# RARAF

# THE RADIOLOGICAL RESEARCH ACCELERATOR FACILITY

**An NIH-Supported Resource Center**

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The Radiological Research Accelerator Facility, an NIH-funded Biomedical Technology Resource Center, provides CRR members and the radiation research community at large with advanced irradiation techniques using charged particle and neutron beams. RARAF serves as the radiation core for the Columbia Center for High Throughput Minimally Invasive Radiation Biodosimetry. RARAF staff also support CRR researchers with design of irradiations and with dosimetry.

**Research Using RARAF**

The "bystander" effect – the response of cells that are not directly irradiated but are in close contact with, nearby, or only in the presence of irradiated cells – has been the focus for many of the biological studies at RARAF over the past two decades. Both the Microbeam and the Track Segment Facilities continue to be utilized in various investigations of this response to radiation exposure. This year the number of biological experiments investigating the mechanism(s) by which the bystander effect is transmitted has declined somewhat, with newer technological developments on the Super Microbeam displacing user availability. The track segment facility and our neutron capabilities remain in operation providing users spatially averaged particle irradiation for studies of cell populations.

**Experiments**

Listed in Table I are the experiments performed using the RARAF accelerator (a 5.5 MV Singletron; High

***Table I.*** *Experiments Run at RARAF January 1 - December 31, 2016*

| Exp No. | Experimenter | Institution | Exp. Type | Title of Experiment | Shifts Run |
|---|---|---|---|---|---|
| 110 | Tom K. Hei | CRR | Biol. | Identification of molecular signals of alpha particle-induced bystander mutagenesis | 4.5 |
| 113 | Alexandra Miller | AFRRI | Biol. | Role of alpha particle radiation in depleted uranium-induced cellular effects | 3.5 |
| 165 | Helen Turner | CRR | Biol. | Mouse/blood irradiation using IND spectrum neutrons | 2 |
| 172 | Susan Bailey | Colorado State University | Biol. | Targeted telomeric damage and the persistent DNA damage response | 1 |
| 173 | Ekaterina Dadachova | Albert Einstein College of Medicine | Biol. | Comparison of fungal cell susceptibility to external alpha particle beam radiation versus alpha particles delivered by $^{213}$Bi-labeled antibody | 1 |
| 174 | Gordana Vunjak-Novakovic | Columbia University | Biol. | Micro proton induced x-ray emission of bone/cartilege grown on artifical scaffolds | 1.5 |
| 175 | Constantinos Broustas/ Sanjay Mukherjee | CRR | Biol. | Mouse/blood irradiation using IND spectrum neutrons | 3 |
| 178 | Alejandro Carabe-Fernandez | University of Pennsylvania | Phys. | Microdosimetric and radiobiological characterization of new Si-based microdosimeters using particle microbeams | 2 |
| 179 | John Ng | Cornell University | Biol. | Effect of LET on immunotoxicity | 46.5 |

Voltage Engineering Europa) between January 1 and December 31, 2016 and the number of shifts each was run in this period. Half shifts are assigned when experimental time is shared among several users (e.g., track segment experiments) or when experiments run for significantly more or less than an 8-hour shift. Use of the accelerator for experiments was 47% of the regularly scheduled time (40 hours per week). Nine different user experiments were run during this period. Three experiments were undertaken by members of the CRR, supported by grants from the National Institutes of Health (NIH), the National Cancer Institute (NCI), the National Institute of Allergies and Infectious Diseases (NIAID) and the National Institute of Biomedical Imaging and Bioengineering (NIBIB). Six experiments were performed by external users, supported by grants and awards from the Department of Energy (DoE), the Department of Defense (DoD), the NIH, the National Aeronautics and Space Administration (NASA), the National Science Foundation (NSF), the National Cancer Institute (NCI), and internal funding from Cornell University. One of these experiments was a collaboration between RARAF/CRR staff and an outside user. Brief descriptions of these experiments follow.

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

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

Dr. Miller also instituted a study using her SEAC cell line in a comparison study of the RARAF neutron spectrum irradiator and the reactor neutron spectrum irradiator at AFRRI. This intercomparison work is being supported by AFRRI for their systems analysis for comparison to other facilities. Results of this work will be shared with RARAF and the broader community to further the understanding of the effects of differing neutron energy spectra.

**Helen Turner** and **Constantinos Broustas** made use of our neutron spectrum irradiation system to study the effects on mice and human peripheral blood samples. This work is supported by the Columbia-based Center for Medical Countermeasures against Radiation (CMCR) for the development of biodosimetry tools for a radiologic event. The mice were irradiated with up to 2 Gy of neutrons and comparison mice were given up to 4 Gy of x-rays using the Westinghouse orthovoltage x-ray system. The human blood samples were given up to 2 Gy of neutron spectrum dose and 4 Gy of x-rays. Some mice were also given 1 Gy of neutrons and then a secondary dose of x-ray to simulate a mixed field. The mice were sacrificed and blood was collected and scored for micronucleus and γH2AX foci, or global gene expression levels were measured, to determine dose response. The animals were also held in metabolic cages for collection of urine and feces for metabolic process variation determinations performed at Georgetown University.

**Susan Bailey** from Colorado State University works on the effects of telomere length and damage on the health and viability of cells. She makes use of the RARAF microbeam to target and irradiate telomeres in the cells. The work performed this year focused on

telomere degradation following targeted nuclear irradiation. The experiment was used also by the RARAF staff as a baseline imaging test for the imaging of telomeres using the labels of interest to Dr. Bailey with the new super resolution microscope, as that facility will become available for use early next year.

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

**Gordana Vunjak-Novakovic** uses our charged particle microbeam facilities for particle-induced x-ray emission (PIXE) analysis of cartilage-bone interfaces looking at chemical compositions of the two materials as they interface and progress through the life cycle. The change of concentration of calcium in both materials through the development of arthritis is of high interest in arthritis care and prevention. This past year, the neutron microbeam line has been modified to allow for this work to be performed at that endstation. This allows higher beam currents on the target for more rapid data acquisition. Samples from both sacrificed animals and laboratory constructs on artificial scaffolds are being measured. The design of the artificial scaffolds could lead to the ability to make bone and cartilage replacements in the labs grown from a patient's own stem cells for joint reconstruction and repair.

**Alejandro Carabe-Fernandez** of the University of Pennsylvania is developing silicon 3D radiation microsensor arrays, capable of quantifying deposited energies within micron-sized targets. Compared to traditional TEPCs, these detectors do not require a gas supply, operate at low voltages, are light and easily portable and have a fast response. The goal of this project is to use the targeting ability of the microbeam to characterize individual microsensors within the microdosimeter array. So far Dr. Carabe-Fernandez has irradiated two device prototypes on the Permanent magnet Microbeam and is developing a new detector array that can be more easily interfaced with the microbeam endstation. The goal would then be to characterize the response of different microdosimeter configurations (diameter, depth, and pitch) representing different cell types, and to derive relative biological effectiveness (RBE) from mechanistic biophysical models (e.g. MKM and LEM). The experimental RBE (relative biological effectiveness) obtained from clonogenic assays of individual cells exposed to the microbeam will also be obtained and compared to that obtained from the microsensors. This will allow investigators to: 1) characterize the microdosimetric properties of each individual microsensor as well as study crosstalk between the sensors in an array; 2) validate the microsensors as viable instruments to calculate RBE; 3) determine new features required to develop current microsensor technology to a new generation that allows more precise RBE measurements.

**John Ng** of Cornell University has expanded his work significantly with the help of the RARAF staff. Building on his significant experience in clinical cancer treatment, his project is looking for immune response signals from cells after irradiations using particles of different LET. The aim is to determine effects of targeted radiotherapy with specifically chosen particle energies that can be combined with immunotherapy to increase the efficacy of both for the treatment of many types of cancers, particularly the more radioresistant strains. This study has focused on a mammary tumor cell line that was developed at Cornell University for the study of immune response, in particular, the relocation of calreticulin from the endoplasmic reticulum to the cell membrane and the release of HMGB-1 and ATP into the intercellular matrix/media. These three responses are indicative of immune system triggering responses to irradiation from these cells. The experiment makes use of the RARAF track segment irradiator as a source for particles of different LET (from 10 to 160 keV/µm). The studies this year explored the higher end of the LET range (65-160 keV/µm). The results are promising in that they show a peaked response in all three assays at ~110 keV/µm. We are in the process of confirming these results. We also look forward to further exploring low LET (10, 25 and 40 keV/µm) and expanding these studies to other cancer and normal tissue cell lines.

**Development of Facilities**

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

- Focused particle microbeams
- IND spectrum neutron source
- Advanced imaging systems
- Targeting and manipulation of cells
- New cell analysis tools
- Small animal systems
- FLASH irradiation system
- UV sterilization systems

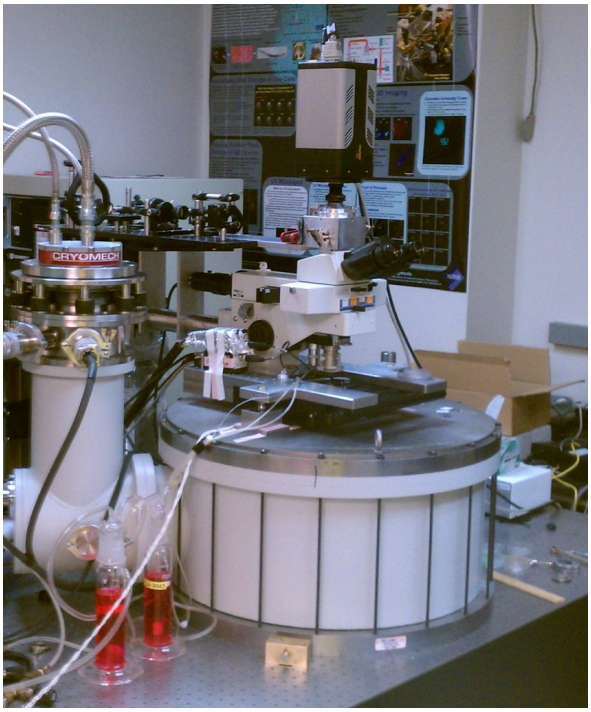


***Figure 1.*** *Super Microbeam solenoid and microscope endstation installed at RARAF.*

*Focused particle microbeams*

Prior to installation of the Solenoid magnet (see below), the electrostatically focused microbeam was consistently operated with a 1-2 µm diameter beam and 0.5 µm when called for by a particular experiment. The electrostatic microbeam focusing system was retired as part of the continued development of our Super Microbeam, which occupies the same location as the electrostatic system and use the microscope endstation developed for the microbeam system.

The Super-Microbeam development continued with the installation of the superconducting solenoid focusing system at the end of the microbeam beamline. This is the Phase 1 development of the Super Microbeam, using the solenoid as the only focusing element. A reconstruction of the microscope endstation (Fig. 1) was required as the spool of the electrostatic system and the solenoid housing are not of the same physical size. The system was assembled throughout the Summer and Fall of 2016 and initial beam testing was begun in late October. The current beam size is 3.5 µm with further alignment optimization underway. The ultimate size of the beam for Phase 1 will be 250 nm, which we plan to achieve early in 2017.

During the redevelopment of our electrostatic/Super Microbeam system, the permanent magnet microbeam (PMM) was used as our primary charged particle microbeam. This system is also our microbeam endstation for the development of our Flow and Shoot (FAST) microfluidics irradiation system, the capillary electrophoresis (CE) system, and the automated cell picking system. The PMM has all of the irradiation capabilities of the electrostatic microbeam except the sub-micron beam spot size. The PMM also does not have the potential for electrical breakdown from failures of the vacuum window, making it an ideal initial testbed for all our new technologies.

*IND spectrum neutron source*

The Improvised Nuclear Device (IND) spectrum irradiator was completed in 2014 and has been extensively used during the past two years. This year saw the irradiation of mice, fresh human whole blood samples, and plated cell lines.

This fast neutron irradiation source was designed to generate the neutron spectrum seen from the "Little Boy" atomic bomb dropped at Hiroshima at 1.5 km from ground zero. This field is generated through the reactions $^{9}Be(d,n)^{10}B$ and $^{9}Be(p,n)^{9}B$ using a mixed beam of monoatomic, diatomic and triatomic protons and deuterons. The RARAF Singletron uses a gas mixture of hydrogen to deuterium of 1:2 which feeds into the RF plasma ion source. This irradiator is on the 0º beam line as any bending of the beam to get to a target would separate the 6 different beams, preventing generation of the spectrum.

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

The base irradiation dose rate has been calibrated to deliver 0.25 Gy of neutrons in 10 minutes (with a gamma-ray contribution of an additional 1%). This dose rate allows the delivery of 1 Gy in under 1 hour.

*Advanced imaging systems*

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

Real-time imaging

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

Multi-photon microscope with the UV microspot

The multi-photon microscope was developed several years ago and integrated with the charged particle microbeam irradiator. This microscope, through the long

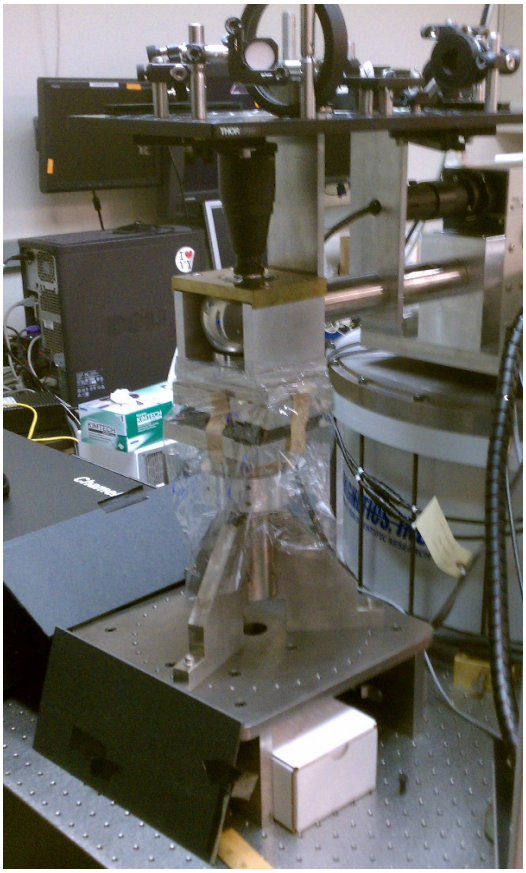

***Figure 2.*** *New endstation support spine. The extra stand at the bottom was required by the height of the new solenoid. The optical bench on the top is the same as previously installed for the multi-photon system.*

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

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

STED

We are developing a Stimulated Emission Depletion (STED) super resolution microscope system with optical resolution of 75 nm in combination with our super

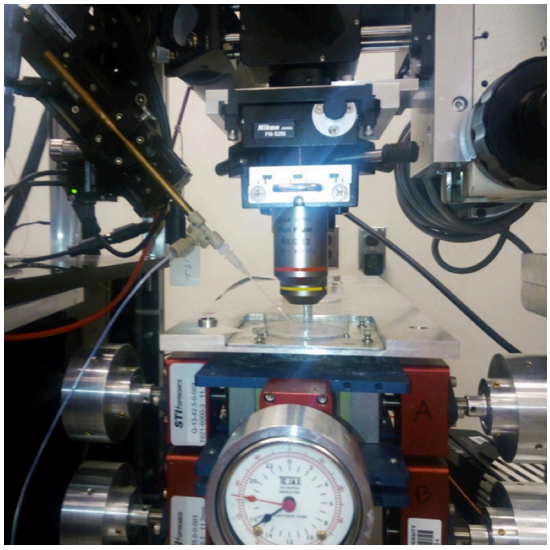

***Figure 3.*** *The single cell picker, mounted on the permanent magnet microbeam endstation.*

microbeam to achieve compatible imaging resolution and beam spot size. The STED system at RARAF builds on the multi-photon microscope using it as the primary excitation laser. A second continuous-wave (CW) laser is added coaxial with the multi-photon laser. Using polarization optics, the second laser projects a donut shaped point spread function around the excitation spot of the multi-photon system. With proper selection of the second laser wavelength and sufficient intensity, the second laser will deplete the fluorescent states around the excitation spot allowing fluorescence from the center of the donut, which will be reduced to nanometer sizes.

The STED development continues on the microbeam endstation (Fig. 2). The STED imaging project was paused during the reconstruction of the microbeam endstation for the Super Microbeam work. The preservation of the installed optical pathways allows the restarting of STED imaging early in 2017. We have made the decision to go to time gated gSTED and have begun purchasing the required equipment for this upgrade.

*Targeting and manipulation of cells*

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

Cell picker

Picking individual cells that are adhered to a microbeam irradiation dish is one of the methods to isolate cells from a microbeam dish and dispense into microfluidic single cell analysis devices. Previously this

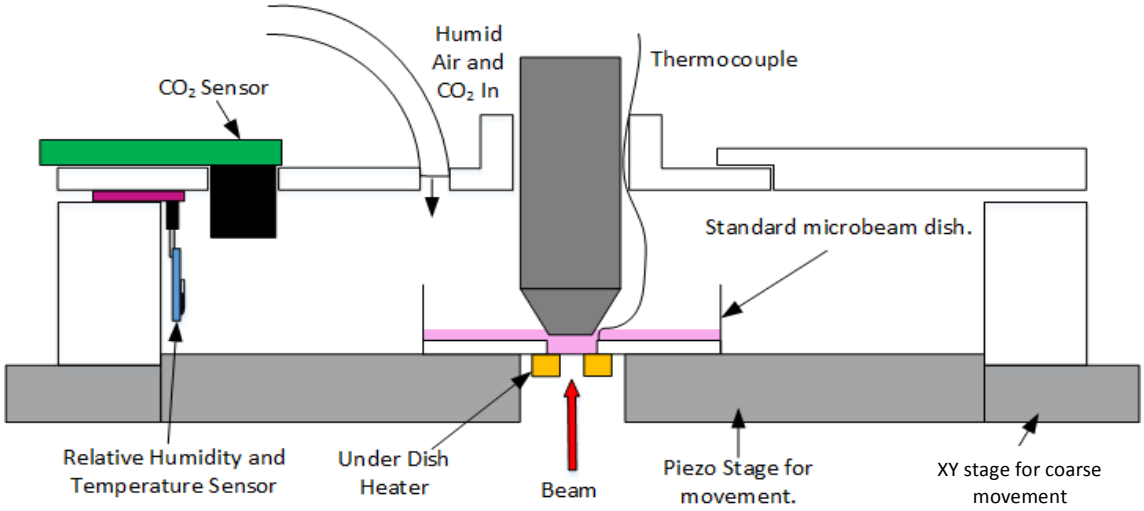


***Figure 4.*** *A schematic of the AMOEBA device shows the 3D printed case surrounding the microbeam dish and microscope objective. Sensors for $CO_2$, temperature, and humidity all continuously monitor environmental conditions. The control software controls inputs to the system to maintain the desired conditions. The AMOEBA is specifically designed to work within the current microbeam irradiation protocol, including the use of standard microbeam dishes.*

capability was incorporated in the Permanent Magnet Microbeam endstation (Fig. 3) as a semi-automated device that is part of the microbeam control software, and includes joystick control of robotic motion of a microcapillary. The microcapillary is brought next to a cell on a microbeam dish, trypsin is dispensed and the cell is aspirated into the capillary. In the past year we have worked to improve the workflow of the cell picker. We optimized the picking conditions including the amount of liquid on the cells, staining method, and imaging setup, as well as the general technique of locating a cell, then dispensing trypsin, and then aspirating a single cell. Our current push is to improve our picking speed and the efficiency of picking a single cell.

Cell dispenser

Development of the single cell dispenser has continued with a focus on improving electrical signal quality and testing a complete system with cells. We have improved the electrical signal quality, which is used to detect a cell passing over the microelectrodes within the device, by making the connection to the electrode more mechanically robust. This robust connection reduces the noise and makes triggering off of a cell detection event easier. Testing of the dispenser system has moved from using beads to using cells in suspension. In order to test cells for an extended period without them losing shape (due to their death since they are out of an incubator), we chemically fixed a batch of suspended cells. We also applied a crystal violet dye to the fixed cells to enable us to view them easily both within the microfluidic device and within a dispensed droplet. We started preliminary testing of the complete system and are currently evaluating the ability of the system to eject a single cell autonomously.

MicroFACS

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

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

AMOEBA

Significant development of the Automated Microbeam Observation Environment for Biological Analysis (AMOEBA) system in the past year has made it a functional biology support system at RARAF (Fig. 4). The system, which typically monitors and controls temperature, pH, and humidity on a microbeam endstation, is a modular configuration that can be adjusted for any number of experimental conditions. The system is run through custom software that can monitor multiple inputs simultaneously and make appropriate changes to control the environment. A 3D printed shroud fits around the existing endstation and defines the volume around a microbeam dish with environmental control. The AMOEBA allows for long term experiments where cells can be exposed using the microbeam and continuously observed for over 36 hours. The system's performance was verified through the observation of cellular division, which is an indicator of cell health, during an extended observation on the endstation. Users who wish to use the AMOEBA for their microbeam experiments can work

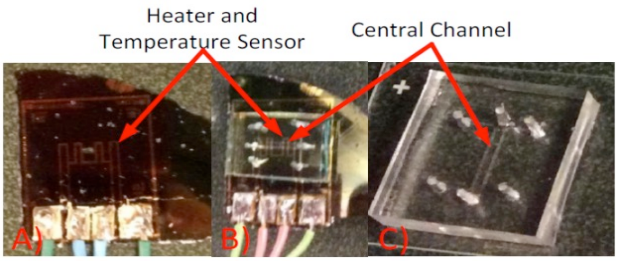


***Figure 5.*** *Three photographs of prototype microAMOEBA systems show progress in integration of silicon components with PDMS. Temperature sensors and heaters (A, B) were fabricated by patterning metal on the silicon surface. The PDMS structure (B, C) creates a controlled microfluidic environment for cell culture and irradiation.*

with the RARAF team to configure the AMOEBA for their needs.

The microAMOEBA (Fig. 5) is similar to the AMOEBA, which has been designed to work around the existing microbeam irradiation protocol, because it also has the goal of carefully controlling the environment during a microbeam experiment. The microAMOEBA is unique because it aims to specifically control the microenvironment around cells with the added goal of enabling faster changes of controlled parameters than would be possible with the AMOEBA. The microAMOEBA's significantly reduced control volume makes this possible. The microAMOEBA is designed to operate using the same control software and modules as the AMOEBA system, while the sensors and actuators for the system are made within a microfluidic system. Our goal is to construct the microAMOEBA using a silicon substrate, which can contain all necessary electrical connections and a thin window to allow the microbeam to reach the cells, and an attached microfluidic structure made of PDMS. The PDMS not only acts as the cell culture chamber, but it also allows for control of the dissolved oxygen level through controlled diffusive transport. Ruthenium dye is being examined for use as a fluorescent reporter of dissolved oxygen within in the system. We continue to characterize the dissolved oxygen control parameters and limitations in an effort to enable fast changes within the microenvironment.

Another key factor that the system has been designed to control is temperature. We have shown the ability to control temperature on the silicon substrate, which is the surface that cells are grown on, through a range of temperatures from 37° C up to 60° C. Extensive testing has been performed, culturing cells within the sealed microfluidic environment. HeLa cells have been maintained within a microfluidic device for multiple days.

*New cell analysis tools*

CE-LIF

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

In the CE-LIF system at RARAF (Fig. 6) the grounded end of a 50 µm bore capillary is brought to the cell using the semi-automated cell picker. Once a cell is aspirated into the capillary, 20-30 kV is applied between the grounded end of the capillary and the Laser Induced Fluorescence (LIF) system, enclosed in a light tight insulating box. This results in two superimposed flow modalities experienced by the analytes: (1) Electrophoretic flow, responsible for separating the analytes by charge and Stokes radius; (2) Electroosmotic flow, which drives the buffer and analytes (regardless of polarity) toward the detector. The electroosmotic flow is

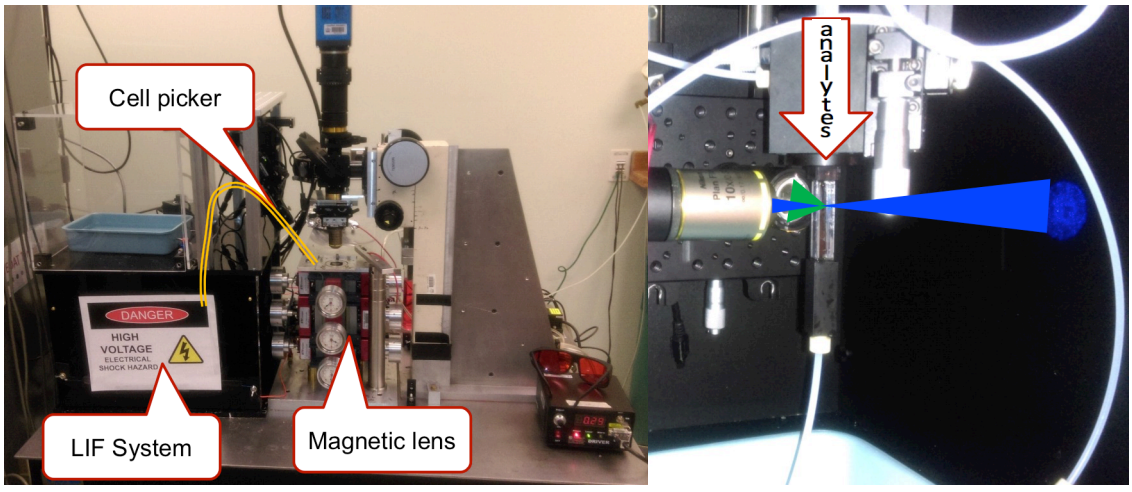


***Figure 6.*** *The CE-LIF system mounted on the Permanent Magnet Microbeam (left). The 360 µm diameter capillary has been highlighted in orange for clarity. The right panel shows a close up of the LIF system. An analyte stream flowing out of the capillary intersects an excitation laser beam (blue). Fluorescence emission light (green) is collected perpendicularly into an optical fiber, and detected by a high sensitivity spectrometer.*

much stronger than the electrophoretic flow, ensuring that all analytes will reach the detector. In the LIF system, the analytes are hydrodynamically focused into the path of a laser, with the light collected perpendicularly and detected by a high-sensitivity spectrometer. We have recently acquired a deep cooled Bayspec spectrometer, providing highly sensitive detection of fluorescent molecules.

Mouse Phantom

The anatomically accurate mouse phantoms continue to be used in various capacities around the CRR. A crucial development in the past year has been the comparison of the mouse phantom performance in a sample irradiation with a computer model of the same radiation. Two common irradiations performed in the CRR, both using the Small Animal Radiation Research Platform (SARRP), were used to compare modelling with the physical phantom: a lung irradiation using a 3 mm square collimator and an abdominal irradiation using a 5 mm square collimator. The physical models were tested using radiochromic film strategically placed within the phantoms. The computer simulation was performed in MCNP, and included the phantom as well as the SARRP. A comparison of the resulting radiation dose map, specifically in regions of very low dose outside of the target region, showed very good agreement between the physical models and the simulation. These results confirmed that this unique phantom is a good tool to accurately assess dose distribution.

The mouse phantoms were also used to help assess neutron dosimetry for the CMCR projects. The phantoms were loaded into exactly the same position as the mice used in this experiment, thus allowing us to confirm that a uniform dose was received through the body of the mouse while it was rotated around the neutron source.

*FLASH Irradiator System*

To study the possible beneficial effects of ultrahigh dose-rate proton irradiations on complications affecting normal tissue after radiation therapy, we assembled the ultra-high dose rate irradiator. It allows us to deliver short charged particle pulses carrying therapeutic doses of several tens of Gray to a 0.12 $in^2$ area. With this irradiation setup we can precisely define the time of the charged particle pulse (ranging from less than a millisecond to several minutes) delivered to a sample, therefore achieving dose-rates of up to several hundred Gray per second. Development of unwanted radiation-induced late effects was initially investigated using a full thickness EpiAirway lung tissue model from MatTek that consists of normal human tracheal/bronchial epithelial cells co-cultured with normal human stromal fibroblasts. Biological endpoints that were investigated are histological and inflammatory markers of the onset of inflammation and fibrosis in the tissue models used. The ability to change the dose rate by several orders of magnitude with the same setup, makes it possible to compare the tissue response to ultrahigh dose-rate proton irradiations with the response to irradiations done using conventional therapeutic dose-rates (2 Gy/min).

*UV Sterilization Systems*

Scientists at RARAF continue to explore the use of far-UVC light as a tool for killing bacteria and viruses. While conventional germicidal lamps, most notably at 254 nm, are very effective at killing pathogens, they are also harmful to humans. The deep ultraviolet sterilization (DUVS) work performed at RARAF aims to utilize specific very short wavelength UVC (207 nm) as a safe means of pathogen elimination. The theory of this work centers on the limited penetration of short wavelength UV radiation. UVC light with a wavelength in the range of 200-225 is strongly absorbed by proteins, thus its ability to penetrate biological materials is very limited. The very short half value distance means that while the light can penetrate bacteria and viruses, which are typically smaller than 1 μm, it cannot penetrate the human stratum corneum (the outer dead-cell skin layer, thickness 5-20 μm), nor the ocular cornea (thickness ~500 μm), nor even the cytoplasm of individual human cells. Recent work in this area has aimed to both verify the effectiveness of UVC at eliminating pathogens and to show that exposure to these wavelengths does not pose a significant threat to human health.

The efficacy of DUVS for inactivation of aerosolized influenza viruses is one project currently underway. A special exposure chamber has been engineered to control aerosol generation, DUVS exposure time, and collection of exposed materials. The system has controls for input pressure, which controls aerosol generation rate, humidity, which effects droplet size, and volumetric flow rate, which controls the exposure time and also droplet size. The goal is to simulate virus aerosolization through human coughing, sneezing, and speaking. Tests with various DUVS exposure methods are underway.

Another project testing DUVS is the application of UVC to the penetration site of implants or catheters, which are prone to infection. Current tests incorporate transmitting laser generated UVC light with an optical fiber and then applying the UVC through an optical diffuser. The diffuser could be placed within the penetration site alongside any implant or catheter and provide a means of sterilization. Current tests of bacteriological killing of MRSA are being performed in vitro and have demonstrated the ability to inactivate the bacteria. Work continues to quantitate the efficacy of this approach and to explore in vivo application with animal trials.

Tests of the safety and efficacy of 222 nm light have also been a focus in the past year. Numerous experiments have been performed using mice that have simulated wounds or surgical site infections. The wounds have been exposed to UVC light to determine its effectiveness for infection control. Additional tests using cells in culture and artificial human tissue models were performed in vitro and were essential in demonstrating the safety of

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

| | |
|---|---|
| Radiobiology and associated dosimetry | 26.5% |
| Radiological physics and chemistry | 1% |
| On-line facility development and testing | 21.5% |
| Safety system | 5% |
| Accelerator-related repairs/maintenance | 8.5% |
| Other repairs and maintenance | 2.5% |
| Off-line facility development | 72% |

DUVS over long-term exposures. Additionally, preliminary tests to examine the safety of UVC light regarding cataract formation were initiated and continue into the coming year.

**Singletron Utilization and Operation**

Table II summarizes accelerator usage for the past year. The nominal Singletron availability is one 8-hour shift per weekday (~248 days per year), however the accelerator is frequently run well into the evening, often on weekends, and occasionally 24 hours a day for experiments or development. Total use for experiments and development this year was 59% of the regularly available day shifts.

Accelerator use for radiobiology and associated dosimetry was about 70% of the year before and below the average for the last 5 years. About 76% of the use for all experiments was for track segment irradiations, 8% for charged particle microbeam irradiations, and 16% for neutron irradiations. Approximately 79% of the experiment time was for studies proposed by external users, and 21% was for internal users.

On-line facility development and testing was about 21.5% of the available time, primarily for development and testing of the Super Microbeam solenoid focusing system. Significant time was also dedicated to the multiple microfluidic and analysis tools using the PMM endstation. This is about average for the last five years and slightly more than the previous year.

The accelerator was opened twice in 2016 for ion source replacements. This maintenance requires ~5 days for a full turn around cycle and was scheduled over weekends for minimal interference with experiment and development schedules. With the new DREEBIT Heavy Ion Source development proceeding, we expect to have significant accelerator maintenance and openings in the coming year for this accelerator enhancement.

**Training**

*REU*

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

The 2016 REU participant at RARAF was Connor Crickmore from St. Edmund Hall, Oxford University. Connor worked with David Welch and Manuela Buonanno to examine the efficacy of deep ultraviolet light to inactivate aerosolized influenza virus. Much of the 10-week program involved modifying the experimental aerosol chamber. Controls were added for adjusting the relative humidity within the chamber, which is crucial for creating aerosol particles of appropriate sizes to mimic human coughing, sneezing, and breathing. Additional improvements to the experimental setup allowed for easy switching from the test configuration to the experimental configuration, therefore simplifying the experimental procedure. The aerosol system was parametrically characterized independent of any UV light with the goal of determining how to control droplet size distributions. After gathering and analyzing experimental data, a set of good operating conditions was established and preliminary tests running influenza virus through the aerosol chamber were performed. Work with aerosol sterilization using various deep UV light sources is ongoing.

*Group Training*

In addition to training individuals at RARAF, staff members also participate in training courses presented at other facilities as a means of introducing microbeam concepts and experiments to a broader audience. This year, Andrew Harken lectured on "High/low LET microbeams" at the NASA Space Radiation Summer School, Brookhaven National Laboratory, Upton, NY, on June 11, 2016.

*Microbeam Training Course*

The fifth RARAF Microbeam Training Course "Single-Cell Microbeams: Theory and Practice" was given May 16-18, 2016. There were nine students (Fig. 7) participating, listed in Table III. Dr. Marcelo Vazquez returned as the director of the Microbeam Training Course.

The course followed the same schedule as in previous years, with lectures and hands on laboratories for the design, operation, and use of charged particle microbeams. The course spanned three days opening with lectures on day one, hands-on laboratory experience on day two, and concluding lectures on day 3.

A main feature of the course is the experimental design done by each of the students as if they were proposing to come to RARAF to do an experiment. The

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

| *Name* | *Position* | *Affiliation* |
|---|---|---|
| ***Jason Annkah*** | Ph.D. Student | Dept. of Medical Physics & Biomedical Engineering, University College, London |
| ***Jerome Lacombe*** | Postdoctoral Researcher | Center for Applied NanoBioscience & Medicine, Univ. of Arizona |
| ***Luis Spitta*** | Research Scientist | Institute of Aerospace Medicine, German Aerospace Center (DLR), Cologne, Germany |
| ***R. Ileng Kumaran*** | Postdoctoral Researcher | Cold Spring Harbor Laboratory |
| ***Han Xu*** | Ph.D. Student | Dept. of Physics and Radiation Laboratory, Univ. of Notre Dame |
| ***Rob Hinshaw*** | Ph.D. Student | Medical Engineering and Medical Physics, Harvard-MIT Health Sciences & Technology |
| ***Tao Ye*** | Ph.D. Student | Center for Ion Beam Applications, National University of Singapore |
| ***Veljko Grilj*** | Postdoctoral Researcher | Columbia University, Center for Radiological Research |
| ***Prenkumar Saganti*** | Professor | Dept. of Physics, Prairie View A&M and the NASA Center for Applied Radiation Research, Prairie View, Texas |

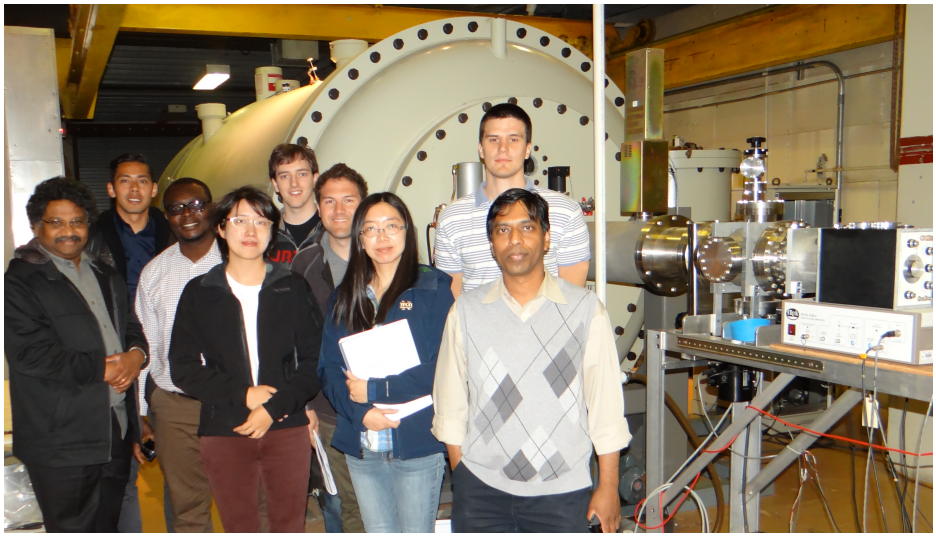

***Figure 7.*** *Students at the fifth RARAF Microbeam Training Course.*

students work with the RARAF staff to devise potential experiments and then present their proposals at the end of day 3 as a final demonstration of what they have learned from the course about the nature of microbeams and their potential applications.

**Dissemination**

*Web site*

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

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

*Virtual training course*

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

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

The on-line curriculum material consists of audio podcasts and the same handouts that the face-to-face students received. The audio of each podcast is synched with the accompanying PowerPoint slides (viewable on a video iPod, tablet, PC or Mac, or smart phone), as well as a PDF version of the slides. High-resolution video (720p, with audio) was also used to document demonstrations of all aspects of a microbeam experiment, from making microbeam dishes to irradiating cells and performing online analyses. After extensive editing, this resulted in about 4½ hours of video footage. Additional material is added to the on-line course for new course presentations or lecturers.

The on-line training course can be accessed through the RARAF website (www.RARAF.com) and YouTube channel (http://www.youtube.com/user/RARAFcourses). The videos can be viewed on any Internet-enabled device supporting YouTube format.

*Tours*

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

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

**Personnel**

The Director of RARAF is Dr. David Brenner, the Director of the Center for Radiological Research (CRR). The accelerator facility is daily managed and operated by Dr. Gerhard Randers-Pehrson and Dr. Guy Garty, the Co-Associate Directors of RARAF.

Dr. Charles Geard, a Senior Biologist Emeritus, continues to visit RARAF frequently, lending his considerable expertise.

Dr. Gerhard Randers-Pehrson, a Senior Research Scientist, has extensive experience in accelerator physics. He oversees operation of the Singletron, and all aspects of accelerator development.

Dr. Guy Garty, an Associate Professor at CUMC, is developing the CE-LIF system. Dr. Garty is also PI of the Center for High Throughput Minimally Invasive Radiation Biodosimetry's Radiation Core and PI of a NIAID-sponsored contract for developing an automated dicentric assay.

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

Dr. Andrew Harken, an Associate Research Scientist, is responsible for the Super Microbeam development with STED imaging. He is also the project lead on the microFACS system.

Dr. Manuela Buonanno, an Associate Research Scientist in radiation biology, collaborates with many of the external users and performs assays using diverse biological systems.

***RARAF Staff:*** *(front row, l-r) Guy Garty, David Brenner (Director) Manuela Buonanno, Sofia Barbieri, Malek Haj Tahar, (back row) Veljko Grilj, David Welch, Andrew Harken, Christian Siebenwirth.*

Dr. David Welch, an Associate Research Scientist, is responsible for the development of new microfluidic tools and interfaces for microfluidic irradiation tools. His expertise in microfluidics has been of considerable assistance in the development of our microfluidics applications.

Dr. Veljko Grilj, a new Postdoctoral Research Scientist, Joined RARAF in 2016. Dr. Grilj is responsible for assisting Dr. Harken with the Super Microbeam development. He is also responsible for working with Drs. Ponnaiya and Buonanno, and operating the accelerator for our outside user experiments.

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

Two postdoctoral Research Scientists and a visiting PhD student joined RARAF at the beginning of 2017:

Dr. Christian Siebenwirth, a Postdoctoral Research Scientist, will be responsible for the DREEBIT Heavy Ion Source accelerator development project.

Dr. Malek Haj Tahar, a Postdoctoral Research Scientist, will be responsible for assisting the modeling of RARAF ion beam systems. He will be taking the lead in the development of a new small animal irradiation therapy system as a potential future direction.

Ms. Sofia Barbieri, a Ph.D. Candidate at the University of Pavia in Italy, has joined us for 6 months and will be working on the microFACS project computer programming and user interface. She will also continue her Ph.D. work looking at H2AX focus formation with respect to particle LET. ■


*(l to r, from Top): Pi day celebration at the CRR - Nils Rudqvist, Sally Amundson, Lubomir Smilenov, Mashkura Chowdhury, and Aesis Luna. Sally Amundson, Margaret Zhu, and Tom Hei. David Brenner and Tom Hei. Lance Redford. Helen Turner and Gerhard Randers-Pehrson. David Welch, Robert Morton, and Vladimir Ivanov.*