

Single-Particle/Single-Cell Ion Microbeams as Probes of Biological Mechanisms

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(Invited Paper)

Abstract—An ion microbeam is a very narrow beam of charged particles, typically protons, alpha particles, or heavier, of micrometer/submicrometer size, corresponding to cellular/subcellular dimensions. Together with integrated techniques for locating live cellular or subcellular targets, they allow rapid sequential irradiation of these targets. This review covers both the technology involved in modern single-cell microbeams, as well as some current applications. The recent explosion of interest in microbeams was initially driven by interest in the domestic radon problem, in which target cells are exposed either to zero or one alpha particle. Microbeams allow cells to be individually irradiated with exact numbers of particles. As microbeams were built, refined, and used, the biological questions that were addressed with them have considerably broadened, to encompass many aspects of damage signal transduction. Two areas in particular have attracted much interest: One is the use of microbeams to address the sensitivity of subcellular targets, such as the cytoplasm or mitochondria. The other reflects the ability of the microbeam to irradiate some cells, but not others, allowing a direct investigation of the so-called bystander effect, where signals from irradiated cells can apparently cause biological responses in neighboring unirradiated cells.

Index Terms—Accelerators, biological effects of radiation, imaging, particle beams.

I. INTRODUCTION

WHAT HAPPENS to a mammalian cell, or a neighboring cell, when it is struck by ionizing radiation? This basic question has led to the development of single-particle single-cell microbeam facilities for studying the biological effects of particle irradiation. In order to study the effects of ionizing radiation on single cells, such as, the bystander effect [1], it is practical to have a device that can locate single-cell and subcellular targets and deliver a prescribed specific dose [2] to each target. A minimum delivery of exactly one ion per cell nucleus demonstrates the precision of a single-particle single-cell ion microbeam and is particularly applicable to studying effects from radon exposure [3]. Particle accelerators with specialized ion optics or collimators are capable of forming

charged-particle microbeams with subcellular resolution, where the beam size is smaller than a cell nucleus. Coupled to an endstation with imaging, location, and positioning techniques, a microbeam is a powerful tool for controlled cell-irradiation experiments.

Where cell-irradiation studies date back to studies in the 1950s [4], pioneers in the field of single-cell ion microbeam irradiation include groups at Columbia University [5], the Gray Cancer Institute (GCI) [6], [7], and the Pacific Northwest National Laboratory [8]. Since that time, the number of planned and operational microbeam facilities has significantly increased. To provide a cohesive example of a microbeam facility, this review article will first concentrate on the development of light ion microbeam technology at the Radiological Research Accelerator Facility (RARAF), Columbia University and subsequently provide technical summaries of similar facilities. For a review on heavy ion microbeam facilities and their applications, refer to the companion paper in this issue by Funayama *et al.*

II. RARAF—A MICROBEAM FACILITY

Microbeam technologies at RARAF are geared toward cell-irradiation experiments involving the bystander effect, genomic instability, and adaptive response. Researchers at RARAF have developed a series of microbeam systems: Microbeam I, Microbeam II, and the permanent magnetic microbeam (PMM), propagating those technical aspects that have proven useful from one microbeam generation to the next. The following overview of RARAF microbeams demonstrates merging novel concepts with successful established design.

A. Microbeam I

Initial microbeam development at RARAF was based on a vertical collimated beam from a model D1, 4.2-MV High Voltage Engineering (HVE) Van de Graaff accelerator with a duoplasmatron ion source [5]. This particle accelerator, built in 1949, was originally the injector for the Cosmotron project at Brookhaven National Laboratory and was moved to Nevis Laboratories in 1980, where it was used to pioneer single-cell single-particle microbeam studies at RARAF. The duoplasmatron is a hot-cathode magnetic ion source, which typically produces ions of hydrogen and helium isotopes [9]. In the duoplasmatron design, an innovative electromagnet-electrode

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combination forms plasma primarily at the exit hole of the ion source [10].

The first-generation collimated microbeam at RARAF, now decommissioned and referred to as Microbeam I, was initially used for biology experiments starting in February 1994. Apertures for beam collimation consisted of a 2-mm-diameter aperture located 1.3 m before a final collimating pair of apertures. The final consecutive collimating apertures were 5- and 6- μm -diameter laser-drilled holes in 12.5- μm -thick stainless steel sheets, separated by 300 μm . The 6- μm -diameter aperture acted as an antiscatter element, to limit the particle irradiation to an area smaller than the nucleus of a human cell. Nevertheless, a small fraction of the particles (8%) arrived outside of the prescribed target region. This is a general characteristic of all collimated microbeams and is overcome by using a focusing system. Two actuators were used to adjust the alignment of the final collimator, which was mounted in a spherical gimbal. This decommissioned collimator assembly is currently located at the McMaster Accelerator Laboratory (McMaster University, Hamilton, ON, Canada) for microbeam development in that facility.

Accurate cell imaging and targeting were facilitated through a program written for real-time image analysis and stage positioning. Developed in Visual Basic under the Windows NT operating system, the irradiation algorithm was essentially: 1) with a low-magnification objective lens, acquire a series of adjacent images for coarse cellular location, and 2) under high magnification, reimage each region that contains at least one cell in the coarse image, then locate, position, and irradiate cells. For each cell to be irradiated, a mechanical stage moves the cell target to the coordinates of the ion beam, previously determined by imaging the profile of laser transmission through the final collimator. Tried and established on Microbeam I, this control program has served as the base for further generations of microbeam system development at RARAF.

Each microbeam experiment includes an irradiation protocol with 1) a prescribed number of particles (as low as one) per targeted cell, 2) a percentage of cells to be targeted, and 3) targeting modes: uniform targeting, subcellular, site-specific targeting (nucleus or cytoplasm), and patterned targeting, such as stripes on tissue [11]. This protocol is realized by deflecting the beam once the prescribed number of particles has been detected by an ion counter; different particle detection technologies are in use at the various microbeam facilities, as discussed below.

With Microbeam I, the ions exited a vacuum beamline through a 3.8- μm -thick polypropylene window and traversed a 100- μm -thick air gap prior to irradiating cells plated on a 3.8- μm -thick polypropylene membrane. After penetrating the cells, these ions had sufficient range to then enter an ion counter filled with P10 gas (90% argon and 10% methane) [5]. This particle detector design included an optically transparent, 2.5- μm -thick mica entrance window to allow in-line mounting at the end of the high-magnification objective. As well, a side passage in the detector blew humidified air with 5% CO_2 over the cells to keep them moist. The ion detection signal propagated through a preamplifier and amplifier combination and on through a single-channel analyzer and scaler. The gate

of the scaler attached to the input of a high-voltage amplifier (Technisches Büro S. Fischer, Ober-Ramstadt, Germany), where the output drove electrostatic deflection plates to shutter the ion beam. The shutter (stainless steel plates: 53.3-cm length, 5.7-cm height, and 1.3-cm gap) was positioned approximately 6.4-m upstream from the exit window.

Several design aspects became RARAF standards since the successful operation of Microbeam I. For one, a vertical ion beam for irradiation has a practical advantage when working with plated cells covered by a very thin nominally uniform layer of medium. Also, cell-dish transfer between the biologist and the microbeam operator is expedited by having a biology workbench and incubator in the same room and adjacent to the irradiator. For biology experiments, such as mutation and oncogenic transformation studies [1], [3], [12]–[15], available high throughput of about 11 000 cells per hour was vital for obtaining statistically relevant numbers of irradiated samples.

B. Microbeam II

The essential change from Microbeam I to Microbeam II was to progress from a collimated beam to a focused ion beam on a dedicated beamline with a double-focusing magnet. The Microbeam II endstation incorporates a Nikon Eclipse 600FN microscope that rests in a kinetic mount and is also attached to a pivot arm for online and offline positioning. Sample illumination is regulated with an adjustable light-guide-coupled shuttered UV lamp. Ion beam scatter at the vacuum exit window is minimized by using a 100-nm-thick SiN exit window. A precision xyz -stage incorporated into the endstation of Microbeam II comprises two components: a custom coarse xy -stage, designed and constructed in-house, coupled to a three-axis piezoelectric-actuated fine-motion stage, the LP-200 low-profile nanopositioner from Mad City Laboratory (Madison, WI) [16].

A microbeam formed with focusing elements brought a promise of a smaller beam spot size, which matched the biological interests of targeting subcellular components. Electric and magnetic quadrupole fields are the preferred choices of ion optics to focus ion beams, because these fields are reasonable to produce and they can easily be modeled with transfer matrices [17]. For the Columbia University Microbeam II system, a compound lens consisting of two electrostatic quadrupole triplets with “Russian symmetry” was developed [18]. There were several basic reasons for choosing the electrostatic route for focusing over the more prevalent use of electromagnetic lenses, as seen at other facilities. First, electrostatic lenses lack the hysteresis inherent in magnetic lenses, allowing easy change between ion beams of differing linear energy transfer (LET). Also, stable voltage is more readily achieved than stable current, which is required for magnetic systems. The focal properties of electrostatic lenses are conveniently linked to the accelerator’s terminal voltage, allowing easy switching between different ions with the same acceleration potential.

This double lens is essentially a two-stage lens, where each stage has a 1.569-m object distance to the lens assembly entrance and an 8-cm working distance, measured from the lens assembly exit. One of the main features of the multiplet lens

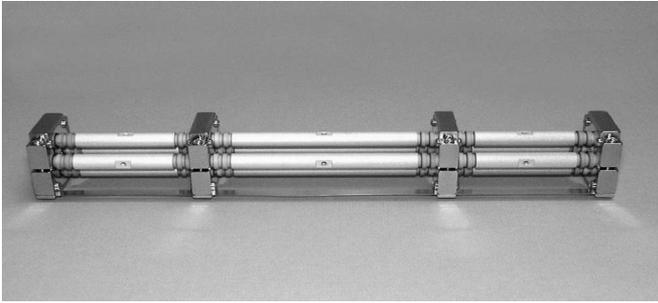


Fig. 1. Picture of one electrostatic quadrupole triplet lens section used on Microbeam II. The rod clamps also define ground plane locations on either side of the electrodes. The total length of each rod is 30 cm.

design being used is that part of the alignment of the electrodes is accomplished by using four 1-cm-diameter 30-cm-long Macor (machinable glass ceramic) rods for each quadrupole triplet. The electrode regions are thin-layered bands of gold evaporated onto the cylindrical surfaces. The pole lengths are such that the operating voltage on each electrode is roughly equal—to ensure no “weak links.” Alignment of successive electrodes relative to each other is assured by their one-piece construction.

For a double quadrupole triplet where the lens strengths are set according to “Russian” symmetry (+A,−B,+C) and (−C,+B,−A), a circular beam spot and equal aberration coefficients in both planes are expected. In our case, the demagnification of this lens is 57X, which is expected to form a 0.5- μm -diameter beam spot.

Coinciding with the development of the double-triplet lens just mentioned, two other geometries were experimentally evaluated. First, an electrostatic quadrupole quadruplet lens was manufactured from four rods of Macor and installed in the Microbeam I beamline. From initial tests with this quadruplet, three design aspects were incorporated to alleviate sparking issues and to improve the ion optics: 1) grooves were added in the sections between electrodes and ground planes to increase the leakage path, 2) ion implantation of the nonelectrode segments enhanced control of the resistance gradients, and 3) a phase-space sweeper was added just prior to the object aperture to eliminate any correlation between particle position and direction. The phase-space sweeper essentially “confuses” the beam by varying voltages on two pairs of orthogonal cylindrical electrodes to continually steer the beam in a nonrepetitive pattern, effectively erasing any ion optical “memory” of upstream components. This electrostatic quadrupole quadruplet provided a beam spot size sufficient for targeting cell nuclei ($\sim 5 \mu\text{m}$).

For evaluating a second geometry, a single electrostatic quadrupole triplet, shown in Fig. 1, was installed in the Microbeam II beamline, where the ion beam is shuttered by applying excessive voltage across two of the four electrodes on the phase-space sweeper. Effectively, the second stage of the double quadrupole triplet lens, the single triplet operated very reliably with few sparks, and it has proven to be quite robust, surviving occasional vacuum excursions. Along with the phase-space sweeper, this lens focused a 6 MeV⁴He⁺⁺ beam down to a diameter of 2 μm . Combining two such electrostatic lenses, the compound triplet lens was installed in the Microbeam II

beamline for biology experiments that began in October 2007 and has achieved a submicrometer beam diameter.

Ion-beam profiles are measured by stepping xy -crossed 3- μm -thick nickel knife edges through the beam at the desired focal plane, while monitoring the ion energies with a solid-state detector. Threshold settings on a single-channel analyzer enable the distinction between the unobstructed ion counts from those that penetrate the knife edges. The ion-beam size is determined using a χ^2 -monitored linear fit to the fraction of obstructed beam between approximately 0.2–0.8. Assuming a largely uniform ion-beam profile, the reciprocal of the fitted slope is the FWHM of the beam distribution. An autofocusing routine that uses the downhill simplex method successively measures ion-beam sizes for combinations of electrostatic lens voltages to optimize the lens focus settings. When the ion-beam focus is determined, its xy -location reference is found for the imaging system by scanning a 4- μm -diameter fluorescent bead from a position over the ion-beam focus in an outward rectangular spiral pattern, using the same energy-loss technique that was used during the autofocus routine. When the fluorescent bead position is determined to be at the center of the ion beam, the solid-state detector is removed, and an image of the bead is acquired, transferring the ion-beam coordinates to the control program.

C. 5-MV Singletron

To focus particle beams to even smaller subcellular dimensions, the energy stability required by the compound electrostatic quadrupole lens exceeded what the original RARAF accelerator could supply. Coincidentally, a 5-MV Singletron from HVE in the Netherlands was purchased to replace the aging Van de Graaff, and the Singletron was installed during summer/fall of 2005. With a terminal potential of 5 MV, higher energy proton beams with LETs below 10 keV/ μm are available for low-LET experiments on both microbeam and broad beam irradiation facilities at RARAF.

The main technical advantage of this new accelerator is the greatly reduced voltage fluctuation. Significant energy fluctuations can manifest as a chromatic aberration effect in the lens. Hence, with lower energy fluctuations, the optics performance is improved. The Singletron generates high voltage through an inductively coupled Cockcroft–Walton style power supply. Compared to the Van de Graaff, which uses a circulating cloth belt to mechanically charge the terminal, high-voltage production on the Singletron employs no moving parts. The Singletron voltage ripple is guaranteed to be no more than 200 V peak-to-peak at 3.75 MV, compared with the several kilovolt voltage fluctuations of the former Van de Graaff.

The Singletron is equipped with a radio frequency (RF) ion source, which can provide ions for acceleration from a choice of hydrogen, deuterium, and helium gas. Standard on single-ended particle accelerators, the RF ion source uses axial-extraction with an axial magnetic field. Ions are created through collisions between neutral gas particles and electrons excited into oscillation by the RF electric field [19], [20]. Plasmas are formed within Pyrex glass bottles, where the material is a poor catalyst for hydrogen recombination, and the canal design includes an insulating sleeve that 1) acts as a virtual anode, 2) protects

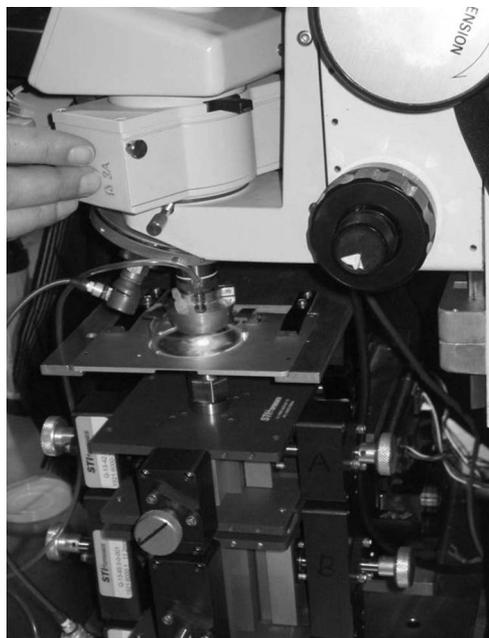


Fig. 2. Photograph of the endstation on the PMM. Knobs on each pole piece allow setting the magnetic quadrupole field strengths.

the metallic cathode, and 3) promotes optimal extraction [21]. A 3.5-MV version of the Singletron with an RF source was extensively evaluated in Leipzig [22].

D. PMM

In addition to Microbeam II, a PMM, based on permanent magnetic quadrupole lenses, was developed on a dedicated beam line at RARAF. The permanent quadrupole lenses (STI Optronics, Inc., Bellevue, WA) form a double triplet Russian quadrupole arrangement designed to have the same pole strengths and configuration as the electrostatic lens system on Microbeam II. Fine tuning of the lens strengths is achieved by physically moving rare-Earth permanent magnets in a shaped yoke—modifying field strength. Similar to an electrostatic lens, a permanent magnet lens also benefits from a small pole gap (due to the lack of large coils) and high stability. The PMM can be operated with particles from either a particle accelerator or from an isotopic source [23], [24]. The final quadrupole triplet lens section sits just below the PMM endstation, as shown in Fig. 2.

Just prior to the decommissioning of the RARAF Van de Graaff accelerator in the summer of 2005, a beam spot size of 20 μm was attained [25]. In the beginning of 2007, after laboratory renovations and a particle accelerator upgrade, the PMM beam line was reassembled, optically aligned and a 20- μm beam was observed again, without touching the magnet adjustments. This confirmed that this type of microbeam can continue to operate without further adjustments, in principle, forever.

The present spot size diameter of 8 μm is larger than the theoretically achievable 5- μm diameter (with a 0.5-mm object aperture). Extensive simulations have shown that this discrepancy may be caused by an addition of 1% octapole moment to the quadrupole lenses. This can be corrected by a fine adjustment

of the permanent magnets, breaking the symmetry that has been imposed up to now. A second factor that was experimentally seen to affect beam spot size is the alignment of the two lenses with respect to each other. Both of these factors are under investigation with the goal of achieving a 5- μm beam. Also, the 1.8- μm -thick aluminum scattering foil originally used to randomize the particle beam entering the object aperture has been replaced with an active magnetic phase-space sweeper, similar to the one employed on the electrostatic microbeam. It is expected that this will reduce the chromatic aberrations and assist in obtaining a smaller spot size. Once fully optimized, the PMM will provide a useful secondary microbeam facility at RARAF and will enable biology to be simultaneously performed with system developments on the electrostatic microbeam.

E. Imaging Modes

Epifluorescence microscopy has been the standard method for imaging cells on the Columbia University microbeams. This works for single cells with extremely low concentrations (50–100 nM) of Hoescht 33342 nuclear stain. However, this imaging technique requires a fluorescent additive and potentially phototoxic UV illumination. At RARAF, technical innovations are integrating multiphoton microscopy and phase-based nonstain cell imaging into the Microbeam II endstation. These techniques are enabling 1) imaging within tissue samples, 2) observing postirradiation cell dynamics, and 3) targeting unstained cells during irradiation experiments.

While results from cell-culture experiments have contributed to studies on low-dose radiation effects, such as the bystander effect, radiation studies using tissue samples have the potential to represent cellular response within organisms [11]. For observing postirradiation cellular dynamics within tissue, multiphoton microscopy offers optical sectioning compatible with live samples and can image at depths of several hundred micrometers. Multiphoton excitation of fluorochromes occurs at the focal point of a tunable mode-locked titanium-sapphire laser source. At RARAF, a custom-built multiphoton microscope conforms to the particular geometrical constraints of an irradiation endstation with an available Nikon Eclipse 600FN microscope at the end of a vertical ion beam [26]. Three-dimensional multiphoton imaging is made possible through sequential optical sectioning, aided by the precision *xyz*-stage.

Two phase-based imaging techniques explored at RARAF for imaging unstained cells are immersion Mirau interferometry (IMI) and quantitative phase imaging (QPI) [27]. Mirau interferometry produces images acquired by compiling a series of interferograms with incremental 1/4 wavelength path-length differences between sampling and reference lengths. IMI was developed at RARAF to image cells in medium; the region between the spot mirror and the beam splitter is backfilled with an immersion fluid that matches the index of refraction of cell-growth medium. With QPI, three reflected-light images are obtained: in focus and slightly above and below the sample plane. These images are then used to solve the light transport equation using Fourier transform-based software (Iatvia Limited, Victoria, Australia). These combined imaging developments

enhance irradiation protocols to accommodate bulk samples and no-UV/no-stain imaging.

III. MICROBEAM FACILITIES WORLDWIDE

While new microbeam facilities continue to enter the field of single-cell irradiation, the groups reviewed here are particularly active contributors to the development of low-LET microbeam irradiation technology.

A. GCI (Northwood, U.K.)

A pioneer in the field of single-cell ion irradiation, the GCI microbeam facility [6], [7], [28] is based on a collimated vertical-up ion beam from a vertical-down 4-MV Van de Graaff particle accelerator, equipped with an RF ion source. An electrostatic shutter for beam blanking is positioned between two 90° magnets that bend the particle beam to horizontal and then to vertical-up. The entire dish is mapped, using Hoechst stains, prior to irradiation. Particles exit a window consisting of 3- μm Mylar backed by an 18- μm -thick transmission scintillator, which pairs with a photomultiplier tube (PMT) placed above the cells for particle detection. During cell irradiations, a dc motorized lead-screw moves the collimator to the bottom of the cell dish to optimize targeting accuracy and then retracts for stage motion clearance. For particle detection, the PMT is placed in a microscope objective mount within a light-tight environment [6].

Having operated with v-groove and capillary collimators, the collimator of choice at GCI is commercially manufactured, thick-walled fused silica tubing with dimensions: 1-mm long, 245- μm outer diameter, and 1- μm inner diameter (SGE, Milton Keynes, U.K.). A miniature precision “lathe” or fiber optic cutter is used to cleave the capillary, which is then delicately placed in a specially designed holder. Capillary alignment is achieved by minimizing the particle energy spread, monitored with a high-resolution silicon surface-barrier detector [7]. With CR-39 track-etch plastic, the targeting accuracy for a 1- μm -diameter bore capillary was measured as: 1) protons hit 90% of targets with an accuracy of $\pm 2 \mu\text{m}$ or 96% of cells with an accuracy of 5 μm , and 2) $^3\text{He}^{2+}$ ions hit 99% of cells with an accuracy of $\pm 2 \mu\text{m}$ [29].

At GCI, the particles are detected by an 18- μm -thick (Bicron BC400) scintillator placed after the collimator and before the cell dish. With this style of before-dish detector, a layer of medium can be kept on the cells during irradiation. Coupled with the PMT, the detection efficiency is near 100%. This scintillator is also beneficial for registering the position of the collimator, by imaging scintillator light, produced during high-current scenarios performed before and after each experiment. Specimens for biological studies at GCI have included tissue samples and cell cultures (with a throughput of up to 10 000 cells per hour) [30]. With a wealth of microbeam experience, GCI is relocating to Oxford University in 2008 and in collaboration with the Surrey Ion Beam Centre (University of Surrey, Guildford, U.K.) they are designing a scanning focused vertical ion nanobeam with an estimated throughput of 100 000 cells per hour [31].

B. CENBG (Bordeaux, France)

The focused microbeam at Centre d’Etudes Nucleaires de Bordeaux Gradignan (CENBG) [32] is based on a 4-MV Van de Graaff accelerator, generating proton or helium nuclei beams of 1–3.5 MeV. These beams are focused using a Russian magnetic quadruplet to less than 1 μm in vacuum or $\pm 5 \mu\text{m}$ in air and can be used for scanning transmission ion microscopy (in vacuum) or cellular irradiations (in air), reaching a throughput of 2000 cells per hour [33], [34].

A unique feature of the CENBG microbeam is the particle detector used to control counted particle irradiations. A low-pressure (10 mBar $i\text{-C}_4\text{H}_{10}$) gas chamber is used as the exit window from the vacuum beamline, providing particle detection before the cells while minimizing beam scattering.

The irradiation stage is designed to deliver particles on cells cultured in a dedicated, vertical, culture flask. Online cell imaging during irradiation is done using an epifluorescent microscope and cellular dyes, although some work has also been done using cells which express fluorescent proteins [33]. Cell positioning is performed using a high precision xyz -stage with a targeting accuracy of $\pm 2 \mu\text{m}$ obtaining 95% of the particles within $\pm 5 \mu\text{m}$.

C. Single Ion Hit Facility (Cracow, Poland)

The single ion hit facility (SIHF) in IFJ PAN Cracow utilizes a Van de Graaff accelerator and RF ion source [35] coupled to two quadrupole doublets, to obtain a 2.5 MeV proton spot size of 10 μm at the sample [36]. The SIHF features a horizontal beamline with sample illumination using a high-brightness LED on the vacuum side of the exit window. This allows online observation and targeting of unstained cells in the transmitted light using a standard microscope objective, immediately prior to irradiation. Particle counting is achieved using a solid-state detector which replaces the microscope objective during irradiation. An additional channeltron detector, collecting secondary emission electrons from the vacuum exit window was found to have insufficient detection efficiency for protons. Beam targeting is performed using a voice coil stage, obtaining a throughput of 1000 to 3000 cells per hour.

D. PTB (Braunschweig, Germany)

The Physikalisch-Technische Bundesanstalt microbeam [37]–[39] is routinely used for the irradiation of living cells using an extremely wide range of LET values—3–200 keV/ μm (protons at 1–20 MeV or helium nuclei at 1–28 MeV). The beam diameter is 2 μm FWHM, achieved by focusing the beam from either a 3.75 MV Van de Graaff generator or a variable energy cyclotron, using an RF ion source and a penning ion source, respectively [40]. The microbeam is obtained using a two stage focusing system, consisting of magnetic quadrupole doublets, with a 90° bend between them. By making use of the energy dispersion of a 90° bending magnet, the beam current can be reduced to a few particles per second using the beam defining slits in the horizontal section, without the spatial resolution being compromised by slit scattering.

The PTB microbeam features a vertical beam, entering the endstation from above. Individual cells are targeted using a fast electrostatic deflector placed immediately in front of the last quadrupole doublet, obtaining a throughput of up to 50 000 cells per hour.

Cell recognition is performed before irradiation. The microscope objective is then replaced with a PMT for detecting particles as they traverse a thin scintillator before impinging on the cells. In analysis following the irradiation, irradiated cells are located by their position with respect to a fiducial marker on the irradiation substrate.

E. LIPSION (Leipzig, Germany)

The Leipzig High-Energy Ion Nanoprobe (LIPSION) [41]–[43] is based on a 3.5-MV Singletron, coupled to an electromagnetic quadrupole quadruplet lens in split Russian geometry [41], achieving spot sizes of 40–100 nm in vacuum and less than 350 nm in air. Although no online cell recognition is done at this time, the LIPSION system has been irradiating cells, with micrometer scale patterns of 2.35-MeV protons and 2-MeV helium nuclei, since 2003 (e.g., [44]). Particle counting is achieved by placing a movable p-i-n diode after the cells.

The cells are irradiated by a horizontal ion beam, in a specially designed vertical holder, which is scanned across the beam using a piezoelectric stage with submicrometer positioning accuracy.

F. INFN (Legnaro, Italy)

The microbeam facility at the Istituto Nazionale di Fisica 518 Nucleare-Laboratori Nazionali di Legnaro (INFN-LNL) [45] is based on a 7-MV Van de Graaff accelerator, delivering protons, deuterons and helium nuclei covering a wide range of LET from 7 to 150 keV/ μm . The microbeam is obtained using micrometer-sized collimators obtaining beam spots of 2–3 μm in diameter. A distinctive feature of the INFN-LNL microbeam facility is that the microcollimator is positioned in air in front of the aluminized Mylar extraction window, facilitating its manipulation and alignment.

Cell targeting is performed using an offline semiautomated phase contrast microscope, eliminating the use of UV and stains. A helicoidal guided translation system allows the biological sample to be automatically moved from the horizontal position under the microscope to the vertical position in front of the microcollimator with $< 2 \mu\text{m}$ precision. The fine movement of the cell dish in front of the beam pipe (and under the microscope during cell recognition and revisiting phases) is achieved with a remotely controlled 2-D translation stage. Particle counting is performed using a silicon surface barrier detector, placed downstream from the sample.

G. LPS (Saclay, France)

The microbeam at the Pierre Süe Laboratory (LPS) [46], [47], CEA Saclay, was designed to study the response of cells to alpha particle exposure, allowing the comparison of the radiological and chemical toxicities of uranium [46]. The

TABLE I
SUMMARY OF FEATURES AT MICROBEAM FACILITIES. IN GENERAL, THE AVAILABLE PARTICLES ARE HYDROGEN AND HELIUM NUCLEI AND PUBLISHED THROUGHPUTS SPAN THOUSANDS TO TENS OF THOUSANDS CELLS/HOUR

Microbeam Facility	Beam Formation	Terminal Potential	Beam Dia. at Target ^a
<i>Vertical-Beam Facilities</i>			
RARAF Microbeam I	Mechanical	4 MV	6 μm
RARAF Microbeam II	Focusing	5 MV	$< 1 \mu\text{m}$
RARAF PMM	Focusing	5 MV	8 μm
Gray Cancer Institute	Mechanical	4 MV	5 μm
PTB	Focusing	3.75 MV ^b	2 μm
Pierre Süe Laboratory	Mechanical	3.75 MV	10 μm
<i>Horizontal-Beam Facilities</i>			
CENBG	Focusing	4 MV	10 μm
LIPSION	Focusing	3.5 MV	350 nm
Lund Nuclear Probe	Focusing	3 MV	5 μm
Single Ion Hit Facility	Focusing	3 MV	10 μm
INFN	Mechanical	7 MV	2–3 μm

^a Note: diameter-measuring protocols vary according to the institute

^b Higher potentials available using a variable energy cyclotron

microbeam features a vertical beam from a 3.75-MV Van de Graaff accelerator, collimated, using a 1-mm-long 5- μm bore silica capillary such that 90% of the particles fall within a 10- μm -diameter circle, entering the sample from below.

At this time, targeting is performed by moving the sample dish above the beam but a system is under development for rapidly moving the capillary using piezoelectric actuators. Automated cell recognition is also under development [47]. Particle counting is achieved using a thin scintillator placed between the exit window and the irradiation dish.

H. Lund Nuclear Probe (Lund, Sweden)

The Lund Nuclear Probe [48]–[50], which has recently started performing biology, is based on a 3-MV single-ended Pelletron with an RF ion source [50], coupled to a focusing system that has four magnetic quadrupole lenses in a split Russian configuration [49]. This lens has reached a spot size of approximately 2 μm in vacuum and around 5 μm in air [51]. The Lund Nuclear microprobe features a horizontal beam, which is targeted on cells in a fixed vertical holder using a magnetic beam steering system [50].

Cell recognition is performed by viewing nonstained cells with visible light and a transmission microscope [51]. Microscope objectives and a particle detector are mounted in a translation stage; particle counting is currently achieved by replacing the microscope objective with a surface barrier detector and detecting particles after they have passed through the cells. Work is underway on a thin silicon detector to be placed in the vacuum system upstream from the cells eliminating the current need for removal of the medium prior to irradiation [52].

IV. CONCLUSION

In recent years, the field of single-cell microbeam irradiation, particularly in terms of the number of facilities, has grown significantly. Radiation biology research at microbeam facilities

continues to flourish as fundamental studies of the interaction of ionizing radiation with cellular matter. With the ability to irradiate subcellular targets, microbeam facilities maintain a crucial role in understanding complex cell-response phenomena, such as, the bystander effect.

This paper considered the microbeam facility at the RARAF, Columbia University, as a model for discussing technical details, including: forming a micrometer-sized ion beam, imaging cell targets, and irradiation control. Supplementary aspects of single-cell microbeam technology were exemplified through reviewing facilities worldwide. Table I summarizes the features of the reviewed facilities. In addition to these facilities, numerous institutions are currently proposing or developing microbeam facilities to further the understanding of cellular response to radiation.

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