# THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

**An NIH-Supported Resource Center**

**WWW.RARAF.ORG**

*Director: David J. Brenner, Ph.D., D.Sc.*
*Associate Director: Gerhard Randers-Pehrson, Ph.D.*
*Manager: Stephen A. Marino, M.S.*

**Research Using RARAF**

For over a decade, many of the biology studies at RARAF, including those involving animals, have examined the "bystander" effect - the response of cells that are not directly irradiated when in close contact with, or are even only in the presence of, irradiated cells. The emphasis of most of the present biological experiments is to determine the mechanism(s) by which the effect is transmitted, primarily via direct gap junction communication through cell membrane contact. Both the Microbeam and the Track Segment Facilities continue to be utilized in various investigations of this phenomenon. Research into bystander effects in 3-D systems continued this past year with the irradiations of *C. elegans* nematodes using a "worm clamp" system adapted for use on the microbeam system.

The experiments performed using the RARAF Singletron between January 1 and December 31, 2011 and the number of shifts each was run in this period are listed in Table I. 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 26% of the regularly scheduled time (40 hours per week), about 1/4 higher than last year. Five different experiments were run during this period. Two experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH). Three experiments were performed by external users, supported by grants and awards from the Department of Defense (DoD), the National Air and Space Administration (NASA), and The Israel Science Foundation. Brief descriptions of these experiments follow.

Hongning Zhou and Tom Hei of the CRR continued experiments to identify the signaling transduction pathways involved in radiation-induced bystander responses (Exp. 110). Using the Microbeam Facility, they observed that cytoplasmic irradiation with $^{4}$He ions could induce mutagenesis in mitochondrial functional human skin fibroblasts, although the mutation induction rate is relatively low compared with nuclear-irradiated cells. However, cytoplasmic irradiation could induce very little, if any, mutagenesis in directly irradiated cells without mitochondrial DNA. Furthermore, using real time quantitative PCR, they found that targeted cytoplasmic irradiation induced a transient increase in mitochondrial DNA contents as a function of time post-irradiation. To detect mitochondrial alteration after cytoplasmic irradiation, small airway epithelia cells labeled with GFP glycoprotein linked to their mitochondrial membranes were irradiated through their

***Table I.*** *Experiments Run at RARAF January 1 - December 31, 2011*

| Exp No. | Experimenter | Institution | Exp. Type | Title of Experiment | Shifts Run |
|---|---|---|---|---|---|
| 110 | H. Zhou, B. Zhang, K. K. Lam, T. K. Hei | CRR | Biol. | Identification of molecular signals of alpha particle-induced bystander mutagenesis | 47.8 |
| 112 | Y. Horowitz | Ben Gurion Univ., Israel | Phys. | HCP and neutron irradiation of LiF:Mg, TI TLD chips | 6.5 |
| 113 | A. Miller | AFRRI | Biol. | Role of alpha particle radiation in depleted uranium-induced cellular effects | 1.0 |
| 152 | B. Ponnaiya, H. Lieberman | CRR | Biol. | The role of Rad9 in mediating global gene expression in directly irradiated and bystander cells and chromosome abnormalities. | 5.2 |
| 153 | C. Zeitlin | Southwest Research Institute | Phys. | Fast neutron detection efficiency of boron-loaded plastic scintillators. | 3.5 |

cytoplasm with $^{4}$He ions. After irradiation, mitochondrial fusion was observed using the RARAF multiphoton imaging system that is available on the microbeam end station. One day post-irradiation, cells were stained with a cell-permeant green-fluorescent dye that is selective for the mitochondria of live cells. When compared to similarly-treated controls, irradiated cells showed a significant reduction in mitochondrial membrane potential, indicating a loss of function. These results indicate that mitochondria play a critical role in cytoplasmic irradiation-induced genotoxicity and impact on our understanding of the cellular response to DNA damage and low dose radiation risk assessment.

Yigal Horowitz of Ben Gurion University, Israel resumed studies of the responses of different types of thermoluminescent devices (TLDs) to high-LET charged particles and monoenergetic neutron irradiation (Exp. 112). TLDs were irradiated with doses of 1 and 100 Gy of 6 MeV neutrons produced using the D(d,n)$^{3}$He reaction. Both TLD-600 ($^{6}$LiF:Mg,Ti) chips, which are sensitive to neutrons and γ rays, and TLD-700 ($^{7}$LiF:Mg,Ti) chips, which are only sensitive to γ rays were irradiated. The lower dose was to determine the sensitivities of the TLDs and the larger dose was to obtain good measurable optically stimulated luminescence (OSL).

The exposure of military personnel and civilians to the alpha emitter and heavy metal depleted uranium (DU) is of concern to the Department of Defense. Alexandra Miller of the Armed Forces Radiobiological Research Institute (AFRRI) continued studies using the Track Segment Facility to evaluate DU radiation-induced carcinogenesis and other late effects using *in vitro* models and to test safe and efficacious medical countermeasures (Exp. 113). Human osteoblast cells (HOS) were irradiated with $^{4}$He ions to evaluate the effect of the biological countermeasure phenylbutyrate (PB) on cell survival, neoplastic transformation, chromosomal aberrations, and global DNA methylation status.

Rad9 has been implicated in a wide range of cellular processes (including the regulation of cell cycle checkpoints and DNA damage repair) that are thought to play roles in the development of tumors. Brian Ponnaiya and Howard Lieberman continued investigations of the effects of Rad9 on radiation-induced changes in chromosome status and gene expression in human and mouse cells directly irradiated or as bystanders (Exp. 152). The three genotypes used in this study were wild-type mouse ES cells, Rad9-/- cells and Rad9-/- cells ectopically expressing the mouse Rad9 gene. Cells were seeded onto double-ring mylar dishes and irradiated with 1 Gy of $^{4}$He ions using the Track Segment Facility. Irradiated and bystander populations were separated 24 hours after irradiation and reseeded into T25 flasks. Chromosome preparations were made at 7 day intervals and metaphases were analyzed for gross chromatid- and chromosome-type aberrations. The data supports previous findings of a role for Rad9 in both genomic instability and bystander responses. In addition, differential expression of chromatid- and chromosome-type aberrations as a function of Rad9 status suggests that Rad9 might play different roles in the appearance of delayed chromosomal aberrations in directly irradiated and bystander cells.

Cary Zeitlin of the Southwest Research Institute, along with several colleagues, began to characterize the efficiency of a neutron spectrometer based on a boron-loaded plastic scintillator (Exp. 153) for use in space. Neutrons are thermalized in the large detector as they lose energy in elastic collisions, primarily with the hydrogen nuclei in the scintillator material. Since these collisions occur extremely rapidly, the energy of the neutron is observed as a single pulse. The low-energy neutrons are often captured by the boron, which has a very large thermal neutron cross section and releases a 1.5 MeV alpha particle a very short time after the pulse from the neutron collisions. The pulse from this alpha particle is of constant amplitude and indicates that the first pulse was caused by a neutron that has given up all its energy. An advantage of this design is the direct measurement of the neutron spectrum. Most other spectroscopy systems require complicated deconvolution programs to determine the neutron spectrum. The detector was irradiated with essentially monoenergetic neutrons with energies from 0.5 to 3.0 MeV and 6 MeV to obtain initial neutron spectra.

**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
- Non-scattering particle detector
- Advanced imaging systems
- Targeting and manipulation of cells
- Small animal systems
- New neutron source

**Focused particle microbeams**

The electrostatically focused microbeam has continued operating very reliably this past year, consistently producing a beam spot 1-2 μm in diameter using a 500 nm thick silicon nitride exit window. A window only 100 nm thick is used when a sub-micron beam spot is desired.

Emphasis on quality control was maintained. We perform a microbeam test run the evening before an irradiation so that the next morning, after the accelerator has warmed up, the charged particle beam is found immediately and has a minimal beam spot diameter. This provides an earlier and trouble-free start for irradiations and consequently a greater throughput.

Twelve new ceramic quadrupole triplet rods were machined in our shop. The rods were then sent to the Institute of High Current Electronics in Russia for implantation of platinum ions to increase the surface resistivity, which reduces ion charge build-up on the insulating sections between the electrodes, and were returned in early 2011. The conductivities of the insulating sections will be measured, the sections carefully masked and the rods sent out to have a gold layer 1 μm thick plated on the electrode sections to make them conducting. The rods then will be tested in vacuum with high voltage using the test fixture described in last year's report. After testing, eight of the rods will be assembled into two quadrupole triplet lenses and the lenses mounted in an alignment tube for insertion into the microbeam beam line, where the voltages on the lens elements will be adjusted to produce a beam spot with a sub-micron diameter.

The permanent magnet microbeam (PMM) uses a compound quadrupole triplet lens system made from commercially available precision permanent magnets. Its design is similar to that of the electrostatic lens system for the sub-micron microbeam, the major difference being that it uses magnetic rather than electrostatic lenses. After tuning, it consistently produced a $^{4}$He beam spot 5 μm in diameter. The quadrupole magnet strengths used to focus the beam were adjusted to produce a focused 4.4 MeV proton beam for development of the Flow And ShooT (FAST) microfluidics system, described below, and for irradiation of *C. elegans* nematodes.

**Focused x-ray microbeam**

We have developed a microbeam to provide characteristic $K_{\alpha}$ 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.

A small x-ray source is produced by bombarding a Ti target with 1.8 MeV protons using an electrostatic quadrupole quadruplet lens to focus the beam to ~50 x 120 μm on the target. The x rays used are emitted at 90° to the proton beam direction. A zone plate is used to focus the x-ray source to a beam spot 5 μm in diameter. The system is 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 charged particle microbeam systems.

A new quadrupole quadruplet lens with an 8mm bore, significantly larger than the 2 mm bore on the previous quadruplet, has been constructed and installed. The new lens has five ground planes, one between each set of electrodes (five), providing better definition of the electric field than the previous lens, which only had ground planes

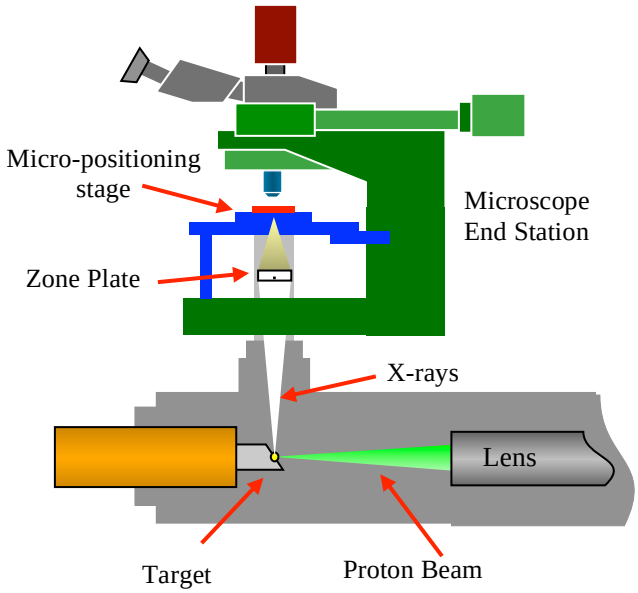


***Figure 1**: Schematic layout diagram of the x-ray microbeam endstation.*

at the ends and between the 2nd and 3rd elements. The increased bore allows much higher proton beam currents to be obtained (>2 μA), greatly increasing the dose rate. We have begun irradiations of cell nuclei with the focused x-ray beam to observe foci formed by single-strand repair proteins tagged with GFP in XRCC1 cells, from our collaborator David Chen at the University of Texas Medical Branch, and γH2AX foci in normal human fibroblasts.

**Neutron microbeam**

Neutrons produced by the $^{7}$Li(p,n)$^{7}$Be 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. 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, which will stop the incident proton beam. Using a 1.886 MeV proton beam, thin samples in contact with the target backing will be exposed to a beam of neutrons 20 μm in diameter having energies from 10-50 keV. This will be the first neutron microbeam in the world.

The facility is being constructed on a dedicated horizontal beamline using a quadrupole quadruplet to focus the proton beam. The microscope, stage, video camera and computer systems already have been assembled and tested. Construction of the support and alignment system for the quadruplet lens and the LiF target has been completed and installation of the beam line is about to proceed.

As soon as the beamline is completed, the voltages on the quadruplet lens will be adjusted to produce a small focused proton beam. In order to measure the beam spot size, a thin Havar metal window will be used in place of the gold target. The protons will pass through this window and the beam spot size will be determined in the same manner as for the particle microbeams: a knife-edge scan using thin Havar strips. Unlike the other microbeams in which the charged particle or x-ray fluence is low, the proton beam current for the neutron microbeam will be at least 1 nA ($6x10^9$ protons/s – too high to count) so an ionization chamber, instead of a solid state detector, will be used to observe the change in the proton beam as it is scanned.

**Non-scattering particle detector**

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

An under-dish detector design was investigated several years ago in which an aluminum electrode was evaporated on one side of a thin silicon wafer and three parallel gold electrodes were evaporated on the opposite side, with only a small horizontal gap between the ends of the electrodes. The prototype detector produced a usable signal but was fragile, even with a 10 μm thickness, and was broken in use. We have been unable to obtain wafers thinner than 10 μm and efforts to thin down these wafers to ~2 μm have been unsuccessful.

Other thin detector designs are being investigated in collaboration with the Mechanical Engineering Department of Columbia University. In one design, an amorphous silicon layer ~1 μm thick, was deposited on the surface of a silicon nitride microbeam vacuum window. The detector behaved as a diode, as expected, however no pulses were observed when the detector was traversed by $^4$He ions. This design may be investigated further since such a thin silicon layer might not absorb enough energy to produce a usable signal; a thicker silicon layer and reduced electronic noise might make this design useful.

**Advanced imaging systems**

We continue to develop new imaging techniques to obtain two- and three-dimensional images of cells without using stain. This is of great importance for the microbeam irradiation facilities in order to avoid damage to the cells, to maintain physiological conditions, and to image thick samples, especially small animals, for targeting and observation.

**SIMI**

Immersion-based Mirau interferometry (IMI) was developed at RARAF by constructing an objective to function as an immersion lens using standard interferometric techniques; however interferometry is very sensitive to vibrations, even as small as a fraction of a wavelength. Although this system provides usable images in a vibration-free environment; on the electrostatic microbeam endstation small vertical motions due to vibrations in the building greatly reduce the image quality, and passive and active systems to reduce these vibrations were unsuccessful.

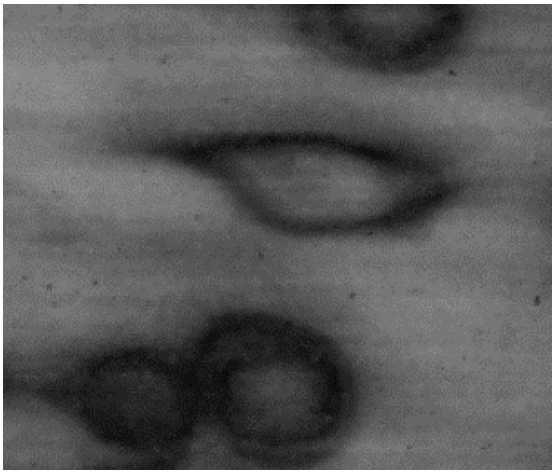

***Figure 2****. Simultaneous Immersion Mirau image of HT 1080 fibrosarcoma cells expressing the XRCC1 gene. The cells were plated on glass slides and imaged in phosphate-buffered saline.*

Recently, 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 by use of 1/8 wavelength (λ/8) waveplates. A polarization beam splitter is used to send the x and y components to form interferograms on a single camera. Since the images are taken simultaneously, there is no effect from vibration. This system is also much faster than Immersion Mirau because only one image is necessary instead of four images at different distances, requiring 3 vertical movements of the stage.

New optical elements were fabricated to adapt the Immersion Mirau objective for Simultaneous Immersion Mirau Interferometry. The small, thin glass discs required (8 mm D, 0.2-0.3 mm thick) were coated, either as spot mirrors or partially reflective (10-85%) beam splitters. This year the discs have been assembled into 2- or 4-piece elements with 1/8λ polycarbonate film waveplates sandwiched between the glass discs. These elements have been installed in the SIMI objective housing, designed and constructed in our machine shop, and the value of the partially reflective beam splitter that produces the best image has been determined. SIMI will be incorporated

into the sub-micron microbeam endstation as an imaging option.

**UV microspot**

A multi-photon microscope was developed and integrated into the microscope of the Microbeam Facility several years ago 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 and *C. elegans*. Two photons delivered very closely together in space and time can act as a single photon with half the wavelength (twice the energy). The longer wavelength of the light beam allows better penetration into the sample while still being able to excite fluorophors at the focal volume and less damage is produced in the portion of the sample not in the focal volume. This system also can be used as a laser "microspot" to induce UV damage in the focal volume of the laser. Several users, both internal and external, have made use of this facility this year, particularly for 3-D imaging.

**Targeting and manipulation of cells**

We have purchased a micro-milling machine and a variety of small mill and drill bits. This system was used to manufacture the single cell dispenser and microfluidics chips for the FAST. It eliminates the need to use Mechanical Engineering Department facilities at the Morningside campus of Columbia.

**FAST**

A Flow And ShooT (FAST) targeting system based on microfluidics is being developed to increase the throughput of the microbeam and to provide irradiation of non-adherent cells, such as lymphocytes, that do not plate on surfaces and therefore do not have stable positions.

Cells moving through a narrow capillary are imaged by a high-speed camera to track their trajectory. The point-and-shoot system is used to aim the particle beam to the projected position of the cell on the trajectory and the particle beam is enabled. The deflection coil currents are changed continuously to follow the path of the cell until the required number of particles is delivered. The final system will be capable of tracking several cells at a time.

We have manufactured polydimethylsiloxane (PDMS) microfluidic chips using soft lithography. The channel has a width of 200 µm and height of 20 µm, so that the cells, when targeted by the microbeam, flow in the immediate vicinity of the exit window. The bottom of the irradiation section of the microfluidic channel is 10 µm thick and the top is 20 µm thick, so particles can reach the cells and the detector above the channel. The flow rate is controlled by a syringe pump.

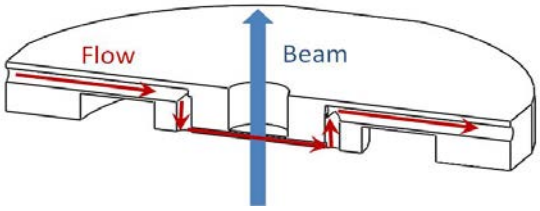


***Figure 3****. FAST microfluidics chip cross section.*

Initial tests with fluorescent beads flowing through the channel and imaging at 25 frames/s resulted in the predicted bead position being within 1 µm of the actual position 90% of the time and within 2.5 µm 98% of the time. A new fast camera has been installed that allows imaging of the irradiation area at up to 1,000 frames/s. The increased imaging speed will make possible more accurate cell targeting and permit a higher flow rate, increasing throughput to as high as 100,000 cells/hr.

During the past year the permanent magnet lens system was tuned to reduce the diameter of the 4.5 MeV proton beam spot and the beam alignment was adjusted to minimize changes in the beam spot size with position when the beam is deflected.

**OET**

A novel cellular manipulation technique is being adapted to irradiate non-adherent cells on the sub-micron microbeam endstation. We are developing an OptoElectronic Tweezer (OET) system, initially developed by our collaborators, the Ming Wu group at Berkeley National Laboratory.

The OET consists of two parallel-plate electrodes. The top electrode is Indium Tin Oxide (ITO), which is transparent and is covered with a 1 µm thick layer of hydrogenated amorphous silicon (a-Si:H) that acts as a photoconductive layer. When light is focused on the surface of the a-Si:H, the conductivity of the layer increases by several orders of magnitude. By patterning a dynamic light image on the electrode, a reconfigurable virtual electrode is created. When the virtual electrode and its opposing plate electrode are biased with an AC voltage, a non-uniform AC field is created.

In the presence of a non-uniform electric field, a dielectric particle (*e.g.*, a cell) will feel a force caused by dielectric polarization (dielectrophoresis, DEP). The conductivity of the fluid in the chamber must be carefully controlled as it will strongly affect the electric field in the fluid layer of the OET device. If the resistance of the fluid layer is less than that of the a-Si:H, then the voltage drop will occur in the a-Si:H layer, and the DEP effect will be reduced in the liquid. The direction of the force is a function of the AC voltage frequency and the fluid conductivity. Below a certain frequency the cells are attracted by the force; at higher frequencies they are repelled.

Initial tests demonstrated the ability to manipulate fluorescent beads, moving them around with an image projected by a laser; tests this year have been performed using computer-generated images projected into the microscope using a standard LCD projector. To better understand the system, the finite element software COMSOL Multiphysics Simulation was used this year to simulate the electric field in the ET device.

**Cell dispenser**

Another cell manipulation device that is under development is a single cell dispenser. The dispenser consists of a microfluidic channel in which selected cells can be dispensed into a multi-well plate. In a system where cells normally travel across a T-intersection, a pressure pulse can eject a droplet containing a single cell as it passes the nozzle. The pressure pulse is generated by applying a pneumatic backpressure and quickly opening and closing a solenoid valve. The size of the droplet is determined by the precise control of the timing of the opening and closing of the valve and the applied liquid backpressure. The valve requires a 12 V 'peak' voltage pulse 150 μs long to open the nozzle, and a hold voltage to keep it open for the specified amount of time. A function generator specifies the length of the pulse and a microprocessor applies the 'peak' and 'hold' voltages to the solenoid. The device is made from a polymethyl methacrylate (PMMA) slab with 100 μm x 50 μm channels directly milled using a micromilling machine.

Presently this system is manual and very laborious. An automated system is being developed to control the dispenser based on the fluorescence of the cells or other selected criteria.

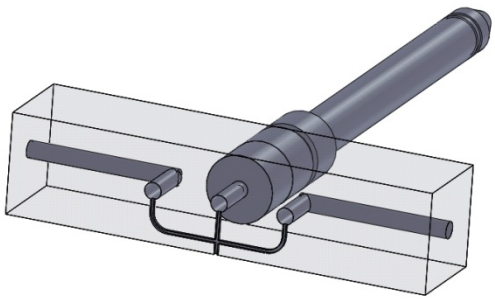

***Figure 4****. CAD model of the dispenser.*

**Small animal systems**

*C. elegans* is a multi-cellular eukaryotic organism that is simple enough to be studied in great detail and is well-established as a research tool. From a practical perspective, it is small enough (~100 μm diameter, ~1 mm long) to be compatible with microbeam irradiation and a wide variety of mutants and transgenics are readily available, as is a large community of *C. elegans* researchers.

Initial irradiations required worms to be anesthetized and manually handled individually, a slow and laborious process. In order to provide high-throughput irradiations, we have developed, in collaboration with the Whitesides group at Harvard University, a microfluidic worm clamp for rapid immobilization of large numbers of live worms for morphological analysis and fluorescence imaging. We have manufactured worm clamps with four channels and a 10 μm thick PDMS bottom to allow charged particle penetration. We have begun using these chips for anesthetic-free irradiation of *C. elegans* worms and have seen that the worms indeed remain immobilized. The clamp design is being expanded to accommodate 16 worms with a possible further expansion to 64 worms

**New neutron source**

Development has begun on a fast neutron source with a broad spectrum that will emulate that of the "Little Boy" atomic bomb at Hiroshima. The neutron spectrum will extend to 10 MeV. A mixed beam of 5 MeV monatomic, diatomic and triatomic protons and deuterons will be incident on a thick beryllium target, producing neutrons from the $^{9}Be(d,n)^{10}B$ and $^{9}Be(p,n)^{9}B$ reactions. The diatomic and triatomic particles break up on contact with the target into individual ions with 2.5 MeV and 1.67 MeV energies, respectively, enhancing the lower-energy portion of the spectrum. In order to produce this mixed ion beam, a gas source with a specific ratio of hydrogen to deuterium will be placed in the terminal of our Singletron accelerator and a new, 0º beam line has been installed. Since this beamline does not involve deflecting the particle beam from the accelerator, there will be no separation of different ions and the full beam from the accelerator will be utilized.

The beryllium targets have been purchased and a high-capacity water-cooling system has been designed and constructed. Studies of the focusing of the ion beam have begun.

Because the area in which this source will be located was not originally intended for such a strong source of neutrons, additional shielding will have to be installed and a radiation survey performed.

**Singletron Utilization and Operation**

Table II summarizes accelerator usage for the past year. The Singletron normally is started between 8 and 9 am and the nominal accelerator availability is one 8-hour shift per weekday (~250 shifts per year); however the

***Table II.*** *Accelerator Use, January - December 2011.*
*Usage of Normally Scheduled Shifts*

| | |
|---|---|
| **Radiobiology and associated dosimetry** | **22%** |
| **Radiological physics and chemistry** | **4%** |
| **On-line facility development and testing** | **14%** |
| **Microbeam Training Course** | **4.5%** |
| **Safety system** | **2%** |
| **Accelerator-related repairs/maintenance** | **0.1%** |
| **Other repairs and maintenance** | **2%** |
| **Off-line facility development** | **40%** |

***Table III**. Students for the first RARAF Microbeam Training Course.*

| Name | Position | Affiliation |
|---|---|---|
| Anne Marie Adamczyk | Postdoctoral Associate | University of Tennessee, Knoxville, TN |
| Manuela Buonanno | Ph.D. student | New Jersey Medical School (UMDNJ), NJ |
| Stefanie Girst | Undergraduate | University of the Armed Forces, Munich, Germany |
| Ying Nie | Asst. Professor | Loma Linda University, CA |
| Mykola Onyshchenko | Postdoctoral Associate | NIH, MD |
| Diana Pignalosa | Scientist | GSI, Darmstadt, Germany |

accelerator is frequently run well into the evening, often on weekends, and occasionally 24 hours a day for experiments or development.

Accelerator use for radiobiology and associated dosimetry was about 25% more than for last year. About 72% of the use for all experiments was for microbeam irradiations, about 12% for track segment irradiations and 16% for neutron irradiations. Approximately 1/3 of the experiment time was for experiments proposed by external users, and 2/3 was for internal users.

On-line facility development and testing was about 14% of the available time, primarily for development and testing of the electrostatically focused microbeam, the x-ray microbeam and the Flow And Shoot (FAST) system. This was significantly less use than for the last number of years because many of our current projects deal with imaging and cell manipulation and therefore do not require the use of the accelerator for much or all of their development.

There was only a fraction of a shift of Singletron maintenance to investigate a small leak in the insulating tank gas. The accelerator was not opened for maintenance or repair this year. This is the lowest amount of time spent on accelerator repair since RARAF was moved to Nevis Laboratories in 1982. The Singletron charging system continues to be very stable and reliable.

**Training**

We continue to participate in the Research Experiences for Undergraduates (REU) project in collaboration with the Columbia University Physics Department. For 10 weeks during the summer students attend lectures by members of different research groups at Nevis Laboratories, work on research projects, and present oral reports on their progress at the end of the program. Among other activities, the students receive a seminar about RARAF and take a tour.

This year Hamin Jeon from Emory University in Atlanta, Georgia participated in the program and worked with Alan Bigelow on UV sterilization of human wounds.

**Microbeam Training Course**

The first RARAF microbeam training course "Single-Cell Microbeams: Theory and Practice" (http://raraf.org/microbeamtraining.htm) was given from


***Figure 5**. The students for the first RARAF Microbeam Training Course at the Singletron accelerator console.*

May 3-5 this year. This three-day course consisted of lectures, demonstrations, and hands-on experience. The course was designed for specific needs (*e.g.*, imaging, designing a microbeam facility). Because of the intimate nature of the course, it was limited to 6 participants.

To assist in the design and operation of the course, we recruited Dr. Marcelo Vazquez of Loma Linda University Medical Center as the Director. Dr. Vazquez has had significant experience from his prior employment by NASA at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL). He helped establish the first NASA Space Radiation Summer School and assisted in running the course for three years. He is familiar with the requirements of educating students on the utilization of specialized irradiation facilities.

Notification of the course was made by e-mail using the contact lists for the 2008 and 2010 Microbeam Workshops. Flyers announcing the course were handed out at the 9th International Microbeam Workshop, July, 2010 in Darmstadt, Germany and at the 56th Annual Meeting of the Radiation Research Society, September 2010 in Hawaii and verbal announcements were made in sessions at these meetings.

**Students**

In response to our notifications, we received 17 applications with CVs. The prospective students covered a wide range of educational levels – from undergraduate to postdoc to scientist - and were from the U.S., Europe, the Middle East, and South America. Candidates were about evenly split in gender and field (physics or radiobiology). The six applicants selected for the course

***Figure 6****. From left to right: Steffi and Ying plating cells on Day 1; Anne Marie optically locating the microbeam beam spot on Day 2; Mykola making a slide for the γH2AX assay on Day 2; Anne Marie, Mykola, Manuela and Diana discussing results with Brian Ponnaiya on Day 3.*

are given in Table III and shown in Figure 5. No fee was charged for the course and some assistance was provided for travel, housing and food.

**Course**

Most of Day 1 involved lectures on the physics and biology of microbeams. The students received a tour of the various RARAF microbeam facilities. There was a demonstration of the procedure for plating cells on the special microbeam dishes, after which the students were divided into pairs with each person plating 3 dishes for irradiation on Day 2. Another demonstration showed the formation of foci in cells with a repair protein tagged with a fluorescent protein. After irradiation, fluorescent spots could be seen forming in seconds directly under the cross-hairs indicating the position of the irradiation. This very visual demonstration was so impressive it actually evoked gasps from the students.

Day 2 consisted mostly of demonstrations and hands-on work by the students. After observing the accelerator start-up and the characterization of the $^4$He beam, the students performed a γH2AX assay on cells that had been irradiated previously by Brian Ponnaiya. Between the steps in the assay, one pair of students alternately prepared their cells for irradiation or actually irradiated cells with the microbeam for micronucleus assay. They each also performed the procedure to optically locate the beam spot. The other pairs either had lunch or observed a demonstration of microbeam irradiation protocols and had a tour of the neutron and broad beam facilities at RARAF.

On Day 3, the students processed their cells for the micronucleus assay and then heard lectures on microbeam developments occurring at RARAF and on biological results obtained by RARAF users. That evening there was a group dinner at which the students received certificates of completion. Each student took home a notebook containing copies of all the slides from the lectures as well as the instructions on all the physics and biology procedures that were demonstrated and that they had performed.

At the end of the course, the students were given a questionnaire on which they rated various aspects of the course and gave comments. Students thought the course was interesting and informative. They gave high ratings to the content, program structure, hands-on activities and demos, relevance, the materials provided, actual irradiation of samples and sample preparation, and interaction with faculty.

A virtual course created from this training course is described in the Dissemination section below. A second course will be given next year in conjunction with the 10$^{th}$ Microbeam Workshop, to be held at Columbia University.

**Dissemination**

The content of our website is updated continually to reflect the current state of research at RARAF. For present users, the current month's accelerator schedule is posted and the Experiment Scheduling Request form can be filled out and printed for submission to request beam time. For prospective new users, there is information about the irradiation facilities at RARAF as well as forms and instructions for proposing new service or collaboration experiments. In the Dissemination section, we offer a list of papers detailing research performed at RARAF and published in peer-reviewed journals. Many of these papers, and almost all recent papers, are available in PDF format free from our web site.

To further disseminate general information about microbeam technology, we are active participants in Wikipedia. We have created or significantly expanded encyclopedic entries for a number of topics, such as microbeam, RARAF, and Mirau interferometry, and have encouraged others in the microbeam community to participate in these efforts as well.

We are developing a virtual microbeam training course, based on the three-day microbeam training course "Single-Cell Microbeams: Theory and Practice" held at RARAF in May of this year. For interested scientists unable to attend the course, our virtual microbeam training course allow users to tailor the curriculum to best meet their needs ─ providing concise summary information for an overview and more detailed knowledge in specific topics of interest. Eleven Powerpoint-based lectures from our three-day training course were recorded and converted into enhanced podcasts. Each podcast consists of audio, synched with the accompanying PowerPoint slides (viewable on a video iPod or a PC), as well as a PDF handout of the PowerPoint slides (http://raraf.org/educationalmaterials.htm).

We plan to augment these podcast lectures with video demonstrations and a virtual tour. High-resolution video (640x480 or higher, with audio) will be used to document

demonstrations of all aspects of a microbeam experiment from making microbeam dishes to irradiating cells and online analysis. The full microbeam training course is scheduled to be completed in time for the International Conference on Radiation Research.

The 10th International Workshop: Microbeam Probes of Cellular Radiation Response will be hosted at Columbia University March 15-17, 2012 by RARAF personnel with Alan Bigelow as the organizer. Over 80 participants from around the world have registered for the three-day Workshop. This workshop provides a forum for the microbeam community to come together and discuss the present and future of microbeam research. The third day of the Workshop will be a tour of the RARAF microbeam facilities. The RARAF web site will be the central repository for information on this meeting (http://raraf.org/meeting/index.html).

**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, retired this year and is now Professor Emeritus. He continues to spend most of the work-week at RARAF.

Dr. Brian Ponnaiya, an Associate Research Scientist, is the biology advisor for RARAF. He presently spends about half his time at the CRR.

Dr. Alan Bigelow, an Associate Research Scientist, is continuing the development of the multiphoton microscopy system, which includes the UV "microspot" irradiation facility, as well as Optical Electronic Tweezers for manipulating cells.

Dr. Guy Garty, a Research Scientist, is developing the Flow And Shoot (FAST) 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.

Dr. Andrew Harken, a Postdoctoral Fellow, is responsible for the x-ray microbeam. He is also working on the imaging of cells without stain using a highly sensitive EMCCD camera.

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 an accelerator-generated neutron source with a spectrum similar to that of the Hiroshima atomic bomb.

Sasha Lyulko, a graduate student in the Physics Department at Columbia University, is involved in developing the Simultaneous Immersion Mirau Interferometry (SIMI) system and also worked on imaging for the NIAID project.

Manuela Buonanno, a Postdoctoral Fellow, began working at RARAF in August.

Michael Grad, a graduate student in the Mechanical Engineering Department, has spent most of his time since September working at RARAF, making microfluidics chips for FAST, developing a single cell dispenser and working on the Optoelectronic Tweezers with Alan Bigelow.

**Recent Publication of Work Performed at RARAF**


1. Bigelow, A.W., Randers-Pehrson, G., Garty, G.,. Geard, C. R, Xu Y., Harken, A.D., Johnson, G.W. and Brenner, D.J. Ion, X-ray, UV and neutron microbeam systems for cell irradiation. AIP Conf. Proc. **1336**, pp. 351-355.
2. Fuks, E., Horowitz, Y.S., Horowitz, A., Oster, L., Marino, S., Rainer, M., Rosenfeld, A. and Datz, H. Thermoluminescence solid state nanodosimetry – the peak 5a/5 dosimeter. Radiat. Prot. Dosim. **143**: 416-426 (2011) PMCID: PMC310827.
3. Garty, G., Grad, M., Jones, B.K., Xu, Y., Randers-Pehrson, G., Attinger, D. and Brenner, D.J. Design of a novel flow-and shoot (FAST) microbeam, Radiat. Prot. Dosimetry **143**: 344-348 (2011) PMCID: PMC3108275.
4. Harken, A.D., Randers-Pehrson, G., Johnson, G.W. and Brenner, D.J. The Columbia University proton-induced soft x-ray microbeam. Nucl. Inst. Meth. B **269**(18): 1992-1996 (2011) PMCID: PMC3146766.
5. Hu, B., Grabham, P., Nie, J., Balajee, A.S., Zhou, H., Hei, T.K. and Geard, C.R. Intrachromosomal changes and genomic instability in site-specific microbeam-irradiated and bystander human-hamster hybrid cell. Radiat. Res. 2011 Nov 11. [Epub ahead of print].
6. Ivanov, V.N., Ghandhi, S.A., Zhou, H., Huang, S.X., Chai, Y., Amundson, S.A. and Hei, T.K. Radiation response and regulation of apoptosis induced by a combination of TRAIL and CHX in cells lacking mitochondrial DNA: a role for NFκB- and STAT3-directed gene expression. Exp. Cell Res. **317**: 1548-1566 (2011) PMCID: PMC2860693.
7. Marino, S.A., Johnson, G.W., Schiff, P.B. and Brenner, D.J. Modification of shirt buttons for retrospective radiation dosimetry after a radiological event. Health Phys. **100**: 542-547 (2011) PMCID: PMC3079536.
8. Mezentsev, A., Ming, L. and Amundson, S.A. Involvement of HNF4A in the low-dose radiation response of a human 3-dimensional tissue model. Radiat. Res. **175**: 677-688 (2011) PMCID: PMC3148653.
9. Miller, A.C. Development of models to study radiation-induced late effects. In "HFM Panel-099 RTG-033 Activity: Radiation Bioeffects and Countermeasures -The Radiation Bioeffects and Countermeasures RTG-033 Final Report", NATO, 2011. ■