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MD Anderson Cancer Center
Making Cancer History®

Nanoscale radiation measurements in mixed radiation fields at the molecular level

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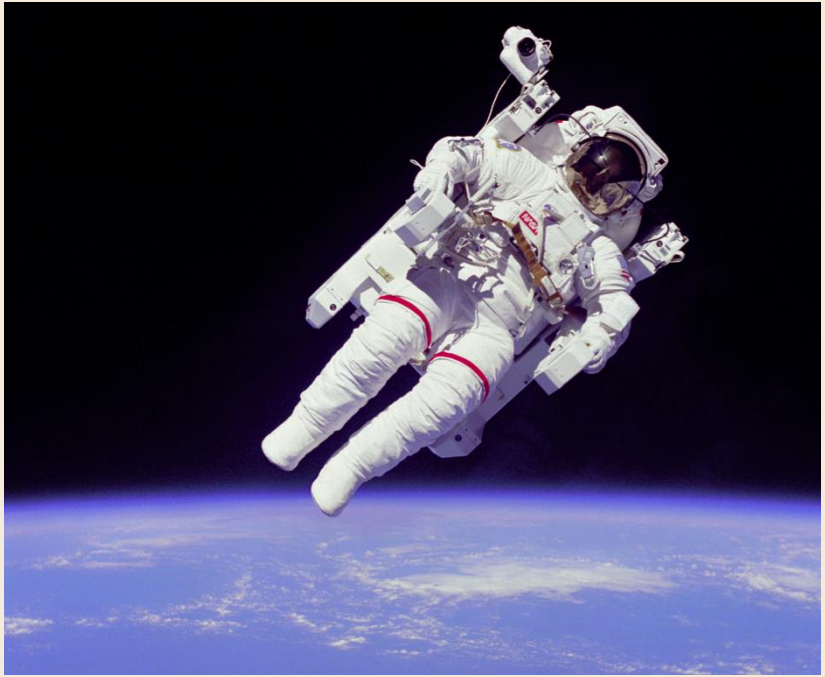
Disclosures

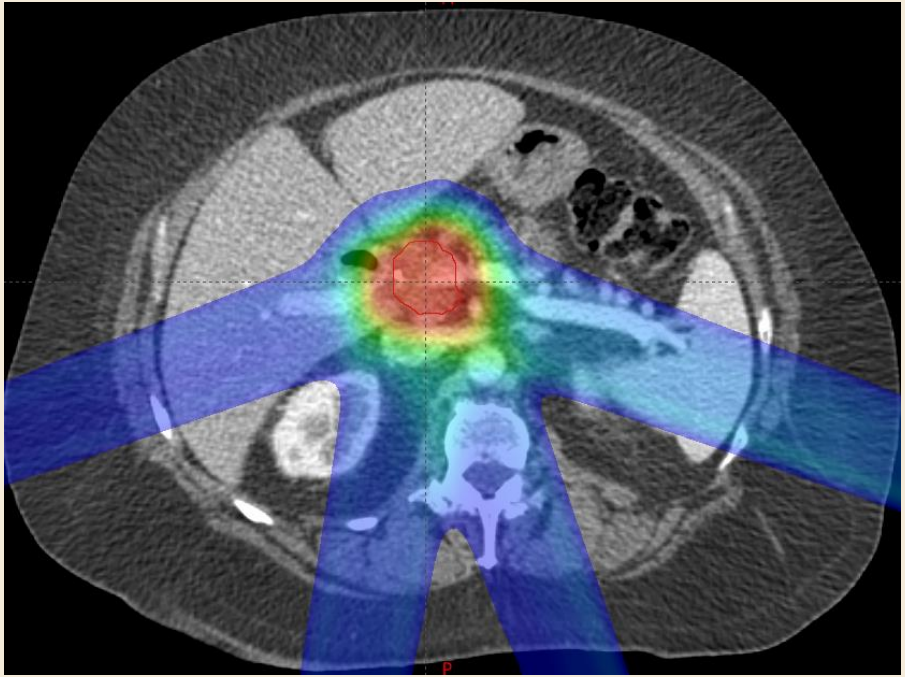
Research funds: Elekta Inc.

High-energy mixed radiation fields

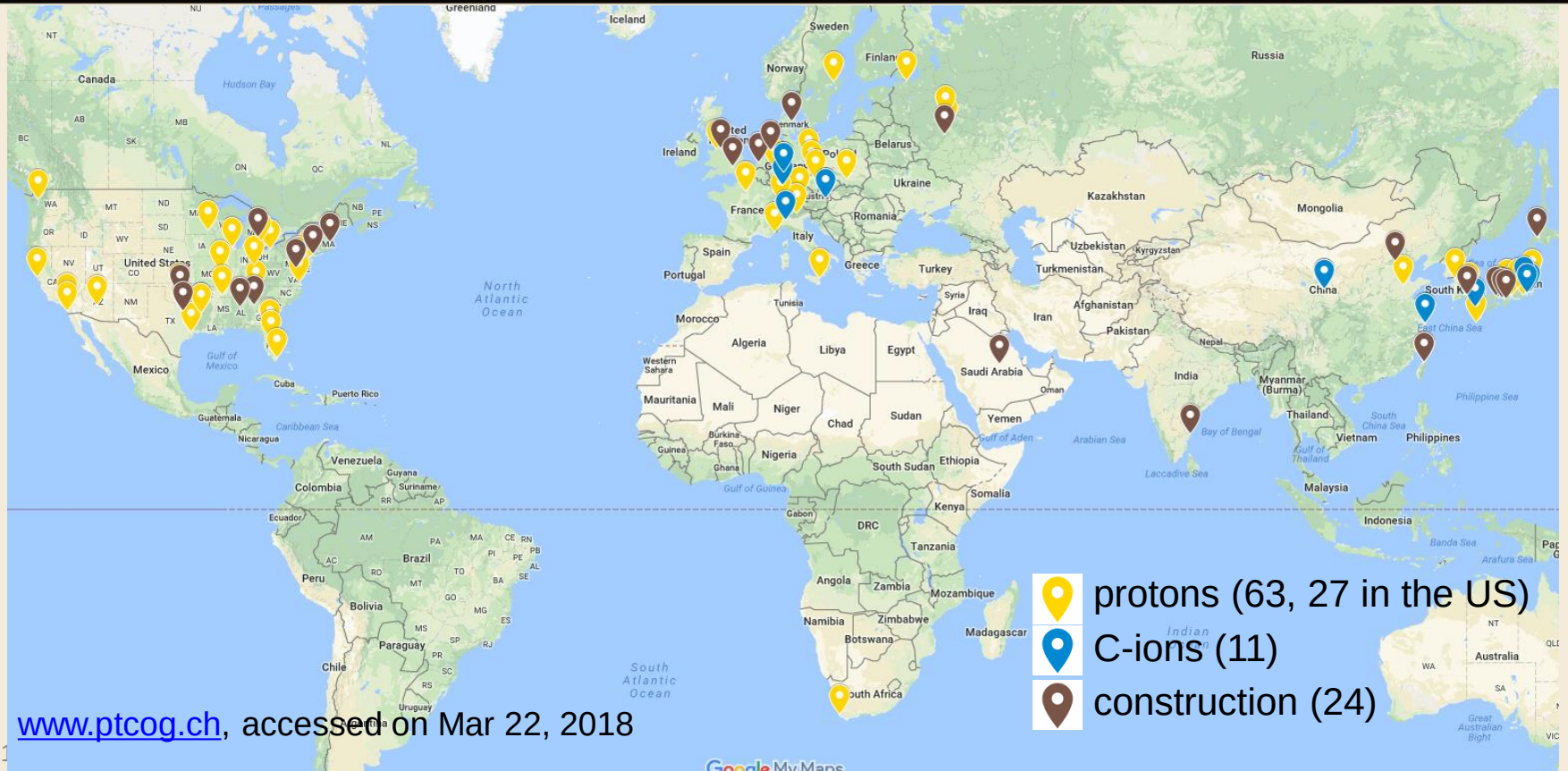
Space radiation environment



C-ion therapy

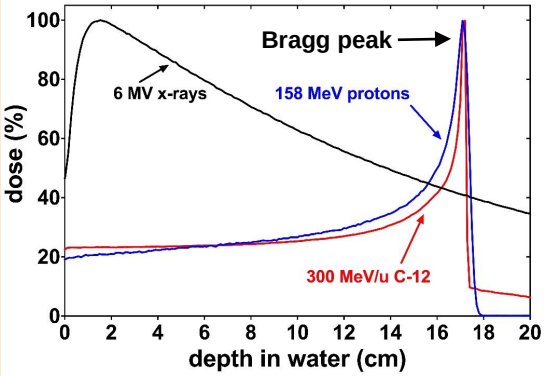


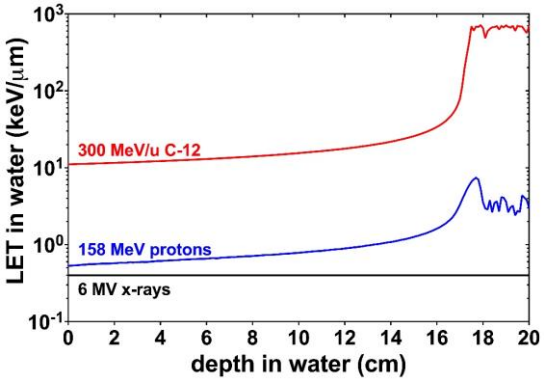
Particle therapy facilities

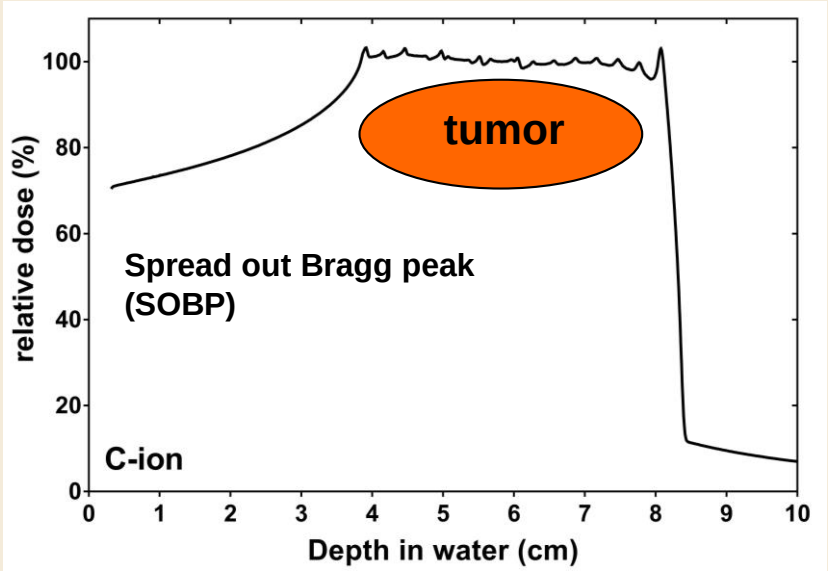


www.ptcog.ch, accessed on Mar 22, 2018

Physical characteristics of particle beams



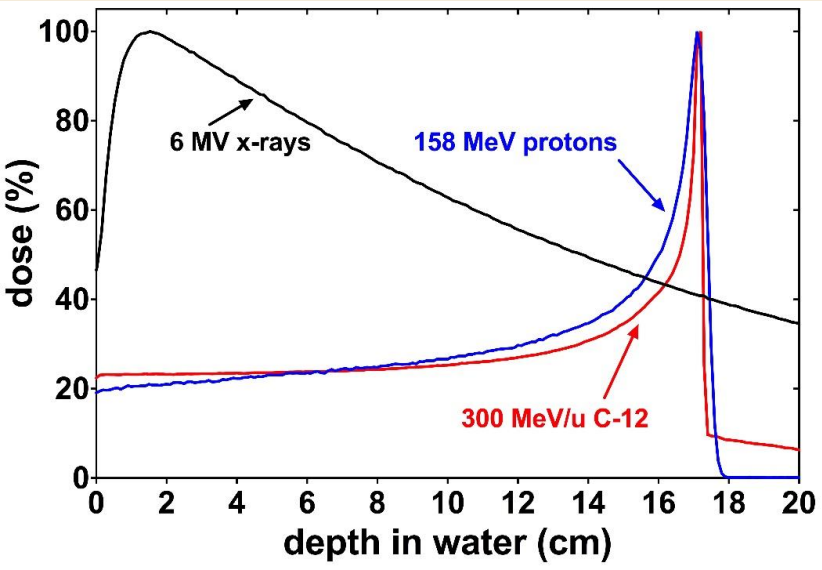


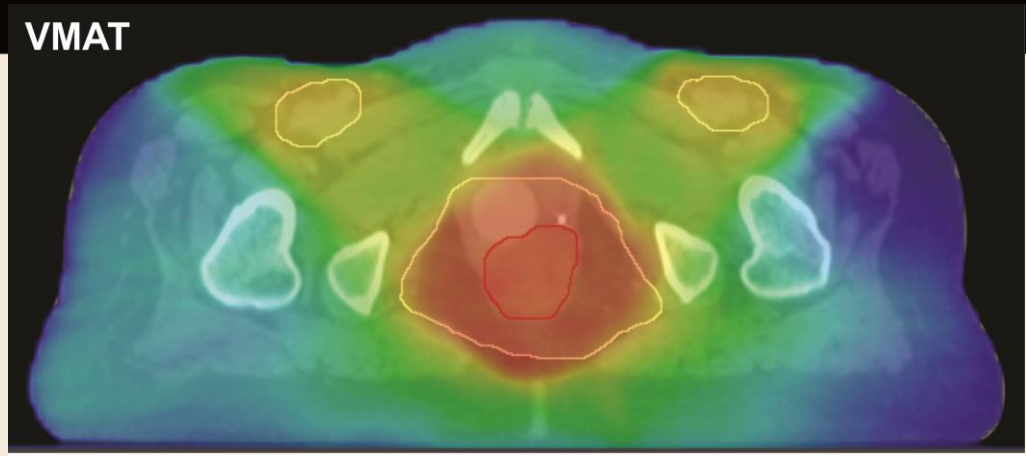


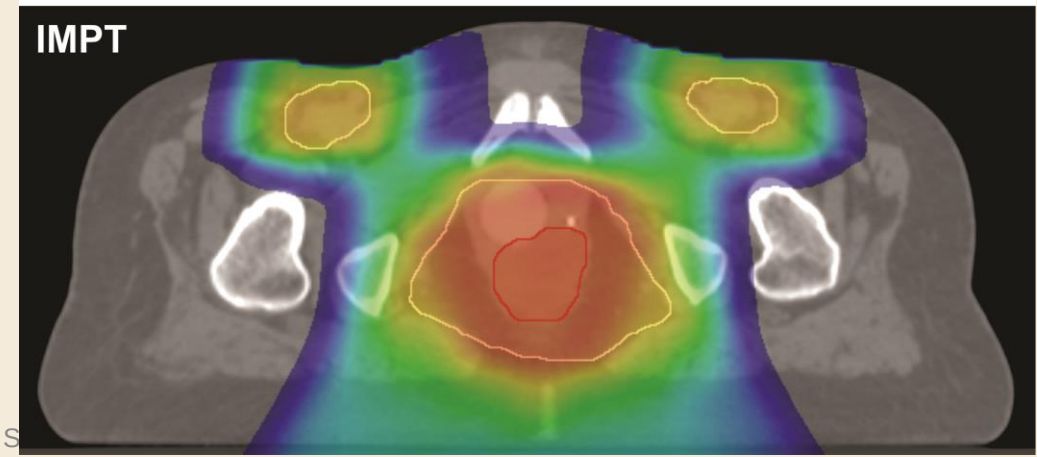
- ✓ **Particle beams stop!**
- ✓ **Sharp distal falloff**
- ✓ **Sharper lateral penumbra**
- ✓ **LET increases with depth**

Physical characteristics of particle beams

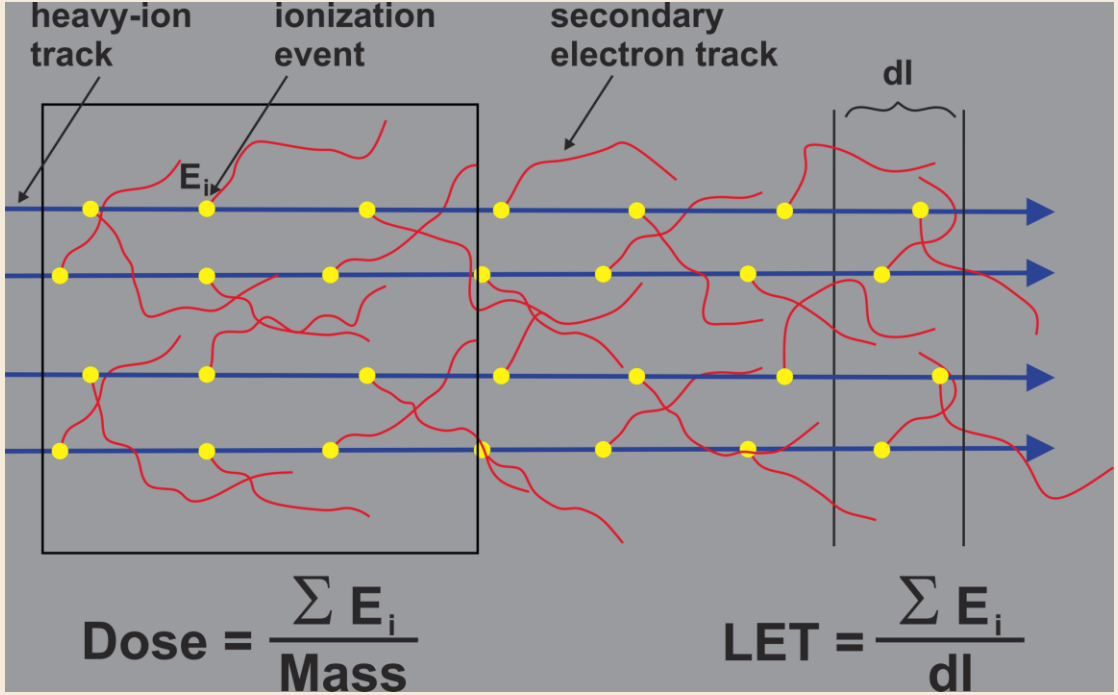
- ✓ Conformal dose distributions
- ✓ Lower integral dose
- ✓ Better sparing of organs at risk
- ✓ Potential to escalate the dose





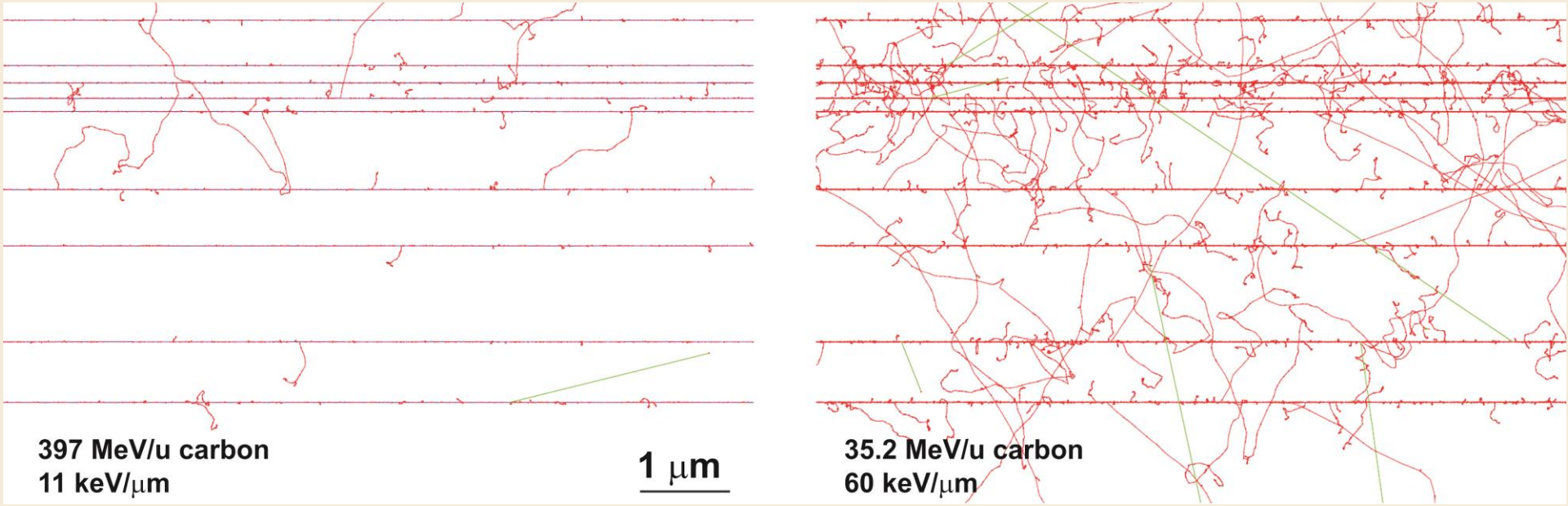


Physical characteristics of particle beams

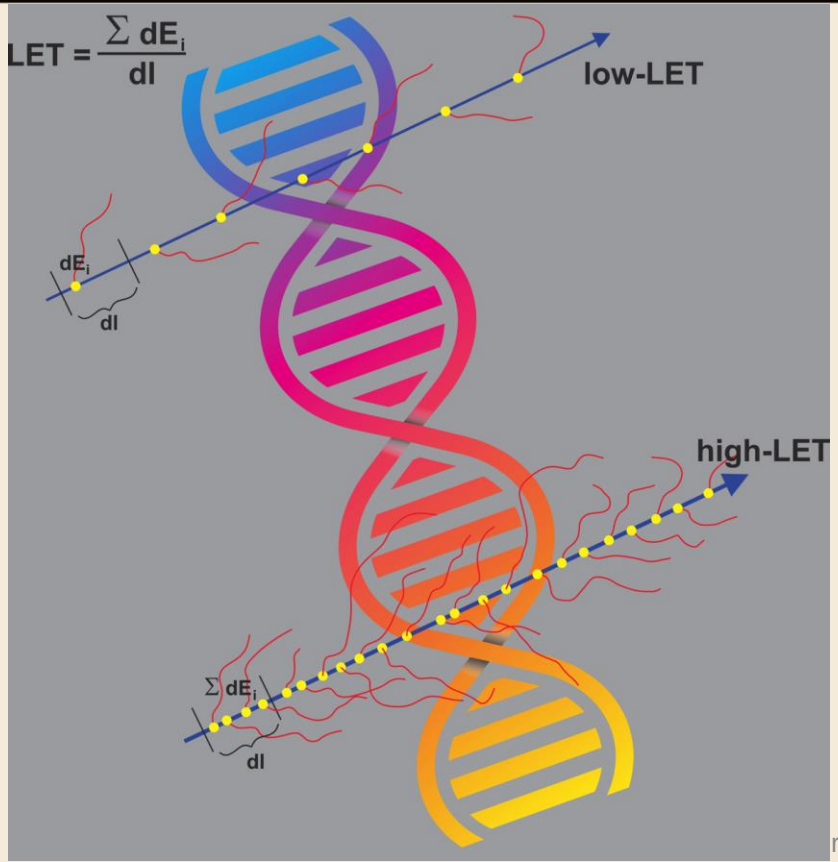


- **Linear energy transfer (LET): Energy transferred per unit length**
- **LET describes the spatial pattern of energy deposition**
- **Units: keV/μm or eV/nm**

Physical characteristics of particle beams

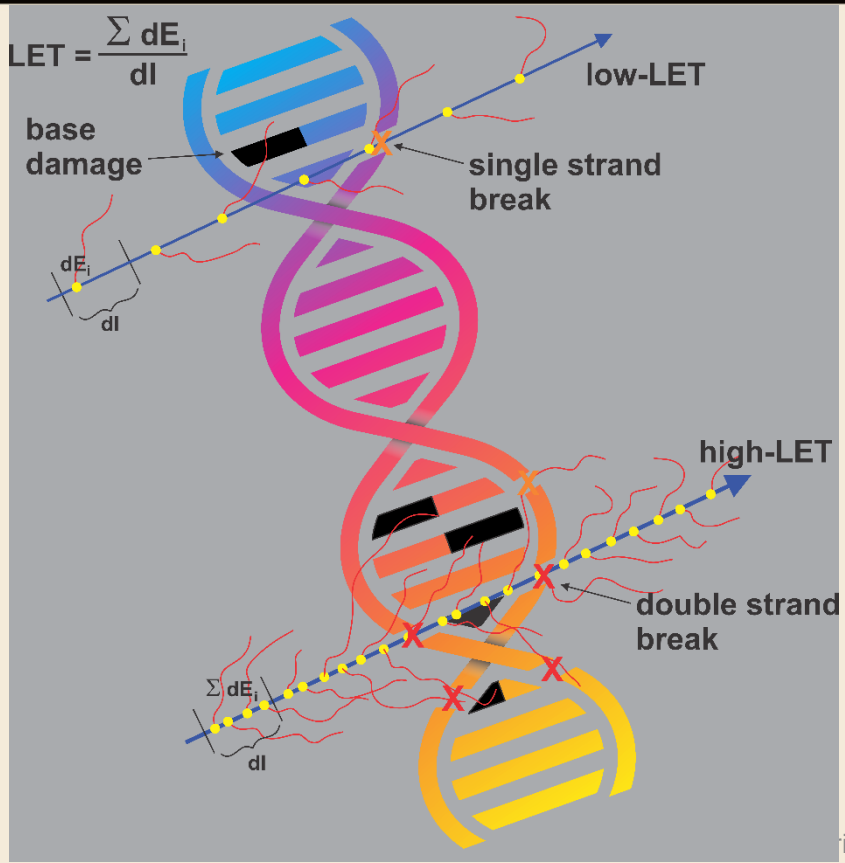


Radiation-induced DNA damage



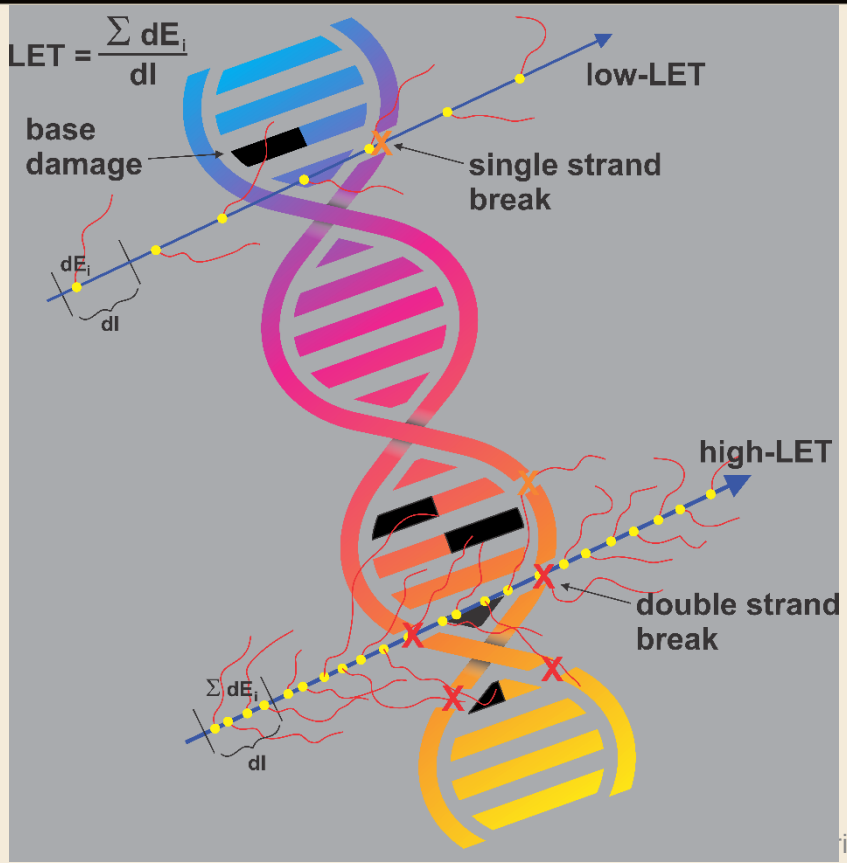
- **Ionizing radiation induces DNA damage**
- **Low-LET**
  - **Sparsely ionizing: mostly indirect DNA damage via reactive oxygen species (ROS)**
  - **DNA damage depends on oxygen status of tumors**
- **High-LET**
  - **Densely ionizing: induces direct DNA damage**
  - **Less dependent on tumor hypoxia**

Radiation-induced DNA damage



- Types of DNA damage
  - Base damage
  - Single strand break
  - Double strand break
- Simple or clustered DNA damage
  - Radiation predominantly produces clustered DNA damage

Radiation-induced DNA damage



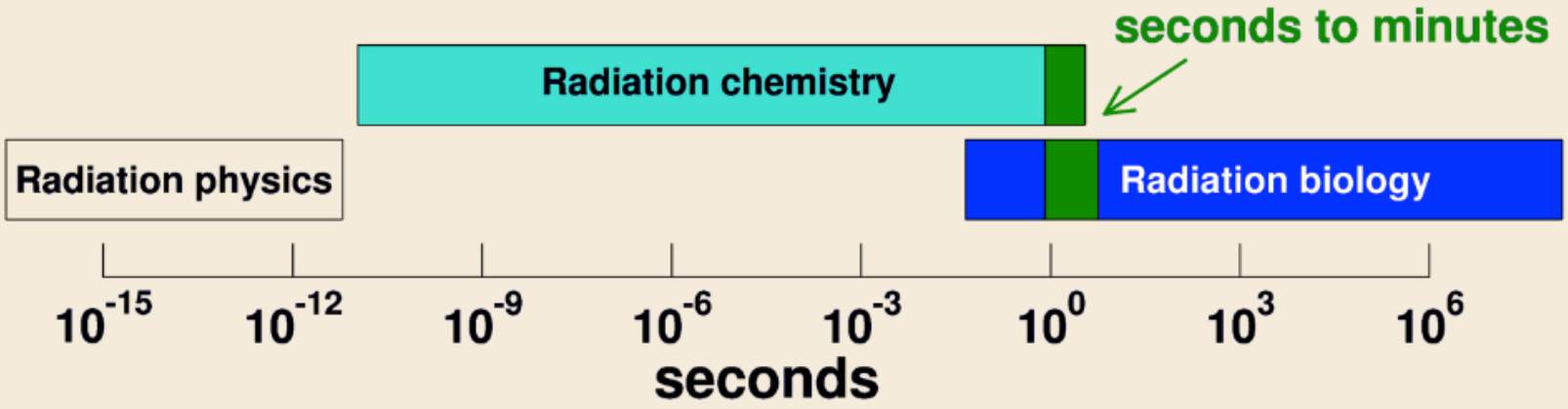
- **Clustered DNA damage: two or more individual lesions within one or two helical turns of DNA**
  - **Clustered non-DSB**
  - **Clustered DSB**
- **Yield of DNA lesions**

	DNA lesions (Gbp^{-1} Gy^{-1})		
	SSB	DSB	DSB cluster
1 keV/μm low-LET	~170	~6	~0.1
50 keV/μm high-LET	~150	~12-16	~0.3

Friedland et al 2017, Sci Rep 7:45161

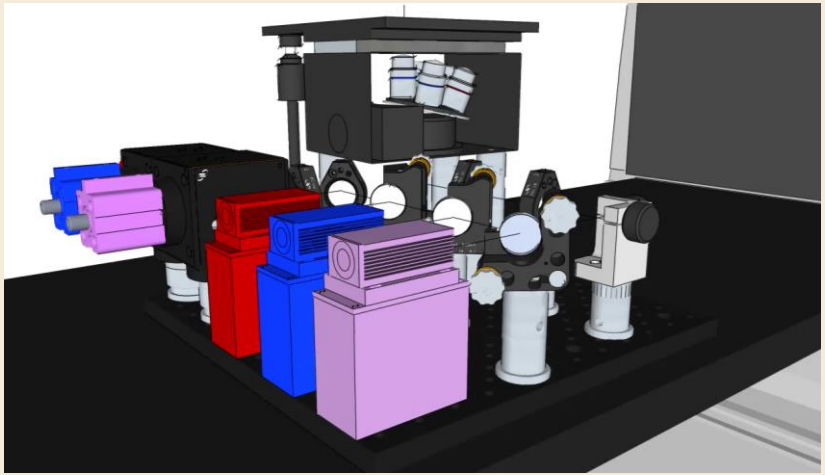
Radiation-induced DNA damage

- **Understand biophysical aspects of radiation to predict response**
- **Understand how LET is related to DNA damage, DNA repair and cell's fate**



- **No techniques are currently available to study early DNA damage response in therapeutic radiation beams**

Live cell imaging in the beam line



McFadden et al 2016, IJROBP 96, 221

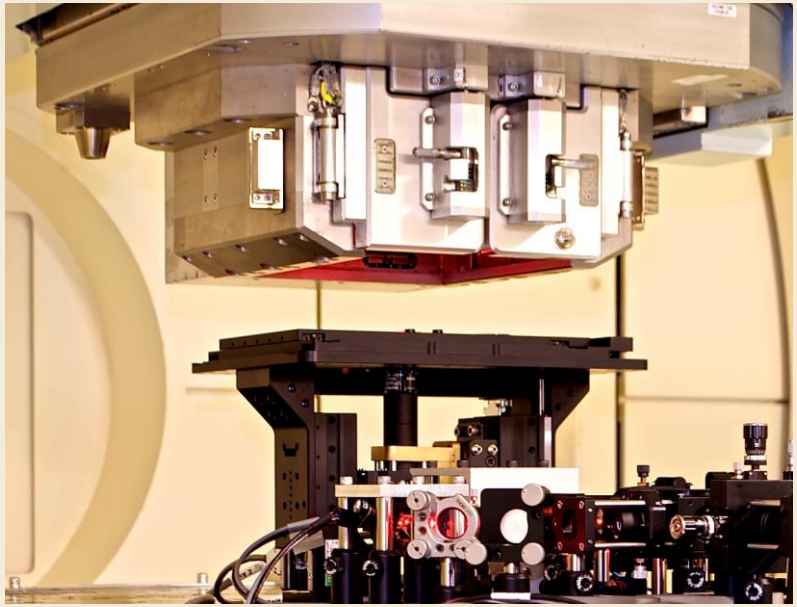
- Designed and constructed a portable confocal microscope
  - Live cell imaging
- Flexible configuration
- Can be shipped to any place
- Can be used in any beam line
  - horizontal beams
  - vertical beams

Time-Lapse Monitoring of DNA Damage Colocalized With Particle Tracks in Single Living Cells

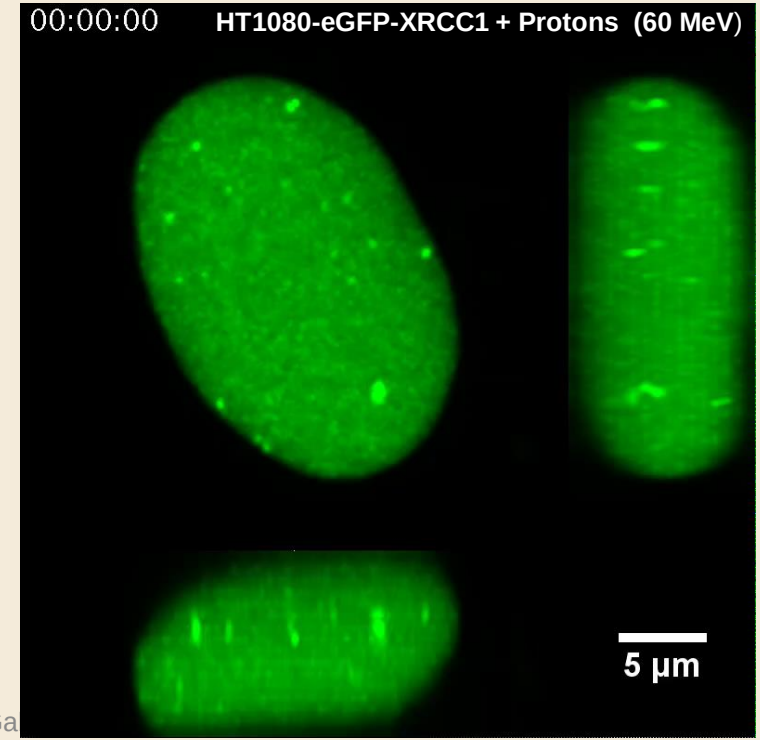
Conor H. McFadden, MSc,* Timothy M. Hallacy, BSc,*,† David B. Flint, BSc,*,‡ Dal A. Granville, PhD,§ Aroumougame Asaithamby, PhD,|| Narayan Sahoo, PhD,* Mark S. Akselrod, PhD,¶ and Gabriel O. Sawakuchi, PhD*,‡

Connor McFadden
Sr Research Engineer

Live cell imaging in the beam line

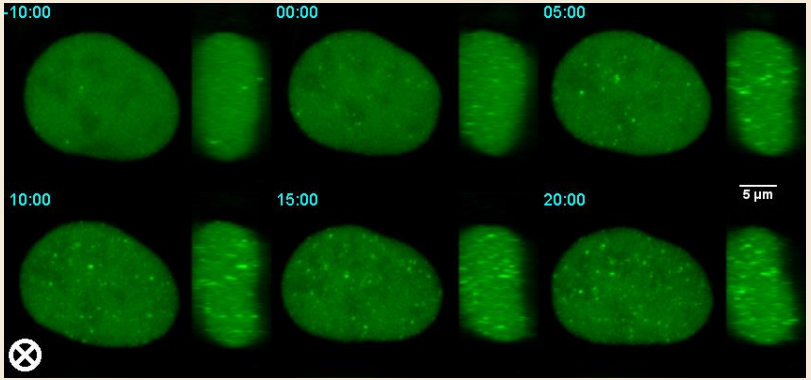


Genetically engineered cells that express a fluorescent probe tagged to a DNA repair protein

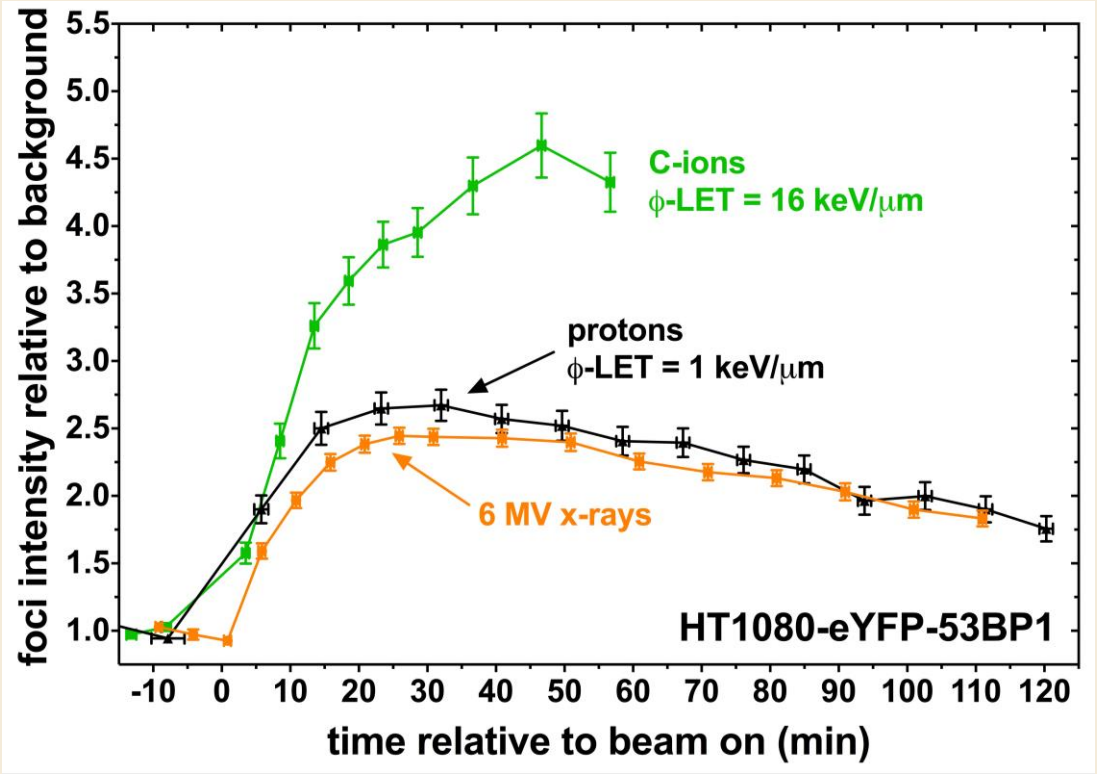


Asaithamby
UTSW

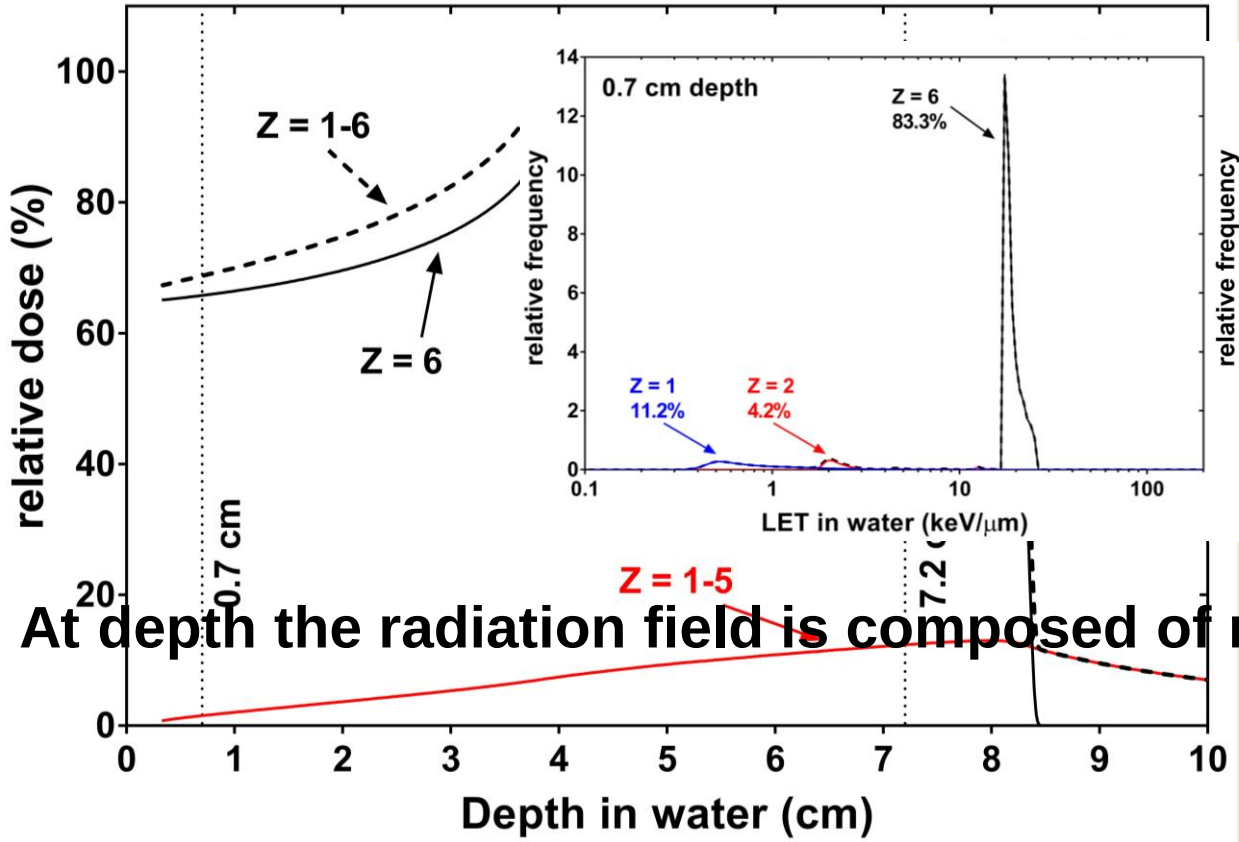
Live cell imaging in the beam line

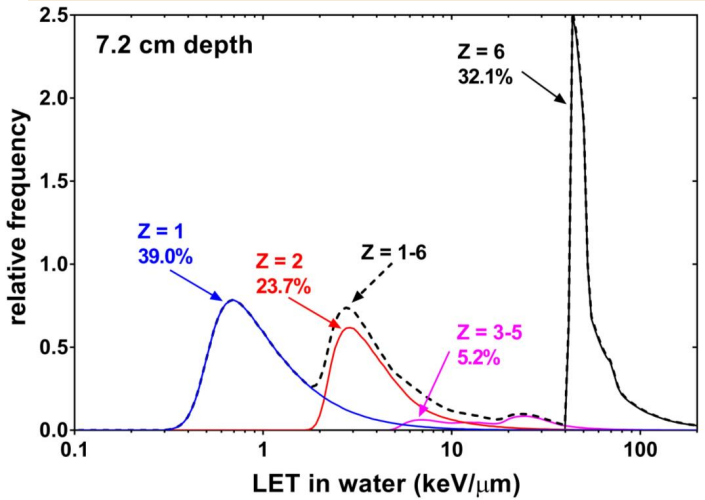


Live cell imaging in the beam line enables to monitor fast spatiotemporal behavior of DNA damage response and repair



C-ion particle spectrum is complex

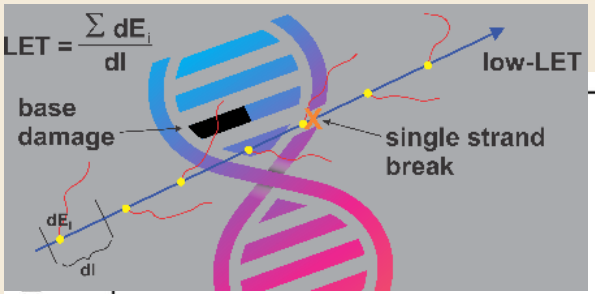


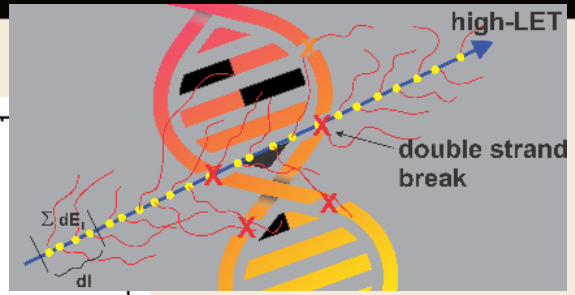


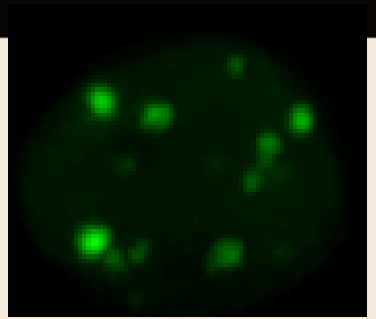
At depth the radiation field is composed of many charges and LETs

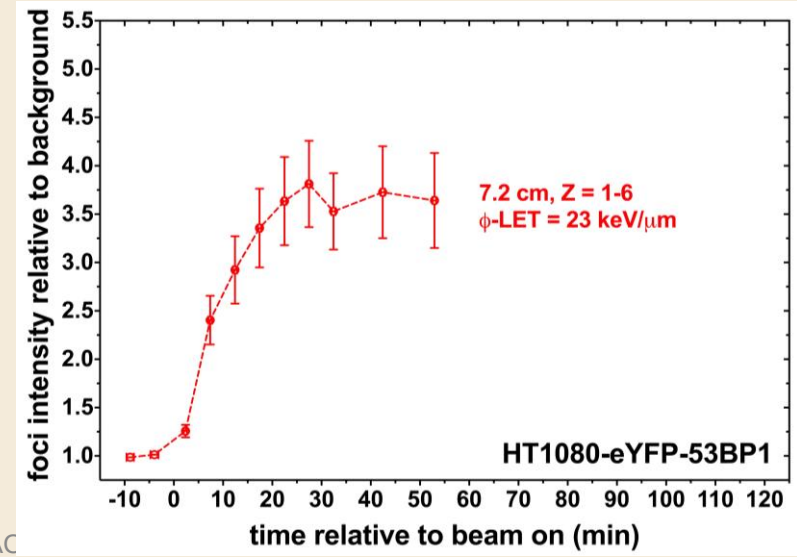
Greilich, DKFZ

C-ion particle spectrum is complex

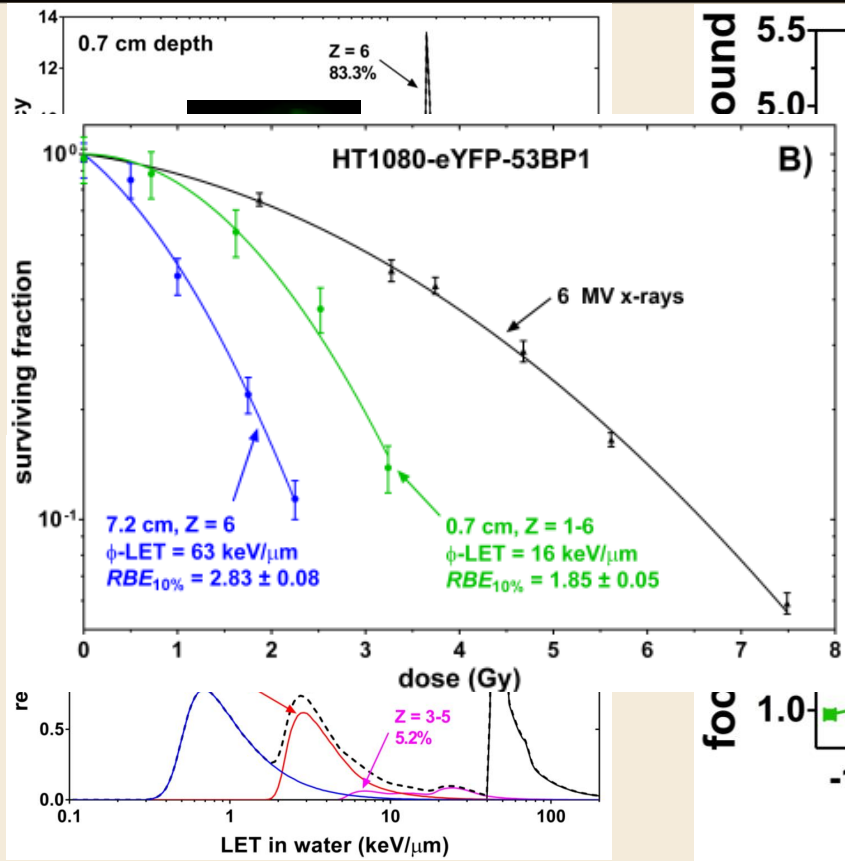


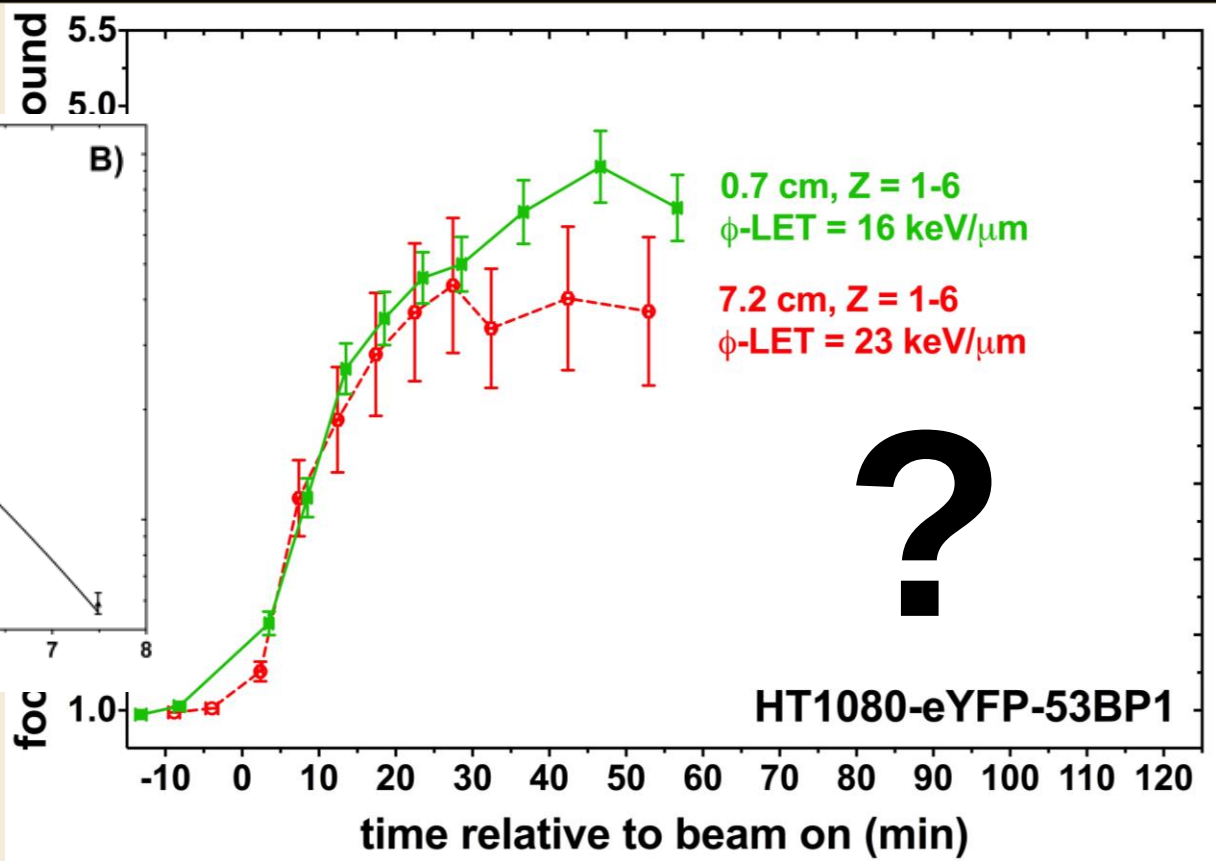






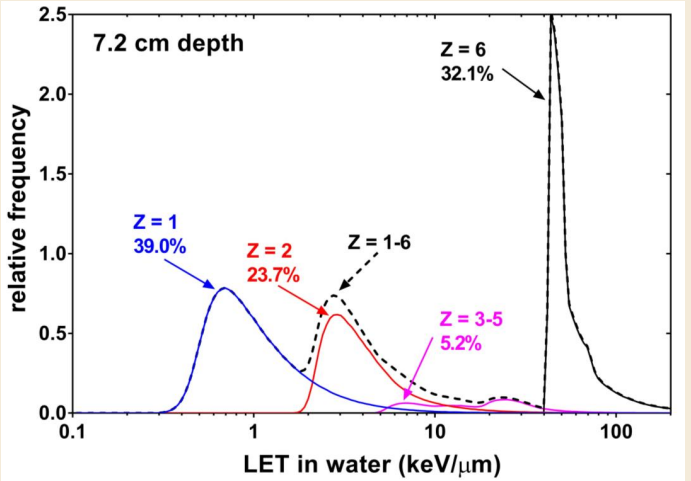
C-ion particle spectrum is complex

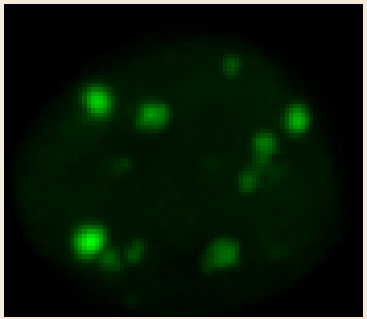




High-resolution radiation measurements in live cells in real time

- Measure radiation with sub-micrometer spatial resolution
- Measure LET or energy deposition of individual tracks in a mixed field
- Link radiation measurements with DNA damage response
- Separate individual DNA damage response according to radiation type





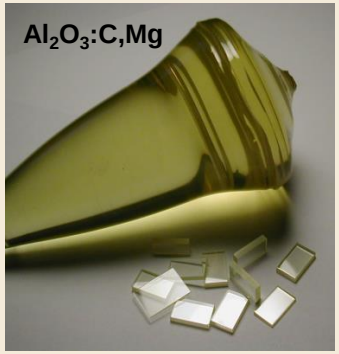
NOT EASY TASKS

High-resolution radiation measurements

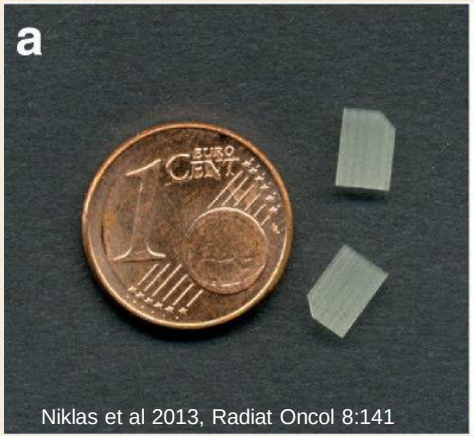
Mark Akselrod
Landauer, Inc.

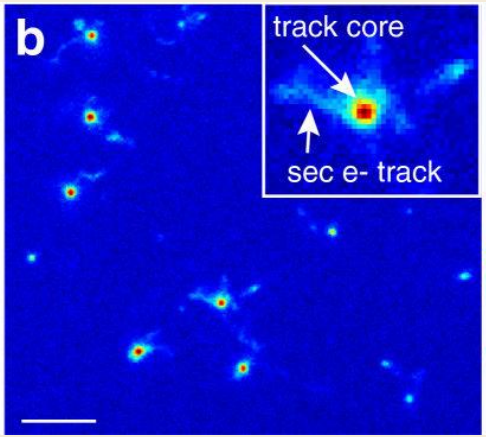
Steffen Greilich
DKFZ

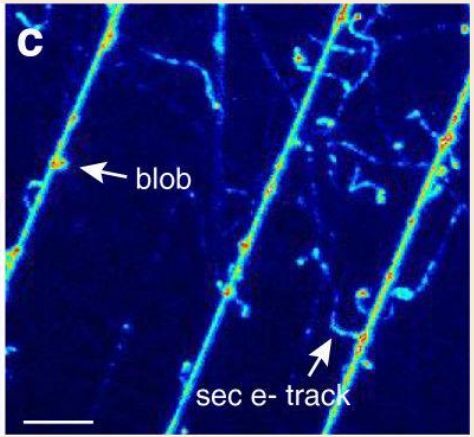
Fluorescence nuclear track detector (FNTD)

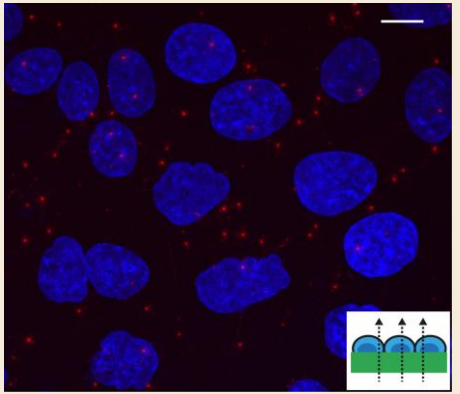


- Biocompatible
- No chemical process
- Compatible with cell imaging



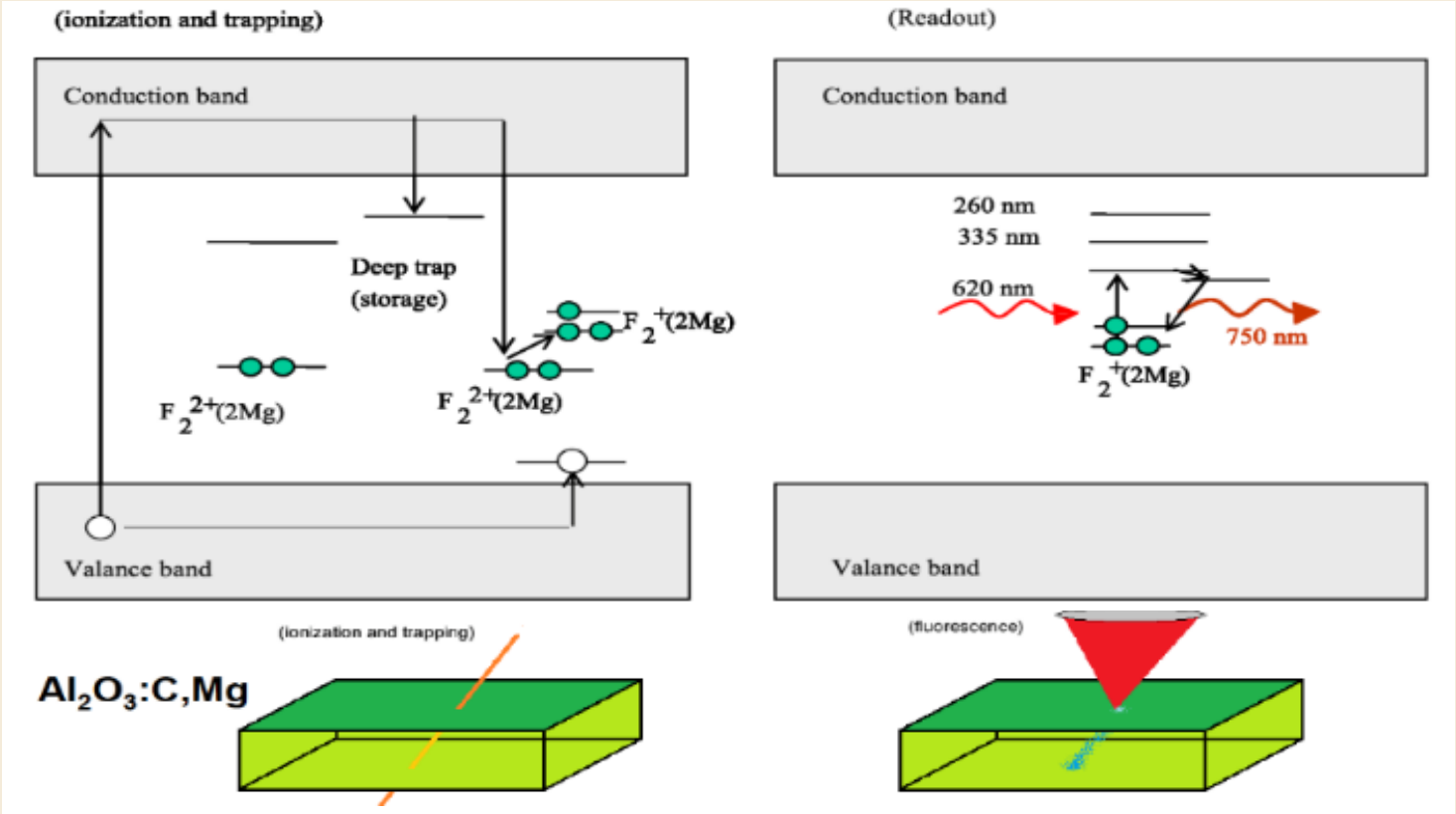






High-resolution radiation measurements

Fluorescence nuclear track detector (FNTD)



High-resolution radiation measurements

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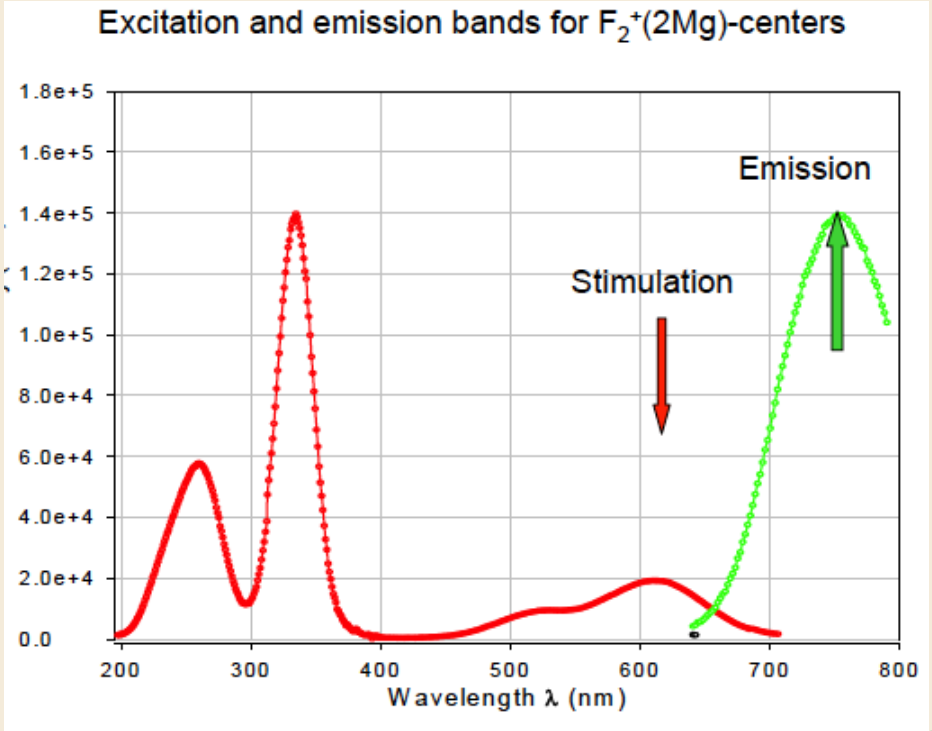
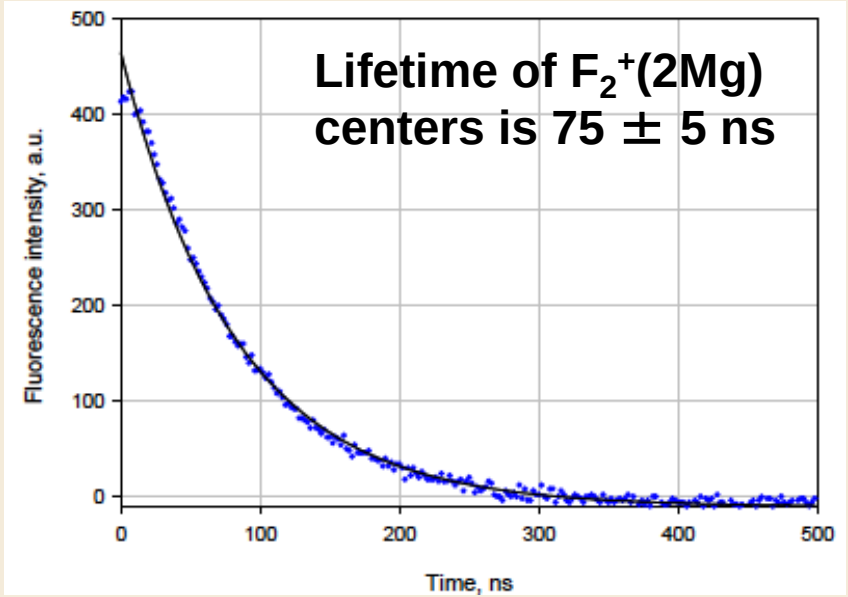


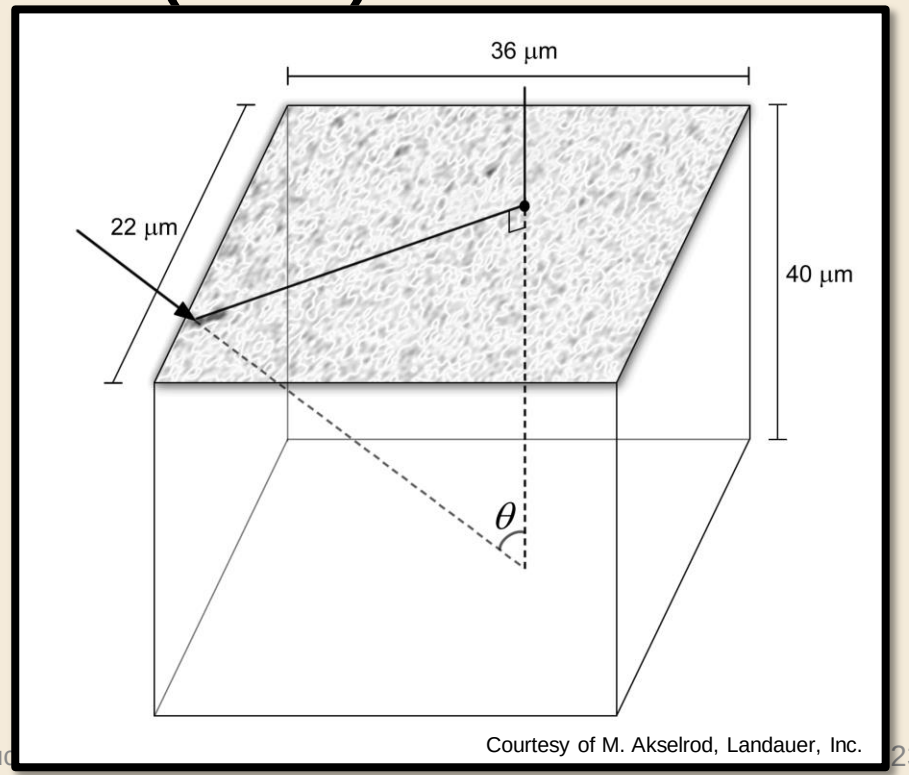


Courtesy of M. Akselrod, Landauer, Inc.

High-resolution radiation measurements

Fluorescence nuclear track detector (FNTD)

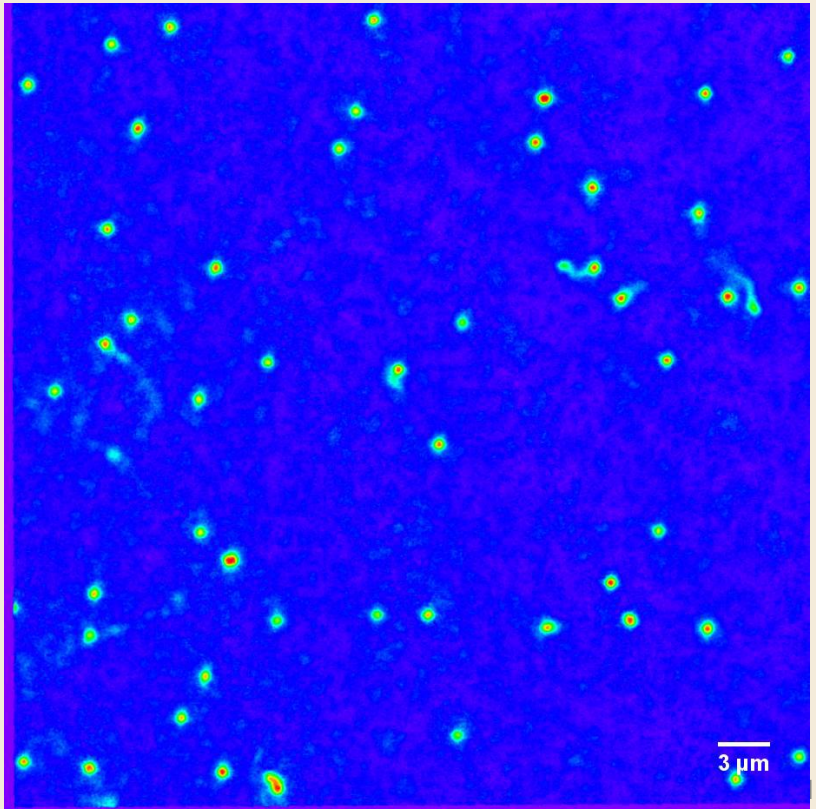
- Reconstruction of confocal stacks allows 3D images of tracks
- Spatial resolution is limited by the method used to readout
    - Confocal microscopy is limited by diffraction
    - Super-resolution microscopy



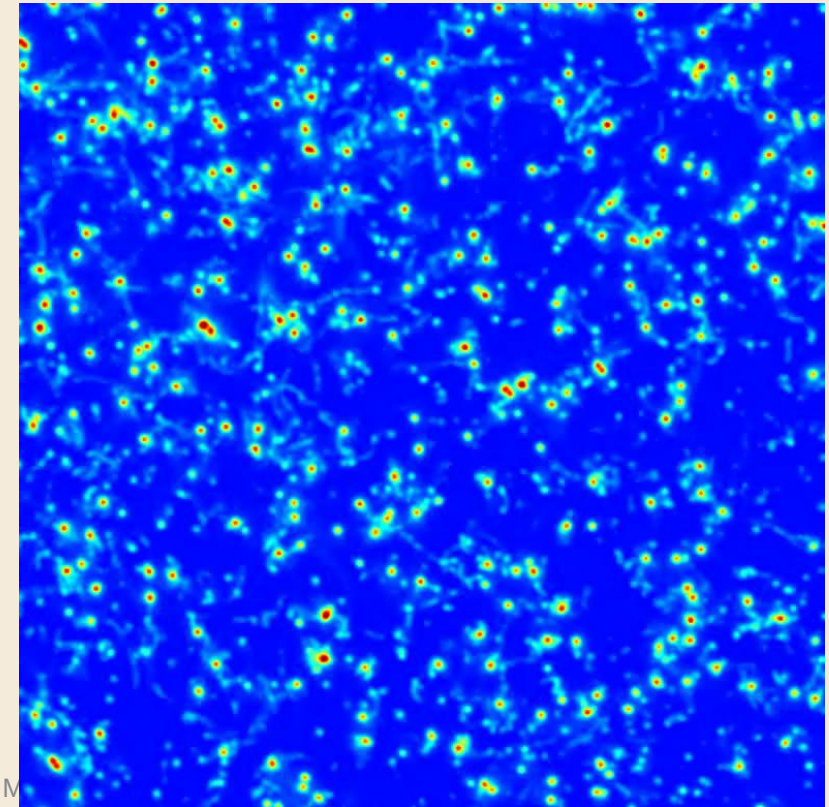
Courtesy of M. Akselrod, Landauer, Inc.

High-resolution radiation measurements

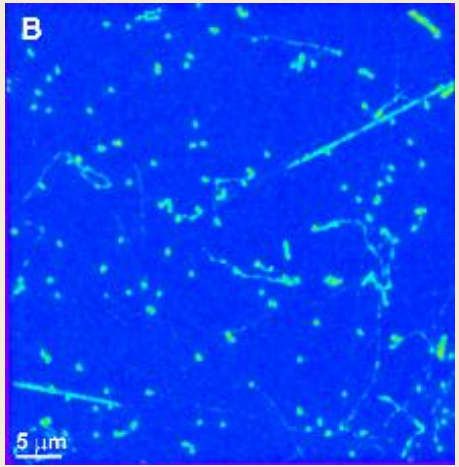
Protons, ~65 MeV, ~1 keV/μm in H_2O

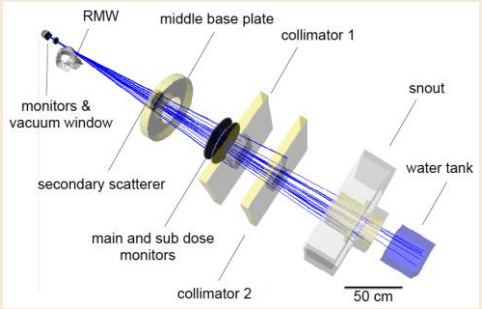


C-ions, ~160 MeV/u, ~20 keV/μm in H_2O



High-resolution radiation measurements

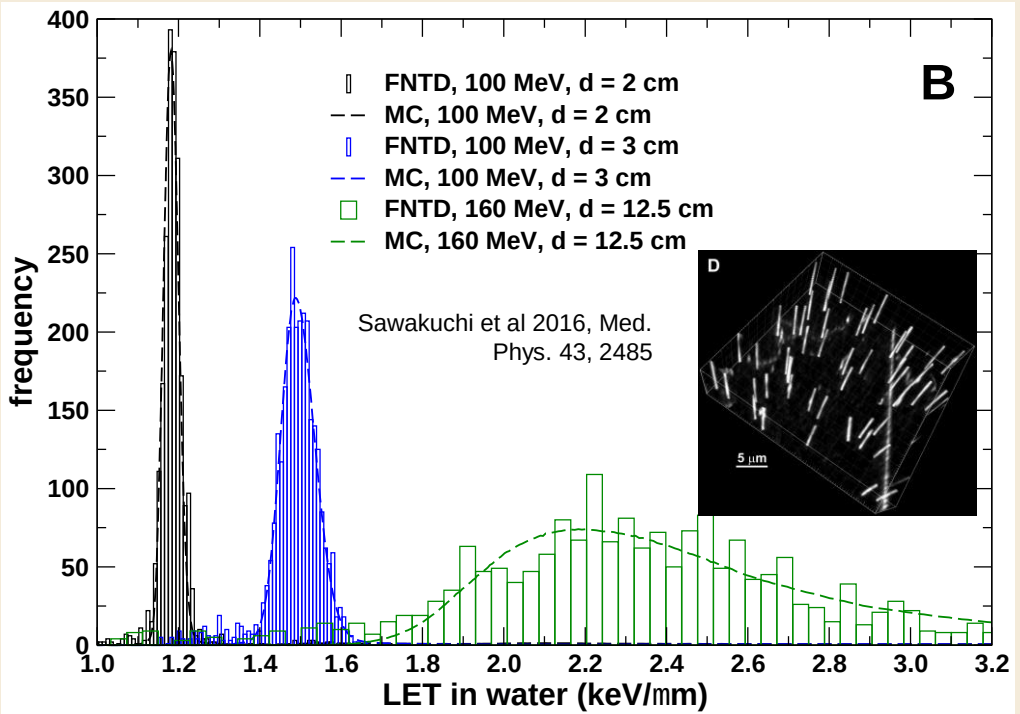




FNTD images

+

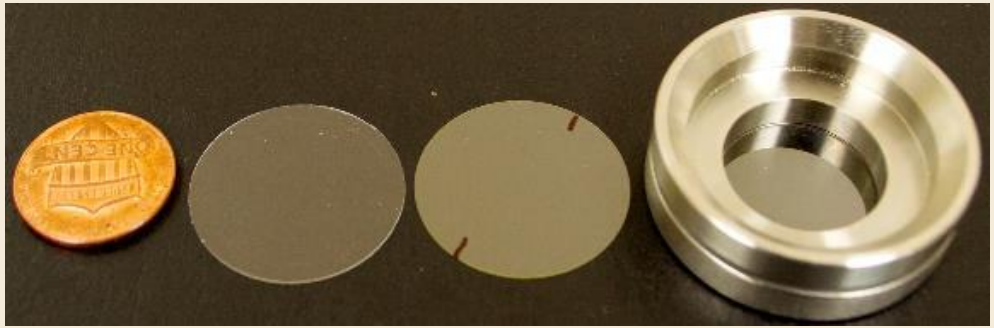
Detailed MC simulations



LET measurements of single tracks

High-resolution radiation measurements in live cells in real time

- Can cut into coverslips
- **Biocompatible!**



glass FNTD FNTD + holder

McFadden et al 2016, IJROBP 96, 221

Time-Lapse Monitoring of DNA Damage Colocalized With Particle Tracks in Single Living Cells

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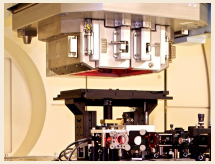
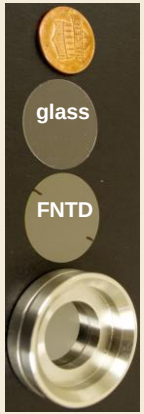
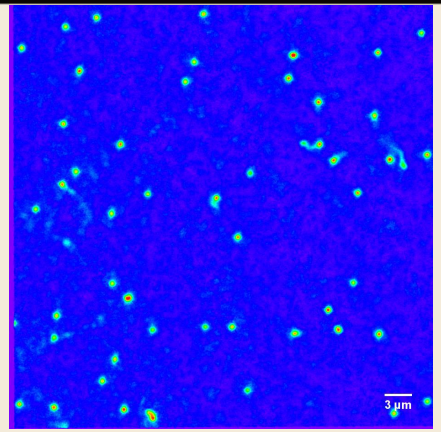
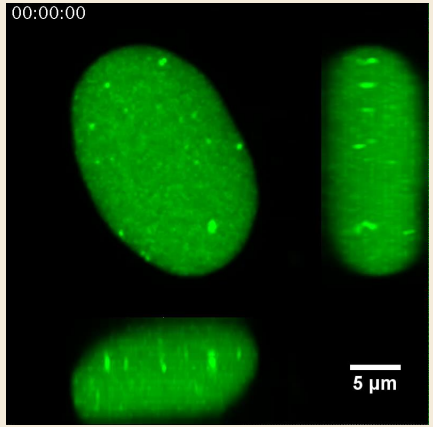
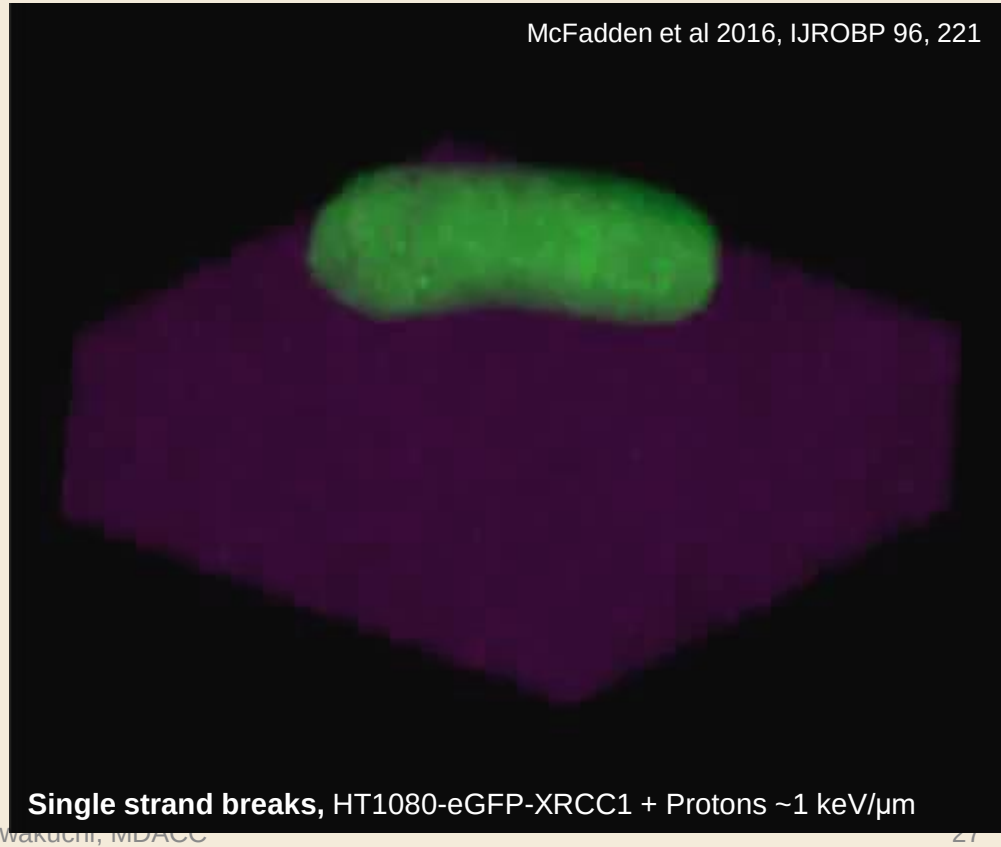
High-resolution radiation measurements in live cells in real time











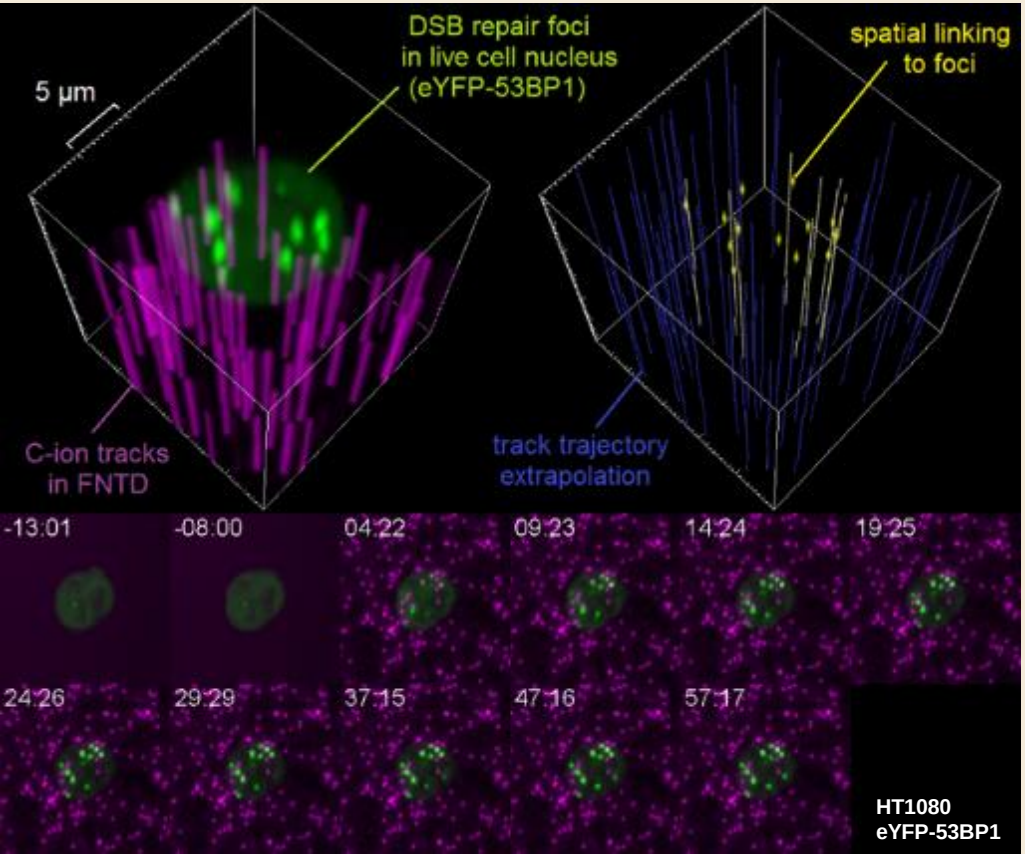
High-resolution radiation measurements in live cells in real time

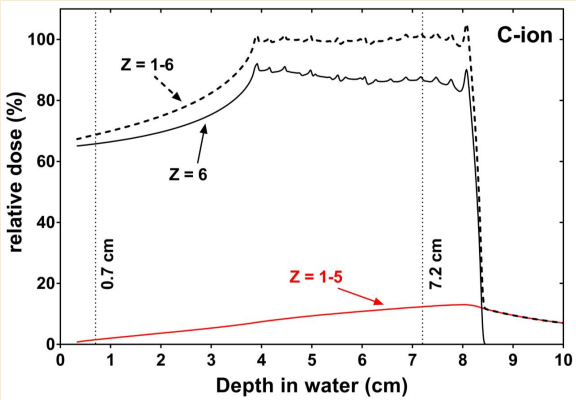
Develop a technique to record particle tracks and early DNA damage response while on the beam line

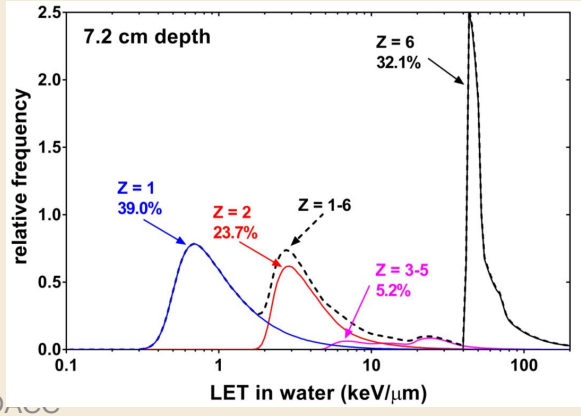
Challenges

Live cell imaging	Confocal microscopy
Nanoscale radiation measurements	Fluorescence nuclear track detectors (FNTD)
Co-localization of DNA damage and track traversals at the molecular level	Confocal + FNTDs

High-resolution radiation measurements in live cells in real time

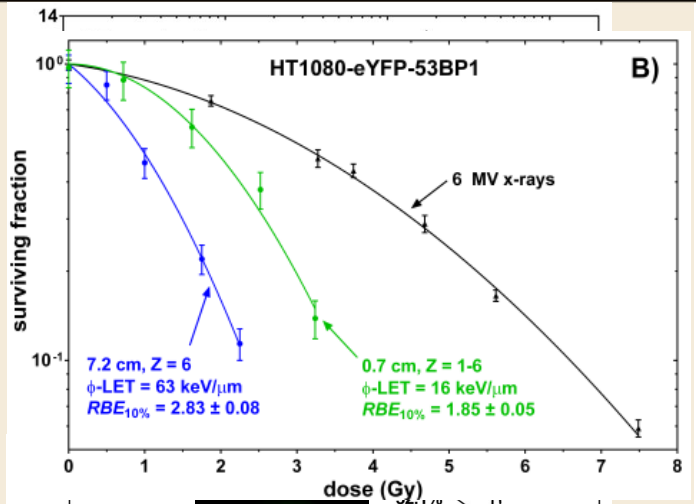


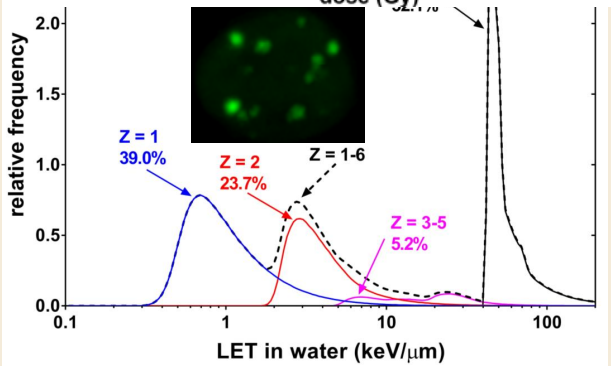


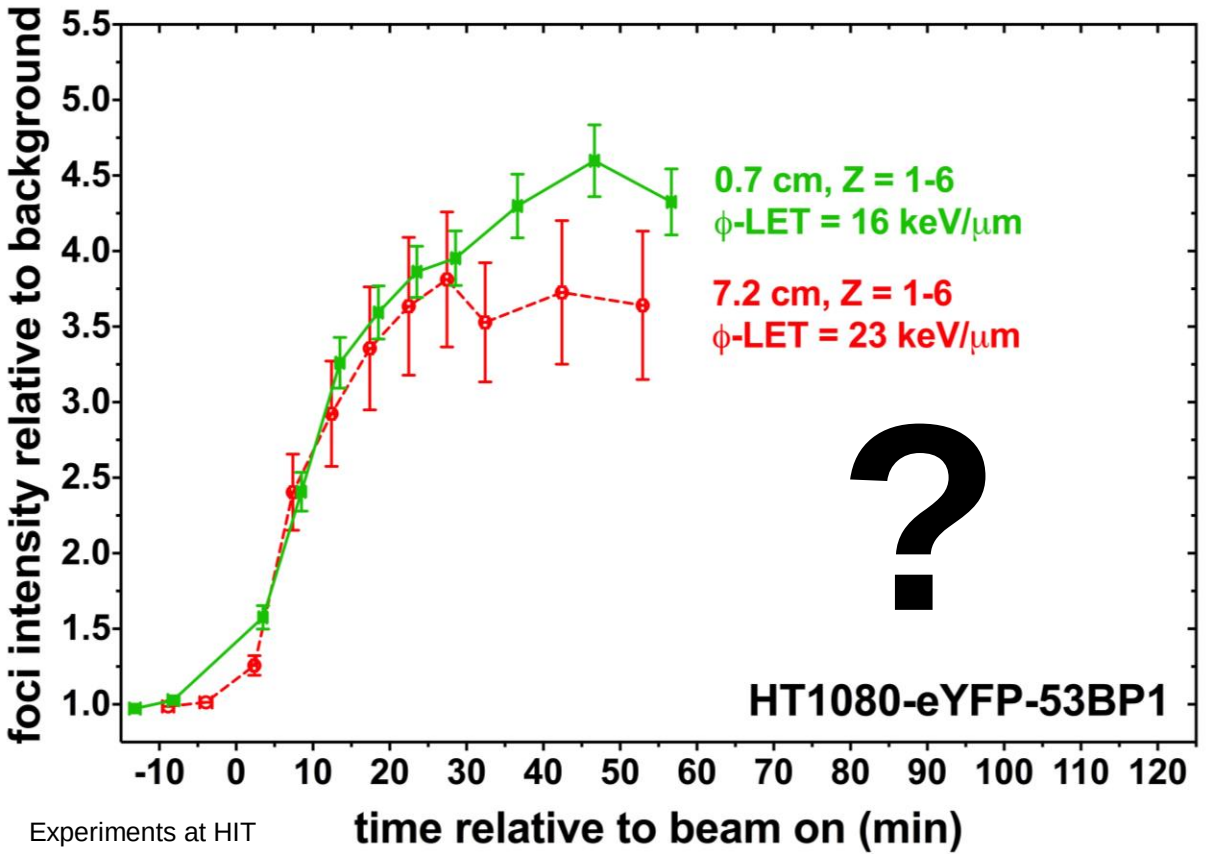


Experiments at HIT in collaboration with Dr Greilich

High-resolution radiation measurements in live cells in real time

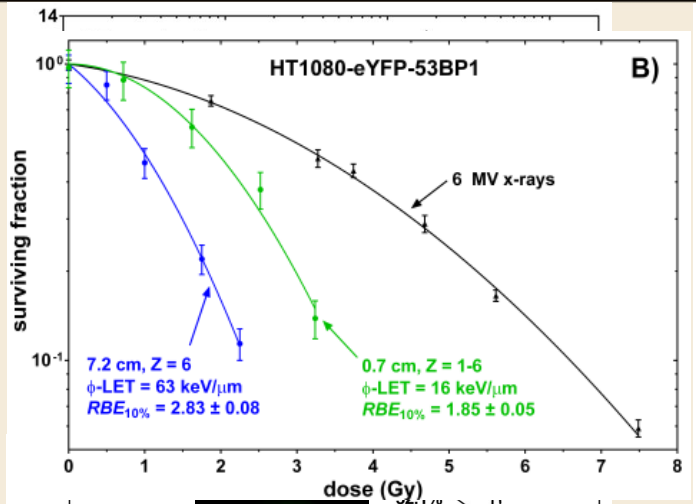


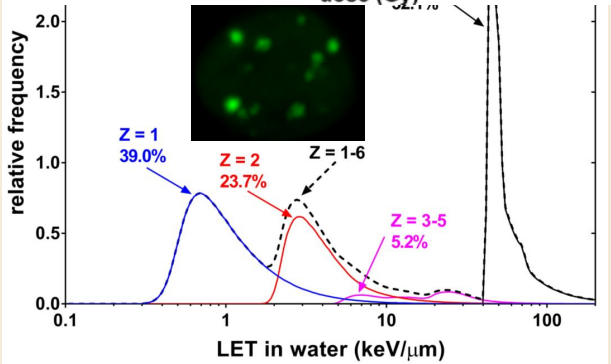


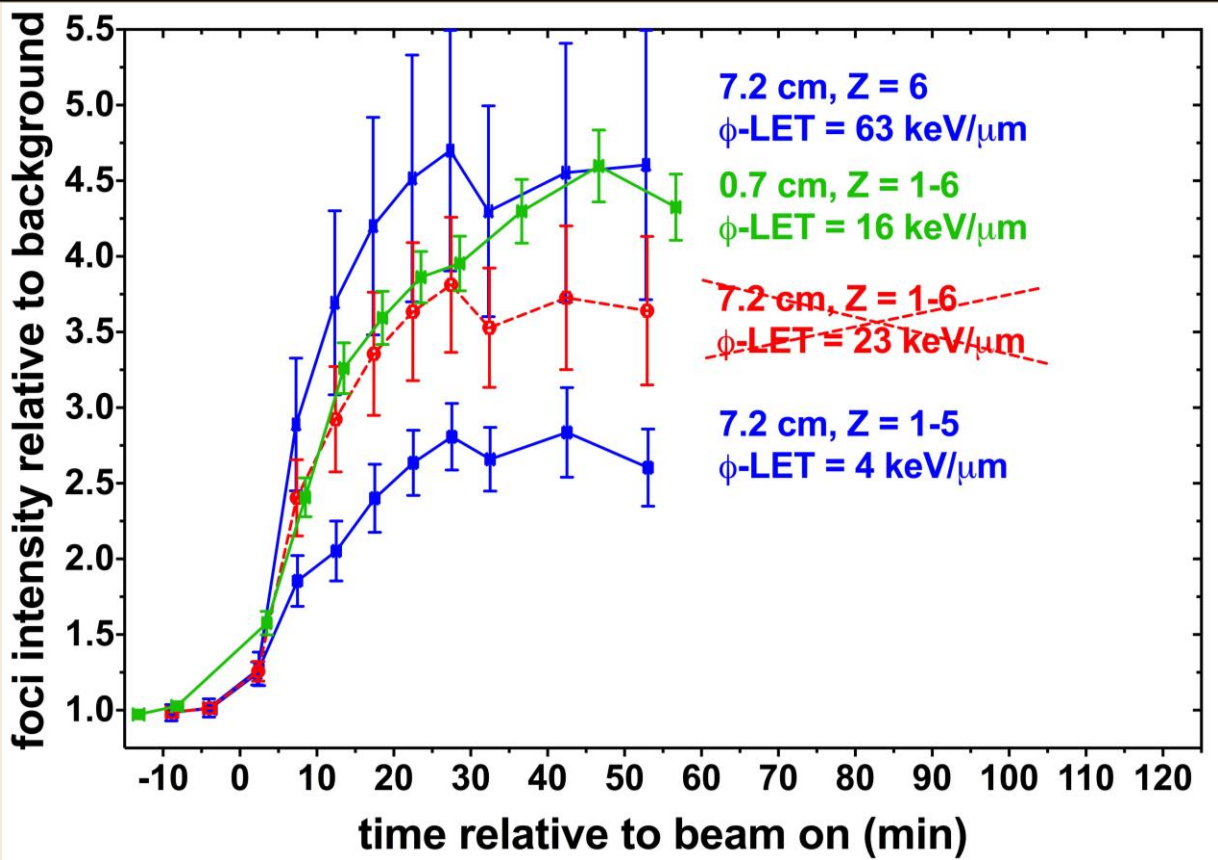


Experiments at HIT

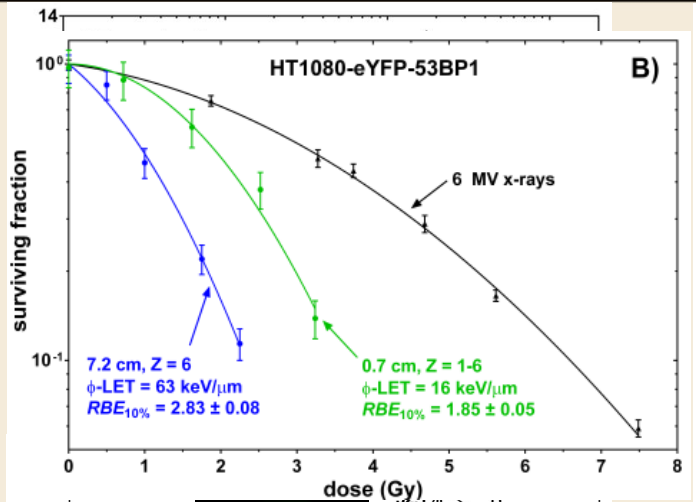
High-resolution radiation measurements in live cells in real time

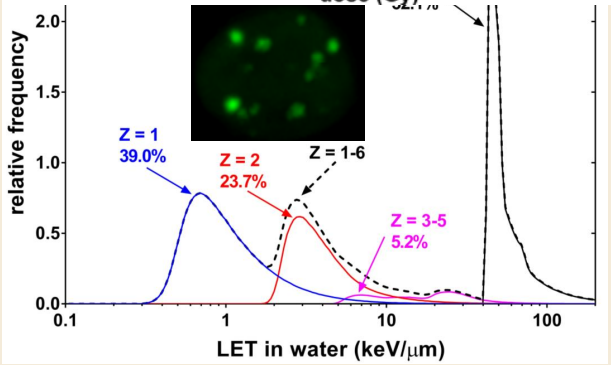


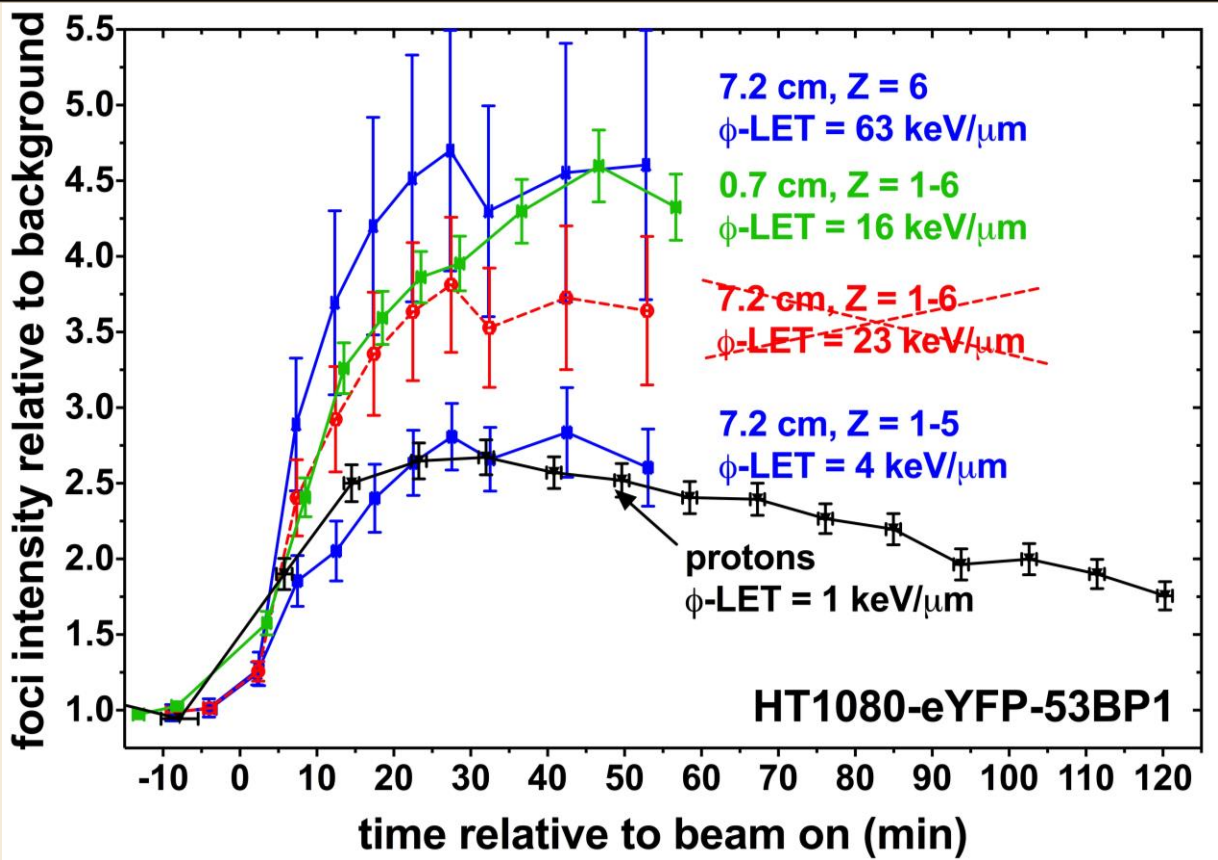




High-resolution radiation measurements in live cells in real time







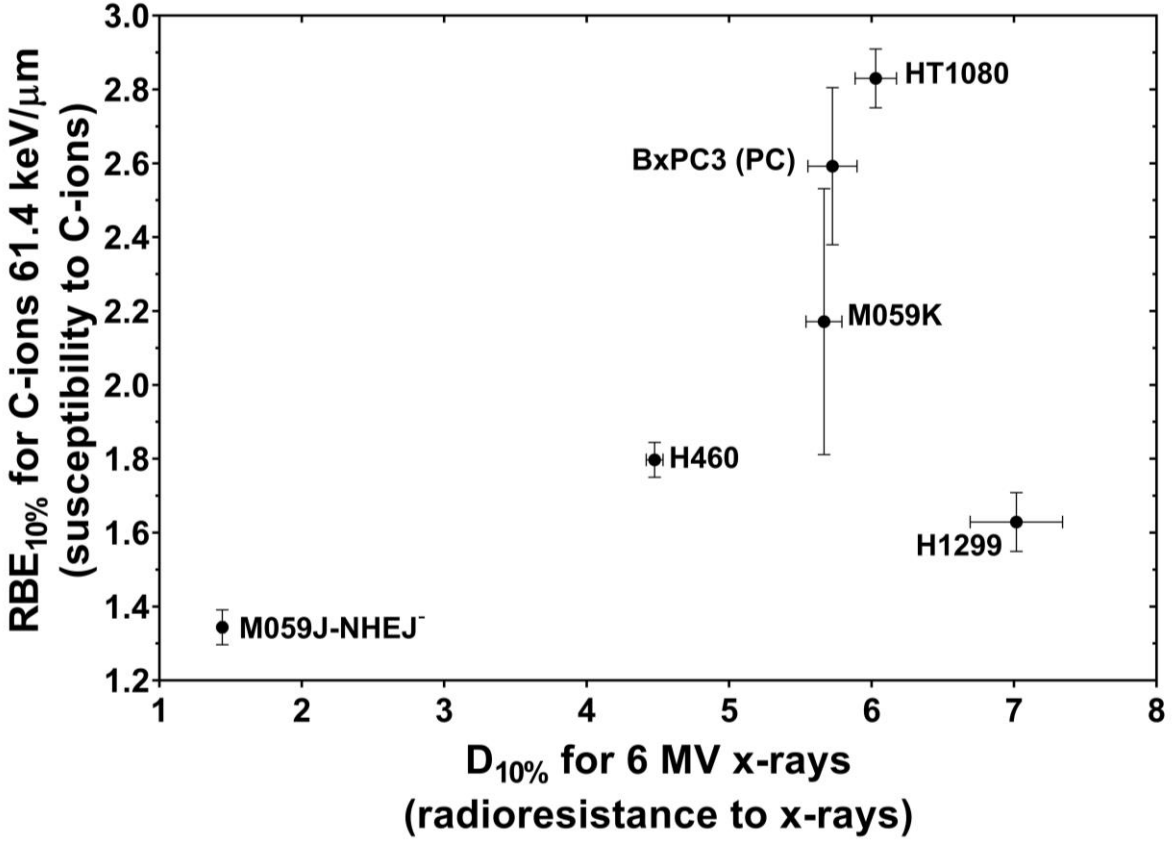
Final remarks

- **We developed a technique for online co-localization of tracks with DNA damage that allows to investigate the effects of mixed field radiation in live cells at the molecular level**

- **Demonstrated that it is possible to assign foci to individual tracks with LET information**

- **We show that a few high-LET lesions may drive survival**

Final remarks



High-LET C-ions

- **Large variation in RBE among cell lines**
- **Response strongly depends**
  - **Cell cycle variability**
  - **DNA repair status**
  - **Chromatin packing**
  - **Microenvironment**
  - **Immune system**
- **Important to separate**
  - **DNA damage**
  - **DNA repair**

Thank you! Questions?

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