THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY (RARAF)

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An NIH-Supported Resource Center

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RESEARCH USING RARAF

The "bystander" effect - the response of cells that are not directly irradiated but are in close contact with, nearby, or only in the presence of irradiated cells, has been the focus for many of the biological studies at RARAF over the past two decades. Both the Microbeam and the Track Segment Facilities continue to be utilized in various investigations of this response to radiation exposure. This year the number of biological experiments investigating the mechanism(s) by which the effect is transmitted has declined somewhat, with newer experiments starting to use the Microbeam Facility to examine damage to sub-cellular structures (e.g., mitochondria, telomeres, and specific chromosomes) and other radiation effects. Research into bystander effects *in vivo* continued this past year with irradiations of the ears of mice.

Experiments

Listed in Table 1 are the experiments performed using the RARAF Singletron between January 1 and December 31, 2015, and the number of shifts each was run in this period. Fractional shifts are assigned when experimental

Table 1. *Experiments Run at RARAF January 1 - December 31, 2015*

Exp No.	Experimenter	Institution	Exp. Type	Title of Experiment	Shifts Run
110	Tom K. Hei	CRR	Biology	Identification of molecular signals of alpha particle-induced bystander mutagenesis	48.5
113	Alexandra Miller	AFRRI	Biology	Role of alpha particle radiation in depleted uranium-induced cellular effects	3
146	Michael Bardash	QEL	Physics	Development of a solid state microdosimeter	1
163	Lubomir Smilenov, Anna Saran	CRR/ENEA	Biology	Mouse ear irradiation of connexin deficient mice	2
164	Lubomir Smilenov	CRR	Biology	Mouse irradiation using IND spectrum neutrons	2
165	Helen Turner	CRR	Biology	Mouse/blood irradiation using IND spectrum neutrons	1
172	Susan Bailey	Colorado State University	Biology	Targeted telomeric damage and the persistent DNA damage response	2
173	Ekaterina Dadachova	Albert Einstein College of Medicine	Biology	Comparison of fungal cell susceptibility to external alpha particle beam radiation versus alpha particles delivered by ^{213}Bi-labeled antibody	3.5
174	Gordana Vunjak-Novakovic	Columbia University	Biology	Micro proton induced x-ray emission of bone/cartilege grown on artifical scaffolds	2
175	Constantinos Broustas	CRR	Biology	Mouse/blood irradiation using IND spectrum neutrons	1
176	Art Pallone	Norwich University	Physics	Development of CCD sensors for ion beam measurements and dosimetry	1
177	Kathryn Held, Hongning Zhou	Harvard Medical School/CRR	Biology	Induction of superoxides in bystander cells	1
178	Alejandro Carabe-Fernandez	University of Pennsylvania	Physics	Microdosimetric and radiobiological characterization of new Si-based microdosimeters using particle microbeams	2
179	John Ng	Cornell University	Biology	Effect of LET on immunotoxicity	2.5

time is shared among several users (e.g., track segment experiments) or when experiments run for significantly more or less than an 8-hour shift. Use of the accelerator for experiments was 37% of the regularly scheduled time (40 hours per week). Fourteen different experiments were run during this period. Four experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH), including the National Cancer Institute (NCI), the National Institute of Allergies and Infectious Diseases (NIAID) and the National Institute of Biomedical Imaging and Bioengineering (NIBIB). Eight experiments were performed by external users, supported by grants and awards from the Department of Energy (DoE), the Department of Defense (DoD), the NIH, the National Aeronautics and Space Administration (NASA), the National Science Foundation (NSF), the National Cancer Institute (NCI), and internal funding from the Georgetown University Department of Radiation Medicine. Two experiments were collaborations between RARAF/CRR staff and outside users. Brief descriptions of these experiments follow.

A group led by Tom Hei of the CRR continued experiments investigating the effects of cytoplasmic irradiation and the radiation-induced bystander effect (Exp. 110). Using the Microbeam Facility, Jinhua Wu investigated mechanisms by which cytoplasmic stimuli modulate mitochondrial dynamics and functions in human small airway epithelial cells (SAECs). Their recent studies have shown that mitochondrial fragmentation induced by targeted cytoplasmic irradiation of human SAE cells is mediated by up-regulation of dynamin-regulated protein 1 (DRP1), a mitochondrial fission protein. To further explore the role of mitochondria in modulating the biological activities of high-LET radiation, autophagy in SAECs was examined. Autophagy was observed as early as 30 minutes after cytoplasmic irradiation with 10 alpha particles and peaked at 4 hours based on LC3B punctae formation. Sequestration of free radicals by DMSO abolished the induction of LC3B punctae formation, suggesting that activation of autophagy is free radical-dependent. Autophagy led to an increase of γ-H2AX foci that was dramatically reduced by choloroquine (CQ) or 3-methyladenine (3-MA), which are known inhibitors for autophagy. The DRP1 inhibitor mdivi-1 also significantly reduced autophagy, indicating that it plays a key role in activation of autophagy. DRP1 knockout HCT116 cells showed little or no autophagy after cytoplasmic irradiation, further confirming its role in autophagy induction. DRP1-dependent up-regulation of autophagy-initiating protein beclin-1 was also observed. Finally, a sustained activation of ERK was detected, suggesting potential involvement of the non-canonical MEK/ERK pathway in regulating autophagy in cytoplasmic irradiated cells.

Alexandra Miller of the Armed Forces Radiobiological Research Institute (AFFRI) continued studies using the Track Segment Facility to evaluate depleted uranium (DU) radiation-induced carcinogenesis and other late effects using *in vitro* models and to test safe and efficacious medical countermeasures (Exp. 113). One objective of this study has been to determine if phenylbutyrate (PB), a histone deacetylase inhibitor and epigenetic effector, can mitigate neoplastic cell transformation induced by different qualities of radiation, and if so, to identify which adverse epigenetic mechanisms are involved and potentially reversed by PB. This also would be of interest for Space missions and alpha particle exposures from accidental releases. Track segment irradiations with ^{4}He ions were performed on human small airway epithelial cells (SAECs) and growth rate, transformation, and genomic instability were quantified. Irradiation of SAECs overcame contact inhibition and caused an increase in transformation frequency and induction of gene amplification, i.e., genomic instability. Treatment with PB following irradiation resulted in a significant suppression of transformation frequency and gene amplification. Studies are ongoing evaluating the impact of PB treatment on changes in DNA methylation caused by irradiation with ^{4}He ions.

In a second part of the study, rodent bone marrow stromal cells were irradiated and co-cultured with unirradiated hematopoietic progenitor cells (FDC-P1). The FDC-P1 cells were monitored for their ability to grow in agar to assess neoplastic transformation. The data have demonstrated that co-culturing irradiated bone marrow stromal cells with FDC-P1 cells causes an increase in neoplastic transformation of FDC-P1 cells that involves the process of cell-cell communication. Additional mechanistic studies have shown that antioxidant processes are also involved in the non-targeted effect in FDC-P1 cells. Further studies with this model are ongoing, and are evaluating the involvement of non-targeted effects in the response to multiple exposures at low doses (5 cGy).

Tests of a solid-state microdosimeter were made by Michael Bardash of QEL, Inc. He has designed and constructed an electronic device with an active area of a few μm^2 and a thickness of less than one μm, on the order of the dimensions of a cell nucleus. The Track Segment Facility was used to irradiate the device with 4 He ions, which, because of their high LET, would deposit enough energy in the very thin device to make a measurable signal. While results were inconclusive, Michael will be returning in 2016 to continue the experiments with improved devices.

We have expanded the mouse ear irradiation protocols this year with a collaboration with Anna Saran of the Laboratory of Radiation Biology and Biomedicine at ENEA, Rome, using mice that are deficient in connexin 43, which she has developed. Using a mouse ear model, we have shown bystander effects induced by microbeam irradiation in an intact living mammal (Figure 1;

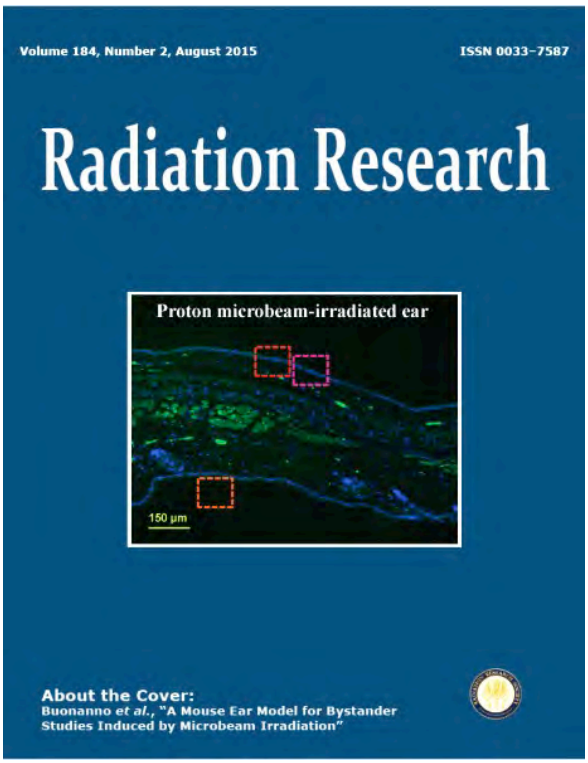

Figure 1. *Publication of in vivo microbeam studies on the mouse ear featured on the cover of Radiation Research. Reproduced here with permission from the journal.*

Buonanno, M., et. al. A Mouse Ear Model for Bystander Studies Induced by Microbeam Irradiation, *Radiat Res.* 2015 Aug;184(2):219-25.) Recent findings suggest gap junctions, which are communicating channels between adjacent cells, may be involved in the in vivo bystander effect and that certain proteins in gap junctions called "connexins" are required. Specifically, connexin 43 (cx43) was identified as critical to the radiation induced bystander effects: knocking out cx43 blocks the bystander effect in vitro. However, knocking out cx43 is embryonically lethal in mice. Dr. Saran has established a mouse model in which one of the two cx43 alleles is defective, leading to substantial reduction in gap junction function. Unlike the connexin 43 knockout mice, these mice are viable and fertile. The $cx43^{+/-}$ mice and their wild type counterpart were shipped from Rome and delivered to RARAF. We followed our mouse ear microbeam irradiation protocol, in which only selected cells of the ear are irradiated with a 30-micron diameter proton microbeam to create clear regions of irradiated and non-irradiated tissue. Thirty minutes after the exposure, both irradiated and non-irradiated ear tissues were examined for evidence of specific radiation-induced biomarkers of DNA damage (i.e. γH2AX foci formation). As expected, irradiation of a small number of cells of the mouse ear elicited a bystander response in both wild type mice and $cx43^{+/-}$ mice, however in the latter, the extent of the response was significantly less.

Lubomir Smilenov, Helen Turner, and Constantinos Broustas made use of our improvised nuclear device (IND)-spectrum neutron irradiation system to study the effects of the IND-spectrum irradiation of mice and blood samples. This work is supported by the Columbia Center for High-throughput Minimally-invasive Radiation Biodosimetry as a NIAID-funded Center for Medical Countermeasures against Radiation. Mice were irradiated with up to 2 Gy of neutrons, with comparison mice given up to 4 Gy of x-rays using the Westinghouse orthovoltage x-ray system at RARAF. Some mice were also given 1 Gy of neutrons and then a secondary dose of x-ray to simulate a mixed field. The mice were sacrificed 1 to 7 days after the exposure, and blood was collected and either scored for micronuclei and γH2AX foci, or processed for global RNA expression measurements. The animals were also held in metabolic cages for collection of urine and feces, which were processed for metabolomics. Human donor blood samples were also exposed to up to 2 Gy of IND-spectrum neutrons and 4 Gy of x-rays and assessed for the same endpoints as the mouse blood.

Susan Bailey from Colorado State University works on the effects of telomere length and damage on the health and viability of cells. She makes use of the RARAF microbeam to target and irradiate telomeres in the cells. The work performed this year focused on telomere degradation following targeted nuclear irradiation. The experiment also was used by the RARAF staff as a baseline test for the imaging of telomeres using the labels of interest to Dr. Bailey with the new super resolution microscope, as that facility will become available early next year.

Ekaterina Dadachova at the Albert Einstein College of Medicine, working with Igor Shuryak of the CRR, has been developing radioimmunotherapy (RIT) for treatment of *Cryptococcus neoformans* infections using ^{213}Bi-labeled antibodies specific to the cryptococcal capsule. She is performing a comparison of fungal cell susceptibility to external α-particle beam radiation versus α particles delivered by the bismuth-labeled antibodies (Exp. 173). Fungi grown to stationary phase in defined minimal medium were suspended in solution. As has been done in past experiments, the solution was formed into a thin layer with a known uniform thickness under a cover slip. The fungi were irradiated with doses of 1 to 80 Gy of 125 keV/µm ^{4}He ions. Results so far indicate that: a) *C. neoformans* is more sensitive to external beam α particles than to external γ rays; b) α particles delivered by the capsule-binding antibodies may be more cytocidal to the *C. neoformans* cells than external beam α particles. This work has expanded in the past year to include proteomic, transcriptomic and metabolomic research into the radioresistance seen in these fungi.

Gordana Vunjak-Novakovic uses our charged particle microbeam facilities for PIXE analysis of cartilage-bone interfaces looking at chemical compositions of the two materials as they interface and progress through the life cycle. The change of concentration of calcium in both

materials through the development of arthritis is of high interest in arthritis care and prevention. This past year, the neutron microbeam line has been modified to allow for this work to be performed at that endstation. This allows higher beam currents on target for more rapid data acquisition. Samples from both sacrificed animals and laboratory constructs on artificial scaffolds are being employed in these studies. The design of the artificial scaffolds could lead to the ability to grow in the lab bone and cartilage replacements from a patient's own stem cells for joint reconstruction and repair.

Art Pallone, working with Alan Bigelow, made use of our microbeam to test CCD sensors as potential alpha particle detectors for ion beam optics and parameterization. Using a pixelated detector, with suitably sized pixels and energy resolution, it is possible to determine the size and particle energy of a charged particle beam. Dr. Pallone is interested in the potential to use simple web cam CCDs as radiation sensors for low cost teaching opportunities and experimental platforms.

Kathryn Held, working with Hongning Zhou of the CRR, used the microbeam facility for gathering preliminary data on the induction of superoxides in bystander cells at early times following irradiation (5-20 minutes). These experiments were successful and we hope Dr. Held will return in 2016 for additional experiments.

Dr. Carabe-Fernandez of the University of Pennsylvania is developing silicon 3D radiation microsensor arrays, capable of quantifying deposited energies within micron-sized targets. Compared to traditional tissue equivalent proportional counters (TEPCs), these detectors do not require a gas supply, operate at low voltages, are light and easily portable, and have a fast response. The goal of this project is to use the targeting ability of the microbeam to characterize individual microsensors within the microdosimeter array. Different microdosimeters of different dimensions (diameter, depth and pitch) representing different cell types will be exposed, and the derived relative biological effectiveness (RBE) from mechanistic biophysical models (e.g. Microdosimetric Kinetic Model (MKM) and Local Effect Model (LEM) will be calculated. The experimental RBE obtained from clonogenic assays of individual cells exposed to the microbeam will also be obtained and compared to that obtained from the microsensors. This will allow the investigators to: 1) characterize the microdosimetric properties of each individual microsensor as well as study crosstalk between the sensors in an array; 2) validate the microsensors as viable instruments to calculate RBE; and 3) determine new features required to develop current microsensor technology to a new generation that allows more precise RBE measurements.

John Ng began preliminary work on the effects of charged particles at different LETs (10 – 50 keV/μm) on immunotoxicity in cancer cell lines. This exploratory work builds off his experience in clinical cancer

Figure 2. *Superconducting solenoid installation. A) Dr. Harken and Mr. Farrell positioning the bottom field restricting plate. B) Delicately lifting the solenoid down to the second mezzanine level at RARAF. C) Drs Randers-Pehrson and Bigelow positioning the solenoid on the restricting plate and table. D) Alignment of the solenoid to the bottom restricting plate. E) Final testing assembly of the solenoid with both restricting plates, cooling lines, monitoring sensors, current lines, and vacuum support connected.*

treatments using targeted particle therapy as one of the main irradiation protocols. The aim is to determine effects of targeted radiotherapy that can be combined with immunotherapy to increase the efficacy of treatment of many types of cancers. The results are promising and John anticipates continuing this project in 2016.

DEVELOPMENT OF FACILITIES

Development continued on a number of extensions of our irradiation facilities and capabilities for imaging and irradiating biological specimens:

- Focused particle microbeams
- Focused x-ray microbeam
- Neutron microbeam
- IND-spectrum neutron source

- Advanced imaging systems
- Targeting and manipulation of cells
- New cell analysis tools
- Small animal systems

Focused particle microbeams

The electrostatically focused microbeam was consistently operated with a 1-2 μm diameter beam and a 0.5 μm diameter beam when called for by an experiment. We have continued the protocol of performing a test run of the microbeam system on the evening preceding an irradiation day. These test runs have become a vital point of development for new techniques and training of operators for the microbeam system.

The Super-Microbeam development continued with the design and purchase of the super conducting solenoid magnet from Cryomagnetics, Inc. We took possession of the solenoid this year and performed verification and field-testing of the magnet (Figure 2). The construction of the Stimulated Emission Depletion (STED) super resolution microscope extension continued with the integration of the laser introduction pathway for the STED depletion laser co-alignment. The interface of the STED development with the multi-photon microscope was begun with a low power alignment laser. The 2W continuous wave (CW) high power laser was purchased and will be installed for imaging in early 2016.

The permanent magnet microbeam (PMM) was used as a secondary charged particle microbeam endstation for the development of our Flow and Shoot (FAST) microfluidic irradiation system, the capillary electrophoresis (CE) system, and the automated cell picking system. The PMM has all of the irradiation capabilities of the electrostatic microbeam except the sub-micron beam spot size. The PMM is also free from the electrical breakdown potential from failures of the vacuum window, making it an ideal initial testbed for all our new technologies.

Focused x-ray microbeam

The x-ray microbeam uses characteristic Ti Kα x rays (4.5 keV) generated by proton-induced x-ray emission (PIXE). PIXE produces a nearly monochromatic x-ray source (extremely low bremsstrahlung) of the characteristic target x-ray energy. This allows these x-rays to be focused using a Fresnel zone plate to a spot size of 5 μm from a proton beam size of ~50 μm in diameter.

The x-ray microbeam is stationed on a dedicated horizontal beamline at RARAF with the x-ray beam focused up in the vertical direction with the same microscope and stage geometry of the charged particle microbeam systems, allowing for easy intercomparison between the microbeam types. While minimally used this year, the x-ray microbeam remains available for users.

Neutron microbeam

The neutron microbeam at RARAF is the world's first microbeam that can irradiate single cells with neutrons. Incident protons near the reaction threshold (1.881 MeV) of the $^{7}Li(p,n)^{7}Be$ reaction generate neutrons that are severely forward coned in the laboratory frame of reference. By placing the target cells close to the lithium target it is possible to limit this cone to a single cell target. The Neutron microbeam uses a proton beam at 1.886 MeV focused to 8 μm on the lithium target. This results in a neutron spot size at the cell targets of 20 μm diameter with neutron energies ranging from 10-50 keV and a dose rate of 27 mGy/min.

The neutron microbeam is located in the accelerator bay at RARAF on a dedicated horizontal beam line. The proton beam is focused using a single quadrupole quadruplet with the spot size measured using an ionization counter and a knife-edge occlusion measurement. The center of the proton beam, visualized using a thin scintillator, is the center of the neutron beam. The proton beam measurements are made with a thin Havar metal window, which is exchanged with the lithium target for diagnostics.

The neutron spot size is measured using CR-39 track-etched plastic coated with a thin layer of lithium carbonate heavily enriched with ^{6}Li. The neutrons interact with the ^{6}Li through the $^{6}Li(p,\alpha)^{3}H$ reaction, producing energetic α and ^{3}H recoils that are easily observable as pits in the etched CR-39 using a microscope.

IND-spectrum neutron source

The improvised nuclear device (IND)-spectrum irradiator was completed in 2014 and was used extensively this past year to irradiate both whole blood and small animals.

This fast neutron irradiation source was designed to generate the neutron spectrum seen from the "Little Boy" atomic bomb at Hiroshima at 1.5 km from ground zero, as representing an energy spectrum and distance of relevance to detonation of an IND. This field is generated through the reactions $^{9}Be(d,n)^{10}B$ and $^{9}Be(p,n)^{9}B$ using a mixed beam of monoatomic, diatomic and triatomic protons and deuterons. The RARAF Singletron uses a gas mixture of hydrogen to deuterium of 1:2, which feeds into the RF plasma ion source. This irradiator is on the 0º beam line, as any bending of the beam to get to a target would separate the six different beams and prevent the spectrum generation.

The neutron spectrum was verified using two proton recoil detection systems. A 2" diameter 2" thick liquid scintillator for energies >1 Mev and a 1.5" diameter spherical gas proportional counter with 3 atmospheres of hydrogen gas for <1 MeV. Using MCNPX-PoliMi Monte Carlo simulations to calculate the exact response functions of the detectors, it is possible to reconstruct the spectrum from the readout of the detectors in the neutron field.

The dose rate has been calibrated to deliver 0.25 Gy of neutrons in 10 minutes (with a gamma-ray contribution of

an additional 1%). This dose rate allows the delivery of 1 Gy in less than 1 hour.

Advanced imaging systems

We continue to develop new techniques to obtain two- and three-dimensional images of cells, reduce UV exposure and improve resolution.

Real-time imaging

Short-term biological effects that happen within seconds to the first few minutes after irradiation set the stage for later effects. Real-time imaging and observation of the short-term effects will give insight to experimenters into their endpoints. Techniques have been developed using our EMCCD camera and our fast switching SOLA LED light source to acquire images with several frames per second to observe the short-term effects of irradiation on a timescale of minutes to hours following irradiation.

Multi-photon microscope with the UV microspot

The multi-photon microscope was developed several years ago and integrated with the charged particle microbeam irradiator. This microscope, through the long wavelength incident laser, allows in depth imaging of 3D tissues and small animals, such as *C. elegans* and zebrafish embryos. This is achieved using the sectioning capability of the multi-photon effect where the photon density increases to generate constructive interference producing a 3D voxel of photons with half the wavelength and twice the energy, which can locally excite fluorophores and/or other fluorescent effects (e.g. auto fluorescence and second-harmonic generation). This 3D voxel is then scanned through a single layer and stepped through the sample using the nanoprecision z-stage, generating a stack of 3D slices of the sample that are reconstructed into 3D images.

If the intensity of the laser is increased, there can be a 3-photon interference at the area of constructive interference, resulting in a voxel with 1/3 of the wavelength (and three times the energy), generating a voxel of UV light that is used as the UV microspot. The UV microspot can induce localized damage within a 3D target.

STED

We are developing a Stimulated Emission Depletion (STED) super resolution microscope system with optical resolution of 75 nm in combination with our super microbeam to achieve compatible imaging resolution and beam spot size. The STED system at RARAF builds off the multi-photon microscope using it as the primary excitation laser. A second CW laser is added in parallel with the multi-photon laser. Using polarization optics, the second laser projects a donut shaped point spread function around the excitation spot of the multi-photon system. With proper selection of the second laser wavelength and sufficient intensity, the second laser will deplete the fluorescent states around the excitation spot allowing emission of fluorescence only from the center of the donut, which will be reduced to nanometer sizes.

The use of STED imaging on live cells using a water immersion lens was shown to be possible using the STED super resolution microscope at Dr. Liao's Lab in the Mechanical Engineering Department. This system, while not optimized for water immersion optics, gave a resolution of <100 nm.

The STED development continues on the microbeam endstation. The second laser introduction pathway has been completed and the initial co-alignment of the STED laser beam with the multi-photon laser has been performed. We have purchased the 2W CW laser for the STED imaging system. It will be installed in early 2016, at which time imaging tests will commence.

Targeting and manipulation of cells

We have the capability to fabricate microfluidic devices in hard plastics, such as acrylic, and soft plastics, such as polydimethylsiloxane (PDMS). The micro-milling machine installed at RARAF has software to produce parts designed using the Solid Works computer-aided design (CAD) program. This system has been used to manufacture the single-cell dispenser and the microfluidic chips for the cell sorter and microFACS systems (described below). Several new microfluidic systems are being developed to target, manipulate, and analyze cells.

FAST

The Flow And ShooT (FAST) microfluidic irradiation system has continued in development. This system flows non-adherent cells, such as lymphocytes, though a microfluidic channel over the microbeam window, where the Point and Shoot magnetic deflector tracks, targets and irradiates the cells as they pass the beam location.

We have modified the design of the FAST chip to allow more reliable and convenient incorporation into the microbeam endstation. Previously, we had tested various all-PDMS or PDMS adhered to thin plastic foil designs and found them to be unreliable. In particular, it was difficult to reliably connect them to other devices (such as a syringe pump or a cell dispenser) while providing clearance for an objective lens and particle detector above and the beamline below.

The new design (Figure 3) involves two silicon pieces that have been selectively etched to leave regions of 1000 nm thick silicon nitride supported in a silicon frame (similar to the silicon nitride exit windows already used for microbeam irradiations). A layer of adhesive, approximately 50 microns thick, is patterned on top of one of the silicon pieces to create a flow channel across the window. The other silicon piece is flipped over to attach to the first chip and act as a lid to the channel. Proper alignment of the windows in each of the two silicon pieces with the defined flow region patterned out of the adhesive creates an ultrathin channel for imaging

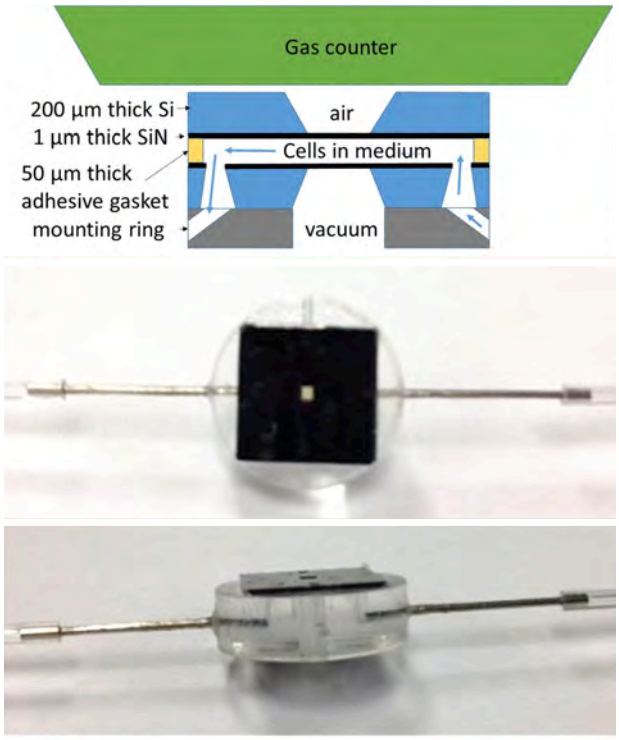

Figure 3. *New design for the FAST microfluidic irradiation system shown in schematic (top), top view (middle) and side view (bottom).*

and irradiation. This design allows for the bottom silicon piece to act as the microbeam exit window for maximum accuracy of the point and shoot irradiations. Furthermore, the thin microfluidic environment has fluidic access ports that integrate into a custom designed snout to eliminate fluidic connections on the top of the chip. The channel and the SiN layers are thin enough for particles to pass completely through the chip and into the gas ionization counter mounted on the microscope objective. This new design has been successfully tested with a wide range of flow rates and has shown good performance with the existing Flow and Shoot irradiation setup.

Cell picker

We have incorporated a semi-automated cell picker into the Permanent Magnet Microbeam endstation. Joystick control of the picker has been integrated into the microbeam software, allowing the user to select an individual cell and remove it from the microbeam dish post irradiation.

Cell dispenser

Development of the automated cell dispenser has continued with a focus on combining the sensing ability, the dispensing mechanism, and controlled fluid flow into a single device. Much of the work up to this point has been in developing each of these separately but many challenges were faced in combining these abilities into a single device. The current iteration of the dispensing chip combines the superior sensing properties of gold electrodes on a glass surface with a polymethyl methacrylate (PMMA) structure that can be machined to stably hold a high-pressure solenoid valve. A layer of double-sided adhesive is patterned and placed between the glass layer and the PMMA layer to define the fluid flow channel while also holding the assembly together.

A key design change in the past year has been in turning the dispensing direction so that it is perpendicular to the horizontal fluid path. The new design dispenses a droplet through the glass slide in a vertical direction using a hole drilled through it, which makes accurate droplet placement easier. The detection mechanism, involving sensing impedance differences across a set of three electrodes, remains the same as in previous versions and is still used to trigger a dispensing event. Work continues with testing for consistent droplet volume and accurate droplet dispensing location. With the improved design we are able to begin testing our ability to reliably dispense a single cell.

MicroFACS

The microfluidic Fluorescence-Activated Cell Sorting (microFACS) system has continued development to combine flow cytometry and sorting with our other microfluidic irradiation and dispensing technologies.

The microFACS system uses Dean vortex drift flow focusing to entrain the samples into a sheath flow focused column for flow cytometry detection in the main channel. The sample is illuminated with a laser through fiber optic coupling with the fluorescent output also detected through fiber optic coupling. The combination of fiber optics and microfluidics will allow for the microFACS to be coupled to the other microfluidic systems in close proximity to the microbeam endstations.

Computerized data acquisition and scoring were integrated into the microFACS during 2015. While still being optimized, this has allowed better quantification of the quality of the data being generated in the microFACS system, and represents a significant advance toward accurate scoring of samples. The design of the flow system to generate lower speeds to match the FAST irradiator and other systems also continued. The channel sizes and length of curved sections for Dean vortex drift flow focusing were adjusted as results of output speed and scoring quality tests dictated.

AMOEBA

An automated system for precise regulation and control of environmental conditions for biological samples before, during, and after microbeam irradiations, our Automated Microbeam Observation Environment for Biological Analysis (AMOEBA) system will establish, maintain and change conditions (e.g. temperature, pH, pCO_2, drug concentrations) in the culture as our microbeam users require. This system will provide feedback for automated fluid-flow control systems for all of the needed parameters through a distributed electronics control packaged.

The AMOEBA is being designed in two comparable systems: the standard AMOEBA for dish based microbeam experiments and the μAMOEBA for microfluidics based irradiation experiments. Initial testing has verified that we can control temperature (37 ± 1 °C) and pH over long periods with minimal feedback circuitry. We have begun using these basic controls for monitoring cell kinetics involving DNA repair, cell-cycle progression and chromosomal domain dynamics.

New cell analysis tools

CE-LIF

We have finished construction and begun testing of our Capillary Electrophoresis – Laser Induced Fluorescence (CE-LIF) system (Figure 4) to provide our users with the capability of measuring reactive oxygen species within individual cells immediately after irradiation. The nanoliter input volumes make this system ideal for single-cell, small-scale biochemical analyses.

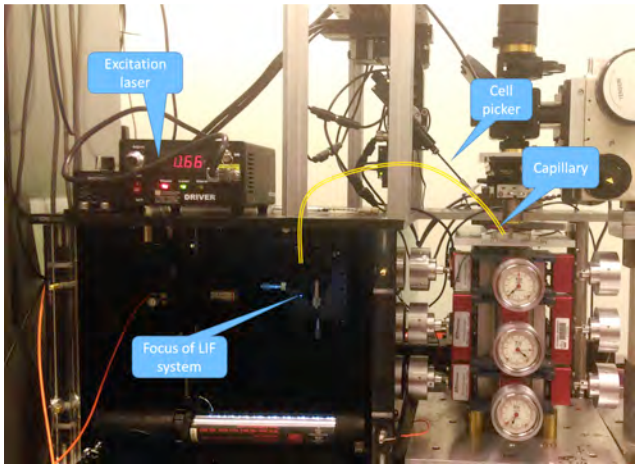

Figure 4. *The current CE-LIF system at RARAF.*

The CE-LIF system at RARAF (Figure 4): The grounded end of a 50 μm bore capillary is brought to the cell using the semi-automated cell picker. Once a cell is aspired into the capillary, 20-30kV is applied between the grounded end of the capillary and the Laser Induced Fluorescence (LIF) system, enclosed in a light tight insulating box. This results in two superimposed flow modalities experienced by the analytes: (1) Electrophoretic flow, responsible for separating the analytes by charge and Stokes radius; (2) Electroosmotic flow, which drives the buffer and analytes (regardless of polarity) toward the detector. The electroosmotic flow is much stronger than the electrophoretic flow, ensuring that all analytes will reach the detector. In the LIF system, the analytes are hydrodynamically focused into the path of a laser, with the light collected perpendicularly and detected by a high-sensitivity spectrometer. We have recently acquired a deep cooled Bayspec spectrometer, providing highly sensitive detection of fluorescent molecules.

Small animal systems

Investigations of radiation-induced bystander effects have been conducted in cell cultures and 3-D systems *in vitro*. The next logical step was to develop and implement microbeam irradiation protocols to study effects in living organisms. We have developed a mouse ear model for *in vivo* bystander studies. With an average thickness of 250-300 μm, this model can be used to investigate radiation-induced bystander effects with a 3-MeV proton microbeam having a range of 134 μm.

Using gentle suction, the ear of an anesthetized mouse is flattened onto the underside of a flat plate of a custom-made holder. The flattened mouse ear is then placed over the microbeam port and cells along a line on the ear are irradiated with the proton microbeam. At chosen times after irradiation, mice are sacrificed and a punch of the ear is collected. Tissues are then fixed, paraffin-embedded and cut in 5-μm sections perpendicularly to the direction of the line of irradiation. The sections are then analyzed for biological endpoints (i.e., formation of repair protein foci, apoptosis) as a function of the distance from the irradiated line.

SINGLETRON UTILIZATION AND OPERATION

Table 2 summarizes accelerator usage for the past year. The nominal Singletron availability is one 8-hour shift per weekday (~248 days per year), however the accelerator is frequently run well into the evening, often on weekends, and occasionally 24 hours a day for experiments or development. Total use for experiments and development this year was 57.5% of the regularly available day shifts.

Accelerator use for radiobiology and associated dosimetry was about 125% that for last year and right at the average for the last 5 years. About 66% of the use for all experiments was for charged particle microbeam irradiations, 22% for track segment irradiations, and 12% for neutron irradiations. Approximately 26% of the experiment time was for studies proposed by external users, and 74% was for internal users.

On-line facility development and testing was about 20.5% of the available time, primarily for development and testing of multiple microfluidic and analysis tools using the PMM endstation. This was about 20% less than

Table 2. *Accelerator Use, January 1 - December 31, 2015*
Normally Scheduled Shifts

Radiobiology and associated dosimetry	35.5%
Radiological physics and chemistry	2.5%
On-line facility development and testing	19.5%
Safety system	2.5%
Accelerator-related repairs/maintenance	0%
Other repairs and maintenance	2.5%
Off-line facility development	54%

the average over the last five years and slightly less than last year due to an emphasis on development not requiring accelerator use and previously developed systems coming into online use.

The accelerator was not opened during the calendar year of 2015. The accelerator was last opened in October of 2014 when the ion source was changed. We anticipate this will need to be performed again in early 2016 and will be timed to coincide with the reconfiguration of the microbeam.

TRAINING

REU

Since 2004 we have participated in the Research Experiences for Undergraduates (REU) project in collaboration with the Columbia University Physics Department. This is a very selective program that attracts highly talented participants. For 9-10 weeks during the summer each student attends lectures by members of different research groups at Nevis Laboratories, works on a research project, and presents oral and written reports on his or her progress at the end of the program. Among other activities, the students receive a seminar about and take a tour of RARAF.

The 2015 REU participant at RARAF was Leah Turner from Lehigh University. Leah worked with David Welch to fabricate a new generation of anatomically accurate mouse phantoms for use in radiation dosimetry studies (Figure 5). Along with the construction of the phantoms, Leah also helped with preliminary studies to analyze the performance of the phantoms using the Small Animal Radiation Research Platform.

Group Training

In addition to training individuals at RARAF, staff members also participate in training courses presented at other facilities as a means of introducing microbeam concepts and experiments to a broader audience. As for the past two years, Gerhard Randers-Pehrson lectured on "High/low LET microbeams" at the NASA Space Radiation Summer School, Brookhaven National Laboratory, Upton, NY, on June 11, 2015.

Figure 5. *Anatomically correct mouse phantom made by our REU student Leah Turner.*

Microbeam Training Course

In 2015, we postponed our Microbeam Training Course for one year in anticipation of the microbeam renovation that began for the replacement of the electrostatic microbeam with the superconducting solenoid Super Microbeam. We will resume the Microbeam Training Course in May of 2016

DISSEMINATION

Web site

The RARAF website design that was created in 2013 provides clear and effective presentation while improving access to content. Functional menus (including a home page rotating-picture menu) were designed to make navigation through the content easy and interesting, with a hierarchical structure from general information, suitable for a general or non-science audience, to more-detailed technical content.

The site contains information on microbeams in general, as well as detailed technical information on our various microbeams; *in-vitro* and *in-vivo* endpoints that we use; details of available on-line and off-line imaging capabilities; microfluidic systems we are developing; other charged particle and neutron irradiation facilities available at RARAF; our on-line training course materials; publications lists; information on RARAF contacts and directions to the facility. The site is periodically updated to include new radiation facilities, cell handling and analysis capabilities, publications and other information.

Virtual training course

We have developed an on-line virtual microbeam training course, based on the three-day microbeam training courses. This on-line course was designed to give interested physicists and biologists who could not attend in person a thorough introduction to microbeam technology.

The goal of the online course, as for the face-to-face course, is to facilitate a better understanding of how microbeams work, what experiments can be performed using a microbeam, why these experiments are of biological interest, and how to design / perform these experiments.

The on-line material consists of audio podcasts and the same handouts that the face-to-face students received. The audio of each podcast is synched with the accompanying PowerPoint slides (viewable on a video iPod, tablet, PC or Mac, or smart phone), as well as a PDF version of the slides. High-resolution video (720p, with audio) was also used to document demonstrations of all aspects of a microbeam experiment, from making microbeam dishes to irradiating cells and performing online analyses. After extensive editing, this resulted in

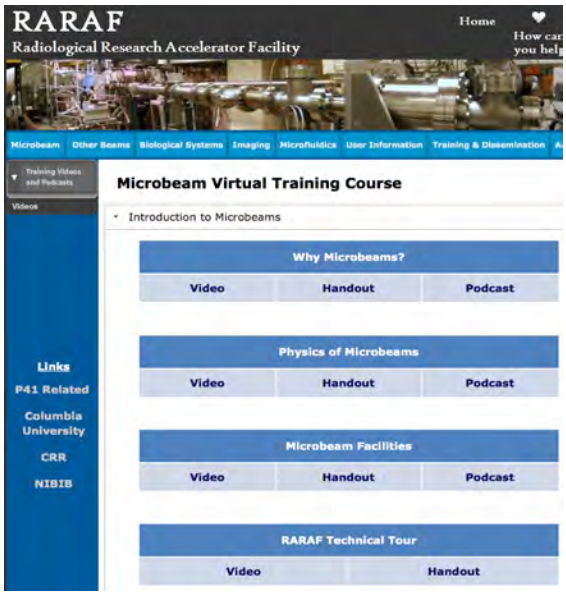

Figure 6. *RARAF webpage for the virtual microbeam training course.*

about 4½ hours of video footage. Additional material is added to the on-line course for new course presentations or lecturers. The on-line training course (Figure 6) can be accessed through the RARAF website (www.RARAF.org) and YouTube channel (http://www.youtube.com/user/RARAFcourses). The videos can be viewed on any Internet-enabled device supporting YouTube format.

Tours

In addition to training students, tours of the Facility provide a general introduction to the research performed at RARAF and the irradiation facilities that are available. This year we gave tours to more than 30 scientists, students, and members of the public.

Figure 7. *Dr. Harken presenting at the Science-on-Hudson public lecture series at the Columbia Nevis Campus. He presented "RARAF: Radiation on the Small Scale Working on Some Big Questions"*

As an example, twelve high school seniors who had been offered priority admission to Columbia as physics majors, some of whom were Columbia I. I. Rabi Scholarship winners, toured RARAF in April along with John Parsons from the Physics Department at Nevis Labs.

Public Presentation

Dr. Harken presented "RARAF: Radiation on the Small Scale Working on some Big Questions" at the new Science-on-Hudson Series of public presentations at the Columbia University Nevis Campus (Figure 7). Science-on-Hudson is a series of public lectures for the Westchester community to come and learn about the work being performed at the Nevis Campus, of which RARAF is a part.

PERSONNEL

The Director of RARAF is Dr. David Brenner, the Director of the Center for Radiological Research (CRR). The accelerator facility is operated by Dr. Gerhard Randers-Pehrson, the Associate Director of RARAF.

Dr. Charles Geard, a Professor Emeritus, continues to visit RARAF frequently and lend his considerable expertise.

Dr. Brian Ponnaiya, an Associate Research Scientist, is the biology advisor for RARAF. He collaborates with many of the external users and coordinates with the CRR, where he spends about half his time.

Dr. Alan Bigelow, an Associate Research Scientist, developed the multiphoton microscopy system, which includes the UV microspot irradiation facility, and worked on the development of the Raman spectroscopy and AMOEBA systems.

Dr. Guy Garty, an Associate Professor, developed the Flow and Shoot (FAST) system and is developing the CE-LIF system. He spends about half his time working on the CRR National Institute of Allergy and Infectious Diseases (NIAID) project, for which he is the project manager.

Dr. Andrew Harken, an Associate Research Scientist, is responsible for the x-ray microbeam. He is also working on the imaging of cells without stain using a highly sensitive EMCCD camera, the STED system for extremely high-resolution spectroscopy, and the microFACS system.

Dr. Yanping Xu, an Associate Research Scientist, is developing the neutron microbeam. He is also developing the accelerator-generated IND-spectrum neutron source.

Dr. Manuela Buonanno, a Postdoctoral Research Scientist in radiation biology, collaborates with many of our external users and performs the assays for the mouse ear microbeam irradiations.

Dr. David Welch, a Postdoctoral Research Scientist, is responsible for the development of new microfluidic tools and interfaces for microfluidic irradiation tools. His

***RARAF Staff**: (front row, l-r) Yanping Xu, Guy Garty, Matt England, Dennis Farrell, David Brenner, (back row, l-r) David Welch, Gerhard Randers-Pehrson, Andrew Harken, Manuela Buonanno, Brian Ponnaiya.*

expertise in microfluidics has been of considerable assistance in the development of our microfluidics applications.

Mr. Matt England is a Ph.D. candidate at the University of Surrey in the U.K. and has joined the RARAF staff this year as the development of the AMOEBA system for microbeam platforms is his Ph.D. dissertation work. He is supervised locally by Drs Bigelow, Garty, and Harken.

Mr. Dennis Farrell has joined the RARAF staff on a part time basis. He is performing microbeam irradiations, serving as the Radiation Safety Officer and providing management support for the RARAF staff.

RECENT PUBLICATIONS OF WORK PERFORMED AT RARAF

Buonanno M, Randers-Pehrson G, Smilenov LB, Kleiman NJ, Young E, Ponnayia B and Brenner DJ (2015) A Mouse Ear Model for Bystander Studies Induced by Microbeam Irradiation. *Radiat Res* **184:** 219-225.

Garty G, Ehsan MU, Buonanno M, Yang Z, Sweedler JV and Brenner DJ (2015) Microbeam-coupled capillary electrophoresis. *Radiat Prot Dosimetry* **166:** 188-191.

Sun H, Olsen T, Zhu J, Tao J, Ponnaiya B, Amundson SA, Brenner DJ and Lin Q (2015) A Bead-Based Microfluidic Approach to Integrated Single-Cell Gene Expression Analysis by Quantitative RT-PCR. *RSC Adv* **5:** 4886-4893.

Sun H, Olsen T, Zhu J, Tao J, Ponnaiya B, Amundson SA, Brenner DJ and Lin Q (2015) A microfluidic approach to parallelized transcriptional profiling of single cells. *Microfluid Nanofluid* **19:** 1429-1440.

Xu Y, Randers-Pehrson G, Turner HC, Marino SA, Geard CR, Brenner DJ and Garty G (2015) Accelerator-Based Biological Irradiation Facility Simulating Neutron Exposure from an Improvised Nuclear Device. *Radiat Res* **184:** 404-410.

Xu Y, Randers-Pehrson G, Marino SA, Garty G, Harken A and Brenner DJ (2015) Broad Energy Range Neutron Spectroscopy using a Liquid Scintillator and a Proportional Counter: Application to a Neutron Spectrum Similar to that from an Improvised Nuclear Device. *Nucl Instrum Methods Phys Res A* **794:** 234-239.

Xu Y, Zhang B, Messerli M, Randers-Pehrson G, Hei TK and Brenner DJ (2015) Metabolic oxygen consumption measurement with a single-cell biosensor after particle microbeam irradiation. *Radiat Environ Biophys* **54:** 137-144. ■