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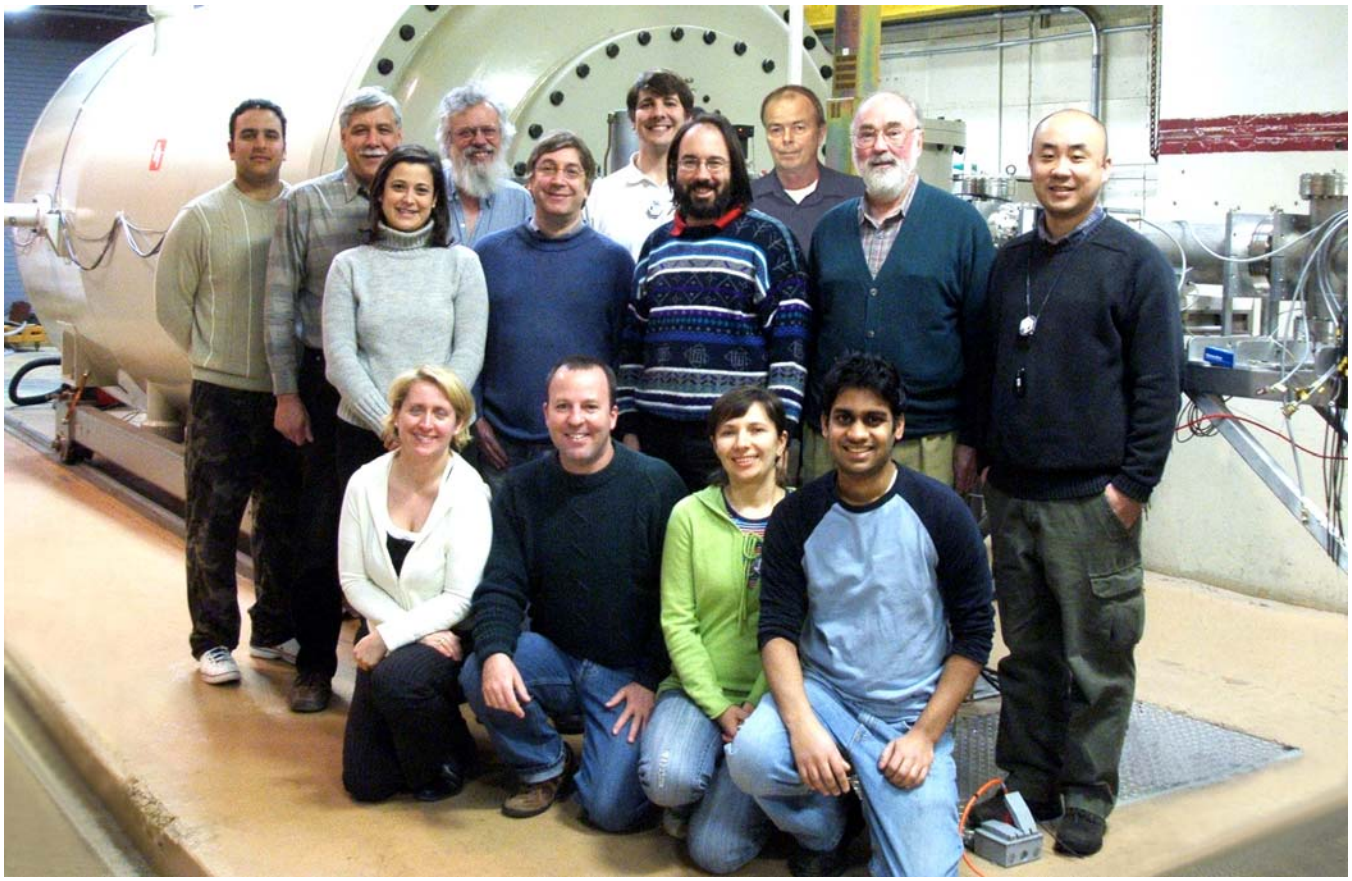
RARAF PROFESSIONAL STAFF



RARAF Staff *(l–r)*: *Front row*: Helen Turner, Alan Bigelow, Sasha Lyulko and Bharat Patel; *2nd* row: Antonella Bertucci, David Brenner, Guy Garty, Charles Geard and Yanping Xu; *3rd row*: Abel Bencosme, Stephen Marino, Gerhard Randers-Pehrson, Andrew Harken and Gary Johnson. Not shown: Brian Ponnaiya, Kenichi Tanaka and Gloria Jenkins-Baker.

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The Radiological Research Accelerator Facility

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

Director: David J. Brenner, Ph.D., D.Sc.
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Introduction

There have been several notable accomplishments at RARAF this year:

- The achievement of a sub-micron focused charged particle beam.
- Completion of the installation and upgrading of the two-color multiphoton system on the Microbeam Facility, the first multi-photon system installed on a microbeam for biological irradiations.
- Microbeam irradiation of small animals (C. elegans nematodes), including the first vertebrate irradiations (Japanese medaka embryos).
- A record amount of use of the accelerator for radiobiology and also for on-line development of facilities and procedures.

Research using RARAF

The "bystander" effect, in which cells that are not irradiated show a response to radiation when in close contact with or even only in the presence of irradiated cells, continues to be the main focus of the biological experiments at RARAF. Almost every biology experiment run this year, including those involving animals, examined this effect. The emphasis 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. 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.

A special type of track segment dish is being used to investigate cell-to-cell communication in the bystander effect using the Track Segment Facility. "Strip" dishes consist of a stainless steel ring with thin (6-μm) Mylar foil glued to one side in which a second dish is inserted. The Mylar foil glued to the 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, with tissue samples irradiated using either helium ions or protons. Imaging systems for the Microbeam Facility have been developed to enable observation and targeting of cells that are not in monolayers. Two animal systems have been irradiated using the Microbeam Facility this year: *C. elegans* nematodes and Japanese medaka embryos. In addition, cultured human tissue samples are being irradiated using the Track Segment Facility.

The experiments performed at RARAF from January 1 through December 31, 2008 and the number of days each was run in this period is listed in Table I. Fractional days are assigned when experimental time is shared among several users (e.g., track segment experiments) or experiments run for more or less than an 8-hour shift. Use of the accelerator for experiments was 56% of the regularly scheduled time (40 hours per week), about 85% higher than last year and the highest use we have attained at Nevis Labs. Thirteen different experiments were run during this period. Eight 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). Five experiments were performed by outside users, supported by grants and awards from the Department of Defense (DoD), NASA, and DoE. Brief descriptions of these experiments follow.

Exposure to ionizing radiation may induce a heritable genomic instability that leads to a persisting enhanced frequency of genetic and functional changes in the progeny of irradiated cells. Burong Hu and Charles Geard of the CRR continued their investigation into whether cytoplasmic irradiation or the bystander effect can also lead to delayed genomic instability (Exp. 103). The charged-particle Microbeam Facility was used for precise nuclear or cytoplasmic irradiation of normal human lung fibroblasts using 6 MeV ^{4}He ions. Their results show that the fraction of metaphase cells involving human chromosome 11 changes (including chromosome 11 rearrangement, entire chromosome 11 deletion and duplication) was significantly higher than that of the controls, not only after nuclear irradiation but also after cytoplasmic irradiation and in the bystander cell group. mBAND chromosome analyses of 15 clonal 1 isolates from each of the control, nuclear and cytoplasmic irradiations as well as the bystander cell group were conducted. The results show that unstable clones involving human chromosome 11 rearrangements arose in three irradiated groups. Clones from the control group remained stable. Further analyses showed there was no large change in the number of unstable clones over the time in culture in each irradiated group. The stability of the individual clones, however, changed with time in culture in some clones from the control and the irradiated groups. These results suggest that genomic instability fol-

Table 1. Experiments Run at RARAF, January 1 - December 31, 2008

Exp. No.	Experimenter	Institution	Exp. Type	Experiment Title	No. Days Run
103	B. Hu C. R. Geard	CRR	Biology	Damage induction and characterization in known hit versus non-hit human cells	14.8
106	B. Ponnaiya C. R. Geard	CRR	Biology	Track segment alpha particles, cell co-cultures and the bystander effect	0.3
110	H. Zhou M. Hong T. K. Hei	CRR	Biology	Identification of molecular signals of alpha particle-induced bystander mutagenesis	55.7
113	A. Miller	AFRRI	Biology	Role of alpha particle radiation in depleted uranium-induced cellular effects	0.2
114	M. Suzuki (H. Zhou)	NIRP, Japan	Biology	Bystander response in primary human bronchial epithelial cells using the G2PCC technique	2.3
133	S. Ghandhi B. Yaghoubian S. Amundson	CRR	Biology	Bystander effects in primary cells	7.3
136	A. Mezentsev S. Amundson	CRR	Biology	Bystander effects in 3D tissues	8.8
139	S. Amundson	CRR	Biology	Signal transduction in cytoplasmic irradiation	9.8
140	L. Han (T. K. Hei)	Shanghai Medical University	Biology	Study of the mechanism of radiation-induced inactivation of the FHIT gene by single cell microbeam irradiation	19.1
141	D. Chen, A. Asaithamby	Univ. of Texas Southwestern Medical Center	Biology	Visualization of recruitment of DNA damage markers to the sites of DNA damage induced by microbeam irradiation	7.3
142	W. Dynan, W. Kuhne (A. Bertucci)	Medical College of Georgia	Biology	Proton irradiation of Japanese medaka embryos *in vivo*	5.5
143	W. Dynan, W. Kuhne	Medical College of Georgia	Biology	Accumulation of PSF p54 (nrb) at sites of human cells *in vitro*	1.5
144	A. Bertucci	CRR	Biology	Microbeam irradiation of *C. elegans*	8.0

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

lowing ionizing radiation exposure is not dependent on direct damage to the cell nucleus.

Brian Ponnaiya and Charles Geard of the CRR continued another study investigating the bystander effect (Exp. 106). The Track Segment Facility was used for broad-beam charged particle irradiations with 125 keV/μm ^{4}He ions to examine genomic instability in irradiated and bystander htert immortalized human bronchial epithelial cells (HBEC-3kt). These cells were cultured on standard single-sided Mylar track segment dishes and irradiated with half the dish covered by a thin metal shield. Cells on the non-covered portion of the dishes were irradiated with ^{4}He ions, while cells on the covered portions of the dishes were bystander cells. Irradiated and bystander populations from each dish were separated and set up in culture. At various times post irradiation G2-PCCs were prepared from each culture. The chromosomes were analyzed by both Giemsa staining (for gross chromosomal aberrations) and mFISH for more subtle alterations (e.g. translocations).

Efforts to identify the cell-to-cell signaling transduction pathways involved in radiation-induced bystander responses (Exp. 110) were continued by Hongning Zhou, Mei Hong and Tom Hei of the CRR. Using the Microbeam Facility to produce a focused 6 MeV ^{4}He beam, they have found that oxidative stress as well as lipid peroxidation is induced after cytoplasmic irradiation. The product of lipid peroxidation, 4-hydroxynonenal, may be able to induce downstream activation of MAPK pathway(s) and, in turn, expression of cyclooxygenase-2. Using the Track Segment Facility for irradiation with 125 keV/μm ^{4}He ions, they have found that PKC is translocated from cytosol to membrane both in directly-irradiated and bystander cells. In addition, free fatty acid content is elevated in both irradiated and bystander cells. Since fatty acids have been shown to induce translocation of PKC from cytosol to membrane, they may serve as possible signal molecules mediating bystander effects. In other experiments, several different human and rodent cell lines were irradiated using both irradiation facilities. The preliminary data indicate that mitochondrial function is critical for the radiation-induced bystander effect.

Exposure to depleted uranium (DU) during military operations or a terrorist attack is a concern for military personnel and civilians. The long-term health effects of low-dose high-LET radiation exposures are not well known. Furthermore, development of pharmacological countermeasures to low-dose radiological contamination is important for the health and safety of both military and civilian populations. 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 low-dose radiation- or DU-induced carcinogenesis using *in vitro* and *in vivo* models and to test safe and efficacious medical countermeasures (Exp. 113). A third goal of this study is to identify biomarkers of both exposure and disease development. New approaches in her laboratory have enabled her to add an additional goal, which is to identify the mechanisms involved in these processes. These experiments include survival, neoplastic transformation, mutagenicity, genomic instability, and genotoxicity studies. Recently she has begun to evaluate the effect of LET on radiation-induced leukemia.

Masao Suzuki of the National Institute of Radiological Science, Japan in collaboration with Hongning Zhou of the CRR resumed a study to determine whether alpha particle irradiation in the cytoplasm can induce a bystander response in primary human bronchial epithelial cells using the G2PCC technique (Exp. 114). A fraction of the cells were irradiated in the nucleus with 6 MeV ^{4}He ions using the Microbeam Facility. The cells were then accumulated in the G2 phase of the cell cycle and the process of premature chromosome condensation was used to observe chromatin aberrations.

A group led by Sally Amundson of the CRR continued three experiments concerning radiation-induced gene expression profiles in primary human fibroblast and epithelial cell lines using cDNA microarray hybridization and other methods. The first experiment, performed by Shanaz Ghandhi and Benjamin Yaghoubian, involved use of the Track Segment Facility for comparison of gene expression responses to direct and bystander irradiation (Exp. 133). Human fibroblast cells (IMR90) and epithelial cells: (HBEC-3KT and SAEC) were plated on "strip" dishes (described above) for direct-contact bystander irradiations. The cells were irradiated with 0.5 Gy of 125 keV/μm ^{4}He ions and assayed for micro-nucleus formation. Using the fibroblast model, they have identified potential genes and pathways that are being validated by real-time PCR. They continue to work on the identification of potential genes of interest in epithelial cells from microarray studies.

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) precisely imitates the structure of the epidermis. It 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* (e.g. for biodosimetry) 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. The tissue samples are grown on membranes on the end of cylindrical plastic holders. Plastic discs have been constructed that fit in the dish openings in the irradiation wheel and have small holes to provide precise alignment of the feet that are around the bottom edges of the tissue holders. A hole in the middle of each disc is fitted with two stainless steel half-discs that have a precise 0.001" (25 μm) space between them and are thick enough to stop the charged particles. This provides a narrow line of irradiation across the center of the entire sample. 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 used the Microbeam Facility to irradiate either the nuclei or the cytoplasm of normal human fibroblasts with 6 MeV ^{4}He ions and extracted RNA with the goal of performing global gene expression profiling 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 had initial success in amplifying and labeling small quantities of RNA (from about 2000 cells per sample) and has hybridized this amplified RNA from microbeam experiments to whole genome microarrays. This work is continuing in order to obtain a sufficient number of biological repeats for meaningful data analysis. She is beginning to validate the microarray results for individual genes using quantitative real-time PCR.

Ling Han of the Second Military Medical University, Shanghai, China, in collaboration with Dr. Tom Hei of the CRR, completed an experiment to determine the expression, injury and signal transduction of the FHIT (Fragile Histidine Triad) gene (Exp. 140). The Microbeam Facility was used to irradiate cells in the nucleus, the cytoplasm or the culture medium with 6 MeV ^{4}He ions. In other irradiations, only a fraction of the cells were irradiated and the co-cultured unirradiated (bystander) cells were examined. FHIT gene function was studied at different stages over 50 generations after irradiation and the role FHIT plays in cell transformation was examined in any transformed cells detected.

David Chen and Aroumougame Asaithamby of the University of Texas Southwestern Medical Center initiated an experiment to observe 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 conducted the first vertebrate irradiations using the RARAF Microbeam Facility (Exp. 142). Japanese medaka fish 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 and shipped to RARAF. At the time of irradiation embryos were at Stage 27-28 (representing the 24 – 30 somite stage). 4.5 MeV protons were delivered at fluences of 10,000 or 20,000 protons to areas of the brain using beam diameters of 25, 50 and 100 μm. The protons have a range of only ~280 μm and stop in the embryos. The embryos were then 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. The average number of TUNEL-positive cells for the 50 μm beam treatment group was 62±16 for embryos irradiated with 10,000 protons, and 185±36 for embryos irradiated with 20,000 protons. Statistical analysis conducted using a t-test indicates that the difference between the 20,000-proton group and the other two (including controls) were significant ($p<0.01$). Similar analyses will be completed for the treatments using a 25 and 100 μm beam diameter. Future work will address response using later stage embryos and incorporate the use of histological sectioning and staining to investigate the presence of a peak of tissue damage corresponding to the proton Bragg peak. Additionally, cellular and DNA damage effects will be investigated in neighboring regions not traversed by the charged particle beam.

In another experiment, William Dynan of the Medical College of Georgia irradiated the nuclei of two human cell lines using the Microbeam Facility (Exp. 143). The purpose of this experiment was to investigate the role of polypyrmidine tract binding protein-associated splicing factor (PSF) and p54(nrb) in the repair of radiation-induced DNA damage. PSF and p54(nrb) each contain tandem RNA-recognition motifs (RRMs) and interact to form a stable complex, which has multiple functions in RNA biogenesis. The PSF p54 complex enhances DNA end-joining in vitro, suggesting that these proteins might also be involved in DNA double-strand break repair. HeLa and HCT 116 cells transfected with mCherry-Ku80 (positive control), PSF-dsRed and p54-AcGFP were irradiated in the nucleus with 100 and 200 6-MeV ^{4}He ions. Using fluorescent microscopy, the mobilization of the PSF and p54 to the site of DNA double-strand breaks can be monitored.

The first animal irradiations using the Microbeam Facility were performed for an experiment by Antonella Bertucci of the CRR (144). For this project the strain SJ4005 zcls4 hsp-4::gfpV was used, which has a transcriptional reporter for the hsp-4 gene. Under normal conditions, the green fluorescent protein (GFP) expression is most prominent in the spermatheca, the tail and pharynx. Transcription of the hsp-4 gene is induced in the gut and in the hypodermis upon endoplasmic reticulum (ER) chemical stress. Young adult *C. elegans* were exposed to a 3 MeV proton microbeam with a 1μm diameter beam spot. Animals were placed in specially designed microbeam dishes under anesthesia and irradiated with different numbers of particles. Each worm was exposed in one or more anatomical regions (tail, pharynx or spermatheca). Samples were collected after exposure and re-cultured for GFP expression evaluation at 24 hours. Initial analysis of the results indicates that worms exposed to proton microbeams delivered at precisely targeted regions elicit a distal bystander effect characterized by a GFP over-expression after 24 hours.

Development of Facilities

This year our development effort continued on a number of extensions of our facilities:

- Development of 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 April, the compound electrostatic quadrupole triplet lens system installed at the end of 2006 was used to produce a sub-micron diameter focused ^{4}He ion beam at RARAF for the first time! A beam diameter of ~0.8 μm can now be obtained routinely. The beam can be used to target sub-nuclear structures in cells. The major goal of the focused lens development now has been achieved.

The original quadrupole triplet lens used for the microbeam from 2003 to 2007 and another quadrupole triplet that was assembled in December have been inserted in a second lens alignment tube. This tube has been modified for the installation of the deflection coil for the Point and Shoot system and will replace the existing lens system in February, 2009. The point and Shoot system, will direct the microbeam to the target position rather than moving the cells to the beam, greatly decreasing the time required.

The permanent magnet microbeam (PMM) uses a compound quadrupole triplet lens made from commercially available precision permanent magnets. It is similar to the one designed for the sub-micron microbeam, the major difference being that it uses magnetic rather than electrostatic lenses. Because the magnet strengths are essentially fixed, only a single energy (5.3 MeV) proton or ^{4}He ion beam can be focused.

The quadrupole magnet strengths used to focus the beam have been adjusted to produce 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 0.3 mm in diameter, a

beam of 5.3 MeV ^{4}He ions has been focused into a spot 5 μm in diameter (a demagnification of x60, compared to the theoretically attainable x100). A miniature Hall probe will be used to map the magnetic fields of the lenses to look for aberrations and determine the octupole moment of the lenses, both of which would interfere with focusing.

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 was used to test the Point and Shoot system.

Non-scattering particle detector

Currently the RARAF microbeam irradiator delivers a precise number of particles to thin samples by counting the particles traversing them using a gas proportional placed immediately above the cells. To irradiate samples thicker than the range of the incident ions, a completely non-scattering particle detector is necessary upstream of the samples. The Lumped Delay Line Detector (LD^2) is a novel 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. If the capacitance is set such that the propagation velocity of the pulse equals the projectile velocity, the pulses capacitively induced in all segments by the passage of a single charged particle will add coherently, resulting in a fast electron pulse at each end of the delay line.

The detector constructed at the end of 2007 was placed in a horizontal beam line for testing with a ^{4}He ion beam. The noise in the Amptek low-noise pre-amplifier is still large enough that the signal pulse, which consists of only 125 electrons, cannot easily be seen. In addition, there are reflections when an electronic pulse is provided to the detector, indicating a mismatch between the impedance of the detector and the input of the preamplifier.

Efforts are being made to cool the input FET on the detector amplifier to reduce the inherent thermal noise and enhance the signal-to-noise ratio. Calculations using the computer program AIMSPICE to simulate the electronic behavior of the LD^2 are underway to determine the best termination system for the signal from the detector in order to reduce "ringing" and signal loss. One of the prototype detectors will be taken to the Edwards Accelerator Laboratory in Athens, Ohio in March to be tested with a pulsed particle beam. A single nanosecond pulse will contain more than 1,000 protons and provide a signal at least 500 times larger than will be produced by a single He^{++} ion, making it much easier to tune the signal termination and determine the signal amplitude.

After the full-length detector is tested, it will be mounted between the two electrostatic lenses in the electrostatic microbeam and become the standard detector for all microbeam irradiations.

Advanced imaging systems

Development continued on new imaging techniques to view cells without using stain and to obtain three-dimensional images of unstained cells.

The immersion-based Mirau interferometric (IMI) objective has been designed 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. A custom Mirau objective was constructed in our shop in 2007 and several beamsplitters of different reflectivity (5-85%) were combined with spot mirrors into separate modules so that they can easily be interchanged in the lens.

Interferometry is very sensitive to vibrations, even as small as a fraction of a wavelength. A vibration-free environment provides usable images with this system; however on the Microbeam II 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.

The feasibility of a new approach using Simultaneous Immersion Mirau Interferometry has been demonstrated to overcome the vibration problem. 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.

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 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.

Initially a Chameleon (Coherent Inc.) tunable titanium sapphire laser was the source for the multi-photon excitation. This year the laser was upgraded to a Chameleon Ultra II, which has a wider 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 an array of photomultiplier tubes (PMTs). The system has been used to observe a GFP-tagged XRCC1 DNA single-strand break repair protein in real time for the experiments by David Chen (exp.141).

A housing that enables use of two PMTs, and therefore the acquisition of simultaneous images from two fluorophors, has been constructed and mounted on the microbeam microscope. Two PMTs allows investigation of fluorescence resonance energy transfer (FRET). Molecules labeled with two fluors normally widely separated can change their con-

formation by phosphorylation, positioning the two fluors near each other. In this close proximity, the emission from one fluor can excite the other, changing the ratio of emissions from the two fluorophors, giving a measure of the amount of phosphorylation in the sample. Initial experiments to observe FRET initiated by microbeam irradiation have begun.

Another potential use for the multi-photon system is fluorescent recovery after photobleaching (FRAP). 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.

Targeting of cells

During irradiation, cells to be irradiated are moved to the beam position using the microbeam stage. When a collimated microbeam was being used, this was necessary but relatively time-consuming. 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 used to observe the cells during irradiation much faster than moving the stage.

We are developing 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). This magnet system has been used for the microbeam facility at Gesellschat für Schwerionenforschung (GSI), Darmstadt, Germany. Two Kepco BOP power amplifiers are used to drive the coils. A short section of beam line was constructed around which the coil was placed and the assembly was mounted just below the upper quadrupole triplet on the PMM for preliminary testing. The deflection of the beam is linear with coil current and does not affect the beam spot size. A similar coil has been mounted in the lens tube for a second compound electrostatic quadrupole triplet, just below the second lens, and will be installed in the Microbeam II beamline in February.

Focused X-ray microbeam

We are developing an X-ray microbeam to provide characteristic K_α X rays generated by proton-induced emission (PIXE) from Ti (4.5 keV). 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. Charged particle beams can generate nearly monochromatic X rays because, unlike electrons, they have a very low bremsstrahlung yield.

At the suggestion of one of the members of our Advisory Committee, in 2007 we changed from a transmission design, in which the X rays used are emitted in the direction of the proton beam, to a reflection design, in which the X rays used are emitted at 90° to the proton beam direction. This eliminated several problems inherent in the previous design. 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.

A small X-ray source (~20 μm D) will be 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. A zone plate will be used to focus the X-ray source to a beam spot 1-2 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 zone plate has been received and assembled in its mounting structure. Preliminary measurements were performed using a proton beam in Microbeam II focused to 10 μm to produce X rays from a thin Ti foil. The zone plate structure was mounted above the foil and scanned with an aperture to determine the X-ray beam spot size. A beam spot ~14 μm in diameter was measured, which was expected for the test geometry.

The beam line, including the electrostatic lens, has been assembled and initial tests of the focusing of the proton beam are underway. The microscope and micropositioning stage have been assembled and will soon be put in place. A focused X-ray beam should be available this spring.

Neutron microbeam

Calculations and preliminary measurements have been undertaken for a 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 target. The backing material will be Au or Pt, ~15-17 μm thick. Using a 1.890 MeV proton beam, thin samples in contact with the target backing should be exposed to a beam of neutrons 10-12 μm in diameter.

Initial measurements using CR-39 track etch plastic and, in collaboration with Mark Akselrond and Jeff Sykora of Landauer, Inc., fluorescent nuclear track detectors (FNTDs) are being performed to demonstrate the restricted nature of the neutron emission.

Singletron Utilization and Operation

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

Accelerator use for radiobiology and associated dosimetry was about 85% higher than last year and was the highest level of use since RARAF has been at Nevis Labs, about 20% higher than the previous record. About 74% of

Table 2. Accelerator Use, January–December 2008
Usage of Normally Scheduled Days

Radiobiology and associated dosimetry	56%
Radiological physics and chemistry	0%
On-line facility development and testing	62%
Safety system	2%
Accelerator-related repairs/maintenance	3%
Other repairs and maintenance	2%
Off-line facility development	30%

the use for all experiments was for microbeam irradiations and 26% for track segment irradiations. Demand for the Microbeam Facility has increased because it enables selective irradiation of individual cell nuclei or cytoplasm, making it essential for many of the experiments examining the bystander effect. Because of the relatively low number of cells that can be irradiated in a day, microbeam experiments usually require significantly more beam time than broad beam (track segment) irradiations to obtain sufficient biological material, especially for low probability events such as mutation and bystander effects.

Use of the Track Segment Facility was 22% of the experiment time, about the same as last year. Irradiation times for individual samples are usually 30 seconds or less, so that multiple experimenters, as many as 5, 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 one day every other week.

For the first time since 2000, there was no utilization of the accelerator for radiological physics. The experiment of last year by Elenea Aprile examining the response of a liquid xenon detector has likely finished and the irradiations of TLDs by Yigal Horowitz have paused for the time being.

On-line facility development and testing surged to 62% of the available time, a new record, 40% higher than last year and 30% higher than the previous record. This includes development and testing of the electrostatically focused microbeam, development of the PMM, the Point and Shoot system, the LD2, the X-ray microbeam, the neutron microbeam and development of new biological techniques (e.g. FRET).

Approximately 26% of the experiment time was used for studies proposed by outside users, about 30% less than what was used last year and 25% less than the average for the last five years.

Online development use increased by about 40% over last year and was at a record level, about 30% higher than the previous record. In addition to beam tests and development of the electrostatically focused microbeam, considerable effort was expended on minimizing the beam spot diameter for the permanent magnet microbeam (PMM) and developing the X-ray microbeam.

There were only 8 shifts of Singletron maintenance and repair time this year, less than 2/3 that of last year. This is equal to the lowest it has been in the last 20 years. We reinstalled the GVM that was sent at the end of 2007 to the manufacturer, High Voltage Engineering Europa (HVEE), for repair. There was also a replacement of some of the column resistors and diodes damaged by a vacuum excursion that occurred when a microbeam exit window broke. The accelerator charging power supply is so stable that we continue to run without terminal voltage regulation by setting the charging current to obtain the desired terminal voltage. The ion source has run very reliably and required no maintenance this year.

Because the beam energy acceptance is so narrow for the electrostatic microbeam and the PMM, beam intensity decreases rapidly as the terminal potential changes by less than a kilovolt. In 2007 a remote computer terminal was installed in the Microbeam II lab to allow the accelerator terminal voltage to be controlled from both the console and the lab. This year a remote computer terminal was installed for the PMM.

The Use of Wikis for Scientific Dissemination at RARAF

Much dissemination of information today involves electronic media, for example, online journals (including electronic publication of new articles), WIKIs, blogs, and web sites. The RARAF web site is already a significant repository of information about our microbeam and the technology we are developing. The content of our website is continually updated to reflect the current state of the research at RARAF and to answer questions our users may have. We offer useful information such as the current month's accelerator schedule, keep users apprised of the status of new developments at RARAF through our New Developments section, and also offer a list of papers detailing research performed at RARAF and published in peer-reviewed journals (many available in PDF format free). While we continue to keep the website up-to-date, as discussed below, we plan to expand significantly our online information dissemination program.

A wiki is a page or collection of web pages on a topic designed to enable anyone who accesses it to contribute or modify content. Wikis can be used to create collaborative websites. Our goal is to disseminate information about microbeam technology to the general public and to the microbeam community through Wikipedia, an online encyclopedia. We have already created an encyclopedic entry for "microbeam," (Fig. 1) and we will continue to add content to the microbeam wiki topics of interest to the microbeam community. Our hope is to encourage others to take up the mantle as well.

Some terms that do not exist or have only very limited entries in Wikipedia, but would be useful, include:

- Bystander effect
- RARAF
- Center for Radiological Research
- *In vitro* oncogenic transformation
- Mutagenesis
- Chromosomal aberration
- Mirau optics
- Multiphoton microscopy

Creative use of online media can improve exposure of our field, aid in recruitment of new scientists, train those

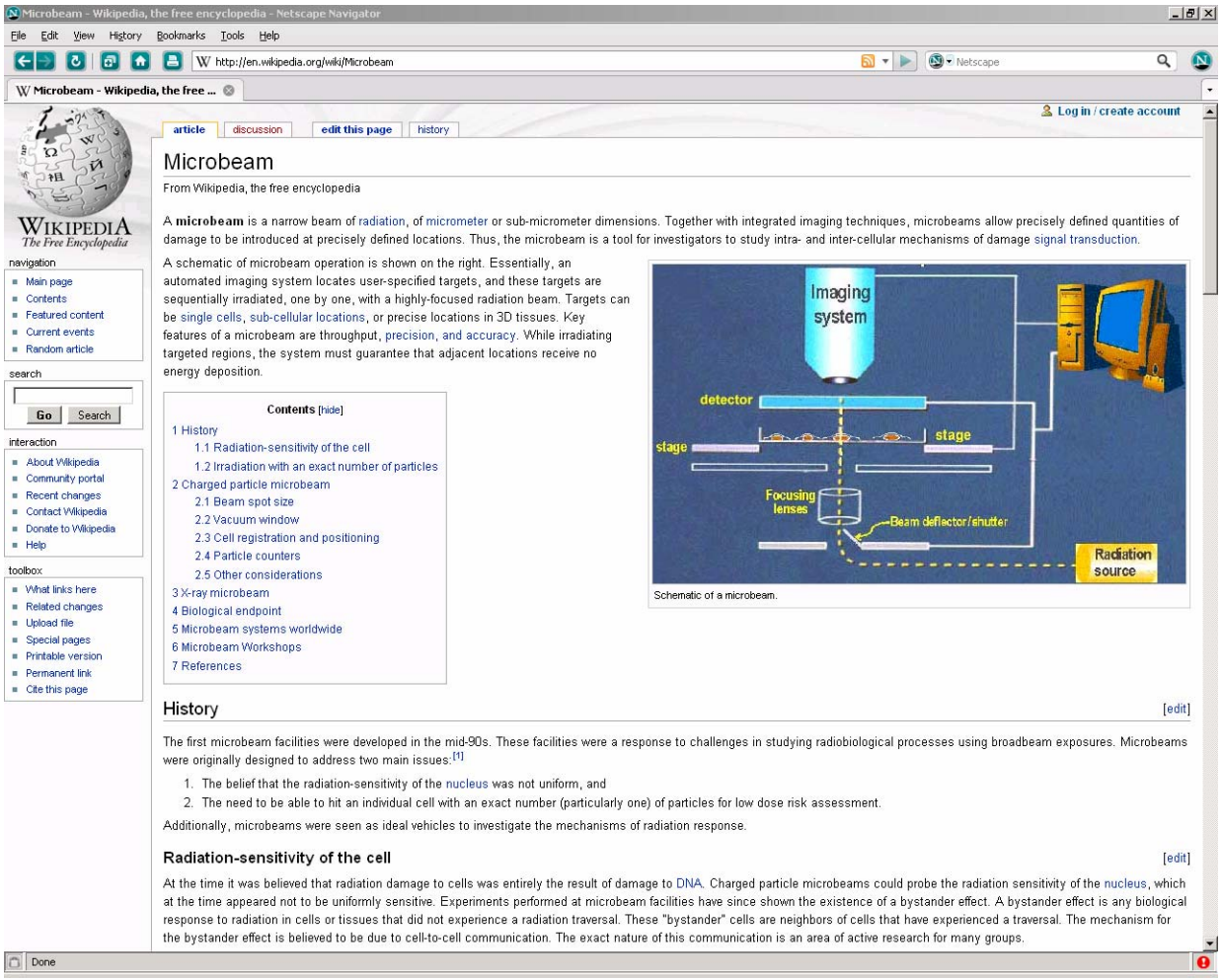

Fig. 1. Encyclopedic entry for "microbeam" from Wikipedia.

already in the field, and rapidly disseminate information to accelerate the pace of new discoveries.

Training

This summer we again participated in the Research Experiences for Undergraduates (REU) project in collaboration with the Columbia University Physics Department. Students attend lectures, work on a research project and present an oral report on their progress at the end of the 10-week program. Andrew Durocher from Wheaton College in Massachusetts worked with Gerhard Randers-Pehrson on a procedure to unfold the energy spectrum of an intense pulsed X-ray microbeam from the piled-up pulses in a gas proportional counter.

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 and the Senior Biologist for the P41 grant that is the major support for RARAF, 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, an Associate Research Scientist, is developing an inductive detector (LD^2) for single ions and the permanent magnet microbeam (PMM). 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 methods to image cells without stain and spends about half her time working on 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.

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.

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

- Dr. Brian Ponnaiya is an Associate Research Scientist performing experiments using the Track Segment irradiation facility. He now 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 about half her time at RARAF. She is also working on the development of FRAP and FRET capabilities for the Microbeam Facility.
- Dr. Antonella Bertucci, a Postdotoral Fellow, arrived in March and spends about half her time at RARAF. She has been working with Helen Turner on the NIAID project and is performing microbeam experiments using *C. elegans* nematodes.

Kenichi Tanaka, a Staff Associate who arrived in August, 2007 from Hiroshima University, Japan for a one-year visit, worked with Gerhard Randers-Pehrson on the detection of explosives. He terminated his visit in March in order to accept a position at the University of Hokkaido.

Julia Schaefer, an undergraduate student from Berufsakademie Karlsruhe (the University of Cooperative Education), Germany, arrived at the end of December for a three-month visit. She is working with Helen Turner on part of the NIAID high-throughput biodosimetry program. She will submit a 25-page report to her university on her learning experience at RARAF.

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**, id. 045807, 2009.
2. Bailey SM. Michael Fry Research Award lecture: Telomeres and double-strand breaks - all's well that "ends" well. *Radiat Res* **169**:1-7, 2008.
3. 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.
4. Bigelow AW, Brenner DJ, Garty G and Randers-Pehrson G. Single-particle / single-cell ion microbeams as probes of biological mechanisms (Review article). *IEEE T. Plasma Sci.* **36**: 1424-31, 2008.
5. 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.
6. Bigelow AW, Geard CR, Randers-Pehrson G and Brenner DJ. Microbeam-integrated multiphoton imaging system. *Rev Sci Instrum* **79**:123707, 2008.
7. Ghandhi SA, Yaghoubian B and Amundson SA. Global gene expression analyses of bystander and alpha particle irradiated normal human lung fibroblasts: Synchronous and differential responses. *BMC Med Genomics* **1**:63, 2008.
8. Hei TK, Zhou H, Ivanov VN, Hong M, Lieberman HB, Brenner DJ, Amundson SA and Geard CR. Mechanism of radiation-induced bystander effects: a unifying model. *J Pharm Pharmacol* **60**:943-50, 2008.
9. Horowitz YS, Horowitz A, Oster L, Marino S, Datz H and Margaliot M. Investigation of the ionisation density dependence of the glow curve characteristics of LiF:Mg,Ti (TLD-100). *Radiat Prot Dosimetry* **131**:406-13, 2008.
10. Randers-Pehrson G, Johnson GW, Marino SA, Xu Y, Dymnikov AD and Brenner DJ. The Columbia University sub-micron charged particle beam. *Nucl. Instr. Meth. A* (submitted 2008).
11. 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.
12. Zhou H, Ivanov VN, Lien YC, Davidson M and Hei TK. Mitochondrial function and nuclear factor-kappaB-mediated signaling in radiation-induced bystander effects. *Cancer Res* **68**:2233-40, 2008. ■