An overview of RARAF:
From broad beams to microbeams, single proteins to small animals,
where we have been to where we are going.

Andrew Harken
March 28, 2013
An overview of RARAF

- History of RARAF
- Broad beams
- Microbeams
- Imaging
- Microfluidics
- Where we are going
The Radiological Research Accelerator Facility

- **Who are we?**
  - We are a Biomedical Technology Resource Center (P41 BTRC) under the National Institute of Biomedical Imaging and Bioengineering (NIBIB) through the National Institute of Health (NIH)

- **What do we do?**
  - RARAF is a multidisciplinary facility designed for the delivery of known quantities of radiation to target samples with micrometer precision using a single-cell/single-particle microbeam irradiator.
The Center for Radiological Research

- Founded 1915 to study applications of radiation in medicine
- Early developments:
  - Dose (≡Energy/mass)
  - “Controlled” Radiation therapy
- Today:
  - Biological consequences of radiation exposures.
- RARAF is the “physics arm” of the CRR

Dr. Gioacchino Failla (1891 - 1961)
RARAF: History

- In the mid-1960’s, Drs. VP Bond (Brookhaven) and HH Rossi (Columbia CRR) want a monoenergetic neutron source to study biological effects, measure dosimetry and develop microdosimetry.

- RARAF opens at Brookhaven 1967
  - 4 MV Van de Graaff Accelerator
  - Original injector for the Cosmotron 2 GV collider at Brookhaven
RARAF Accelerator
Van de Graaff at Brookhaven National Laboratory 1949
RARAF moved out of Brookhaven in 1980 to make space for the ISABELLE p-p collider .....which was never completed.
RARAF Accelerator Move from Brookhaven

Nevis 200 MeV cyclotron partially disassembled and being entombed
RARAF Accelerator move from Brookhaven

Van de Graaff stored at Nevis and being positioned

RARAF literally built around the accelerator!
RARAF Accelerator Replacement 2005

Van de Graaff before removal
RARAF Accelerator Replacement 2005

New Accelerator going in new back door
RARAF Accelerator Replacement 2005

Singleton, baseplate & quadrupole

HV power supply & resonator coil
Our 5.5 MV Singletron Accelerator from High Voltage Engineering Europa (HVEE)

Interior of Singletron
Terminal with shell removed
Our 5.5 MV Singletron Accelerator

Electrodes & diode stacks with spark gaps

Cockcroft–Walton charging system ± 100V
Our 5.5 MV Singletron Accelerator

RF ion source
Our 5.5 MV Singletron Accelerator

- **Available source gases**
  - Helium \(^{4}\text{He}+, ^{4}\text{He}++\) \(^{4}\text{He}++ = \text{simulated alpha}\)
  - Hydrogen \(^{1}\text{H}+, ^{1}\text{H}_2+, ^{1}\text{H}_3+\)
  - Deuterium \(^{1}\text{D}+, ^{1}\text{D}_2+, ^{1}\text{D}_3+\)
  - Nitrogen \(^7\text{N}+ \text{to} ^7\text{N}+5\)
  - Helium-3 \(\text{Not currently installed}\)
Beam Lines in RARAF

1. Microbeam
2. UV Microspot

Permanent Magnet Microbeam

X-ray microbeam

Track Segment Facility
Neutron Facility
RARAF Irradiation Modes

**Particles:**
- Charged particles
  - Broad beam
  - Microbeam
- Neutrons
  - Broad beam
    - Monoenergetic
    - Spectrum irradiator
  - Microbeam

**Photons:**
- X-rays Microbeam
- UV Microspot

**Offline Sources:**
- Cs-137 irradiator
- 250 kV x-rays
Broad beams: Where we started

**Particles:**
- Charged particles
  - Broad beam
  - Microbeam
- Neutrons
  - Broad beam
    - Monoenergetic
    - Spectrum irradiator
  - Microbeam

**Photons:**
- X-rays Microbeam
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**Offline Sources:**
- Cs-137 irradiator
- 250 kV x-rays
Monoenergetic Neutrons

- Proton or deuterium beam on to deuterium and tritium make neutrons
  - $T(d,n)^4He / T(p,n)^3He / D(d,n)^3He$
- Angular location of the sample determines final neutron energy

Target

Neutron system installed at Nevis
Charged Particle Irradiators:

- **Broad Beam**
  - Track segment
  - Beam spread out into uniform line
  - Dishes are passed through the irradiation zone
  - Time of passage averaged with beam rate = dose
  - Lots of cells (100's of thousands+)
  - Cells get irradiated in both nucleus and cytoplasm (average number of particles in each area)
Low dose radiation risk estimation

Increased risk

Dose

Domestic radon exposure $<<1 \alpha/\text{cell}$

Coal miner data $>>1 \alpha/\text{cell}$

Microbeam Experiments!

Broad beam experiments $\sim 1 \alpha/\text{cell}$ (poisson)
Microbeams: Where We Are

**Particles:**
- Charged particles
  - Broad beam
  - Microbeam
- Neutrons
  - Broad beam
    - Monoenergetic
    - Spectrum irradiator
  - Microbeam

**Photons:**
- X-rays Microbeam
- UV Microspot

**Offline Sources:**
- Cs-137 irradiator
- 250 kV x-rays
What is a Single-Cell Microbeam?

- A single-cell microbeam can deposit ionizing radiation damage in micrometer or sub-micrometer sized regions of cells.
A quantitative example of inter-cellular damage communication: Bystander Responses

Damage is expressed in “bystander” cells, which are *near* to an irradiated cell, but have *not themselves received any energy deposition*.
Low-dose risk estimation and the bystander effect

- Where bystander responses have been quantitated, they have shown saturation.
- In such cases, extrapolating linearly from low to very low doses could underestimate the risk at very low doses.

Based on mutation data from the RARAF microbeam.
Microbeams represent the most direct way to study intercellular damage response

- Produces DNA damage in defined cells, while guaranteeing that adjacent cells are not hit
- Can study effects in the adjacent cells

Blue-stained nuclei: HIT cells
Red-stained cytoplasm: NON-HIT cells
How to make a microbeam?

Imaging system

Moveable stage

Particle detector

Focusing lenses

Beam deflector/shutter

Accelerator
Foci formation at DNA damage site

HT-1080 cells with GFP-tagged XRCC1 SSB repair protein

Cells, courtesy of David J. Chen
Painting “NIH” on a cell nucleus with gfp-tagged XRCC1 repair foci, using our 0.6 mm microbeam

Cells, courtesy of David J. Chen
Example of a microbeam

The Permanent Magnet Microbeam (PMM)
Quadrupole lens

Compression stronger than expansion

Magnetic

Electrostatic
Double triplet lens

\begin{figure}
\centering
\includegraphics[width=\textwidth]{double_triplet_lens}
\caption{Illustration of a double triplet lens with dimensions and parameters.}
\end{figure}
How small can we go?

<table>
<thead>
<tr>
<th>Year</th>
<th>Technique</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td>Pinhole aperture</td>
<td>10</td>
</tr>
<tr>
<td>2001</td>
<td>Focused: Single quadrupole quadruplet</td>
<td>5</td>
</tr>
<tr>
<td>2007</td>
<td>Focused: Single quadrupole triplet</td>
<td>1.3</td>
</tr>
<tr>
<td>Today</td>
<td>Focused: Compound quadrupole triplet</td>
<td>0.5</td>
</tr>
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</table>
Endstation

- Imaging
- Detector
- Piezoelectric stage
- Beamline
Other Microbeams
Multiphoton Microscope

Possible to target a mitochondrion in a live small airway epithelial cell

UV Microspot

- **UVA / UVB / UVC**
  - 3.45 - 5.47 eV
  - 227 - 360 nm

- **Three-photon**
  - UVA / Visible
  - 2.30 - 3.65 eV
  - 340 - 540 nm

- **Two-photon**
  - Visible / IR
  - 1.15 - 1.82 eV
  - 680 - 1080 nm

- **Single-photon**
  - Classification
  - Energy Range
  - Wavelengths

- **Multiphoton Mode**
  - Form of laser microbeam
  - Multiphoton processes produce a micro-volume of UV radiation
  - Can deliver “spot” damage, an advantage over all other microbeam systems

Use Imaging Tool as an Irradiator

<table>
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<tr>
<th>Multiphoton Mode</th>
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<td>227-360 nm</td>
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<tr>
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</tr>
</tbody>
</table>

Fluorescein
OGG1 – View 6
Irradiation: 5 horizontal line scans (~50 mJ delivered to cell nucleus)

Observation: faint response, signal remains in nucleus.

Cells, courtesy of David J. Chen
Soft X-ray microbeam
Zone Plate

- 120 μm diameter
- 50 nm outer ring spacing
- 1st order transmission efficiency at 4.5 keV 12.5% or better
- 10 mGy/sec delivered to sample with a 5 μm spot
PIXE Soft X-ray Microbeam:

- Proton spot size
  120 μm x 50 μm

- X-ray Spot size:
  5 μm x 5 μm

- Present dose rate
  10 mGy/sec
  (10 photons absorbed)
Irradiation Results

AG1522 cell stained for γ-H2AX
Fixed 30 minutes post irradiation
Lens Chamber

Nanopositioning Stage

Zone Plate Holder

Target

protons →
A neutron microbeam?

- 30 keV Neutrons cannot be focused
- Use a kinematic trick!
  - $^7\text{Li}(p,n)^7\text{Be}$ has threshold at $E_p = 1.880 \text{ MeV}$
  - At $E_p = 1.880 + \delta$
    - Momentum at CM very low
    - Momentum in Lab strongly forward peaked

Requires tight control of beam energy
Neutron Microbeam

- Neutron microbeam visualized with $^6$Li coated CR-39 track etch detector

Sept 2012: 90 µm
Feb 2013: 70 µm
Design goal: 20 µm
Imaging
To target cells or sub-cellular components they must be imaged.

Most common approach is with fluorescent labeling.
Fluorescent labeling is often OK, but not always.
Cytotoxicity, Mutagenicity and DNA damage by Hoechst 33342.

Durand RE, Olive PL.

We use 50-100 nM
Cell survival with various UV intensities

We use < 0.5 mW/cm$^2$
Rapid EMCCD image acquisition at the microbeam

Image quality maintained with EMCCD, but with a major decrease in UV exposure
SIMI: Simultaneous Immersion Mirau Interferometry

HT1080 fibrosarcoma cells imaged with SIMI in PBS
Microbeam-Integrated Multiphoton Imaging System

- To provide 3D imaging capability for RARAF microbeam users, which is integrated with the microbeam irradiation system
- To record post-irradiation dynamics in cells, tissue samples, and small organisms
Principle of multiphoton imaging
Light-tight detector housing (2 PMT channels)

Scan Head

Beam Expander

Switch Mirror

Scan Lens

Attenuator

Chameleon Ultra II Ti:S Laser (680-1080nm)

Instrument Designer
Gary Johnson
Examples of 3-D tissue imaging at the RARAF microbeam

- **In vitro**
  - Human Umbilical Vein Endothelial Cell tissue
    - YOYO-1 stain (green)
    - Auto-fluorescence (blue)

- **In vivo**
  - Wild type *C. elegans* pharynx
    - Auto-fluorescence (blue)
    - Second Harmonic Generation (red)
Animal models for the microbeam

- A lot of interesting biology happens in 3D systems

Requirements for small animal irradiation:

- Thin sample - Proton penetration 200-300 μm
- Optically clear – to enable targeting
- Well established system – need good endpoints
In vivo microbeam irradiation of worms

Worms have green fluorescent protein expressed in response to stress
• Microbeam irradiation of either atrium or ventriculum
• To investigate repopulation of non-irradiated cells in lethally exposed areas
• Studies just initiated (Dr. K. Targoff)
Mouse irradiations

We have designed and built a holder to position the flattened mouse ear over the microbeam port.
Mouse irradiations
Microfluidics on the Microbeam
Why microfluidics

*Microfluidics* is the science and technology of systems that manipulate minute amounts of fluids, using sub-millimeter microchannels.

*Microfluidics provides:*

- High throughput/automation of single cell handling
- A host of single cell analysis devices
**FAST (Flow-And-Shoot Technology)**

- **Currently**
  - Cells *attached* to microbeam dishes, and either:
    - Dish *moved* to bring cellular target over the microbeam
    - Beam moved to shoot cellular targets (point & shoot)

- **Proposed**
  - Cells *flowing* through a microfluidic channel
    - Cells targeted by Point & Shoot as they flow by
    - Cells dispensed to user device
FAST (Flow-And-Shoot Technology)

Mounted on Permanent Magnet Microbeam

- 5µm diameter beam
- Point & Shoot
- 5.3 MeV He$^{++}$/protons
- 1000/sec
Real time tracking

- Images the flowing cells
- Center of cell located **in real time** frame to frame
- Future position predicted using:

\[
X_{i+1} = X_i + \frac{X_i - X_{i-1}}{T_i - T_{i-1}} \times (T_{i+1} - T_i)
\]

- New position
- velocity
- Old position
- Actual time
With Cells

- CRL4025 - Human endothelial cell line (trypsinized)
- GFP expressed throughout the cell.
Optical manipulation of cells

- Why do we want this?
  - Parallel Manipulation of cells
    - Controlling distance between cells before, during and after irradiation
    - Handling groups of cells in parallel
  - Manipulating cells in suspension
Optical manipulation of cells
How does it work?

- Optical tweezers system:
  - Transparent conductive top electrode
  - Photoconductive bottom electrode
  - Dynamic light source

Light pattern generator
Optical manipulation of cells
How does it work

- Light pattern generated on photoconductive electrode
- AC is applied
  - Electric fields are formed
- Fields repel cells
  - No physical barriers!
Optical manipulation of cells
How does it work

- Light pattern generated on photoconductive electrode
- AC is applied
  - Electric fields are formed
- Fields repel cells
  - No physical barriers!
  - Illumination pattern can be changed dynamically.
    - Cells can be boxed in
    - Cells will track pattern
How does this work?
Optical manipulation of cells
preliminary data with beads

20 micron non-fluorescent beads
Microfluidic irradiation of worms

We have:

- Built worm clamps with thin (~10 µm) bottoms, to allow microbeam penetration
- 4 channels/clamp

Worm irradiations now routinely performed in clamps
Where are we going?
Aiming at still smaller targets

- At present our imaging capabilities and our microbeam targeting capabilities roughly match (as they should)
- Both are around 300 - 400 nm
- Imaging limits are because of the Abbe diffraction limit
- Microbeam diameter limits are because of inherent spherical and chromatic aberrations from our electrostatic focusing lenses
We want to target specific areas on a single chromosome
We want to target transcription sites

Solovjeva L et al. Mol. Biol. Cell 2005
We want to target mitochondria
Current imaging on the Microbeam

Resolution ~400 nm

Resolution ~250 nm
The Abbe diffraction limit $d_{\text{min}} \approx 200 \text{ nm}$

$$d_{\text{min}} = \frac{\lambda}{2 \times NA}$$

$$NA \leq n (1 - 1.5)$$

$$360 \text{ nm} \leq \lambda \leq 800 \text{ nm}$$
The Particle Beam Focusing Limit

Limiting aperture diameter (microns)

Microbeam Diameter (microns)

Expected microbeam spot size vs. pre-lens aperture size, for different count rates

10
1
0.1
10 100 1000
0.5

Aberration limit

Where we are now

Sept ‘08

March 09

Nov. ‘07

March 09
Breaking through both the aberration limit and the diffraction limit on the microbeam
Getting to the limit

Stray magnetic fields can deflect the beam

Opening/closing the door
Moved beam by several microns!
The Super Microbeam

We currently use quadrupole electrostatic lenses to provide the strong fields necessary for focusing.

These electrostatic lenses provide a lower limit on how small a diameter we can make the microbeam, due to their intrinsic spherical and chromatic aberrations.

In principle, solenoid lenses can provide lower spherical and chromatic aberrations, and consequently superior spatial resolution.
A solenoid has many coils of wire carrying DC current

The field is strong and uniform in the center and weak and divergent on the outside.

Ions entering a solenoid spiral around the field lines and are periodically refocused onto the axis.

Head-on view
When the field strength and the length of the solenoid are selected appropriately, the ions make one partial turn and then focus beyond the far end of the solenoid.
Double superconducting magnet solenoid lens design

Predicted beam spot: 75 nm
Super Resolution Microscopy

- Super Resolution microscopy is needed for targeting at the 70 nm resolution for the super microbeam

- We have chosen to use STimulated Emission Depletion (STED)
How does STED work?

- Fluorescence limited to the sub-diffraction spot by a depletion ‘donut’ surrounding the excitation focus
- Depletion happens by de-exciting the fluorophores stimulating them to emit at a longer wavelength – STimulated Emission Depletion
- Requires STED intensity >> Fluorophore saturation intensity

\[
\frac{1}{\lambda} = \frac{1}{2 \cdot NA} \sqrt{1 + \frac{I_{STED}}{I_{Fsat}}} \]

STED Resolution
Super Resolution STED at RARAF

- Excitation laser – existing multiphoton system
  - Provides laser path, introduction, and detection capabilities
  - Broad range of excitation wavelengths for multi-color STED

- STED laser
  - Coupled on optical bench just before laser scan head
Live cells require media immersion ultimate resolution ~70 nm – right on par with Super Microbeam

GFP-tagged mitochondria imaged at Mechanical Engineering STED Laboratory
Continuing Microfluidics: 
Single-cell microfluidics-based qRT-PCR

- Microfluidic handling to enable near-simultaneous qRT-PCR analysis by parallel processing
Check us out!
RARAF – The People

Director: David Brenner
Assoc. Director, Chief Physicist: Gerhard Randers-Pehrson
Facility Manager: Steve Marino

Physics:
Alan Bigelow
Guy Garty
Yanping Xu
Andrew Harken
Sasha Lyulko

Biology:
Brian Ponnaiya, Chief Biologist
Manuela Buonanno
Charles Geard-Emeritus Chief biologist

External Advisory Committee

<table>
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<tr>
<th>EAC Member</th>
<th>Institution</th>
<th>Expertise</th>
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</thead>
<tbody>
<tr>
<td>Dr. Fred J. Burns, Chairperson</td>
<td>NYU</td>
<td>Radiation carcinogenesis</td>
</tr>
<tr>
<td>Dr. Frederick Maxfield</td>
<td>Weill Cornell Medical College</td>
<td>Microscopy / imaging</td>
</tr>
<tr>
<td>Dr. Jacqueline Yanch</td>
<td>MIT</td>
<td>Accelerator physics, radiation biology</td>
</tr>
<tr>
<td>(New Member)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dr. David A. Boothman</td>
<td>UT Southwestern</td>
<td>Radiation-induced repair and protein induction</td>
</tr>
<tr>
<td>Dr. Albert J. Fornace Jr.</td>
<td>Georgetown</td>
<td>Radiation-induced genomics / metabolomics</td>
</tr>
<tr>
<td>Dr. Eric J. Hall</td>
<td>Columbia</td>
<td>Radiation biology</td>
</tr>
<tr>
<td>Dr. Marcelo Vazquez</td>
<td>Loma Linda</td>
<td>Charged particle radiation biology, training</td>
</tr>
</tbody>
</table>
Thank you