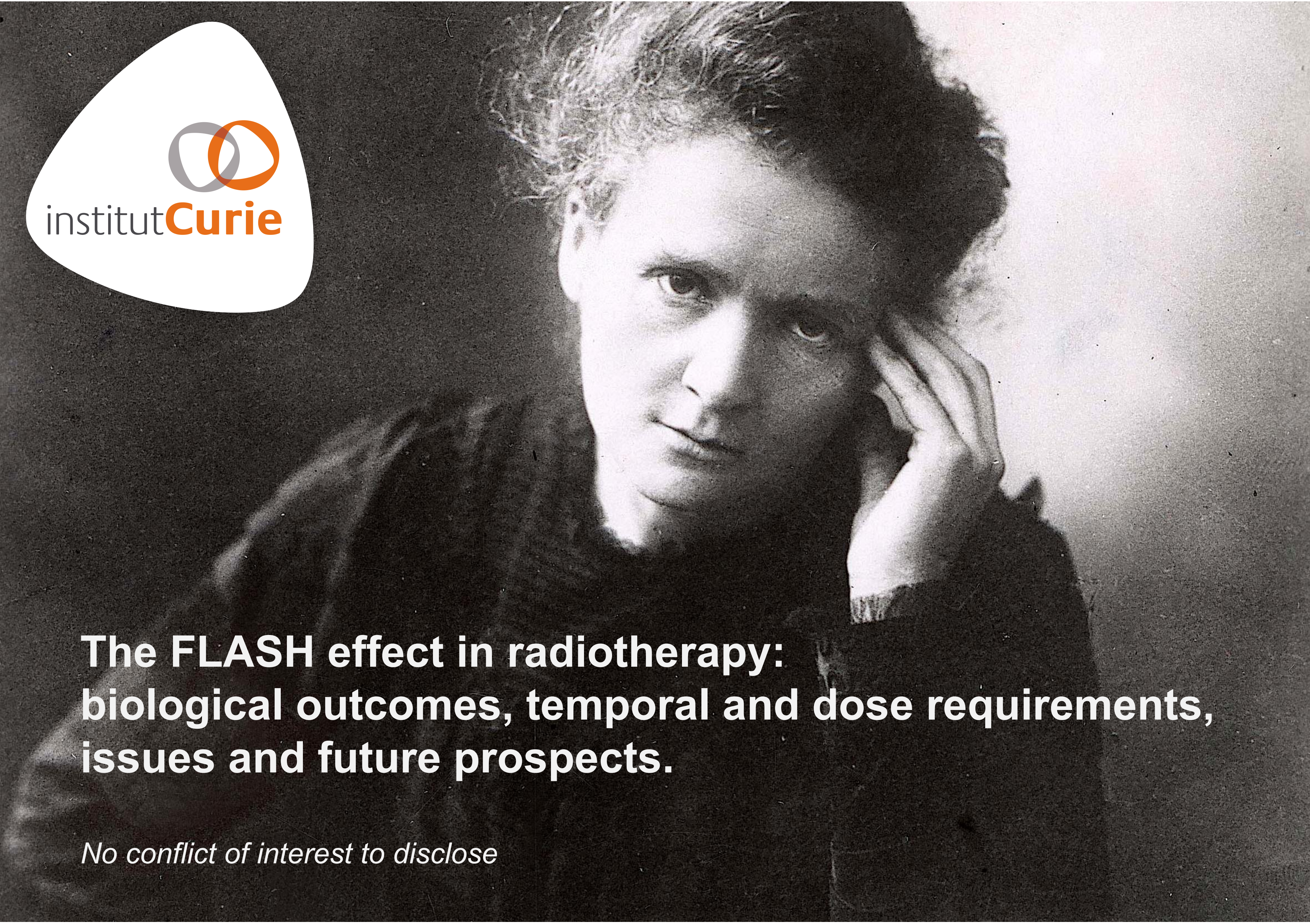


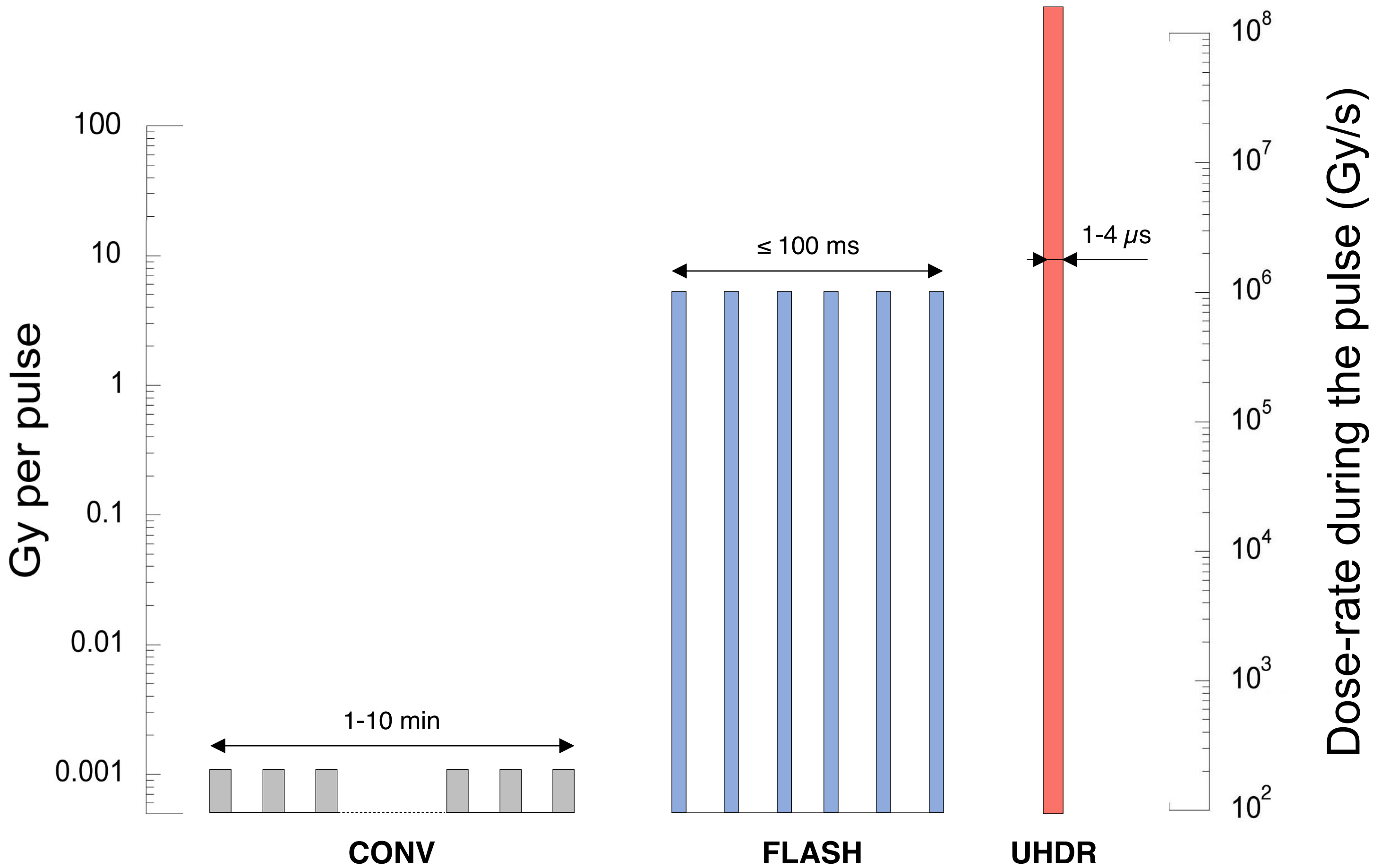
# The FLASH effect in radiotherapy: biological outcomes, temporal and dose requirements, issues and future prospects.

*No conflict of interest to disclose*

Vincent Favaudon – Institut Curie-CdR, Orsay, France

LLR, December 14th, 2020

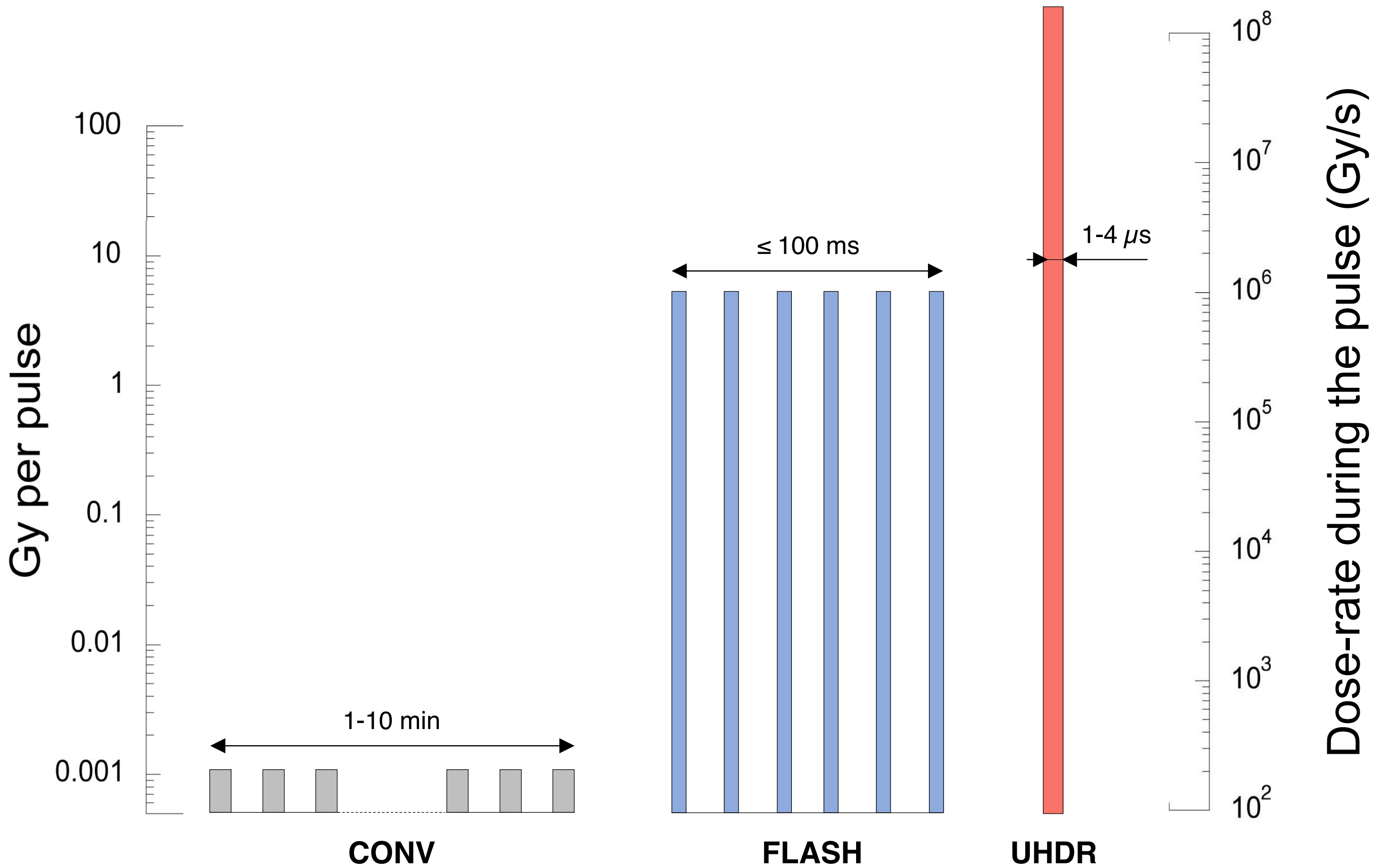
# Temporal structure of energy deposition in the conventional *vs*. FLASH modalities




**2 Gy per day x 15-32 days**

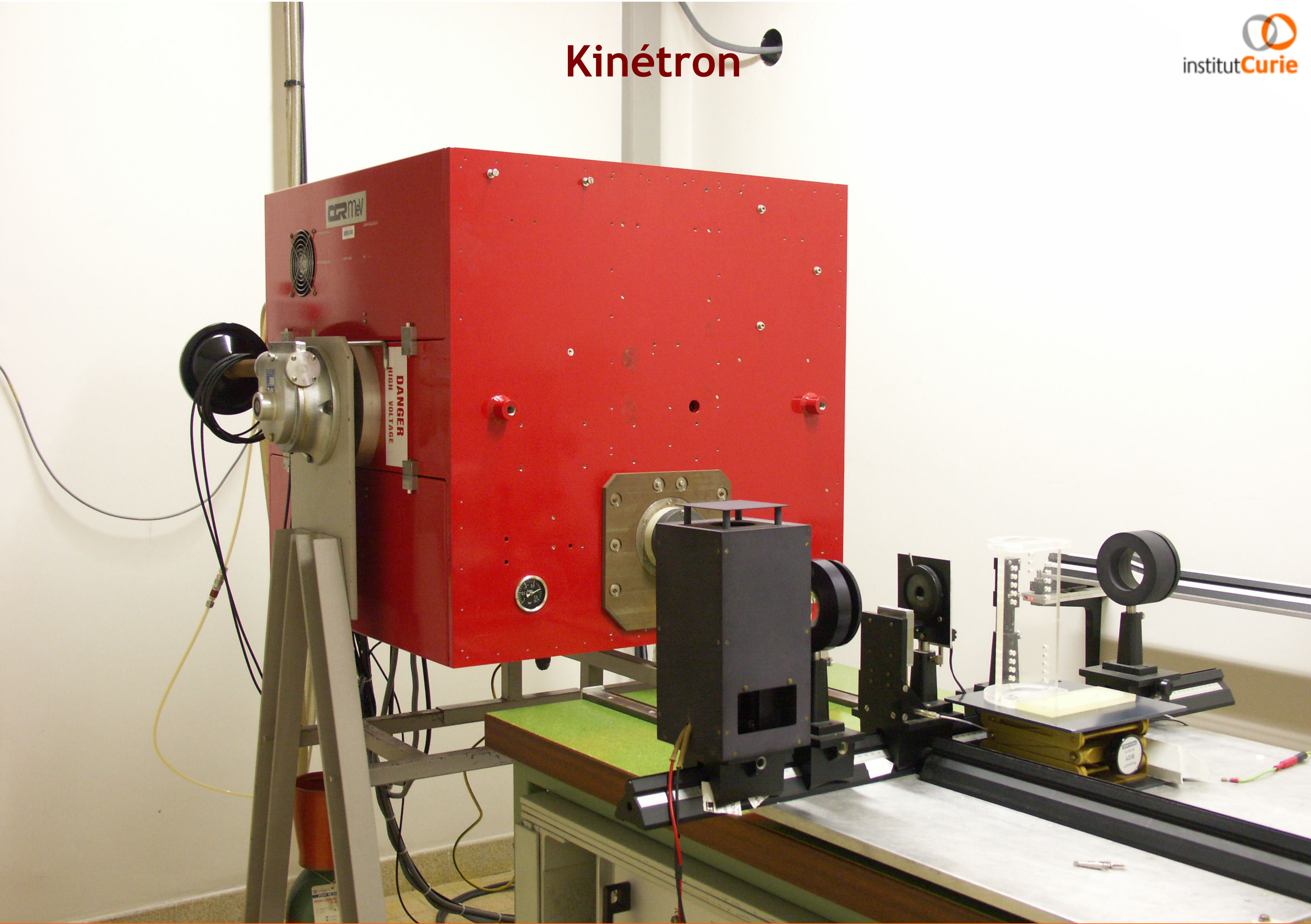
**Single-dose**

# Temporal structure of energy deposition in the conventional *vs*. FLASH modalities




**The FLASH effect stems from a combination of high doses in a single, short session at ultrahigh intra-pulse dose-rate (IPDR).**

# Kinétron



# Specifications

LINAC “Kinétron” (1987)

3.9-5.1 MeV electrons

Triode electron gun

Thermoionic cathode

Pulse width 0.05 - 2.2 µs

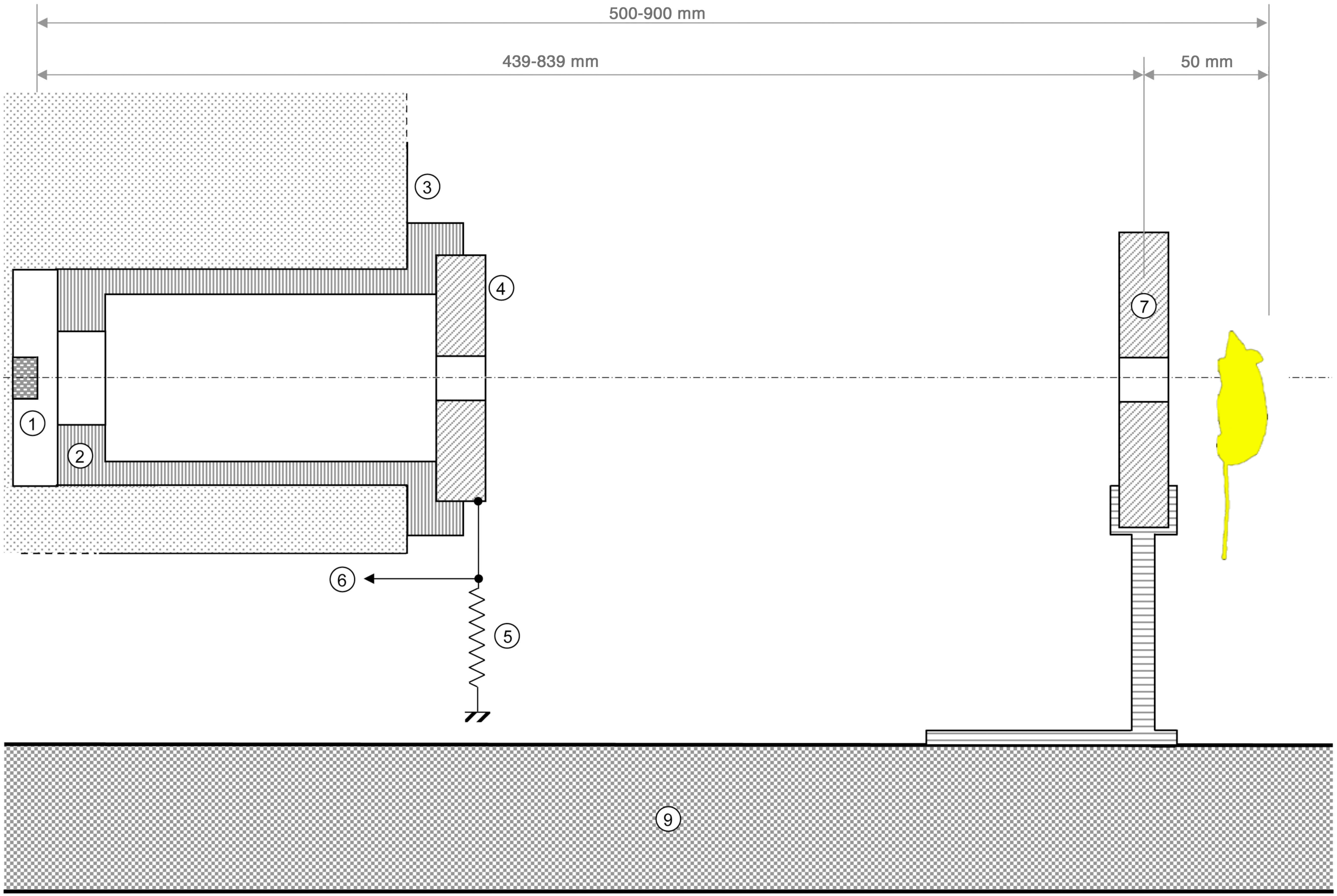
Repeat frequency 0.1 - 200 Hz

Peak current 0.01 - 200 mA (whole emission lobe)

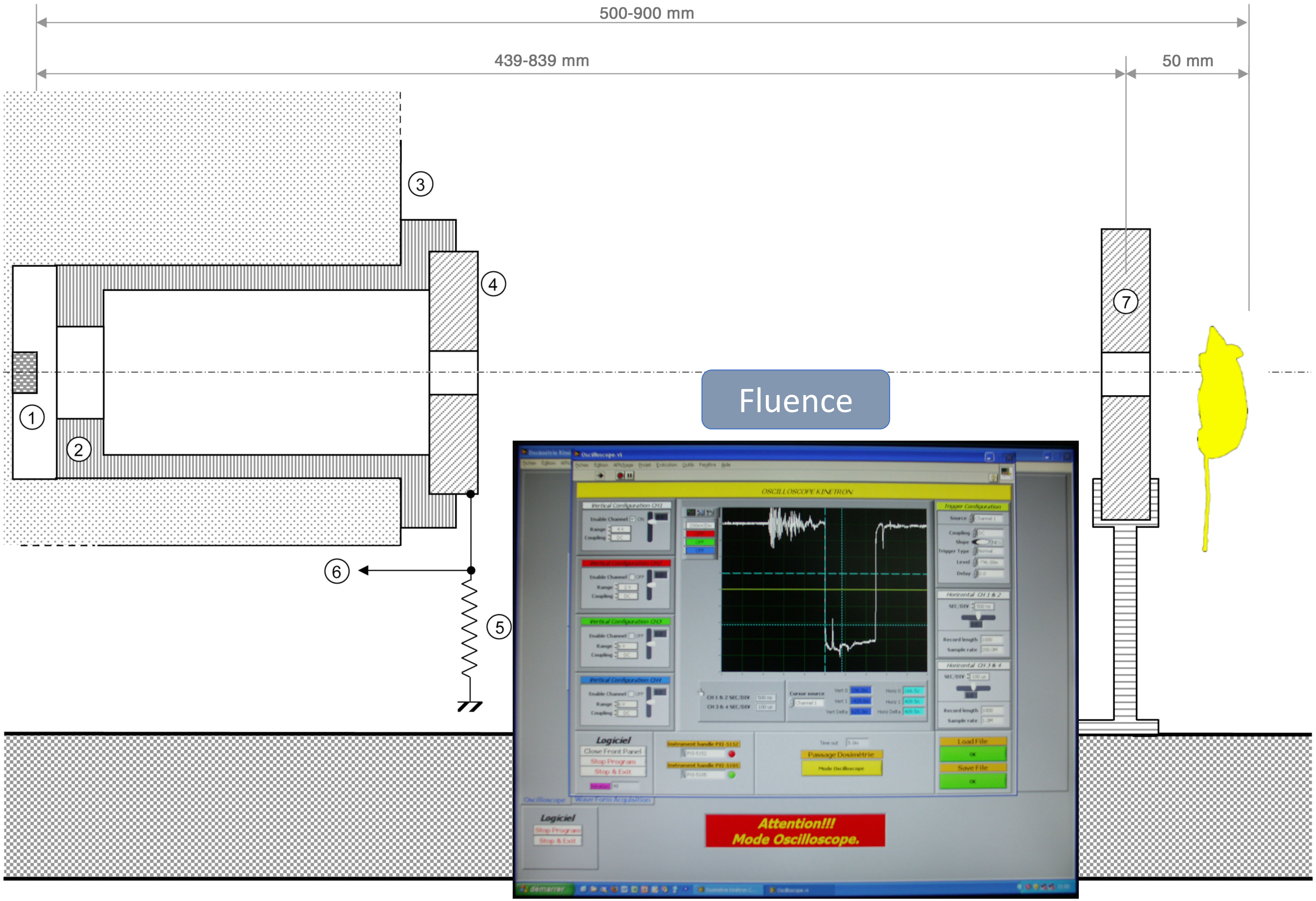
Dose per pulse 0.001 - 50 Gy

Maximum IPDR ≈ 3 $10^7$ $Gy.s^{-1}$

# Setup



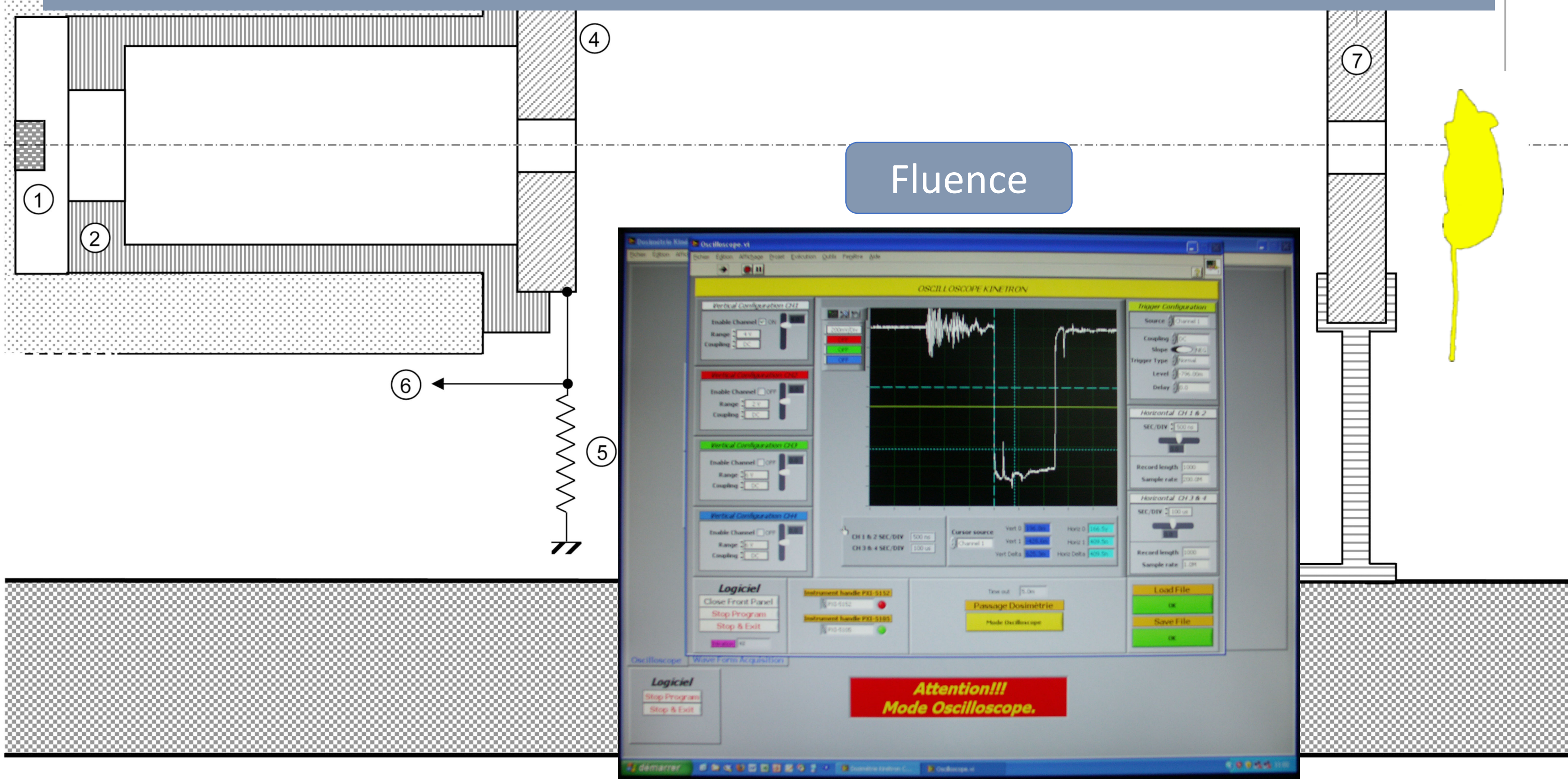
# Setup


500-900 mm
439-839 mm
50 mm
1
2
3
4
5
6
7
Fluence
OSCILLOSCOPE KINETRON
Logiciel
Attention!!!
Mode Oscilloscope.

# Setup

Other available dosimetric methods:

- Methyl viologen radical: submicrosecond, real-time optical detection
- Mallet-Cerenkov light : nanosecond, time-resolved dosimetrey
- Gafchromic EBT3 films (leucomalachite green)
- LiF pellets (thermoluminescence)
- Low dose-rate: Markus ionisation chamber



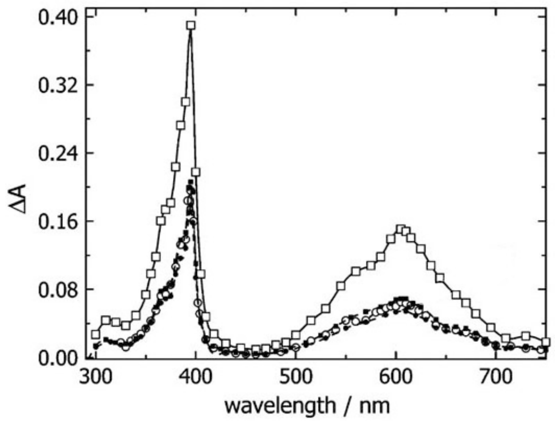
# Methyl viologen radical cation ($N_2O$-$HCOO^-$) as a time-resolved dosimeter in water

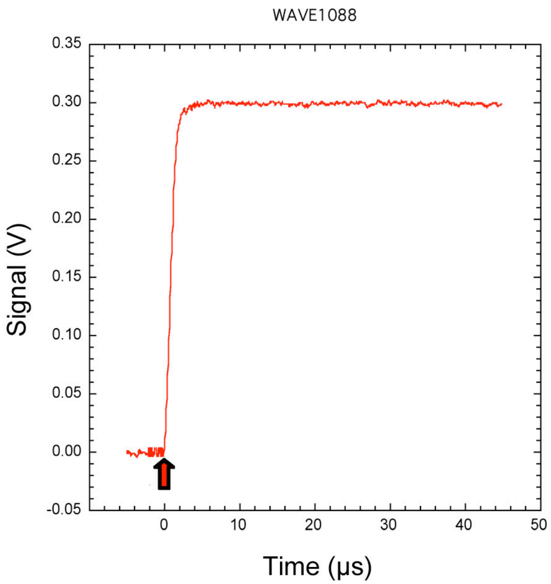
*Das et al. (2003) J Phys Chem **107**: 5998-6006*

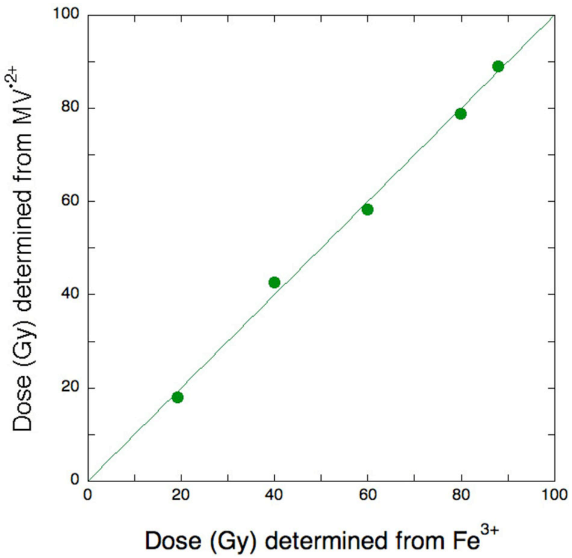
*100 mM formate / $N_2O$ sat.*

*$G = 0.625\ \mu mol.J^{-1}$*

*$\varepsilon_{603} = 13\ 300\ M^{-1}.cm^{-1}$*

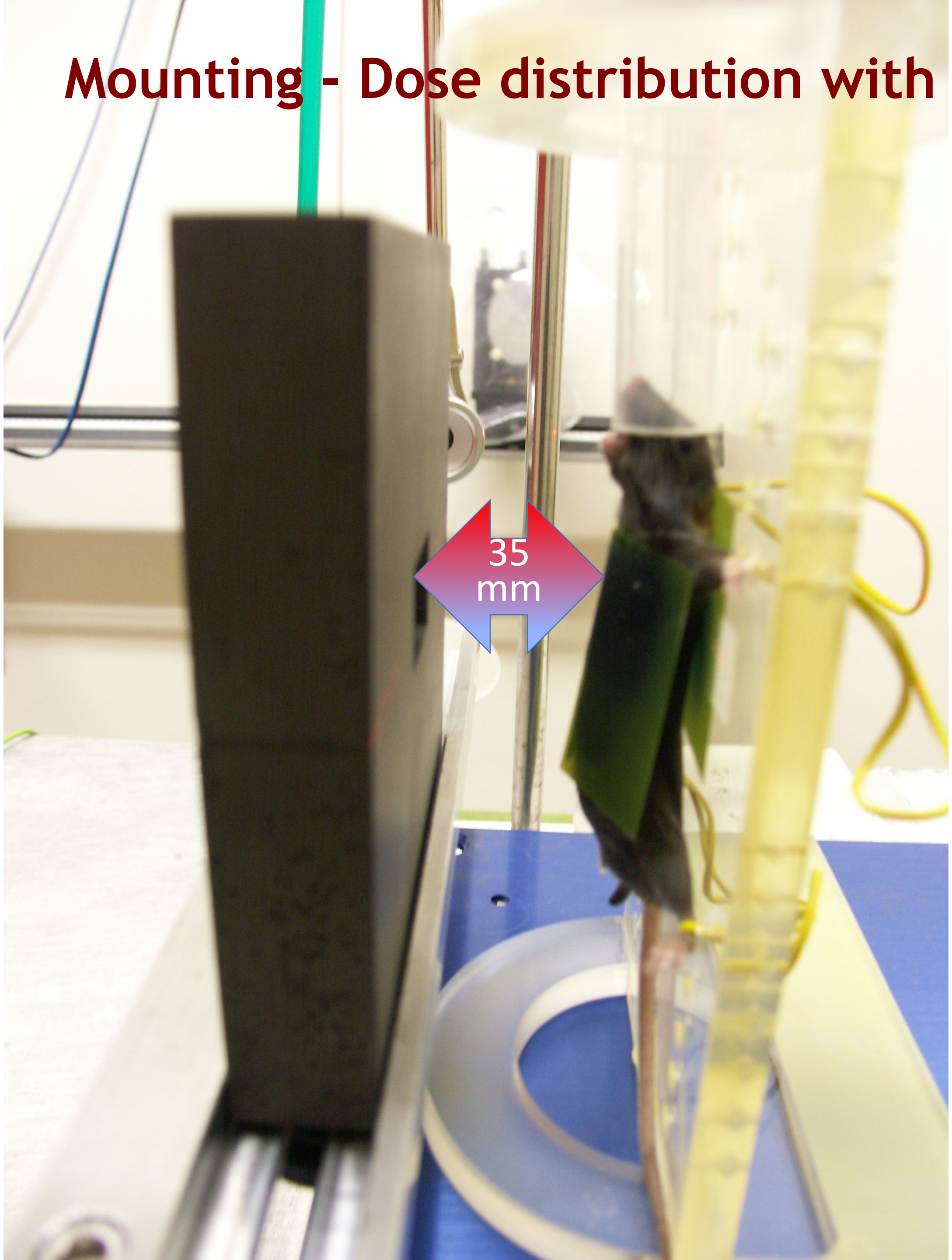


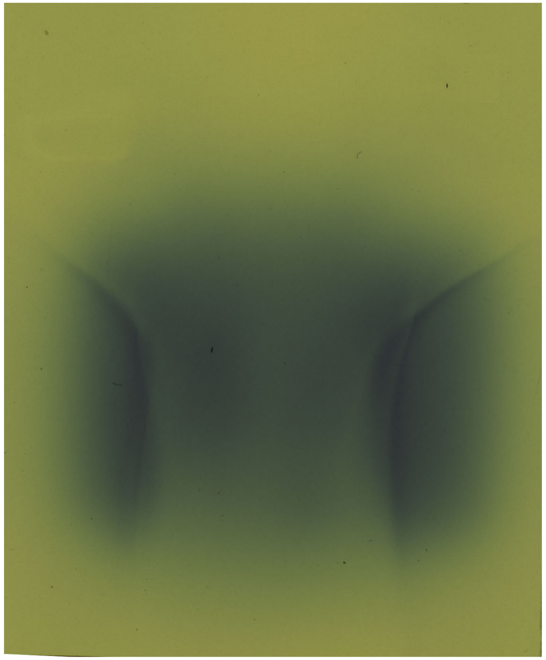



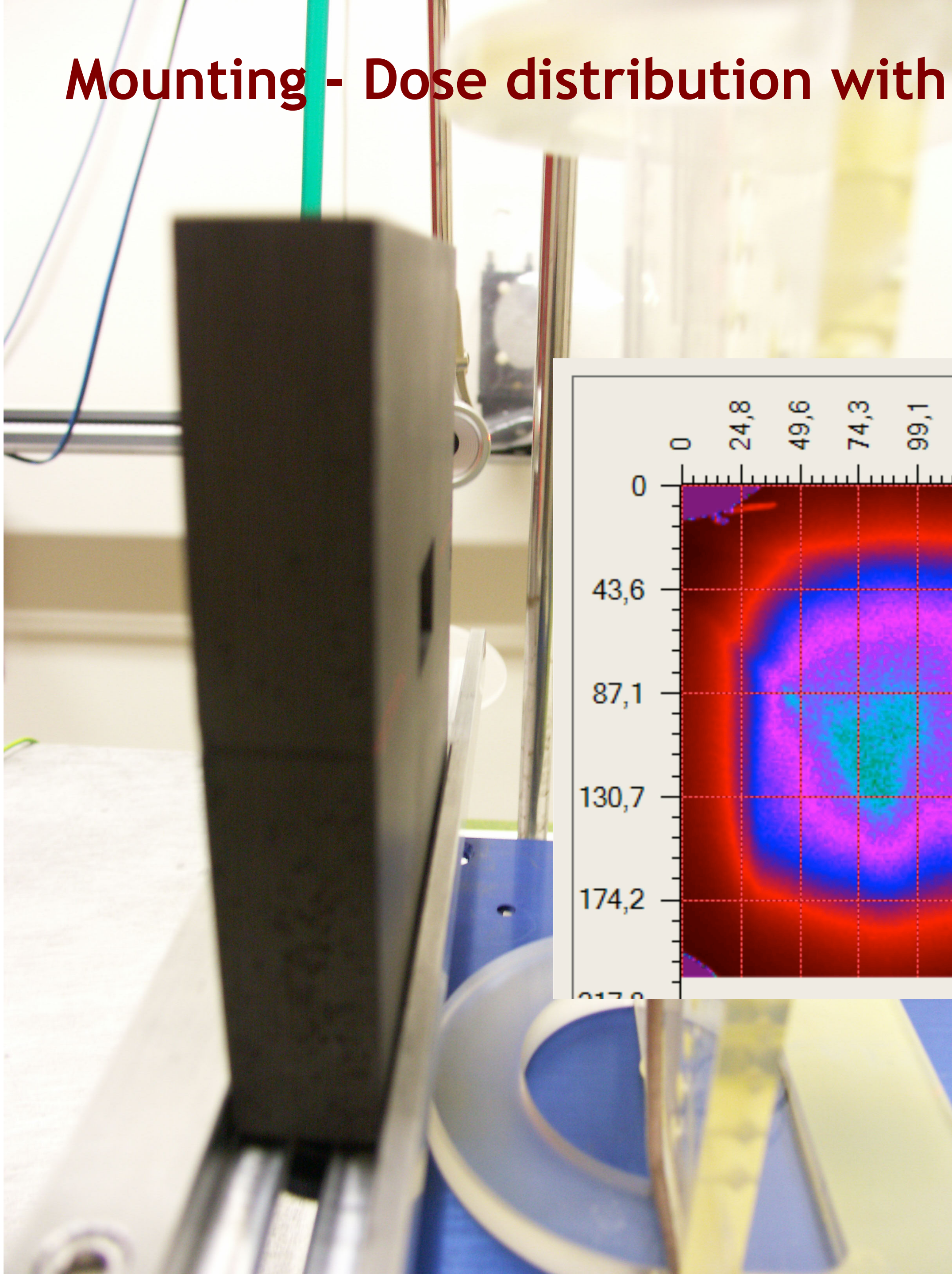
# Mounting - Dose distribution with 4.5MeV electrons

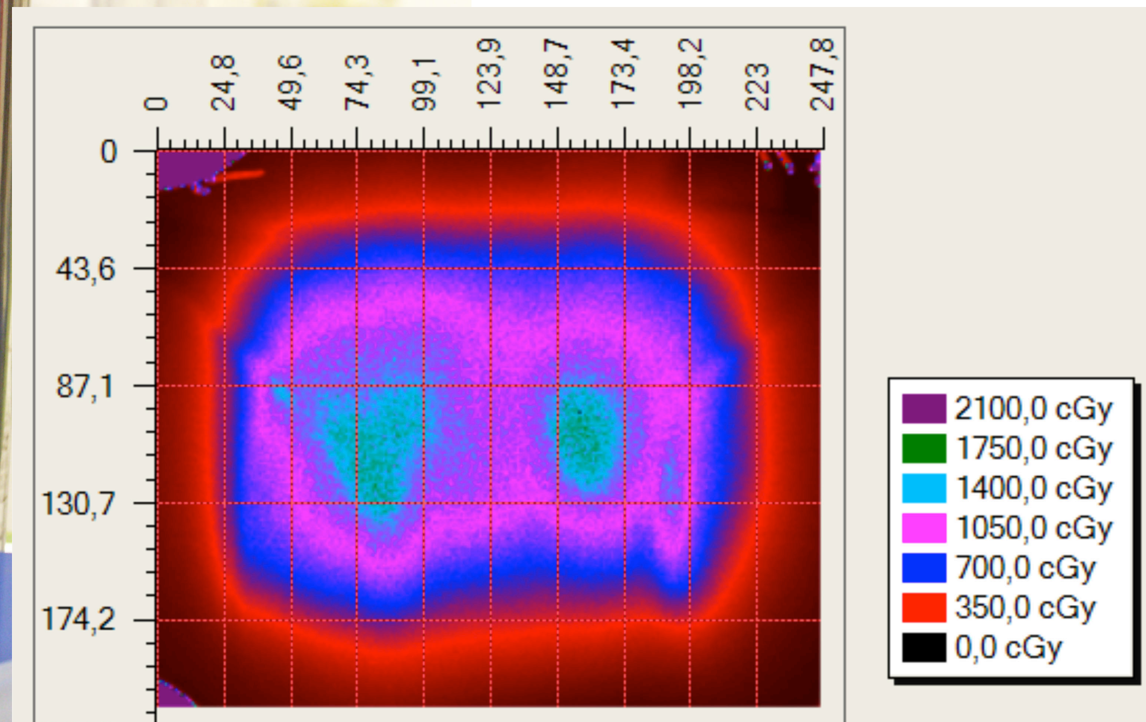




# Mounting - Dose distribution with 4.5MeV electrons





# Radio-induced lung fibrosis in C57BL/6J mice
# 15 Gy in single dose (bilateral thorax irradiation)

**Conventional dose-rate (CONV)**
1 mGy/pulse
IPDR ≈ 3 $10^2$ Gy/s
➤ Beam-on time 5 min

**Ultrahigh dose-rate (FLASH)**
≥ 1 Gy/pulse
IPDR ≈ $10^7$ Gy/s
➤ Beam-on time < 100 ms

1 h – 2 h - 24 h

**Apoptosis**

8 – 16 – 24 – 32 - 36 weeks

**Pneumonitis**
**Inflammation**
**TGF-β activation**
**Lung fibrosis**

**Hair depigmentation**
**Skin necrosis**

# Lung fibrosis – Histology

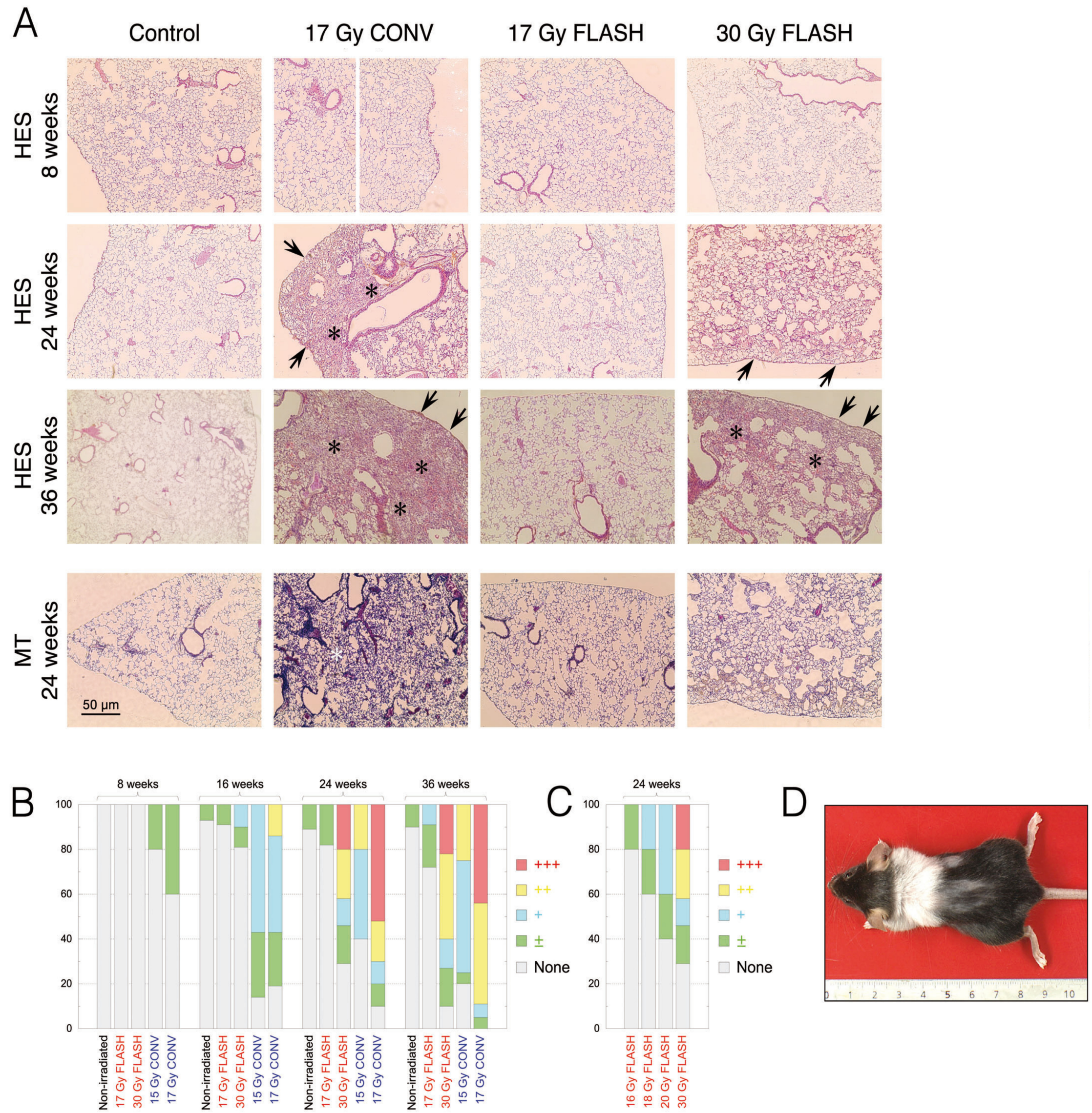


**No fibrosis after FLASH irradiation at doses known to trigger lung fibrosis in C57BL/6J mice.**

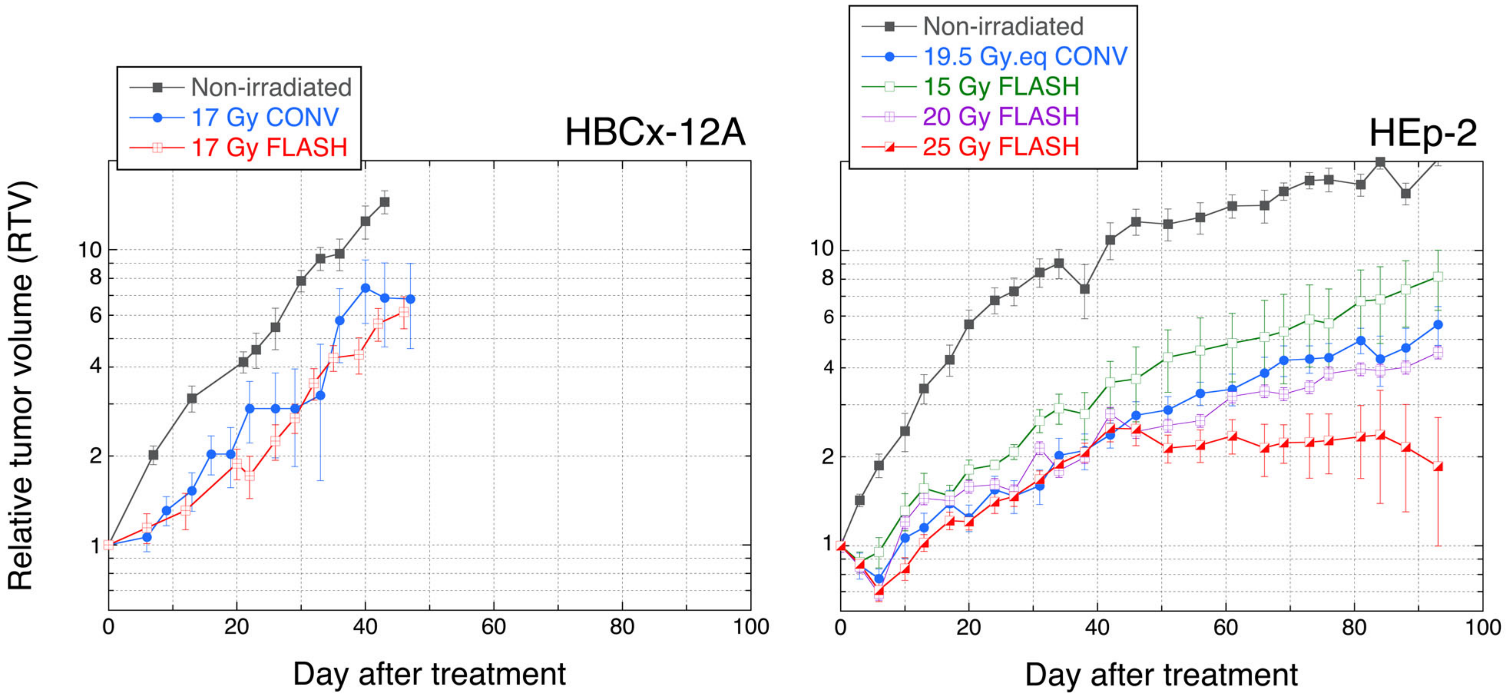
**30 Gy FLASH is nearly equivalent to 17 Gy CONV in terms of fibrogenesis (IPDR ≈ $10^7$ Gy/s).**

**In the skin 30 Gy FLASH spares keratinocytes, not melanocytes (complete de*pig*mentation without *necrosis*).**

**TGFβ, blood capillaries...**



# Cure of tumor xenografts




**FLASH is as efficient as CONV irradiation to cure tumors.**

**Full control of Hep-2 tumors without cutaneous lesions after 25 Gy FLASH.**

# Cure of tumor xenografts

Non-irradiated

**FLASH specifically spares normal tissue from radio-induced complications whilst preserving the antitumor efficiency**

- **Zlobinskaya et al. (2014) Radiat Res 181: 177-183.**
- **Diffenderfer et al. (2020) Int J Radiat Biol 106: 440-448.**
- **Montay-Gruel et al. (2020) Clin Cancer Res (*in press*).**
- **Levy et al. (2020) BiorXiv (*in press*).**

**FLASH is as efficient as CONV irradiation to cure tumors.**

**Full control of Hep-2 tumors without cutaneous lesions after 25 Gy FLASH.**

# The sparing effect of FLASH operates in all normal tissues

| Organ (species) | Publications | Outcomes |
|---|---|---|
| Lung (mouse) | Favaudon et al. (2014) Sci Transl Med 6: 245ra93<br>Fouillade et al. (2020) Clin Cancer Res 26: 1497 | Inflammation / Fibrosis<br>Stem cells / Senescence |
| Brain (mouse) | Montay-Gruel et al. (2017) Radiother Oncol 124: 365<br>Simmons et al. (2019) Radiother Oncol 139: 4<br>Alaghband et al. (2020) Cancers 12: 1671<br>Montay-Gruel et al. (2020) Radiat Res 194 (online) | Neural stem cells / Cognition<br>Cognition<br>Neural inflammation / Cognition<br>Astrogliosis / Cognition |
| Gut (mouse) | Diffenderfer et al. (2020) Int J Radiat Oncol Biol Phys 106: 440<br>Soto et al. (2020) Radiat Res (online)<br>Levy et al. (2020) bioRxciv (online) | Crypt stem cells / Function<br>Crypt stem cells / Function<br>Crypt stem cells / Function |
| Skin (mouse)<br><br>Skin (pig) | Favaudon et al. (2014) Sci Transl Med 6: 245ra93<br>Soto et al. (2020) Radiat Res (online)<br>Vozenin et al. (2019) Clin Cancer Res 25: 35 | Depigmentation / Ulceration<br>Ulceration / Regeneration<br>Ulceration / Regeneration |
| Bone marrow (mouse) | Chabi et al. (2020) Int J Radiat Oncol Biol Phys (online) | Hematopoiesis / Stem cells |
| Zebra fish embryos | Beyreuther et al. (2019) Radiother Oncol 139: 46 | Embryo development |
| Normal human cells *in vitro* | Buonnano et al. (2019) Radiother Oncol 139: 51<br>Fouillade et al. (2020) Clin Cancer Res 26: 1497 | Radio-induced senescence<br>DNA damage, clonogenicity |
| Nasal planum (cats) | Vozenin et al. (2019) Clin Cancer Res 25: 35 | Cure of spontaneous carcinoma |
| Skin (humans) | Bourhis et al. (2019) Radiother Oncol 139: 18 | Treatment of a first patient with FLASH-RT |

Published OnlineFirst December 3, 2019; DOI: 10.1158/1078-0432.CCR-19-1440

CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

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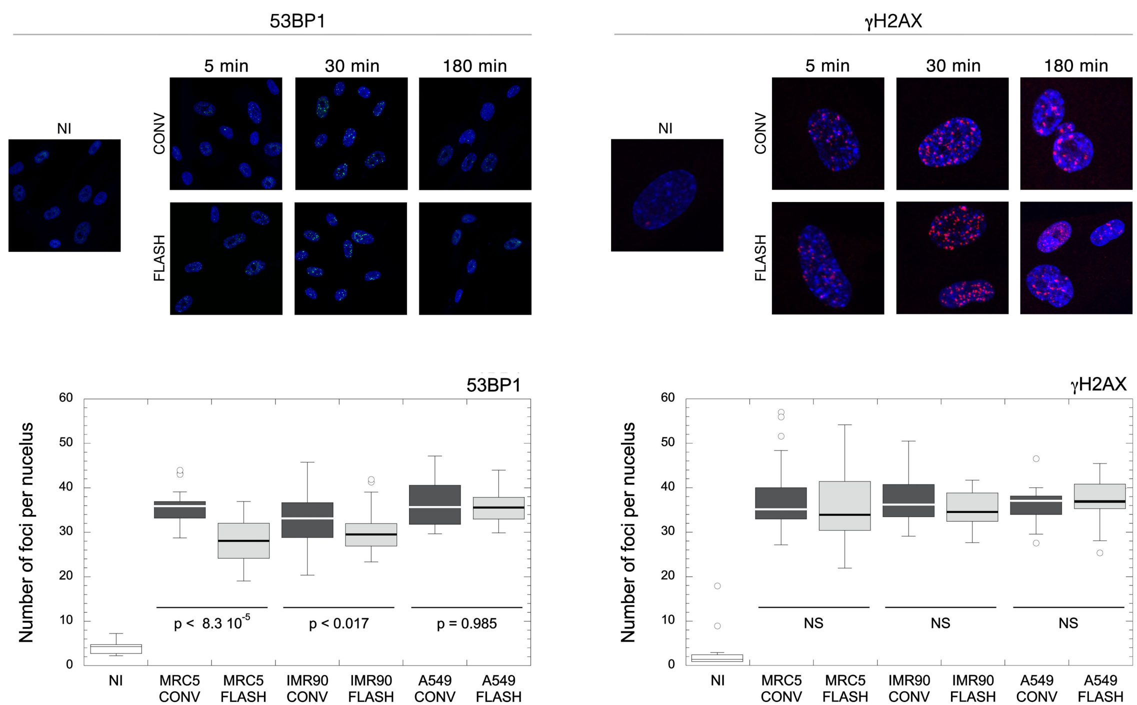
# FLASH Irradiation Spares Lung Progenitor Cells and Limits the Incidence of Radio-induced Senescence

Charles Fouillade[1], Sandra Curras-Alonso[1,2], Lorena Giuranno[3], Eddy Quelennec[1], Sophie Heinrich[1,4], Sarah Bonnet-Boissinot[1], Arnaud Beddok[1], Sophie Leboucher[5], Hamza Umut Karakurt[2], Mylène Bohec[6], Sylvain Baulande[6], Marc Vooijs[3], Pierre Verrelle[7,8], Marie Dutreix[1], Arturo Londoño-Vallejo[2], and Vincent Favaudon[1]

- **FLASH elicits less early foci of 53BP1 than CONV in normal cells only.**



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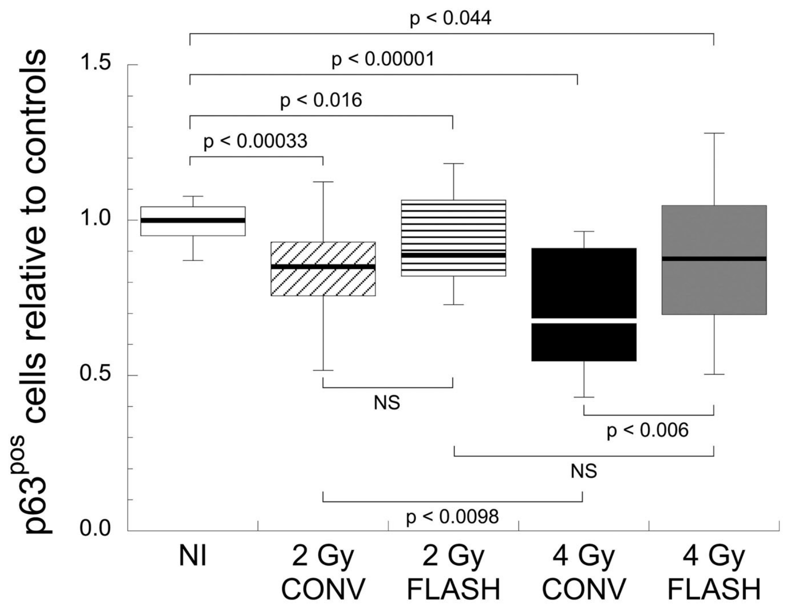
- **FLASH spares normal human lung stem cells *in vitro* from radio-induced cell death**



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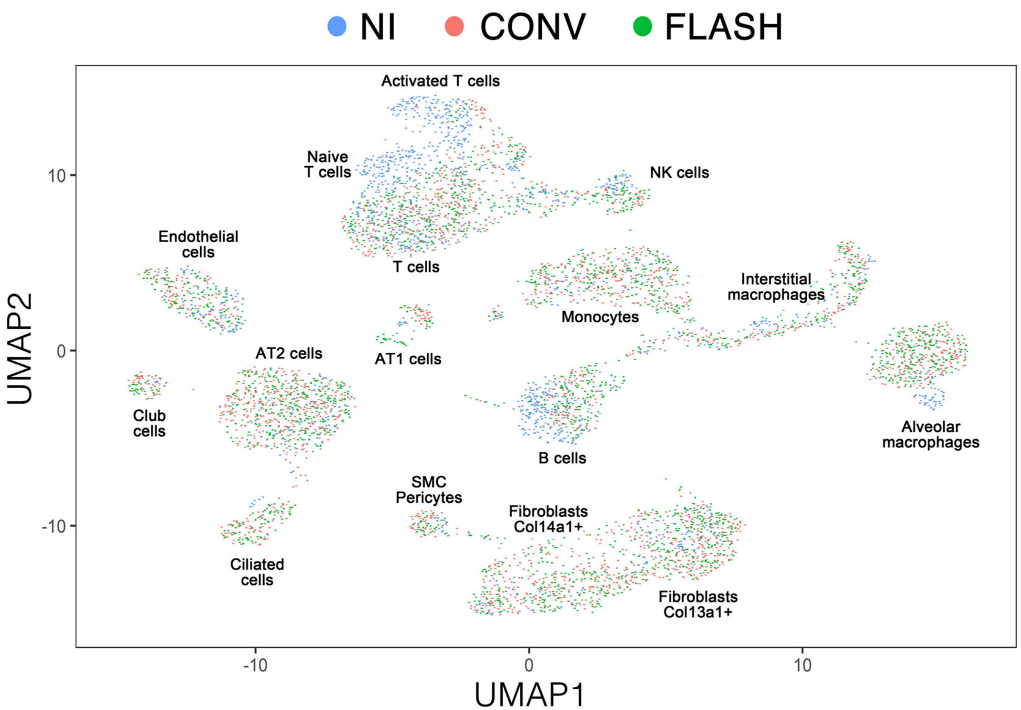
- **sc-RNAseq allows identifying the nature and localisation of progenitor cells in irradiated mouse lung evolving to fibrosis.**



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CLINICAL CANCER RESEARCH | TRANSLATIONAL CANCER MECHANISMS AND THERAPY

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# FLASH Irradiation Spares Lung Progenitor Cells and Limits the Incidence of Radio-induced Senescence

Check for updates

Charles Fouillade[1], Sandra Curras-Alonso[1,2], Lorena Giuranno[3], Eddy Quelennec[1], Sophie Heinrich[1,4], Sarah Bonnet-Boissinot[1], Arnaud Beddok[1], Sophie Leboucher[5], Hamza Umut Karakurt[2], Mylène Bohec[6], Sylvain Baulande[6], Marc Vooijs[3], Pierre Verrelle[7,8], Marie Dutreix[1], Arturo Londoño-Vallejo[2], and Vincent Favaudon[1]

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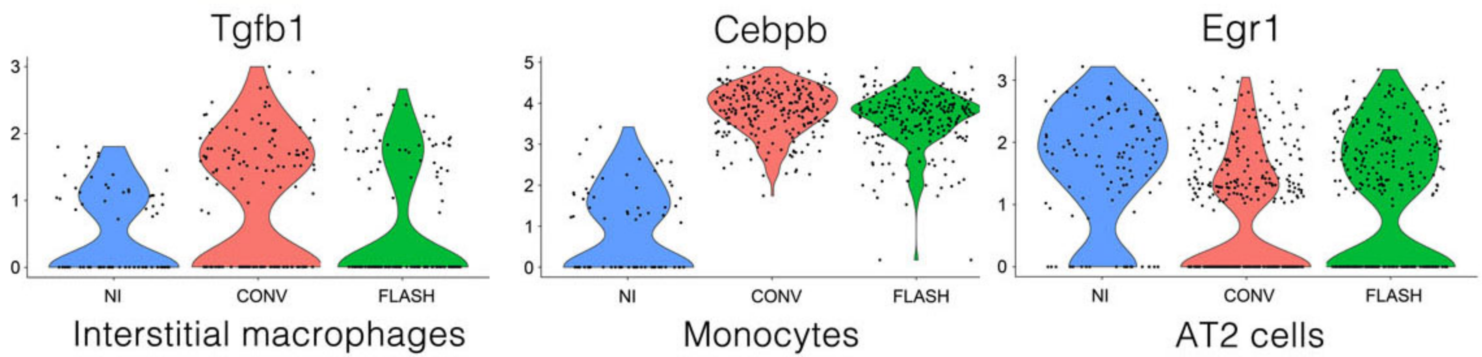

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# FLASH Irradiation Spares Lung Progenitor Cells and Limits the Incidence of Radio-induced Senescence

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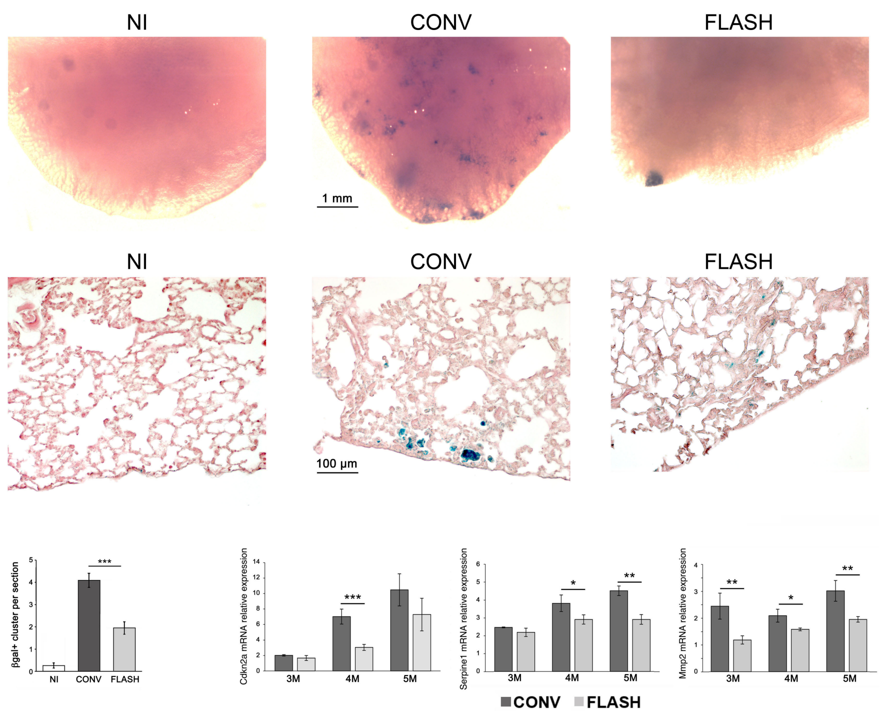
- **FLASH spares irradiated lung from radio-induced senescence and lowers the expression of pro-inflammatory factors (SA-β-galactosidase assay).**



# Increasing the partial pressure of oxygen abolishes the FLASH effect in mouse brain

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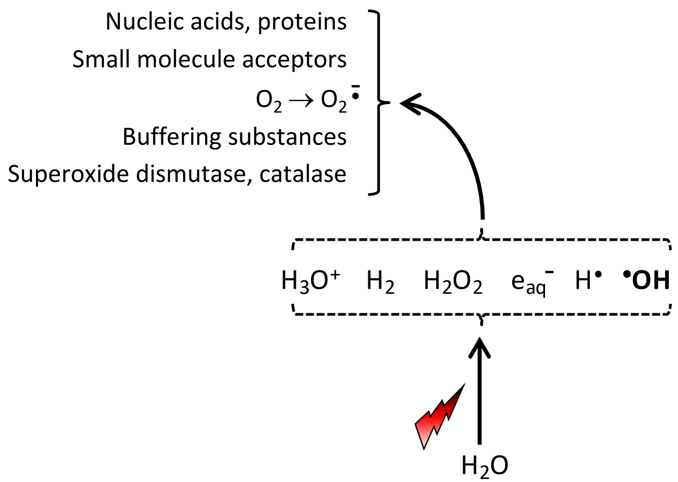
## Experimental

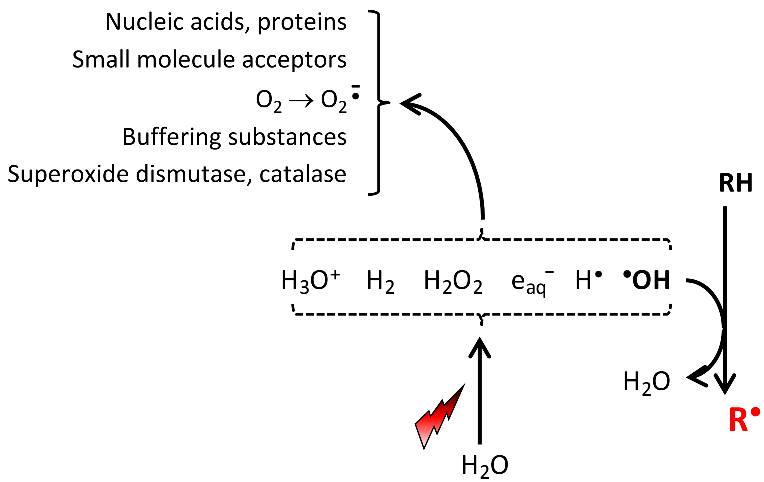
| Publication | Outcome |
|---|---|
| *Montay-Gruel (2019) Proc Natl Acad Sci USA 116: 10943* | Cognition / Inflammation |
| *Simmons et al. (2019) Radiother Oncol 139: 4* | Cognition / Dendritic spines |

## Computational

| Publication | Model |
|---|---|
| *Spitz et al. (2019) Radiother Oncol 139: 23*<br>*Pratx et al. (2019) Int J Radiat Oncol Biol Phys 105: 190*<br>*Petersson et al. (2020) Int J Radiat Oncol Biol Phys 107: 539*<br>*Abolfath et al. (2019) Med Phys (in press)* | Oxygen depletion |
| *Labarbe et al. (2020) Radiother Oncol (in press)* | Radical recombination |

CHUV UCI institutCurie iba Stanford University INDIANA UNIVERSITY SCHOOL OF MEDICINE UNIVERSITY OF OXFORD THE UNIVERSITY OF TEXAS MD Anderson Cancer Center IOWA Penn UNIVERSITY of PENNSYLVANIA


Nucleic acids, proteins
Small molecule acceptors
$O_2 \rightarrow O_2^{\bullet-}$
Buffering substances
Superoxide dismutase, catalase
$H_3O^+$ $H_2$ $H_2O_2$ $e_{aq}^-$ $H^\bullet$ $^\bullet$**OH**
$H_2O$


Nucleic acids, proteins
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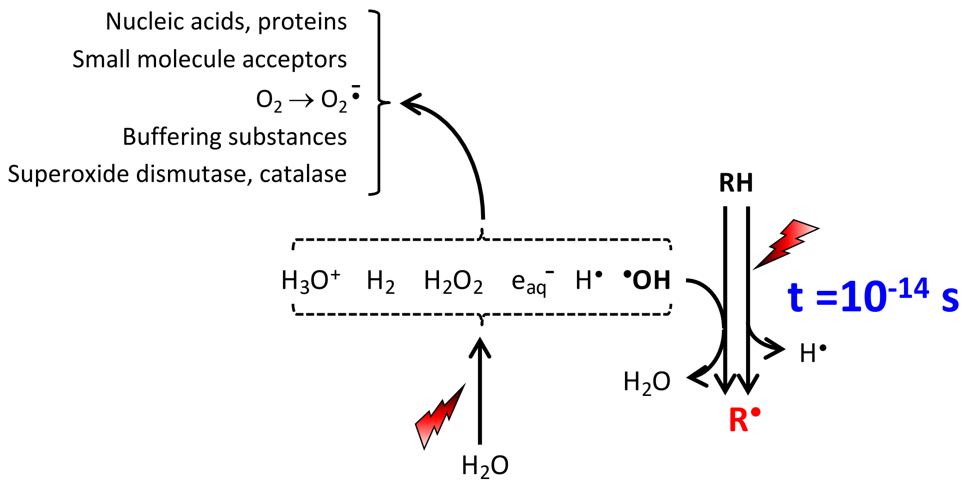
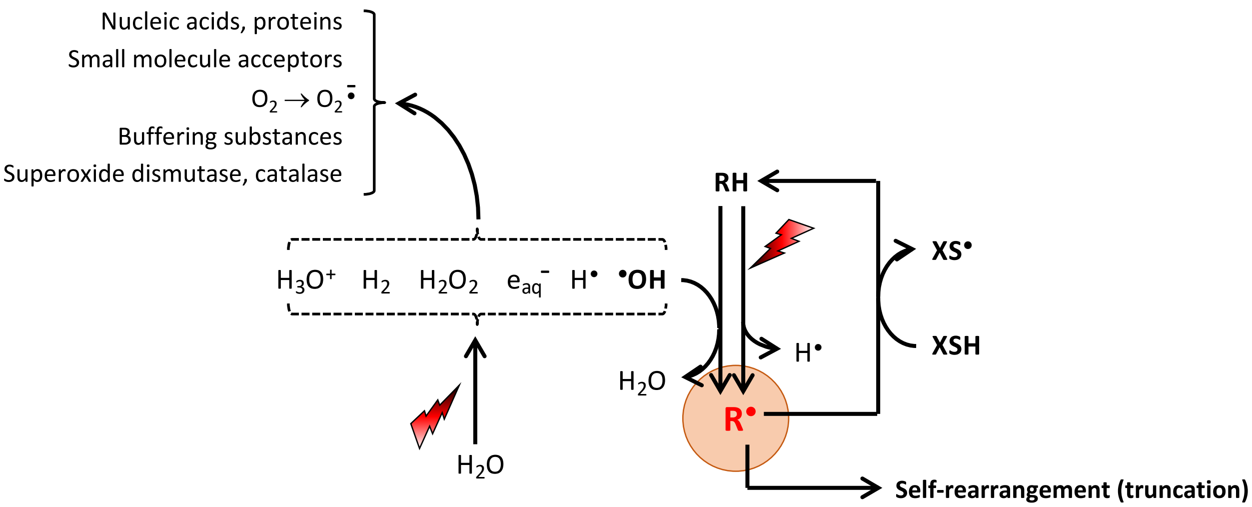
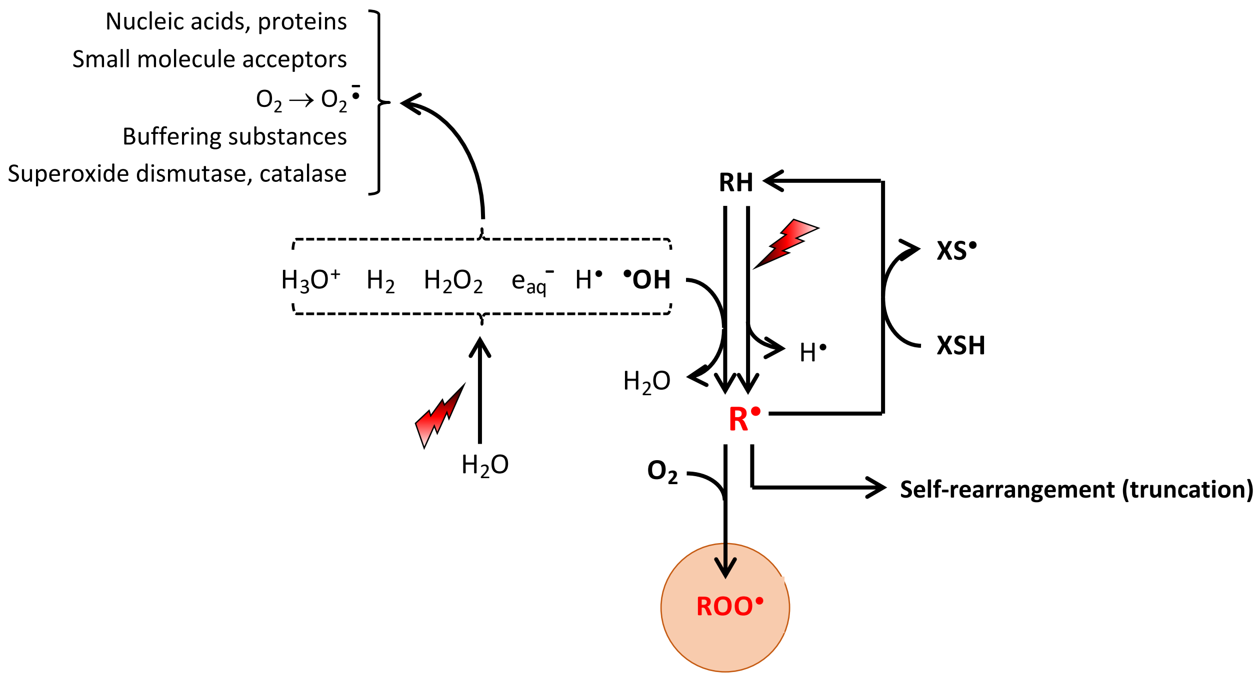
$O_2 \rightarrow O_2^{\bullet -}$
Buffering substances
Superoxide dismutase, catalase
$H_3O^+$ $H_2$ $H_2O_2$ $e_{aq}^-$ $H^\bullet$ $^\bullet OH$
RH
$H_2O$
$R^\bullet$
$H_2O$

**$t = 10^{-9} - 10^{-6}$ s**

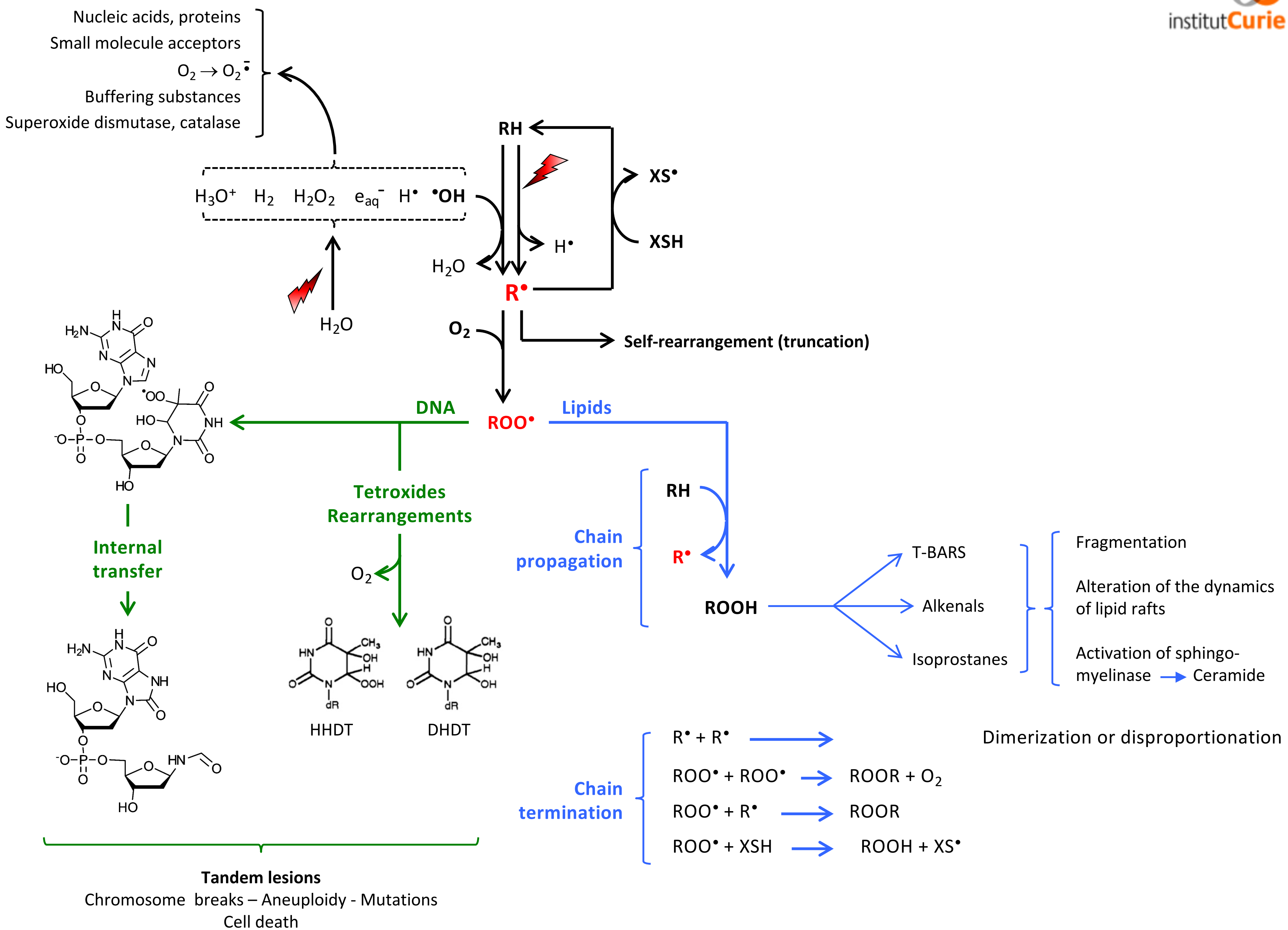

Nucleic acids, proteins
Small molecule acceptors
$O_2 \rightarrow O_2^{\bullet -}$
Buffering substances
Superoxide dismutase, catalase
$H_3O^+$ $H_2$ $H_2O_2$ $e_{aq}^-$ $H^\bullet$ $^\bullet OH$
$H_2O$
RH
$H_2O$
$H^\bullet$
$R^\bullet$
$t = 10^{-14}$ s



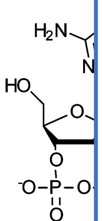

$t_{1/2} \approx 5$ ms




$t_{1/2} \approx$ **1-10 s**


Nucleic acids, proteins
Small molecule acceptors
O2 → O2•−
Buffering substances
Superoxide dismutase, catalase
H3O+ H2 H2O2 eaq− H• •OH
H2O
RH
XS•
XSH
H•
H2O
R•
O2
Self-rearrangement (truncation)
DNA
ROO•
Lipids
Tetroxides
Rearrangements
O2
HHDT
DHDT
Internal transfer
RH
R•
Chain propagation
ROOH
T-BARS
Alkenals
Isoprostanes
Fragmentation
Alteration of the dynamics of lipid rafts
Activation of sphingo-myelinase → Ceramide
R• + R•
Dimerization or disproportionation
ROO• + ROO• → ROOR + O2
ROO• + R• → ROOR
ROO• + XSH → ROOH + XS•
Chain termination
Tandem lesions
Chromosome breaks – Aneuploidy - Mutations
Cell death

Nucleic acids, proteins
Small molecule acceptors
$O_2 \rightarrow O_2^{\bullet -}$
Buffering substances
Superoxide di



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Radiotherapy and Oncology xxx (xxxx) xxx

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journal homepage: www.thegreenjournal.com

Original Article

# A physicochemical model of reaction kinetics supports peroxyl radical recombination as the main determinant of the FLASH effect

Rudi Labarbe [a], Lucian Hotoiu [a,*], Julie Barbier [a], Vincent Favaudon [b,1]

[a] Ion Beam Applications S.A. (IBA), Louvain-la-Neuve, Belgium; [b] Institut Curie, Inserm U 1021-CNRS UMR 3347, University Paris-Saclay, PSL Research University, Centre Universitaire, Orsay Cedex, France

In
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of the dynamics
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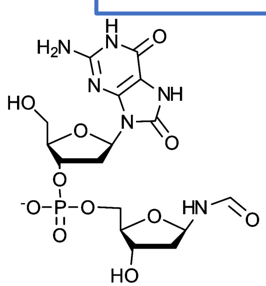
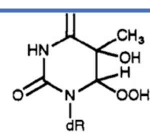
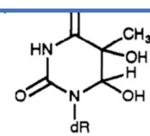




HHDT



DHDT

Isoprostanes

Activation of sphingo-
myelinase → Ceramide

**Chain termination**

| | | |
|---|---|---|
| $R^\bullet + R^\bullet$ | → | Dimerization or disproportionation |
| $ROO^\bullet + ROO^\bullet$ | → | $ROOR + O_2$ |
| $ROO^\bullet + R^\bullet$ | → | ROOR |
| $ROO^\bullet + XSH$ | → | $ROOH + XS^\bullet$ |

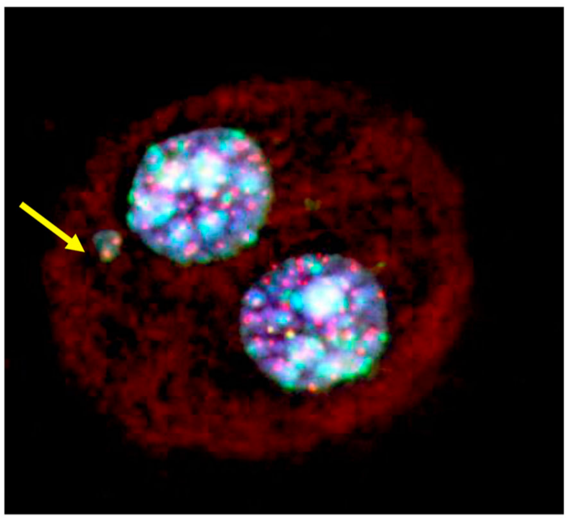
**Tandem lesions**
Chromosome breaks – Aneuploidy - Mutations
Cell death

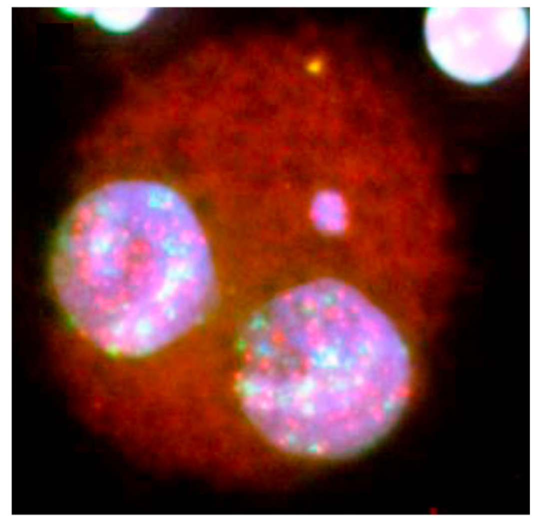
Radiat Environ Biophys (2011) 50:253–263
DOI 10.1007/s00411-011-0353-1

ORIGINAL PAPER

# Dose rate effect on micronuclei induction in human blood lymphocytes exposed to single pulse and multiple pulses of electrons

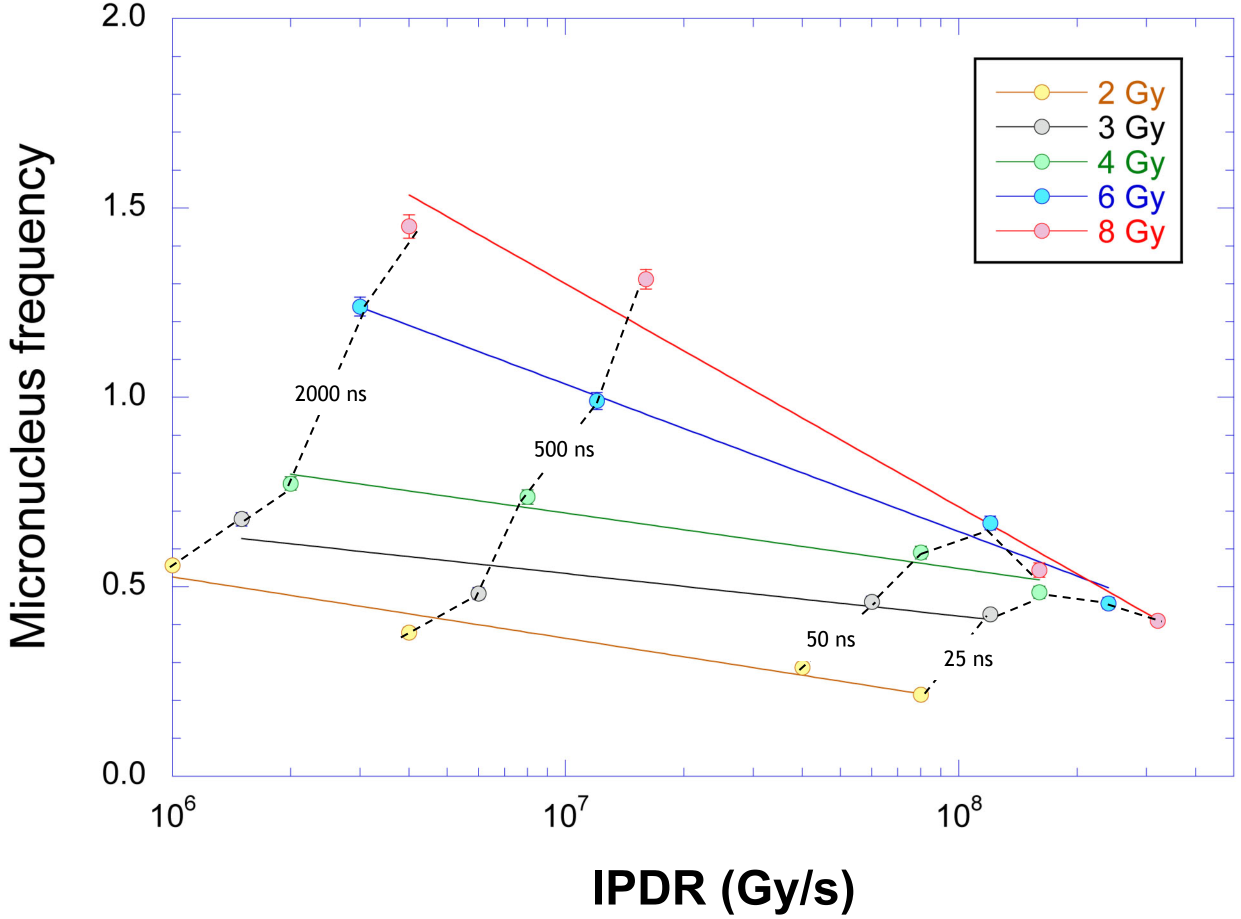
**Santhosh Acharya · N. N. Bhat · Praveen Joseph · Ganesh Sanjeev · B. Sreedevi · Y. Narayana**



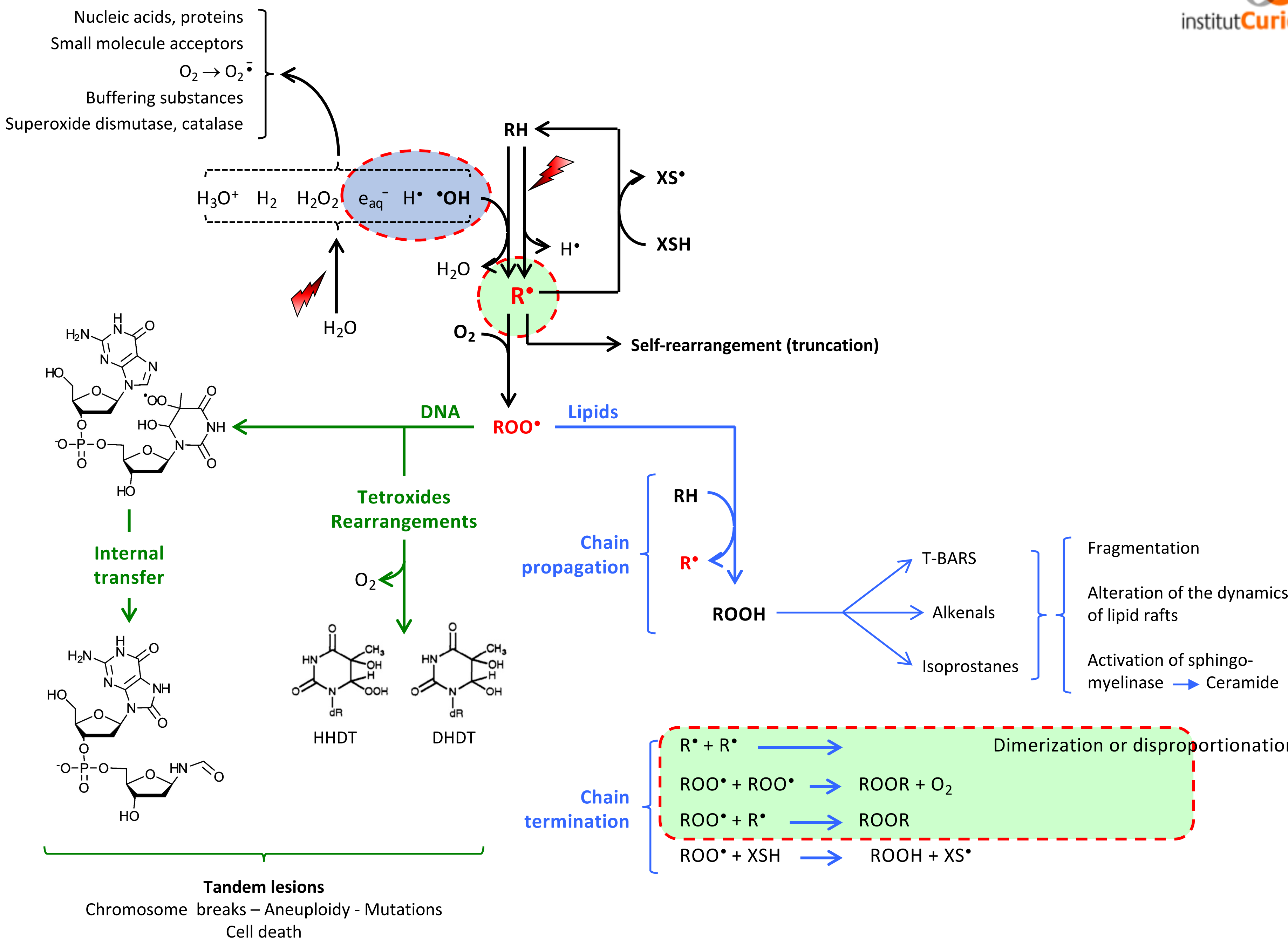


Cytokinesis block for precise determination of acentric chromosomes

# The micronucleus frequency decreases in inverse ratio to the IPDR




Drawn from Acharya *et al.* (2011) Radiat Environ Biophys 50: 253-263.




institutCurie

# Institutions involved in FLASH studies (2020)

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**France**
institut Curie · École Vétérinaire Alfort · Institut du thorax Curie - Montsouris · cea · Gustave Roussy Cancer Campus Grand Paris · ESRF The European Synchrotron · MICEN VET

**Switzerland**
CHUV · Paul Scherrer Institut PSI · EPFL · TUM · CERN

**Germany**
HZDR · PTB

**Netherlands**
Antwerp · umcg Groningen

**England**
University of Oxford · NHS Barts Health NHS Trust · Manchester 1824 The University of Manchester

**USA**
UCI · Indiana University School of Medicine · Iowa · Penn University of Pennsylvania · Stanford University · Johns Hopkins University · The University of Texas MD Anderson Cancer Center · Columbia

**China**
清華大学 Tsinghua University · 東吳大學 Soochow University

# Industrial projects - IORT

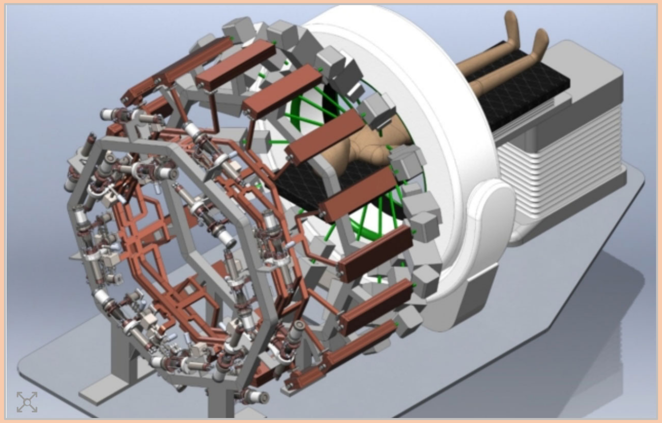



PMB-Alcen (Fr)



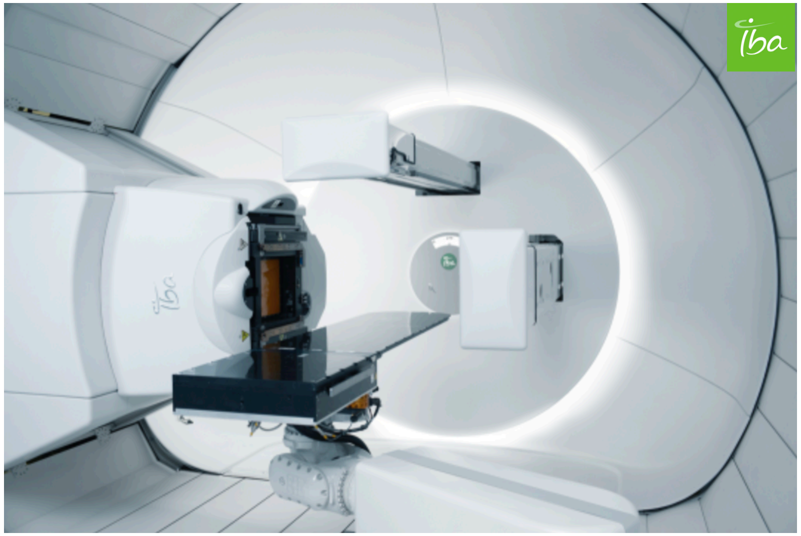
Sordina IORT (It)


Mobetron (CA)



Stanford (CA)

# Industrial projects - Protons and VHEE




Protontherapy (IBA)


Very High Energy Electrons

**Institut Curie (Orsay)**
**Inserm U 612**
Charles Fouillade
Marie-France Poupon
Mano Sayarath
Jean-Michel Lentz
Frédéric Pouzoulet
Eddy Quelennec
Arnaud Beddok
Sarah Bonnet-Boissinot
Janet Hall
Vincent Favaudon
*Animal care facilities*
*Histology platform*

**Inserm U 1196**
**Institut Curie, Orsay**
Pierre Verrelle

**Institut Curie (Paris)**
**Inserm U 900**
Isabel Brito
Philippe Hupé

**Inserm U 1021 – CNRS UMR 3347**
**Institut Curie, Orsay**
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Sophie Heinrich
Lorena Giuranno
Pierre-Marie Girard
Marie Dutreix
Vincent Favaudon

**Inserm U 3244**
**Institut Curie, Paris**
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Hamza-Umut Karakurt
José-Arturo Londono-Vallejo

**CNRS UMR 3348**
**Institut Curie, Orsay**
Sophie Leboucher

**ICGex Platform**
**Institut Curie, Paris**
Mylène Bohec
Sylvain Baulande

**ProtoFlash Group**
**Radiation Oncology, Institut Curie, Paris & Orsay (ICPO)**
Pierre Verrelle
Ludovic de Marzi
Annalisa Patriarca
Valentin Calugaru
Farid Goudjil
Alejandro Mazal

**Institut Gustave Roussy, Inserm U 1030 (Villejuif)**
Virginie Monceau
Benoît Petit
Jean Bourhis
Marie-Catherine Vozenin
*Animal care facilities*
*Veterinary anapath team*

**National Veterinary School Pathology lab. (Maisons-Alfort)**
Laura Caplier
Jean-Jacques Fontaine
*Veterinary anapath team*

**Dept Radiotherapy, Grow-School, Maastricht, Netherlands.**
Marc Vooijs

**INSA-Lyon**
**Platform of Functional Lipidomics**
Nathalie Bernoud-Hubac
Patricia Daira
Baptiste Fourmaux

**INAC/SCIB**
**CEA Grenoble**
Jean-Luc Ravanat

**Laboratory of Radio-Oncology**
**CHUV, Lausanne**
Pierre Montay-Gruel
Marie-Catherine Vozenin

**IBA Research Group**
**Louvain-la-Neuve, Belgium**
Rudi Labarbe
Lucian Hotoiu
Julie Barbier

New horizon in therapy & treatment

# FRPT

FLASH Radiotherapy & Particle Therapy

VIENNA, AUSTRIA

1-3 DECEMBER 2021

## SAVE THE DATE: 1-3 DECEMBER 2021

KENES GROUP

NHS The Christie NHS Foundation Trust

MANCHESTER 1824 The University of Manchester

Biophysics

CHUV

EMPIR EURAMET

The EMPIR initiative is co-funded by the European Union's Horizon 2020 research and innovation programme and the EMPIR Participating States

UHDpulse

InspireProject

Integrating proton research across Europe

Horizon 2020

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