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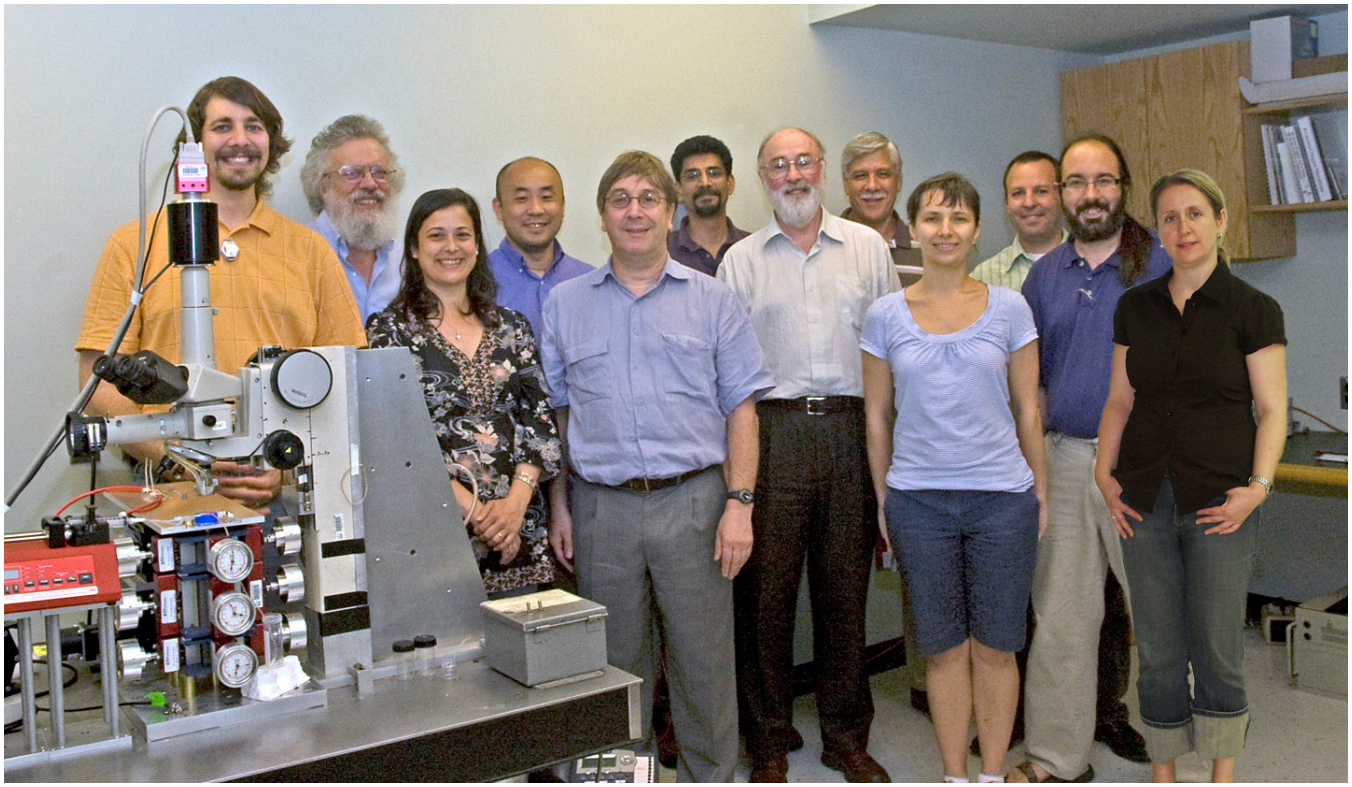
RARAF PROFESSIONAL STAFF



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The Radiological Research Accelerator Facility

AN NIH-SUPPORTED RESOURCE CENTER – WWW.RARAF.ORG

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Research using RARAF

The response of cells that are not directly irradiated when in close contact with or are even only in the presence of irradiated cells continues to be the main focus of the biological experiments at RARAF. For the past several years, the majority of biology studies, including those involving animals, has examined this "bystander" effect. The emphasis of many of the present experiments is to determine the mechanism(s) by which the effect is transmitted, primarily for direct gap junction communication through cell membrane contact. Experiments with tissue and animal systems are being performed to observe the types and magnitudes of the responses in 3-D systems. Both the Microbeam and the Track Segment Facilities continue to be utilized in various investigations of this phenomenon. The single-particle Microbeam Facility provides precise control of the number and location of particles so that irradiated and bystander cells may be distinguished, but is somewhat limited in the number of cells that can be irradiated. The Track Segment Facility provides broad beam irradiation that has a random pattern of charged particles but allows large numbers of cells to be irradiated quickly.

A special type of cell dish is used to investigate cell-to-cell communication in the bystander effect using the Track Segment Facility. These "strip" dishes consist of a stainless steel ring with thin (6-μm) Mylar foil glued to one side into which a second, slightly smaller dish is inserted. The Mylar foil glued to this inner dish has alternate strips of the Mylar removed. Cells are plated over the combined surface and are in contact. The Mylar on the inner dish is thick enough (38 μm) to stop the charged particles (^{4}He ions) and the cells plated on it are not irradiated.

Interest in irradiation of 3-D systems continued this past year. Cultured tissue samples were irradiated with either helium ions or protons using the Track Segment Facility. Two animal systems have been irradiated using the Microbeam Facility this year: *C. elegans* nematodes and Japanese medaka embryos. A fixture has been designed and a prototype constructed that would allow microbeam irradiation of the very thin ears of hairless mice.

The experiments performed at RARAF from January 1 through December 31, 2009 and the number of shifts each was run in this period are listed in Table 1. Fractional shifts are assigned when experimental time is shared among several users (e.g., track segment experiments) or when experiments run for more or less than an 8-hour shift. Use of the accelerator for experiments was 1/3 of the regularly scheduled time (40 hours per week), about 40% lower than last year, which was by far the highest use we have attained at Nevis Labs. Eleven different experiments were run during this period. Seven experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH), the National Aeronautics and Space Administration (NASA), and the Department of Energy (DoE). Four experiments were performed by external users, supported by grants and awards from the Department of Defense (DoD), NASA, and DoE. Brief descriptions of these experiments follow.

Burong Hu and Charles Geard of the CRR concluded their investigation into the role of protein kinase C epsilon in the bystander effect (Exp. 103). The Track Segment Facility was used for irradiation of normal human dermal fibroblasts (NHDF) using ^{4}He ions with an LET of 125 keV/μm. The cells were plated on opposite sides of double-sided dishes with a 1 cm separation between the Mylar surfaces, so that bystander cells were not in contact with the irradiated cells. The gap between the two cell surfaces was filled with medium, thereby stopping the ^{4}He ions from irradiating one layer of cells. It was demonstrated that the PKC epsilon signaling pathway is activated in bystander cells and that PKC epsilon may be a potential molecular target for suppressing the effects of radiation in healthy bystander cells during radiotherapy.

Hongning Zhou, Mei Hong, Bo Shen, and Tom Hei of the CRR continued their efforts to identify the cell-to-cell signaling transduction pathways involved in radiation-induced bystander responses (Exp. 110). In some experiments a fraction of the cells were irradiated either in the nucleus or the cytoplasm using the Microbeam Facility focused 6 MeV ^{4}He beam. In other experiments, cells were plated on "strip" dishes and irradiated using 125 keV/μm ^{4}He ions from the Track Segment Facility. The aim is to identify the roles of 4-HNE (4-hydroxynonenal) and COX-2 in mediating cytoplasmic-irradiation induced mutagenesis, interleukin 33 as an important signal transmitter in the radiation-induced bystander response, and the role of succinate dehydrogenase subunit C in the radiation-induced bystander effect. Preliminary data show that cytoplasmic irradiation can increase mitochondrial content after irradiation, and mitochondrial-deficient cells produce less bystander mutations after cytoplasmic irradiation.

Alexandra Miller of the Armed Forces Radiobiological Research Institute (AFFRI) continued studies using 125 keV/μm ^{4}He ions from the Track Segment Facility to evaluate depleted uranium radiation-induced carcinogenesis using *in vitro* and *in vivo* models and to test safe and efficacious medical countermeasures (Exp. 113). Other goals of this study are the identification of biomarkers for both exposure

Table 1. Experiments Run at RARAF, January 1 - December 31, 2009

Exp. No.	Experimenter	Institution	Exp. Type	Experiment Title	Shifts Run
103	B. Hu C. R. Geard	CRR	Biology	Damage induction and characterization in known hit versus non-hit human cells	0.4
110	H. Zhou, M. Hong B. Shen, T. K. Hei	CRR	Biology	Identification of molecular signals of alpha particle-induced bystander mutagenesis	43.2
113	A. Miller	AFRRI	Biology	Role of alpha particle radiation in depleted uranium-induced cellular effects	0.5
133	S. Ghandhi S. Amundson	CRR	Biology	Bystander effects in primary cells	4.1
136	A. Mezentsev S. Amundson	CRR	Biology	Bystander effects in 3D tissues	7.0
139	S. Amundson	CRR	Biology	Signal transduction in cytoplasmic irradiation	7.6
141	A. Asaithamby D. Chen	Univ. of Texas Southwestern Medical Center	Biology	Visualization of recruitment of DNA damage markers to the sites of DNA damage induced by microbeam irradiation	1.0
142	W. Dynan W. Kuhne (A. Bertucci)	Medical College of Georgia	Biology	Proton irradiation of Japanese medaka embryos *in vivo*	0.8
144	A. Bertucci	CRR	Biology	Microbeam irradiation of *C. elegans*	5.0
145	P. Grabham	CRR	Biology	Effects of high-LET particles on human cell morphology and behavior	3.0
146	M. Bardash	QEL	Physics	Development of a solid state microdosimeter	4.0

Note: Names in parentheses are members of the CRR who collaborated with external experimenters.

and disease development and the mechanisms involved in these processes. These experiments included cell survival, neoplastic transformation, mutagenicity, genomic instability, genotoxicity and radiation-induced leukemia.

Sally Amundson of the CRR is leading a group that continued three experiments investigating radiation-induced gene expression profiles in primary human fibroblast and epithelial cell lines using cDNA microarray hybridization and other methods. The first experiment (Exp. 133), by Shanaz Ghandhi and Lihua Ming, involved use of the Track Segment Facility for comparison of gene expression responses to direct and bystander irradiation. Human lung fibroblast cells (IMR90) and human skin fibroblasts were plated on "strip" dishes for direct-contact bystander irradiations. The dishes were irradiated with 0.5 Gy of 125 keV/μm ^{4}He ions and cells were assayed for micro-nucleus formation and gene expression and western blot analysis was performed. A role for interleukin-33 in the signal transmission for the bystander effect was identified.

The second experiment (Exp. 136), performed by Alexandre Mezentsev, involved irradiation of artificial human tissue samples using the Track Segment Facility. Tissue model Epi-200 (MatTek) is composed of ~20 layers of cells, which represent keratinocytes at different stages of differentiation. The goal of this project is to reproduce tissue response to ionizing radiation *ex vivo* and characterize the effects of low and high doses. The tissues were irradiated with protons having an initial LET of ~10 keV/μm or ^{4}He ions having an initial LET of ~73 keV/μm, either over the entire tissue surface or in a narrow line (~25 μm) across the diameter using a thin slit-shaped collimator. Two types of procedures were performed: isolation of total RNA and immunohistochemistry. The RNA provides quantification of gene expression by Microarray analysis and validation by quantitative real-time PCR. Microarray results are analyzed by computer. The analysis includes gene ontology procedures and network analysis, which normally has a graphical output representing the specific responses to the ionizing radiation. Tissue samples are also fixed in formalin, embedded in paraffin, and sectioned parallel to the line of irradiation for immunohistochemistry and counterstaining. This provides characterization of proteins of interest and describes their role in post-irradiation events, such as transcriptional regulation, contribution to cell signaling mechanisms and gap junction signaling.

In the third experiment, Sally Amundson continued using the Microbeam Facility to irradiate either the nuclei or the cytoplasm of primary human lung fibroblasts with 6 MeV ^{4}He ions and extracted RNA to perform global gene expression analysis in order to gain a better understanding of the

cell signaling that arises from radiation damage to the cytoplasm and which damage response pathways require direct damage to DNA (Exp. 139). She has completed survival experiments and has tested several methods for recovery and amplification of the small amounts of RNA obtained from small numbers of cells. Despite initial excitement, she has not obtained good results from the Cells to CT direct lysis qPCR approach. However, the amplification for microarray analysis appears to be working well, with good R-squared values for repeated amplifications of the same sample. Results of the experiments so far have been unexpected, and she is now performing repeated experiments and additional controls. This work was focused on half-hour and four-hour post-exposure time points.

David Chen and Aroumougame Asaithamby from the University of Texas Southwestern Medical Center continued a study of the responses of cells after microbeam irradiation (Exp. 141). HT1080 human fibrosarcoma cells expressing different types of DNA damage sensing and repair factors were irradiated in the nucleus with 6 MeV ^{4}He ions. The cells contain a red fluorescent protein (RFP) reporter attached to the 53BP1 gene and a green fluorescent protein (GFP) attached to the XRCC1 gene. Cells were irradiated singly and observed for up to 2 hours to monitor the recruitment of the DNA-damage sensing and repair factors to the sites of DNA damage.

William Dynan and Wendy Kuhne of the Medical College of Georgia continued using the RARAF Microbeam Facility (Exp. 142) to irradiate Japanese medaka fish embryos. Medaka embryos were selected because of their small size (~1.2 mm D) and their optically clear chorion, which makes them easy to view on the microbeam endstation. Fertilized embryos were collected from CAB wild-type breeding adults. At the time of irradiation embryos were at Stage 27-28 (representing the 24–30 somite stage). Fluences of 10,000 or 20,000 4.5 MeV protons were delivered to areas of the brain using a beam diameter of 60 μm. The protons have a range of ~280 μm and stop in the embryos. After irradiation, the embryos were subjected to a fluorescent *in situ* terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay to detect DNA fragmentation, which is characteristic of apoptotic cells. A confocal laser scanning microscope was used to collect images using a 3 μm step size. Three-dimensional renderings of the Z-stack images were created and analyzed for the presence of TUNEL-positive cells.

Irradiations of *C. elegans* nematodes (144) using the Microbeam Facility were continued by Antonella Bertucci of the CRR. A new transgenic strain of *C. elegans* was used that has both a Green Fluorescent Protein (GFP) transcriptional gene reporter for heat shock protein-4 (HSP-4) and a Red Fluorescent Protein (RFP) gene reporter for dopaminergic neurons. Young adult *C. elegans* hermaphrodites were exposed to a 3 MeV proton microbeam with a 1μm diameter. Each worm was exposed in the left or right posterior deirid neuron (PDE). Samples were collected after exposure and re-cultured for GFP expression evaluation 24 hours post irradiation. Analysis of the results indicates that microbeam irradiation of specific neurons is capable of inducing both local and distal GFP over-expression in the *C. elegans* posterior intestine. Quantification of stress response using the software QuantWorm developed in our laboratory showed a threefold increase in worms irradiated with 75 protons compared to control worms.

Peter Grabham of the CRR initiated a study of high-LET particles on human cell morphology and behavior using the Microbeam Facility (Exp. 145). Neuron cells in culture were irradiated with 6 MeV ^{4}He ions and observed *in situ* to monitor the movement of labeled mitochondria up and down the neuron axons, which is indicative of axonal transport. Unfortunately, the physiological conditions for the cells could not be maintained adequately for periods greater than 1 hour. Modifications to the Microbeam end station are being designed to ensure that the cells will maintained at 37º C and the amount of cell medium is kept constant despite evaporation so that observation can continue for many hours.

Tests of a solid state microdosimeter were made by Michael Bardash of QEL Inc. (Exp. 146). 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 Microbeam and Track segment Facilities were 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 he did not obtain proof of principle, he obtained responses that were promising enough to submit a request for a Phase II Small Business Incentive Research (SBIR) grant.

Development of Facilities

Development continued on a number of extensions of our facilities:

- Focused accelerator microbeams
- Non-scattering particle detector
- Advanced imaging systems
- Targeting of cells
- Focused X-ray microbeam
- Neutron microbeam

Development of focused accelerator microbeams

In February, as scheduled, the lens assembly with the coils for the Point and Shoot system was installed in the Microbeam II beamline, in place of the original compound triplet lens. The new lens system was able to focus the particle beam to <1 μm D and the original system was kept in storage as a spare. This new compound lens worked well until November, when a computer control problem tried to force the lens high voltage supplies to their maximum levels (+/- 30 kV). On one section of the upper triplet, the gold plating on the surfaces that face the beam on each of two rods was removed in a area ~1 cm D, probably by a discharge from the nearby rods having the opposite polarity. There were smaller damage spots on all four rods. No evidence was observed of any breakdown of the insulating sections between the high voltage and ground. The original compound triplet system was put back in the Microbeam II beamline. Shortly thereafter, the lens began experiencing voltage instabilities as evidenced by vacuum pressure spikes as well as spikes in

the charging current from the power supplies. A number of combinations of the three triplet lenses and permutations in their positions (upper, lower) were tried to address the sparking problems. Strangely, the voltage stability seemed to be better just after the rods were under vacuum than when they had been there for a while and the vacuum pressure was lower. These efforts were ongoing at the end of 2009. New ceramic rods for triplet lenses will be machined, implanted with platinum ions and plated with gold. The rods will be used to replace damaged rods or assembled into new lenses.

The permanent magnet microbeam (PMM) uses a compound quadrupole triplet lens made from commercially available precision permanent magnets. Its design is similar to that for the lens system for the sub-micron microbeam, the major difference being that it uses magnetic rather than electrostatic lenses.

The quadrupole magnet strengths used to focus the beam have been adjusted to produce essentially the minimum beam spot diameter using micrometric screws to retract and extend the individual magnets of each quadrupole. Using a phase space "sweeper" and an object aperture 0.3mm in diameter, a beam of 5.3 MeV ^{4}He ions has been focused into a spot 5 μm in diameter.

The adjusting knobs for the individual magnets in the system lack position indicators. Multi-turn dials will be installed on the knobs so that fine adjustments can be made for additional tuning of the beam spot size to its minimum diameter. In addition, major changes in field strength will be able to be made relatively easily since the magnet positions can be set readily to specific locations very accurately. The lens system will be tuned to focus a 6.0 MeV ^{4}He beam to match the beam most frequently used for the electrostatically focused microbeam. It will also be tuned for a 4.5 MeV proton beam for development tests of the Flow and Shoot system.

The end station for the PMM has been tested and is ready to be used. The PMM will be used primarily for cell irradiations when the electrostatic system is unavailable because of development or repair and for the development of the Flow and Shoot system.

Non-scattering particle detector

Presently the RARAF microbeam endstation delivers a precise number of particles to thin samples by counting the particles traversing them using a gas proportional counter placed immediately above the cells. Because the ^{4}He ions have a very short range (~50 μm), the medium over the cells must be removed to count the ions. To irradiate samples thicker than the range of the incident ions or to allow cell medium to remain in place during irradiations, a very thin particle detector is necessary upstream of the samples.

The Lumped Delay Line Detector (LD^2) was proposed as a novel non-scattering particle detector consisting of 250 silver cylinders, each 3 mm long with a 2.2 mm inside diameter, connected by inductors and capacitively coupled to ground. Theoretically, if the capacitance and inductance are set such that the propagation velocity of the pulse equals the charged particle velocity, the pulses capacitively induced in all segments by the passage of a single ^{4}He ion would add coherently, resulting in a fast electron pulse at each end of the delay line.

Calculations were made using the computer program AIMSPICE to simulate the electronic behavior of the LD^2 to determine the best termination system for the signal from the detector in order to reduce "ringing" and signal loss. The simulation required the addition of electrons at each of the cylinders; it did not simulate the charge arising from induction by the passing charged particle.

The detector was placed in a horizontal beam line for testing with a ^{4}He ion beam, but after many trials no pulses were observed. Considerable effort was made to reduce noise to enable the detection of the small signals that were expected. One of the prototype detectors was taken to the Edwards Accelerator Laboratory in Athens, Ohio in March and tested with a pulsed particle beam. A single nanosecond pulse contained more than 1,000 protons and provided a signal at least 500 times larger than would be produced by a single He^{++} ion. Unfortunately no signal was observed from the detector. Development of this detector has been abandoned for the time being.

Development resumed of another type of under-dish detector design that was initially investigated several years ago. A thin silicon wafer has an aluminum electrode evaporated on one side and three gold electrodes evaporated on the opposite side, with only a small gap between the ends of the electrodes (Fig. 1). The center gold electrode collects the charge in the diagonal region between it and the electrode on the opposite side. The other two gold electrodes are fguards to reduce noise. Because the gap between the electrodes is much larger than the thickness of the wafer, the capacitance, and therefore the noise, is much lower than it would be if the electrodes overlapped. The initial energy of the ions used for irradiation will be raised to compensate for the energy loss in the silicon. Because of scattering in the detector, it cannot be used when sub-micron beam spots are required.

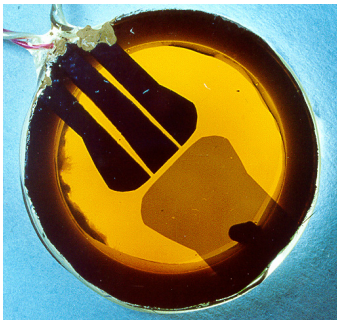

Fig. 1. Photo of the thin silicon detector.

Advanced imaging systems

New imaging techniques to view cells without using stain and to obtain three-dimensional images of unstained cells are of great importance in order to maintain the physiological conditions of the cells on the microbeam facilities.

Immersion-based Mirau interferometry (IMI) was developed at RARAF. An objective was constructed to function as an immersion lens using standard interferometric techniques by acquiring successive images at four positions with sub-wavelength separations using the vertical motion of the microbeam stage. It uses 540 nm (green) light for imaging and therefore does not induce UV damage in the cells. Interferometry is very sensitive to vibrations, even as small as a fraction of a wavelength. This system provides usable im-

ages in a vibration-free environment; however on the electrostatic microbeam endstation vertical motions due to vibrations in the building greatly reduce the image quality. Passive and active systems to reduce the vibrations were unsuccessful. A Fourier technique was investigated to remove the effects of the vibrations, but did not improve the images sufficiently.

In 2008, the feasibility of a new approach to overcome the vibration problem using Simultaneous Immersion Mirau Interferometry (SIMI) was demonstrated. Polarized light is split into equal components in the x and y planes, one of which undergoes a phase shift of 90º using a λ/8 waveplate. A polarization beam splitter is used to send the x and y components to form iterferograms on two separate cameras. Since the images are taken simultaneously, there is no effect from the vibration, however even a slight misalignment or miscalibration between the two cameras creates a discrepancy between the corresponding interferograms that reduces the quality of the final image. This year a further refinement of this system was developed in which both images are obtained by a single camera using a calcite beam displacer instead of a beam splitter. Such beam displacers separate the input beam into two orthogonally polarized beams, but the output beams are parallel and so it can be used in applications where a 90° phase shift is not possible.

A multi-photon microscope was developed for and integrated into the microscope of the single-particle Microbeam Facility in 2007 to detect and observe the short-term molecular kinetics of radiation response in living cells and to permit imaging in thick targets, such as tissue samples. Two photons delivered closely together in space and time can act as a single photon with half the wavelength (twice the energy). This method has the advantages that the longer wavelength of the light beam allows better penetration into the sample while still being able to excite the fluorophor at the focal volume and less damage is produced in the portion of the sample not in the focal volume.

The laser was upgraded to a Chameleon Ultra II, which has a wide range of wavelengths (680 to 1080 nm), increasing the available range of effective wavelengths for the two-photon effect so that red fluorescent protein (RFP) can be imaged. The light available from the laser can penetrate to depths of about 100 microns in a biological sample by varying the Z-position of the specimen stage. Light emitted from the specimen is selectively deflected by a series of dichroic mirrors to a pair of photomultiplier tubes (PMTs). Three-dimensional images can be obtained by making scans at several depths in the sample using the Z-motion of the microbeam stage. The series of two-dimensional images can be assembled into a single 3-D image. The system was used to observe a GFP-tagged XRCC1 DNA single-strand break repair protein in real time for the experiments by David Chen (Exp.141).

Another potential use for the multi-photon system is fluorescent recovery after photobleaching (FRAP), which was requested by the Chen group. Fluorescent foci that are formed in a cell nucleus can be "erased" by extended exposure from the multiphoton laser, which bleaches the fluorophors. The cells can then be observed to determine the time course of foci reforming.

The multiphoton system can also be used as a laser "microspot" to induce UV damage in the focal volume of the laser spot, a capability that some users have requested. A 3-D image of single-strand damage induced by the laser spot is shown on the home page of the RARAF web site (raraf.org). The Columbia crown logo shown there is the result of the fluorescence of tagged repair proteins at sites of damage caused by the microspot in a single cell nucleus and imaged by multiple laser scans of the cell at different depths.

Targeting of cells

The targeting capability of the electrostatic microbeam system was tested using HT1080 human Fibro Sarcoma cell nuclei containing GFP-tagged XRCC1. For each irradiation, the location of the beam spot was superimposed on the cell image and the location of the focus produced by the charged particle beam was observed as it formed and compared to the targeted location. As a demonstration of targeting control, the letters "NIH" were written as a series of foci in a single cell nucleus.

During irradiation, cells to be irradiated are moved to the beam position using the microbeam stage. This was necessary but relatively time-consuming when a collimated microbeam was being used. A focused microbeam is not restricted to a single location on the beam exit window and therefore can be deflected magnetically or electrostatically to any position in the field of view of the microscope much more rapidly than moving the stage, thereby increasing throughput.

We have developed a "Point and Shoot" targeting system for microbeam irradiation based on a wide-field magnetic split-coil deflector system from Technisches Büro Fischer (Ober Ramstadt, Germany). Two Kepco BOP power amplifiers provide the currents used to drive the coils. A coil assembly has been mounted around a short section of beam line just below the upper quadrupole triplet on the PMM. The deflection of the charged particle beam is linear with coil current and does not affect the beam spot size. A similar coil was mounted just below the second lens in the lens tube for a compound electrostatic quadrupole triplet, and was installed in the electrostatic microbeam beamline in February. The power supplies used to drive this coil were unstable, making the effective beam spot size increase, becoming larger as the deflection (and therefore current) increased. It is believed that the absence of the steel tube that is in the field of the PMM system is causing a change in the inductance and capacitance of the coil, causing the oscillations. A steel tube will be inserted into the opening of the coil to eliminate the instability.

A new targeting system based on microfluidics is being developed to increase the throughput of the microbeam. In the Flow And ShooT (FAST) system, cells moving through a narrow capillary are imaged by a high-speed camera to track their trajectory. The charged particle beam is deflected using the Point and Shoot coil to the position of the cell on the trajectory and the particle beam is enabled. The deflection coil currents will be changed continuously to follow the path of the cell until the requested number of particles is

delivered. The system will be capable of tracking several cells at a time. In addition to increasing the speed of the irradiations, this system will be able to irradiate non-adherent cells such as lymphocytes that do not plate on surfaces and therefore do not have stable positions.

Focused X-ray microbeam

We have developed an x-ray microbeam to provide characteristic K_α x rays generated by proton-induced x-ray emission (PIXE) from Ti (4.5 keV). Charged particle beams can generate nearly monochromatic x rays because, unlike electrons, they have a very low bremsstrahlung yield. Higher x-ray energies are not feasible due to Compton scattering; we are limited to x-ray energies where the predominant mode of interaction is photoelectron absorption.

A small x-ray source (~20 μm D) is produced by bombarding a Ti target with high-energy protons using the quadrupole quadruplet lens used for our first focused microbeam, reducing the requirements on the subsequent x-ray focusing system. The x rays used are emitted at 90° to the proton beam direction. This eliminated several problems inherent in the original transmission design in which the x-ray beam was obtained on axis with the proton beam. A zone plate is used to focus the x-ray source to a beam spot 2 μm in diameter. The zone plate has a radius of only 120 μm, an outmost zone width of 50 nm, and a demagnification factor of ~11. The system has been mounted on its own horizontal beam line on the 1st floor of RARAF and the x-ray beam is oriented vertically, so that the geometry of the microscope and stage is the same as for our other microbeam systems.

The endstation, consisting of the microscope, micropositioning stage and EMCCD camera have all been assembled. These items have been designed so that they can be switched readily between the PIXE x-ray microbeam and the Energetiq Technology Inc. low-energy (300-400 eV) x-ray microbeam that is based on a nitrogen plasma as the x-ray source. Since the two sytems are located very near each other, they can share the same endstation.

Neutron microbeam

Neutrons produced by the $^7Li(p,n)^7Be$ reaction are emitted only in a forward conical volume when the proton energy is just above the reaction threshold (1.881 MeV). The half-angle of this cone is dependent on the proton energy and increases with increasing energy. Thin samples placed very close to the thin, neutron-producing target layer will be irradiated by neutrons in a restricted area. A focused proton microbeam 5 μm in diameter will be incident on a 1 μm thick lithium fluoride target. The backing material will be 20-μm thick Au, selected for its high density and thermal conductivity. Using a 1.890 MeV proton beam, thin samples in contact with the target backing will be exposed to a beam of neutrons 10-12 μm in diameter having energies from 10-60 keV.

In collaboration with Mark Akselrond and Jeff Sykora of Landauer, Inc., initial measurements using fluorescent nuclear track detectors (FNTDs) have been made that demonstrate the restricted nature of the neutron emission. A proton beam collimated to 1 mm D was used to produce neutrons restricted to a 2 mm diameter 2 mm from the target.

A test system is being constructed on a horizontal beamline using a quadrupole quadruplet to focus the proton beam to ~10 μm D for further development of the system. This beam line eventually may be used for the neutron microbeam endstation.

Singletron Utilization and Operation

Table 2 summarizes accelerator usage for the past year. The Singletron is started between 7 and 7:30 a.m. on most days from September through June and between 8 and 9 am the rest of the year. The accelerator is often run well into the evening, frequently on weekends, and occasionally 24 hours a day for experiments, development and repair. The nominal accelerator availability is one 8-hour shift per weekday (~250 shifts per year).

Accelerator use for radiobiology and associated dosimetry was about 55% of that for last year - which was the highest level of use since RARAF has been at Nevis Labs. About 2/3 of the use for all experiments was for microbeam irradiations and 1/3 for track segment irradiations.

Use of the Track Segment Facility was 1/3 of the experiment time, about a 50% higher fraction than last year. Irradiation times for individual samples are usually 0.5-5 minutes, so that multiple experimenters, as many as 5 or more, can be run in a single shift, sometimes using different LETs and even different types of ions in the same day. Because the facility is used so efficiently, cell irradiations typically are scheduled only one day every other week. Radiological physics resumed with the testing on the Microbeam and Track Segment Facilities of a solid state microdosimeter designed by QEL, Inc.

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On-line facility development and testing was 40% of the available time, about the same as the average for the previous 5 years. This was primarily for development and testing of the electrostatically focused microbeam, the PMM, the x-ray microbeam, and the neutron microbeam.

Approximately 17% of the experiment time was used for experiments proposed by external users, about 2/3 of what was used last year and also about 2/3 the average for the last five years.

There were 30 shifts of Singletron maintenance and repair time this year. In addition to routine maintenance of the

Table 2. Accelerator Use, January–December 2009
Usage of Normally Scheduled Days

Radiobiology and associated dosimetry	29%
Radiological physics and chemistry	2%
On-line facility development and testing	40%
Safety system	2%
Accelerator-related repairs/maintenance	12%
Other repairs and maintenance	3%
Off-line facility development	30%

ion source there was a leak in a thermo-mechanical gas valve for the ion source and a leak in a seal in the ion source. The Singletron charging system continues to be very stable and reliable

Training

In collaboration with the Columbia University Physics Department, we again participated in the Research Experiences for Undergraduates (REU) project this past summer. Students attended lectures by members of different research groups at Nevis Laboratories, worked on research projects, and presented oral reports on their progress at the end of the 10-week program. Nina Bahar from New York University worked with Guy Garty on the development of a method to perform rapid focusing of a high-speed micro-photographic system. A fluorescent bead is viewed through a cylindrical lens. The direction and amount of elongation of the image indicates whether the lens focus must be increased or decreased and by what amount. This eliminates the need for multiple images to determine the correct focal distance, increasing the speed of the system.

Personnel

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

Dr. Charles Geard, the former Associate Director of the CRR, continues to spend most of each day at RARAF.

Dr. Alan Bigelow, an Associate Research Scientist, continues the development of the multiphoton microscopy system that uses a fast Ti-sapphire laser for three-dimensional imaging and as a "microspot" irradiation facility.

Dr. Guy Garty, a Research Scientist, is developing the permanent magnet microbeam (PMM) and the Flow and Shoot system. He spends about half his time working on the National Institute of Allergy and Infectious Diseases (NIAID) project, for which he is the project manager.

Sasha Lyulko, a graduate student in the Physics Department at Columbia University, is involved in developing the Simultaneous Immersion Mirau Interferometry system and also spends about half her time working on imaging for the NIAID project.

Dr. Andrew Harken, a Postdoctoral Fellow, is developing the x-ray microbeam and the Point and Shoot targeting system and is working with Guy Garty on the PMM. He is also working with Energetiq Technology Inc. on a soft x-ray microbeam that is based on a nitrogen plasma source.

Dr. Yanping Xu, a Postdoctoral Fellow, has been working on the development of a neutron microbeam. He is also working on the NIAID project, developing a method for the determination of the number of lymphocytes in blood samples using light absorption and scattering.

Dr. Stephane Lucas, a visiting professor, arrived in September, 2009 on a sabbatical leave from the University of Namur, Belgium. He is staying until May, 2010 and has been working with Andrew Harken on the soft x-ray microbeam project.

Several biologists from the CRR have office space at the facility and use the RARAF biology laboratories to perform experiments:

- Dr. Brian Ponnaiya, an Associate Research Scientist, has undertaken the role of biology advisor for RARAF that had been that of Charles Geard. He presently spends much of his time at the CRR.
- Dr. Alexandre Mezentsev, an Associate Research Scientist, is working with cultured tissue systems and spends some of his time at RARAF.
- Dr. Helen Turner, an Associate Research Scientist, is working on the NIAID project and spends some of her time at RARAF. She is also working with Alan Bigelow on the development of FRAP and FRET capabilities for the Microbeam Facility.
- Dr. Antonella Bertucci, a Postdotoral Fellow, spends part of her time at RARAF. She has been working with Helen Turner on the NIAID project and is performing microbeam experiments using *C. elegans* nematodes.

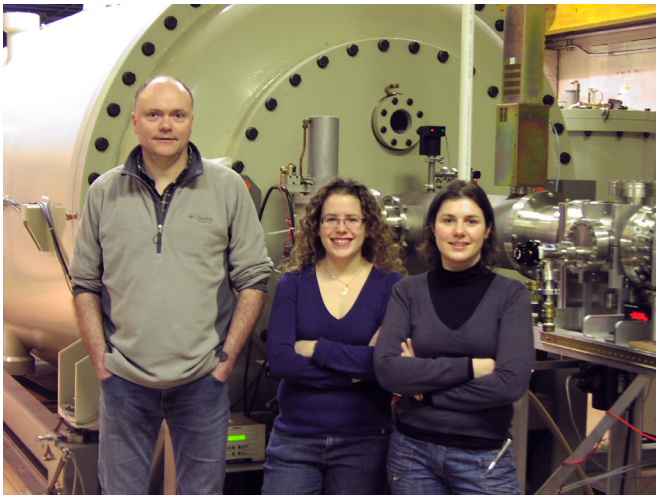
Julia Schaefer, an undergraduate student from Berufsakademie Karlsruhe (the University of Cooperative Education in Karlsruhe), Germany, arrived at the end of December, 2008 for a three-month visit. She worked with Helen Turner on part of the NIAID high-throughput biodosimetry program and returned to Germany in March, 2009.

Recent Publications of Work Performed at RARAF

1. Aprile E, Baudis L, Choi B, Giboni KL, Lim K, Manalaysay A, Monzani ME, Plante G, Santorelli R and Yamashita M. New measurement of the relative scintillation efficiency of xenon nuclear recoils below 10 keV. *Phys. Rev. C* **79**: 045807, 2009.
2. Bertucci A, Pocock RD, Randers-Pehrson G and Brenner DJ. Microbeam irradiation of the *C. elegans* nematode. *J Radiat Res (Tokyo)* **50 Suppl A**: A49-54, 2009.
3. Bigelow A, Garty G, Funayama T, Randers-Pehrson G, Brenner D and Geard C. Expanding the question-answering potential of single-cell microbeams at RARAF, USA. *J Radiat Res (Tokyo)* **50 Suppl A**: A21-8, 2009.
4. Fuks E, Horowitz YS, Oster A.L, Marino S, Rainer M, Rosenfeld A. and Datz H. Thermoluminescence solid state nanodosimetry – the peak 5a/5 dosimeter. *Radiat. Prot. Dosim.* (submitted 2009).
5. Garty G, Grad M, Jones BK, Xu Y, Xu J, Randers-Pehrson G, Attinger D and Brenner DJ. Design of a novel Flow-And-ShooT (FAST) microbeam. *Radiat. Prot. Dosim.* (submitted 2009).
6. Hei TK, Ballas LK, Brenner DJ and Geard CR. Advances in radiobiological studies using a microbeam. *J Radiat Res (Tokyo)* **50 Suppl A**: A7-A12, 2009.
7. Hei TK, Zhou H and Ivanov VN. The Yin and Yang of low dose radiobiology. *Radiation Health Risk Sciences.* Springer, Tokyo, pp.135-142, 2009.
8. Hu B, Shen B, Su Y, Geard CR and Balajee AS. Protein kinase C epsilon is involved in ionizing radiation induced bystander response in human cells. *Int J Biochem Cell Biol* **41**:2413-21, 2009.

9. Ivanov VN, Zhou H, Ghandhi SA, Karasic TB, Yaghoubian B, Amundson SA and Hei TK. Radiation-induced bystander signaling pathways in human fibroblasts: a role for interleukin-33 in the signal transmission. *Cell Signal* **22**:1076-87, 2010.
10. Kovalchuk O, Zemp F, Filkowski J, Altamirano A, Dickey JS, Jenkins-Baker G, Marino SA, Brenner DJ, Bonner WM and Sedelnikova OA. MicroRNAome changes in bystander three-dimensional human tissue models suggest priming of apoptotic pathways. *Mol. Cancer Res.* (submitted 2009).
11. Marino SA, Johnson GW, Schiff PB, Brenner DJ. Modification of shirt buttons for retrospective radiation dosimetry after a radiological event. *Health Phys.* (submitted 2009).
12. Miller AC. Development of models to study radiation-induced late effects. *NATO RTG Technical report 2010*, (in press).
13. Randers-Pehrson G, Johnson GW, Marino SA, Xu Y, Dymnikov AD and Brenner DJ. The Columbia University Sub-micron Charged Particle Beam. *Nucl Instrum Methods Phys Res A* **609**:294-9, 2009.
14. Schettino G, Johnson GW, Marino SA and Brenner DJ. Development of a method for assessing non-targeted radiation damage in an artificial 3D human skin model. *Int J Radiat Biol*. [Epub ahead of print] 2010.
15. Su Y, Meador JA, Geard CR and Balajee AS. Analysis of ionizing radiation-induced DNA damage and repair in three-dimensional human skin model system. *Exp Dermatol*, 2009 July 23. [Epub ahead of print].
16. Zeitlin CJ, Maurer RH, Roth DR, Goldsten JO and Grey MP. Development and evaluation of the combined ion and neutron spectrometer (CINS). *Nucl. Inst. Methods B* **267**: 125-38, 2009.
17. Zhou H, Hong M, Chai Y and Hei TK. Consequences of cytoplasmic irradiation: studies from microbeam. *J Radiat Res (Tokyo)* **50 Suppl A**: A59-65, 2009. ■

Seminar speaker, Dr. Douglas Spitz from the University of Iowa pictured with Dr. Charles Geard (left) and Dr. Tom Hei (right).



Investigators from the University of Namur in Belgium visited the Radiological Research Accelerator Facility Nevis Laboratories. (L-r): Professor Stephané Lucas and graduate students Helene Riquier and Anne-Catherine Wera.