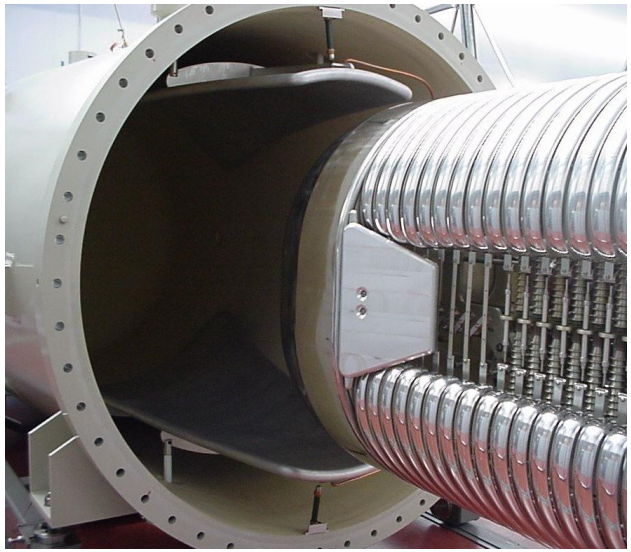
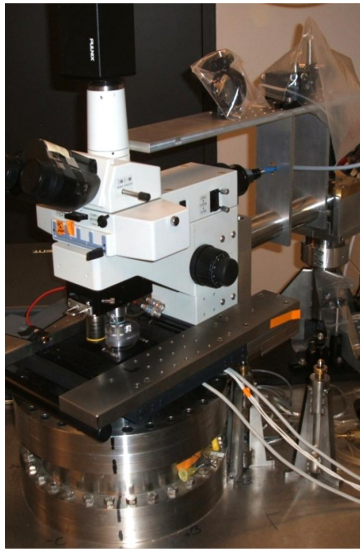
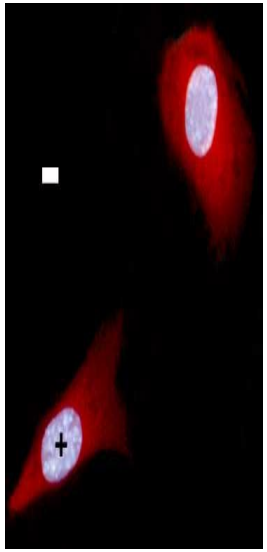
An overview of RARAF:

From broad beams to microbeams, single proteins to small animals, where we have been to where we are going.







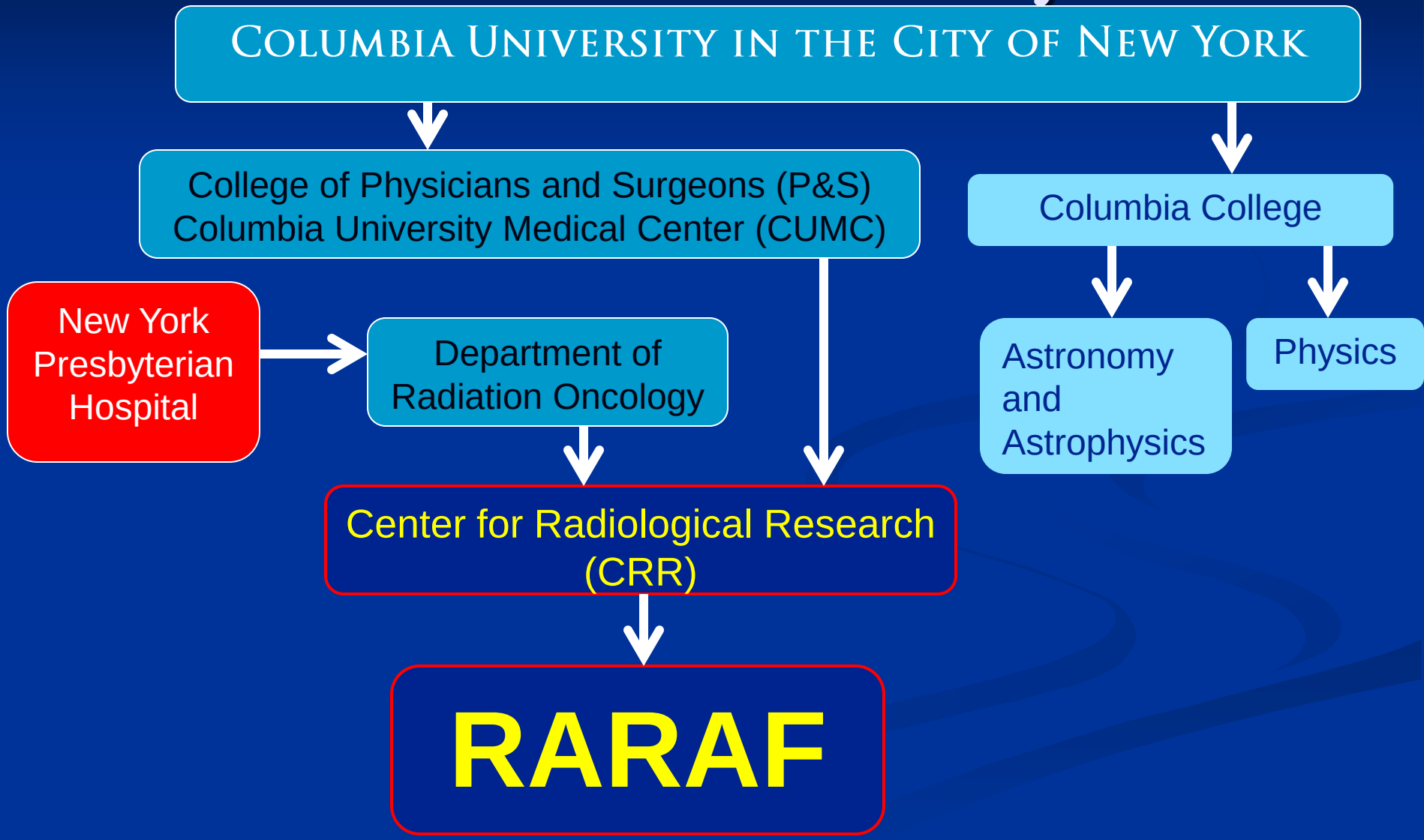
Andrew Harken

March 28, 2013

An overview of RARAF

- History of RARAF
- Broad beams
- Microbeams
- Imaging
- Microfluidics
- Where we are going

The Radiological Research Accelerator Facility



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

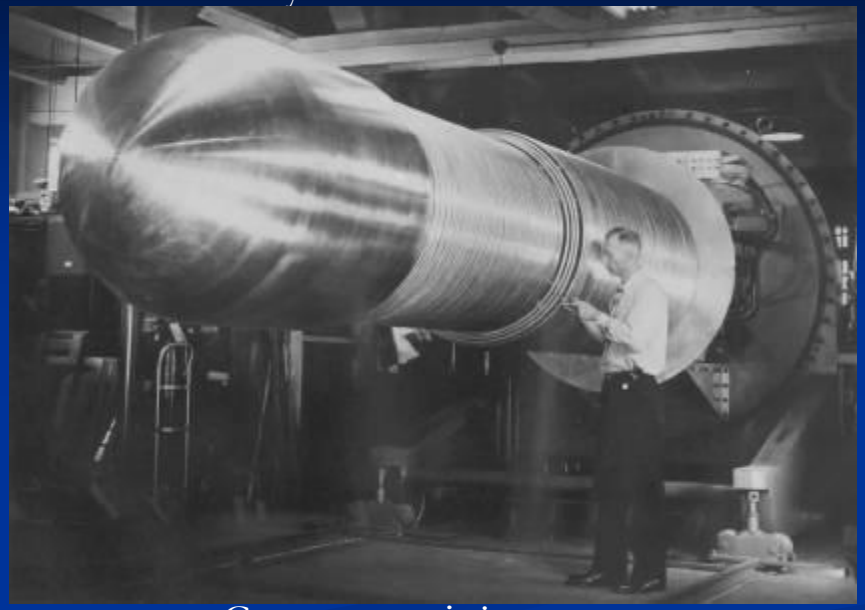
Dr. Gioacchinno Failla
(1891 - 1961)

RARAF: History

- In the mid-1960's, Drs. VP Bond (Brookhaven) and HH Rossi (Columbia CRR) want a monoenergtic 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

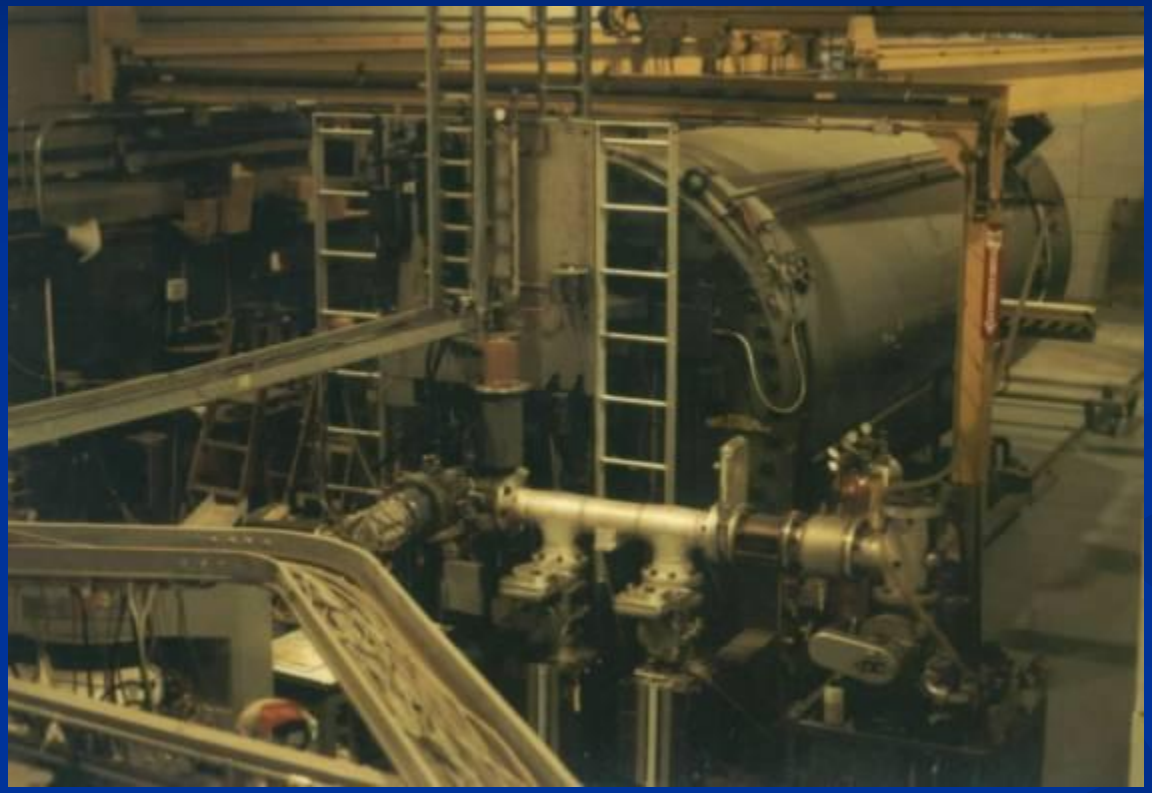


Cosmotron injector

Cosmotron

RARAF Accelerator

Van de Graaff at BNL before move to Nevis



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

Singletron, baseplate & quadrupole

HV power supply & resonator coil

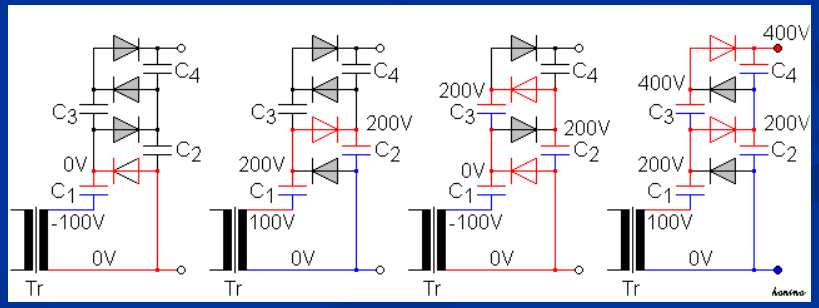
Our 5.5 MV Singletron Accelerator from High Voltage Engineering Europa (HVEE)

Interior of Singletron

Terminal with shell removed

Our 5.5 MV Singletron Accelerator

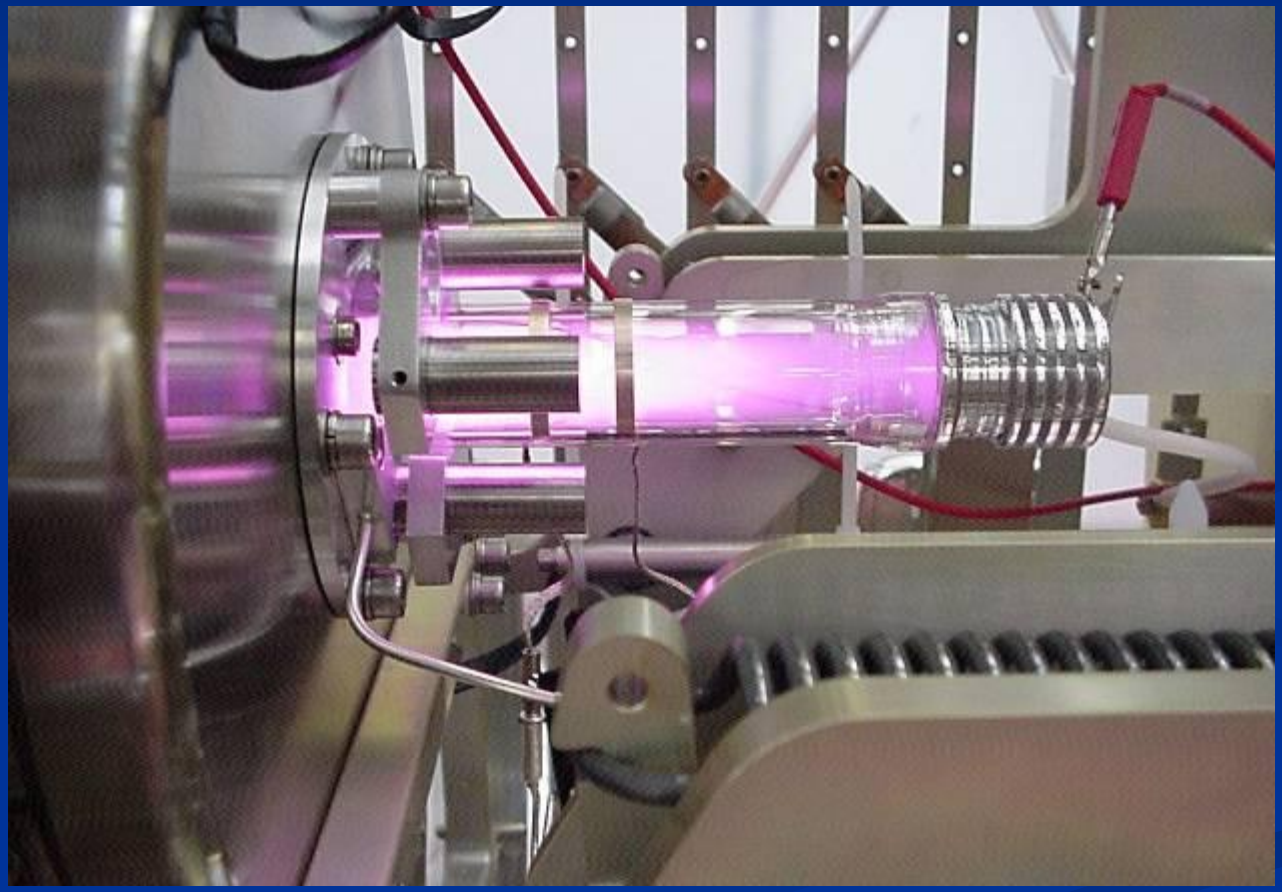
Electrodes & diode stacks with spark gaps



Cockcroft–Walton charging system ± 100V

Our 5.5 MV Singletron Accelerator

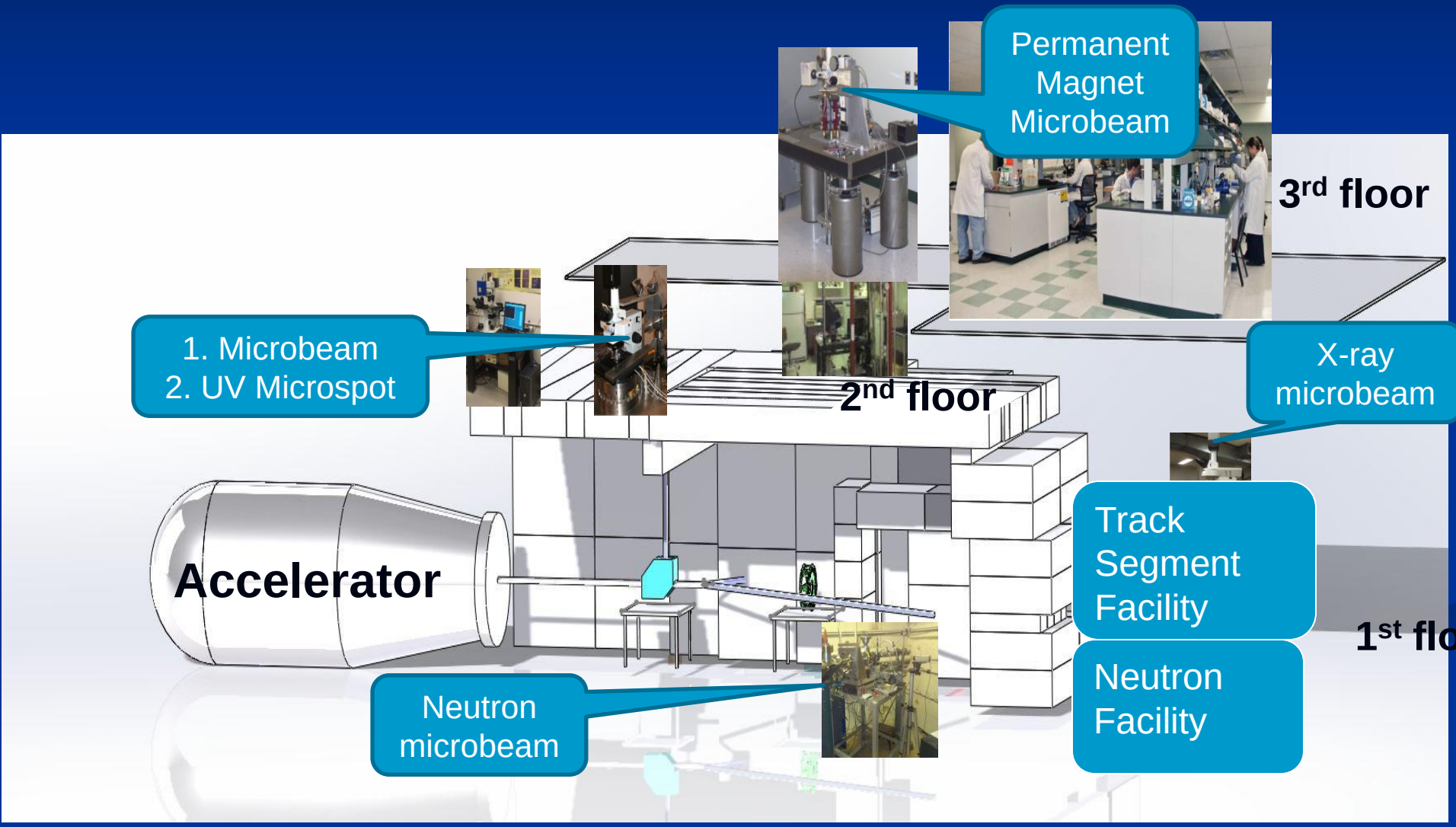
RF ion source



Our 5.5 MV Singletron Accelerator

- Available source gases
  - Helium ($^4He+$, $^4He++$) ($^4He++$ = simulated alpha)
  - Hydrogen ($^1H+$, $^1H_2+$, $^1H_3+$)
  - Deuterium ($^1D+$, $^1D_2+$, $^1D_3+$)
  - Nitrogen ($^7N+$ to $^7N+5$)
  - Helium-3 (Not currently installed)

Beam Lines in RARAF



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
- UV Microspot

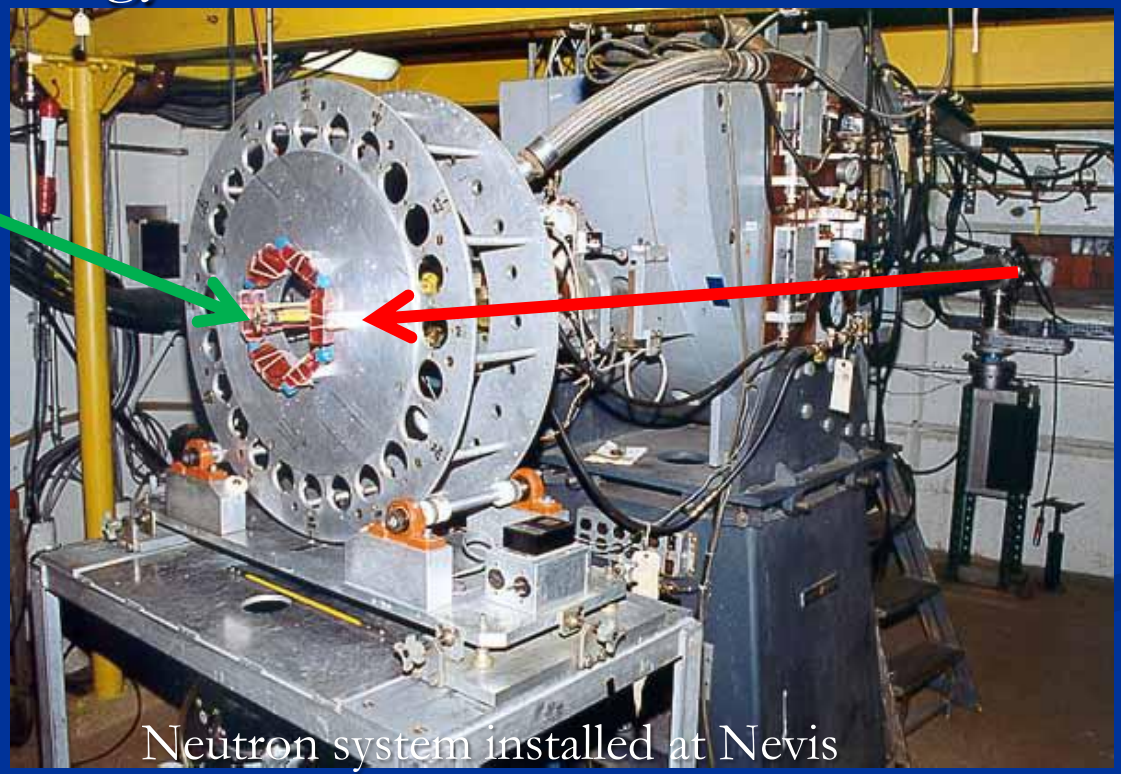
Offline Sources:

- Cs-137 irradiator
- 250 kV x-rays

Monoenergtic 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

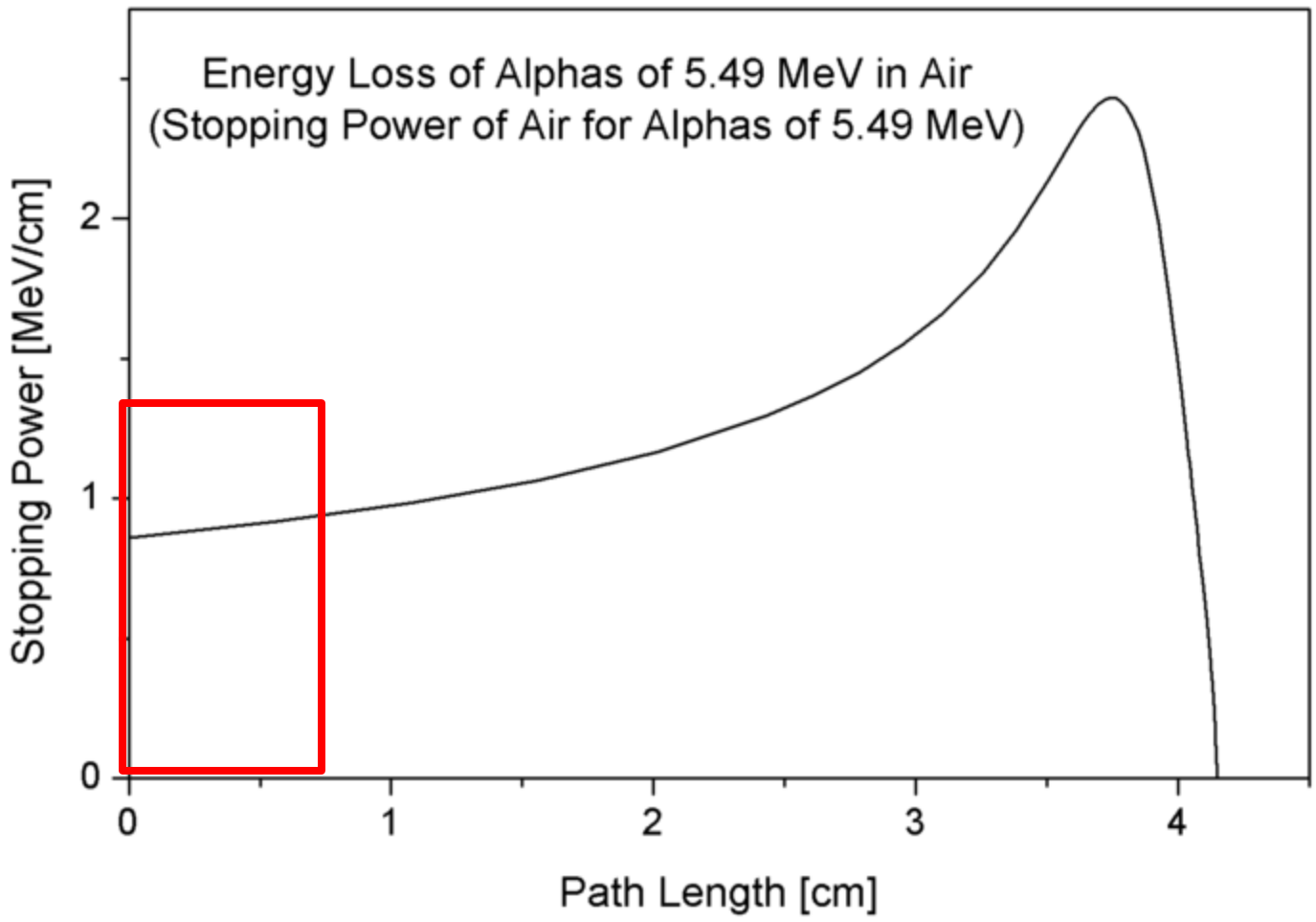
Target



Neutron system installed at Nevis

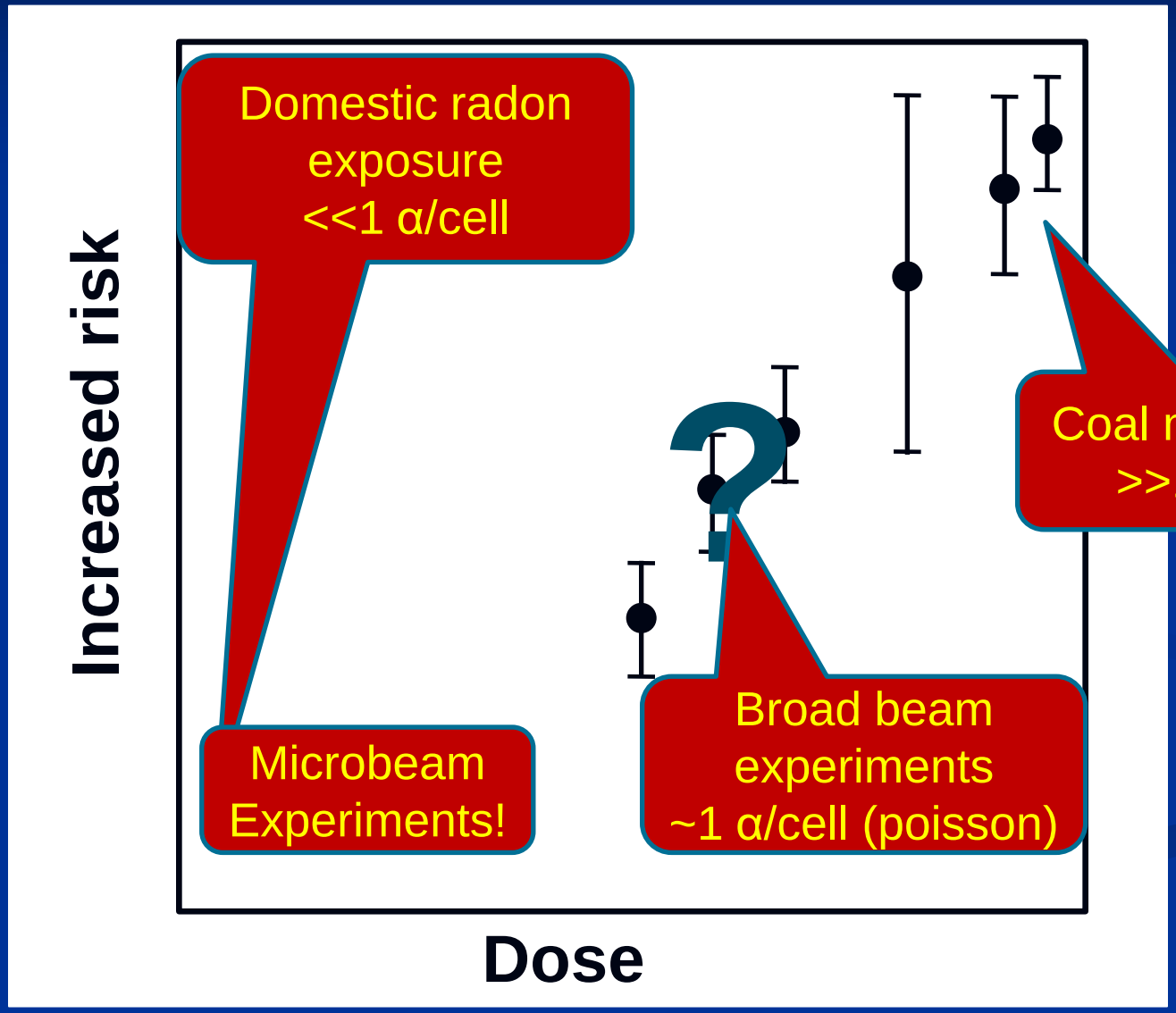
Charged Particle Irradiators:

- T



particles in each area)

Low dose radiation risk estimation



Microbeams: Where We Are

Particles:

- Charged particles
  - Broad beam
  - Microbeam
- Neutrons
  - Broad beam
    - Monoenergetic
    - Spectrum irradiator
  - Microbeam

Photons:

- X-rays Microbeam
- UV Microspot

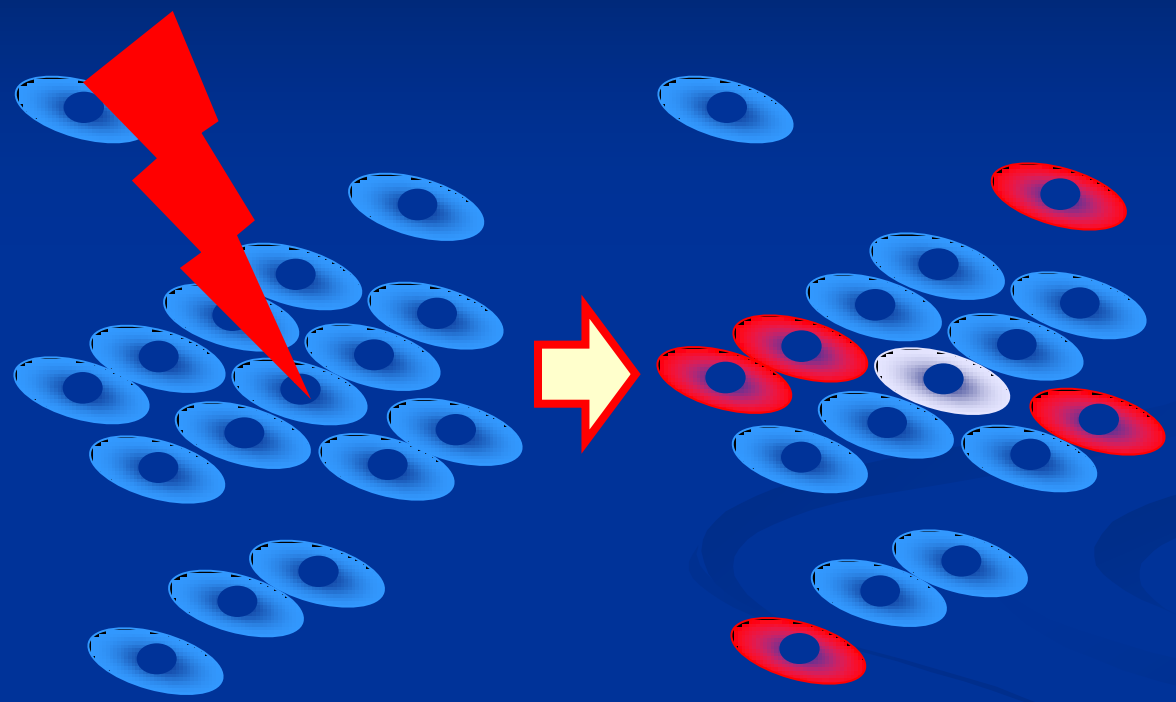
Offline Sources:

- Cs-137 irradiator
- 250 kV x-rays

What is a Single-Cell Microbeam?

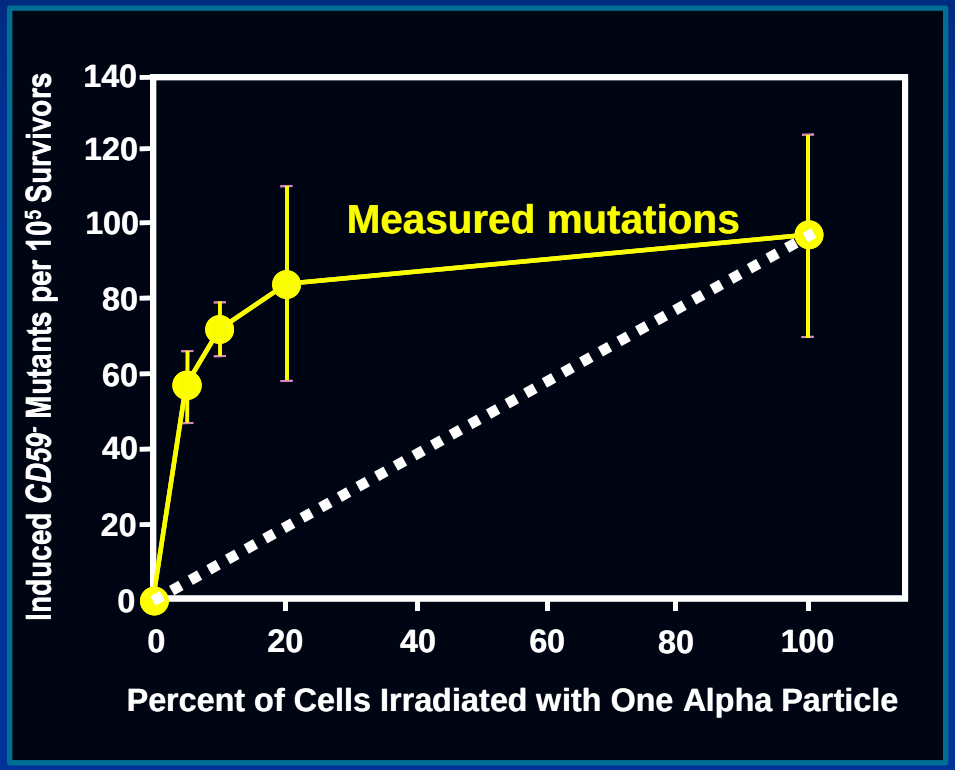
- A single-cell microbeam can deposit ionizing radiation damage in micrometer or sub-micrometer sized regions of cells
- Allows investigation of intra- and inter-cellular mechanisms of stress response

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

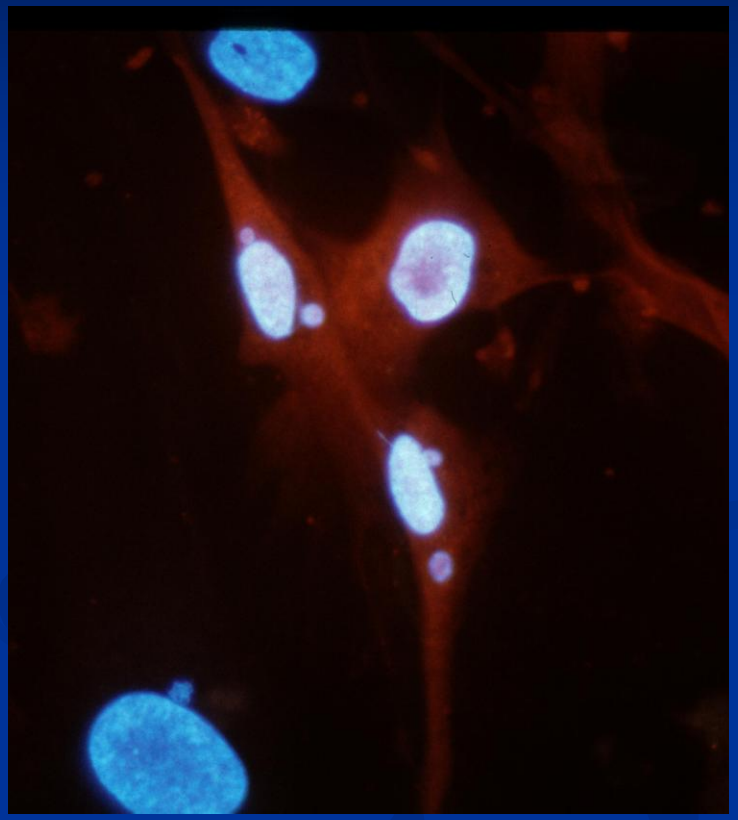


- Where bystander responses have been quantitated, they have shown saturation
- In such cases, extrapolating linearly from low to very low doses could underestimate the risk at very low doses.

Based on mutation data from the RARAF microbeam.
Zhou et al PNAS 98, 14410-5 (2001)

Microbeams represent the most direct way to study inter-cellular damage response

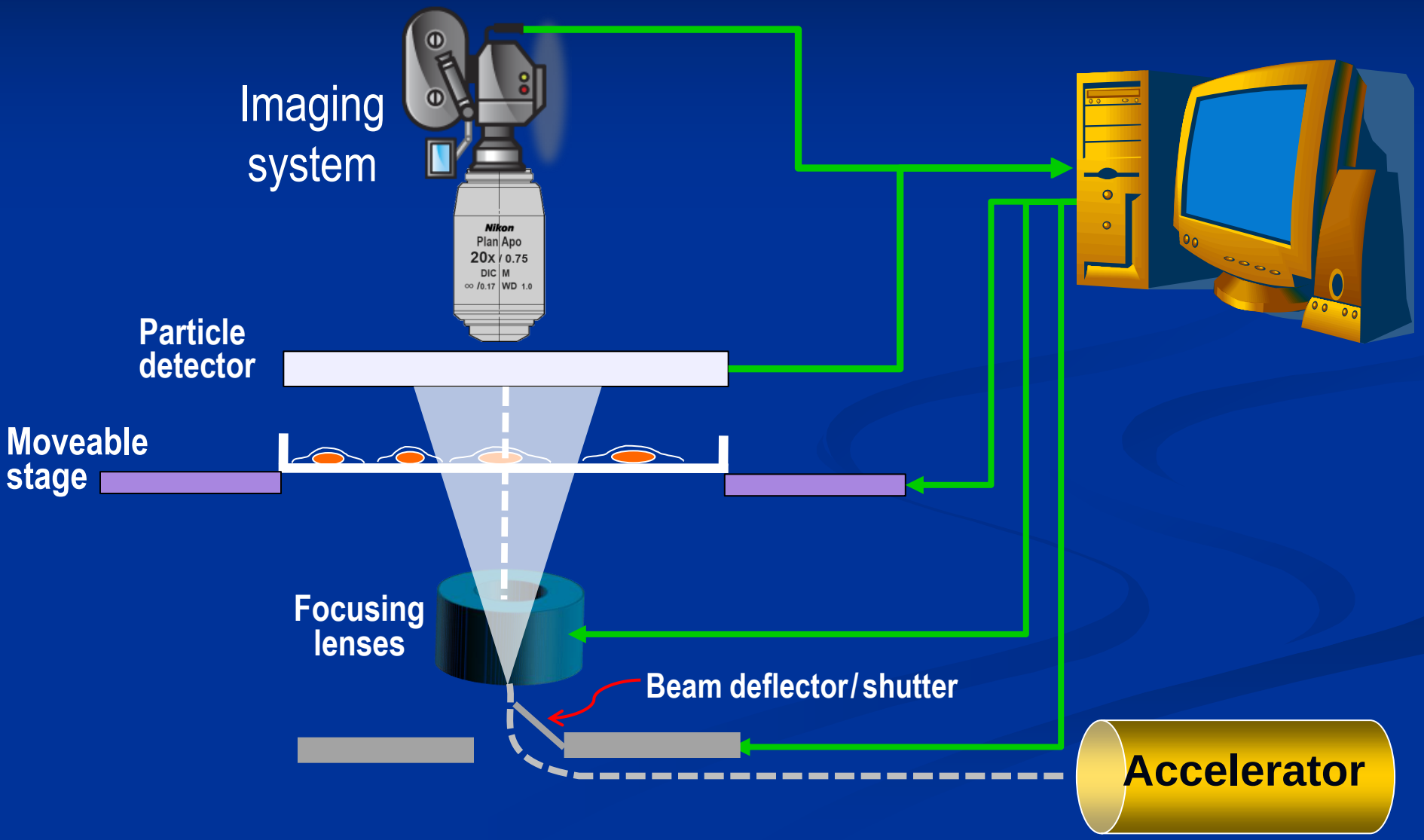
- Produces DNA damage in defined cells, while guaranteeing that adjacent cells are not hit
- Can study effects in the adjacent cells



Blue-stained nuclei:
HIT cells

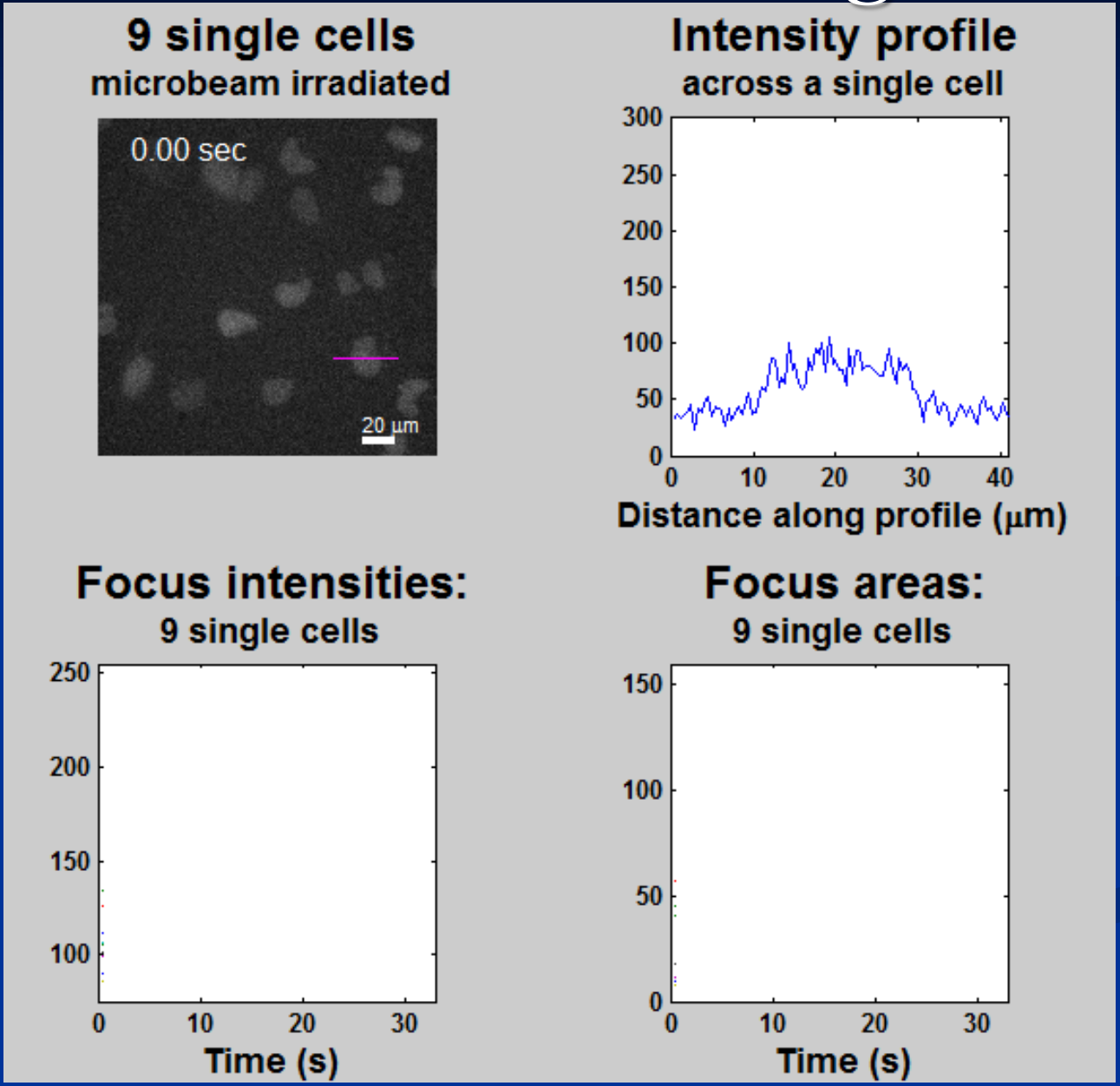
Red-stained cytoplasm:
NON-HIT cells

How to make a microbeam?



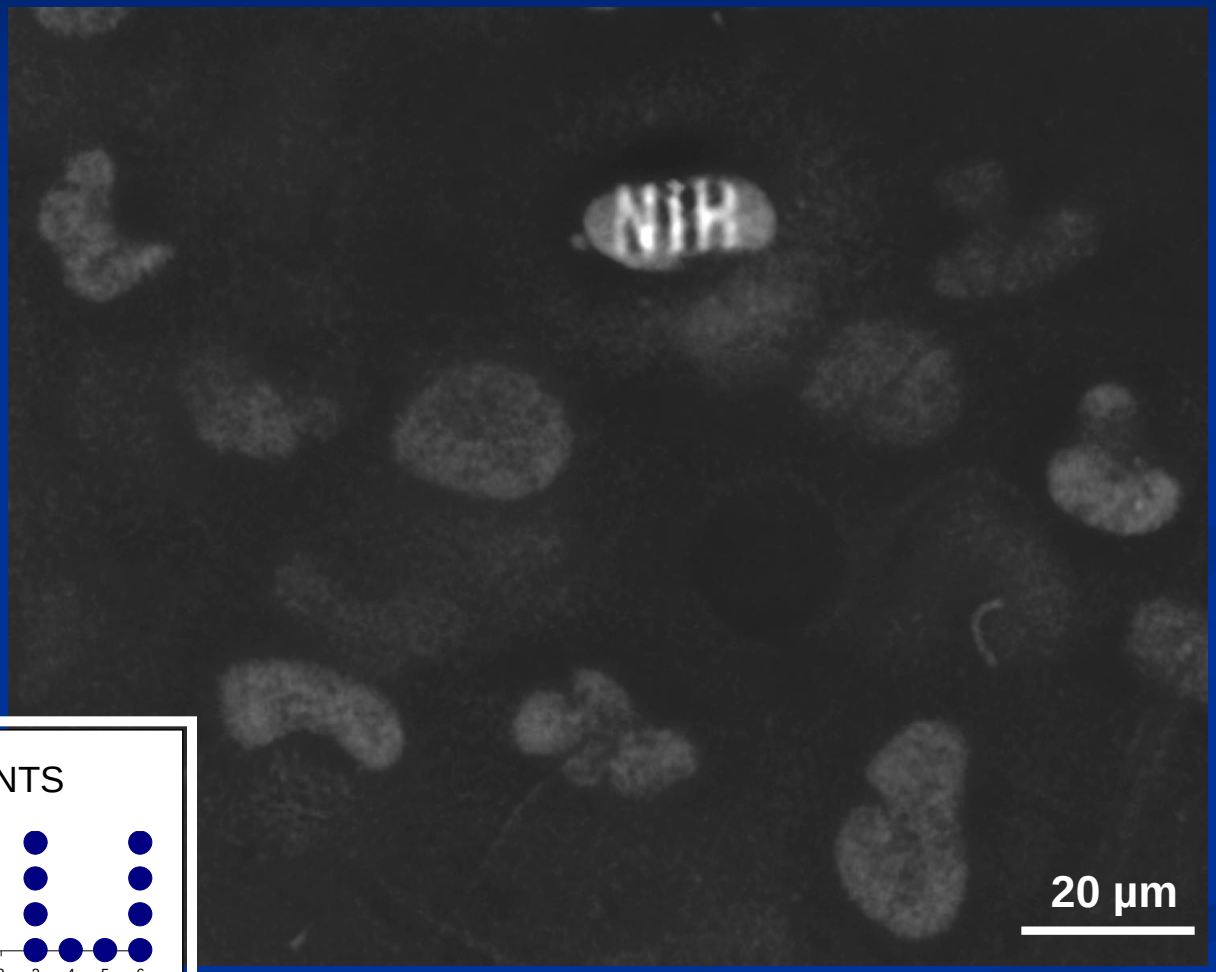
Foci formation at DNA damage site

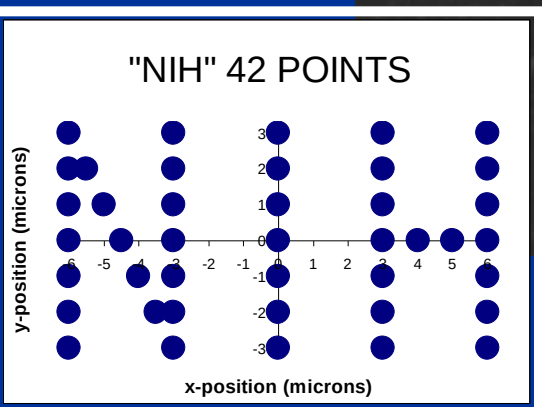
HT-1080 cells with
GFP-tagged XRCC1
SSB repair protein



Cells, courtesy of David J. Chen

Painting “NIH” on a cell nucleus with gfp-tagged XRCC1 repair foci, using our 0.6 mm microbeam

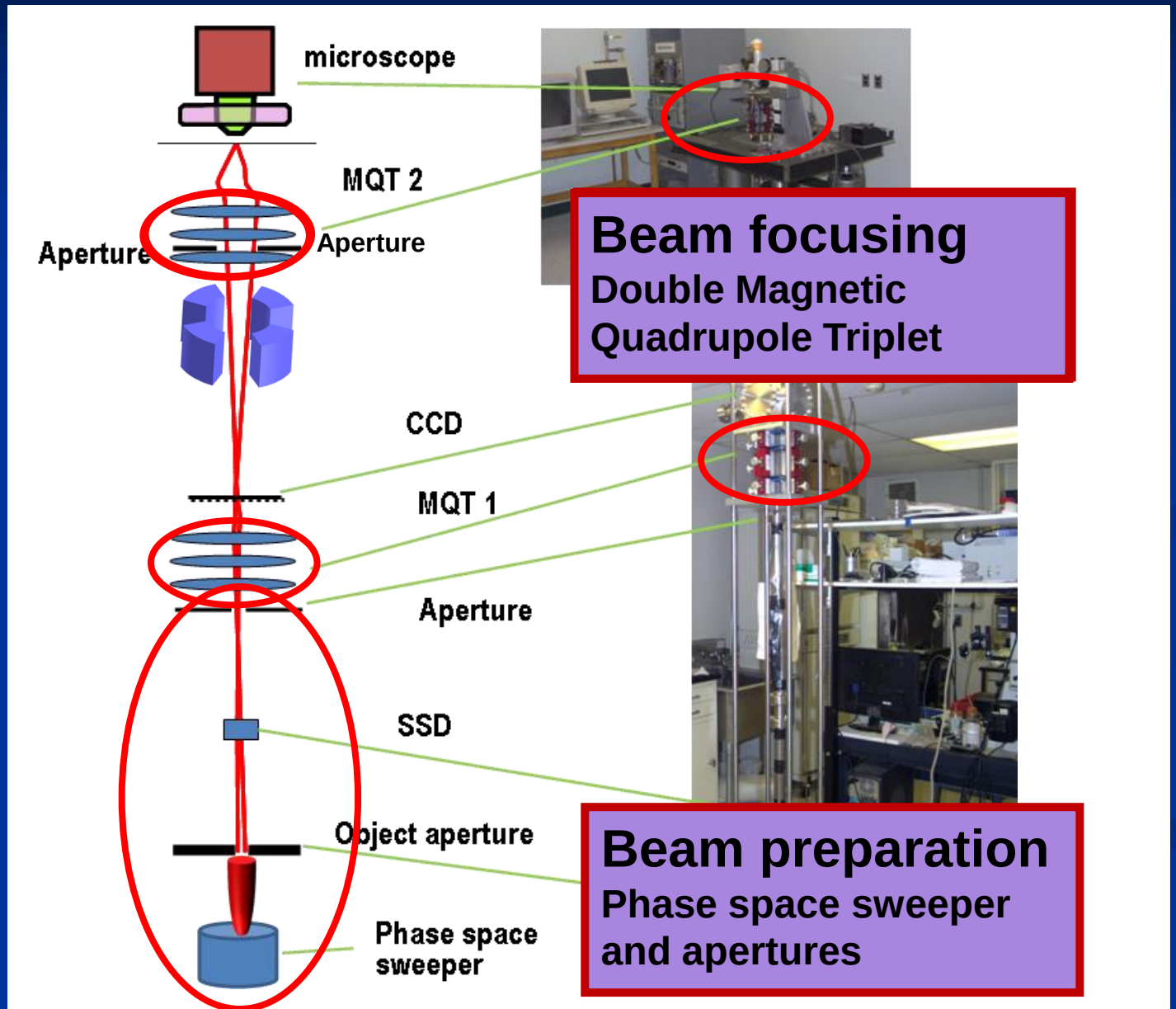




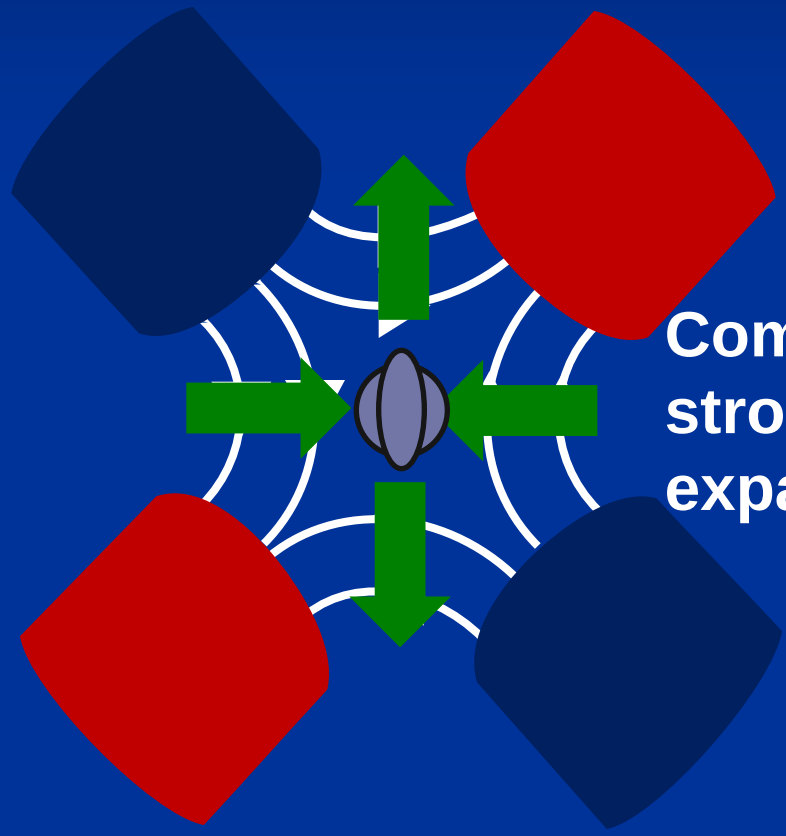
Cells, courtesy of David J. Chen

Example of a microbeam

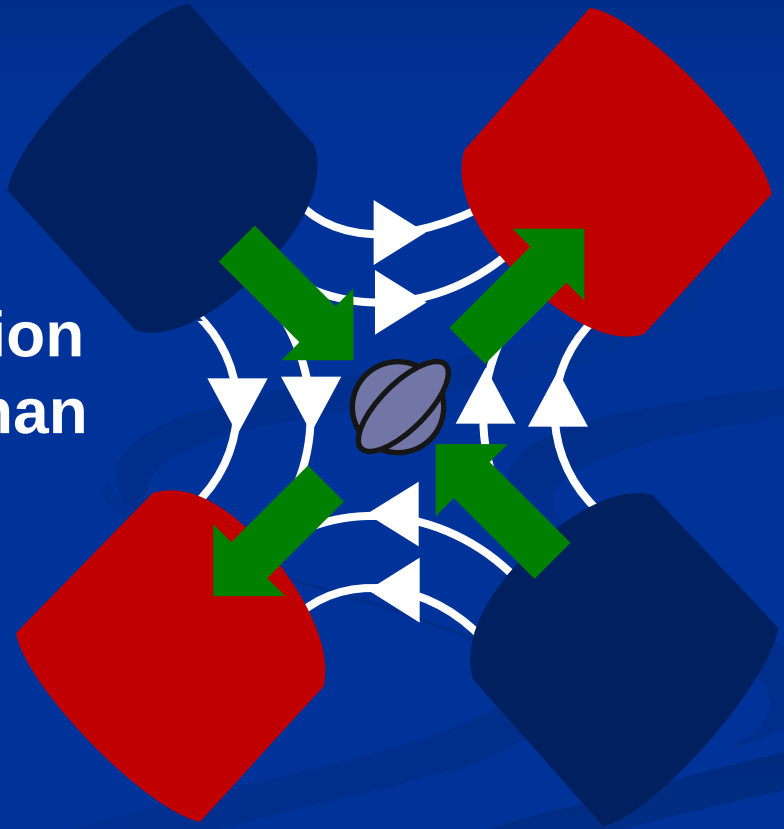
The Permanent Magnet Microbeam (PMM)



Quadrupole lens



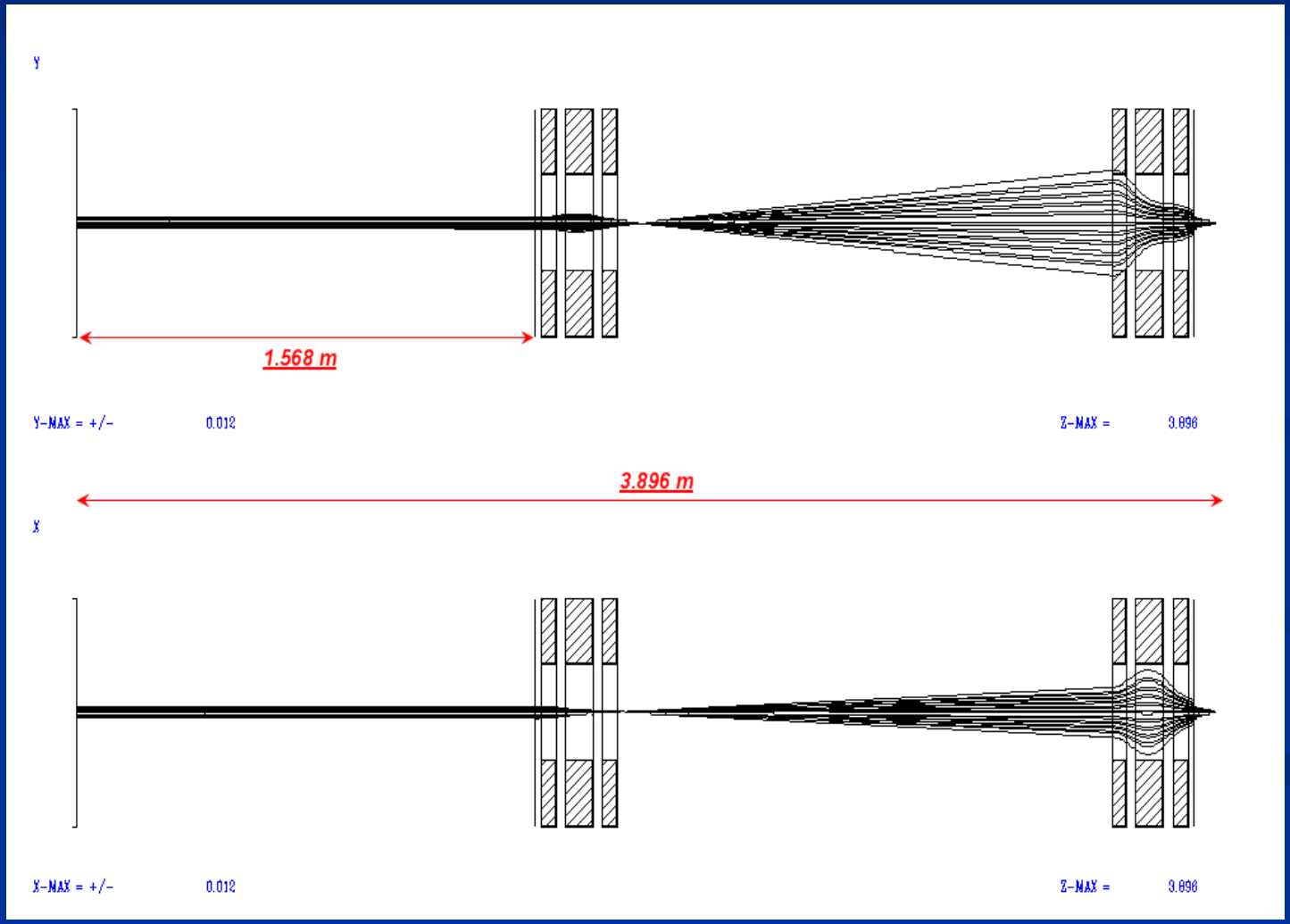
Compression stronger than expansion



Magnetic

Electrostatic

Double triplet lens



How small can we go?

Year	Technique	Diameter (μm)
1996	Pinhole aperture	10
2001	Focused: Single quadrupole quadruplet	5
2007	Focused: Single quadrupole triplet	1.3
Today	Focused: Compound quadrupole triplet	0.5

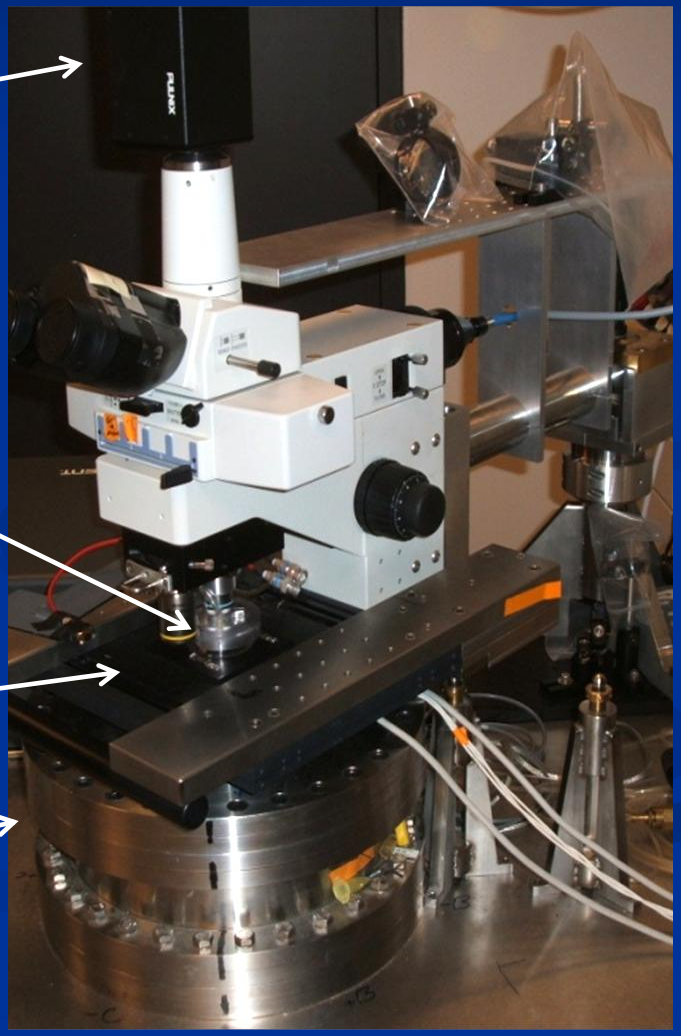
Endstation

Imaging

Detector

Piezoelectric stage

Beamline

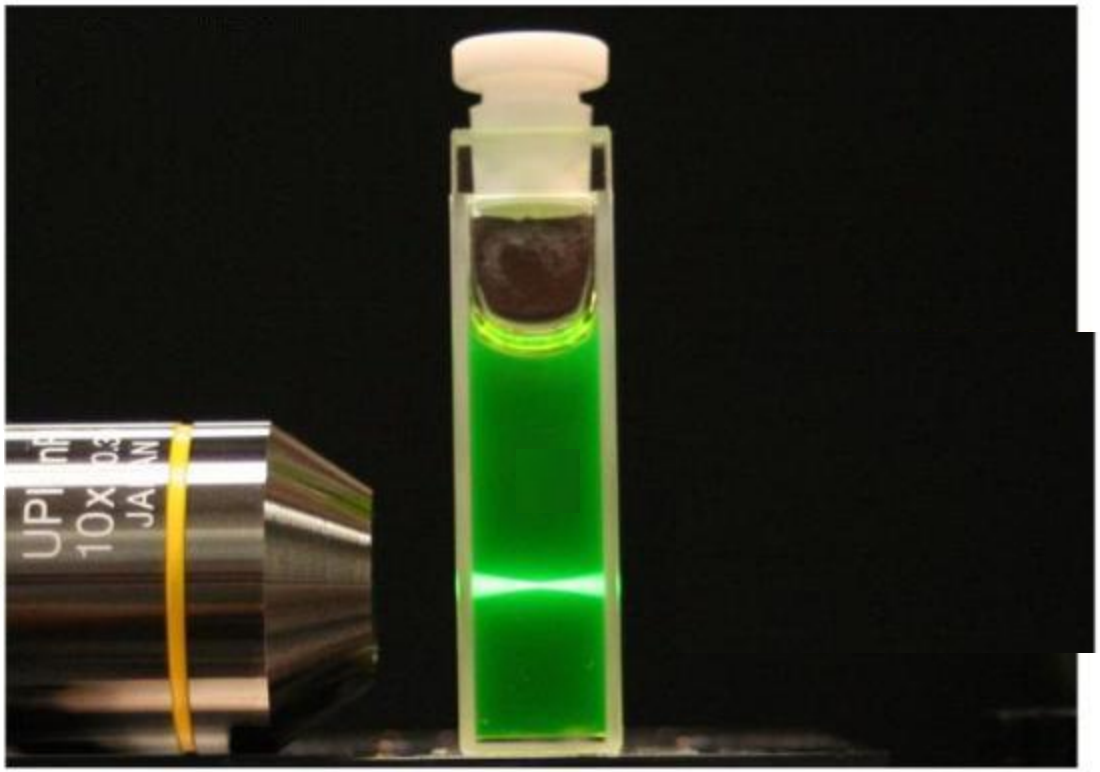


Other Microbeams

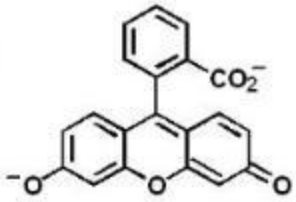
UV Microspot

Multiphoton Microscope

Use Imaging Tool as an Irradiator



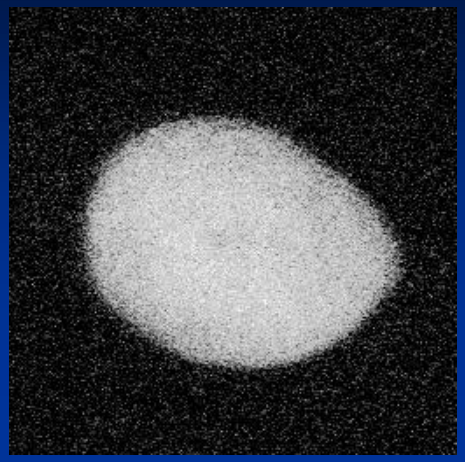
Fluorescein



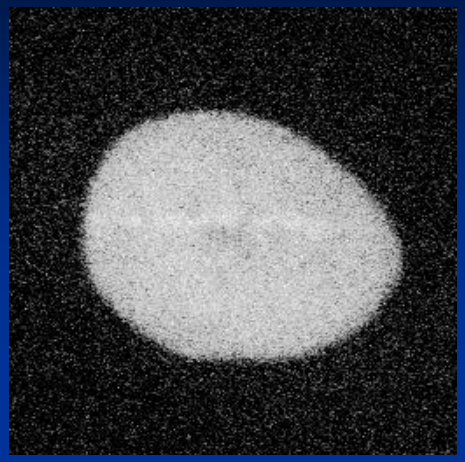
Two-photon	340-540 nm	2.30-3.65 eV	UVA / Visible
Three-photon	227-360 nm	3.45-5.47 eV	UVA / UVB / UVC

OGG1 – View 6

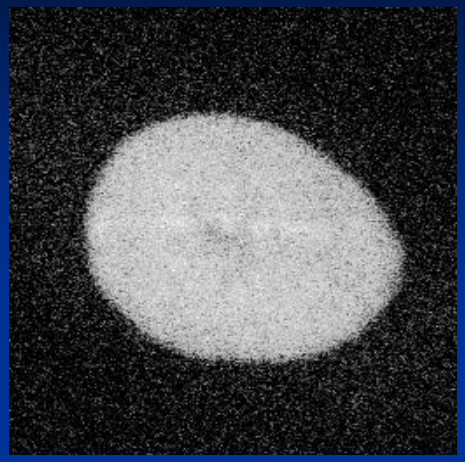
Irradiation: 5 horizontal line scans (~50 mJ delivered to cell nucleus)



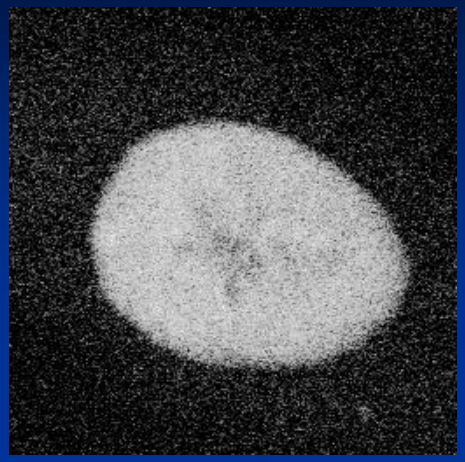
Before



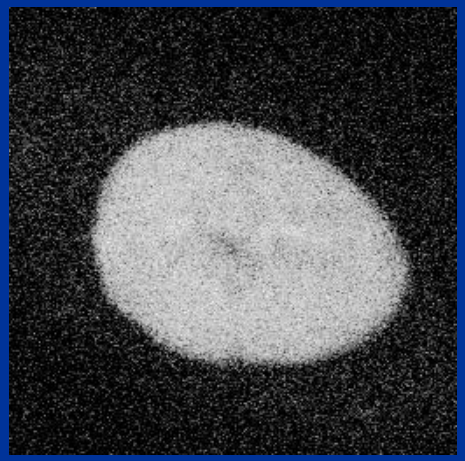
After 1 min.



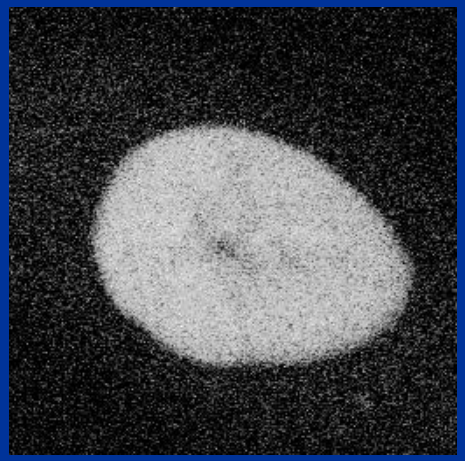
After 2 min.



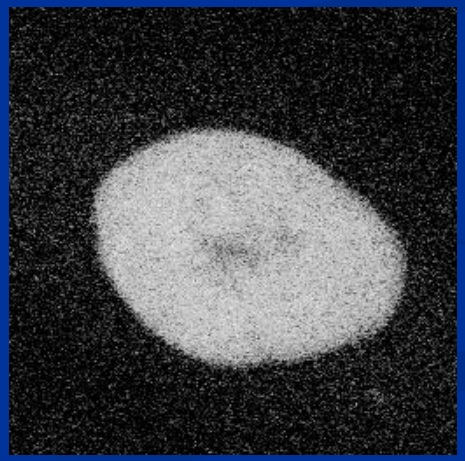
After 3 min.



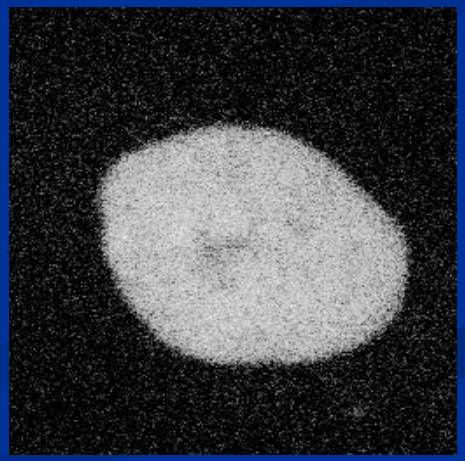
After 4 min.



After 5 min.

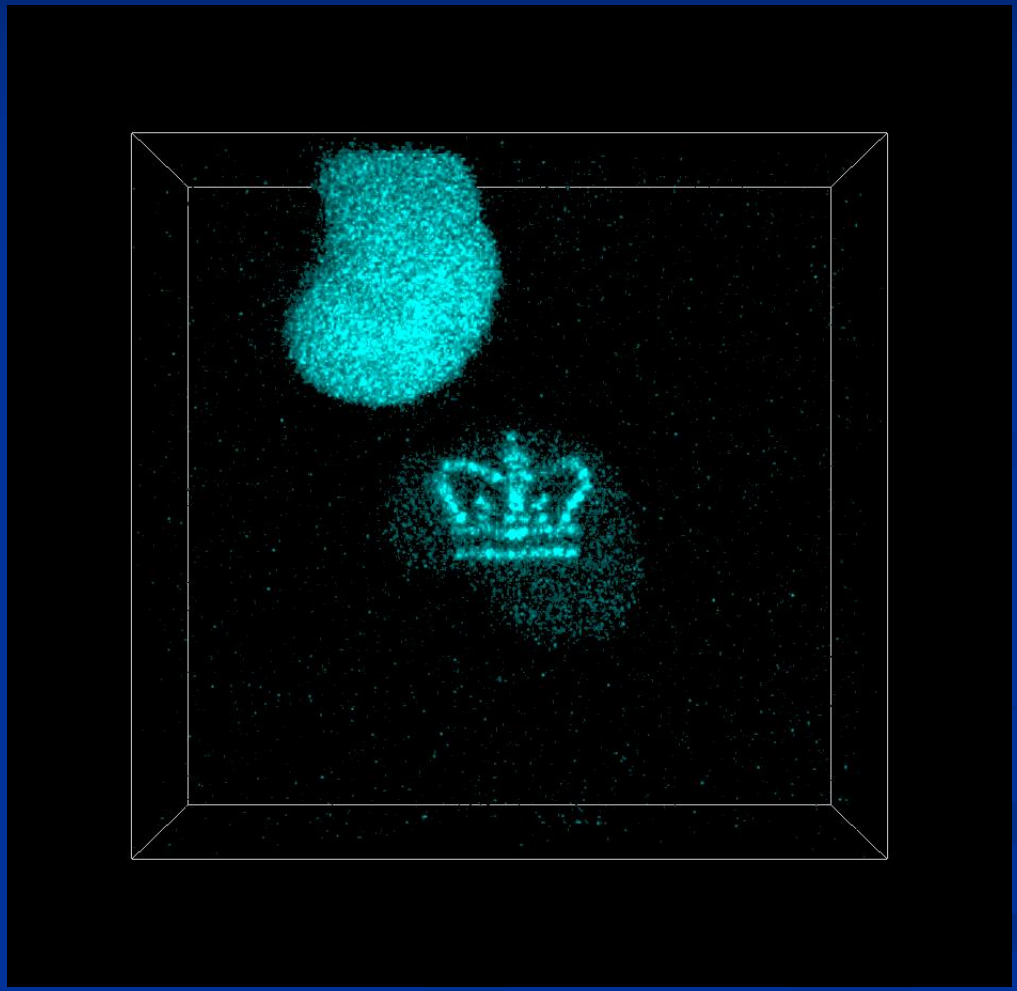


After 17 min.



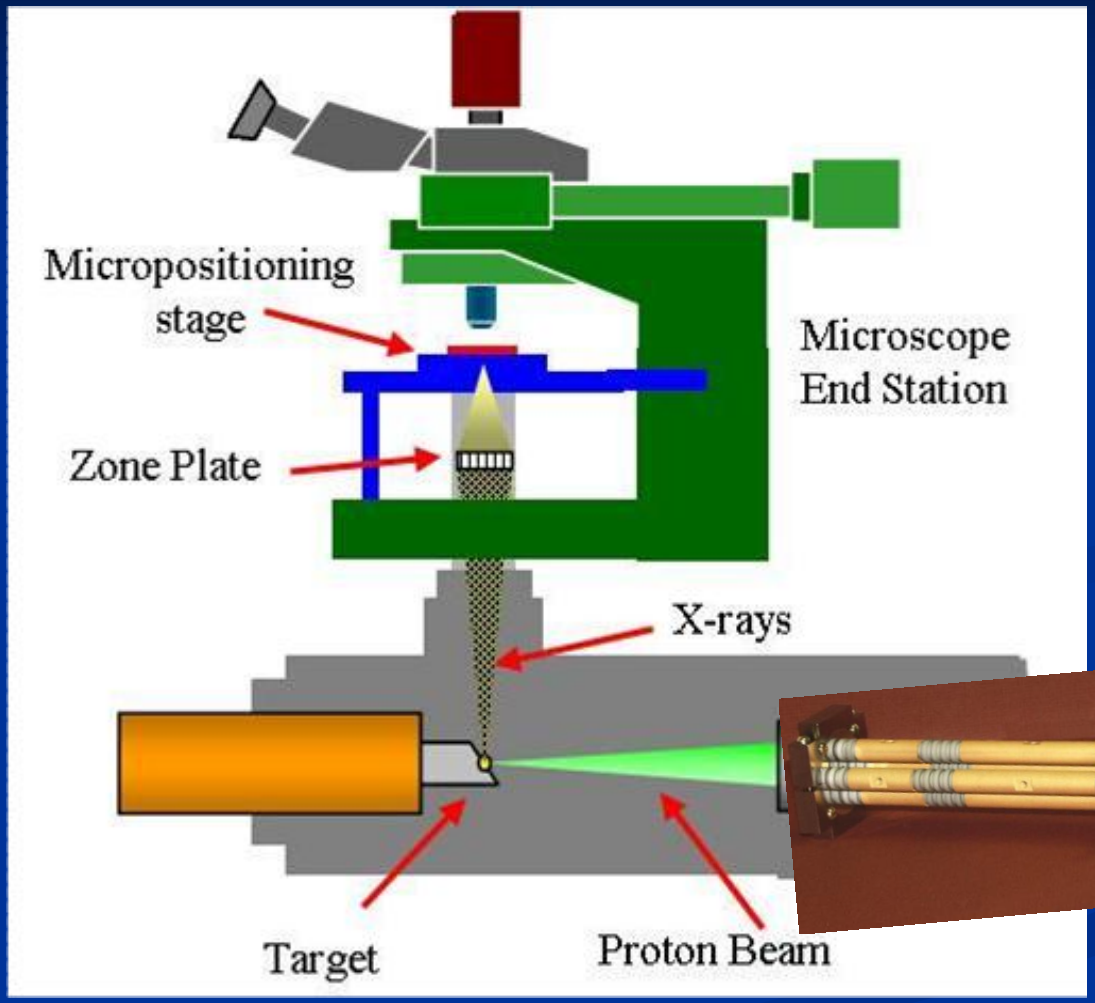
After 28 min.

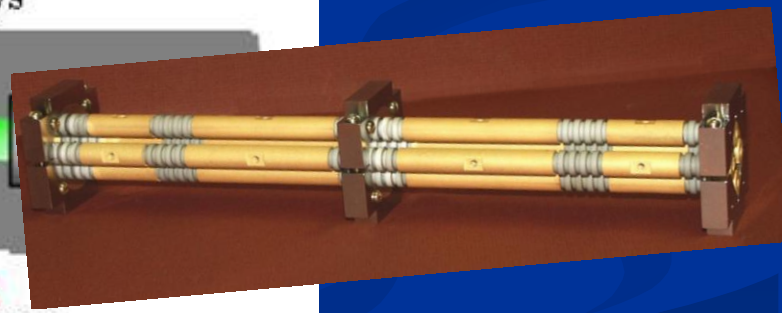
Cells, courtesy of David J. Chen



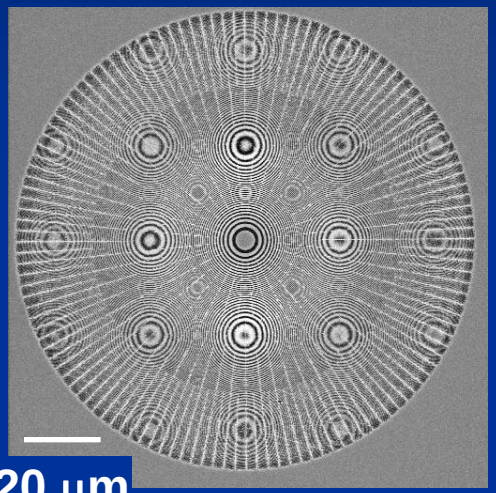
Cells, courtesy of David J. Chen

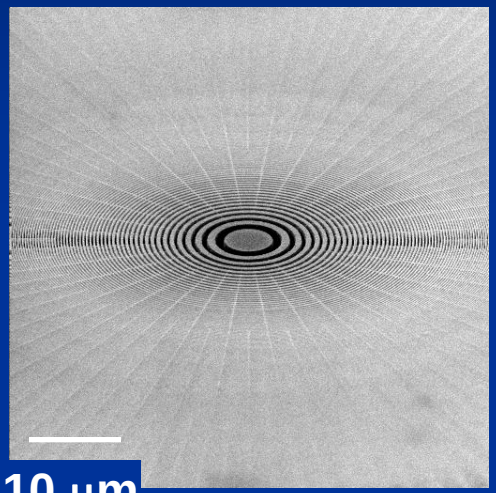
Soft X-ray microbeam

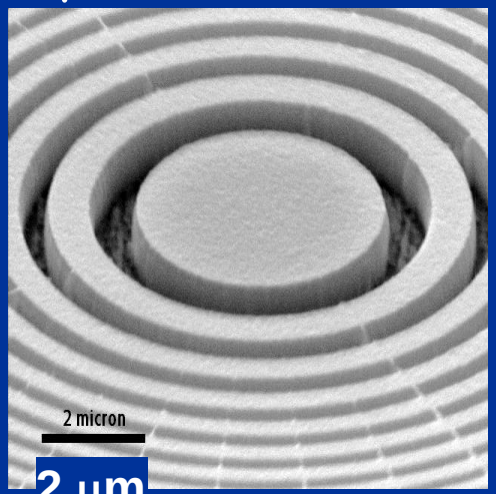


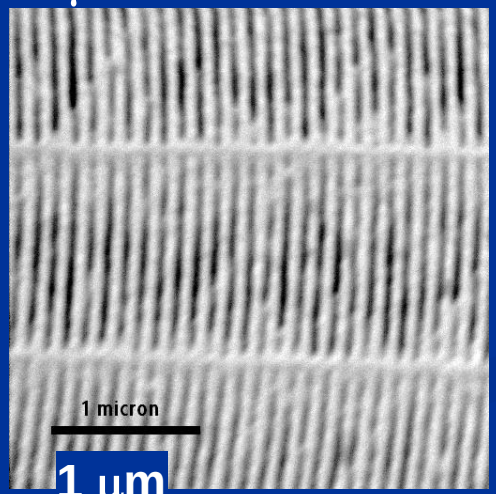


Zone Plate







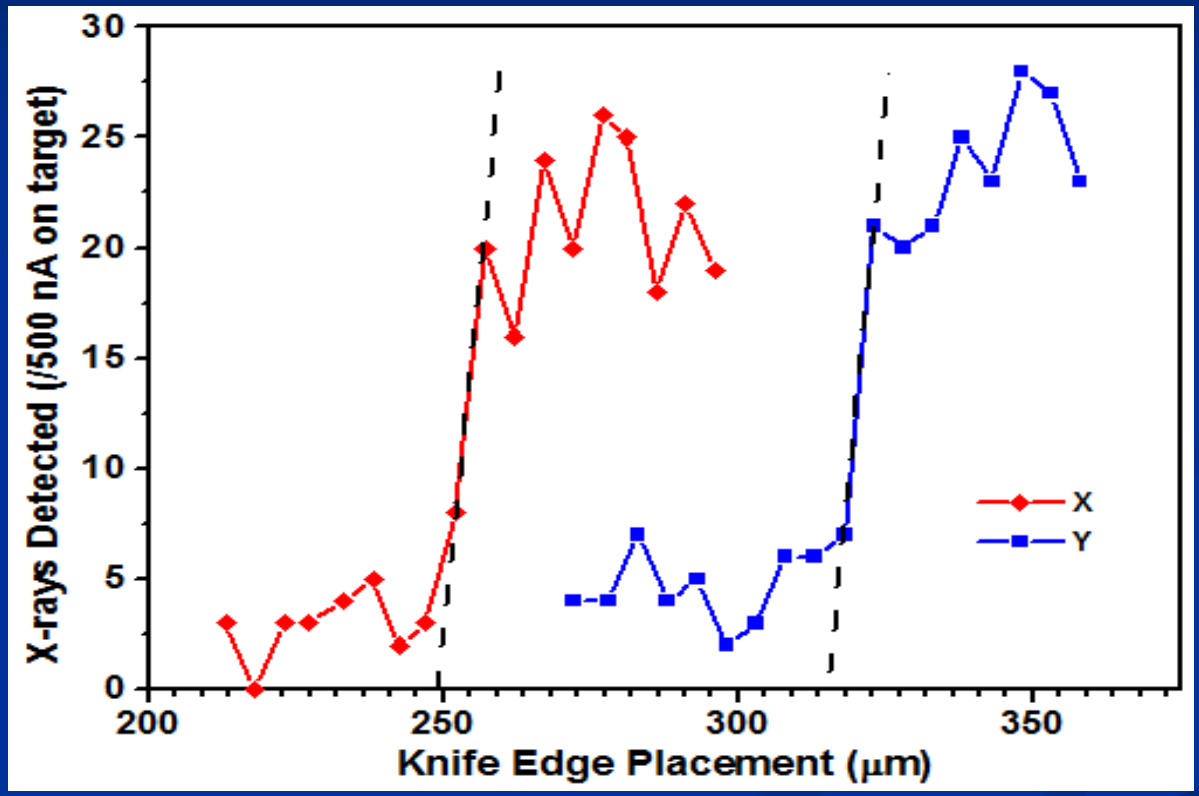


- 120 μm diameter
- 50 nm outer ring spacing
- 1st order transmission efficiency at 4.5 keV 12.5% or better

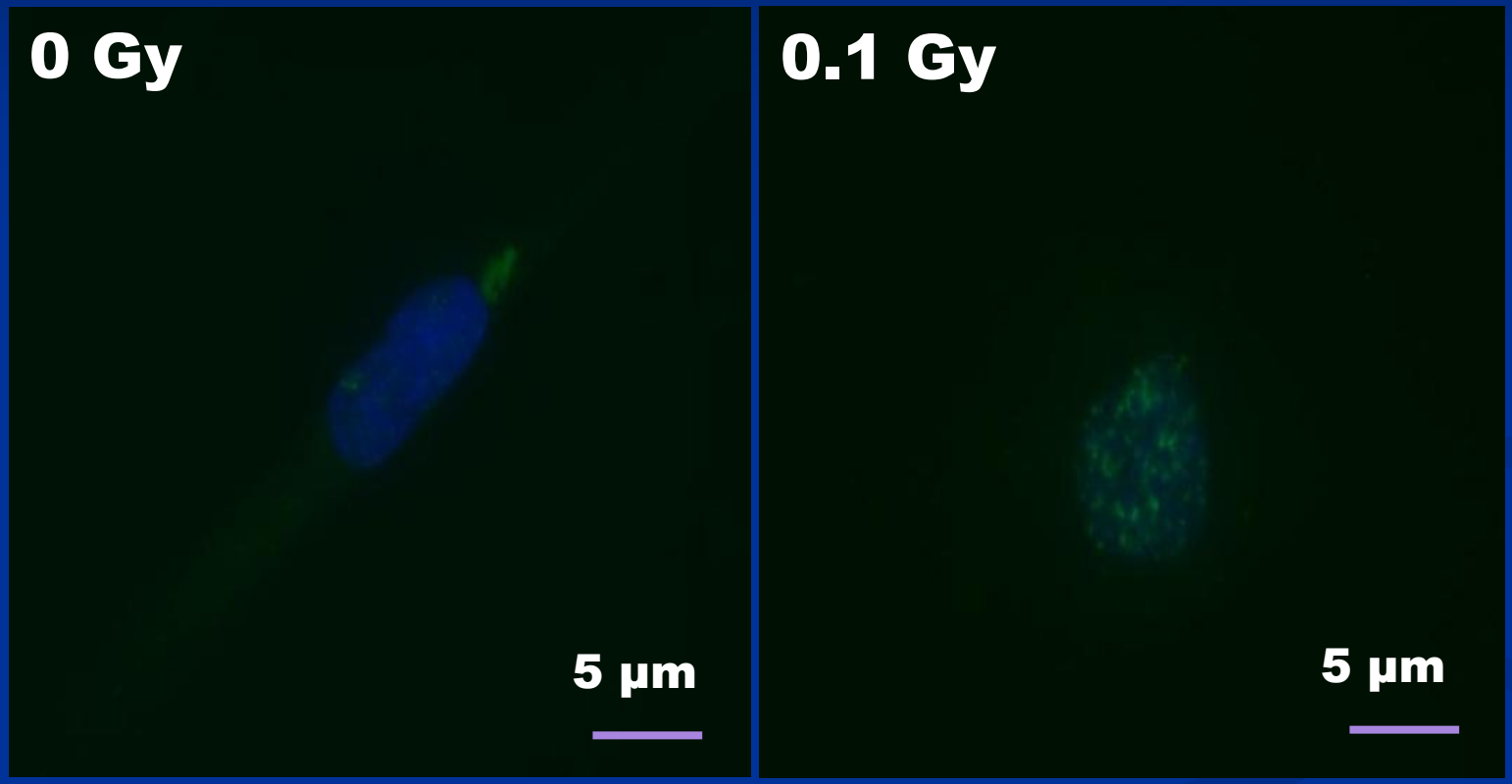
- 10 mGy/sec delivered to sample with a 5 μm spot

PIXE Soft X-ray Microbeam:

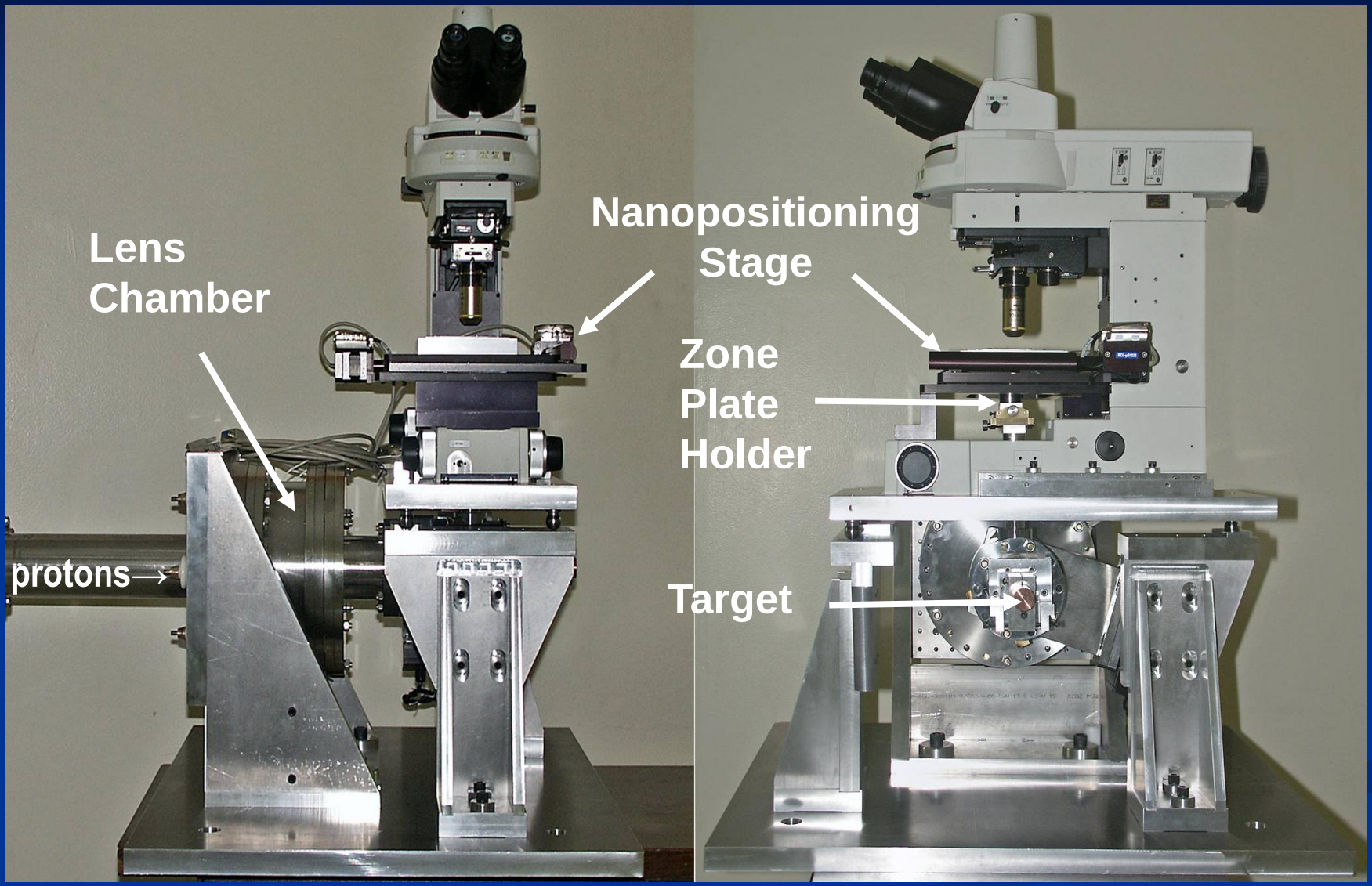
- Proton spot size
  120 μm x 50 μm
- X-ray Spot size:
  5 μm x 5 μm
- Present dose rate
  - 10 mGy/sec
  - (10 photons absorbed)



Irradiation Results



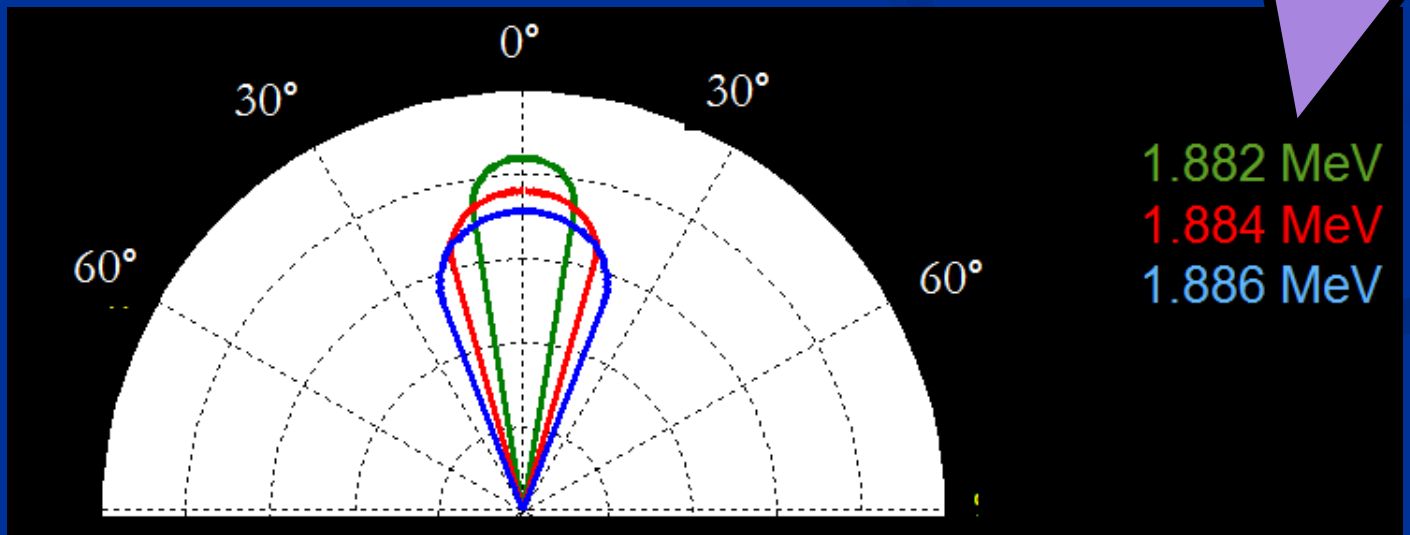
AG1522 cell stained for γ-H2AX
Fixed 30 minutes post irradiation


Lens
Chamber
Nanopositioning
Stage
Zone
Plate
Holder
protons→
Target

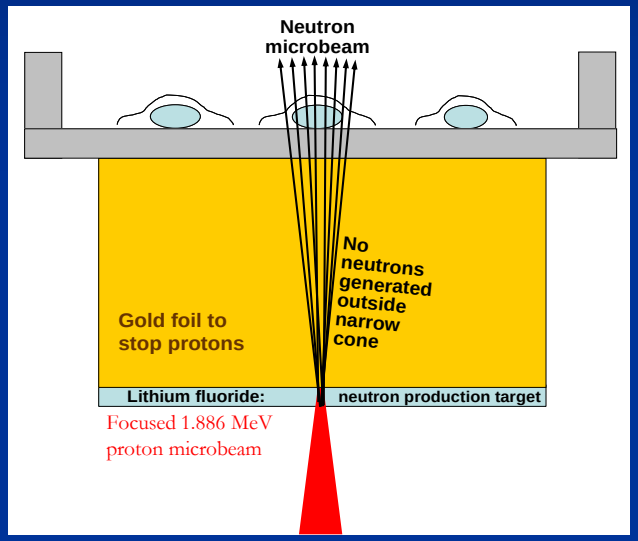
A neutron microbeam?

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

Requires tight control of beam energy



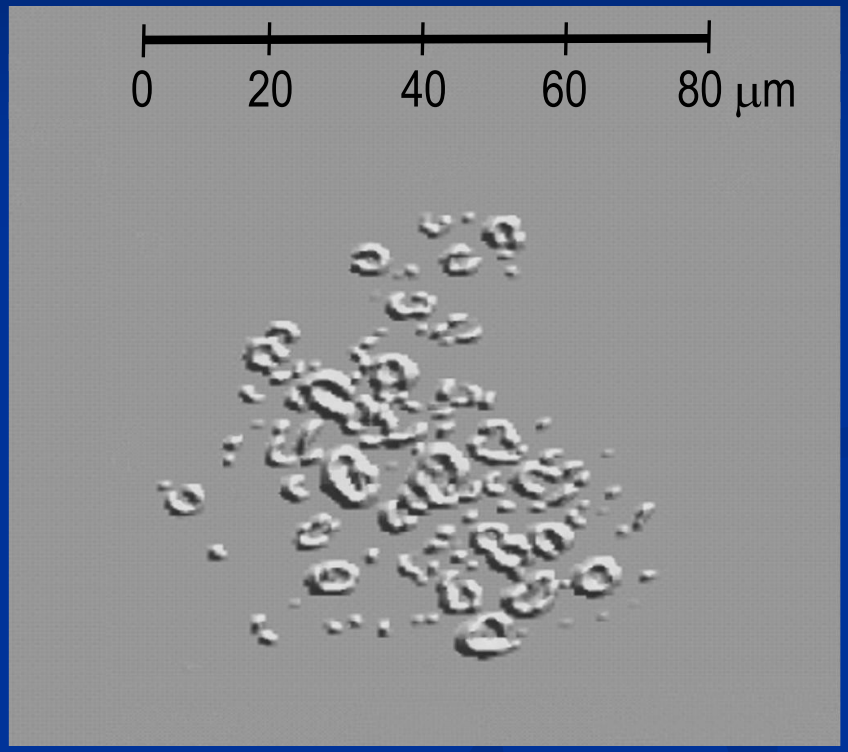
Neutron Microbeam



Sept 2012: 90 μm

Feb 2013: 70 μm

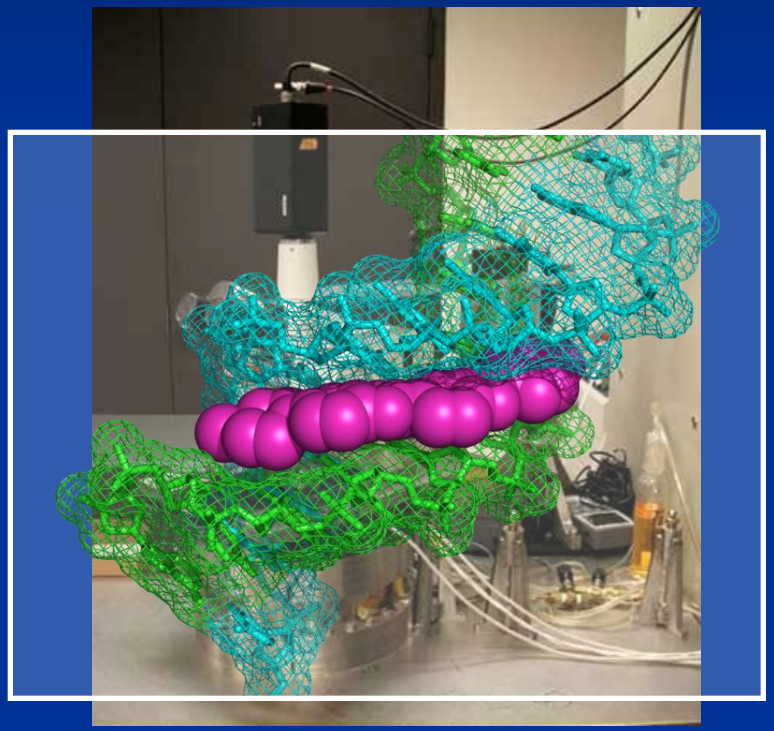
Design goal: 20 μm

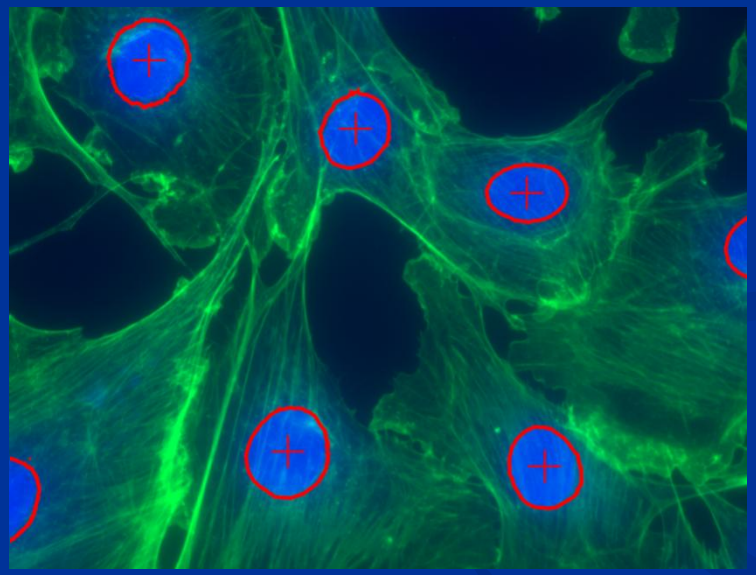


Neutron microbeam visualized with ^{6}Li coated CR-39 track etch detector

Imaging

To target cells or sub-cellular components they must be imaged





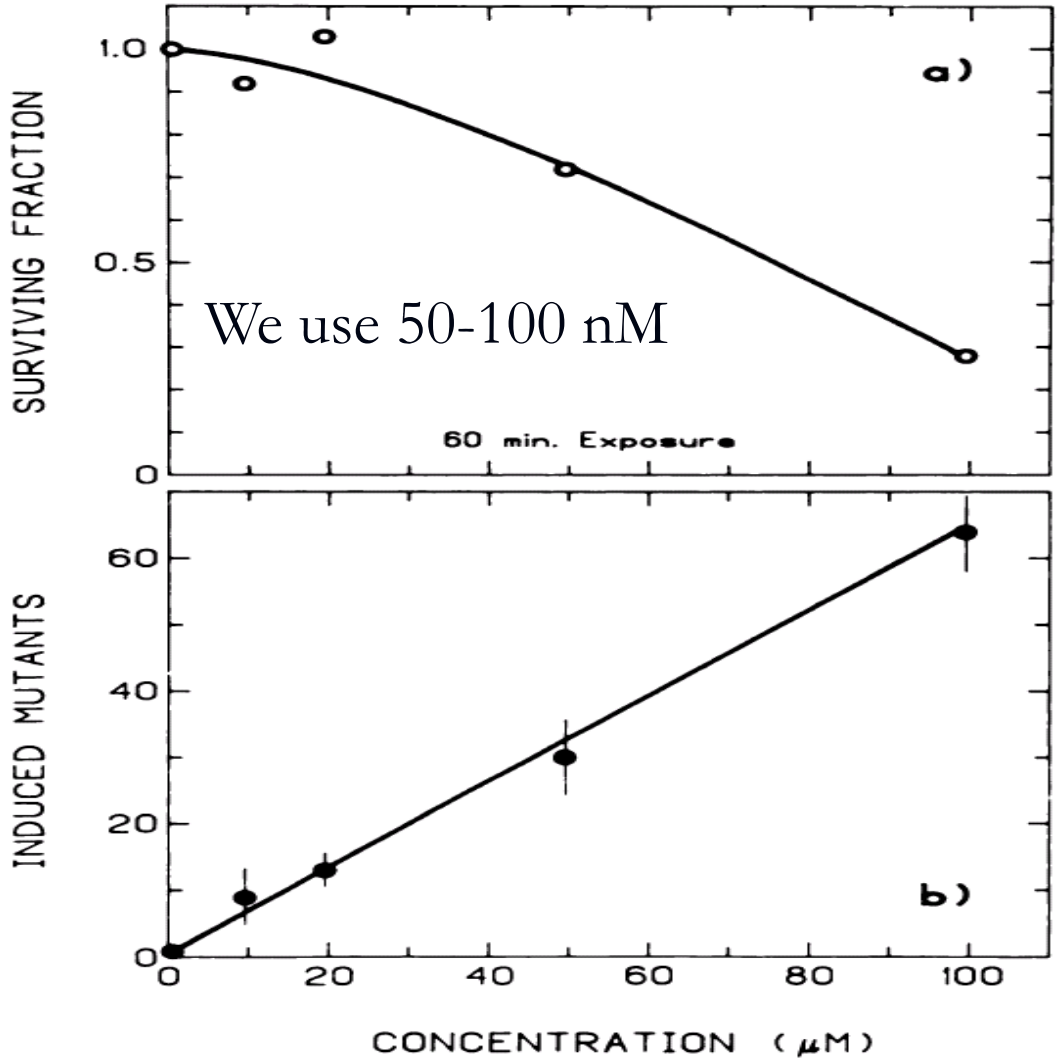
Most common approach is with fluorescent labeling

Fluorescent labeling is often OK, but not always

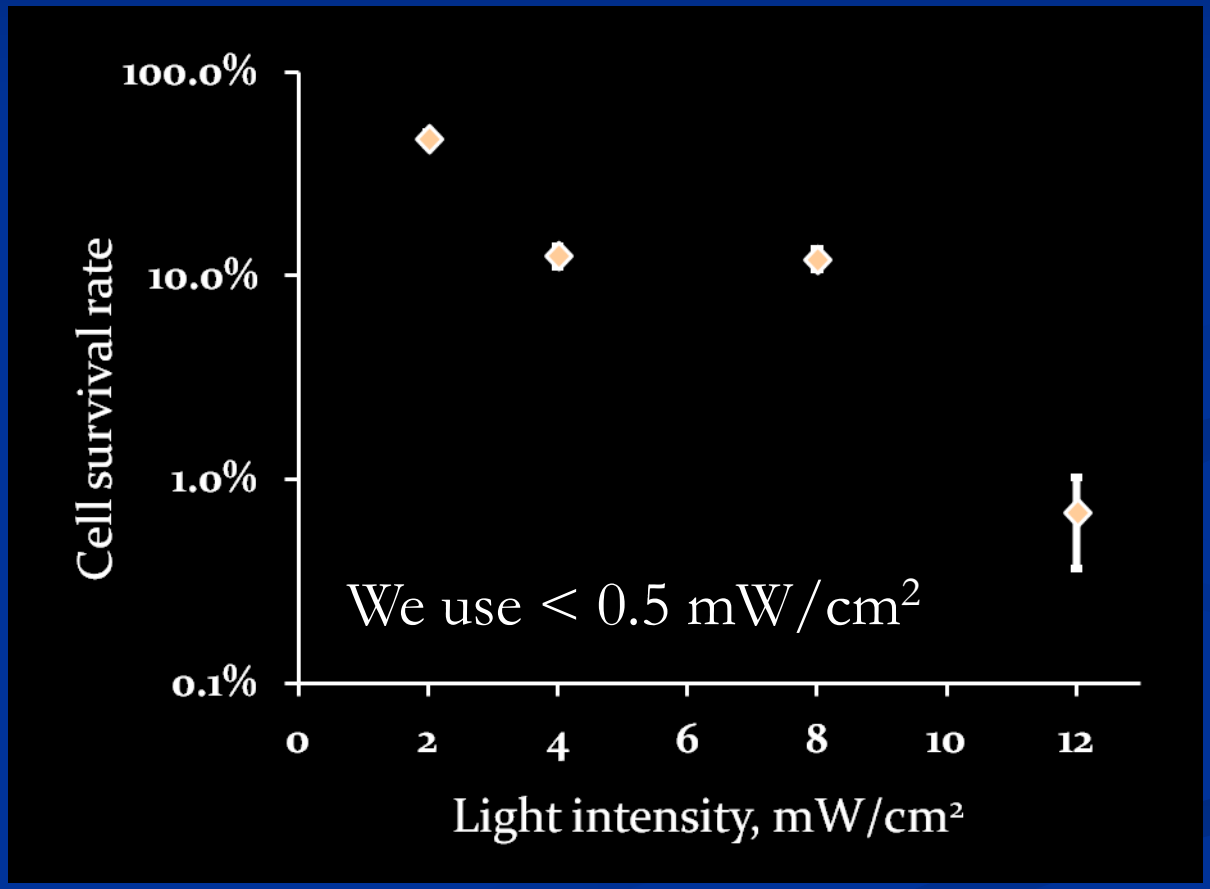
J Histochem Cytochem. 1982 Feb;30(2):111-6.

Cytotoxicity, Mutagenicity and DNA damage by Hoechst 33342

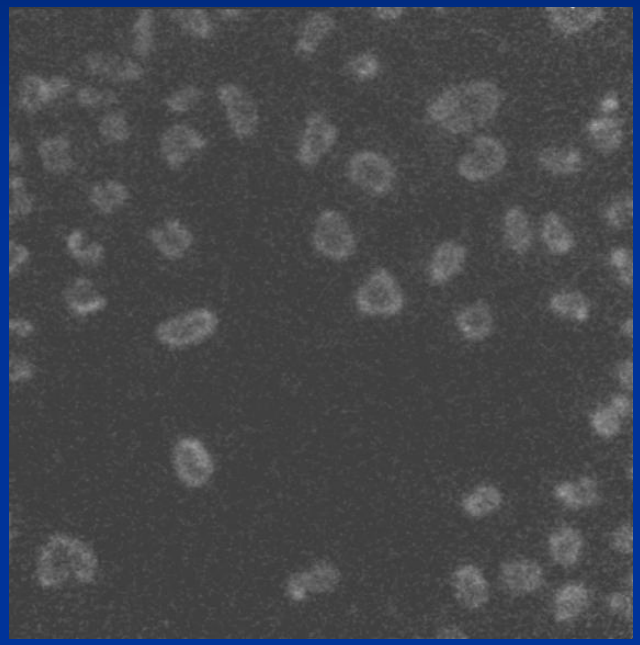
Durand RE, Olive PL.



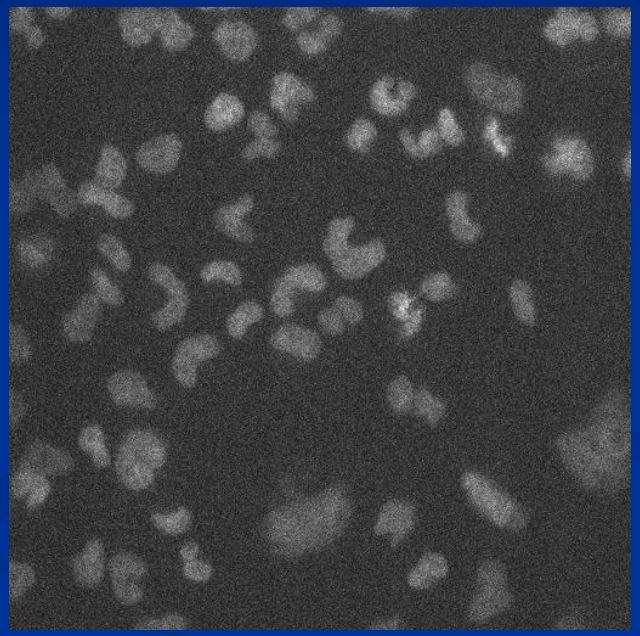
Cell survival with various UV intensities



Rapid EMCCD image acquisition at the microbeam



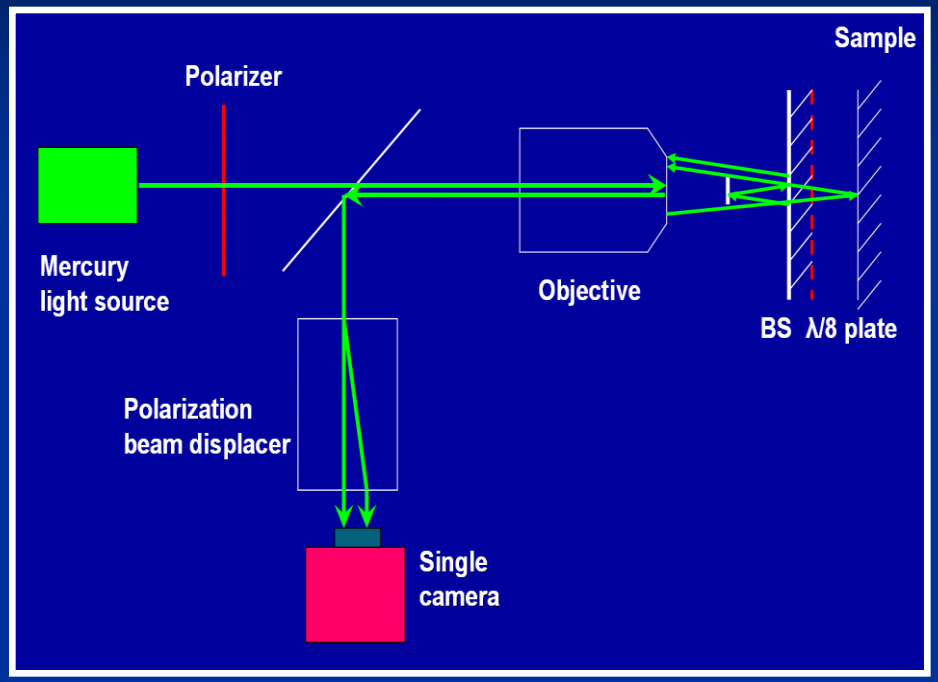
Standard Intensified CCD
500 msec exposure

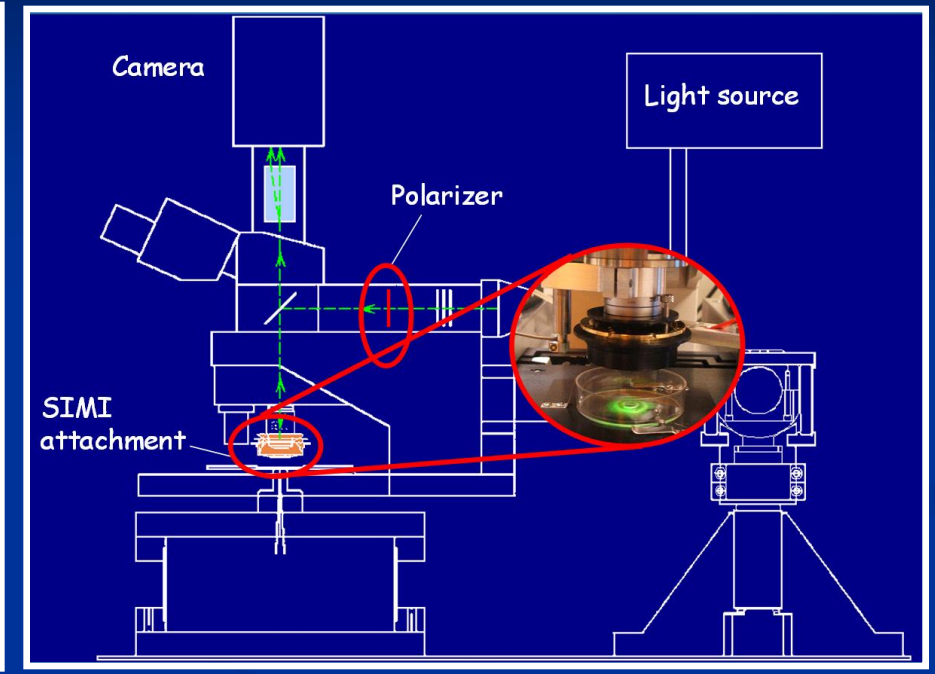


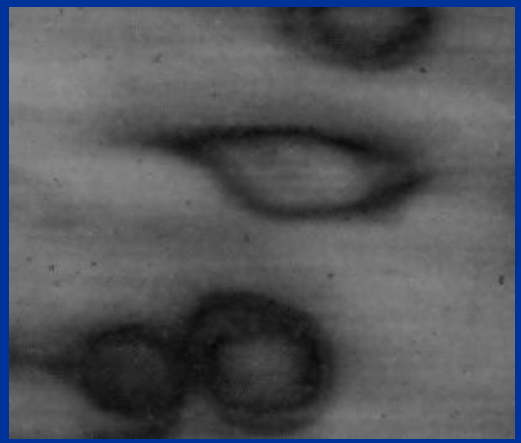
EMCCD
0.001 msec exposure

Image quality maintained with EMCCD, but with a major decrease in UV exposure

SIMI: Simultaneous Immersion Mirau Interferometry





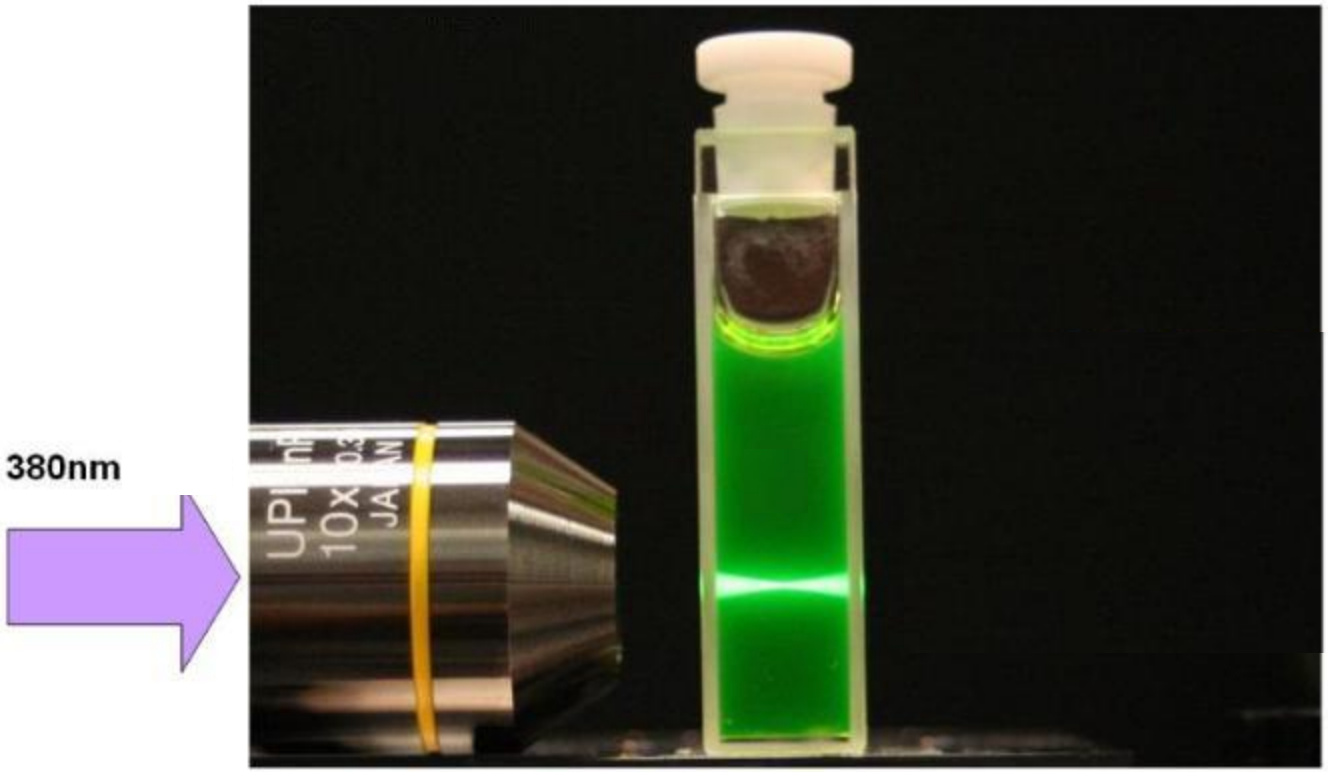


HT1080 fibrosarcoma cells imaged with SIMI in PBS

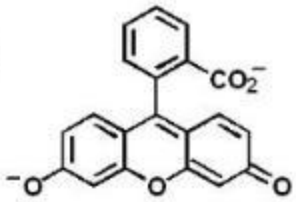
Microbeam-Integrated Multiphoton Imaging System

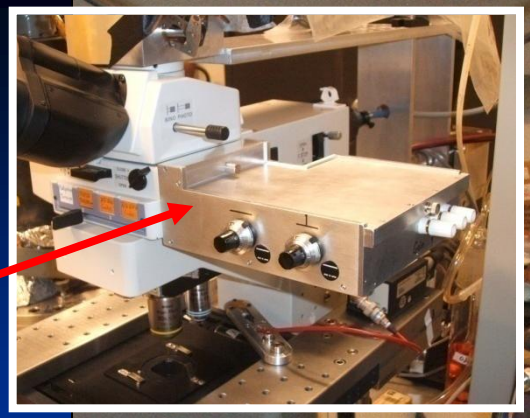
- To provide 3D imaging capability for RARAF microbeam users, which is integrated with the microbeam irradiation system
- To record post-irradiation dynamics in cells, tissue samples, and small organisms

Principle of multiphoton imaging



Fluorescein





Light-tight detector housing (2 PMT channels)

Switch Mirror

Scan Lens

Scan Head

Attenuator

Beam Expander

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

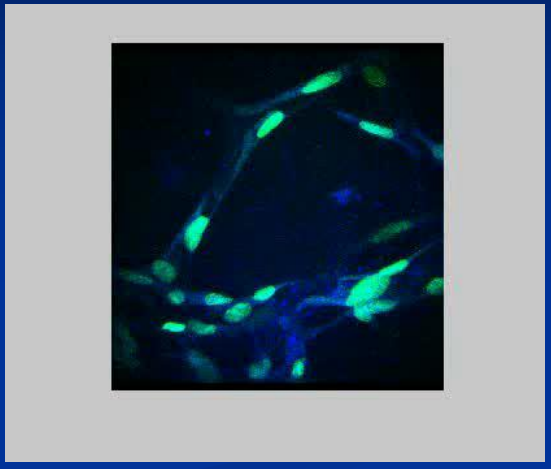
Instrument Designer
Gary Johnson

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)

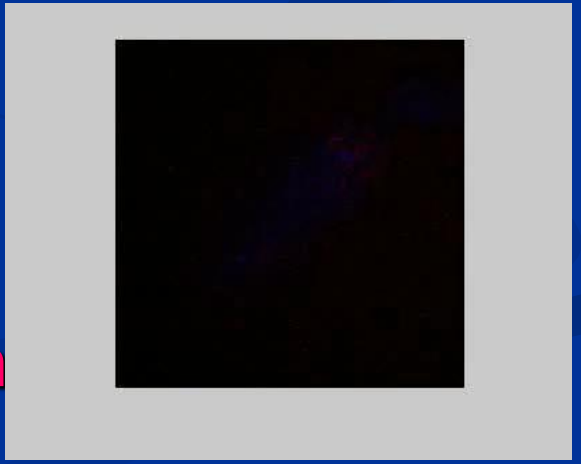


Z-stack rotations

- *In vivo*

  Wild type *C. elegans* pharynx

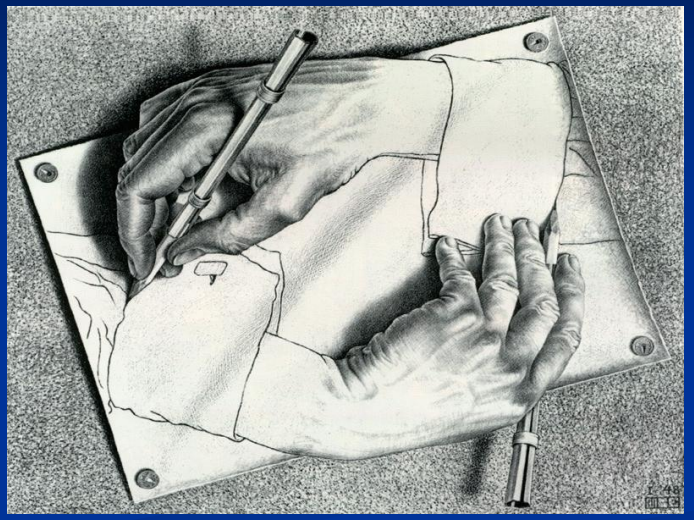
  - Auto-fluorescence (blue)
  - Second Harmonic Generation (red)



Sequence of optical sections

Animal models for the microbeam

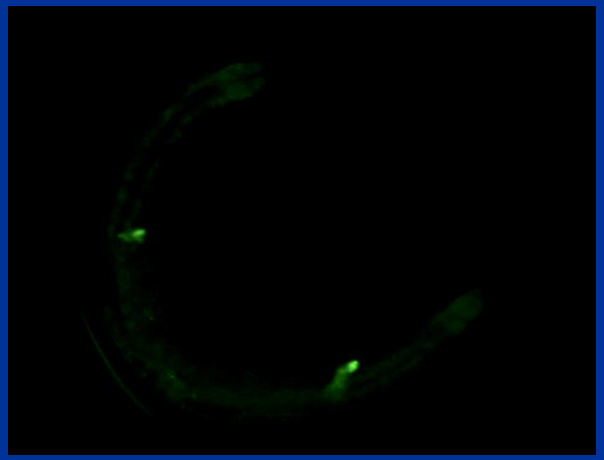
- A lot of interesting biology happens in 3D systems

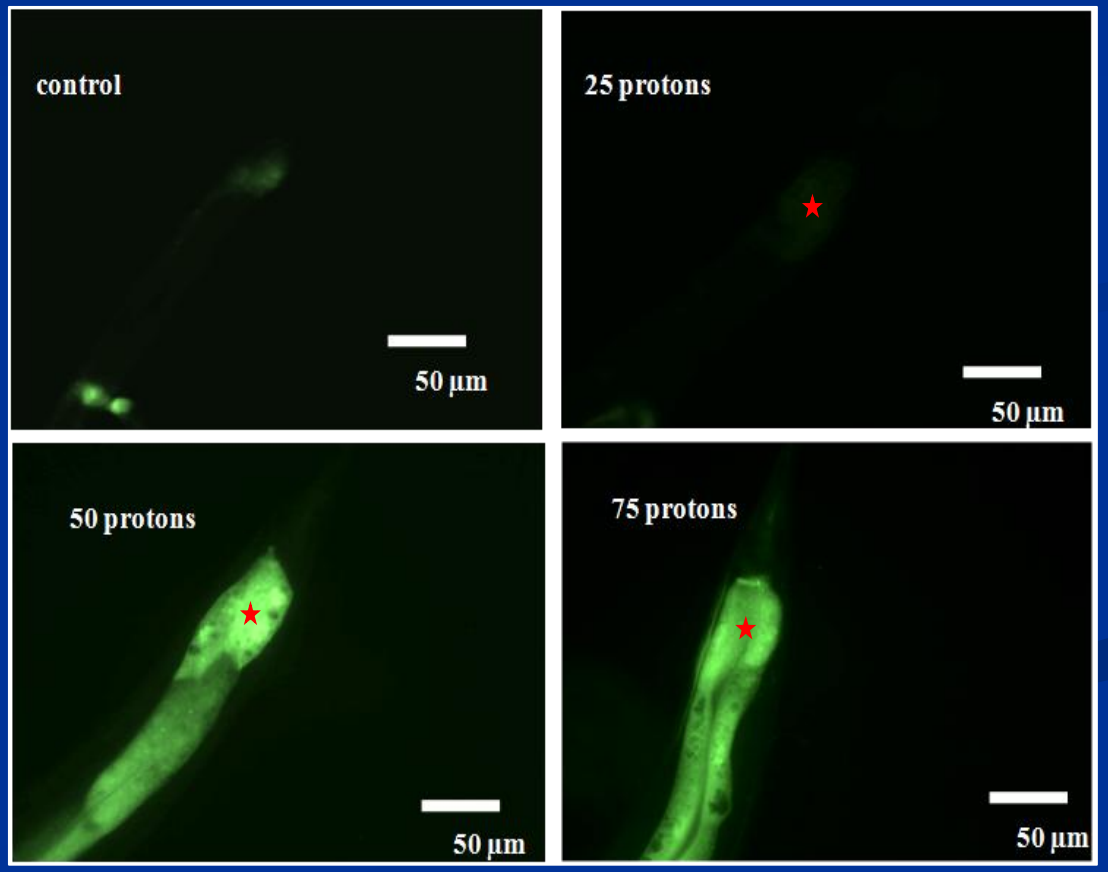


- Requirements for small animal irradiation:
  - Thin sample - Proton penetration 200-300 μm
  - Optically clear – to enable targeting
  - Well established system – need good endpoints

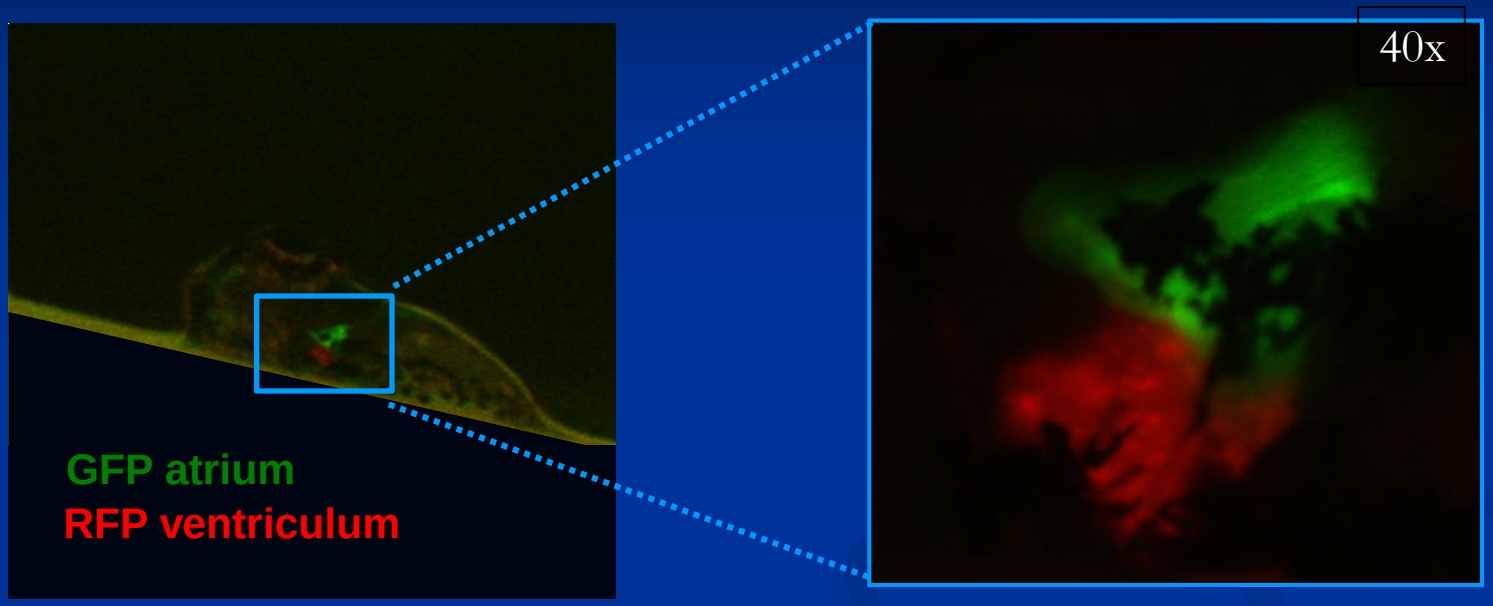
In vivo microbeam irradiation of worms

Worms have green fluorescent protein expressed in response to stress





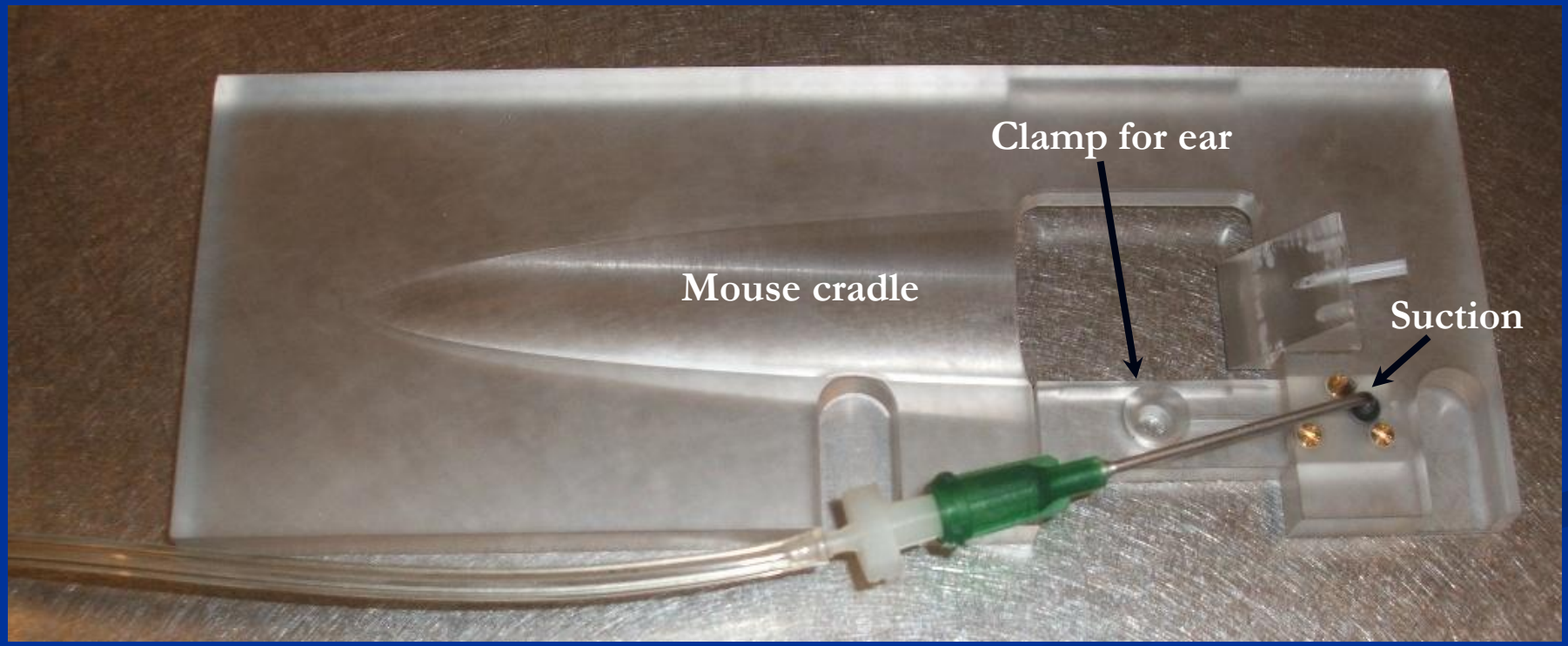
Zebrafish embryos



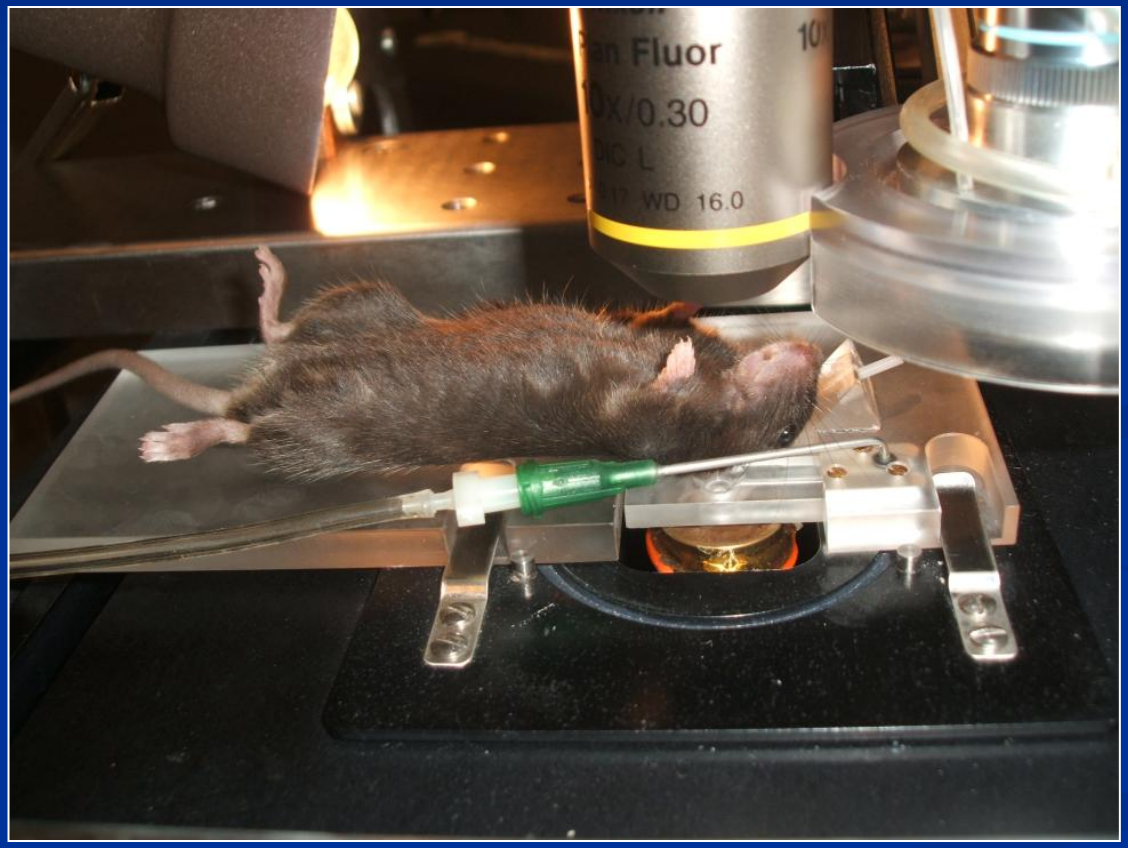
- Microbeam irradiation of either atrium or ventriculum
- To investigate repopulation of non-irradiated cells in lethally exposed areas
- Studies just initiated (Dr. K. Targoff)

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

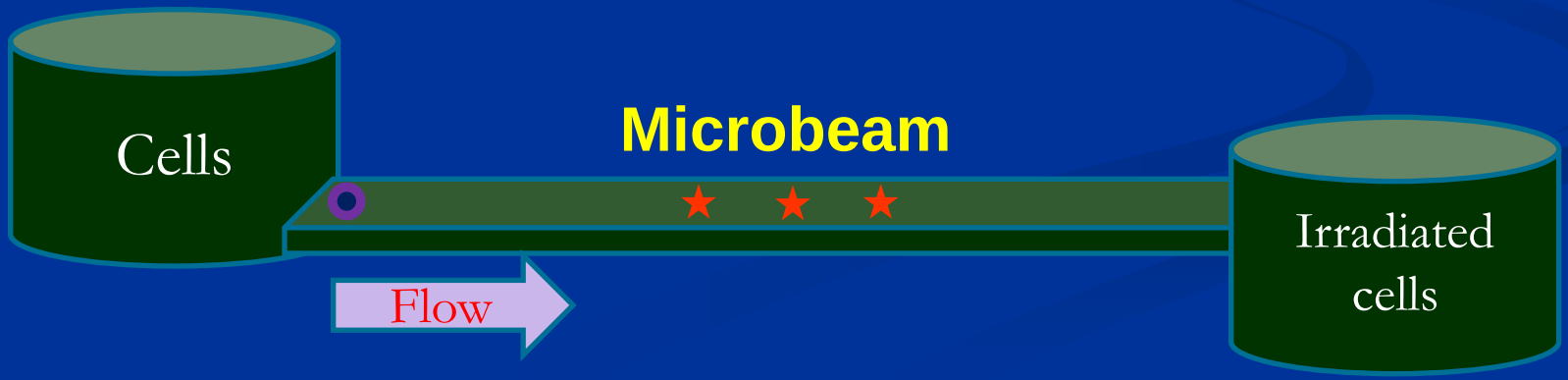
  Cells attached to microbeam dishes, and either:

  - Dish moved to bring cellular target over the microbeam
  - Beam moved to shoot cellular targets (point & shoot)

- Proposed

  Cells flowing through a microfluidic channel

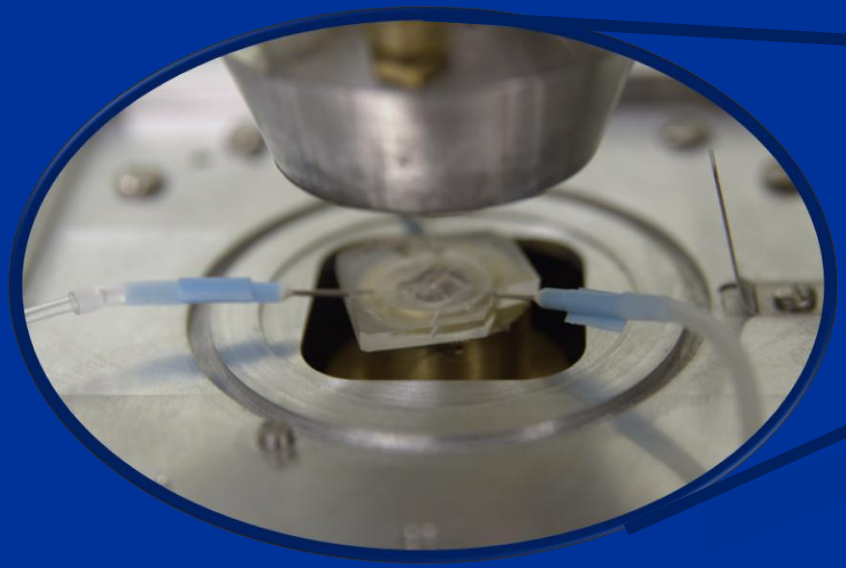
  - Cells targeted by Point & Shoot as they flow by
  - Cells dispensed to user device

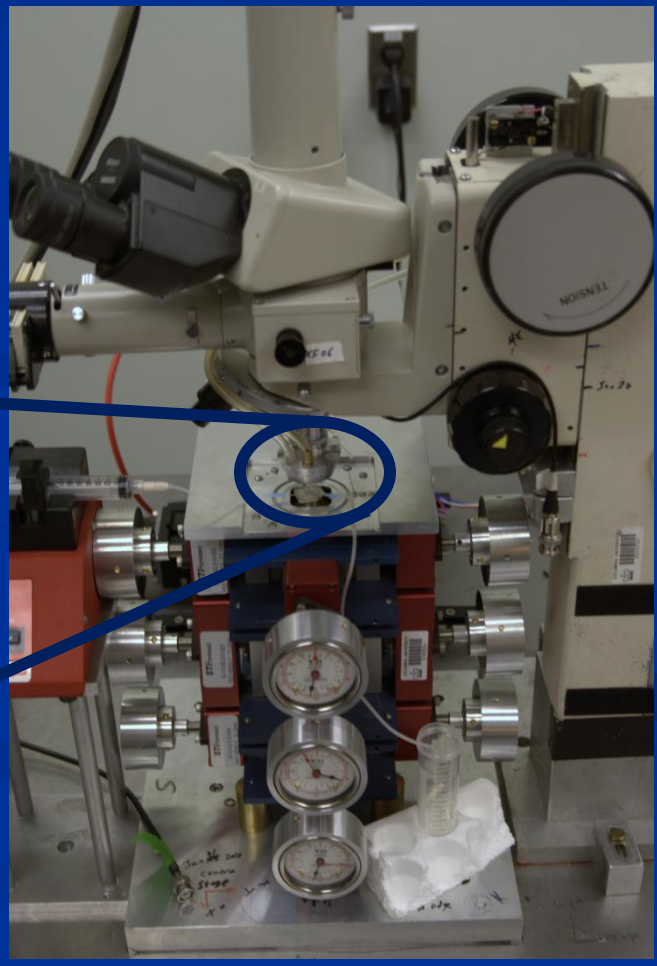


FAST (Flow-And-Shoot Technology)

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:

New position

velocity

$$X_{i+1} = X_i + \frac{X_i - X_{i-1}}{T_i - T_{i-1}} \times \left(T_{i+1} - T_i\right)$$

Old position

Actual time

With Cells

- CRL4025 - Human endothelial cell line (trypsinized)
- GFP expressed throughout the cell.

Optical manipulation of cells

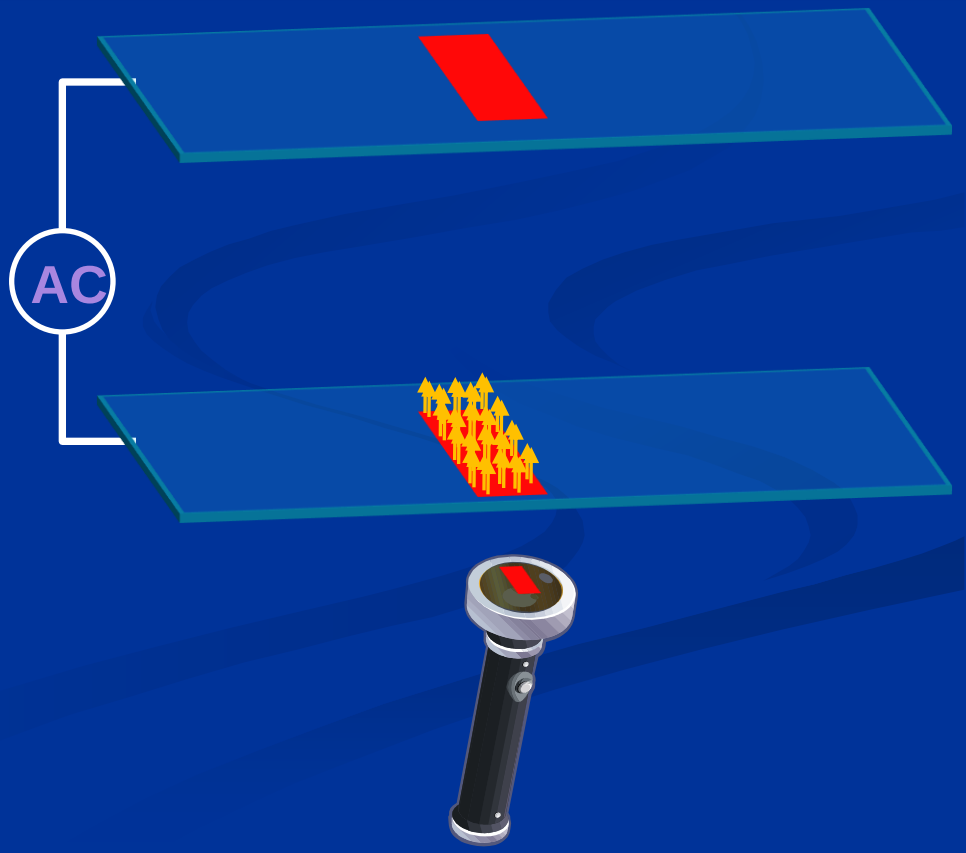
- Why do we want this?
  - Parallel Manipulation of cells
    - Controlling distance between cells before, during and after irradiation
    - Handling groups of cells in parallel
  - Manipulating cells in suspension

Optical manipulation of cells
How does it work?

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

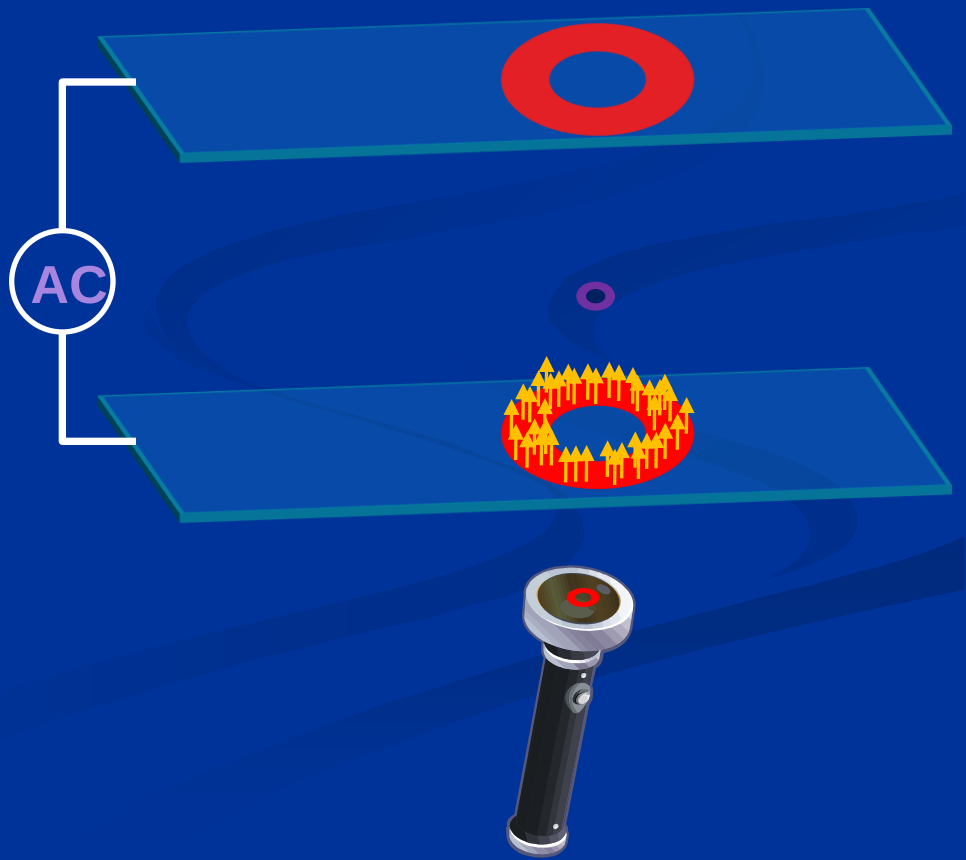
Optical manipulation of cells
How does it work

- Light pattern generated on photoconductive electrode
- AC is applied
  - Electric fields are formed
- Fields repel cells
  - No physical barriers!

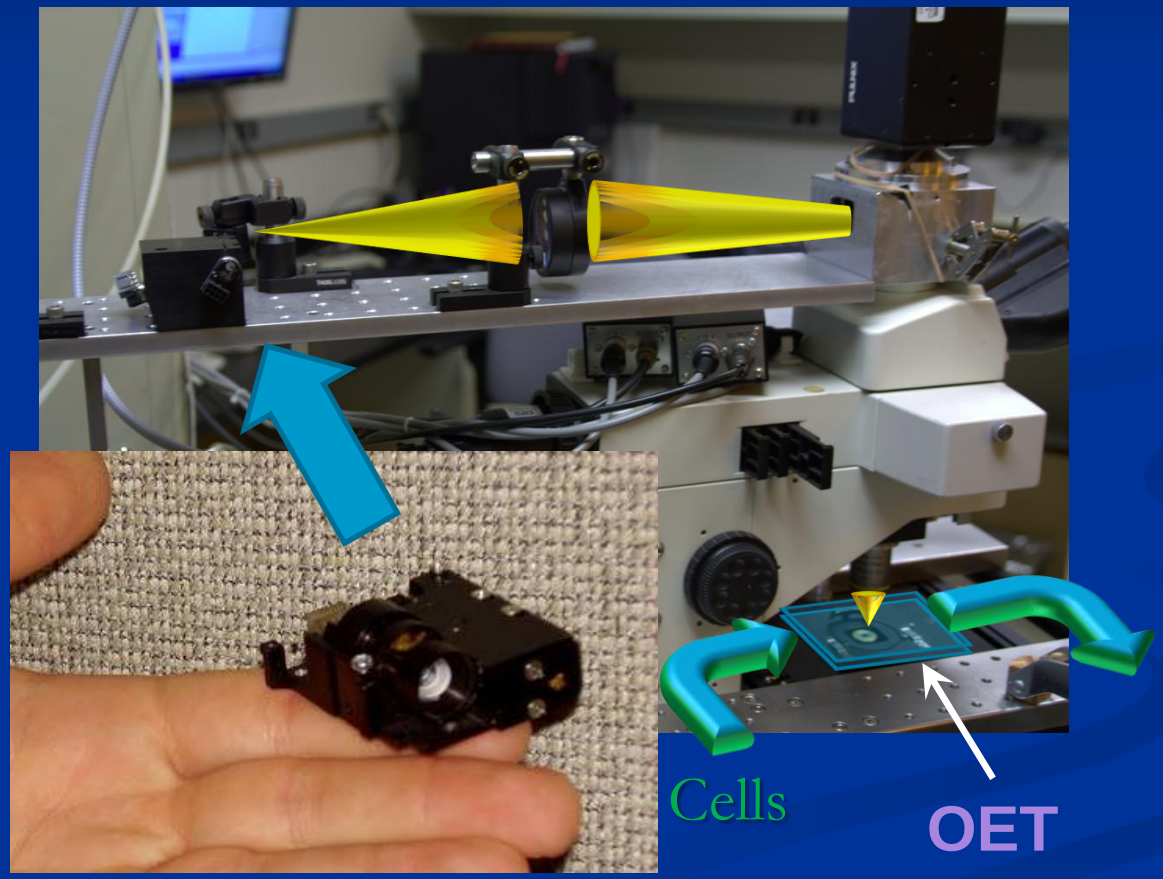


Optical manipulation of cells
How does it work

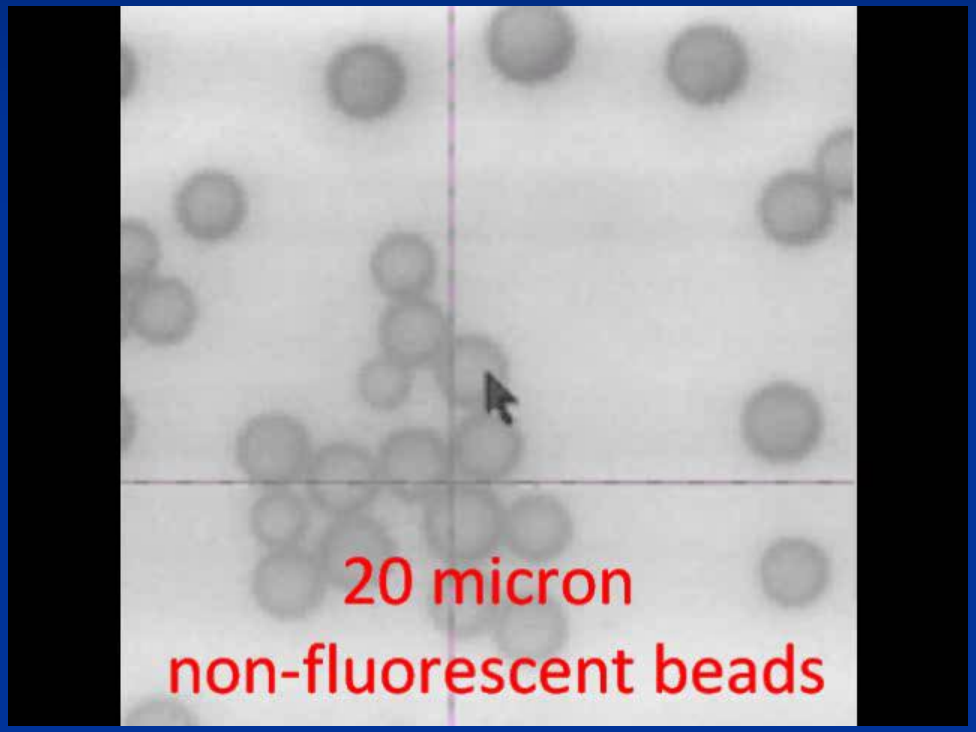
- Light pattern generated on photoconductive electrode
- AC is applied
  - Electric fields are formed
- Fields repel cells
  - No physical barriers!
  - Illumination pattern can be changed dynamically.
    - Cells can be boxed in
    - Cells will track pattern

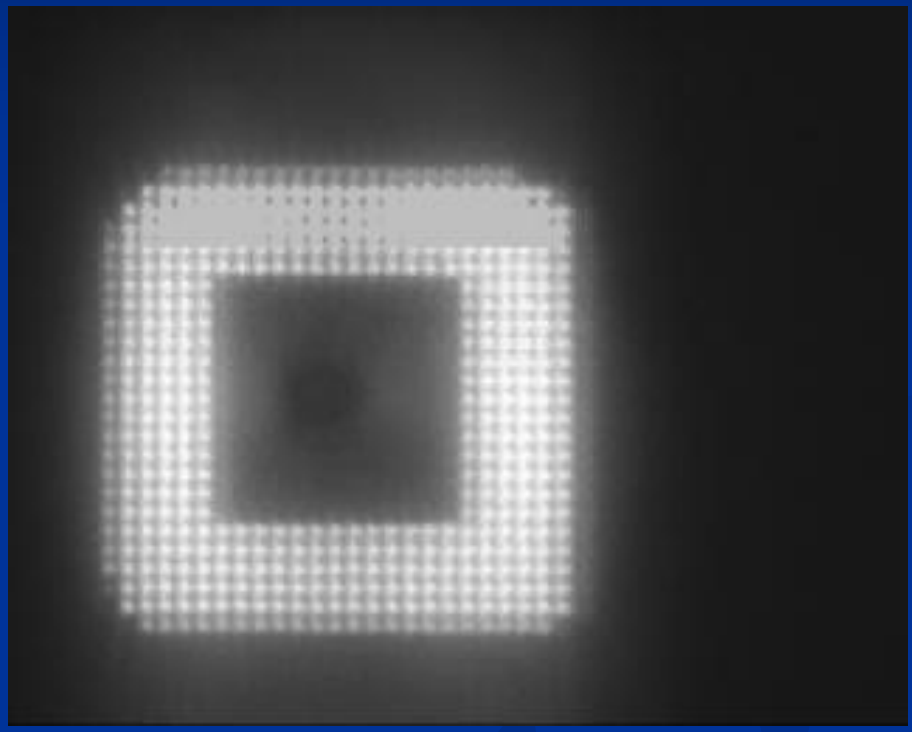


How does this work?



Optical manipulation of cells
preliminary data with beads

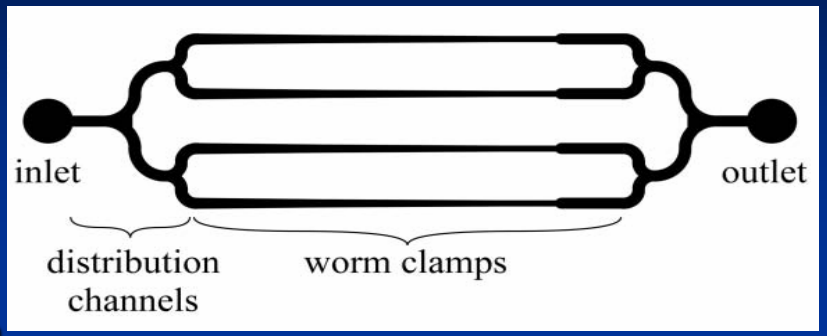


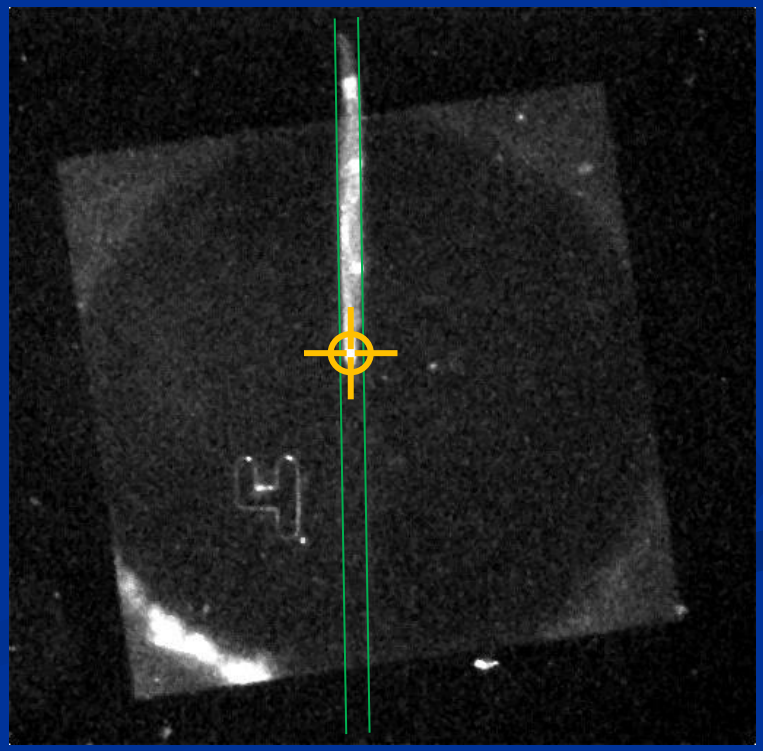


Microfluidic irradiation of worms

We have:

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





Worm irradiations now routinely performed in clamps

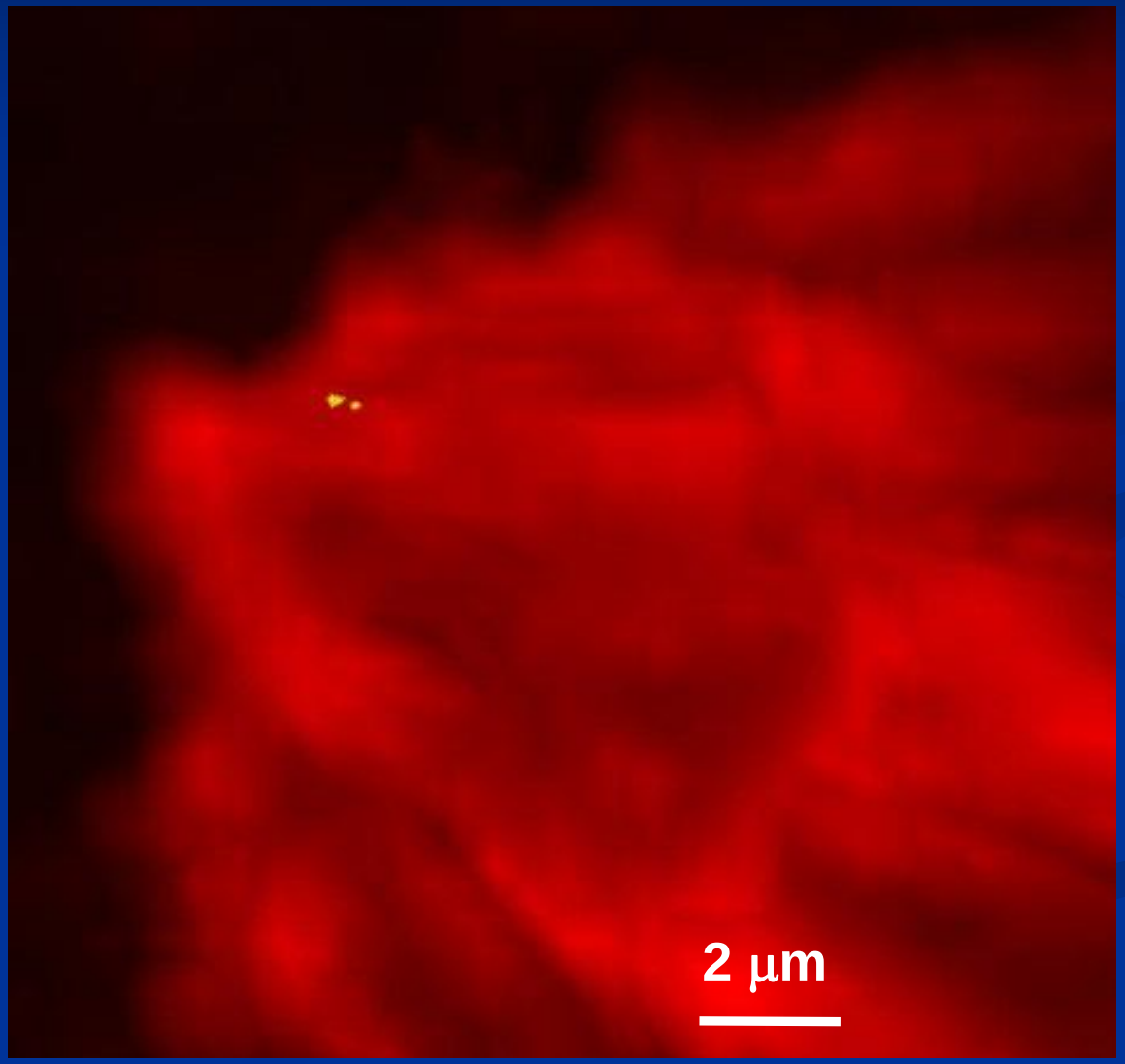
Where are we going?

Aiming at still smaller targets

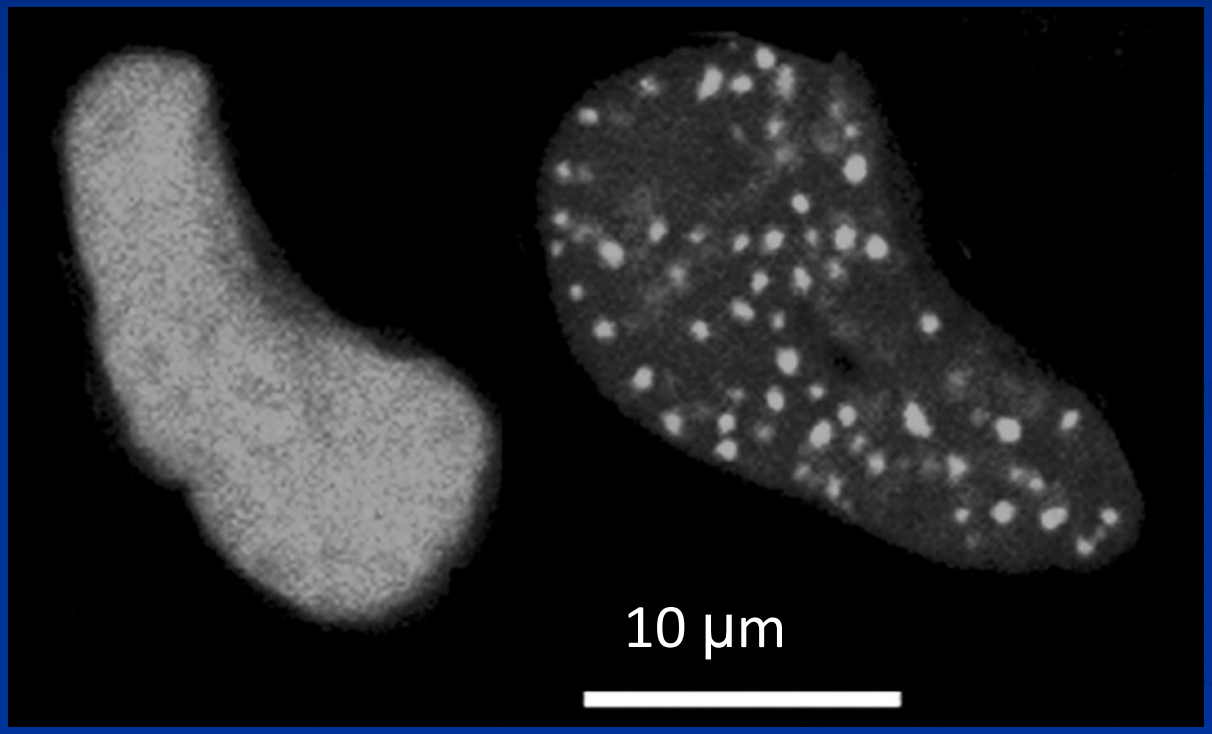
- At present our imaging capabilities and our microbeam targeting capabilities roughly match (as they should)
- Both are around 300 - 400 nm

- 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

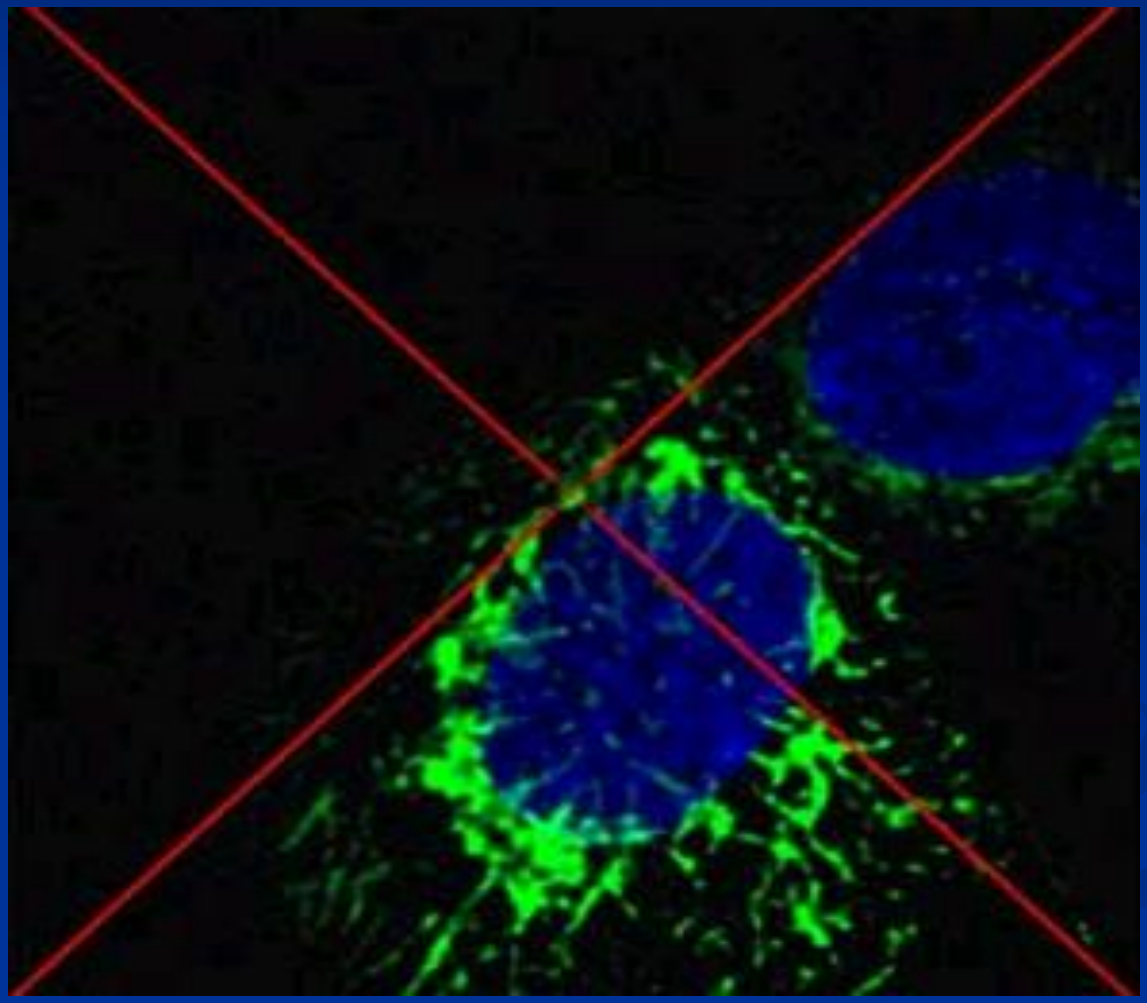


We want to target transcription sites

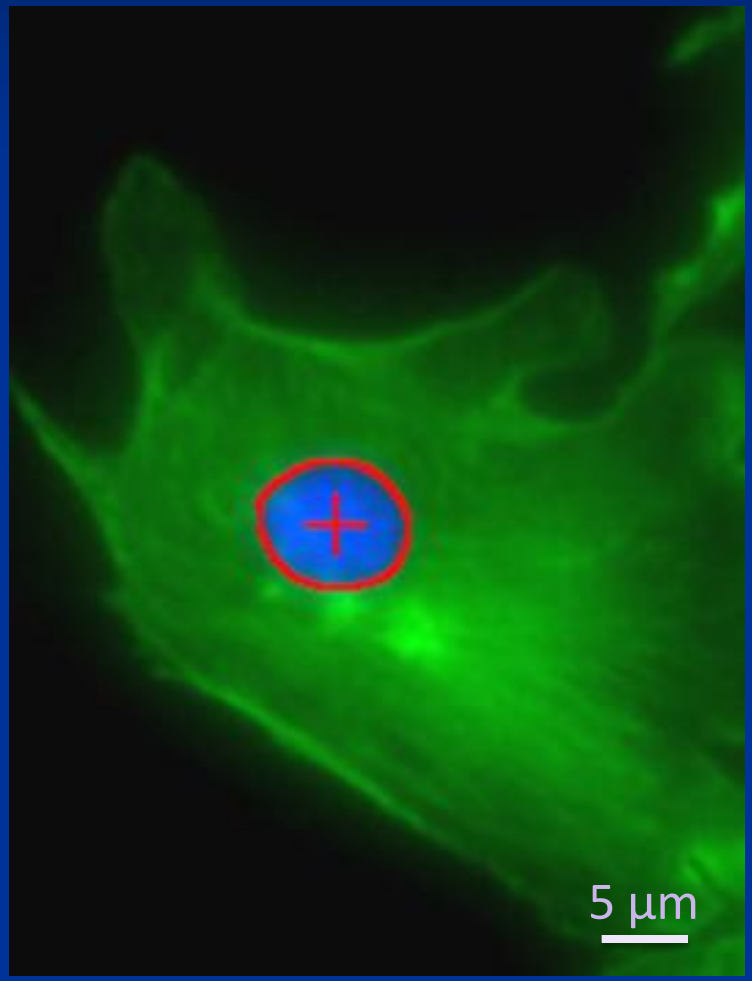


Solovjeva L et al. Mol. Biol. Cell 2005

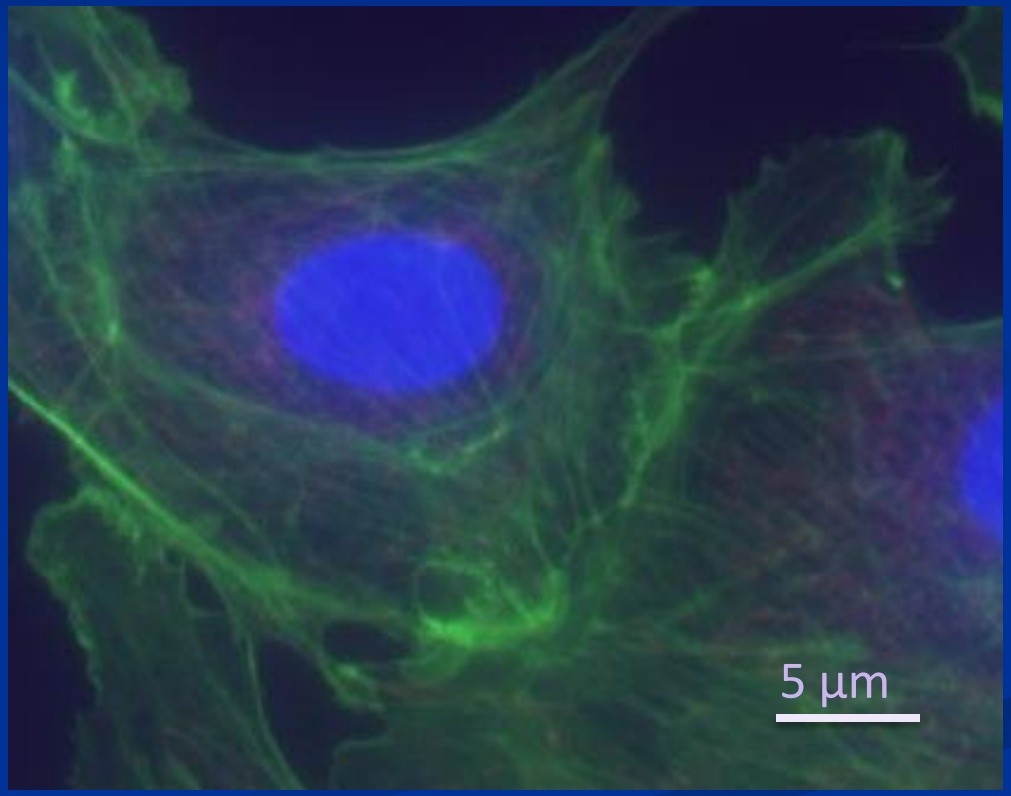
We want to target mitochondria



Current imaging on the Microbeam

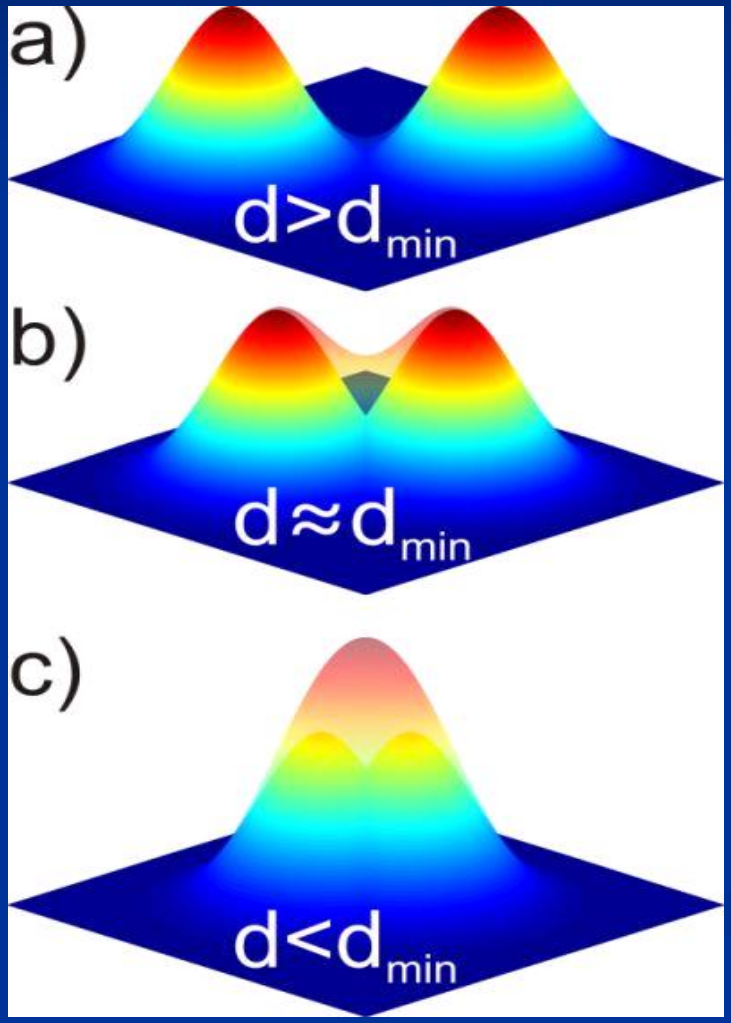


Resolution ~400 nm



Resolution ~250 nm

The Abbe diffraction limit d_{min}~200 nm

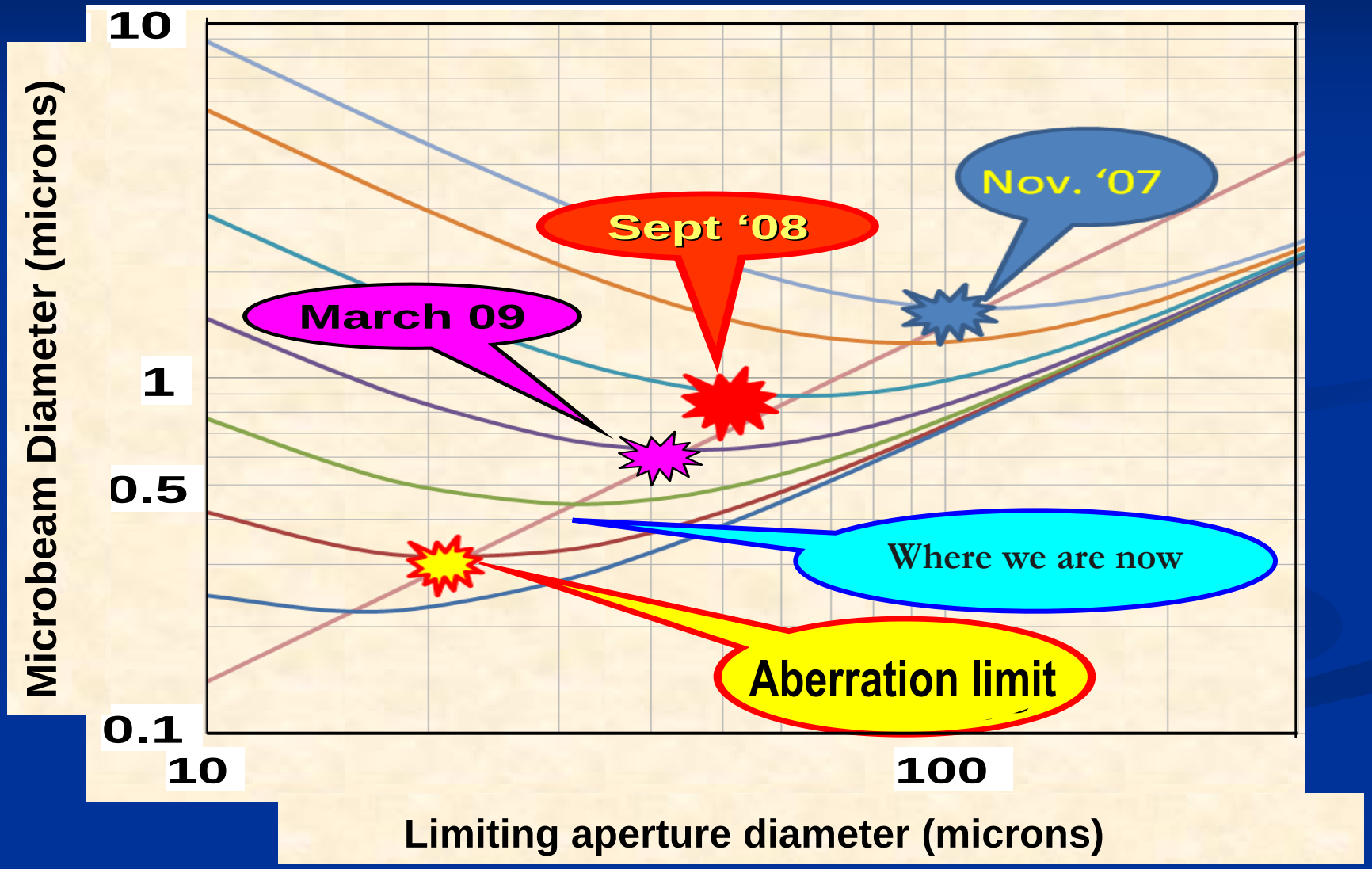


$$d_{min} = \frac{\lambda}{2 \times NA}$$

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

$$360\ nm \leq \lambda \leq 800\ nm$$

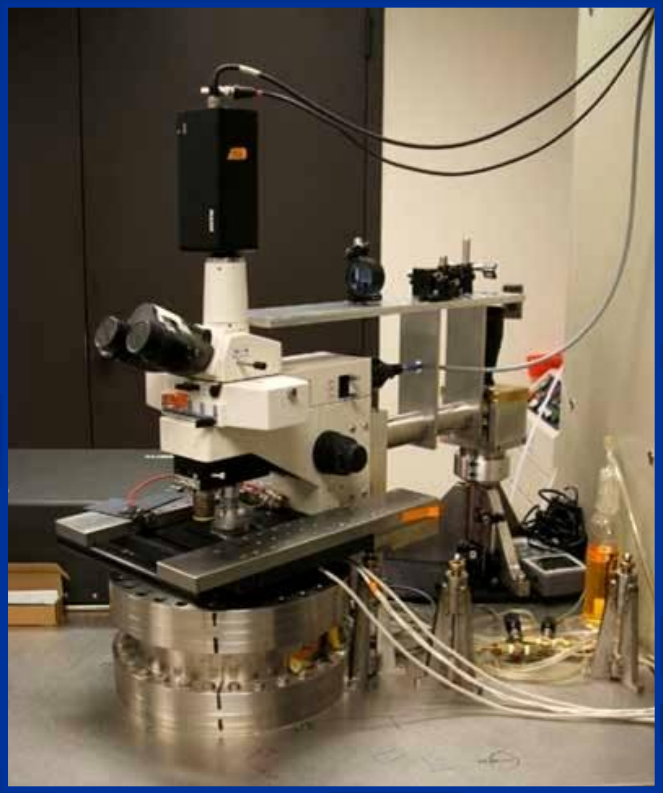
The Particle Beam Focusing Limit



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

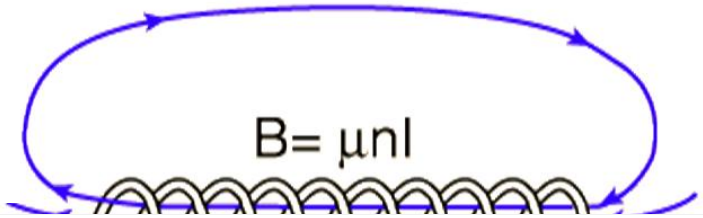
These electrostatic lenses provide a lower limit on how small a diameter we can make the microbeam, due to their intrinsic spherical and chromatic aberrations

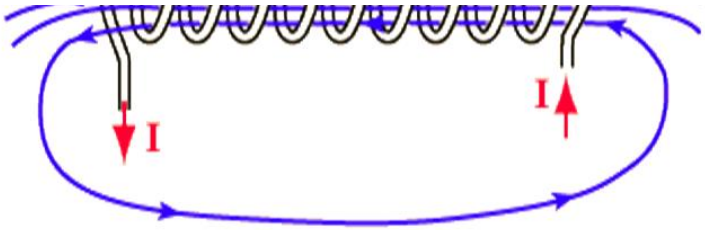
In principle solenoid lenses can provide lower spherical and chromatic aberrations, and consequently superior spatial resolution

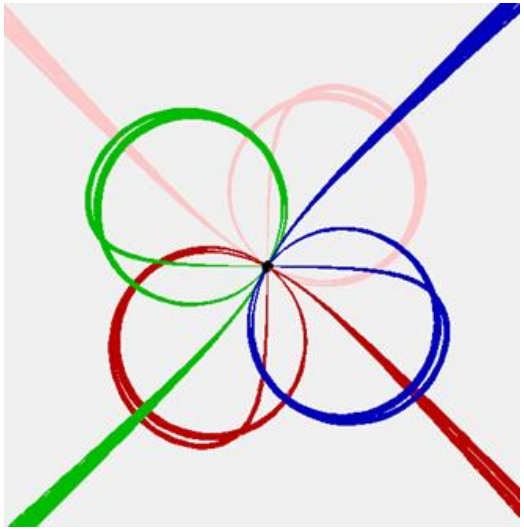
A solenoid has many coils of wire carrying DC current

The field is strong and

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

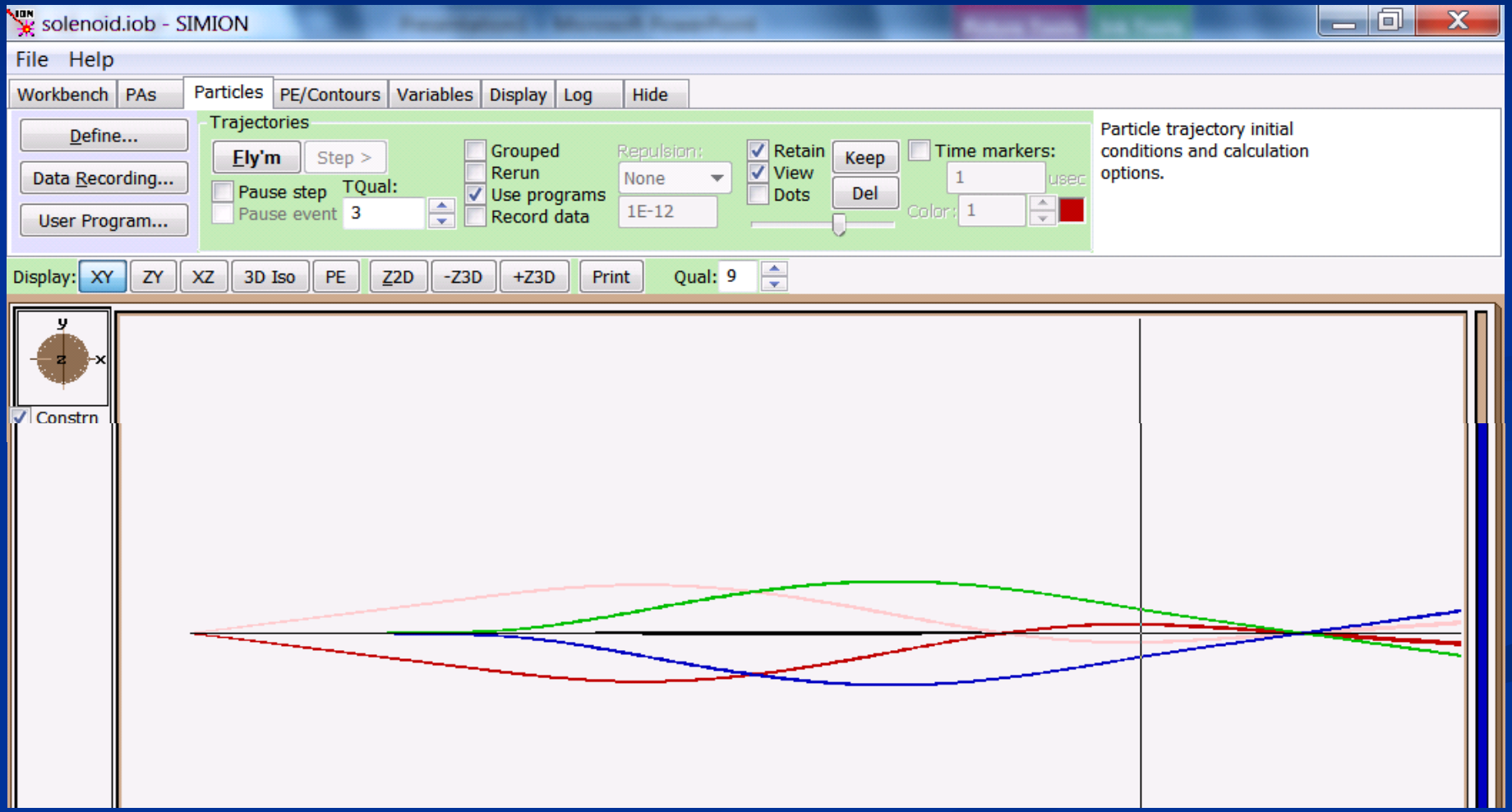






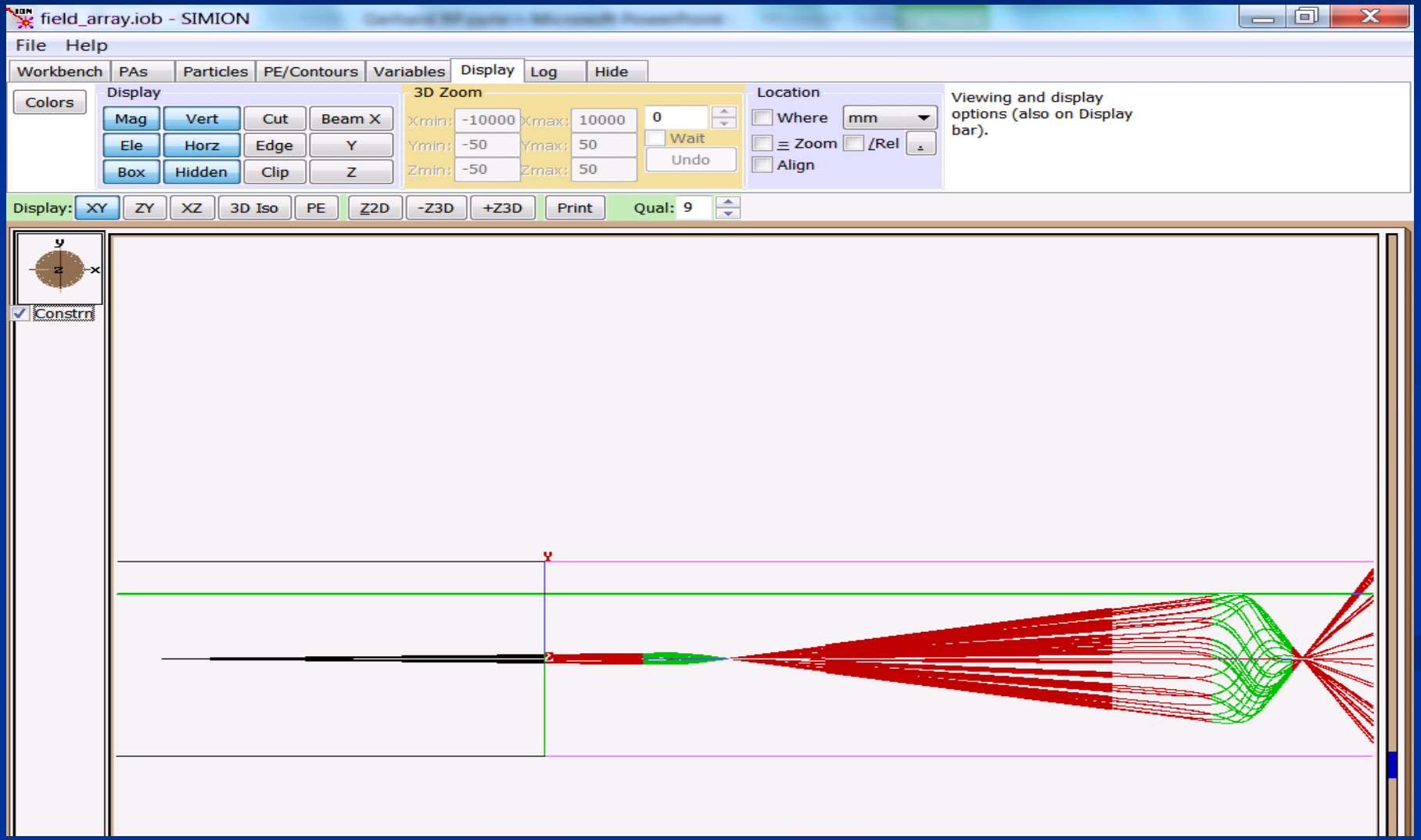
Head-on view

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 need for targeting at the 70 nm resolution for the super microbeam

- We have chosen to use STimulated Emission Depletion (STED)

How does STED work?

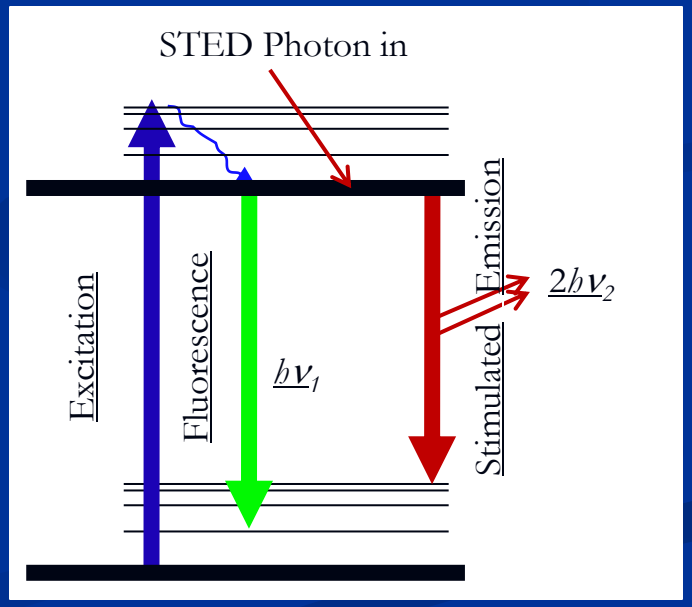
- Fluorescence limited to the sub-diffraction spot by a depletion 'donut' surrounding the excitation focus
- Depletion happens by de-exciting the fluorophores stimulating them to emit at a longer wavelength – **ST**imulated **E**mission **D**epletion

Excitation

- Requires STED intensity >> Fluorophore saturation intensity

STED Resolution

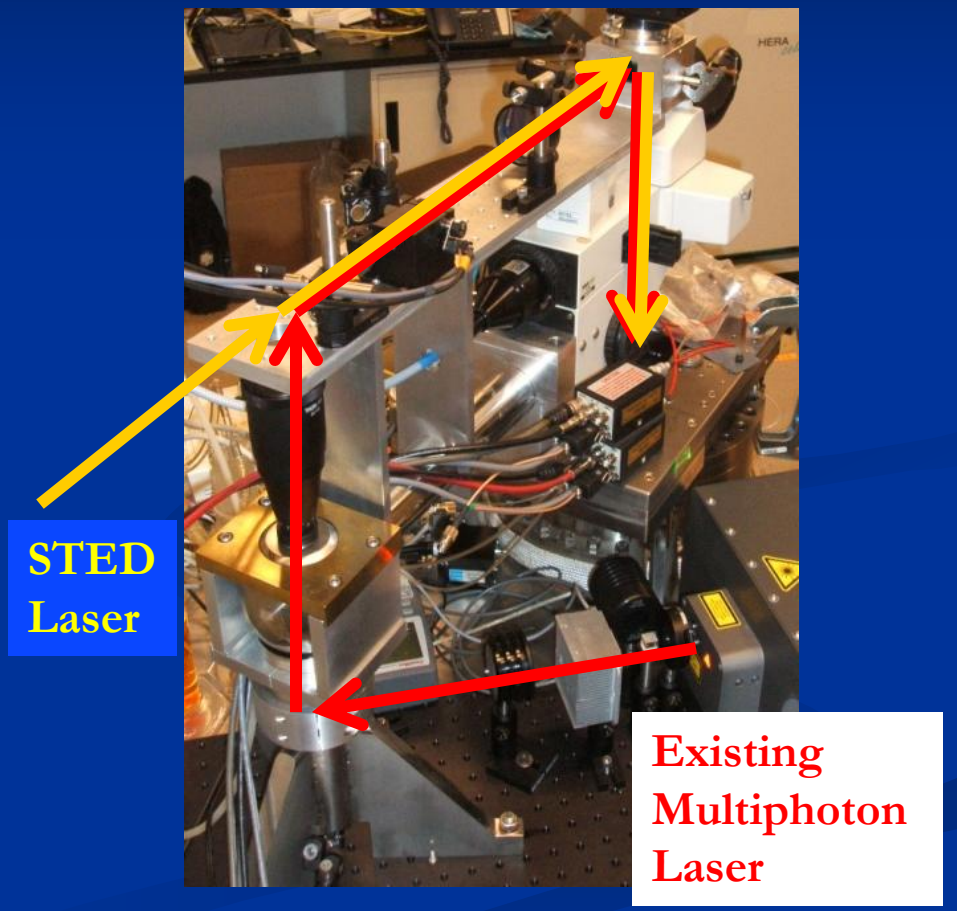
$$d = \frac{1}{\sqrt{1 + I_{STED}/I_{Fsat}}} \frac{\lambda}{2NA}$$



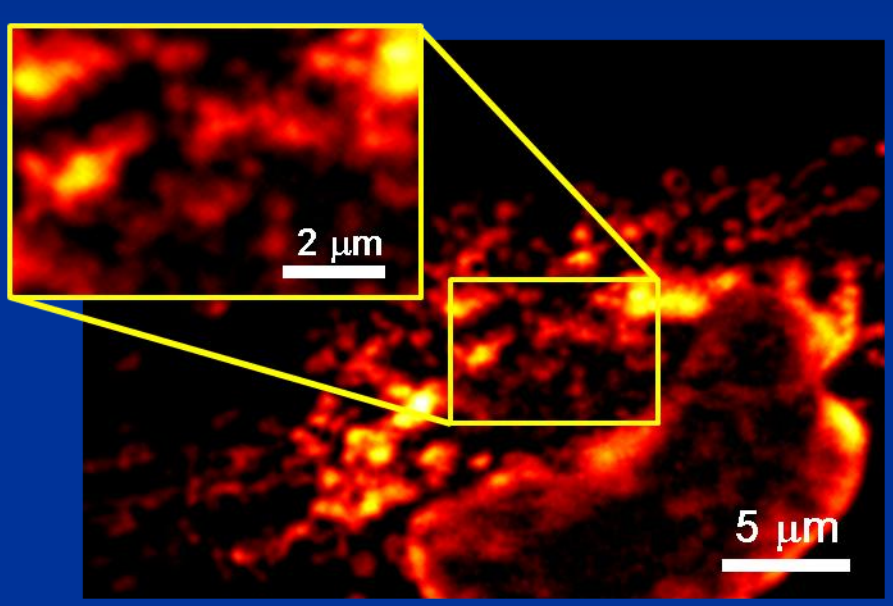
Super Resolution STED at RARAF

- 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

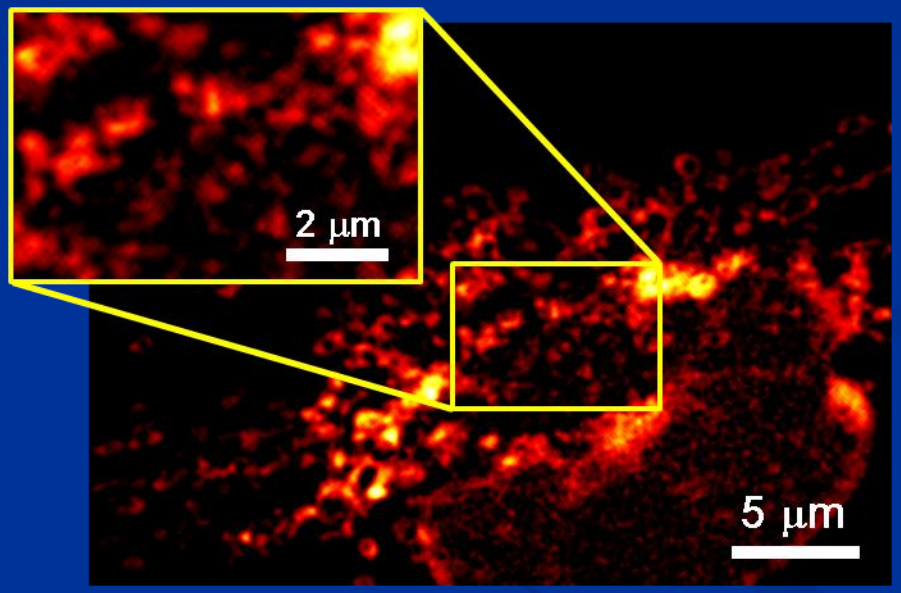
Current microbeam endstation



Live cells require media immersion ultimate resolution ~70 nm
– right on par with Super Microbeam



STED - Off

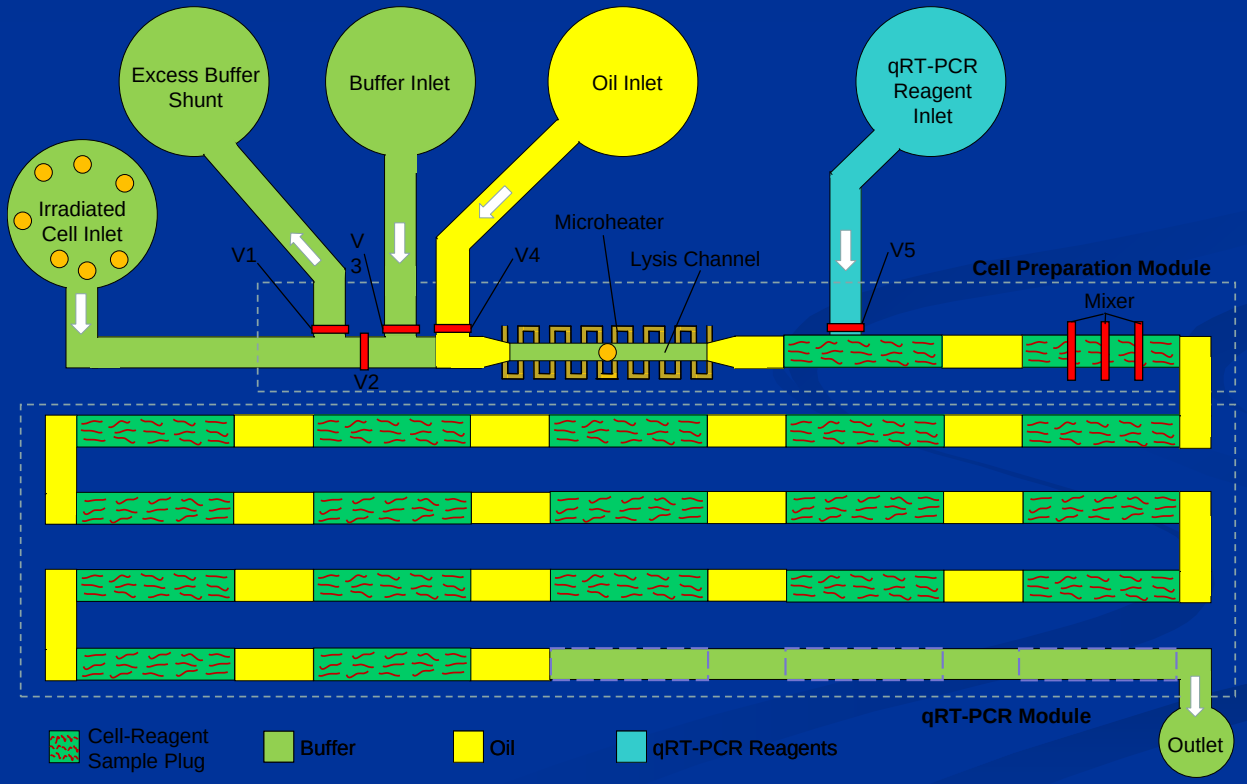


STED - On

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*



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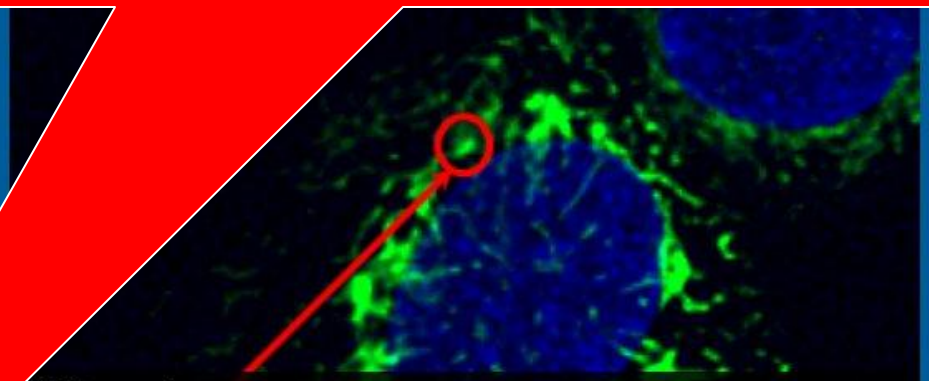

UV Microspot

Form of laser microbeam. Multiphoton processes produces a micro-volume of UV radiation. Delivers "spot" damage, an advantage over all microbeam systems

chnical Tour

Current Accelerator Schedule (PDF)

Latest News and Events

2013 Microbeam Training Course [Poster]

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

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Dr. Eric J. Hall	Columbia	Radiation biology
Dr. Marcelo Vazquez	Loma Linda	Charged particle radiation biology, training

Thank you