Published in final edited form as:

AIP Conf Proc. 2010 August 8; 1336: 351–355. doi:10.1063/1.3586118.

Ion, X-ray, UV and Neutron Microbeam Systems for Cell Irradiation

A.W. Bigelow, G. Randers-Pehrson, G. Garty, C.R. Geard, Y. Xu, A.D. Harken, G. W. Johnson, and D.J. Brenner

Center for Radiological Research, Columbia University 630 West 168th Street, 11th floor, New York, NY 10032

Abstract

The array of microbeam cell-irradiation systems, available to users at the Radiological Research Accelerator Facility (RARAF), Center for Radiological Research, Columbia University, is expanding. The HVE 5MV Singletron particle accelerator at the facility provides particles to two focused ion microbeam lines: the sub-micron microbeam II and the permanent magnetic microbeam (PMM). Both the electrostatic quadrupole lenses on the microbeam II system and the magnetic quadrupole lenses on the PMM system are arranged as compound lenses consisting of two quadrupole triplets with “Russian” symmetry. Also, the RARAF accelerator is a source for a proton-induced x-ray microbeam (undergoing testing) and is projected to supply protons to a neutron microbeam based on the ${}^7\text{Li}(p, n){}^7\text{Be}$ nuclear reaction (under development). Leveraging from the multiphoton microscope technology integrated within the microbeam II endstation, a UV microspot irradiator – based on multiphoton excitation – is available for facility users. Highlights from radiation-biology demonstrations on single living mammalian cells are included in this review of microbeam systems for cell irradiation at RARAF.

Keywords

Radiation biology; microbeam; x-ray; UV; neutron

INTRODUCTION

Single-cell / single-particle microbeam irradiation technology at Columbia University’s Radiological Research Accelerator Facility (RARAF) has evolved continuously starting with the user facility’s initial aperture-based microbeam system¹ installed in February 1994. RARAF’s first microbeam system pioneered numerous radiobiology studies in particle-induced DNA damage and repair mechanisms. The dominant interest among RARAF’s users is basic research involving the bystander effect², where non-targeted cell nuclei manifest damage similar to targeted cell nuclei. In investigating cell-signaling pathways such as those potentially associated with the bystander effect, cell biologists at RARAF pursue fundamental understanding of low-dose effects. Highlights from radiation-biology demonstrations on single living mammalian cells are included in this review of microbeam systems for cell irradiation at RARAF.

MICROBEAM SYSTEMS AT THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

Microbeam platforms at Columbia University’s Radiological Research Accelerator Facility (RARAF) have evolved from an initial aperture-based microbeam (decommissioned) to an array of micron-sized irradiation probes. Our microbeam platforms are: microbeam II

(electrostatic focusing system), the Permanent Magnetic Microbeam (PMM), an x-ray microbeam, the neutron microbeam (under development), and the UV microspot. All these systems have a common irradiation protocol that involves imaging, targeting and irradiating. In short: 1) cells are imaged and their center-of-gravity coordinates are registered within the control program, 2) cells are accurately positioned at the irradiation location, 3) cells are irradiated with a prescribed radiation dose, and 4) the next cell is brought to the irradiation location.

Microbeam II

RARAF's principal microbeam system, microbeam II, uses an electrostatic quadrupole lens system capable of producing a submicron-diameter focused beam in air³. The electrostatic lens system is a compound quadrupole triplet lens system designed with Russian symmetry by A. Dymnikov⁴. Incentives for developing a microbeam system with a focused ion beam were: a) to overcome the penumbra or halo artifact associated with aperture-based microbeam systems and b) to improve beam rate at target. While a sharp submicron beam spot is compatible with irradiating sub-cellular structures, we demonstrate the horizontal-plane positioning stability of the vertical microbeam II ion beam in the section below.

Microbeam II Demonstration—To demonstrate the resolution of the microbeam II irradiator at RARAF, the letters “NIH” were irradiated onto a single live cell nucleus. The cells for this demonstration were HT1080 human Fibro Sarcoma cell nuclei containing GFP-tagged XRCC1 (single-strand DNA repair protein). These cells were provided by David Chen, UT Southwestern, Dallas, Texas; they were plated on microbeam dishes and kept under physiological conditions during the irradiation and imaging phases. A single cell nucleus was irradiated in an “NIH” dot-matrix stage-motion pattern with ~200 6-MeV alpha particles per spot. The pattern was reproduced via precision stage motions to irradiation locations. Typical spacing between points along each letter was 1 μm .

As DNA damage occurred in the cell nucleus, XRCC1 repair protein formed foci at the damage sites. Immediately after the irradiation, a Z-stack of 21 wide-field fluorescent images was acquired using a water-dipping objective (60X 1.0NA), and a step size of $\Delta Z = 0.5 \mu\text{m}$, to reveal GFP concentrations in the nucleus exposed with the “NIH” pattern and in the neighboring (control) cell nuclei. Autoquant software was used to deblur the images in a post-processing step – result shown in Figure 1.

Permanent Magnetic Microbeam

The permanent magnetic microbeam (PMM)⁵ shown in figure 2 is a second focused charged-particle microbeam developed at RARAF. It uses permanent magnetic quadrupole lenses (STI Optronics, Inc., Bellevue, WA) whose strengths are tunable by varying the insertion of permanent magnets into the shaped yokes⁶. Originally designed as a stand-alone microbeam with a ²¹⁰Po source, the system remains tethered to its accelerator-based test source of ions following media attention of the ²¹⁰Po-linked assassination of Alexander Litvinenko in November 2006⁷ that led to heightened security and reduced availability of polonium. The use of an accelerator-based ion source allows for higher beam rate and, due to reduced chromatic aberrations, a smaller spot size than would be available with a ²¹⁰Po source. With the PMM fully functional as a second charged-particle microbeam line at RARAF, its design concept as a stand-alone microbeam is still valid and potentially more economical for radiation-research laboratories because it excludes purchase of a particle accelerator and support electronics. Contrary to electrostatic or electromagnetic lenses, the PMM requires neither high precision voltage/current supplies nor cooling. The use of permanent magnets also allows a much tighter pole-face gap, providing better optical properties compared to electromagnetic lenses.

Presently, the PMM is used for cell-irradiation experiments when development projects occupy the microbeam II endstation or beamline. Also, the PMM is used for development of point & shoot technology as well as microfluidics-based flow and shoot technology (FAST). Impetus for a point & shoot device is increased throughput. Pointing the beam with electromagnetic steering coils to cellular targets within a frame of view relies on electronic switching, which is inherently faster than moving a mechanical stage. Toward that end, a wide-field magnetic split-coil deflector system (Technisches Büro Fischer, Ober Ramstadt, Germany) was integrated into the PMM beamline. FAST developments are in collaboration with Dr. Daniel Attinger's Laboratory for Microscale Transport Phenomena, Department of Mechanical Engineering, Columbia University. In-house software is in development for grabbing images with a high-speed camera of cell targets flowing through a microfluidic channel in order to track and predict the time and position of individual cells as they enter an irradiation zone to be irradiated by the point & shoot system.

Room stability is a challenge for the PMM. The endstation was built in a 3rd floor laboratory, where beam position can shift by several microns as personnel move about the room. At present, the solution for this instability is to have both the microbeam operator and the biologist remain in a fixed location during the 20-minute long irradiation, while additional personnel are requested not to approach known locations where their presence would affect the ion beam position. Meanwhile, designs for a reinforced floor are being developed.

X-Ray Microbeam

In adding a soft x-ray microbeam for low linear energy transfer (LET) irradiation of cells at RARAF⁸, the category of microbeam radiation has expanded beyond charged particles to include electromagnetic radiation. Our approach to x-ray production is through particle-induced x-ray emission (PIXE). In a two-stage process, an accelerator-produced proton beam impinges on a titanium target, embedded in a cooled copper rod, to produce x-ray emission, some of which is focused to a micron-sized diameter using a Fresnel zone plate diffractive optic (Zoneplates, Inc., London, UK). Cells are positioned at the x-ray focal point with an irradiation endstation modeled from the RARAF standard design developed on the microbeam II system.

In our two-stage process to produce an x-ray microbeam, the first stage involves focusing a proton beam and the second stage involves focusing x-rays, as depicted in figure 3. In the first stage, an electrostatic quadrupole quadruplet lens focuses protons from an object distance of 5.77 m to an image distance of 14 cm, where a 100 micron diameter proton spot impinges on a titanium target face cut at a 70-degree angle to the proton incidence. With this geometry, part of the 4.5 keV K α titanium characteristic x-rays are emitted in the vertical direction toward the zone plate and appear to originate from a 100 $\times$ 35 micron elliptical spot. In the second stage of x-ray microbeam production, x-rays exit the vacuum system through a beryllium window into a helium-filled chamber housing a Fresnel zone plate optic. The zone plate and order-selecting apertures are aligned through horizontal positioning adjustments on their mounts while optimizing x-ray fluence measurements with an ionization detector. The focused x-ray beam profile is measured using a knife-edge scan similar to the one used to determine beam profiles in the charged particle microbeams³, the main difference being that here we monitor the beam fluence rather than the pulse height as the knife edge is scanned across the beam.

Currently, the available x-ray spot size is 8 $\times$ 3 microns, which is sufficient to irradiate a single cell nucleus. With an available dose rate of 1 mGy/s, initial biology experiments, expected to commence in Fall 2010, will focus is on low-dose and low-dose-rate applications, for example, bystander effect and adaptive response studies.

Neutron Microbeam

Complementing RARAF's microbeam irradiation platforms for charged particles and electromagnetic radiation, we are developing a revolutionary neutron microbeam that will have the capacity to deliver neutrons to cellular targets. The rationale for developing a neutron microbeam is to study exposure effects by neutrons with energy comparable to those within commercial nuclear reactors (< 50 keV), where personnel might be exposed.

In our design, neutrons are formed through a ${}^7\text{Li}(p, n){}^7\text{Be}$ nuclear reaction, which has a threshold at 1.881 MeV. An electrostatic quadupole quadruplet lens (positioned with object and image distances 2.5 m and 0.55 m, respectively) focuses 10 nA of 1.886 MeV protons to a 10 micron diameter spot at the lithium target where the nuclear reaction occurs – schematic shown in figure 4. The near-threshold reaction provides a relatively high neutron yield, as well as a very narrow forwardly-peaked angular distribution⁹ with fluency parameters compatible with cellular irradiation: 21 degree maximum neutron angle, 16 micron neutron beam diameter at cell dish, and 31 keV mean neutron energy. The projected dose rate is 4.5 mGy/s. A thin Au/Al backing foil on the lithium target design stops forward-moving protons and removes them from the neutron beam.

Size of the neutron microbeam is determined by imaging alpha particle tracks on a customized thin ${}^6\text{Li}_2\text{CO}_3$ -coated fluorescent nuclear track detector (FNTD) placed at the location for cellular irradiation. Alpha particles are produced through the ${}^6\text{Li}(n, \alpha)$ reaction in the FNTD coated layer. Alpha-particle tracks in FNTDs can be imaged with laser scanning microscopy techniques; the excitation wavelength is either 335 nm or 620 nm and the emission wavelength is 750 nm. In order to avoid the long turnaround time required for commercial FNTD imaging, we have obtained optics and a photomultiplier tube to allow in-house FNTD imaging with RARAF's multiphoton microscope¹⁰. The Titanium Sapphire laser excitation source of the multiphoton microscope is tunable over a wavelength range 680 – 1080 nm. Though this wavelength range excludes excitation at 620 nm, it offers three-photon excitation when the laser is tuned to 1000 nm – about three times the 335 nm excitation wavelength of an FNTD.

UV Microspot

Fulfilling requests from our facility users, we are integrating a UV microspot irradiator into our microbeam system. With our combination of a UV microspot and a charged-particle microbeam, RARAF is emerging as a unique facility for work that requires both photon and particle irradiations on the same platform. Our users have expressed needs to use an integrated UV microspot facility in two modes: 1) as a stand-alone UV microspot irradiator, and 2) as a probe in concert with ion-beam irradiation experiments.

Principles of Operation—Traditional UV laser microbeam design involves an elongated laser path of exposure. Our system utilizes multiphoton excitation to produce a micro-volume of effective UV radiation. What makes our UV microspot unique is that it is integrated within the Microbeam II charged-particle cell-irradiation platform to provide a cocktail of photon and particle irradiations within one system.

Leveraging off of existing equipment, the tunable titanium-sapphire (Ti:S) laser from our multiphoton microscope¹⁰ has the ability (through the multiphoton process) to produce a microspot of UV radiation at the focal point of the laser beam. In the two-photon process, two photons with a corresponding wavelength of 720 nm, for instance, can have a superposition of energy and act as one photon with a corresponding wavelength of 360 nm. This process is optimized at the focal point of a laser where the incident photons have a high probability of temporal and spatial coincidence.

The Ti:S laser for our multiphoton microscope covers a wavelength range of 680–1080 nm. These long wavelengths are minimally damaging to a tissue sample and, at the laser's focal point, the two-photon and the three-photon processes effectively provide (340–540 nm) and (227–360 nm), respectively. With this tool, we can implant a microspot of UV radiation into a tissue sample with $0.65 \times 2.8 \mu\text{m}$ full width half maximum (FWHM) resolution and to depths of up to several hundred microns.

UV Microspot Demonstration—To demonstrate the resolution of RARAF's new UV microspot irradiator, the Columbia University crown logo was irradiated onto a live single cell nucleus. The cells for this demonstration were HT1080 human Fibro Sarcoma cell nuclei containing GFP-tagged XRCC1, described in the Microbeam II Demonstration section above. They were plated on Petri dishes and kept under physiological conditions during the irradiation and imaging phases.

Of the two cell nuclei visible in the multiphoton microscopy image shown in Figure 5, the lower cell nucleus was irradiated using a Titanium Sapphire laser tuned to 976 nm, which acts as 488 nm and 325 nm in the two- and three-photon modes, respectively. The crown pattern was produced via precision stage motions to 59 irradiation locations. Each location received 16 mW of laser power for 1 second to an elliptical volume $0.65 \mu\text{m}$ radial by $2.8 \mu\text{m}$ axial FWHM. Typical spacing between points was $1 \mu\text{m}$.

As DNA damage occurred in the cell nucleus, XRCC1 repair protein formed foci at the damage sites. Following irradiation, multiphoton microscope z-stack imaging ($\Delta Z = 1 \mu\text{m}$, Z range = $15 \mu\text{m}$, $\lambda = 976 \text{ nm}$, $\sim 1 \text{ sec/frame}$, 10X averaging) revealed GFP concentration in the nucleus exposed to the crown pattern and in the neighboring cell nucleus. With a 60X water-dipping objective, the image size is $58.1 \mu\text{m}$ wide by $55.8 \mu\text{m}$ high. To improve image quality, AutoQuant, an image deblurring program, was used to surpass point-spread-function limitations.

Acknowledgments

Supported by the National Institute of Biomedical Imaging and Bioengineering under Grant: NIBIB 2 P41 EB002033-14.

References

1. Randers-Pehrson G, Geard CR, Johnson G, Elliston CD, Brenner DJ. Radiation Research. 2001; 156(2):210–214. [PubMed: 11448243]
2. Ponnaiya B, Jenkins-Baker G, Brenner DJ, Hall EJ, Randers-Pehrson G, Geard CR. Radiation Research. 2004; 162(4):426–432. [PubMed: 15447040]
3. Bigelow AW, Brenner DJ, Garty G, Randers-Pehrson G. IEEE Transactions on Plasma Science. 2008; 36(4):1424–1431.
4. Dymnikov AD, Brenner DJ, Johnson G, Randers-Pehrson G. Review of Scientific Instruments. 2000; 71(4):1646–1650.
5. Garty G, Ross GJ, Bigelow AW, Randers-Pehrson G, Brenner DJ. Radiation Protection Dosimetry. 2006; 122(1–4):292–296. [PubMed: 17189277]
6. Gottschalk SC, Dowell DH, Quimby DC. Nucl Instr & Meth A. 2003; 507:181–185.
7. Harrison J, Leggett R, Lloyd D, Phipps A, Scott B. Journal of Radiological Protection. 2007; 27(1): 17–40. [PubMed: 17341802]
8. Harken A, Randers-Pehrson G, Brenner D. Journal of Radiation Research. 2009; 50(Suppl A):A119.
9. Lee CL, Zhou XL. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms. 1999; 152(1):1–11.

10. Bigelow AW, Geard CR, Randers-Pehrson G, Brenner DJ. Review of Scientific Instruments. 2008; 79(12):123707. [PubMed: 19123569]

FIGURE 1.
“NIH” written on a single cell nucleus with the RARAF microbeam. A tribute to our primary funding agency and a demonstration of our microbeam resolution.

FIGURE 2. Photograph of the PMM endstation. Indexing knobs were added to the permanent magnet manipulators for improved tuning precision. The syringe pump in the lower left corner controls flow for FAST.

FIGURE 3.
Schematic layout diagram of the x-ray microbeam endstation.

FIGURE 4.
Schematic of Columbia University's neutron microbeam concept.

FIGURE 5.

The Columbia University Crown logo irradiated by UV microspot onto a single cell nucleus.