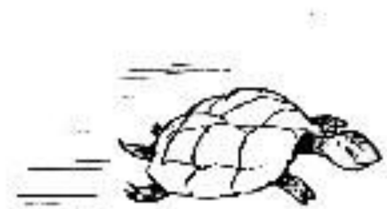
Biochemistry with low energy ions: tools and perspectives

Marco Durante

Napoli, 17.1.2018

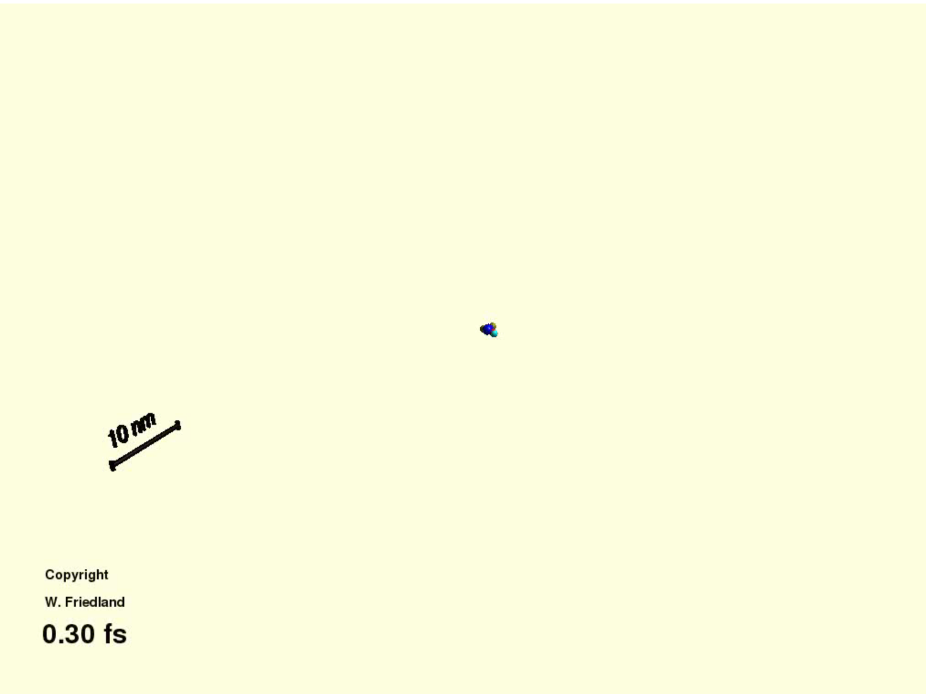
Biomedical applications at low-energy accelerators

- Dosimetry for particle therapy (see Paolo Russo lecture, 18.1 at 9 am)
- Radiobiology research for protection and therapy (see Lorenzo Manti lecture, 18.1 at 9:25 am)
- Low-energy tool as a tool in chemical and molecular biology (this talk!)

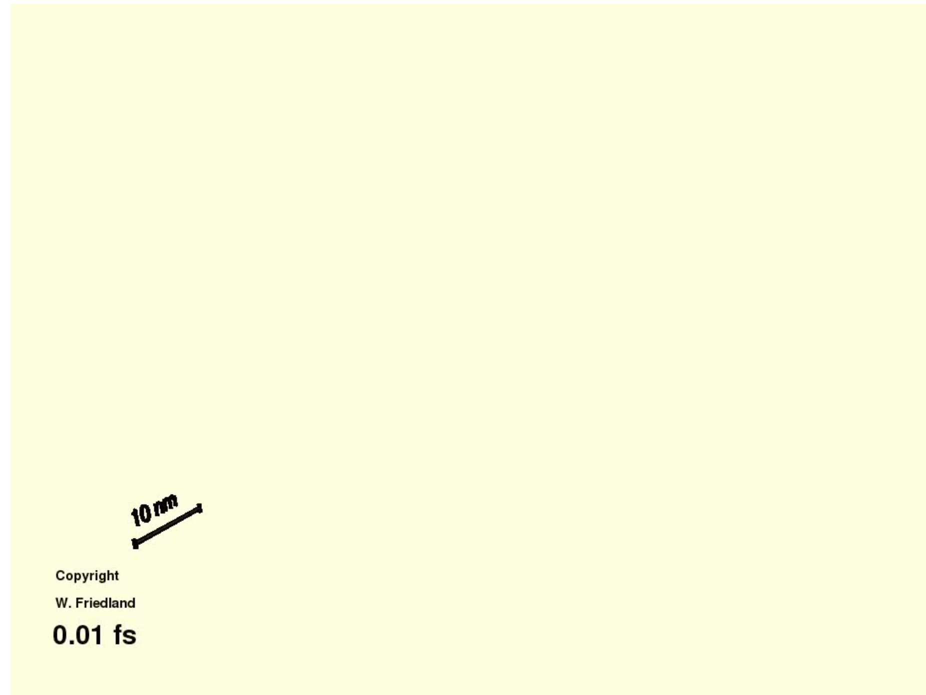


Low vs. high-energy ions at the same LET(=140 keV/μm)

α-particles, 2 MeV



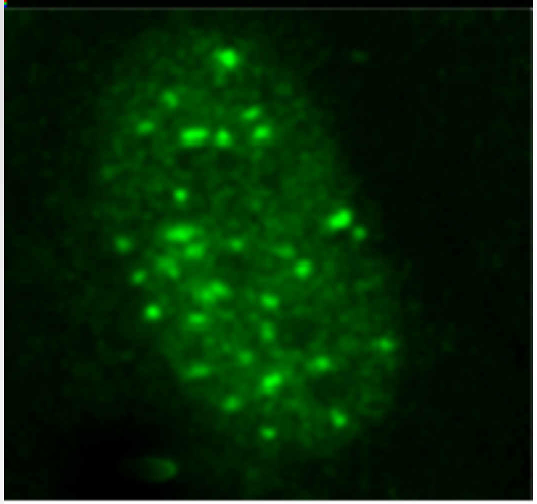
Fe-ions, 1 GeV/n



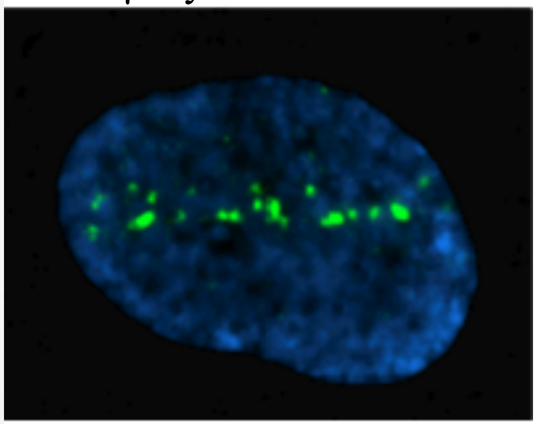
courtesy of Werner Friedland

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

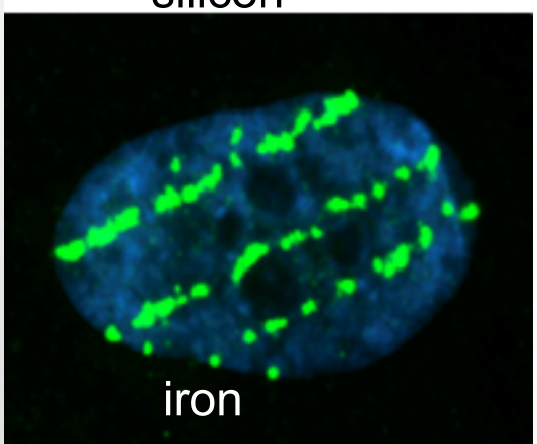
Bio-tracks

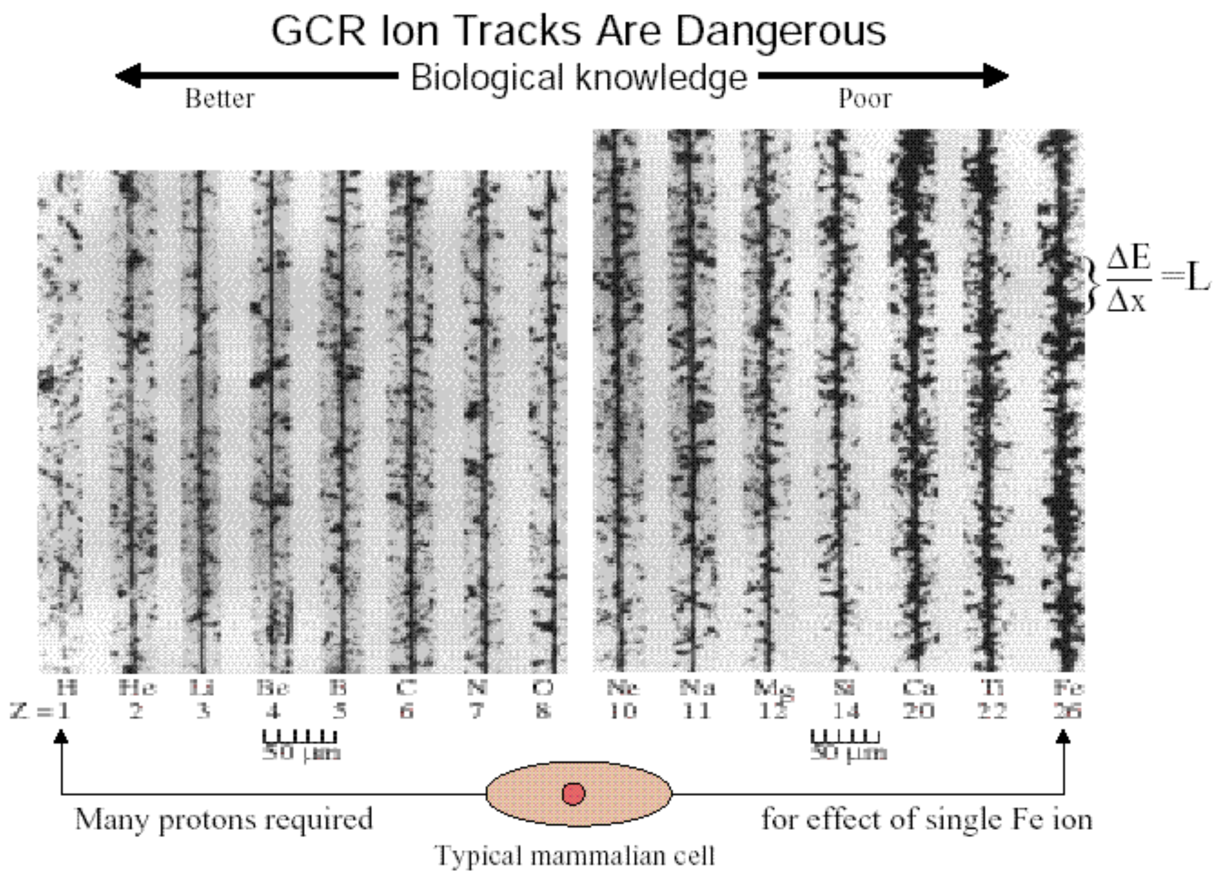


γ-rays

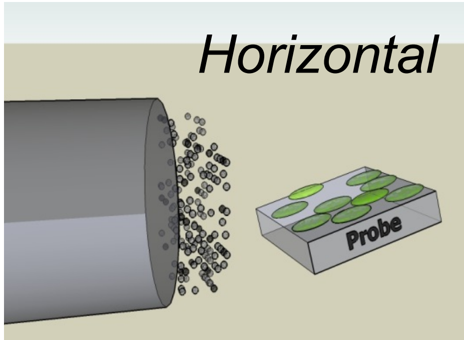


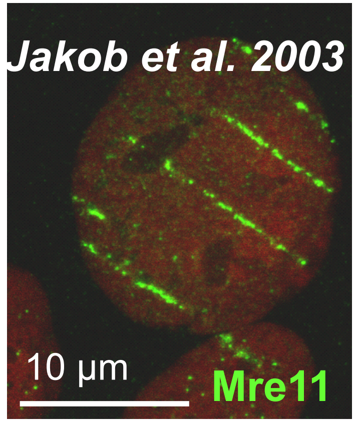
silicon

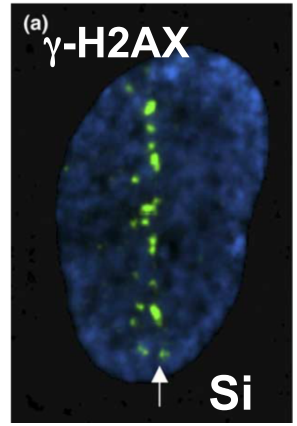




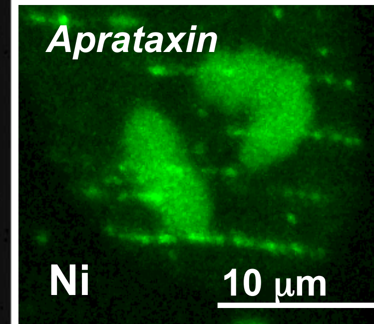
Cucinotta and Durante, *Lancet Oncol.* 2006





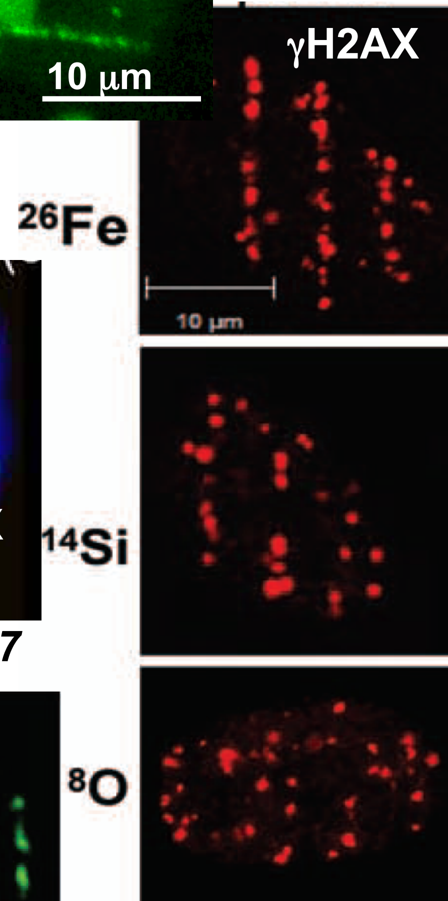


Desai et al. 2005

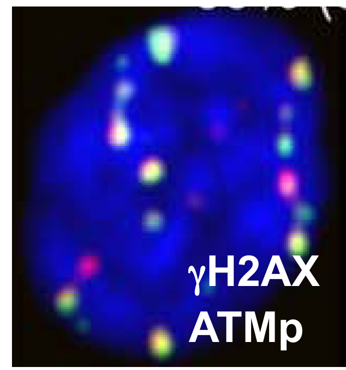


Gueven et al 2004

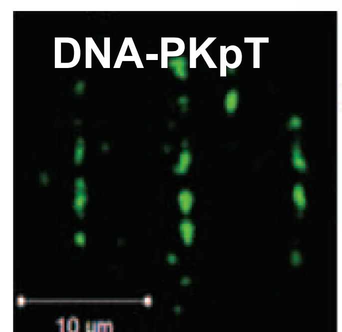
different repair proteins
different lesion densities (LET)
different cell lines

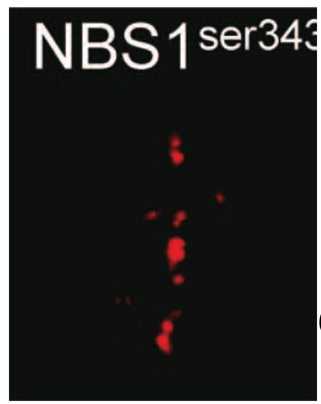


Asaithamby et al. 2008

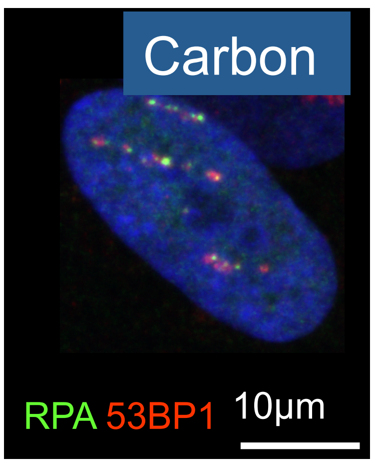


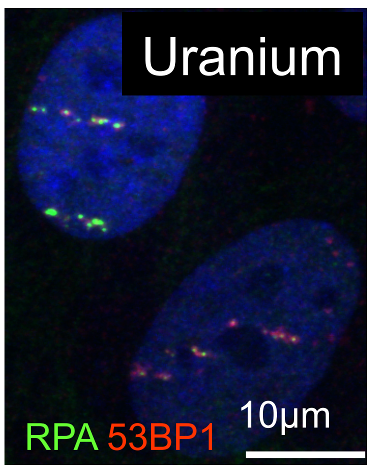
Costes et al. 2007





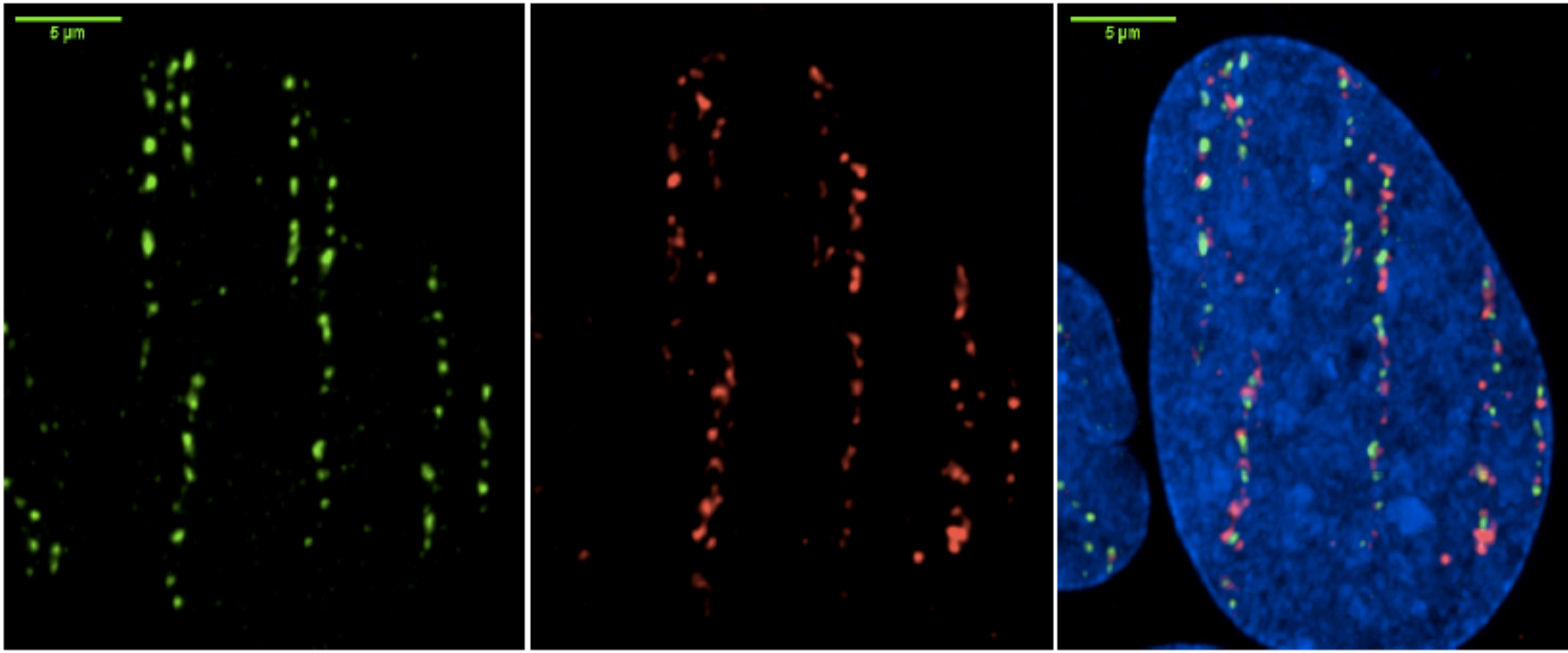
Takahashi et al, 2008





Jakob et al. 2009

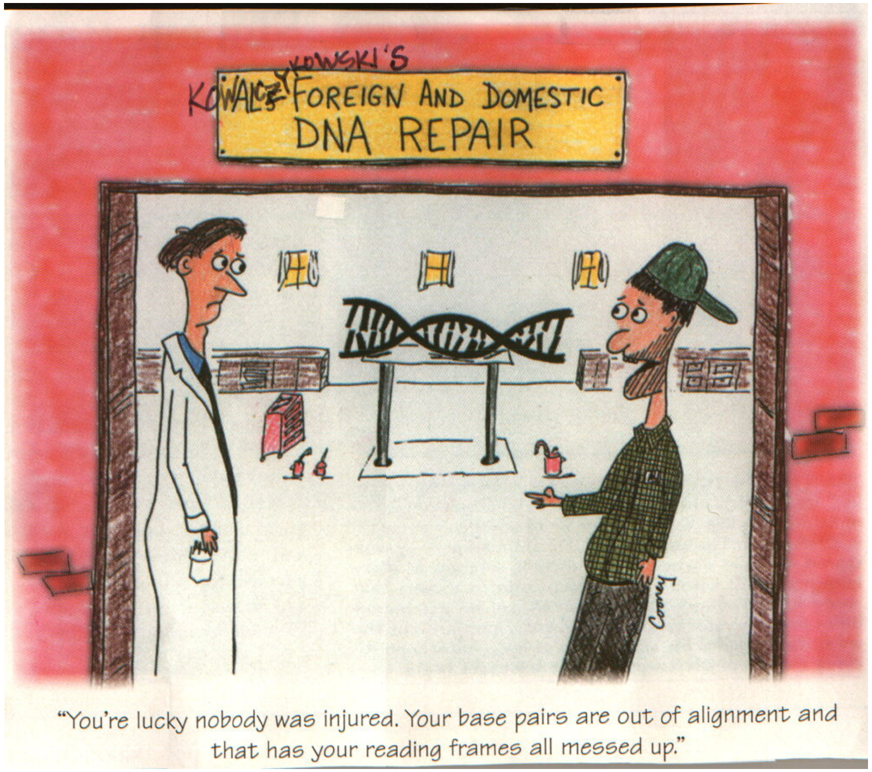
Co-localization of different repair proteins



RPA

53BP1

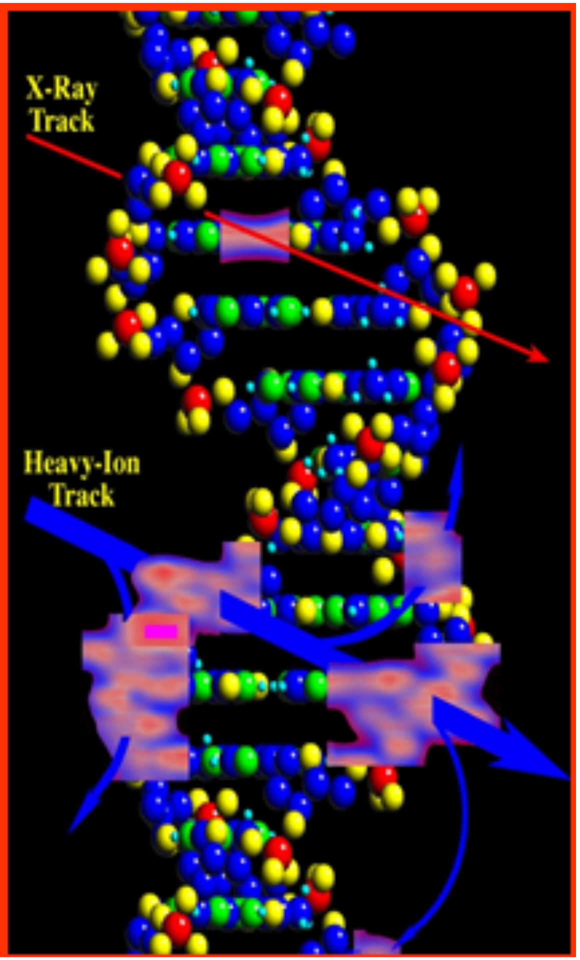
Overlap



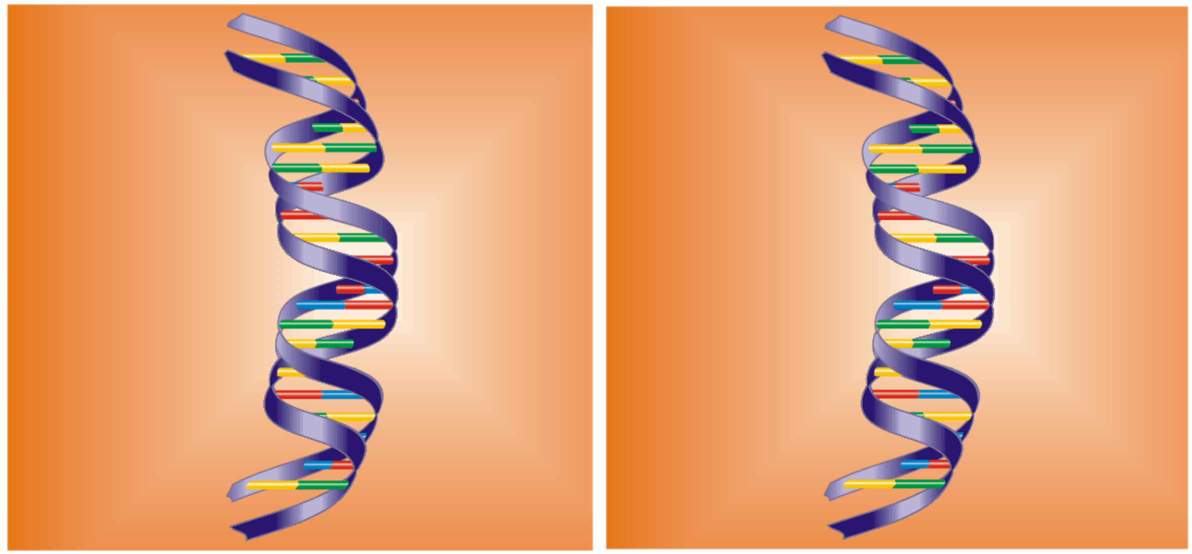
DNA repair: aging, cancer, genetic syndromes, …….

The most unkindest cut of all

(W. Shakespeare, Julius Caesar, Act 3)

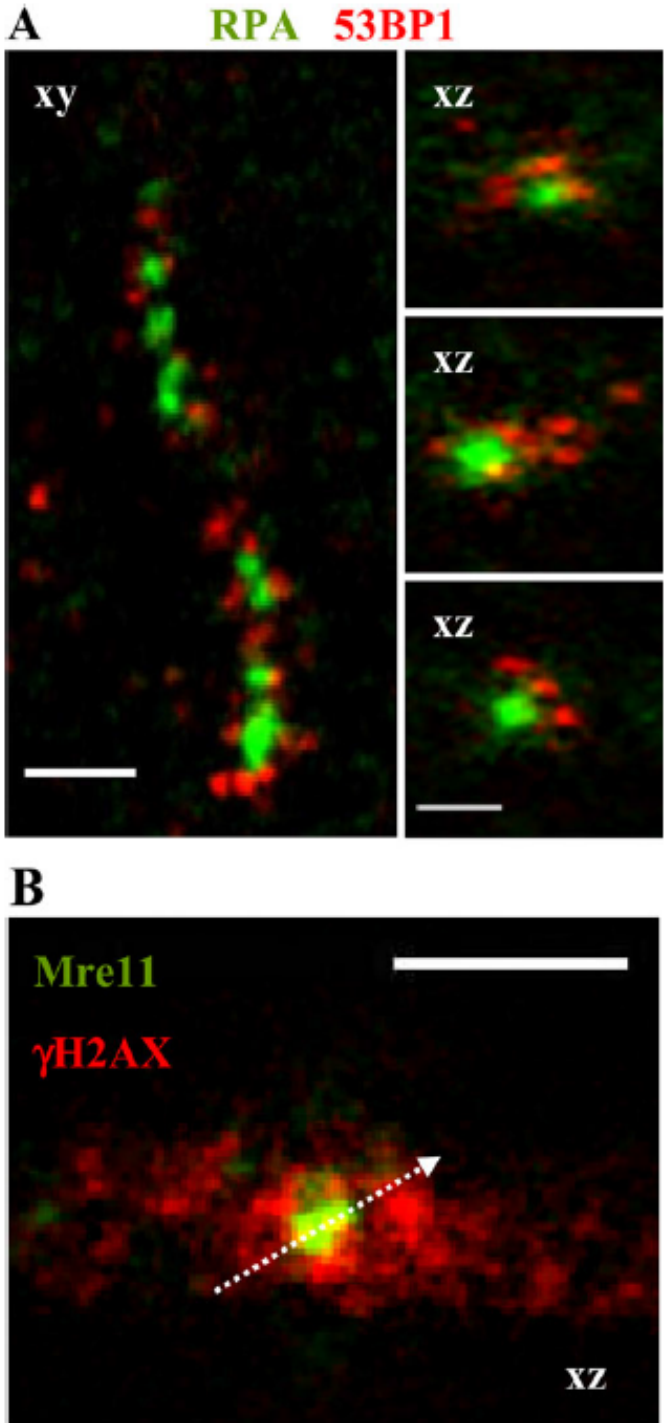


Courtesy of NASA

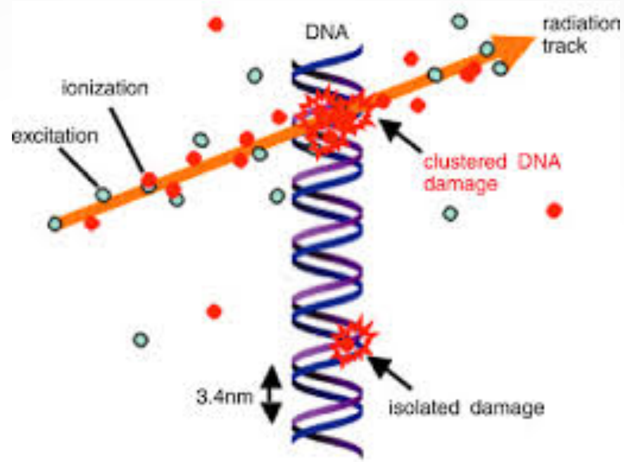


Courtesy of Valeria Conte

Courtesy of Nicole Averbeck



Clustered DNA breaks



Goodhead, *Int. J. Radiat. Biol.* 1994

Biological dose estimation of UVA laser microirradiation utilizing charged particle-induced protein foci Mutagenesis pp. 1–9, 2010

J. Splinter[1], B. Jakob[1,*], M. Lang[2], K. Yano[3], J. Engelhardt[2], S. W. Hell[2], D. J. Chen[3], M. Durante[1,4] and G. Taucher-Scholz[1]

nature COMMUNICATIONS

ARTICLE

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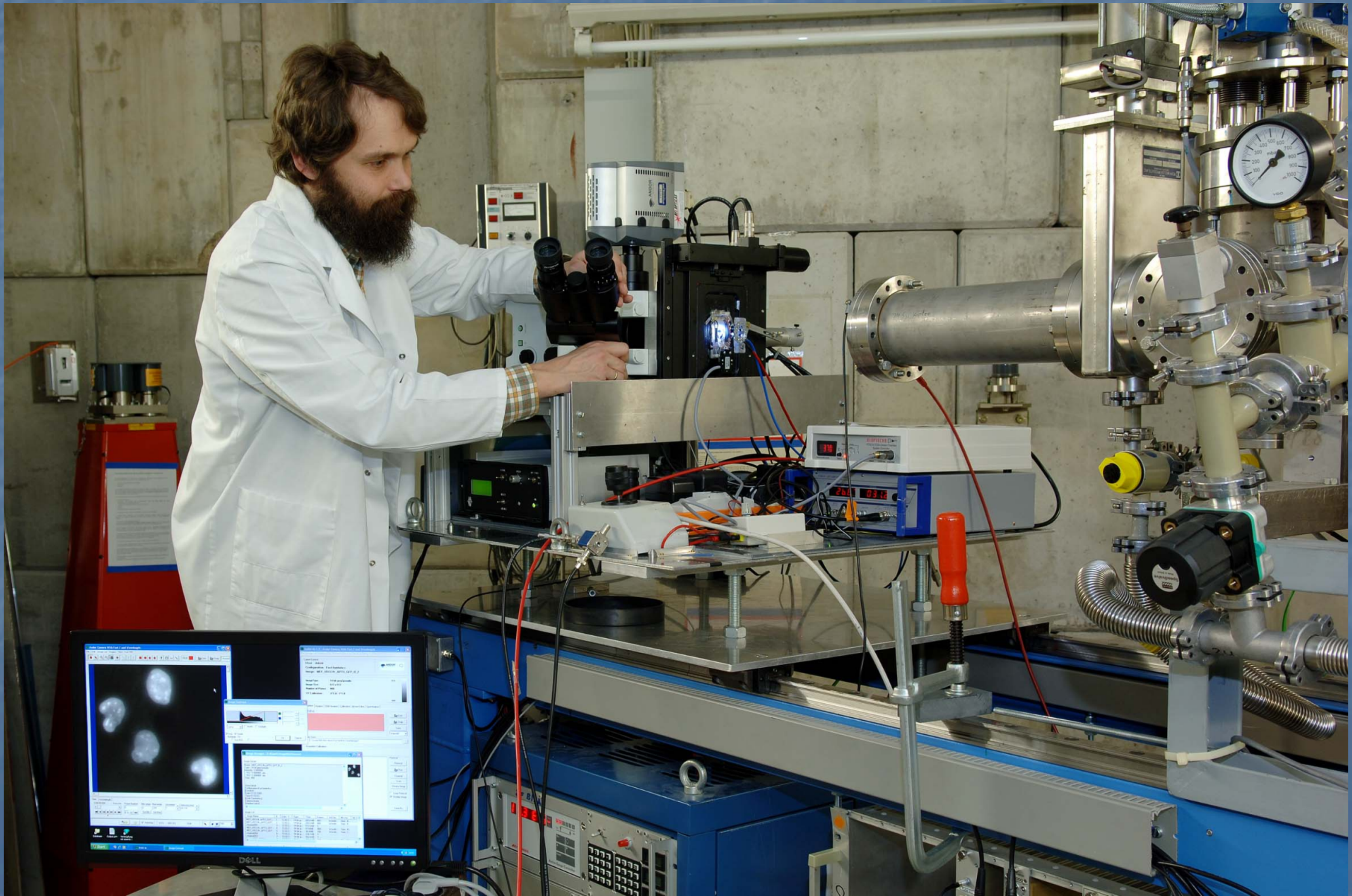
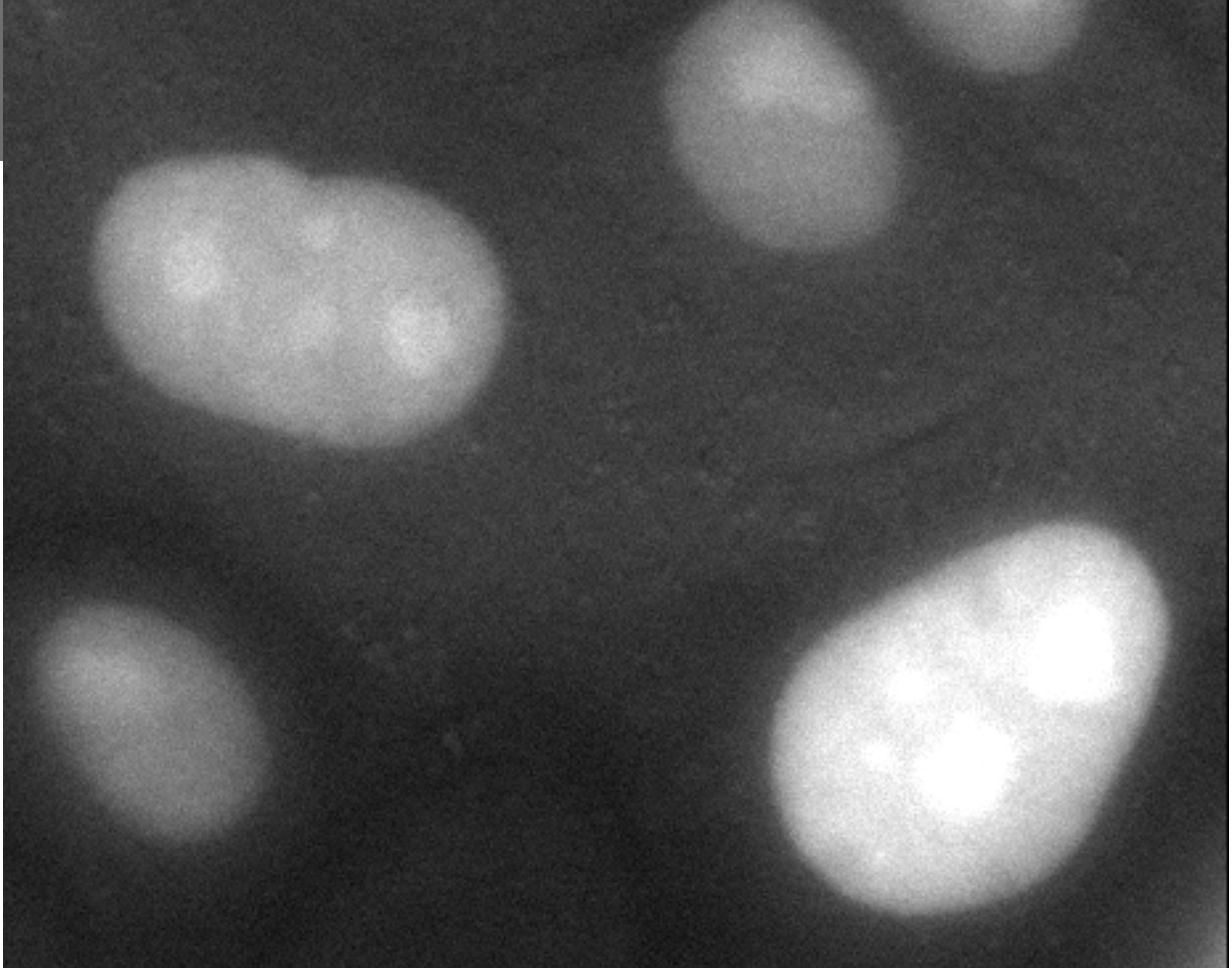
Identification of the elementary structural units of the DNA damage response

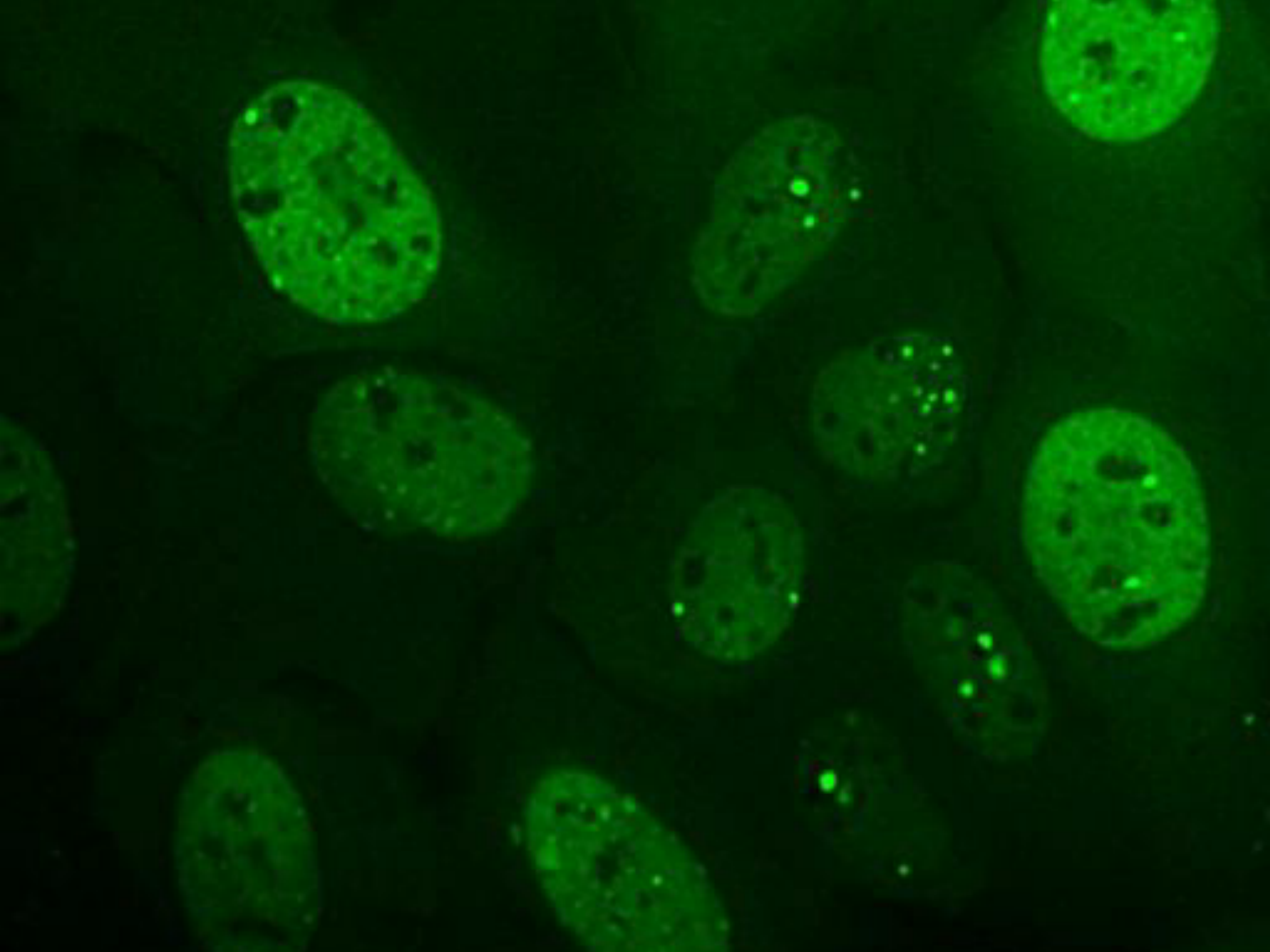
Francesco Natale[1,*], Alexander Rapp[1,*], Wei Yu[1,†], Andreas Maiser[2], Hartmann Harz[2], Annina Scholl[1], Stephan Grulich[1], Tobias Anton[2], David Hörl[2], Wei Chen[3], Marco Durante[4,†], Gisela Taucher-Scholz[4], Heinrich Leonhardt[2] & M. Cristina Cardoso[1]

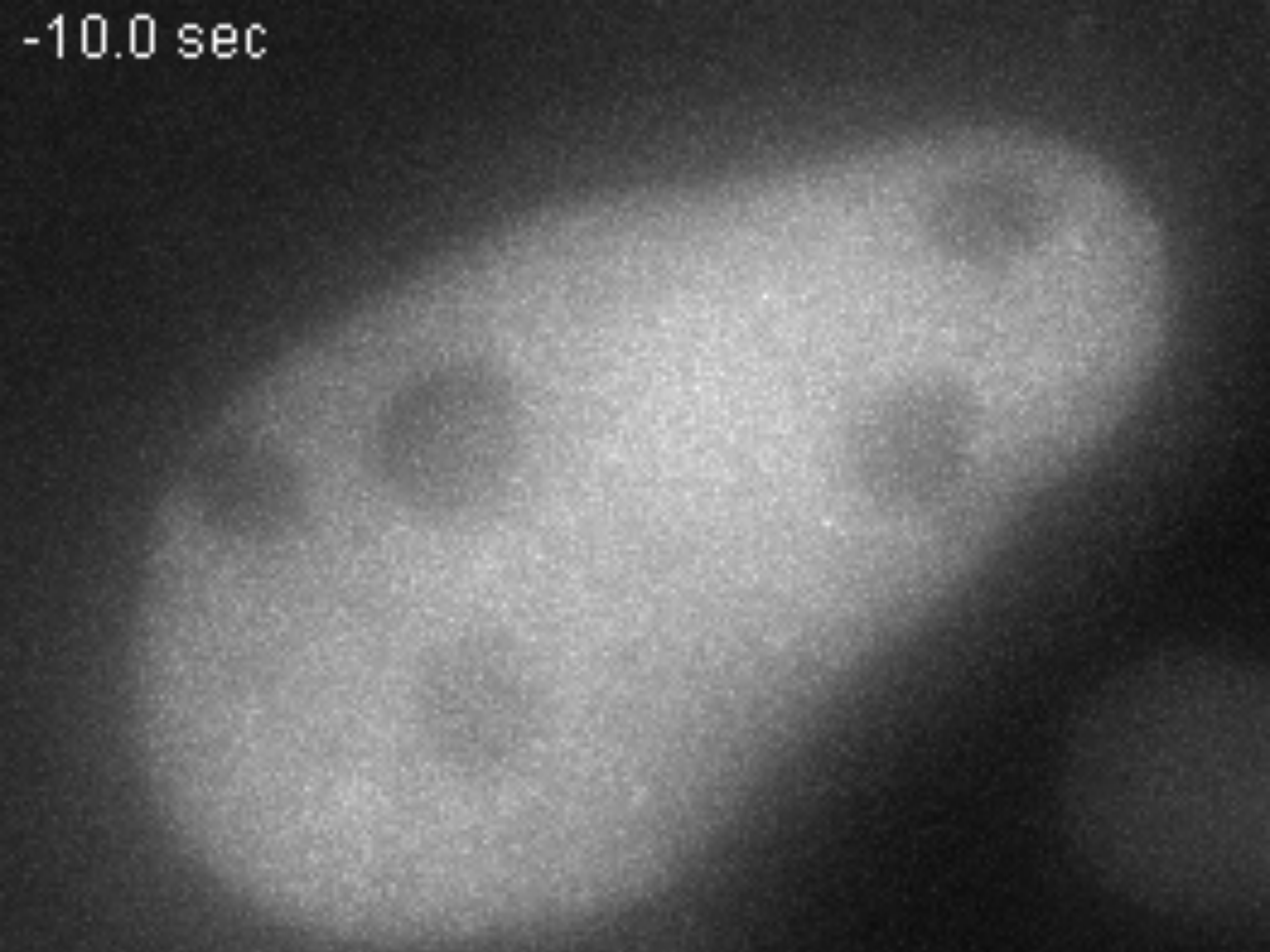
Beamline live cell imaging



GSI



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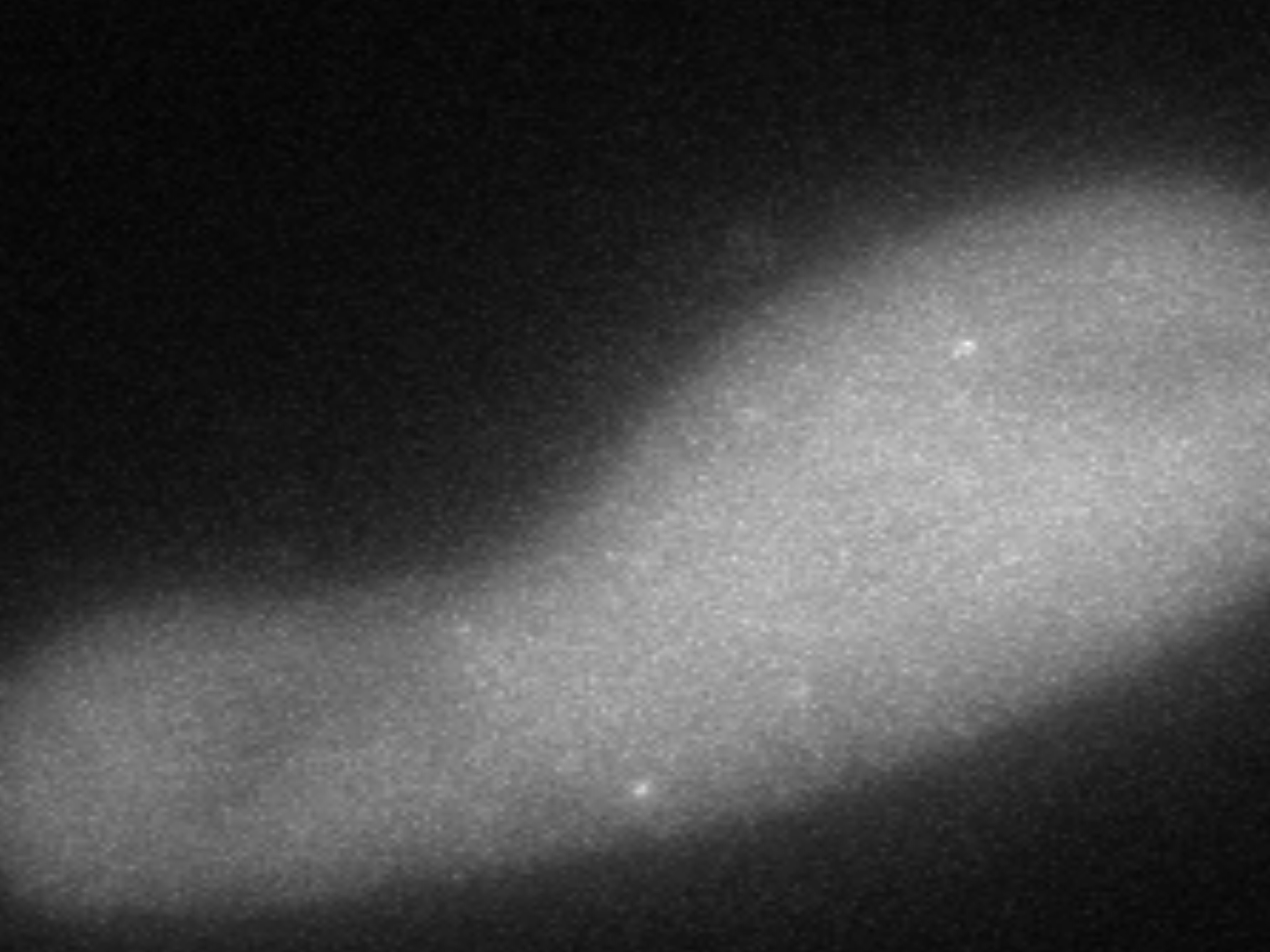


Live cell microscopy analysis of radiation-induced DNA double-strand break motion

B. Jakob[a], J. Splinter[a], M. Durante[a,b,1], and G. Taucher-Scholz[a]

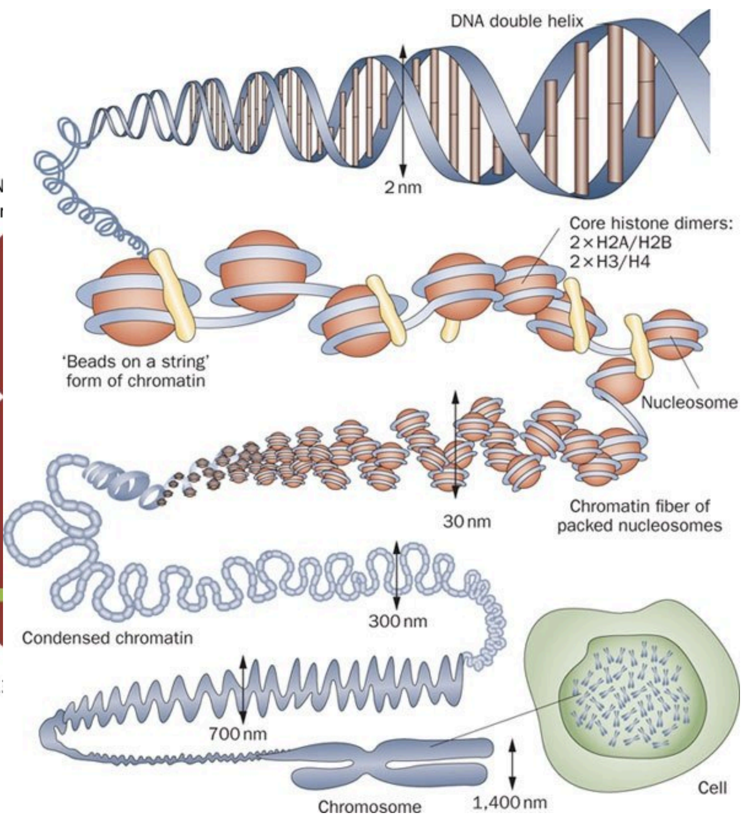
[a]Department of Biophysics, GSI Helmholtzzentrum für Schwerionenforschung, Planckstrasse 1, D-64291 Darmstadt, Germany; and [b]Institut für Festkörperphysik, Technische Universität Darmstadt, D-64289 Darmstadt, Germany

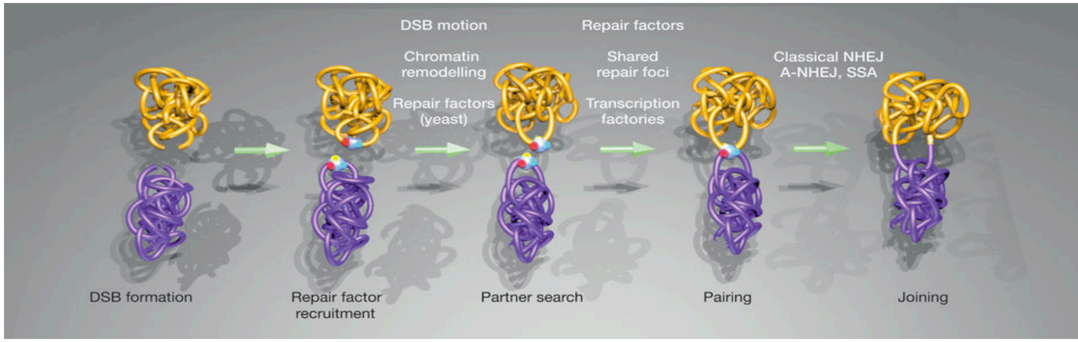
Edited by Philip C. Hanawalt, Stanford University, Stanford, CA, and approved January 13, 2009 (received for review October 31, 2008)

We studied the spatiotemporal organization of DNA damage processing by live cell microscopy analysis in human cells. In unirradiated U2OS osteosarcoma and HeLa cancer cells, a fast

tin was described to occur after γ-rays or local UV-laser irradiation (15, 16). Furthermore, Aten *et al.* (11) discussed the formation of repair clusters after α-particle irradiation, which

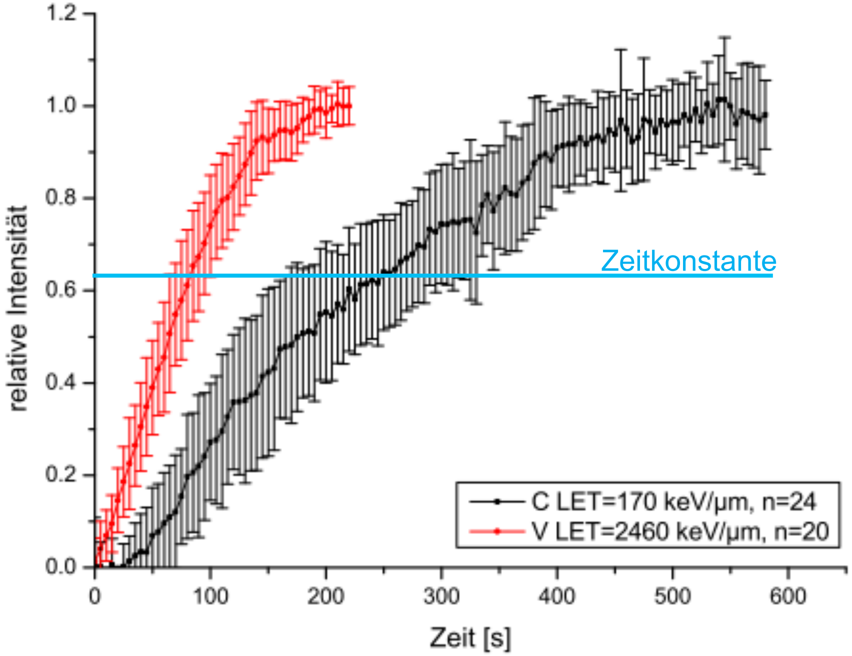
N
Chro

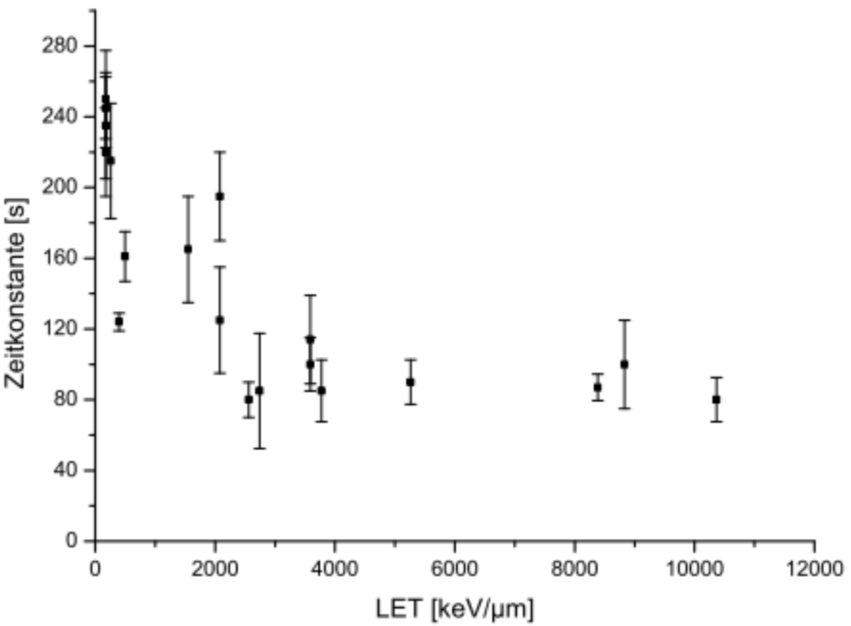




NBS1 accumulation at DSBs after heavy ion irradiation

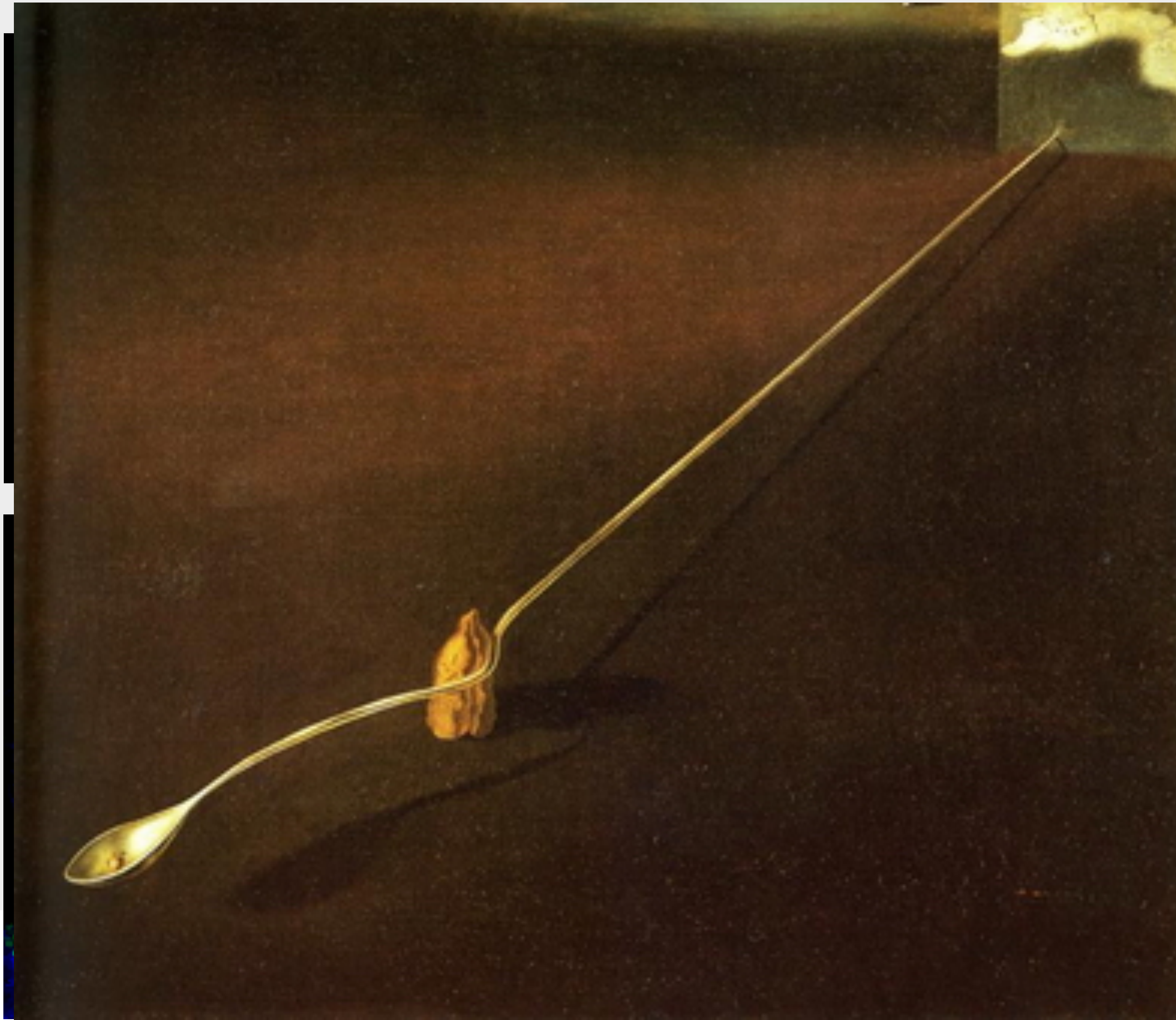
TECHNISCHE UNIVERSITÄT DARMSTADT

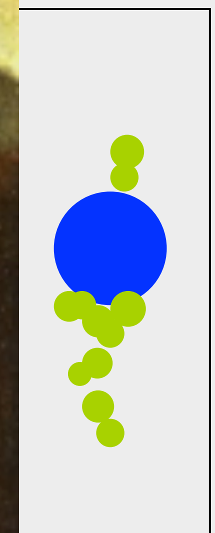




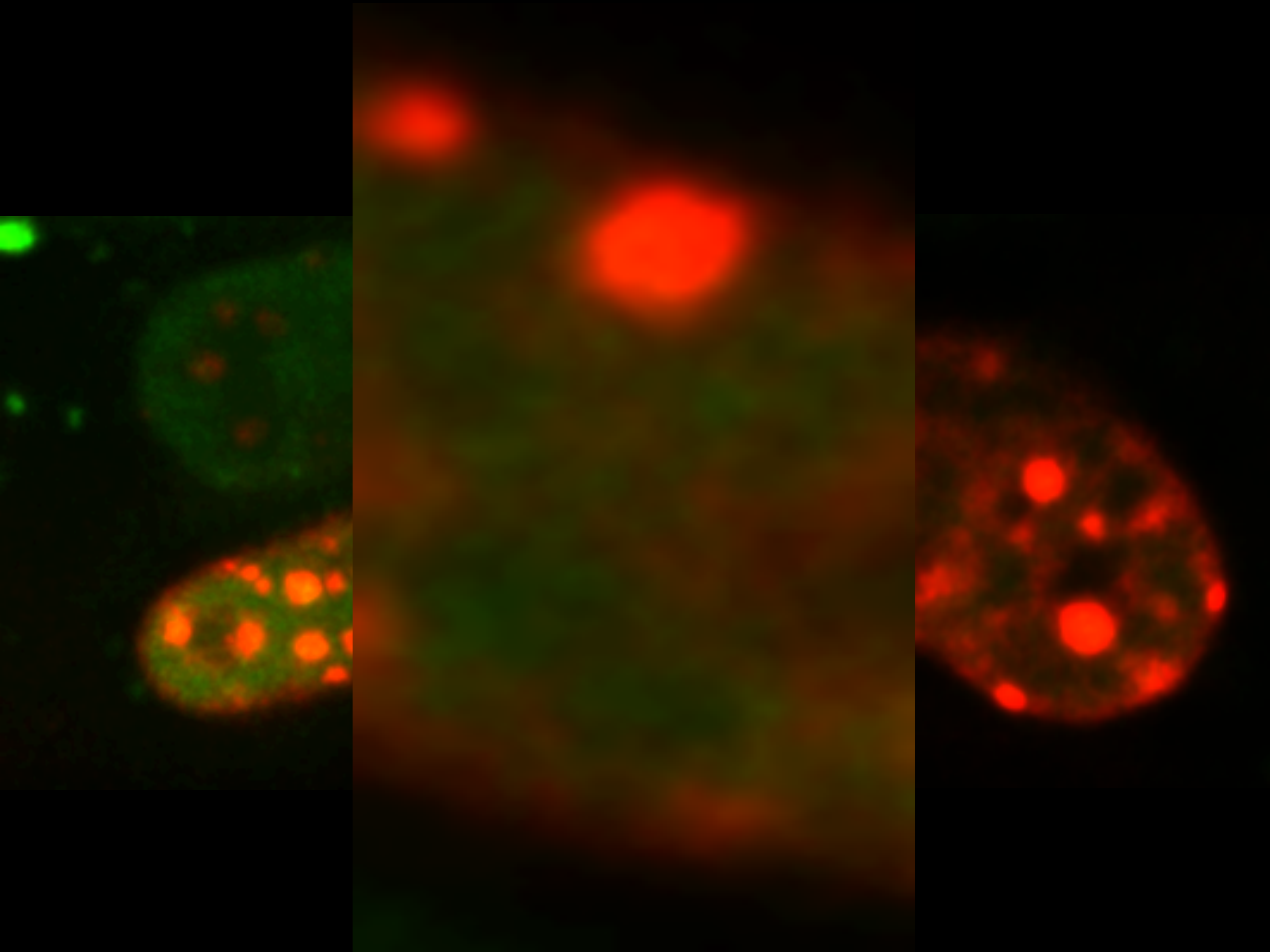
- Faster recruitment with heavy ions
- Saturation at very high-LET

HZE tracks in euchromatin and heterochromatin: „and the crooked shall be made straight“ (*Isaiah*, 40:4)

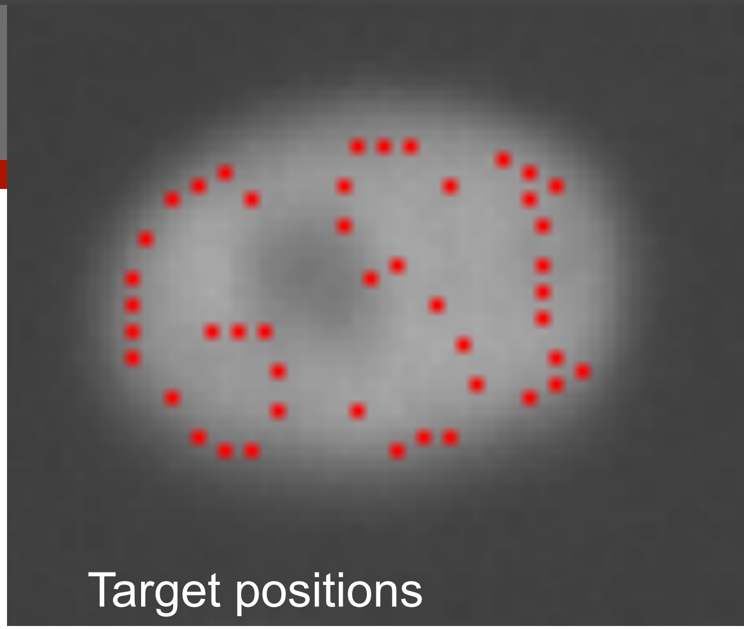


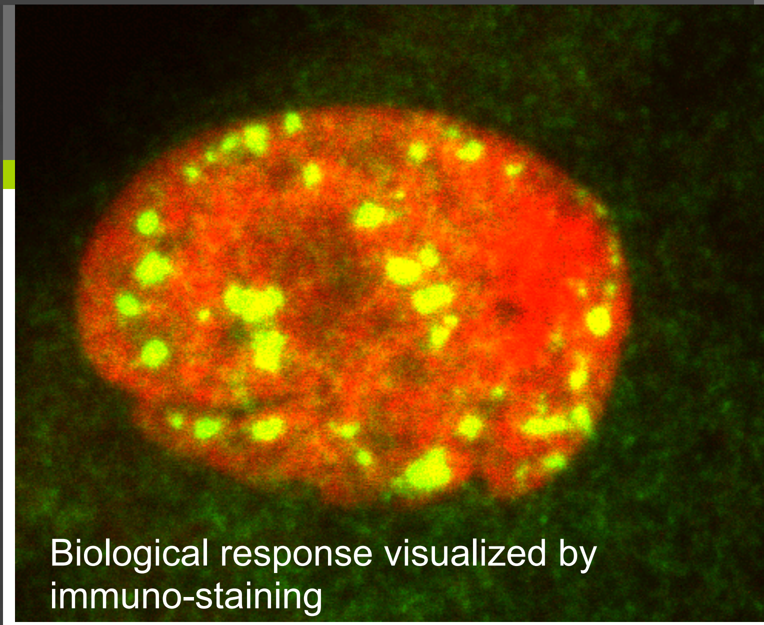


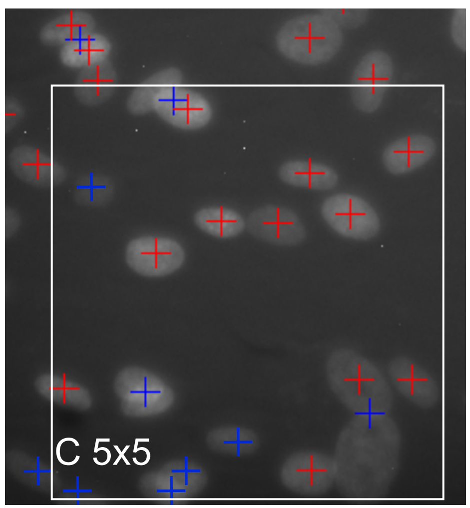
ids Res. 2011

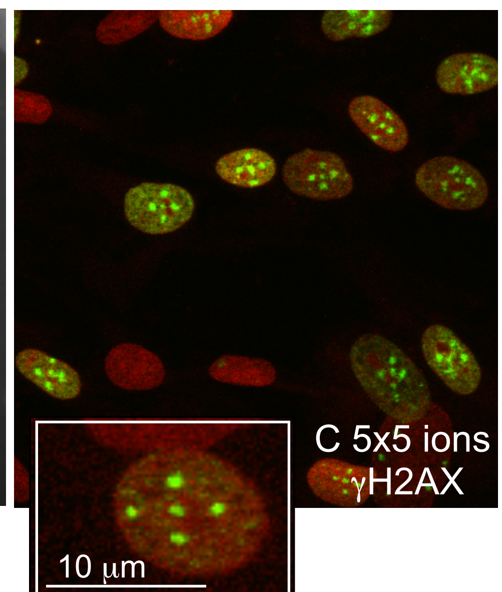


Microbeam - Irradiation of single cells



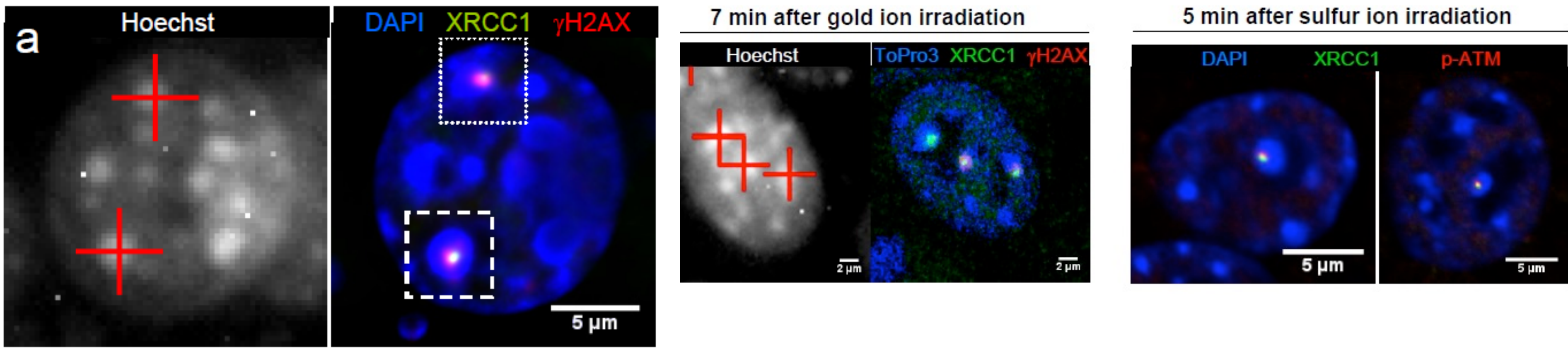


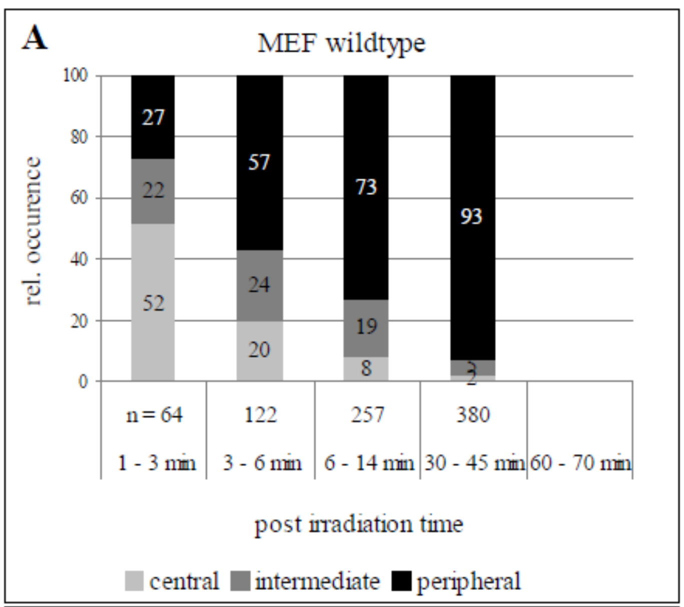


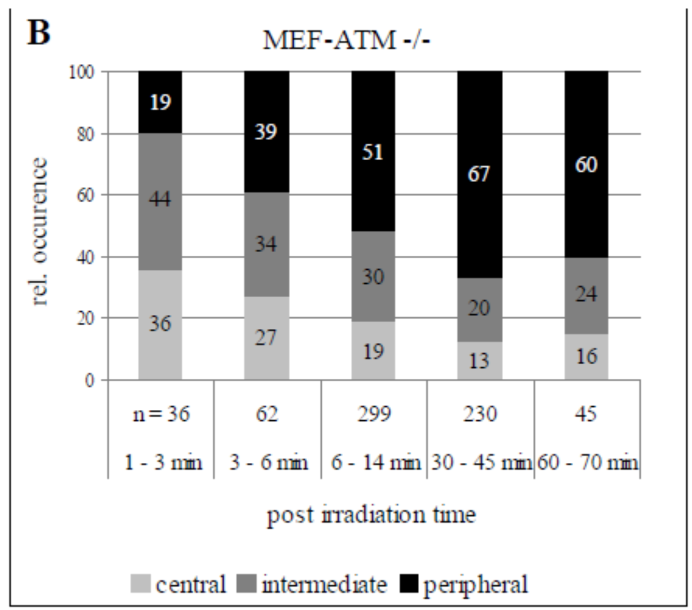


M. Heiß et al.
Radiat. Res. (2006)

Hetrochromatin targeting with the heavy-ion microbeam

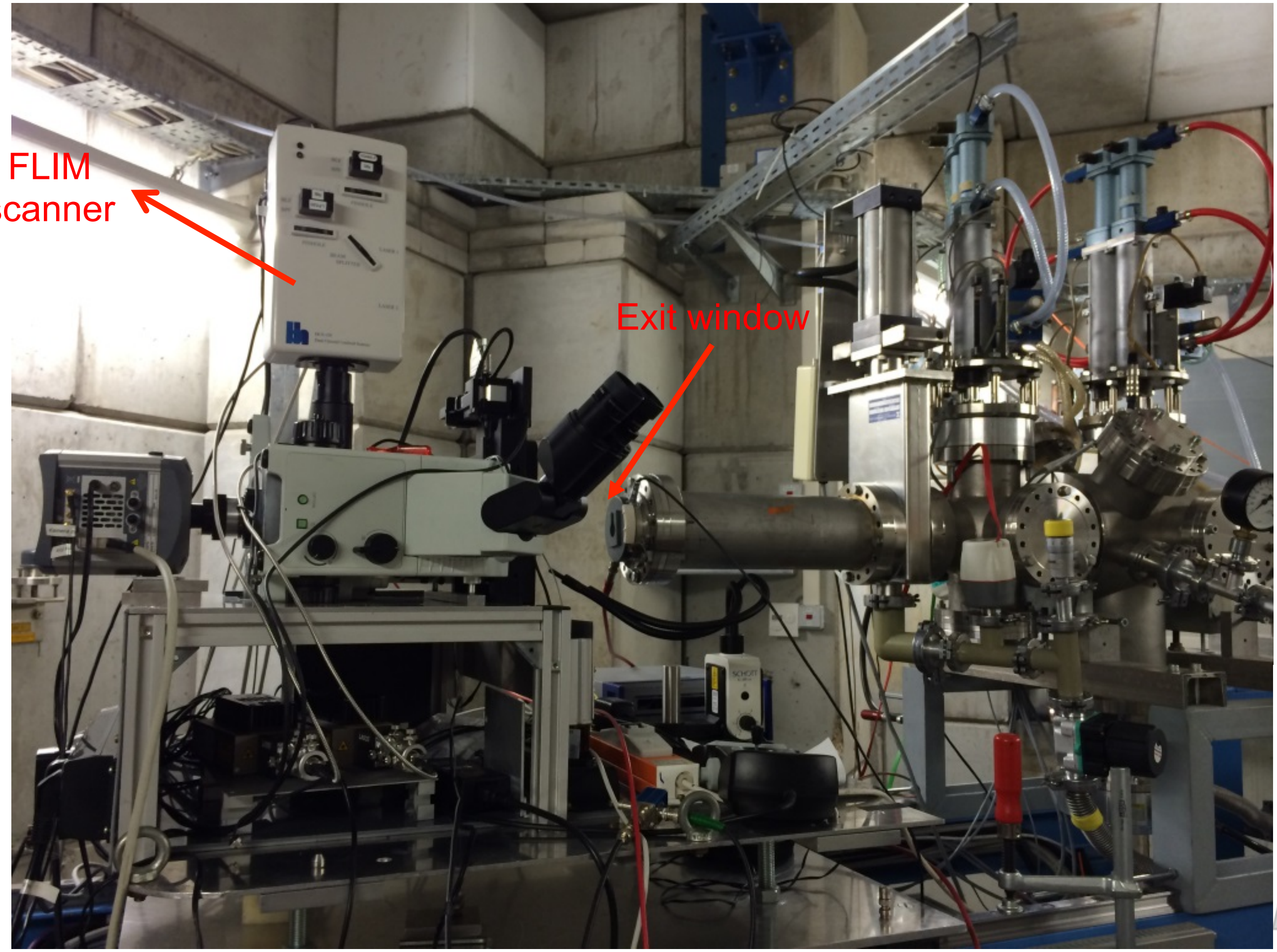


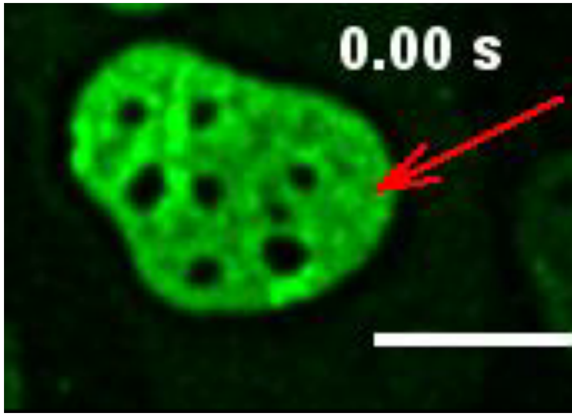


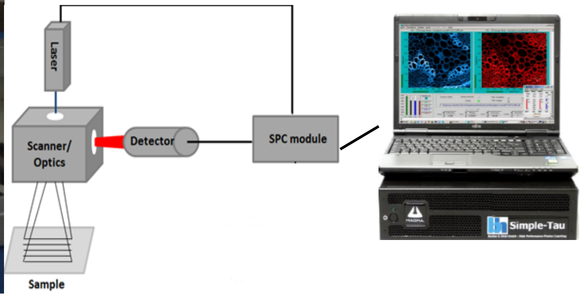


Markus Beuke, Ph.D. thesis 2012

Experimental setup

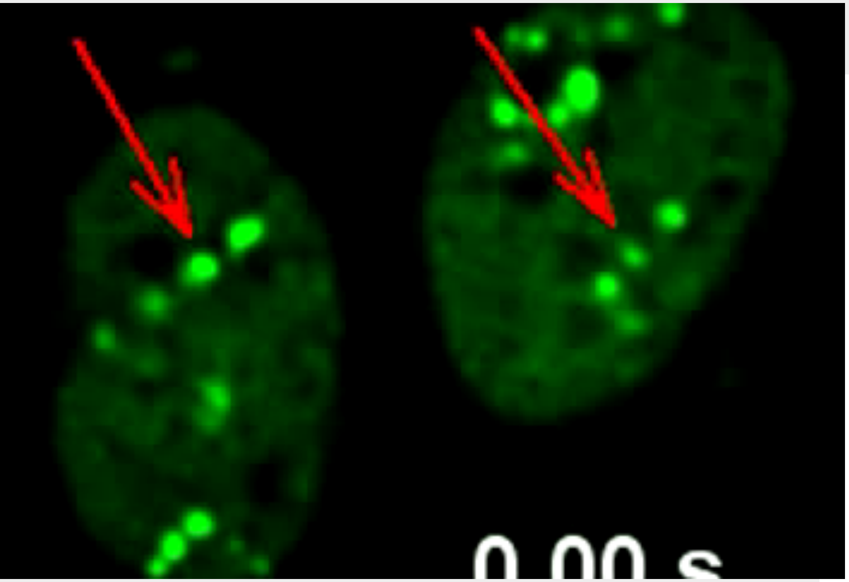


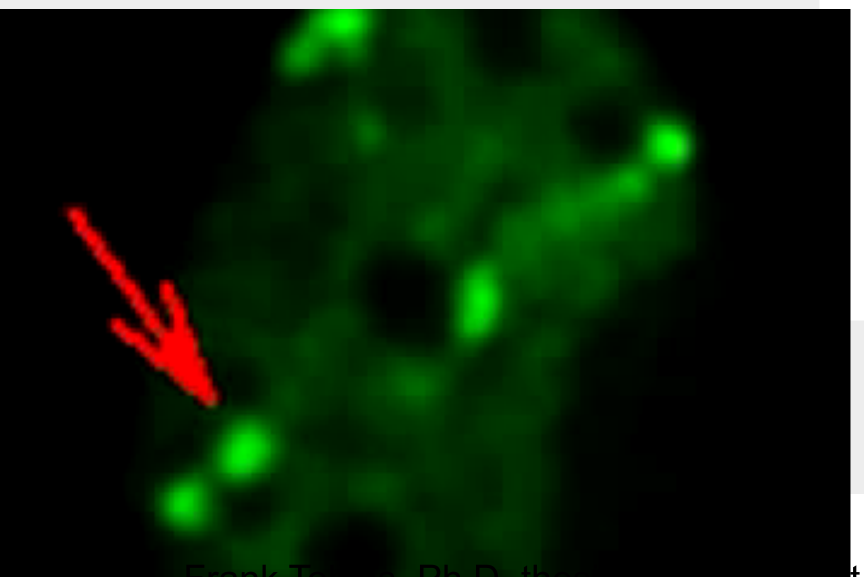


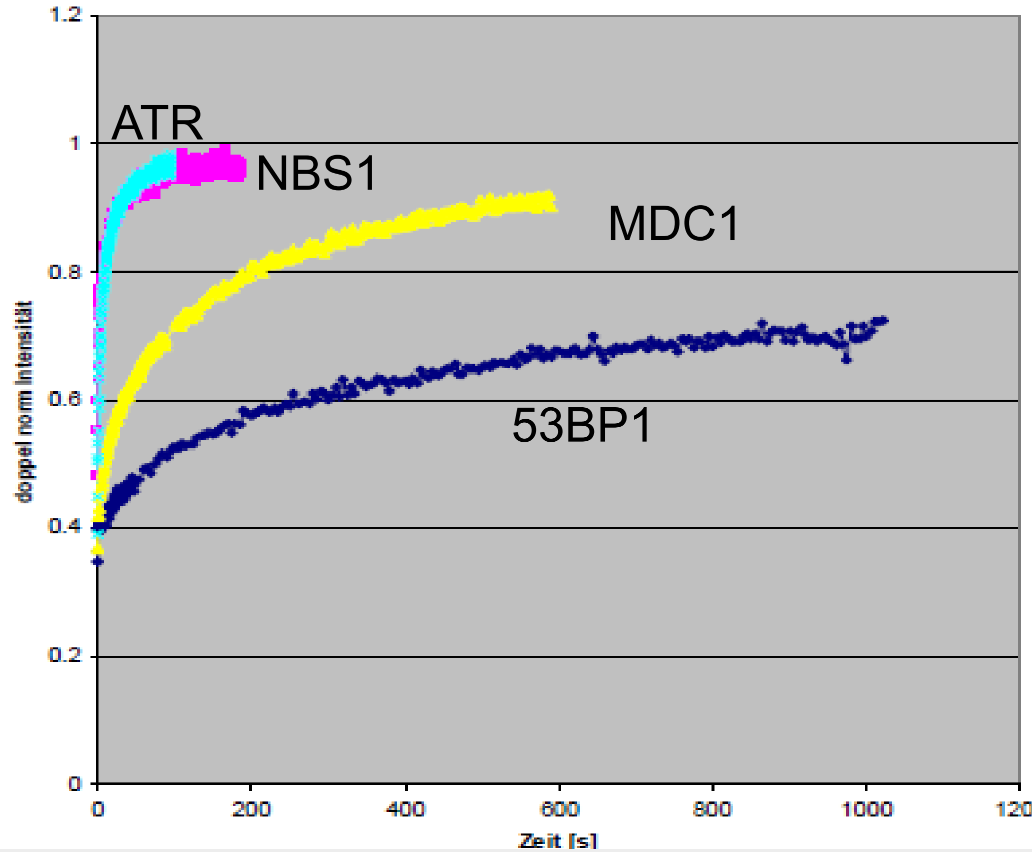


Schematic illustration of FLIM setup

FRAP on streaks created by C-ions

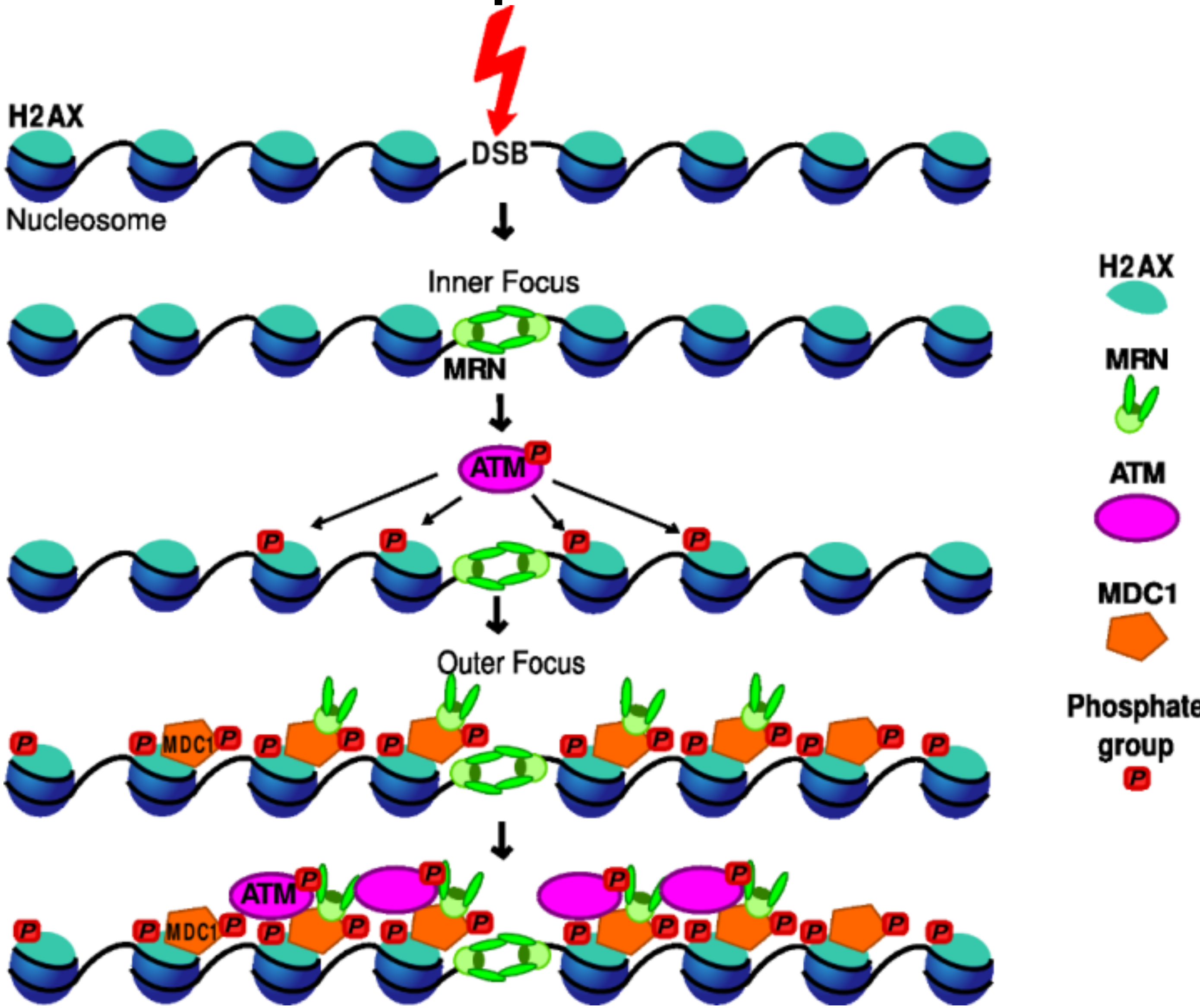




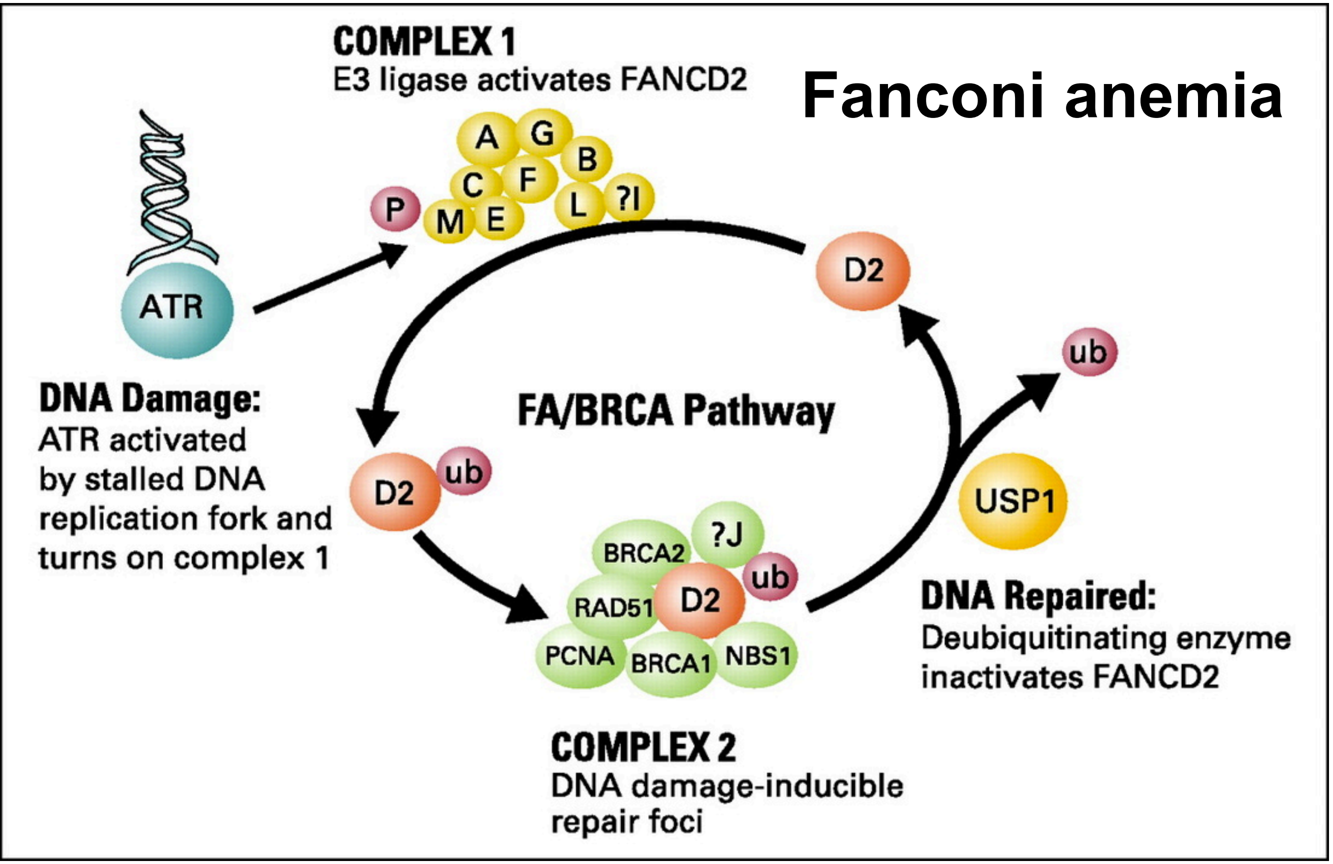


Frank Tobias, Ph.D. thesis TU Darmstadt 2012

Two qualitatively distinct interactions by which MRN complex binds to the DSB



1. MRN (MRE11/ Rad50/NSBS1) binds directly to DNA ends (**inner focus**)
2. Size of the inner focus is proportional to the number of DSBs
3. ATM phosphorylates H2AX
4. MDC1 is recruited to γH2AX
5. MRN binds to MDC1 (**outer focus**)
6. The number of binding sites in the outer focus is independent of the number of DSBs

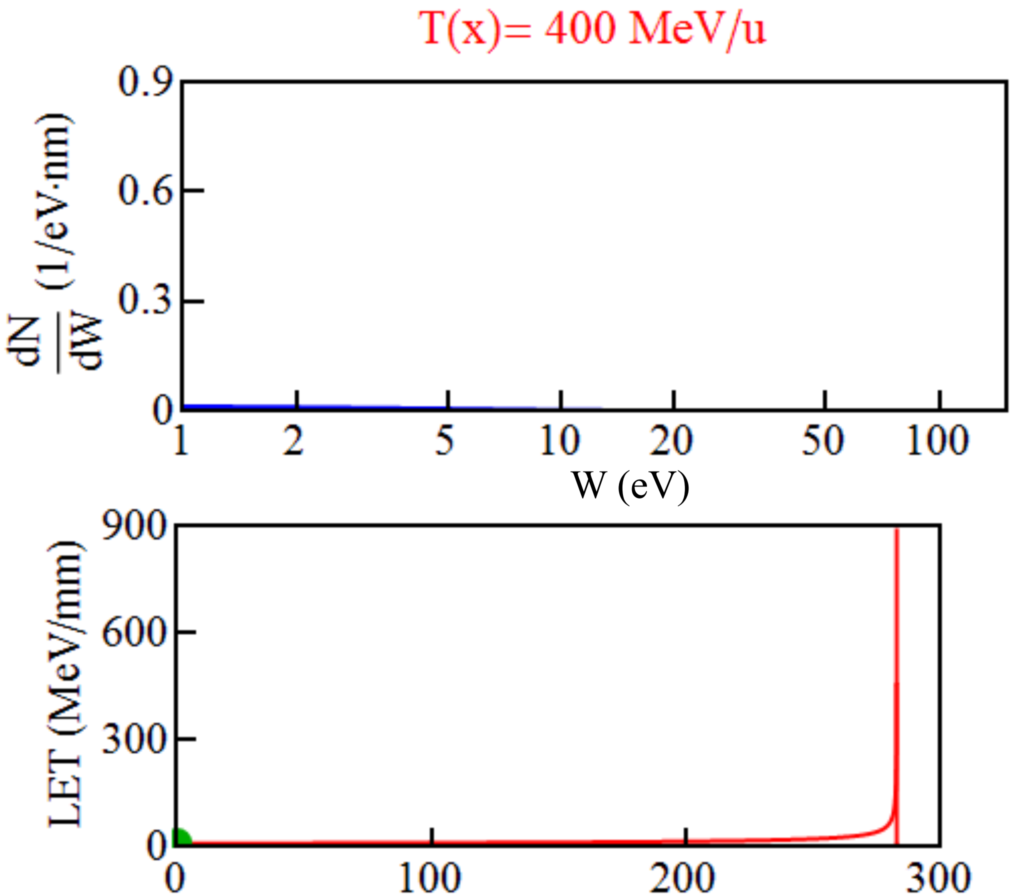


- A rare (1-5 in a million) autosomal recessive genetic disorder characterized by bone marrow failure
- It is caused by a defect in a cluster of approximately 20 proteins (FANC) involved in DNA repair
- Because mist patients develop cancer, the syndrome is very much studied in cancer research
- FANC turnover at sites of DNA damage is not known, and would be important to understand the role of protein quantity
- In Italy, 50% of the patients are from Campania

Low energy ion beams applications in Chemistry

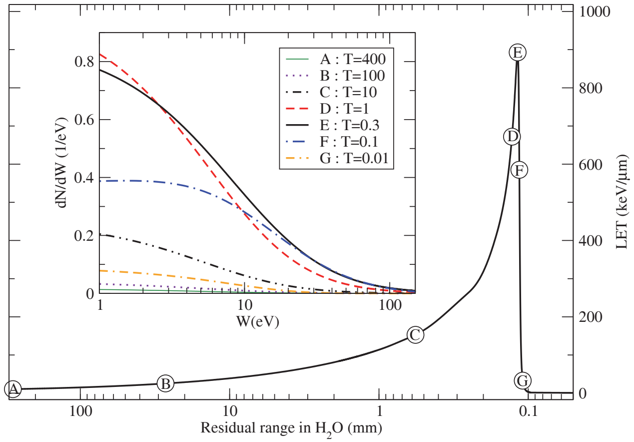
- Mechanistic studies of fundamental processess of ion interactions with atoms/molecules at Bragg Peak
  - Low energy electrons
  - Molecular Fragmentation studies
- Molecular Astrophysics
- Pulse Radiolysis
- Metallic Nanoparticles
- Ion implantation

Secondary Electrons produced by an ion along a Bragg Peak

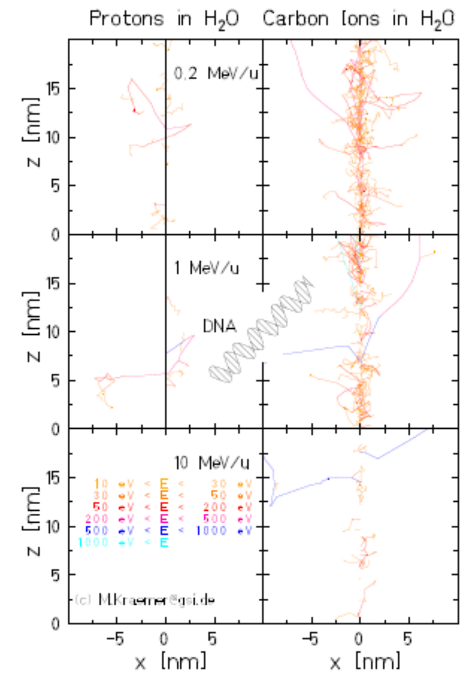


T_0=400 MeV/u penetration depth, x(mm)

Scifoni,et al., 2009

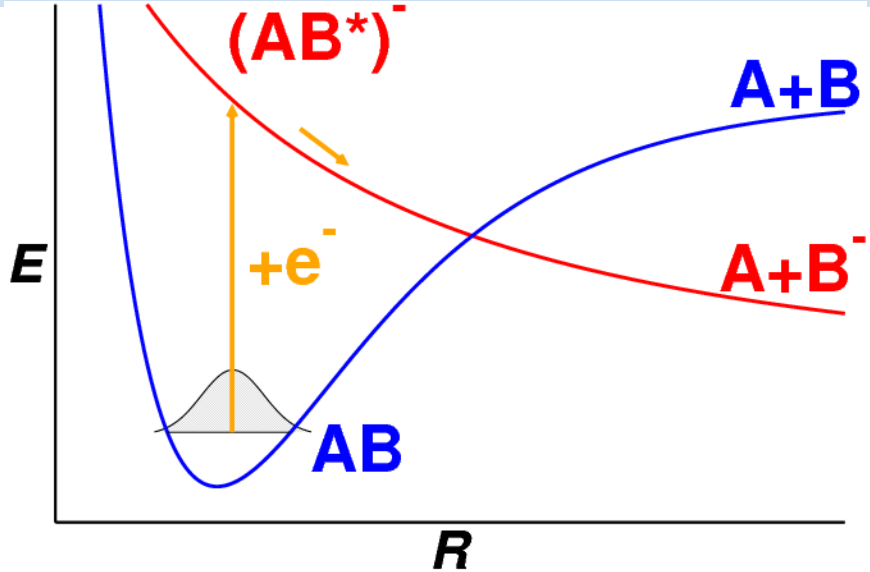


Scifoni Mod Phys Lett 2015

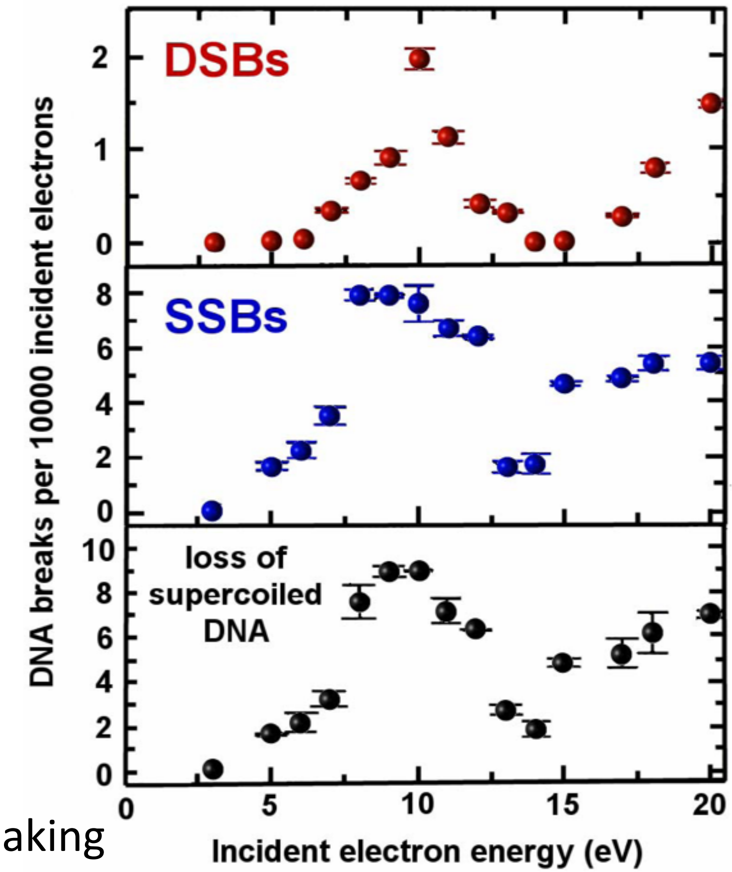


Kraemer et al JPCS 2012

Low energy Dissociative Electron Attachment (DEA)



$$AB + e^- \rightarrow (AB^*)^- \rightarrow A + B^-$$



An electron attack to a molecule with a resonant energy , **lower** than the dissociation threshold, can lead to a dissociative negative state that evolves towards bond breaking

Many experiments (**Sanche**) and quantum collisional studies on selected DNA components (**Gianturco, Fabrikant**) confirm the presence of these resonances, at low energy, and The resonant mechanism has **comparable** effect to higher energy mechanism

Science FREE WITH REGISTRATION

M.A. Huels, B. Bouda¨ıffa, P. Cloutier, D. Hunting, L. Sanche, Science 287, 1658 (2000)
JACS 125, 4467 (2003)

DEA on DNA components

PNAS

DNA strand breaks induced by near-zero-electronvolt electron attachment to pyrimidine nucleotides

Xiaoguang Bao[†], Jing Wang[‡], Jiande Gu[†‡§], and Jerzy Leszczynski[‡§]

[†]Drug Design and Discovery Center, State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 201203, People's Republic of China; and [‡]Computational Center for Molecular Structure and Interactions, Department of Chemistry, Jackson State University, Jackson, MS 39217

Edited by Henry Schaefer III, University of Georgia, Athens, GA, and accepted by the Editorial Board February 23, 2006 (received for review December 2, 2005)

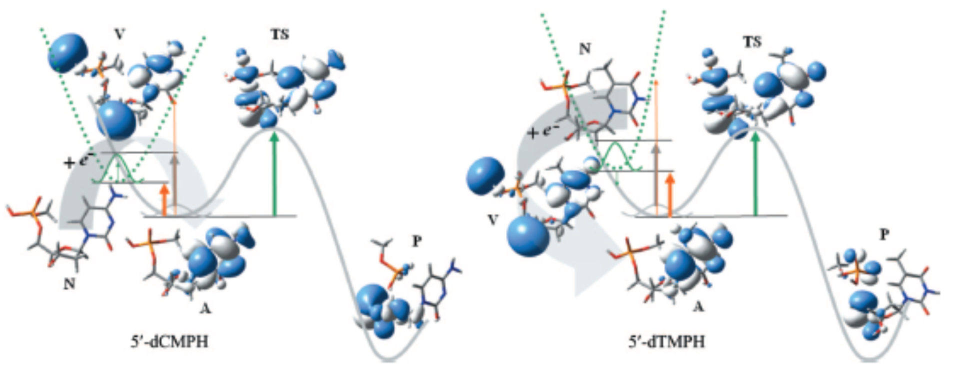
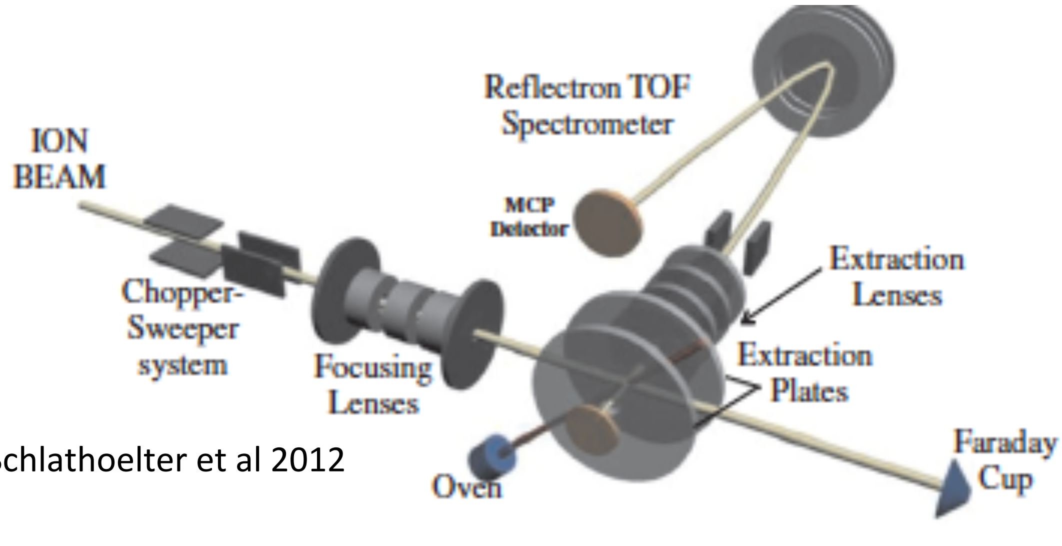


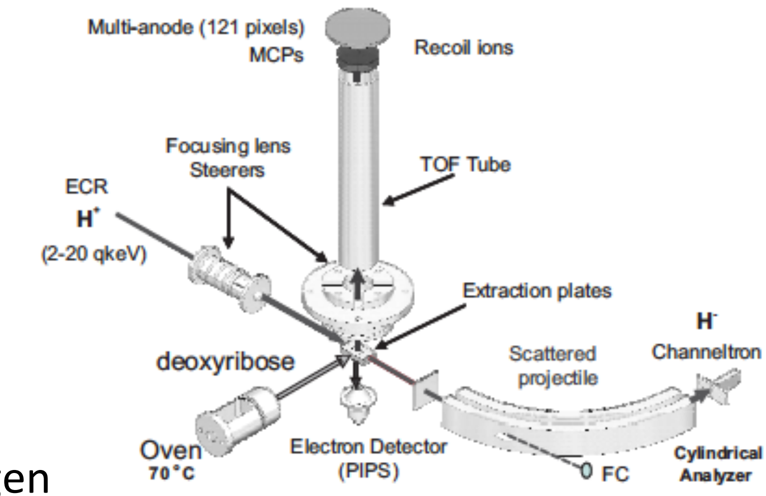
Fig. 2. The distribution of the unpaired electron along the LEE-induced C_5—$O_{5'}$ bond-breaking pathway of the nucleotides. A green dotted line represents the potential energy surface of neutral 5′-dCMPH or 5′-dTMPH; a gray solid line represents the potential energy surface of radical anion 5′-dCMPH⁻ or 5′-dTMPH⁻. A thin green arrow stands for VAE; a thin orange arrow is for VDE; a thick orange arrow is for ZPE-uncorrected EA_{ad}; a thick gray arrow is for ZPE-corrected EA_{ad}; and a thick green arrow is for the activation energy. N, neutral species; A, stable radical anion; V, electron vertical attached radical anion; TS, transition state; and P, bond-broken product.

Molecular fragmentation



Schlathoelter et al 2012

KVI Groeningen



LASIM Lyon

- Different Mass Spectrometer setups allow Ion induced radiation damage study on the molecular level

Example: Water

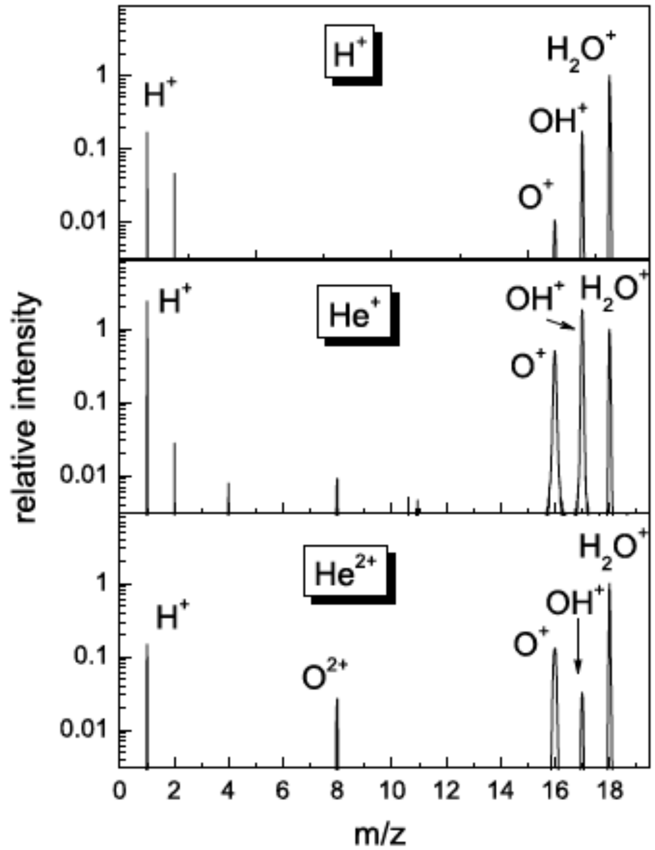
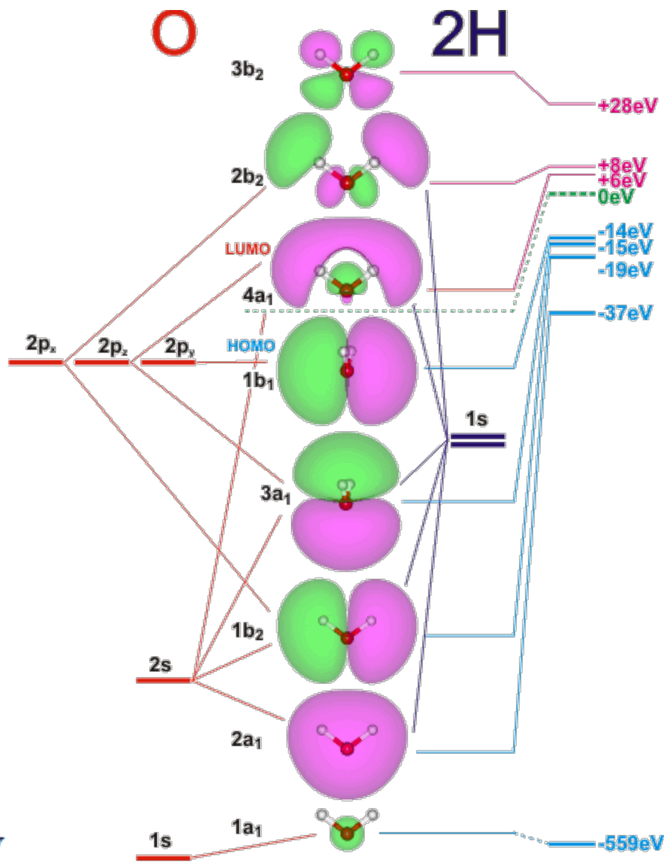


Figure 4.3: *Fragmentation spectra of water molecules after collisions with H^+, He^+ and He^{2+} at 6 keV total energy of the projectile.*

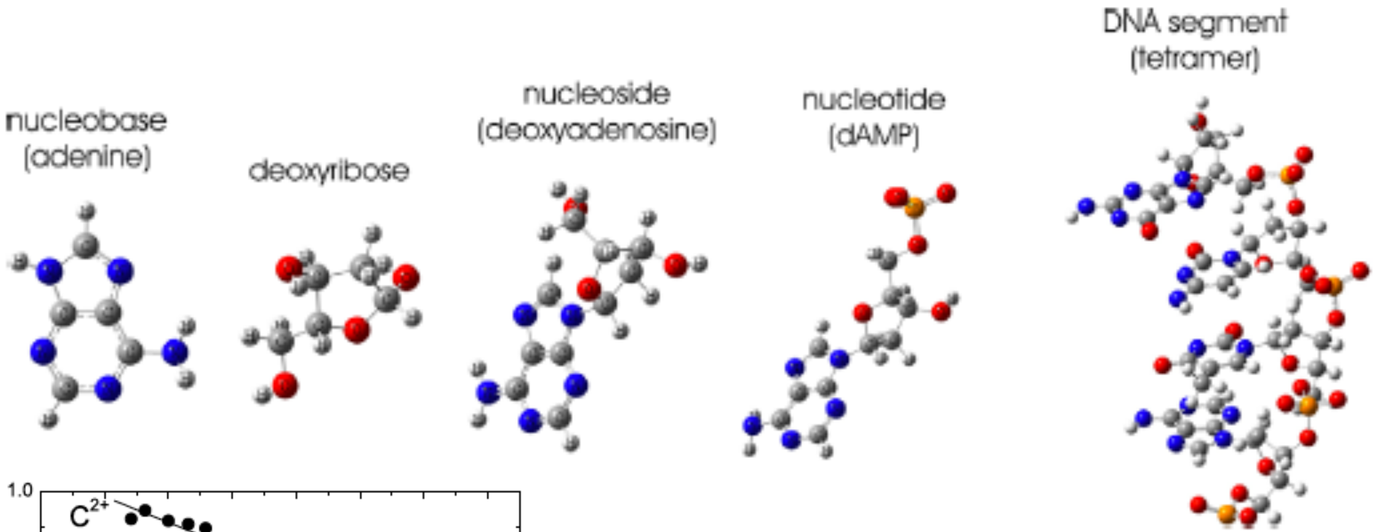


Alvarado et al. J. Phys. 2006
Jahnke et al. Nat. Phys. 2010

Water Molecular Orbitals

DNA components

T Schlathölter *et al* Physica Scripta 2007



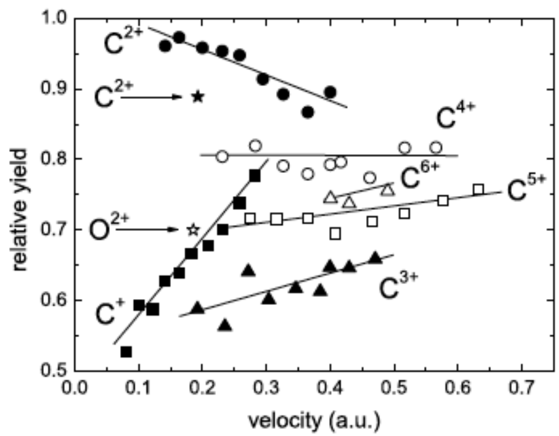
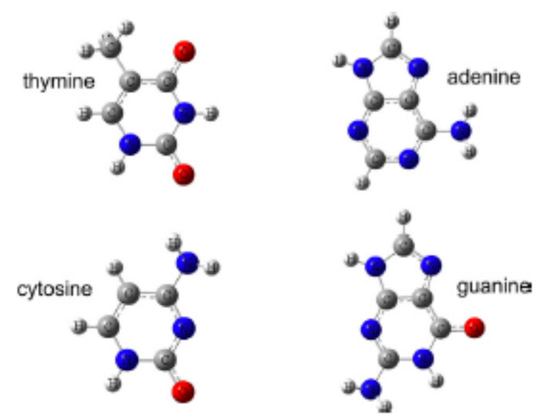
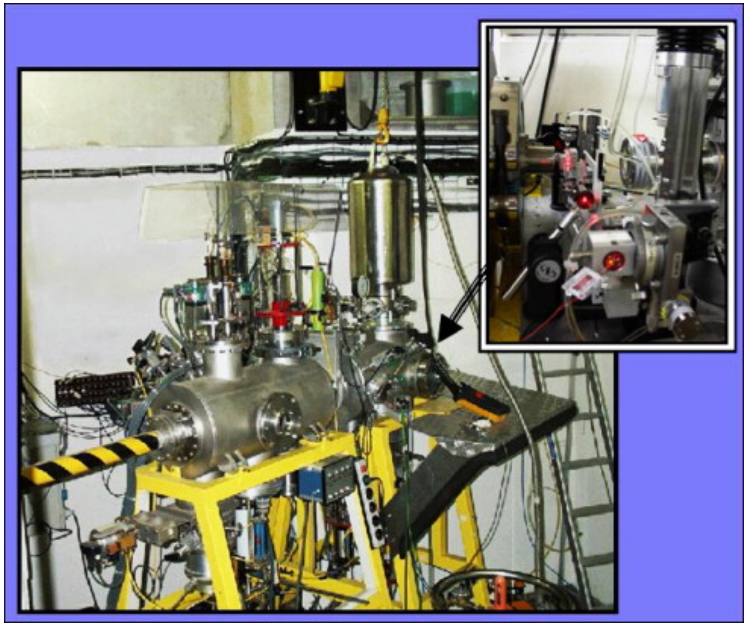


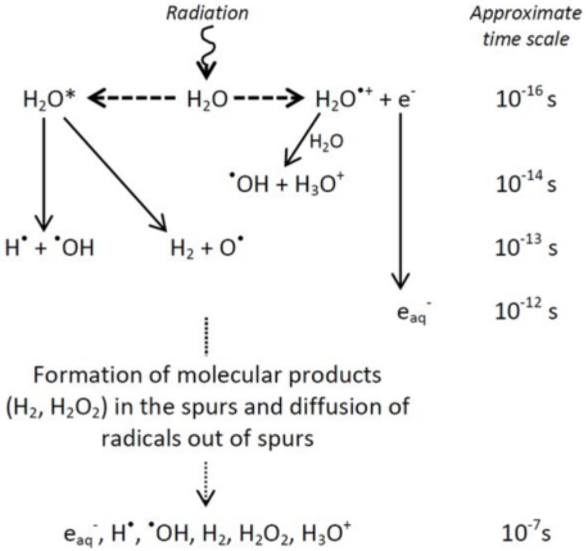
Figure 4. Relative uracil fragmentation cross-sections for interaction with C^{q+} [4].

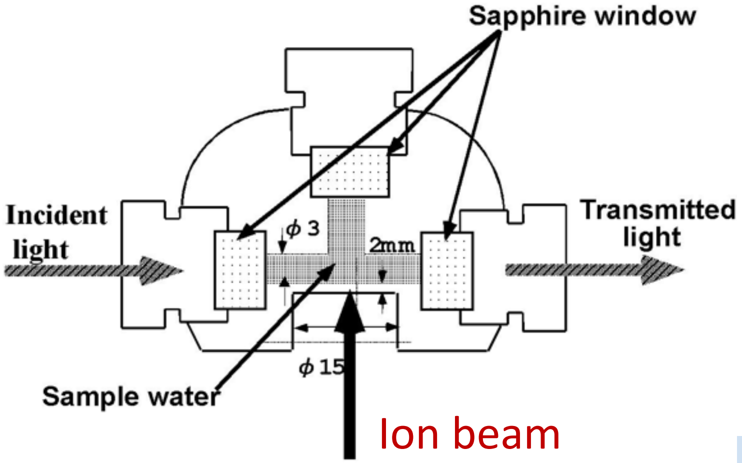


Pulse radiolysis

- Tandem type ion accelerators are indicated for pulse radiolysis studies
- Study of chemical evolution with high temporal resolution (ps and beyond)

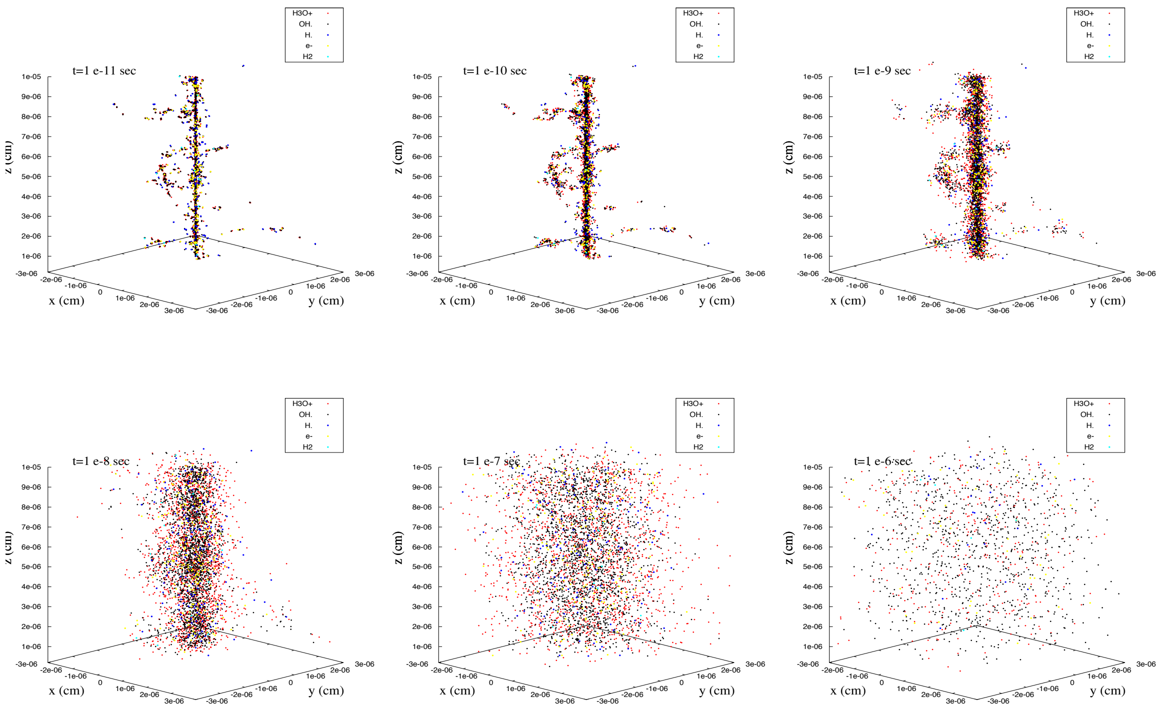


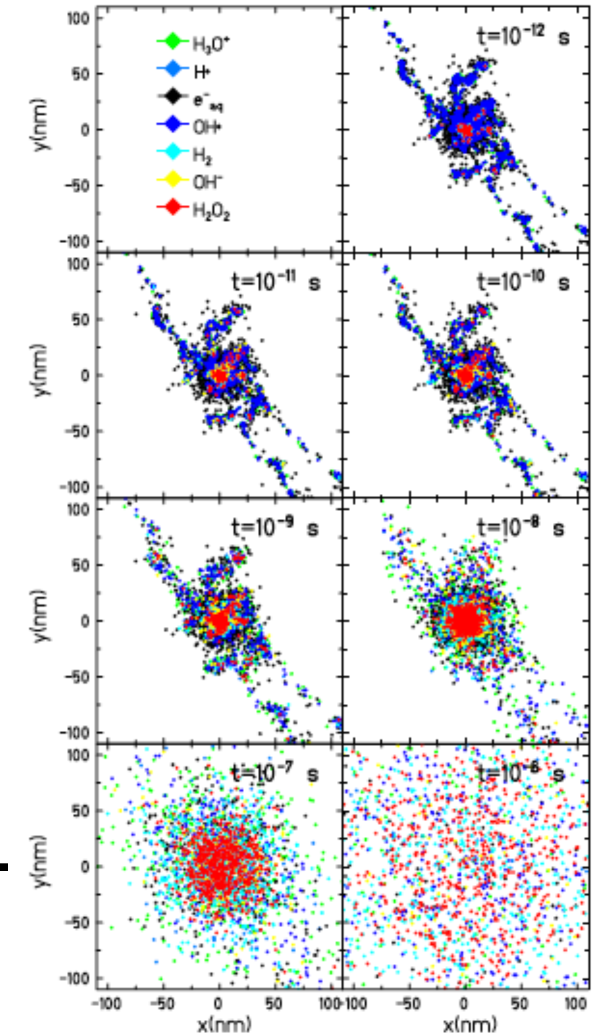




Ion induced radical species evolution

radical diffusion ^{12}C 3 MeV/u (TRAX simulation, D.Boscolo)



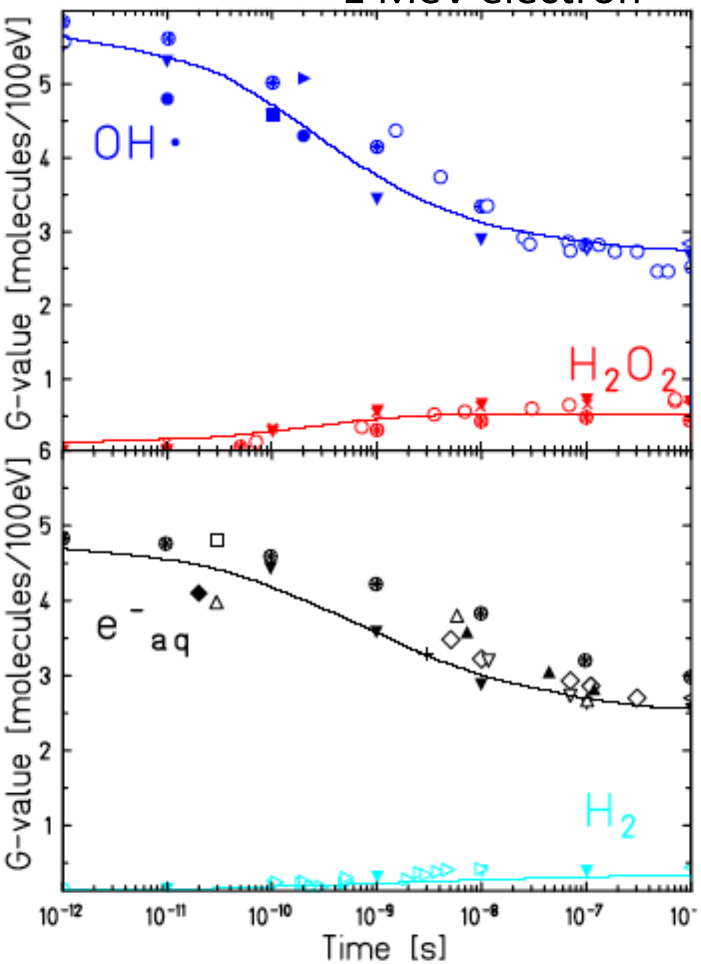


Boscolo, Krämer, Durante, Fuss, Scifoni *Chem Phys Lett* (subm)

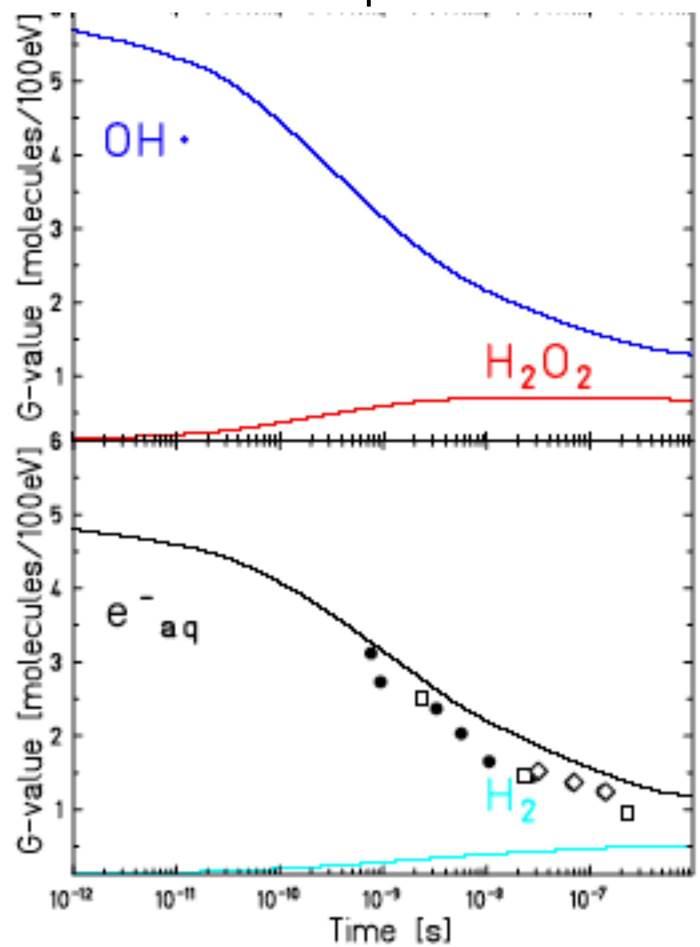
Trento Institute for Fundamental Physics and Applications

Verification with PR data

1 MeV electron



3 MeV proton



Boscolo, Krämer, Durante, Fuss, Scifoni *Chem Phys Lett* (subm)

Il Mattino > Mangia e Bevi > Le News

San Marzano e Corbarino, alleati contro i tumori intestinali

di Luciano Pignataro

0

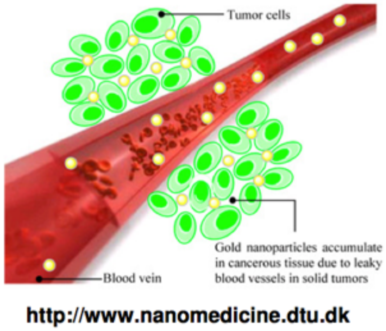
Che i pomodori facciano bene alla salute è cosa nota. Ma la ricerca continua a regalare grandi sorprese. Protagonisti il San Marzano e il Corbarino, potenti alleati della lotta al cancro. Un nuovo studio ha dimostrato infatti che il trattamento con estratti totali di queste due varietà di pomodoro inibisce la crescita e le caratteristiche maligne delle cellule di cancro gastrico.

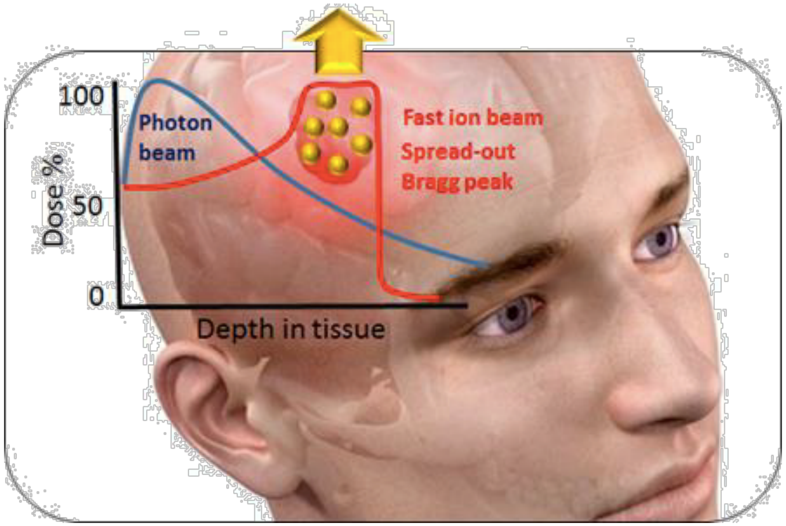
Lo studio, apparso venerdì sul Journal of Cellular Physiology, si è concentrato sul cancro gastrico, che è il quarto tipo di cancro più diffuso al mondo. Lo sviluppo di questa patologia è associato sia a cause genetiche che ad infezioni sostenute da Helicobacter pylori ma soprattutto ad abitudini alimentari errate, come l'eccessivo consumo di prodotti affumicati e salati. Gli autori principali dello studio, Daniela Barone e Letizia Cito, del gruppo di ricerca diretto da Antonio Giordano presso l'Istituto Nazionale Tumori di Napoli, Fondazione Pascale, CROM, hanno esaminato gli effetti del San Marzano e del Corbarino.

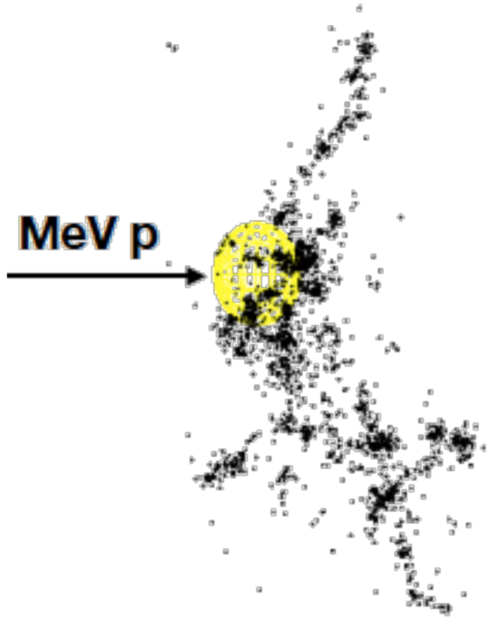
Antitumoral potential, antioxidant activity and carotenoid content of two Southern Italy tomato cultivars extracts: San Marzano and Corbarino

Gold nanoparticles as proton sensitizers

The addition of Gold NP has beeen suggested as a possibility to radiosensitize tumors, thanks to the possibility to selectively targeting tumor cells and the enhancement in electron production







Au secondary electrons verification

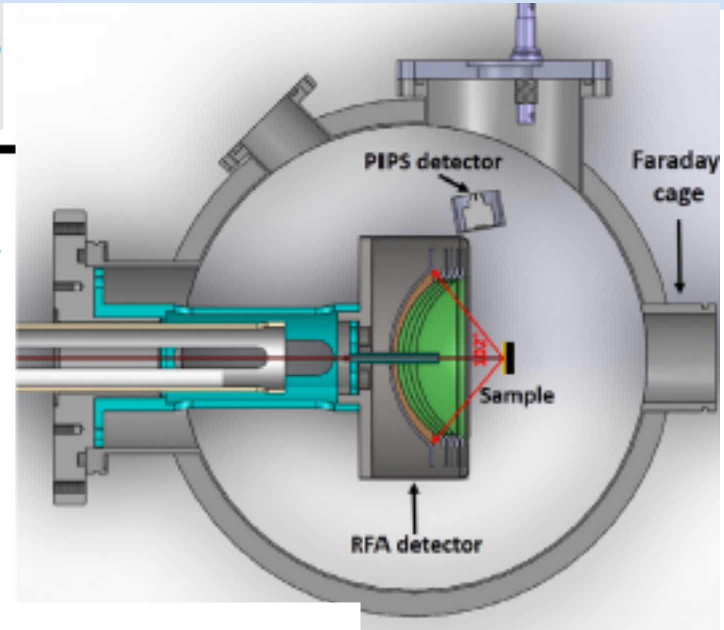
ELSEVIER

Nuclear Instruments and Methods in Physics Research B

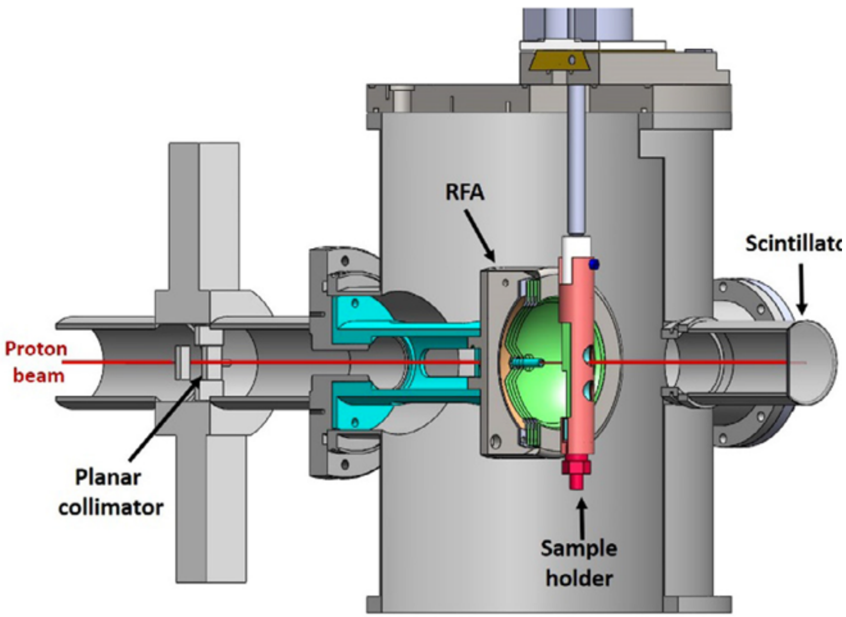
journal homepage: www.elsevier.com/locate/nimb

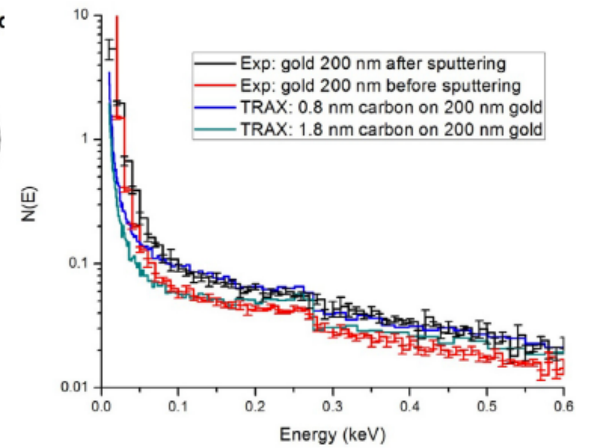
Backscattered electron emission after proton impact on carbon and gold films: Experiments and simulations

F. Hespeels[a], A.C. Heuskin[a], E. Scifoni[b,c], M. Kraemer[c], S. Lucas[a,*] (2017)



Retarding Field Analyzer (RFA).





Retarding field analyzer allows to Collect Spectra of secondary electrons By scanning the retarding field potential

Altais 2-MV TANDEM accel @ Namur

Ion implantation

Generating defects in solids, through ion beams, for i.e. semicond device realization

Light ions/at higher energy → more electronic stopping

Heavier ions/at lower energy → more nuclear stopping

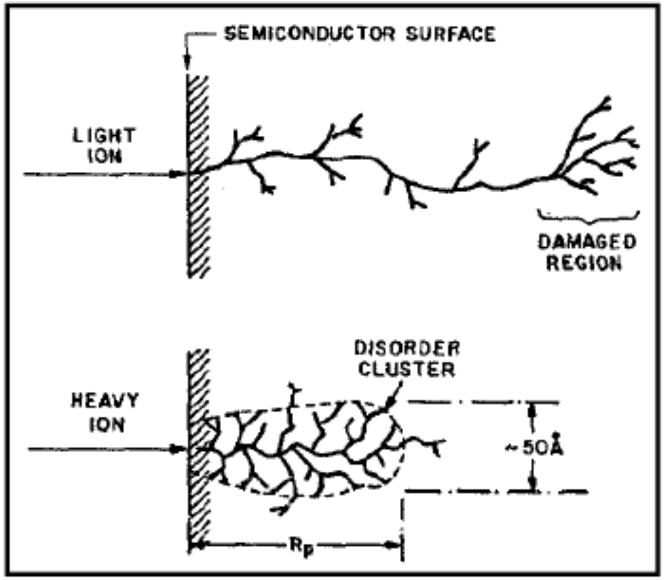
EXAMPLES

Implanting into Si:

H^+ ⇨ Electronic stopping dominates

B^+ ⇨ Electronic stopping dominates

As^+ ⇨ Nuclear stopping dominates



Professor N Cheung, U.C. Berkeley

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Conclusions

- Low-energy accelerators are a powerful tool in biochemistry
- Investments in infrastructures (e.g. microscopes for FLIM, mass spectrometry for molecular fragmentation etc.) are necessary to attract users from chemistry and biology
- Such a facility would be unique in Italy and likely to attract users from all over Europe