Development of a Charged Particle Microbeam for Single-Particle Subcellular Irradiations at the MIT Laboratory for Accelerator Beam Applications

DOE Project DE-FG07-981D13643

Final Report

Principal Investigator: J.C. Yanch

*Department of Nuclear Engineering and Whitaker College of Health Sciences and Technology,
Massachusetts Institute of Technology, Cambridge, M*

I. Introduction

The development of a charged particle microbeam for single particle, subcellular irradiations at the Massachusetts Institute of Technology Laboratory for Accelerator Beam Applications (MIT LABA) was initiated under this NEER award. The microbeam apparatus makes use of a pre-existing electrostatic accelerator with a horizontal beam tube. During year 2 of the award a 90° bending magnet was designed, purchased and installed in order to render the charged particle beam vertical. A dedicated endstation for biological irradiations was developed and installed above the bending magnet. The entire apparatus measures less than 4 m so that the microbeam can be completely housed in a single room. The He^{++} or H^+ charged-particle beam is delimited using either a slit or a single-hole collimator assembly. A system for particle counting will be performed below the cell dish via plastic scintillator in combination with two photomultiplier tubes (PMTs) counting in coincidence mode has been developed and tested off line. Control software, developed in-house, manipulates all aspects of the hardware including the accelerator, beam line components, vacuum systems and all subsystems of the biological endstation.

II. Accelerator and Beamline

The microbeam utilizes a 1.5 MV single stage electrostatic accelerator capable of generating a variety of particle types and charge states. The accelerator, designed by Newton Scientific Inc. (Cambridge, MA) is very compact, measuring only 1.8 m in length and 0.77 m in diameter (see Figure 1). The accelerator comprises an accelerating column that contains the accelerating tube, the high voltage power supply, and the high voltage terminal assembly including the ion source and associated electronics. The ion beam is continuously injected at low energy (15-30 keV) into the accelerating tube from the radio frequency ion source located in the high voltage terminal. The beam is accelerated and focused by the electrostatic field of the accelerating tube and will attain a final maximum energy of 1.5 MeV (single-charged ion) or 3.0 MeV (doubly-charged ions).

The particle beam emerging from the accelerator travels down a 1.2 m long beam tube. This beam tube houses x-y steering plates for beam deflection and a magnetic quadrupole triplet. In the microbeam's original configuration (a horizontal beam), this magnet was designed to provide

Figure 1. Photograph of entire microbeam system. Biological Irradiations are performed in the light-tight endstation (upper right) mounted immediately above the 90 degree bending magnet.

a highly-focused (1 micron) beam spot in vacuum for proton-induced x-ray emission (PIXE) and other surface analysis methods. Since a vertical beam was considered optimum for biological experiments, however, the focusing capability of the triplet is not utilized in the present configuration of the microbeam. Instead, this magnet is used simply to somewhat defocus the ion beam prior to its entry into a 90° bending magnet. The bending magnet serves two purposes. First, the ion beam now becomes vertical which allows cell dishes to be positioned in the horizontal orientation. This is desirable if irradiation times are long requiring some amount of medium to remain on the cells. Second, since the bending of ions in a magnetic field is a function of the charge on the ions, the magnet allows us to selectively transport the ions of choice into the vertical orientation. For instance, for the generation of helium beams, the magnet ensures the transport of 3.0 MeV He^{++} ions to the cell-irradiation endstation, and the rejection of 1.5 MeV He^{+} ions which would result in detection of a particle (via interaction with the plastic scintillator located below the cell dish) but only partial or no cell irradiation due to the limited range of these particles.

III. Cell Irradiation Endstation

Subsystems of the cell irradiation endstation are housed in a 49 x 49 x 31 cm³ light-tight box mounted immediately above the 90° bending magnet (see Figure 2). The cell irradiation endstation comprises the following subsystems: a collimator or slit assembly to delimit the beam, a 2-dimensional motorized stage, a plastic scintillator, light guide and two photomultiplier tubes for particle counting, a specially designed cell dish, UV objective with z-motion for focussing, dichroic mirror, and a CCD-camera for cell visualization. A light source is mounted on the outside of the endstation box; light passes from the source through a 3.2 cm diameter tube to interact with the dichroic mirror. The deflection plates, motorized stage, PMTs, light source and CCD camera are interfaced with a PC for computer control of all aspects of cell irradiation.

III.1 Slit and Collimator:

Beam delimiting is performed using either a slit or a single-hole collimator. The slit is a laser-drilled aperture (Lenox Laser Inc, Glen Arm, MD), 1.8 μm wide and 1 mm long drilled in 45 μm -thick stainless steel and glued to a 9.5 mm diameter mount. This mount can be removed

Figure 2 (a) Schematic illustration showing the subsystems comprising the biological endstation. (b) Photograph showing the inside of the biological endstation.

and the collimator assembly installed in its place. The collimator is made from 285 μm -diameter fused silica tubing with a 1.5 μm -diameter bore. Collimators are cut from the tubing in lengths of approximately 1 mm and inserted into a mounting assembly that also holds the single particle counting apparatus (light guide, plastic scintillator and two photomultiplier tubes). Efforts are underway to characterize the 'beam size' generated by the collimator in its mount using track-etch detectors (CR-39 and LR-115).

Characterization of the radiation pattern produced by the slit has been performed using specially fabricated radiochromic film and demonstrates a beam width of approximately 3 microns. This is described in more detail in section IV.

III.2 Particle Counting:

The particle detection system is located between the collimator and the cell substrate. This configuration allows detection of particles regardless of the thickness of medium covering the cells, and in addition permits experiments in which it is desirable to have the particle stop within the cell. However, given the relatively low energy of the ion beam, a very thin transmission type detector is necessary to minimize energy loss of the particles to a few hundred keV so that sufficient residual range remains for irradiation of the cell through the cell dish substrate. A thin plastic scintillator (Alpha Spectra, Inc., Grand Junction CO) has been chosen as it has a very fast decay time and is available in 5 micron thicknesses. To minimize false positive

Figure 3. The single particle counting system under development employs 2 photomultipliers collecting light created in a thin plastic scintillator optically coupled to a optical light guide.

and negative rates in the thinnest possible scintillator, co-incidence detection of scintillator photons has been adopted. Experiments performed to optimize light collection led to a design using a Lucite light guide into which 2 PMTs are positioned. The scintillator is optically coupled to a slight inset in the light guide using optical cement and the PMTs are coupled to the light guide using optical grease. The light guide, optical cement, scintillator, and optical grease all have similar indices of refraction. Signals from the PMT are fed first into pre-amps and amplifiers, then a timing single-channel analyzer and finally into a co-incidence analyzer. Investigations performed off-line with a low-activity alpha-source have demonstrated that total counting efficiency can be improved by coating the light guide with a reflective paint and by other strategies to enhance the coupling of the PMTs with the light guide and scintillator. Efficiencies of 96% have been attained and work is underway to improve this further.

III Microbeam Control System

Operation of a charged-particle microbeam requires a high level of computer control; the various components and subsystems that compose the microbeam must be coordinated and controlled by software that acts as the interface between the physical hardware and the user. Discussions with microbeam groups at Columbia University and at the Gray Laboratory (UK) lead to the conclusion that while commercial software for control of a microbeam apparatus is a quick solution, no off-the-shelf package provides all required functionality. In addition, the lack of access to source code from a commercial package restricts the ability to generate additional experimental capability. The decision was therefore made to develop the entire control system in-house.

III.1 Overview of Control Software:

The control software is responsible for controlling the entire functioning of the LABA microbeam including accelerator start-up and shutdown and manipulation of all beamline components including the subsystems of the endstation devoted to cellular irradiation. Written in

a proprietary language (Pyramid Technical Consultants, Waltham, MA) similar to C++ and running under the Windows NT operating system, the control software provides an interface within which parameters for the hardware can be both set and routinely monitored by the user. In addition, the software allows the user to input parameters necessary for specification of a particular cell irradiation experiment. A third function of the software is for automated protection

Figure 4: Screen capture illustrating the accelerator control portion of the control system. The user can navigate through the system using the buttons on the right side of the screen.

of the accelerator in the event of unexpected and unsafe increases in pressure or terminal voltage.

Overall control of the various subsystems is broken into logical groups with common functionality being grouped into distinct graphical user interfaces. Navigating through this

interface allows the user to constantly monitor the status of the accelerator, vacuum systems and magnets during all phases of the experiment. A distinct screen on the computer display is dedicated to each subsystem of the control software. For instance, the accelerator operation interface (see Figure 4) permits manipulation of several hardware elements within the high voltage terminal in order to power up the accelerator, adjust particle energy and vary beam

Figure 5: Screen capture identifying the graphical user interface of the LABA microbeam control system. Notice the results of the cell-finding routine as cell centroids are identified with a dot and abnormal objects are labeled with an X.

current. A screen devoted to the vacuum system displays pressures at various locations within the system and allows the user to change the status of vacuum pumps and gate valves. Another interface is used to set parameters associated with all steering and deflection plates as well as focussing and bending magnets. Accessing each interface is possible from any other via a single

mouse click. This multi-page approach allows the user to interact with a simplistic interface regardless of the complexity of the overall control system. Data important for the overall integrity of the system, such as pressures in the accelerator tank, the beamline and the roughing line, are displayed on all pages so that these can be continually monitored by the operator.

Lying underneath this graphical interface is a high level of automation and a network of safety interlocks, which are implemented to ensure both ease of use and the safety of components within the high voltage terminal. This automation is responsible for tasks such as tuning the bending magnet current to the energy of the charged particles so that it produces the exact magnetic field required to direct the horizontal particle beam vertically through the beam collimator.

Incorporation of automated interlocks also supplies a significant level of protection for the accelerator since user response times are not rapid enough to prevent catastrophic arcing due to increased pressures in the accelerator tank. If pressures in the accelerator exceed a given value, safety interlocks within the control software immediately respond by setting the terminal voltage to 0 thus avoiding an arc which could seriously damage or destroy the digital electronics housed within the high voltage terminal. Similarly, critical gate valves in the beam line cannot be opened unless required settings for the vacuum pumps meet specific conditions.

III.2 Cell Identification:

Cells to be irradiated are plated on specially constructed dishes comprised of thin polypropylene affixed to a stainless steel ring. After co-incubation with a fluorescent dye, the cell dish is attached to a 2-D stage and subsequently exposed to a fluorescent light source. A CCD camera observes the magnified fluorescent images of the cell dish, and a Matrox Meteor2 graphics board located in a Dell Optiplex Workstation captures the resulting video signal. The captured image is displayed on the computer screen and, using thresholding techniques, cells (and potentially other fluorescing objects) are identified. For each identified object, several parameters (pixel count, elongation, centroid position, etc) are logged, and averages of these various parameters are constantly maintained so that the routine can more accurately identify what is a

cell and what is debris or potentially the overlap of two or more cells. If the software identifies an object that deviates substantially from these averages, then the software requests that the user manually locate the center(s) of these abnormal objects using the mouse pointer (see Figure 5). Once all objects have been located, the 2-D stage moves the cell dish to the next position in preparation for another image acquisition. Successive image captures are intentionally overlapped slightly to ensure that subsequent concatenation within the control software does not miss or double count a cell that might be positioned at the edge of a given image. This routine is repeated until all cells on the cell dish have been located

Given the level of automation in the cell-finding routine, a built-in method of evaluating this routine's ability to accurately identify cells on the dish was developed. Using this method, the user can test the accuracy of the cell-finding routine against their own ability to locate cells on a given captured image, ensuring that each cell has been identified and also that no fluorescing debris is mistakenly labeled as a cell. This test can be repeated periodically for different users, cell types, fluorescent dyes, etc.

In the future, more automation and testing of this type will be implemented throughout the control software (see Figure 6). Once fully implemented, the software will present the user with a variety of experimental capabilities such as irradiating a predetermined percentage of all cells found on the dish or dividing the dish into a user-defined number of regions and irradiating only the cells within certain regions.

IV. Characterization of the Microslit Collimator for Subnuclear Irradiation

The precision that microslit irradiation provides is of key importance to radiobiological studies for a number of reasons: 1) it allows the experimenter to precisely control the amount of radiation deposited in a given cell, 2) it allows investigation into the distribution of radiosensitivity across a nucleus, permitting varying levels of radiation intensity, and 3) defined

subnuclear distribution of radiation dose allows visualization of damage repair protein localization and the time evolution of DNA damage repair¹.

Figure 6: Flowchart describing the control software and subsequent hardware communication used to automate the irradiation of cells with the LABA microbeam

750 keV protons were generated using the 1.5 MeV charged-particle accelerator at the MIT LABA. The microslit was constructed via laser drilling by Lenox Laser Corporation (Glen Arm, MD USA), and consists of a 45 micron thick stainless steel substrate with a 1.8 micron x 1mm slit in the center, mounted on a vacuum assembly. Normal Human Fibroblast cells obtained from the Radiation Oncology Department at Massachusetts General Hospital (Boston, MA USA) were maintained in Dulbecco's modified minimum essential medium with 4.50 $\mu\text{g}/\text{ml}$ glucose, supplemented with 10% fetal bovine serum (Sigma), 10 $\mu\text{g}/\text{ml}$ streptomycin and 10 $\mu\text{g}/\text{ml}$ penicillin (Mediatech CellGro), and 10mM HEPES Buffer (Hyclone).

IV.1 Cellular Irradiation:

Irradiation dishes were prepared using a custom-made stainless-steel tray, onto which a 4

micron thick polypropylene film was affixed. After assembly and UV sterilization, the film was treated with poly-L-lysine (Sigma) to enhance cell adhesion. Exponentially growing cells were trypsinized into single-cell suspensions and transferred to the irradiation dishes, such that the cells after adhesion would be 90-100% confluent. Cells were incubated for a period of 12 hours and then irradiated at room temperature (22°C). Irradiations lasted 5-30 min, depending on area

Figure 7: The LABA 'slit' collimator: schematic, electron micrograph, and photograph

Figure 8: LR-115 film irradiated using the slit collimator (40X; color adjusted).

irradiated and total dose delivered. At times ranging from 10 minutes to 2 hours after irradiation, cells were washed with 22°C 1X PBS and then fixed with 100% methanol on ice.

Beam dimensions at the midline of the irradiated cells were determined by extrapolating the aspect ratio of the slit (25:1) through the intervening distance and adding the contribution from radial straggle caused by interactions with the intervening material (1.4 microns of mylar, a conservative air gap, 4 microns of polypropylene, and the cells themselves) as modeled by the SRIM 2003 code.^{2,3} The calculated beam profile was determined to be 2.4 ± 0.18 microns, subsequently verified by irradiation of LR115 track-etch film.⁴

Dose was delivered in 2 micron swaths over 5-10mm lengths, such that multiple “stripes” were delivered over the area of the cell dish. Dose in the irradiated areas was approximately 300 Gy, while unirradiated areas received no dose. Dose was calculated using LR115 film to determine proton fluence and the SRIM 2003 code was used to determine dose per proton.

IV.2 DNA Damage Assay

To visualize DNA damage and the localization of the DNA damage response, immunocytochemical techniques were used. After fixation, cells were permeabilized for 5 min. Cells were incubated in blocking solution for 1 hr and then treated with anti-hMre11 (Oncogen Research Products) or with anti-phosphorylated histone H2AX (Trevigen) and a secondary FITC-conjugated antibody (Sigma) and then counterstained with DAPI. Images were captured under epifluorescence with a charge-coupled device camera and processed using Microsoft Photo Editor.

Treatment with anti-hMre11 allowed visualization of one of the DNA repair proteins associated with the repair of DNA double-strand breaks,^{5,6} and treatment with anti-phosphorylated histone H2AX allows for visualization of the DNA double-strand breaks in chromosomal DNA directly.⁷ Examination of the images obtained (see Figure 9) demonstrated the ability of the microslit in conjunction with the MIT LABA charged-particle accelerator to generate a pattern of radiation dose over a defined subnuclear volume. In the future, this

approach will be used to explore the distribution of radiosensitivity over the cell nucleus and to investigate the dose and time response of DNA damage repair proteins.

Figure 9.

V. Publications Arising from this Work

1. O'Meara J.M., Boisseau R.P., Dart A., Ledoux R.J., Nett W.P. and Yanch J.C. "Single-Particle Detection for the MIT Charged-Particle Microbeam" *Radiation Research* **158**(3), 372-373, 2002.
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3. Folkert M., Albritton J.R., Dart A., Ledoux R.J., Luo C.M., Held K.D. and Yanch J.C. "Design, Characterization and Application of a Charged-Particle Microslit for Subnuclear Irradiation" *Radiation Research* **161**, 100-101, 2004.
4. Albritton J.R., Folkert M., Ledoux R.J., Johnson E.D., Nett W.P., Boisseau R.P. and Yanch J.C. "Development and Testing of Control Software for a Charged-Particle Microbeam" *Radiation Research* **161**, 107-1008, 2004.

VI. References

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