The Radiological Research Accelerator Facility

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

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During this past year, we have achieved several significant accomplishments:

- Irradiation of three-dimensional tissue.
- Our first focused microbeam irradiation.
- Our first microbeam irradiation using protons.
- The first charged particle beam as well as the first focused microbeam in the new microbeam facility.
- Another significant development is a grant from the National Center for Research Resources (NCRR) of the National Institutes of Health (NIH) for the purchase of an accelerator to replace the Van de Graaff that is now over 50 years old.
- Recently, the grant supporting the developmental projects at RARAF has been shifted from the NCRR to the National Institute of Biomedical Imaging and Bioengineering (NIBIB).

Research Using RARAF

Interest continues to remain quite strong in the "bystander" effect, in which only some cells are irradiated and a response is obtained that is greater than would be expected for the fraction irradiated. Several experiments examining this effect were continued and new ones initiated, observing a variety of endpoints to determine the size of the effect and the mechanism by which it is transmitted. Evidence has been obtained for both direct cell-cell gap junction communication through cell membrane contact and indirect, long-range communication through media transfer. In some experiments, the unirradiated cells can be identified due to different staining and scored directly. In other experiments, unirradiated cells are physically separated from the irradiated cells during irradiation. 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 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 and multiple users in a single day.

In Table I are listed the experiments performed at RARAF from May 1, 2002 through October 31, 2003 and the number of days each was run in this period. The reporting period comprises 18 months in order to better align it with the date of the Annual Report. Seventeen different experiments were run during this period, about the same as the average for 1997-2002. Ten experiments were undertaken by members of the CRR, supported by grants from the NIH and the Department of Energy (DOE). Seven experiments were performed by outside users, supported by grants and awards from the NIH, the National Aeronautics and Space Administration (NASA), the National Science Foundation (NSF) and the Ministry of Education, Science, Sports and Culture of Japan. Brief descriptions of these experiments follow.

Investigations involving the oncogenic neoplastic transformation of mouse C3H 10T½ cells (Exp. 73) were continued by Eric Hall, Stephen Mitchell and Richard Miller of the Center for Radiological Research (CRR). Using the microbeam facility, 10% of the cells were irradiated through the nucleus with 2 to 12 helium ions. Cells were plated at densities of approximately 200 and 2000 per dish to try to observe the relative contribution of cell-cell communication to the bystander effect. Cell killing and transformation were greater for the cells plated at the higher density relative to those plated at the lower density. The results imply that gap junction communication has a greater role in the bystander effect than media transfer. The track segment facility was used to investigate whether there is an adaptive response for the bystander effect. Cells (bystanders) were plated on special track segment dishes made by cutting the Mylar surface into several equal strips and removing alternate strips. The Mylar is thick enough to stop the incident ions. This dish is placed inside a second dish that has a complete Mylar surface. Cells are plated on the combined surface. All the cells were given a dose of 250 kVp X rays and after 5 hours the nuclei were irradiated with helium ions. Significant reductions in cell killing and transformation were observed for the bystander cells given X rays relative to those that weren't.

Richard Mauer, David Roth and James Kinnison of Johns Hopkins University continued development of a portable neutron spectrometer system for the energy range 0.5 to 100 MeV (Exp. 89). The spectrometer will be used on the International Space Station and the manned mission to Mars. Emphasis is now on determining the shapes and rise times of the pulses generated by gamma rays and neutrons to perform gamma-ray discrimination. A second detector of borated plastic scintillator to be used on the Messenger probe that will orbit Mercury was irradiated with neutrons for an initial evaluation of the system. In addition, an irradiation with 2.1 MeV neutrons was performed on a charged-coupled device (CCD) to be used to send video signals from the New Horizons probe that will travel past Pluto to the outer asteroid belt. Since the probe will be too far away to use solar panels, there will be an on-board power supply. The effect of neutrons emitted by the power supply on the resolution of the camera was determined.

Sally Amundson of the NIH is investigating radiation-induced gene expression profiles in human cell lines using

Table I.

Experiments Run at RARAF, May 1, 2002–October 31, 2003

Exp. No.	Experimenter	Institution	Exp. Type	Title of Experiment	Days Run
73	S. Mitchell, E.J. Hall	CRR	Biology	Neoplastic transformation of C3H10T½ cells by specific numbers of α particles	27.3
89	R.H. Mauer, et al.	Johns Hopkins U.	Physics	Calibration of a portable real-time neutron spectrometry system	4.5
92	S. Amundson	NIH	Biology	Functional genomics of cellular response to high-LET radiation	1.0
94	B. Ponnaiya, C.R. Geard	CRR	Biology	Single cell responses in hit and bystander cells: single-cell RT-PCR and protein immunofluorescence	8.0
103	G. Jenkins, C.R. Geard	CRR	Biology	Damage induction and characterization in known hit versus non-hit human cells	11.9
106	B. Ponnaiya, C.R. Geard	CRR	Biology	Track segment alpha particles, cell cocultures and the bystander effect	9.0
108	H. Zhou, T.K. Hei	CRR	Biology	Modulation of adaptive response in alpha-particle-induced bystander effects	2.0
109	A. Balajee, C.R. Geard	CRR	Biology	DNA damage induction in microbeam-irradiated cells assessed by the comet assay	3.7
110	H. Zhou, D. Roy, T.K. Hei	CRR	Biology	Identification of molecular signals of alpha particle-induced bystander mutagenesis	14.4
114	M. Suzuki (Zhou)	Ntnl. Inst. of Radiological Sci., Japan	Biology	Bystander response in primary human bronchial epithelial cells using the G2PCC technique	4.0
115	A. Caldwell, R. Galea	Columbia Univ.; Max Planck Inst., Germany	Physics	Proton cooling studies for the development of a muon ion source for the Muon Collider/Neutrino Factory	27.4
116	O. Belyakov	CRR	Biology	Long-range communication phenomena in 3-D human tissue systems	12.9
117	K. Irzynska, O. Belyakov	Jagiellonian Univ., Poland; CRR	Biology	Studies of direct and bystander radiation effects in V79 cells using broad field and microbeam irradiation	1.0
118	R. Kolesnick, G. Perez	MSKCC; Mass. General Hosp.	Biology	Characterization of the radiosensitive target for cell death in mouse oocytes	1.3
119	D. Lawrence	Los Alamos Natl. Lab.	Physics	Calibration of a neutron spectrometer for planetary studies	5.8
120	R. Persaud, T. Hei	CRR	Biology	Determination of the bystander response for low-LET protons	5.0
121	A. Zhu, H. Lieberman	CRR	Biology	The bystander effect in mouse embryo stem cells with a mutant Mrad9 gene	2.9

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

cDNA microarray hybridization and other methods (Exp. 92). She is characterizing the response of a p53 wild-type and knock-out pair of human cell lines. The profiles generated by cells irradiated with 0.43 MeV neutrons are providing insight into the specificity of ionizing radiation responses, as well as revealing potential candidates for high LET-specific responses.

Two studies investigating the bystander effect were continued by Brian Ponnaiya and Charles Geard of the CRR. In one study (Exp. 94), levels of p21 production were measured in individual normal human fibroblasts using immunofluorescent staining. This procedure permits observation of the variation in response of individual cells to radiation instead of just the average response of a large number of cells. From 1 to 100% of the cell nuclei were irradiated with helium ions using the microbeam facility. The second investigation uses the track segment facility for broad-beam charged particle irradiations of human fibroblasts and epithelial cells immortalized with telomerase (Exp. 106). Special cell dishes are made from stainless steel rings with thin Mylar windows epoxied on both sides. Cells are plated on both inner Mylar surfaces and the dish volume is filled with medium, eliminating any possibility of cell-cell contact between cells on opposing surfaces. Cells on one surface are irradiated with 4He ions; cells on the opposite surface are unirradiated because the particles stop in the medium before reaching them. Cells are observed in situ after irradiation with doses from 1 to 10 Gy of 125 keV/μm ^{4}He ions. Plateau phase cells are scored for cell cycle delay and micronucleus production while log phase cells are scored for chromosomal aberrations. It was observed that irradiated fibroblasts can induce micronuclei in bystander fibroblasts, but bystander epithelial cells are refractory to irradiated epithelial cells. Furthermore, epithelial cells are capable of responding to irradiated fibroblasts, which results in the induction of micronuclei in the bystander epithelial cells. Chromosomal analyses of irradiated fibroblast populations and bystander cells at the first cell division post irradiation demonstrated the induction of gross chromosome aberrations in the irradiated population and chromatid aberrations (of the simple type – breaks and gaps) in the bystander population. Elevated yields of similar types of chromatid type aberrations were also observed in both irradiated and bystander fibroblast populations up to 20 population doublings post irradiation.

Charles Geard and Gloria Jenkins of the CRR are studying the bystander effect in several cell lines using the microbeam facility (Exp. 103). Normal human fibroblasts and human mammary epithelial cells were irradiated with helium ions, targeting 1%, 10% and 100% of the cell nuclei. Endpoints for various experiments included micronucleus production in S phase, production of p21 and p53 in the fibroblasts and production of H2AX in the mammary cells. In some of the experiments the bystander cells were stained

with a different dye than the irradiated cells so that they could be distinguished.

Hongning Zhou and Tom Hei of the CRR continued to use the single-particle microbeam facility for two experiments investigating the bystander effect. A study examining adaptive response in bystander effects in human-hamster hybrid (A_L) cells (Exp. 108) was completed. After low-dose X-ray irradiation, 10% of the cells were traversed by 1 or 20 helium ions. There was a decrease in the bystander effect for mutation when neighbor cells are traversed by one particle and a somewhat smaller decrease for traversal by 20 particles. . In addition, they found that the bystander cells showed an increase in sensitivity to a subsequent, challenging dose of X rays. The mutation spectra are being analyzed and should provide some evidence for understanding the mechanism of bystander mutagenesis and adaptive response. With Debasish Roy of the CRR, they are trying to identify the signaling transduction pathways involved in radiation-induced bystander mutagenesis (Exp. 110). Hybrid A_L cells, normal human lung fibroblasts, mitochondrial deficient cells, and other functional deficiency cell lines are irradiated using the microbeam facility. A fraction of the cells is irradiated with a single alpha particle. Initially, the irradiated (stained) cells were separated from the unirradiated cells by a cell sorter and accumulated from experiments over four consecutive days. This method proved logistically to be quite difficult. Presently, the cells are kept *in situ* for 2, 6, 24 or 48 hours after irradiation, thereby increasing the number of cells and the time for interaction. In addition, some experiments have been performed using the track segment facility using the "strip" dishes described for Experiment 73. The mRNA extracted from the cells is analyzed using microarrays. Preliminary data show some gene expression changes in the bystander cells.

Investigations of damage induction in normal human fibroblasts and *Ataxia Telangiectasia* cells (Exp. 109) by Adayabalam Balajee and Charles Geard of the CRR included a search for foci of damage and repair proteins. Cells were irradiated through the nucleus with helium ions using the microbeam facility. The *AT* cells were scored using the comet assay to determine chromosome breakage. The fibroblasts were stained and examined to observe the repair proteins, which should cluster around the helium ion track. Fluorescence of stained irradiated cells was 2-3 times greater than in the controls, implying a significant increase in repair proteins, and formed foci, although the number of foci was not the same as the number of ion traversals in the nuclei.

Masao Suzuki of the National Institute of Radiological Science, Japan is trying to determine whether alpha particle irradiation can induce a bystander response in primary human bronchial epithelial cells using the G2PCC technique (Exp. 114). Ten percent of the cells were irradiated in the nucleus with helium ions using the microbeam facility. The cells then accumulated and were harvestedin the G2 phase of the cell cycle, and the process of premature chromosome condensation was used to observe chromatin aberrations.

Initial studies for the development of a muon ion source (Exp. 115) were undertaken by Alan Caldwell and Raphael Galea, originally at the Physics Department of Columbia University (working at the Nevis Laboratory) and now at the Max Planck Institute in Munich, Germany. Pions produced by high-energy proton bombardment of a target will be allowed to decay into muons that must be forced to travel in the same direction, slowed down (cooled) and bunched with a minimum spread in energy and time. As a first test of the method, low energy protons from the Van de Graaff were used to determine the energy spread and timing. The source will eventually be used for the Muon Collider/Neutrino Factory, whose location is yet undetermined.

An investigation of the bystander effect in three-dimensional model human tissue systems was begun by Oleg Belyakov of the CRR (Exp. 116). Several novel artificial human skin tissue systems were obtained from the MatTek Corporation: epidermis, cornea and tracheal/bronchial epithelium, allowing the modeling of the conditions present *in vivo*. The tissues were irradiated using the microbeam facility with 10 helium ions deposited 17 to 400 locations in a line 8-10 mm long across the sample (20 to 500 μm spacings) and with 2.75 MeV protons at 100-300 μm spacings. Irradiations were also performed using the track segment facility to irradiate samples with 125 keV/μm ^{4}He ions and 12 keV/μm protons with doses that resulted in approximately 0.5 to 2 particles per cell nucleus. The microbeam samples were embedded in paraffin and cut into 5 μm-thick sections to observe the bystander effect as a function of distance. For all experiments, three endpoints were studied: an *in situ* apoptosis assay, epidermal differentiation and a proliferation assay. He has observed a clear bystander response, established dose dependency and studied the role of differentiation vs. damage induction processes.

Katarzyna Irzynska, an undergraduate student from Jagiellonian University in Krakow, Poland performed experiments on direct and bystander effects in V79 cells (Exp. 117). Under the tutelage of Oleg Belyakov of the CRR, cells were irradiated with helium ions using the microbeam facility and 11-keV/μm protons using the track segment facility. Irradiated and bystander cells were scored for "total cellular damage" (TCD), defined as the sum of the fraction of cells with micronuclei and those that became apoptotic.

Richard Kolesnick of the Memorial Sloan Kettering Cancer Center (MSKCC) and Gloria Perez of Massachusetts General Hospital are trying to characterize the radiosensitive target for mouse oocyte killing (Exp. 118). Is it the DNA, the plasma membrane or the cytoplasm? Understanding what the target is would help in the development of protective therapies to prevent the side effects of radiotherapy on female germ cells. For these experiments they are using the mouse strain C57BL/6 because oocytes from these mice show low rates of spontaneous apoptosis. Mature and immature oocytes are irradiated in the DNA, the cytoplasm or the cell membrane using the microbeam facility. Because the oocytes are spherical with a uniform diameter of about 80 μm, they are irradiated with protons because the range of the helium ions is insufficient to penetrate much more than half way through the cells.

David Lawrence of Los Alamos National Laboratory performed a calibration of a neutron spectrometer to be used on the Messenger mission, a space probe for investigations

of the planet Mercury (119). The detector comprises a pair of borated plastic scintillators that were irradiated with monoenergetic neutrons from 0.5 to 2.0 MeV to obtain response functions and efficiencies. The detectors were also tested for angular response.

An investigation of the bystander effect using low-LET radiation (Exp. 120) was begun by Tom Hei and Rudranath Persaud of the CRR. A_L cells were irradiated with 3.1 MeV protons (~12 keV/μm) using the microbeam facility. Approximately 20% of the population was irradiated with 200 protons per nucleus. Preliminary results for this experiment indicate that the protons did not induce a bystander effect. The mutant yield for the control group was 200, whereas for the irradiated group it was 160.

Howard Lieberman and Aiping Zhu of the CRR have begun experiments to investigate the bystander effect in mouse embryo stem cells with a mutation in the Mrad9 gene (Exp. 121), which promotes radiation resistance and helps regulate the cell cycle and apoptosis. Cells plated on the special "strip" dishes are irradiated with 1 to 10 Gy of helium ions using the track segment facility and observed for cell survival, micronucleus production and apoptosis. Cells with the mutated gene show an enhanced bystander effect for some of the endpoints.

Development of Facilities

One of the major recent developments has been the receipt of a grant from the National Center for Research Resources (NCRR) of the NIH for the purchase of an accelerator to replace our present 4.2-MV Van de Graaff, which is over 50 years old and has problems with a vacuum leak in the acceleration tube and an aged charging belt. Many parts for the accelerator are custom-made or no longer commercially available and the voltage regulation is only ±2-4 kV. We have ordered a new 5 MV Singletron accelerator from High Voltage Engineering Europa in the Netherlands that will meet or exceed our present capabilities. The charging system is electronic, similar to that of a Cockroft-Walton, so there are no moving parts (belts, chains) in the charging system. Terminal voltage ripple will be 200 V or less. The higher voltage will provide ion beams with longer ranges and lower LETs. Better voltage stability will result in a smaller beam spot for our double lens system since energy spread in the beam reduces focusing. An RF ion source will produce protons, deuterons, ^{3}He ions, and ^{4}He ions with beam currents of at least 100 μA. Control of valves and power supplies in the terminal is performed through a light link rather than by motors with strings, as in our present system. Terminal parameters are monitored through this light link rather than by a TV camera or monocular. Control of the accelerator is through a computer interface. The accelerator should arrive in May, 2005. Removal of the Van de Graaff and installation of the Singletron will require at least a partial dismantling and reconstruction of the extension on the west side of the building and take 3-6 months.

During the past 18 months, our development effort has increased markedly. Not only was on-line development time increased by more than 60% over last year, but two more people were added to the development team: Greg Ross and Guy Garty. Development continued or was initiated on the microbeam facilities and a number of extensions of their capabilities:

- Assembly of the beam line and end station for the new microbeam facility
- Development of focused microbeams
- Voice-coil and precision z-motion stages
- New microbeam irradiation slides
- Laser ion source
- Secondary emission ion microscope (SEIM) for viewing focused beam spots
- Source-based microbeam
- Non-scattering particle detector
- Advanced imaging systems
- Focused X-ray microbeam

The beam line from the new 90-degree bending magnet into the new microbeam laboratory has been completed. Valves, an ion pump, energy regulation slits and a module with a series of object apertures for the lens have been assembled into the beam line. Also installed is an x-y adjustment for the tube inside the beam line in which the two lenses will be mounted, so that the pair of lenses can be aligned with the beam. In order to assemble the beam line and have access to it, a scaffold that can accommodate two people was erected surrounding the beam line and the 90-degree magnet below it. This proved to be more difficult than anticipated because of the braces for the magnet stand as well as the horizontal beam line and associated components. A hole was made in the ceiling of the microbeam room and a winch mounted on the roof so that the 2.5-m long tube containing the lenses could be lowered into the beam pipe.

Construction of the new microbeam facility on the floor over the exit of the Van de Graaff has been essentially completed. The on-line microscope has been modified so that it can be rotated in and out of place over the irradiation port. When off-line, the movable portion of the microscope sits on its original base and is usable as a standard fluorescence microscope. The camera, image analysis system, and microscope stage have all been integrated into the microbeam irradiation control program. The exit port has a SiN window with a 1-mm square section only 100 nm thick to minimize scattering. This will be more important for the 0.5-μm diameter beam that will be produced by the compound lens system. Until the compound lens is constructed, we will use a single quadrupole quadruplet to produce a beam with a diameter of 3 μm for cell irradiations.

Testing of the single electrostatic quadrupole quadruplet continued, using the existing microbeam facility. A beam approximately 3 μm in diameter was obtained using a lens with rods only having a titanium coating (no gold). A second lens constructed with gold plating over the titanium was also able to produce a beam 3 μm in diameter. This lens was used in the original microbeam facility to perform our first focused microbeam irradiations of cells with helium ions and our first microbeam irradiations using low-LET protons. Unfortunately, the lens has developed a problem and has had to be removed from service for repair. The lens with the titanium-coated rods has been mounted in the alignment tube

for the double lens system and placed in the beam line for the new microbeam facility. It has focused the beam to less than 10 μm diameter. We are still in the process of optimizing the focus and should be able to obtain a beam spot of 3 μm or less and begin microbeam experiments on the new beam line. Measurements are being made of the voltages required to obtain various beam spot geometries when all and only some of the lens elements are used. This information will be used by our consultant, Alexander Dymnikov, at the University of Louisiana to calculate parameters for the double quadrupole triplet lens assembly that will be used to focus the ion beam to a diameter of 0.5 μm. Final machining of the rods for the lens electrodes will start as soon as the results of these calculations are received. For these measurements to be most useful, the energy and energy spread of the beam must be known accurately. A thin nickel target has been constructed and mounted on the T2 beam line to use the extremely narrow (<90 eV) Ni(p,γ) resonance at 1.844 MeV to define the beam energy and energy spread. Eventually, the measurements will be repeated on the microbeam line itself using an extremely thin (0.1 μm) layer of Ni on a thin Pt (2 μm) target backing.

Our custom-designed Voice-Coil Stage (VCS), which uses thin coils in permanent magnetic fields to move the microscope stage, has been integrated into the new microbeam facilities and has been shown to position the stage reliably under normal use to within ±0.4 μm, with a settling time of ~50 ms. Development is continuing to improve its performance. The circuitry for the linear variable differential transformer (LVDT) feedback system for the voice-coil stage has been tested and is being built into a NIM module. This stage will provide both more accurate and faster positioning than the present stage, which is moved by stepping motors. We have purchased a high-precision stage from Mad City Labs in Wisconsin that also has a vertical motion. This stage has a range of motion of 200 μm in the x and y directions and 100 μm in the z direction, with nanometer positioning. This stage exceeds the accuracy needed for the 0.5 μm diameter beam, however because of its limited range of motion in the horizontal plane, we will have to mount it within a coarser stage in order to be able to access the entire surface area on which cells are plated. The vertical motion is required for the imaging techniques described below. The stage will also be used to raise and lower the sample over the exit window during movement to minimize the separation and thereby reduce beam spread due to scattering in the window.

In parallel with our improved spatial properties of the microbeam, it became essential to develop corresponding improvements in the substrate on which cells are grown and imaged. Our prime requirements for improvements are improved optical properties, minimal background fluorescence, and strong adherence to the supporting dish or slide. We are using a thermoplastic polymer called parylene, the generic name for a family of polymers that are formed on surfaces exposed to a rarified gas in a vacuum. Standard plastic microscope slides with ¼"-diameter holes are sandwiched around a glass slide that has been coated with a water-soluble release agent. The slides are plated with parylene using a coating system we have purchased and the assemblies separated by soaking in water. Strong, flat coverings over the holes in the slides have been achieved; however the surfaces have many inclusions. Development is continuing on producing films without the surface defects that make them unsuitable for microscopy.

The laser ion source is nearing completion. The spherical lens to focus the ions on the entrance of the acceleration tube was completed last year. The rotating laser target assembly has been designed and constructed. The mounts for the mirrors and lens to direct and focus the laser beam on the target are being designed. When the mounts are received, testing of the system will begin. An evaporable getter was purchased to replace the titanium sublimation pump that was originally going to be used to maintain the vacuum in the source. Unlike the sublimation pump, the getter doesn't require a large power supply, reducing the amount of space required in the accelerator terminal, the amount of weight the column has to support and the amount of terminal power consumed. The reduction in weight and power consumption made the source much more compatible with the new accelerator that is on order. The terminal of the Singletron is being designed to accommodate the ion source without any modification to our present design. We have decided not to install the source in the Van de Graaff, which could take up to a month for modifications to the terminal and testing, because the accelerator will be decommissioned next April.

As we improve the spatial characteristics of the microbeam system, it becomes increasingly important to be able to assess the beam quality in order to adjust the system to its optimum capabilities. A secondary electron ion microscope (SEIM) has been designed and is currently being constructed. This device will enable us to measure the beam profile and position in real time with sub-micron resolution and sensitivity to single projectile particles (1-5 MeV protons, as well as heavier ions). We expect to interchange the SEIM and the cell-imaging microscope rather frequently. To this end a special mount has been designed and built (see Figure 1). The SEIM design was inspired by the technique of

Fig. 1. The new microbeam irradiation station. On the right is the pivot arm for moving the microscope between the online and off-line positions. It will also be used to move the SEIM online and off line. The stands in the lower middle and right are to support the microscope when in the off-line position. The voice coils stage is in place on the microscope.

photoelectron microscopy (PEM) and we gratefully acknowledge the advice of a world expert in PEM, Dr. Gertrude Rempfer, in finalizing our design. The SEIM is based on secondary electrons emitted by a film on which the ions in the beam are incident. The ejected electrons are focused to form a magnified image on an image-intensified CCD. We have developed a novel "folded" design for the SEIM using a mirror lens to maintain a long path for the electrons with a more compact instrument. Calculations indicate a magnification of ~500 can be achieved, yielding a resolution of 0.1-0.2 μm.

Calculations have been performed for the design of a free-standing microbeam (FSM) based on a small, low activity radioactive alpha-particle emitter (0.1 μCi ^{210}Po) plated on the tip of a wire. Alpha particles emitted from the source are focused into a 5μm spot using a compound magnetic lens made from commercially available permanent magnets, since only a single type and energy of particle will be focused. A compound lens system similar to the one designed for the new microbeam will be used, the only difference being that it will use magnetic lenses, rather than electrostatic lenses. The FSM will replace the accelerator in our original microbeam laboratory and will be fitted with a voice coil stage for placement of the cells to be irradiated. It also will be fitted with the existing electrostatic beam deflector, used to enable fast opening and closing of the beam, enabling single particle irradiations. This facility will be used to perform microbeam irradiations during the period when the Van de Graaff is being removed and the Singletron installed. The design would also be useful for groups that desire to perform microbeam experiments at their home institutions but lack an appropriate accelerator. It is estimated that a complete FSM system, including the microscope, could be built for ~$100k.

To irradiate thick samples, such as model tissue systems or oocytes, or to use particles with very short ranges, such as the heavy ions from the laser ion source, a completely non-scattering upstream particle detector is necessary. A novel particle detector has been designed on the basis of a long series of inductive cells coupled together into a delay line. The Lumped Delay Line Detector (LD^2) will consist of 300 silver cylinders 3 mm long with a 2.2 mm inside diameter connected by inductors and capacitively coupled to ground. The cylinders are glued to a semi-cylindrical tube of dielectric material 1 m long for mechanical support. The dielectric has a semi-cylindrical metal tube around it that can be rotated about its axis to adjust the capacitance. If the individual segment delays are set (by adjustment of the capacitance) such that the propagation velocity of the pulse equals the projectile velocity, the pulses induced in all segments will add coherently, giving a fast electron pulse at one end of the delay line that is 150 times larger than the charge induced on a single cylinder. This easily detectable charge of at least 150 electrons will be amplified to provide the detection pulse for the particle counter. The inductors and the cylinders have been purchased; the rest of the detector parts await machining. It is anticipated that this detector will become the standard detector for all the irradiations on the new microbeam facility.

Development has begun on new imaging techniques to view cells without stain and to obtain three-dimensional images. Two different techniques are being investigated: phase-shifted interference microscopy and quantitative non-interference phase microscopy. In phase-shifted interferometry, images are obtained with a Mirau interferometric objective at a sequence of path (phase) differences between the sample and the lens: 0, l/4, l/2, and 3l/4, where l is the wavelength of the incident light. It is important that the substrate for the cells is an optically flat, reflecting surface. Parylene dishes with aluminum plated on the outside will be used as the substrate. The combined images can be used to produce a topographic image by solving for the phase shifts at each point. The essence of the algorithm for determining the phase shifts is to solve for three variables with an over-determined system of four equations. The Mirau lens has been purchased and the substrate system is being developed. The other method being investigated is a relatively new technique that can generate phase images and phase-amplitude images using a standard microscope. To obtain a quantitative phase image, an in-focus image and very slightly positively and negatively defocused images are collected. The resulting data can be used to yield the phase distribution by Fourier-transform methods. Test images sent to the software manufacturer yielded surprisingly good resultant images. We are evaluating a trial copy of the Fourier transform-based software for generating phase images or phase-amplitude images from the three microscope images. The Mad City stage will be able to provide the vertical motion required by both these methods to obtain the necessary images for different distances between the sample and the lens.

We have investigated expanding the microbeam repertoire to include soft X-rays (Al k_α, 1.49 keV). Microbeam studies with high-energy X-rays or gamma rays are not feasible due to Compton scattering effects, so we are limited to X-ray energies where the predominant mode of interaction is photo-electron absorption. A proton beam will be focused onto an aluminum foil using the compound electrostatic lens. The characteristic X rays produced in the foil will be focused to a diameter of 1 μm using a zone plate with a focal length of 12.7 mm. Calculations performed indicate that a 1 nA proton beam should produce a dose rate of 0.1 Gy/sec of X-rays, adequate for the biological studies envisioned. The end of the microbeam line will be modified so that the target and zone plate can be rotated into or out of the beam path to change irradiation modalities quickly.

Accelerator Utilization and Operation

Accelerator usage is summarized in Table II. Use of the accelerator for radiobiology and associated dosimetry decreased by about 25% over 2001-2002. Only about half the accelerator use for all experiments was for microbeam irradiations. Because of the relatively low number of cells that can be irradiated in a day, microbeam experiments often require considerable beam time to obtain sufficient biological material, especially for low probability events such as transformation and mutation, and therefore normally constitute a large fraction of the experimental use. These changes

Table II.

Accelerator Use, May 2002–October 2003
Percent Usage of Available Days

Radiobiology and associated dosimetry	29%
Radiological physics and chemistry	9%
On-line facility development and testing	48%
Off-line facility development	11%
Safety system	2%
Accelerator-related repairs/maintenance	11%
Other repairs and maintenance	1%

in usage resulted in large part from lens development. Until August 2003, when the new microbeam line was in place and had a lens mounted in it, switching between biology experiments using a collimated beam and development using the lens was very inefficient. Three to four days were lost each time in the changes to the end of the beam line required for the two arrangements. To increase efficiency, no microbeam experiments were performed between January and July 2003, allowing us to fully concentrate on lens development.

Utilization of the accelerator by radiological physics increased greatly this past year, primarily due to the experiment on proton cooling (Exp. 115) that comprised about 20% of all the time used for experiments. On one visit, this experiment ran continuously for 3½ days. As usual, there were no chemistry experiments this reporting period.

Use of the accelerator for online development increased by 60% over last year to almost half of all available time (8 hours/day, 5 days /week, excluding holidays), mainly due to the concentration on development from January to July 2003. For several months, many more than the usual number of extra shifts was worked in the evening, on weekends and holidays, bringing total use of the accelerator to 100% of the normally available time.

Accelerator maintenance and repair time declined by a third over last year but was still somewhat higher than the long-term average due to continued problems in the power supply in the terminal used to spray negative charge on the charging belt. Despite several modifications to the supply to reduce sparking, one of two strings of high voltage diodes in the supply shorts out. The vacuum leak in one of the sections of the acceleration tube is a problem that has troubled us for several years. No replacement of the section is planned because the accelerator will be dismantled in about 15 months to make room for the new one. No major repairs or modifications to the accelerator were performed. Once the new accelerator is installed, we anticipate much less accelerator maintenance, not only because the accelerator will be new, but also because the accelerator will be charged electronically and will have few moving parts (no belt or chains). It has an RF ion source that also should require less maintenance than the Duoplasmatron source we are presently using.

Personnel

The Director of RARAF is Dr. David Brenner. The Van de Graaff accelerator facility is operated by Mr. Stephen Marino and Dr. Gerhard Randers-Pehrson. Our ranks have now swelled to a total of seven physicists, an increase of two.

Dr. Alan Bigelow, now an Associate Research Scientist, is continuing the development of the laser ion source, parylene coatings for microbeam slides and an optical system for 3-dimensional viewing of cells.

Dr. Furu Zhan, a postdoctoral fellow from China, is assisting in running the accelerator, performing microbeam irradiations and developing the facility.

Mr. Kurt Michel, an undergraduate student from Pace University, was a part-time intern until May 2003, assisting with the development of the voice coil positioning stage for the microbeam facility and parylene coatings.

Mr. Greg Ross is a Programmer/Analyst who arrived in January, 2003. He is assisting with various programming tasks and is working on the development of the voice coil stage for the microbeam facility and a neutron target for the bomb detection system.

Mr. Guy Garty, a Staff Associate, arrived from Israel in June 2003. He is working on the development of a focused X-ray microbeam, a source-based microbeam and an inductive detector for single ions.

Biologists from the Center for Radiological Research are stationed at the facility in order to perform experiments:

- Dr. Charles Geard, the Associate Director of the CRR, continues to spend most of each working day at RARAF. In addition to his own research, he collaborates with some of the outside users on experiments using the single-particle microbeam facility.
- Dr. Brian Ponnaiya is an Associate Research Scientist performing experiments using the track segment and microbeam irradiation facilities.
- Ms. Gloria Jenkins, a Biology Technician, performs experiments on the microbeam facility for Dr. Geard.
- Dr. Stephen Mitchell, a Postdoctoral Fellow, continues to perform research involving neoplastic transformation of cells.
- Dr. Oleg Belyakov, another Postdoctoral Fellow, left in June 2003. He was performing experiments on the track segment and microbeam facilities using model tissue culture systems.
- Dr. Richard Miller, an Associate Professor, returned in June 2003 as a Research Scientist, but left again in October. He was working with Stephen Mitchell on experiments involving cell transformation.
- Ms. Allison Groome, an undergraduate student from Pace University working as an intern assisting Drs. Geard and Ponnaiya on a part-time basis, left in 2003.

Recent Publications of Work Performed at RARAF (2002-2003)

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ings for the Seventeenth International Conference on the Application of Accelerators in Research and Industry (CAARI), Denton, Texas, November 12-16, 2002. *AIP Conference Proceedings* **680**:347-50, 2003.

3. Bigelow AW, Randers-Pehrson G and Brenner DJ. Laser ion source for the Columbia University microbeam, 9th International Conference on Nuclear Microprobe Technology and Applications, ICNMTA 2002. *Nuclear Inst and Meth in Phys Res B* **210**:65-9, 2003.
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5. Brenner DJ and Sacks RK. Are bystander effects important? *6th International Workshop on Microbeam Probes of Cellular Radiation Response,* Oxford, UK, March 29-31, 2003.
6. Brenner DJ, Sawant SG, Hande MP, Miller RC, Elliston CD, Fu Z, Randers-Pehrson G and Marino SA. Routine screening mammography: how important is the radiation-risk side of the benefit-risk equation? *Int J Radiat Biol* **78**:1065-7, 2002.
7. Geard CR. Cellular responses to low doses using modern microbeams. Proceedings of the *Workshop on Networking Radiation Sciences in Health, Safety, and the Environment*, McMaster University, Hamilton, Ontario, November 25-26, 2002. To be published in *Radiat Res.*
8. Geard CR, Jenkins-Baker G, Bigelow A, Brenner DJ, Hall EJ, Hei TK, Marino S, Randers-Pehrson G and Ponnaiya B. Single cell gene expression and microbeam irradiation: the concept of the average cell. *6th International Workshop on Microbeam Probes of Cellular Radiation Response,* Oxford, UK, March 29-31, 2003.
9. Geard CR, Ponnaiya B, Jenkins-Baker G, Hei TK, Brenner DJ, Hall EJ and Randers-Pehrson G. Microbeam mediated cellular effects*:* Observations and implications for low dose radiation risk assessment. In *Molecular Mechanisms for Radiation-induced Cellular Response and Cancer Developmen*t, Tanaka, K, Takabataka, T, Fujikawa, K, Matsumoto, T and Sato, F, Eds. Institute for Environmental Science, Japan, p. 82-8, 2002.
10. Hall EJ. How Many Bystander Effects Are There? *6th International Workshop on Microbeam Probes of Cellular Radiation Response*, Oxford, UK, March 29-31, 2003.
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12. Horowitz YS, Oster L, Biderman S and Einav Y. Localized transitions in the thermoluminescence of LiF:Mg,Ti: potential for nanoscale dosimetry. *J Phys D Appl Phys* **36**:446-59, 2003.
13. Katanic J. Electron Spin Resonance Characterization of Human Tooth Enamel Response to Proton, Neutron, and Ultraviolet Radiation, Ph.D. thesis, Purdue University, August, 2003.
14. Kinnison JD, Maurer RH, Roth DR and Haight RC. High energy neutron spectroscopy with thick silicon detectors. *Radiat Res* **159**:154-60, 2003.
15. Maurer RH, Kinnison JD, Roth DR and Goldsten JO. Neutron spectroscopy on the international space station. *AIAA Conference on International Space Station Utilization*, Cape Canaveral, FL, 16 October, 2001. Proceedings published on CD ROM, AIAA paper 2001-5059.
16. Maurer RH, Roth DR, Kinnison JD, Goldsten JO, Gold RE and Fainchtein R. Mars Neutron Energy Spectrometer (MANES): an instrument for the Mars 2003 lander. *Acta Astronaut.* **52**:405-10, 2003.
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20. Zhou H, Randers-Pehrson G, Geard CR, Brenner DJ, Hall EJ and Hei TK. Interaction between radiation-induced adaptive response and bystander mutagenesis in mammalian cells. *Radiat Res* **160**:512-6, 2003.
21. Zhou HN, Randers-Pehrson G, Waldren C and Hei TK. Radiation induced bystander effect and adaptive response: implication for low dose radiation risk assessment. *Advances Space Research* (in press).