Phase I: Development of a Charged Particle Microbeam for Targeted and Single Particle Subcellular Irradiation

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Introduction

The overall objective of this NEER project is the development of a system for high-resolution irradiation of cells. Our goal is to produce a charged-particle beam spot of roughly 1 micron which will allow dose delivery at the subcellular level. Single (or multiple) particle irradiation will also be possible, allowing us to experimentally duplicate the radiation environments of occupational and environmental exposures. In these situations virtually no cell receives more than one hit.

To develop this technology we are taking advantage of an existing proton accelerator and charged particle microbeam, originally developed for surface analysis via proton-induced x-ray emission (PIXE). To enable biological irradiations and long-term evaluation of biological consequences we are developing a specialized endstation to replace the existing PIXE hardware. This endstation will incorporate cell visualization capabilities, the capability of monitoring single hits and a long-term visualization system (camera and software) that will permit evaluation of the route of cell death following irradiation under different conditions.

Development of several subsystems has been undertaken during Phase I of this work. These include: i) investigation of materials to serve as both vacuum window and cell growth substrate, ii) scintillator and photomultiplier combinations for determination of single particle transversals, iii) testing of a helium source for alpha particle beam production and iv) implementation of computer control of the charged particle microbeam. In addition to these planned Phase I investigations, we also have investigated the feasibility and cost-effectiveness of incorporating a collimator-based irradiation system in addition to the focused-beam irradiation system planned for eventual installation. Each of these investigations is described below.

1. Objective: To investigate materials capable of simultaneously serving as vacuum window and cell growth substrate.

Accomplishments: Our current plan is to plate cells on a thin film that will also be used as the vacuum window between the microbeam and atmosphere. Charged particle detection (i.e. single particles) will be carried out via a scintillator (and PMT) placed after the cells. This will allow us to directly couple a large scintillator film with a PMT thereby obtaining 2π detection efficiency for the photons emitted by the scintillator. A window material strong enough to serve as a vacuum window for the accelerator and sufficiently large to allow a statistically significant number of cells to be plated is necessary. However, the limited range of the low energy protons requires a thin window in order to minimize energy and range straggling. Thus, the thickness of the window needs to be balanced with the desired cell plating area and to be able to hold vacuum.

A literature and world wide web search revealed several materials that were capable of holding vacuum over the desired area. High density materials such as metals were immediately eliminated because of the large degree of proton energy and range straggling. The search then focused on polymer plastic type materials and other low density materials. Polyimides and silicon nitrides were found to have the best material properties for this study in terms of maintaining strength over a sufficient area.

Window Strength and Suitability Calculations: Prior to testing the window under vacuum, rough calculations to determine the material's suitability as a vacuum window were performed. The Fermi National Accelerator Laboratory's guidelines for designing thin windows for vacuum vessels were followed.

The allowable stress on the thin windows is the more stringent of the following:

$S = 0.5F_u$ or $S = 0.9 F_y$, where S is the allowable stress (psi), as in the above equations, F_u is the ultimate tensile strength, and F_y is the yield strength of stress to produce five percent elongation (psi).

Using the Fermilab guidelines Mylar, Kapton, and silicon windows were evaluated over an area of 1.0 mm^2. The required minimum thickness for the three types of materials were also obtained.

The ion transport code TRIM was used to evaluate the energy straggling due to the windows at the calculated thicknesses. Mylar was found to be unsuitable because, at the minimum thickness determined above, the energy loss and percent energy straggling through the window was too large. The silicon and Kapton windows were more favorable.

The minimum thickness of Kapton is 1.1 μm. While Kapton is a relatively strong polyimide, API.3, a polyimide available from Moxtek, Inc. is available in a much smaller thickness (0.15 μm) and purported by Moxtek to be stronger than Kapton. The company has also the ability to custom make variable thickness material. Discussions with Moxtek engineers led to the conclusion that a 0.4 μm version of AP1.3 would maintain vacuum over the desired 1 mm^2 area. Proton energy and range straggling calculations demonstrated its superiority over either Kapton or silicon. However, the cost to create the necessary number of windows was considered too prohibitive and not worth (at least for our preliminary experiments) the reduction in energy straggling that could be achieved.

Cell Plating: V79 cells were chosen as the test cell line for initial experiments because of their relatively short doubling time (10 to 12 hours) and their ability to survive in relatively harsh conditions. In addition, their radioresponsiveness is well understood and documented. [Once the methodology has been fully developed we will also carry out the experiments using other cell lines.] Cell plating efficiency was determined with the materials that would eventually comprise the cell dish: Kapton window, adhesive and stainless steel mount. The three main components of the Kapton washers window were studied independently and in combination. The combined stainless steel, Kapton, and adhesive reduced the plating efficiency to 70% from 85% for a 60 x 15 mm tissue culture dish.

2. <u>Objective</u>: To investigate the feasibility and cost-effectiveness of implementing a collimation-based cell-irradiation system prior to full implementation of the focused-beam system.

<u>Accomplishments</u>: A significant drawback to the proposed approach of using a horizontal beam to irradiate cells is the necessity of maintaining the cell dish in a horizontal position. While this does not present insurmountable difficulties it does lead to the necessity of removing most of the growth medium from the cells prior to installing the dish on the microbeam and therefore to a shorter upper limit on total dish irradiation time. Maintaining horizontal geometry allows us to use the existing microbeam which is based on high precision focusing via a quadrupole triplet lens. We expect this approach to enable us to achieve a smaller beam spot than current biological microprobes which are based on beam collimation.

During Phase I we have investigated the feasibility of simultaneously developing two configurations, namely i) a straight through horizontal beam for high precision subcellular irradiation, and ii) a vertical beam obtained by bending the charged particles through a 90° bending magnet. Design, construction, and testing of an additional precision lens to refocus the non-vertical beam is beyond the financial scope of this project, however, we can take advantage of the vertical geometry for cellular irradiation by collimating the beam. A 1 μm diameter collimator will result in a few μm beam spots (due to penumbra effects). The bending magnet will also allow us to separate between helium ions of different charge states, as described below.

3. <u>Objective</u>: To determine if alpha-particle beams can be generated and transported.

<u>Accomplishments</u>: Helium gas was used in place of hydrogen or deuterium in the ion source and transported through the 4.1 MeV accelerator. Confirmation of alpha particles on target (as opposed to other particles) was carried out by bending the beam through a known angle using the multi-bore switching magnet. A beam current of 250 nA was measured on the beam stop.

This current is more than sufficient (by orders of magnitude) for single particle cellular irradiation.

The RF ion source on the 1.5 MeV machine will generate both $^4He^+$ and $^4He^{++}$. Generation of alpha particle beams of a single species with the 1.5 MeV accelerator is more difficult due to the straight line configuration of optical elements and the lack of an existing element to separate species on the basis of mass or charge. However, with our current plan of incorporating a 90° bending magnet, separation on the basis of charge state will be possible, and alternating between both helium species can be carried out.

While a 1.5 MeV proton has a range of approximately 46 μm in water, a 1.5 MeV alpha particle ($^4He^+$) will travel only 8.4 μm. This range is insufficient to allow particle transversal through cell dish, cell medium layer and scintillator. However, use of the double charged helium results in an increase in particle energy by a factor of two and a range in water of 18.4 μm. Our developments of the cell dish and particle detection combination therefore use 18 μm as the maximum cumulative thickness of materials.

4. Objective: To examine potential scintillator/photomultiplier tube configurations for single particle counting.

Accomplishments: Two different approaches for single particle counting were investigated. The first uses a thin plastic scintillator placed at the beam exit position, below the cell dish. In this configuration, charged particles pass through the scintillator first, then the cell dish and then the cell. Photons created in the scintillator pass through the cell dish and cell and then are detected via a photomultiplier tube. With this scenario a tradeoff must be made between maximizing light output and minimizing energy straggling (and loss) in a thicker scintillator.

The second configuration moves the scintillator to a position above the cells. Placing the scintillator directly on the PMT results in 2π efficiency and removes the restriction of using an extremely thin scintillator. The only potential drawback to this scenario is energy loss in air above the cells (we can place the scintillator/PMT very close to the cell dish) and potential difficulties in simultaneous cell viewing and particle counting. These drawbacks were felt to be minor compared to improved counting efficiency of this scenario. The Hamamatsu PMT has an extremely low dark current (60 cps) and is ideal for this application.

5. Objective: To implement computer control of the charged particle beam relative to a known x-y position in space.

Accomplishments: The first step in evaluating the quality of the beam and the position resolution of the electrostatic scanning plates was to implement computer control of the X-Y beam scan. High speed beam placement control has been added to the beam optics control system developed by Pyramid Technical Consultants. Since the scanning of the beam must also be coordinated with the data acquisition and general control system, it was decided that the most efficient implementation of the scanning and beam diagnostics would be through a dedicated Digital Signal Processor (DSP) that resided in one of the control systems PCs. The DSP chosen is a Bittware Blacktip processor based on a Analog Devices Sharc ADSP 21062 with a 200 kHz analog and digital I/O mezzanine. A software interface between this DSP and the general control system has been developed as part of Phase I. A preliminary version of the beam scanning control algorithms has been implemented on the DSP. An interface board is now being designed between the DSP I/O and the scanning plate amplifiers. Once this electronics is built, the first tests of the beam scanning will take place.