints.

Several research groups have been intensively working on the preparation of microbeams for biological applications.[1–11] In a conventional scheme, a well-focused energetic ion beam is extracted in air via a vacuum isolation window, then injected into a biological cell in water.[5–8] Another straightforward scheme involves passing energetic ions through a micron aperture with or without a thin window at the end.[9–11] Table I summarizes microbeams developed so far for biological cell irradiation. Drawbacks of these schemes are (i) a relatively large cylindrical volume is damaged along the beam trajectory in addition to the targeted point, (ii) serious energy and angular stragglings are induced during passage through the vacuum isolation window and air, which deteriorates the beam quality, and as a result determines the lower limit of the beam size at the target, and (iii) real-time control/monitoring of the bombarding point is not easy even when a micrometer beam is prepared. To overcome such technical but serious problems, we have developed a scheme using a tapered glass capillary with a thin window at its outlet.[12,13] This scheme can realize pinpoint energy deposition and three-dimensional selection of the bombarding point by observing the outlet through a microscope with a precision of a micron or better in an arbitrary position of a living cell or in any liquid object.

Figure 1(a) shows a drawing of the microbeam preparation setup installed on an *X-Y* stage, which is aligned by the upstream micrometers with respect to the beam line. The beam is first trimmed by a square aperture of 1×1 mm^2 formed by four rectangular aluminum plates, then injected into a tapered glass capillary on a capillary holder and passed through a thin window at the outlet of the capillary. This end window enables the whole beam line to be kept under vacuum and, at the same time, a microbeam can be injected into a liquid target in a well-defined position in front of the capillary. The four aluminum plates of the square aperture are electrically isolated with respect to each other and are used to monitor the ion beam current and the lateral position of the beam with respect to the aperture. The capillary is held to the holder via double O rings. The tapered glass capillary is ~50 mm long [see Fig. 1(b)] with inlet and outlet diameters of 0.8 mm and several micrometers, respectively.[12,13] The downstream micrometers are used to align the capillary to the beam axis with a step of 0.5 mrad. The capillary outlet is placed in the focus of an optical microscope, which is installed on a three-dimensional stage. It is noted that the tapered glass capillary scheme described here has several qualitative advantages over conventional microbeam formation schemes: (1) the targeted position is determined by eye under a microscope with a precision of micrometers or better, (2) the beam is directly injected in a liquid target without air in between, and (3) the beam energy can be tuned so that the energy deposition length along the beam direction is well

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TABLE I. Microbeams developed for biological cell irradiation.

Ion (MeV)	Beam ϕ (μm)	Aperture ϕ (μm)	Window thickness (μm)	Cell holder thickness (μm)	Ref.
100/O	0.55	Focusing	7.5/Kapton	6/Mylar	5
3/He	10	Focusing	0.2/Si_3N_4	4/PP[a]	6
58/C	0.7	Focusing	0.2/Si_3N_4	4/PP[a]	7
2/H	10	Focusing	0.5/Si_3N_4	2/Mylar	8
6/He	7.4	6	15/Mylar	4/PP[a]	9
4/H	4	1	3/Mylar	3/Mylar	10
260/Ne	5	5	Air[b]	8/Kapton	11
3/He	4	1.6	7.5/Glass	···	[c]

[a]Polypropylene.
[b]Differential pumping.
[c]Present work.

defined. Thus, the capillary scheme can realize real-time three-dimensional pinpoint bombardment with an accuracy of 1 μm or better. Figures 1(c) and 1(d) show an optical microscope image and a scanning ion microscope (SIM) image near the capillary outlet, respectively. As expected, this window was vacuum tight and the outlet could be safely dipped in liquid.

Ion beams of 3 and 4 MeV He^{2+} from an electrostatic tandem accelerator at Kochi University of Technology were charge state selected by an analyzing magnet, and then transported to the microbeam preparation setup described above. Figure 2(a) shows a microscope image observed by a charge-coupled device (CCD) camera when a 3 MeV He^{2+} beam was injected in a droplet of liquid scintillator (BICRON BC-501A) through a tapered glass capillary with a thin window. A diffuse blue area is clearly visible downstream of the window showing ions were injected into a volume of about μm^3 in the liquid scintillator through the thin window. The open circles and the crosses in Fig. 2(a) show the observed scintillation intensity projected along and perpendicular to the beam axis, respectively. Figure 2(b) shows the simulated energy deposition in the window and in the scintillator by the computational code "stopping and range of ions in matter," SRIM-2006.[14] The red thick line in Fig. 2(a) shows the scintillation intensity distribution evaluated taking into account the conversion efficiency from the energy deposition to the scintillation.[15–17] A tail of the observed scintillation intensity is seen toward the downstream side, which is qualitatively reproduced by the simulation although is considerably wider than the simulation, and even extends into the glass window, indicating that multiple scattering and reflection of scintillation in the scintillator and/or halation of the CCD camera cause the light emission volume to be overestimated. It is noted that the scintillation from borosilicate is negligibly small compared with that from the scintillator.

The focusing factor ξ, the ratio of the beam density at the outlet to that of the inlet was measured as a function of the outlet diameter D_{out} in the range of 1.5–9.6 μm. The transmitted ion current was measured with a Faraday cup covering the capillary outlet. On the other hand, the incident current entering the capillary was evaluated by replacing the tapered capillary with a Faraday cup having the same inlet diameter as the capillary. It was found that ξ increases monotonically like $\xi \propto D_{out}^{-1.3}$, and reached as high as 1000, which is another important aspect of the tapered glass capillary as compared with a conventional scheme employing a simple aperture.

As the second demonstration, a real biological cell, a HeLa cell whose nucleus was labeled with a fusion protein of histone H2B and green fluorescent protein[18] (GFP) was bombarded by a microbeam. The microbeam was prepared by

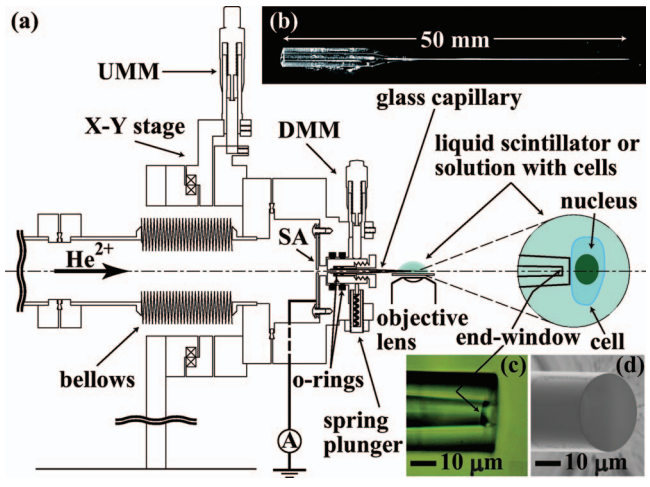

FIG. 1. (Color) (a) Schematic drawing of the microbeam preparation setup. Upstream micrometer (UMM), downstream micrometer (DMM), and square aperture (SA). (b) A photo of a tapered glass capillary, (c) microgram of the capillary outlet, and (d) diagonal image of the capillary outlet by SIM.

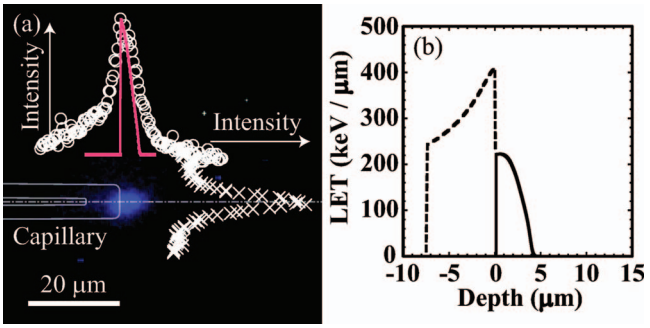

FIG. 2. (Color) (a) Scintillation due to a 3 MeV He^{2+} beam entering a tapered glass capillary with an end-window thickness of 7.5 μm and outlet diameter of 1.6 μm. The thin solid curves show an outline of the capillary. The open circles and the crosses show the projected scintillation intensity along and perpendicular to the beam axis, respectively. The thick red solid line shows the scintillation intensity distribution evaluated taking into account the conversion efficiency from the energy deposition to the scintillation. (b) Show the linear energy transfer (LET) calculated by SRIM-2006 (Ref. 14) with 3 MeV He^{2+} beam entering the capillary with an end-window thickness of 7.5 μm. The dashed and solid lines show the LET in the end window and the liquid scintillator, respectively.

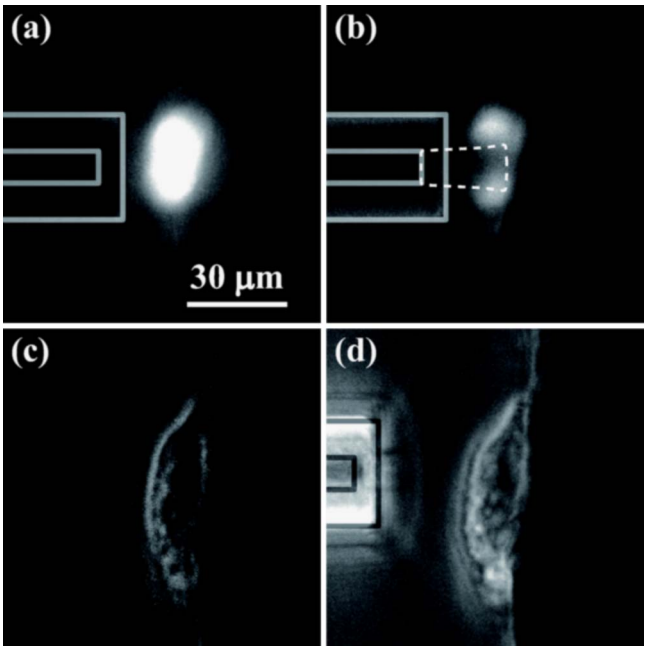

FIG. 3. (Color online) Fluorescent images of a HeLa cell nucleus labeled with histone H2B-GFP (Ref. 18). (a) Before irradiation and (b) after irradiation by 4 MeV He^{2+} beam entering a tapered glass capillary with an end-window thickness of 7.3 μm and outlet diameter of 9.6 μm. (c) and (d) are the phase contrast images of (a) and (b), respectively. Solid and dash lines show an outline of the capillary and a calculation of the energy deposition region by SRIM-2006 (Ref. 14). In (c) and (d), the capillary is away from the cell to prevent sticking together.

4 MeV He^{2+} incident on a tapered glass capillary with an end window of 7.3 μm in thickness and outlet diameter of 9.6 μm [see Figs. 1(c) and 1(d)]. Figures 3(a) and 3(b) show the fluorescence images of the nucleus before and after irradiation for transmitted current of ~100 pA and ~7 s, respectively, which clearly show that the bombarded volume was optically inactivated without affecting global cell morphology [see phase contrast images in Figs. 3(c) and 3(d) before and after bombardment].

In summary, we have demonstrated that a tapered glass capillary with a thin end window can deposit energy in a well-selected volume of microscopic region in a cell. A tool enabling "surgery" in an arbitrary region of a cell is now available, providing a technique to study various functions of intracellular structures independently. According to the simulations performed by SRIM-2006, the bombardment region can further be reduced, e.g., down to 100 nm or so when a 20 keV He^{2+} beam is injected in a tapered capillary with an end-window thickness of 100 nm and an outlet diameter of 100 nm.[19] The combination of a low energy ion injector and an optical microscope would provide a desktop cell surgery system.

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