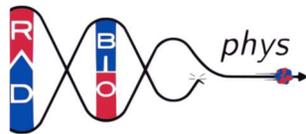


# Fisici nella Radiobiologia applicata

Incontri di Fisica Moderna 2023/2024

Leonardo Lonati

Radiobiology and Biophysics of Radiation Group

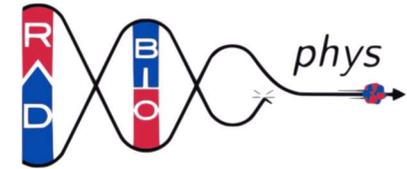


UNIVERSITÀ DI PAVIA  
Dipartimento di Fisica

# Outline

- RadBioPhys: chi siamo
- Background fisico e biologico
- Modelli
  - Sperimentali, Concettuali, Simulati
- Applicazioni:
  - Simulazioni induzione di foci  $\gamma$ H2AX
  - Modellizzazione ciclo cellulare per RT
  - Analisi dati di Comet'assay (TRANSAT)
  - VISAIR Conigli: statistiche con bassi numeri di eventi (VISAIR)

# Laboratorio di Radiobiologia e Biofisica delle Radiazioni - RadBioPhys



[leonardo.lonati@unipv.it](mailto:leonardo.lonati@unipv.it)



[prof.giorgio.baiocco@unipv.it](mailto:prof.giorgio.baiocco@unipv.it)



<http://radbiophys.unipv.eu>



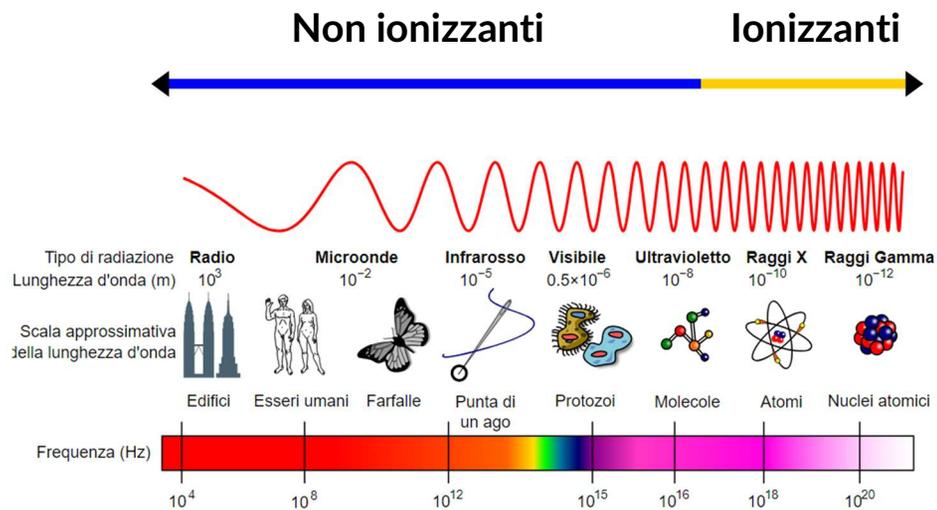
## Membri del gruppo:

Prof. Giorgio Baiocco  
Isabella Guardamagna  
Alice Mentana  
Leonardo Lonati  
Ombretta Iaria  
Andrea Previtali

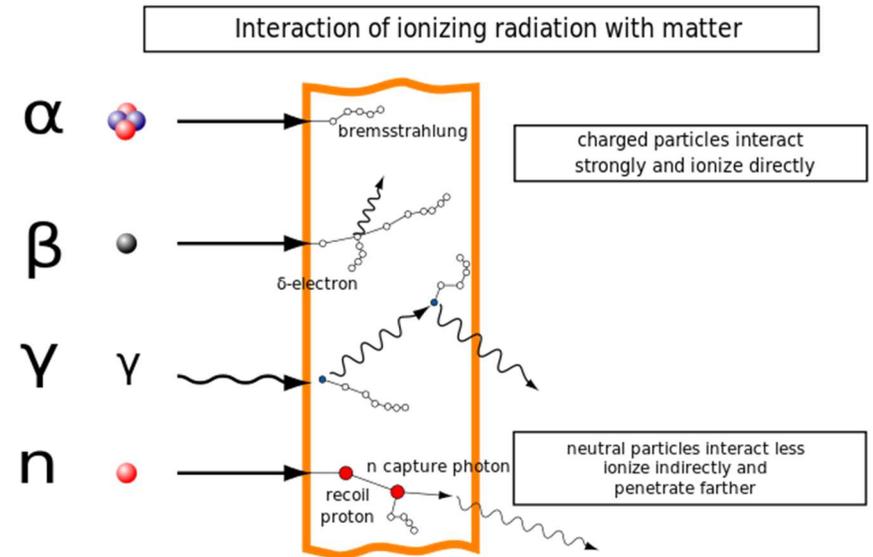


Studi teorici e sperimentali di effetti di radiazioni ionizzanti su strutture biologiche: applicate a radioterapia, diagnostica, radioprotezione.

# Radiazione Ionizzante (IR)



≈ eV: ionizzazioni e eccitazioni



# Qualche grandezza in gioco

Dose assorbita, D (gray, Gy)

$$D = \frac{d\bar{\epsilon}}{dm} \quad \left[ \frac{J}{kg} \right]$$

Dose equivalente,  $H_T$  (sievert, Sv)

$$H_T = \sum_R w_R D_{T,R}$$

Fattore di pericolosità di radiazione,  $w_R$

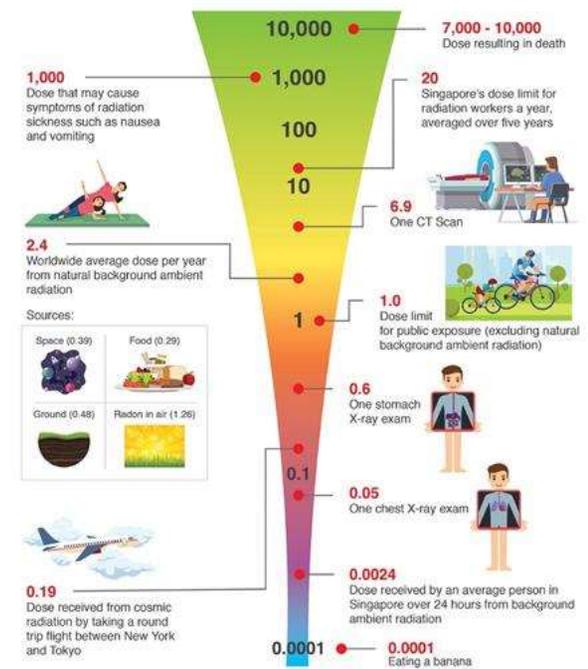
Dose efficace, E (sievert, Sv)

Fattore di sensibilità di tessuti/organi,  $w_T$

$$E = \sum_T w_T H_T = \sum_T w_T \sum_R w_R D_{T,R}$$

## Effective Radiation Dose

(Unit: millisievert = mSv)



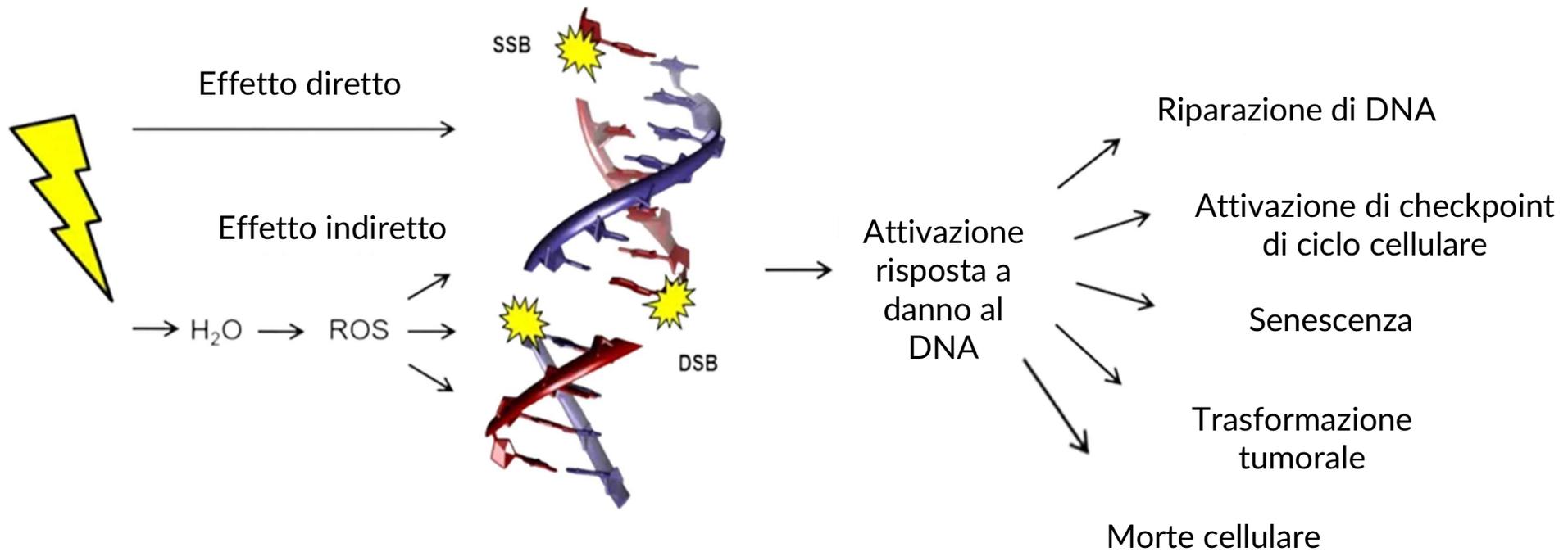
August 2019

# Applicazioni di studio di IR

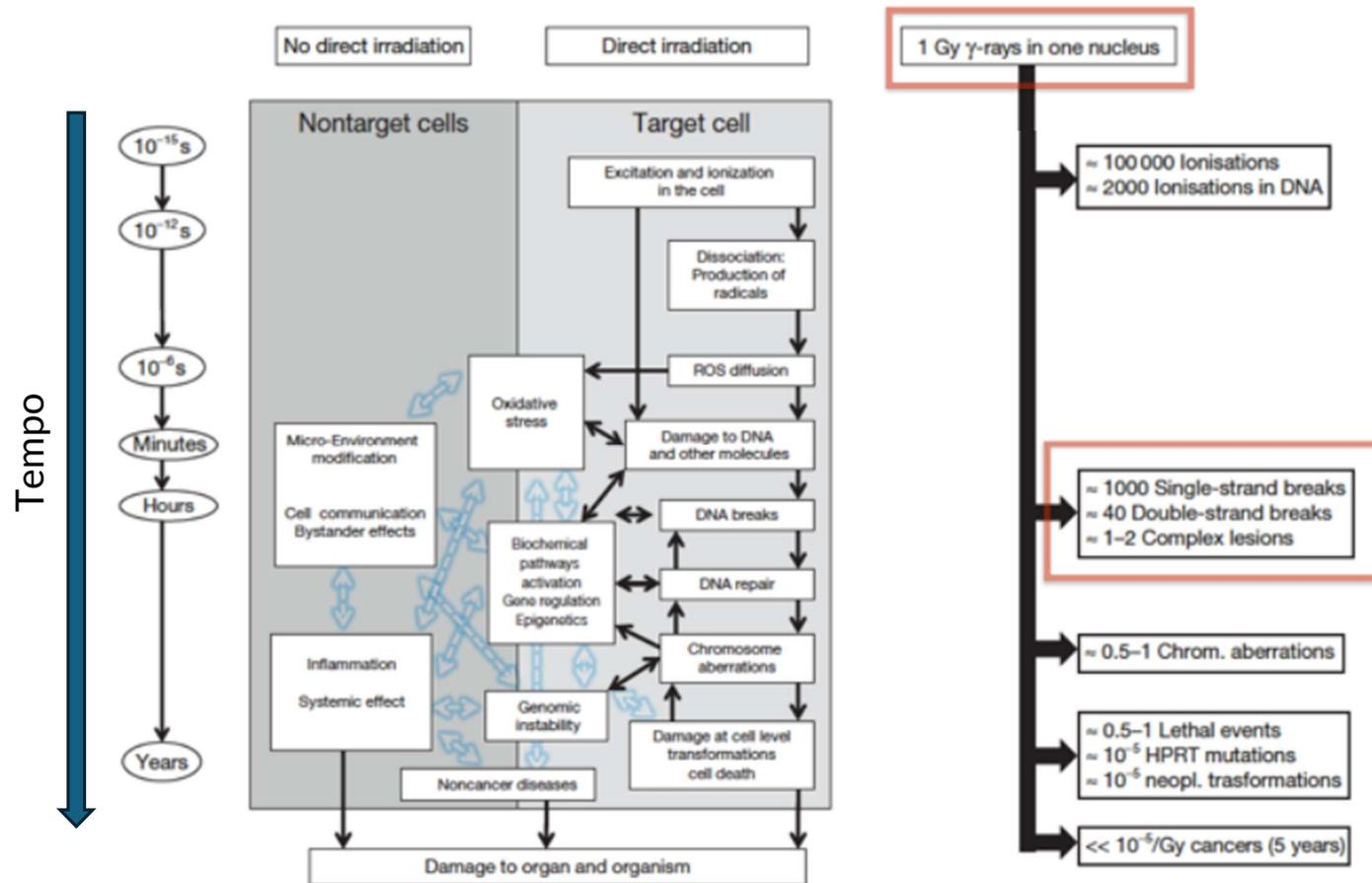
Valutare effetti del rischio da esposizione è fondamentale per:

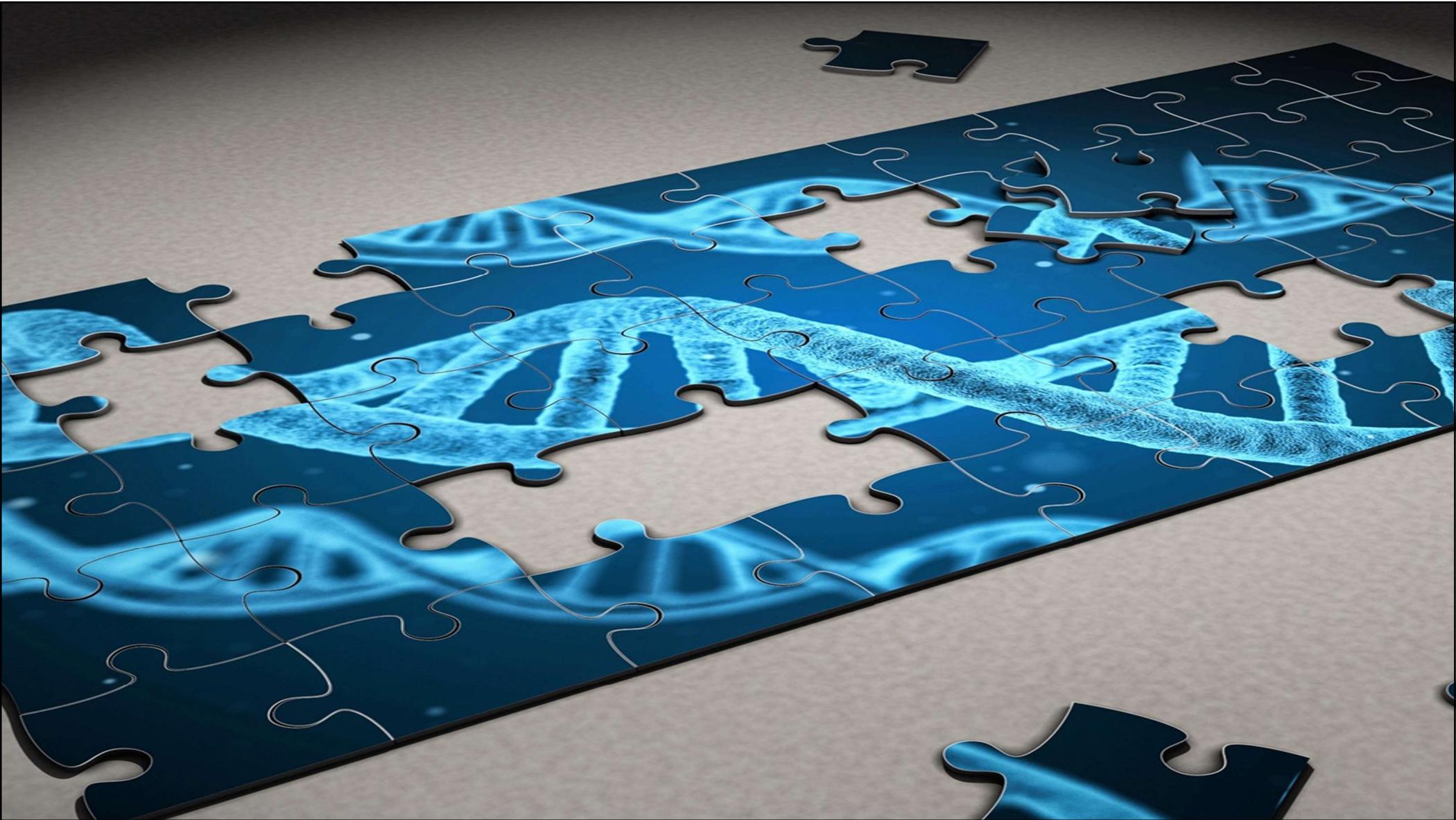
- Ottimizzare usi clinici della radiazione (Diagnostica e Terapia)
- Predire rischio di esposizione a IR in particolare alle basse dosi
- Sviluppo di modelli sperimentali e matematici che predicano l'azione della radiazione su sistemi biologici
  - In particolare su vie di segnalazione e riparazione post-irraggiamento
- Uso di IR come sonda per studiare risposte biologiche a stimoli esterni

# Induzione di danno da IR



# Dinamica temporale di effetti delle IR





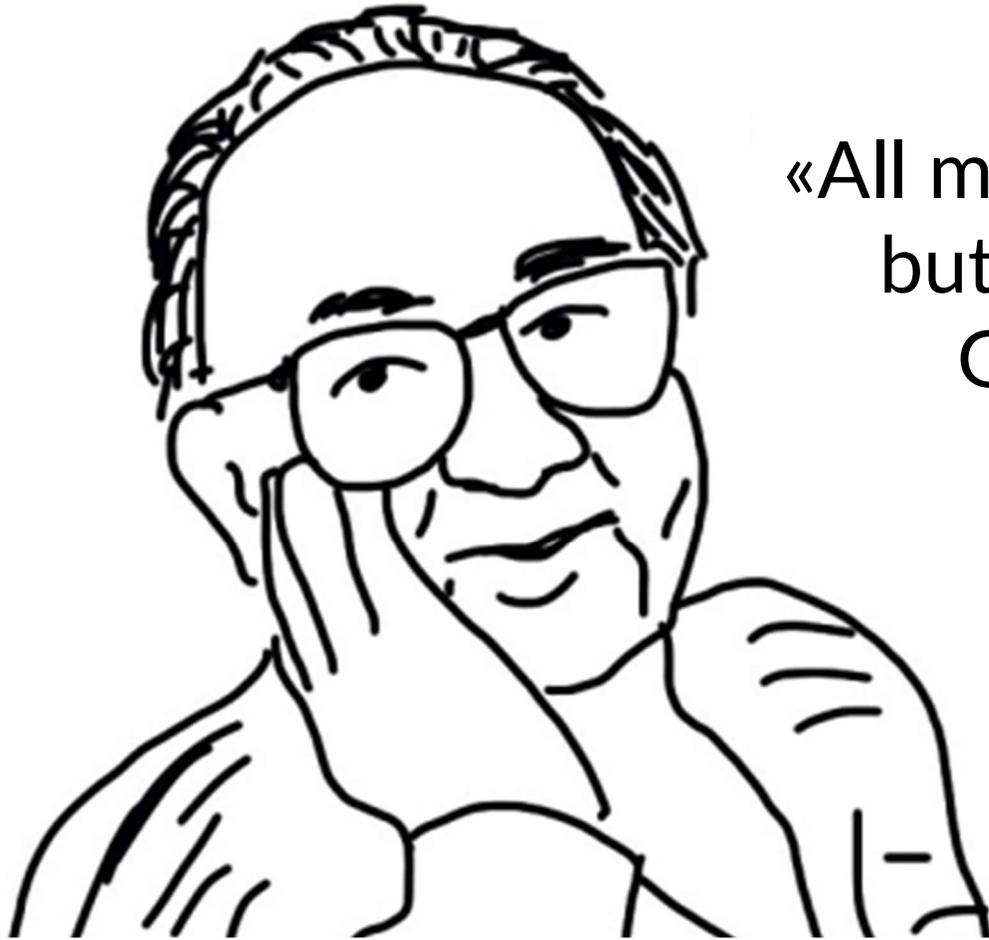
# Modelli!

**Obiettivo: RAPPRESENTAZIONE FORMALE e SEMPLIFICATA** di un sistema o di un fenomeno complesso che vogliamo indagare.

**Concettuali, matematici o sperimentali.**

**Perché usarli in radiobiologia?**

- 1. Predizione:** I modelli ci permettono di fare previsioni su come le radiazioni interagiranno con i tessuti biologici.
- 2. Studio Etico:** Consentono di studiare gli effetti delle IR senza esporre direttamente gli esseri umani.
- 3. Ottimizzazione:** Ci aiutano a ottimizzare le terapie radianti per massimizzare l'efficacia e minimizzare gli effetti collaterali.



«All models are wrong, [...] but some are useful»  
George E.P. Box

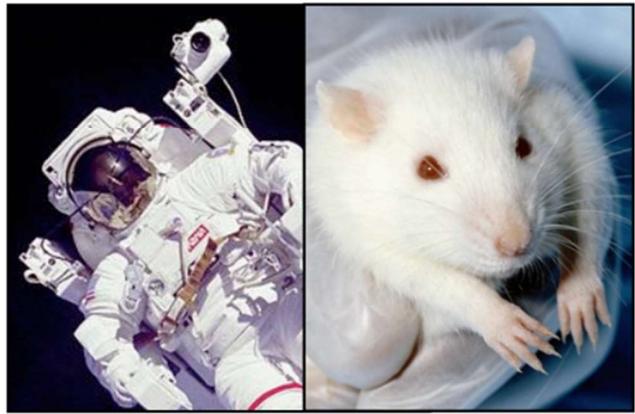
*in silico*



*in vitro*



*in vivo*



# Attività e modelli in *Radbiophys*

- Induzione di Foci da IR
- Modelli computazionali di ciclo cellulare per predire RT
- Dosimetria per radioprotezione da  $^3\text{H}$  (progetti TRANSAT-TITANS)
- Attivazione del sistema visivo post-IR (progetto VISAIR)

# IR-Induced Foci ( $\gamma$ -H2AX)

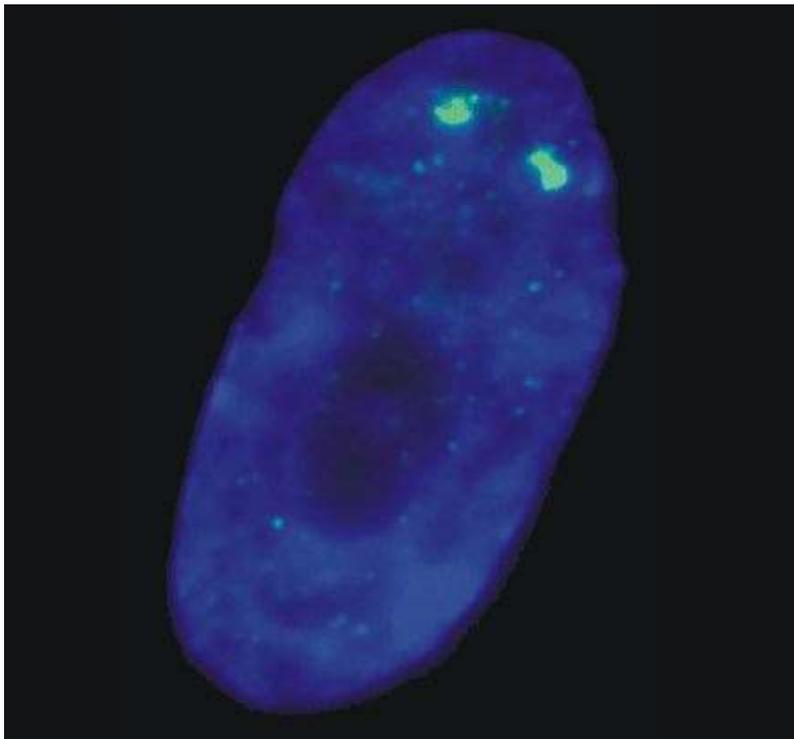


**Istoni:** proteine strutturali del DNA

**Fosforilazione:** modificazione biochimica per marcare e creare un cambiamento funzionale

- Fosforilazione serina 139 dell'istone H2AX.
- Da danno da IR o stress replicative
- Recluta fattori di riparo, creando il cosiddetto IRIF (Ionizing Radiation Induced Foci)
- Fosforilazione si propaga lungo le due estremità del DNA fino a 2Mbps

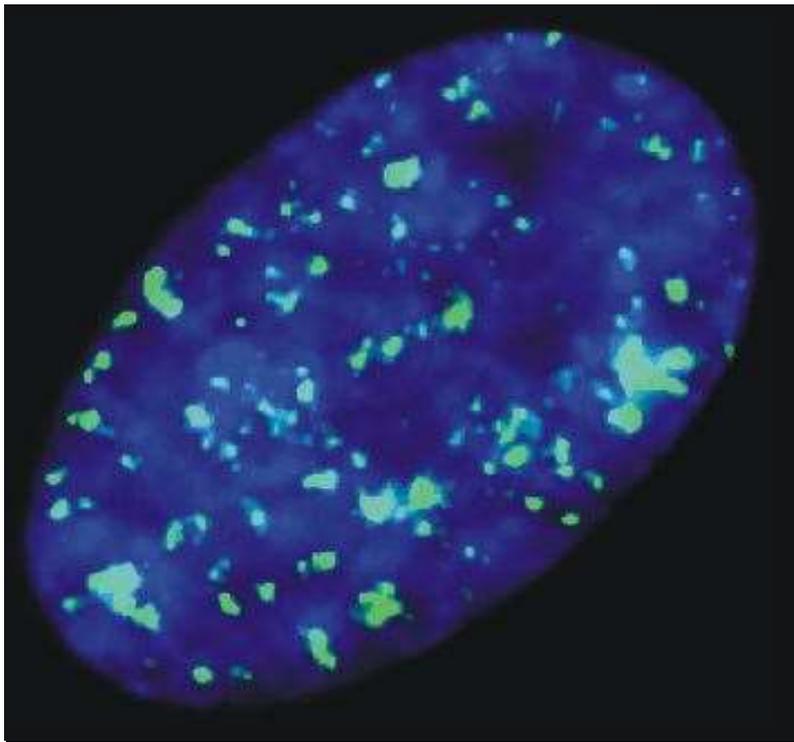
# IR-Induced Foci ( $\gamma$ -H2AX)



Controllo non irraggiato

- Fosforilazione serina 139 dell'istone H2AX.
- Da danno da IR o stress replicative
- Recluta fattori di riparo, creando il cosiddetto IRIF (Ionizing Radiation Induced Foci)
- Fosforilazione si propaga lungo le due estremità del DNA fino a 2Mbps

# IR-Induced Foci ( $\gamma$ -H2AX)

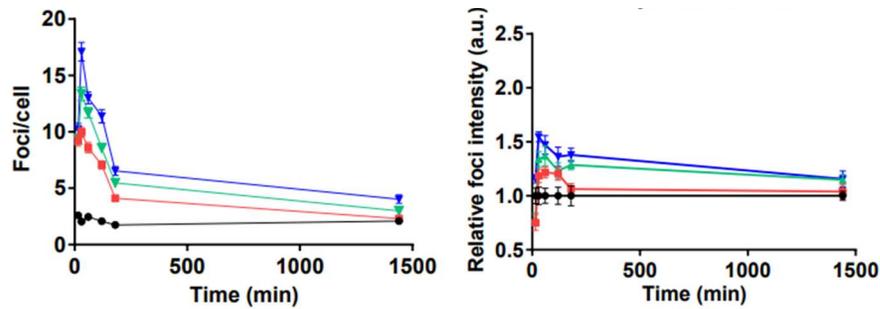


Post-IR di fotoni (2Gy)

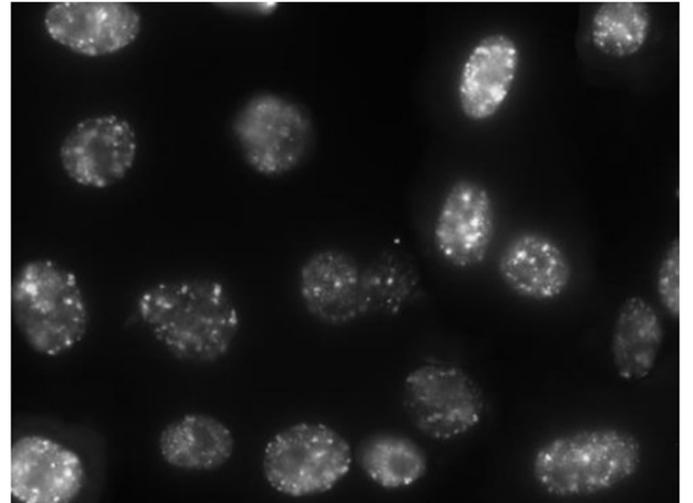
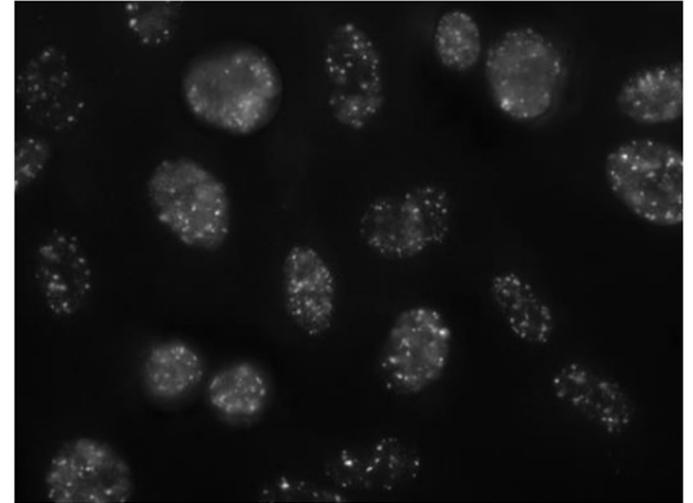
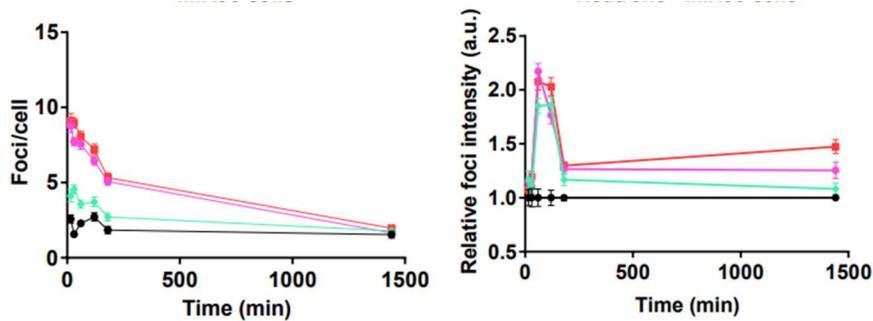
- Fosforilazione serina 139 dell'istone H2AX.
- Da danno da IR o stress replicative
- Recluta fattori di riparo, creando il cosiddetto IRIF (Ionizing Radiation Induced Foci)
- Fosforilazione si propaga lungo le due estremità del DNA fino a 2Mbps

# IR-Induced Foci ( $\gamma$ -H2AX)

fotoni



neutroni



# Perché modellizzare foci?

- Biodosimetria su linfociti
  - Sensibile a basse dosi
  - Limitata ad alte dosi (saturazione)
- Modellizzazione teorica di IRIF permette di superare limiti tecnici sperimentali

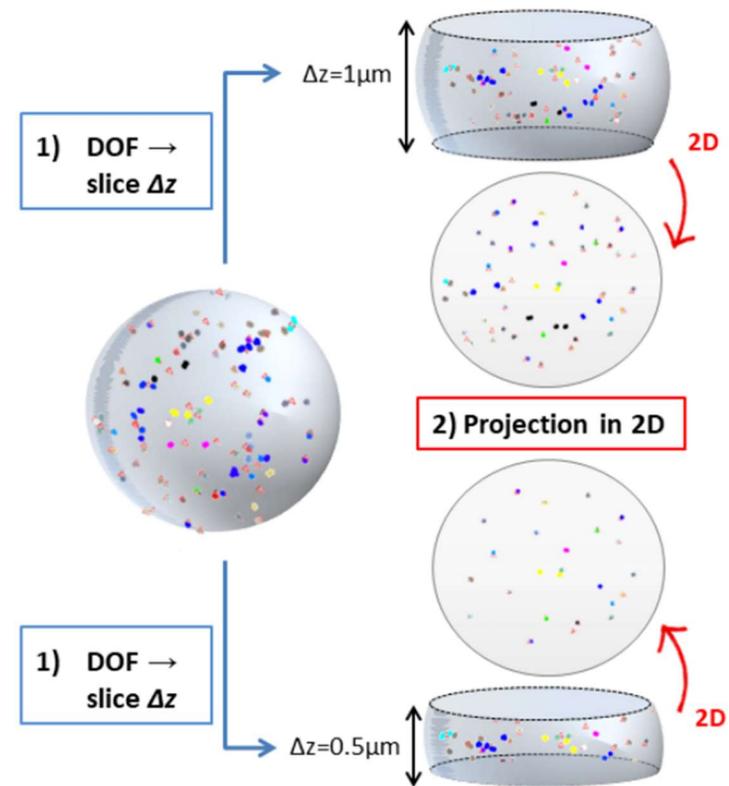
Particelle cariche secondarie da neutroni:

PHITS (transport code) + PARTRAC (track-structure)

# Modello di Induzione di Foci $\gamma$ -H2AX

Riproduzione di punto di vista osservatore a partire da radiazione:

- **PATRAC** struttura di traccia biofisica: distribuzione spaziale 3D di DSBs post-irraggiamento
- Correlazione a distribuzione spaziale di  $\gamma$ -H2AX
- Selezione solo DSBs in una *slice thickness*  $\Delta z$  al centro del nucleo
- Proiezione su asse x-y per correlare a IRIF da immagini 2D
- Clustering algorithm per unire gruppi di DSB in singoli foci

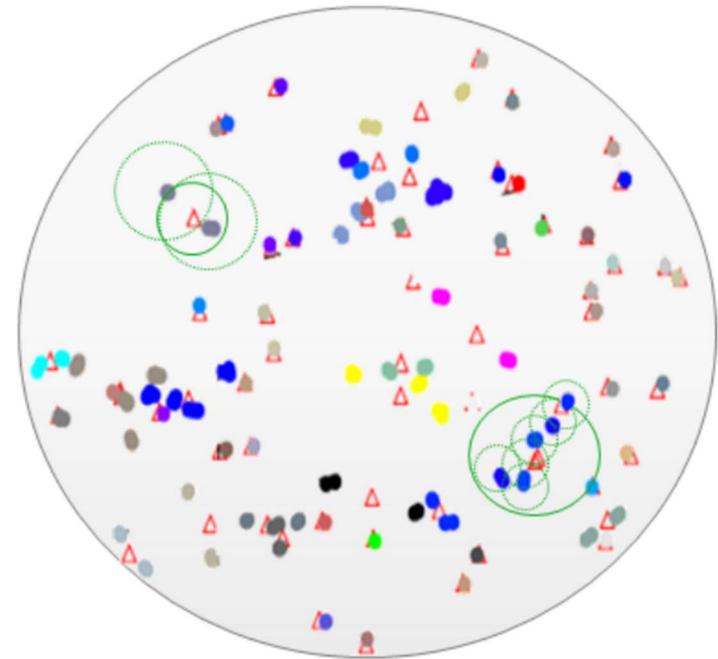


# Modello di Induzione di Foci $\gamma$ -H2AX

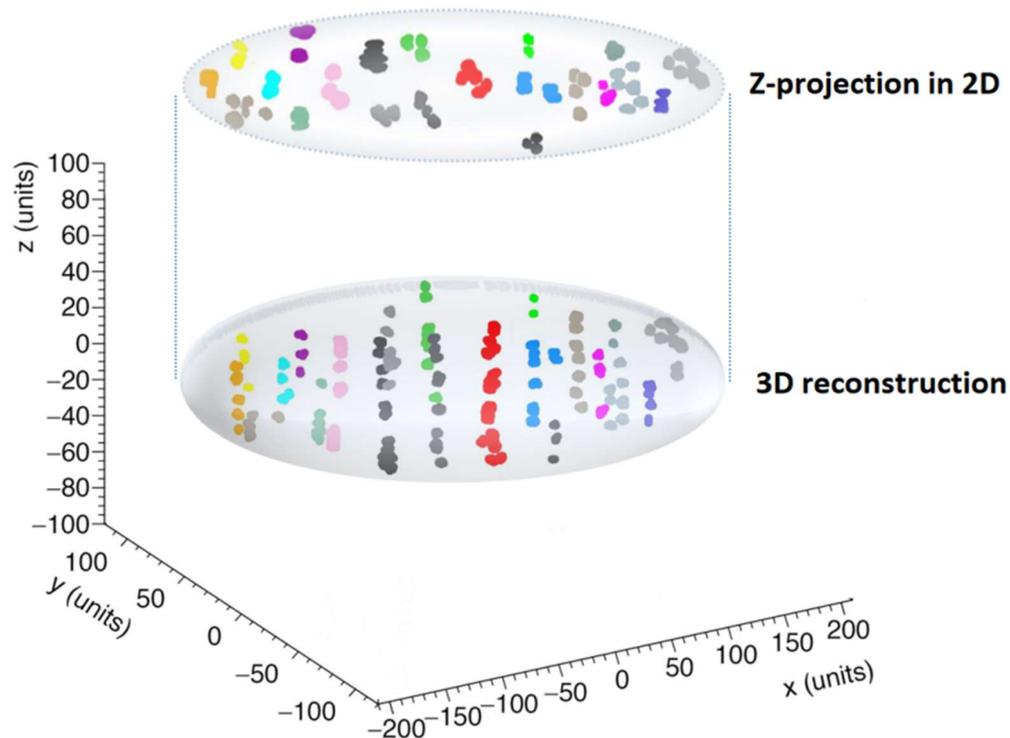
Riproduzione di punto di vista osservatore a partire da radiazione:

- **PATRAC** struttura di traccia biofisica: distribuzione spaziale 3D di DSBs post-irraggiamento
- Correlazione a distribuzione spaziale di  $\gamma$ -H2AX
- Selezione solo DSBs in una *slice thickness*  $\Delta z$  al centro del nucleo
- Proiezione su asse x-y per correlare a IRIF da immagini 2D
- Clustering algorithm per unire gruppi di DSB in singoli foci

3) Clustering -> radius  $r$



# Modello di Induzione di Foci $\gamma$ -H2AX



Article | [Open access](#) | Published: 30 September 2019

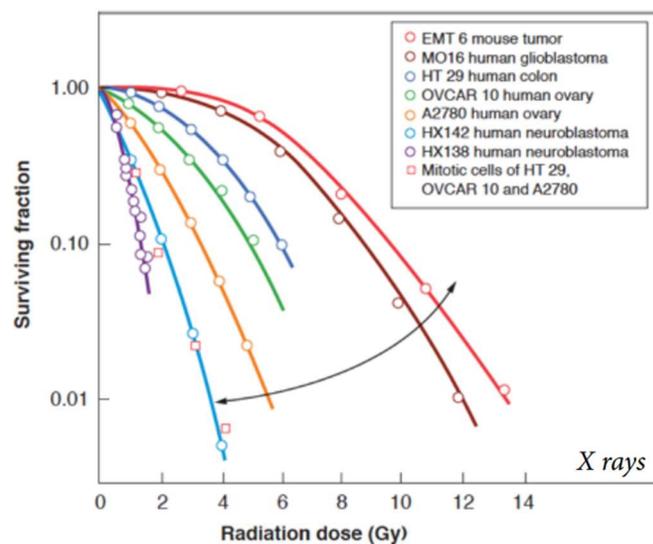
**Predicting DNA damage foci and their experimental readout with 2D microscopy: a unified approach applied to photon and neutron exposures**

Sofia Barbieri , [Gabriele Babini](#), [Jacopo Morini](#), [Werner Friedland](#), [Manuela Buonanno](#), [Veljko Grijj](#), [David J. Brenner](#), [Andrea Ottolenghi](#) & [Giorgio Baiocco](#)

*Scientific Reports* **9**, Article number: 14019 (2019) | [Cite this article](#)

# Applicazione tumori

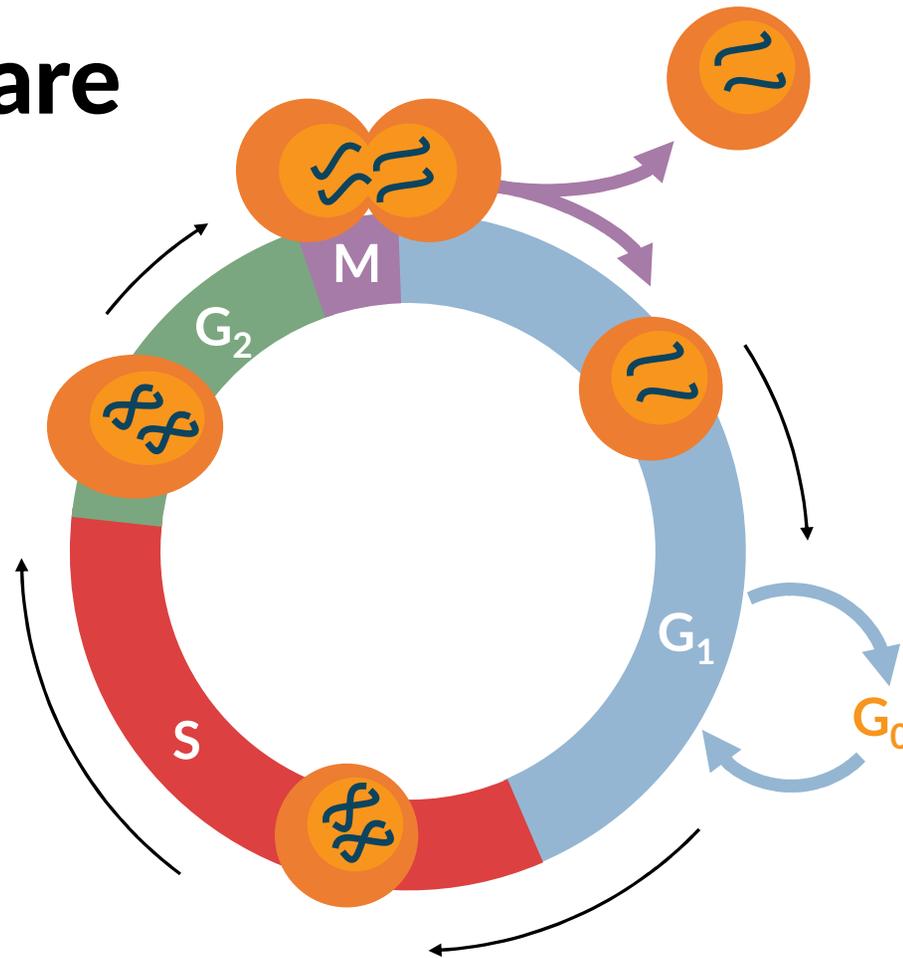
Obiettivo: uccidere cellule tumorali sfruttando IR, Preservando tessuti sani



# Ciclo cellulare

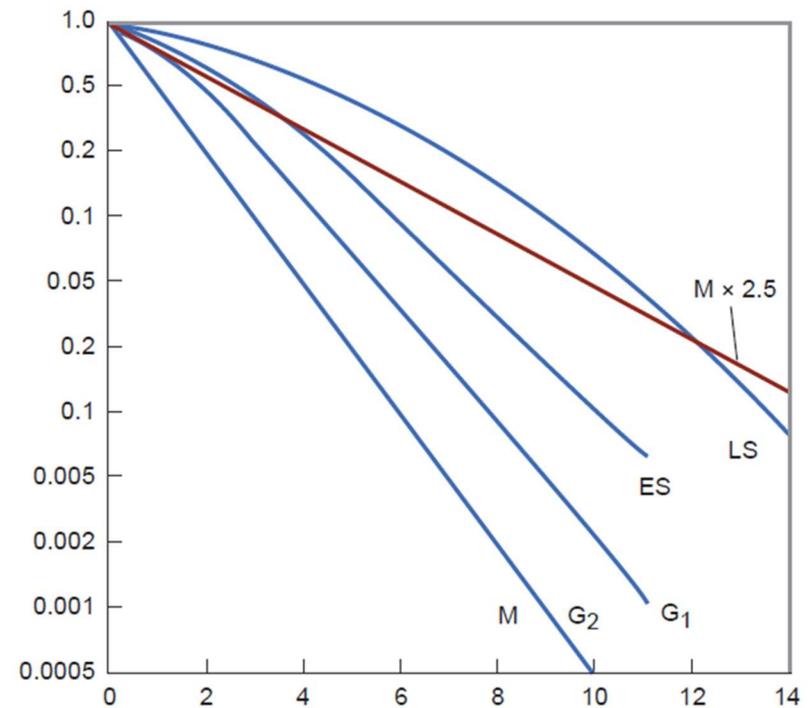
4 phases:  $G_1$ , S,  $G_2$ , M

Progression along the cell cycle regulated by a family of proteins called cyclin-dependent kinases (CDK), activated by cyclins

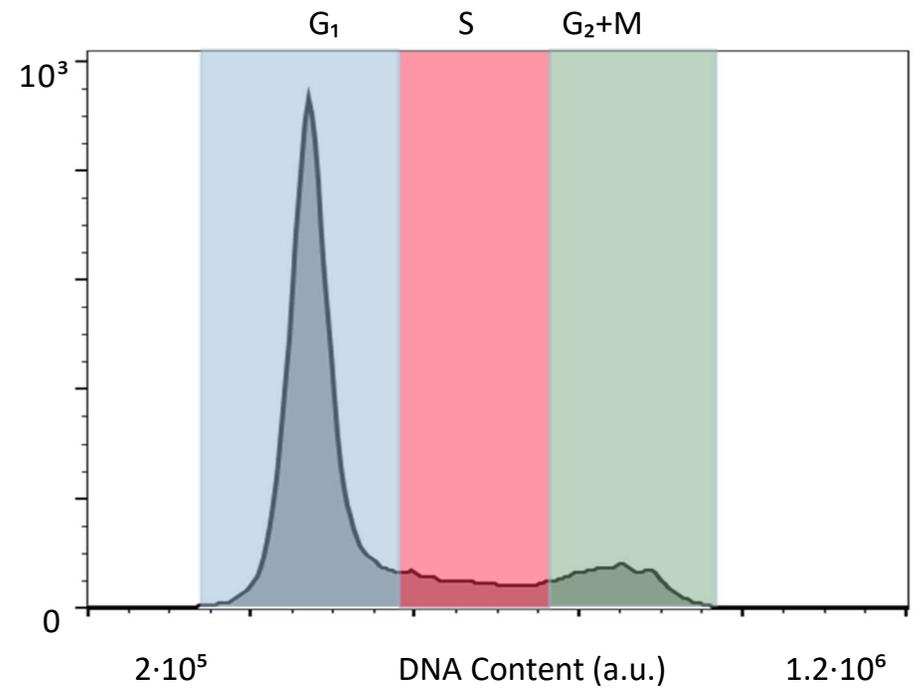
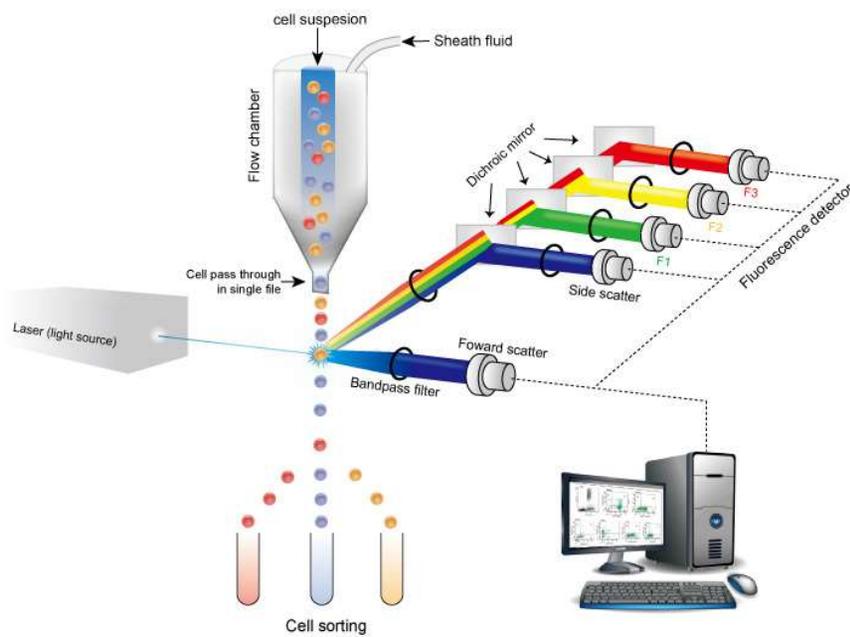


# IR e ciclo cellulare

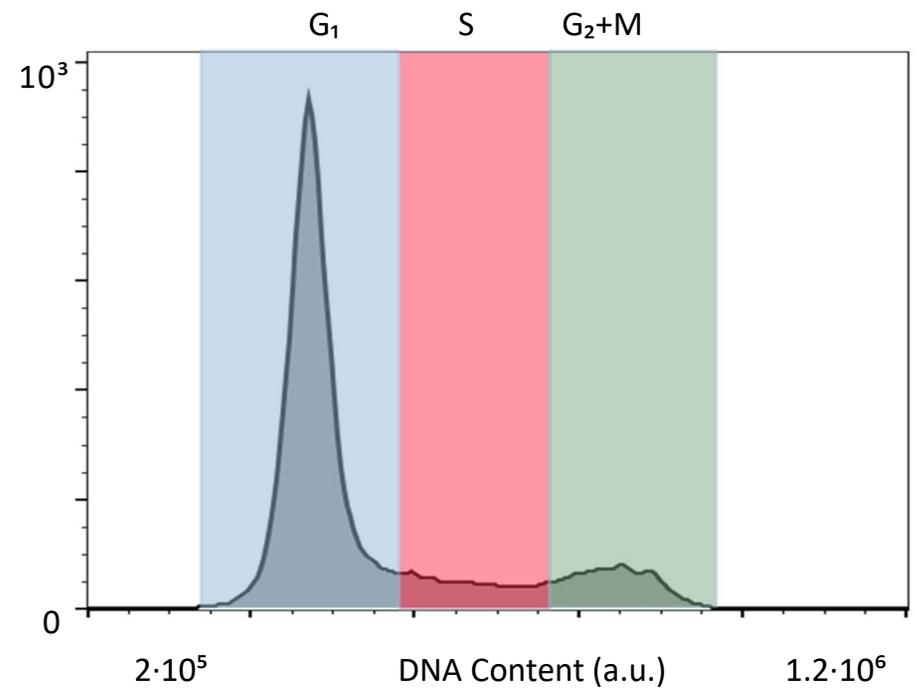
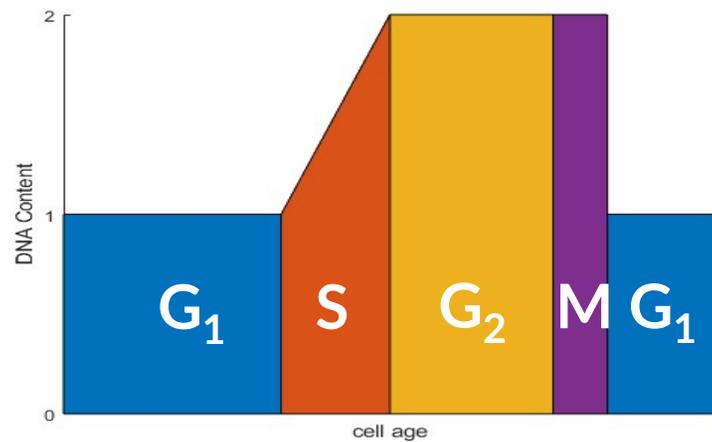
- Cells synchronized in various cell cycle phases prior to IR
- In most cell lines, cells in M- and G<sub>2</sub>-phase are the most radiosensitive
- Resistance increases during the S phase, with maximum in late S (LS)



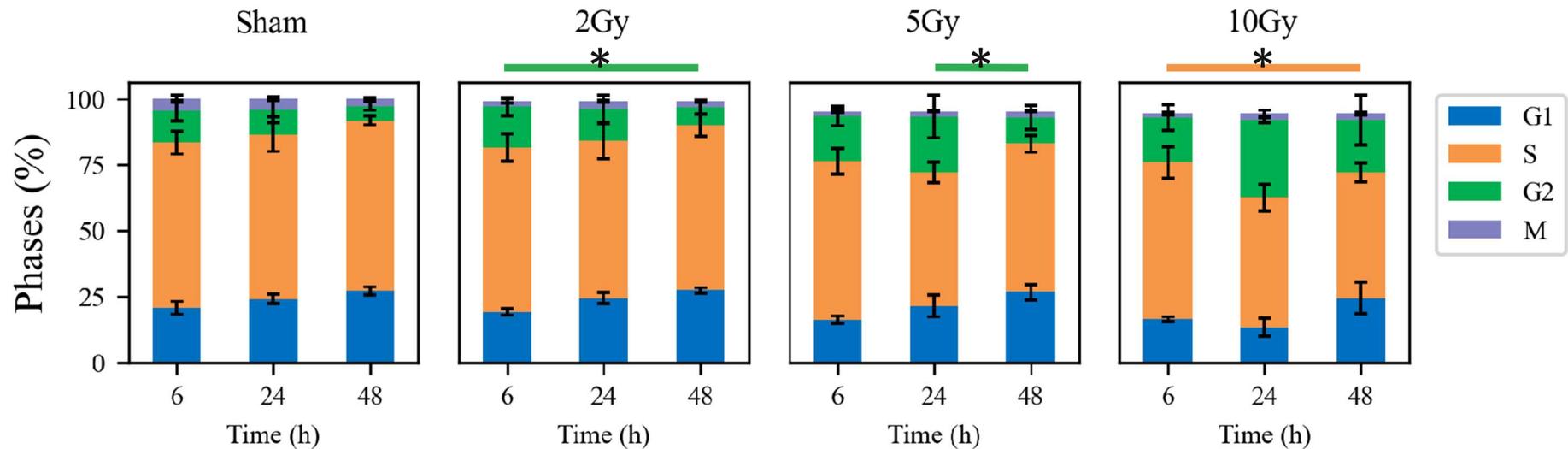
# Misure di citometria a flusso



# Misure sperimentali di citometria a flusso



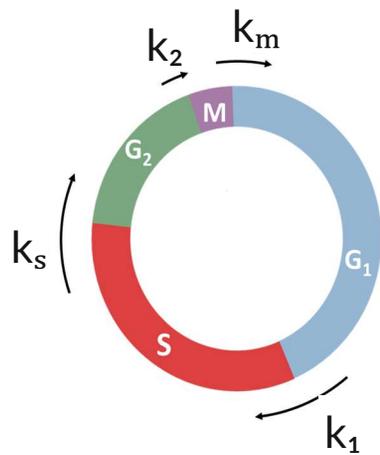
# Misure sperimentali di citometria a flusso



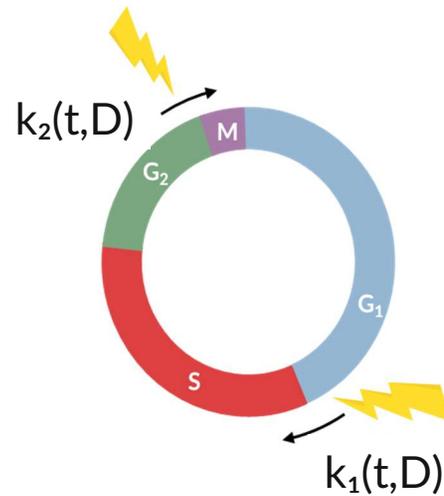
Guardamagna I. et al. "An Integrated Analysis of the Response of Colorectal Adenocarcinoma Caco-2 Cells to X-Ray Exposure". Front Oncol. 2021 Jun 3;11:688919. [doi: 10.3389/fonc.2021.688919](https://doi.org/10.3389/fonc.2021.688919)

# Modello compartimentale di popolazione

SHAM MODEL



IR DISRUPTION



PARAMETERS FIT

$$\chi^2(\vec{\theta}) = \sum_t \sum_{phase} \left( \frac{f_{exp} - f_{mod}}{\sigma_{exp}} \right)^2$$

$$\hat{\theta} = \min_{\vec{\theta}} \left[ \chi^2(\vec{\theta}) \right]$$

# PDE-DNA model feasibility

$$\frac{\partial G_1}{\partial t}(x, t) = 2^2 b M(2x, t) - (k_1 + \mu_{G_1}) G_1(x, t),$$

$$\frac{\partial S}{\partial t}(x, t) = D \frac{\partial^2 S}{\partial x^2} - \mu_S S(x, t) - g \frac{\partial S}{\partial x}(x, t) + k_1 G_1(x, t) - I(x, t; T_S),$$

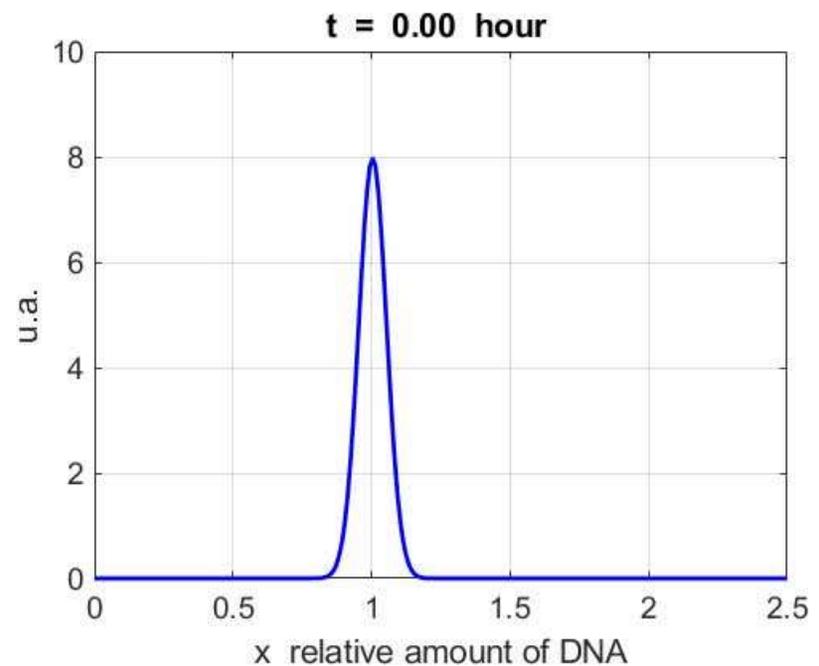
$$\frac{\partial G_2}{\partial t}(x, t) = I(x, t; T_S) - (k_2 + \mu_{G_2}) G_2(x, t),$$

$$\frac{\partial M}{\partial t}(x, t) = k_2 G_2(x, t) - b M(x, t) - \mu_M M(x, t),$$

**Radiation-induced cell cycle perturbations: a computational tool validated with flow-cytometry data**

Leonardo Lonati , Sofia Barbieri, Isabella Guardamagna, Andrea Ottolenghi & Giorgio Baiocco

[Scientific Reports](#) 11, Article number: 925 (2021) | [Cite this article](#)

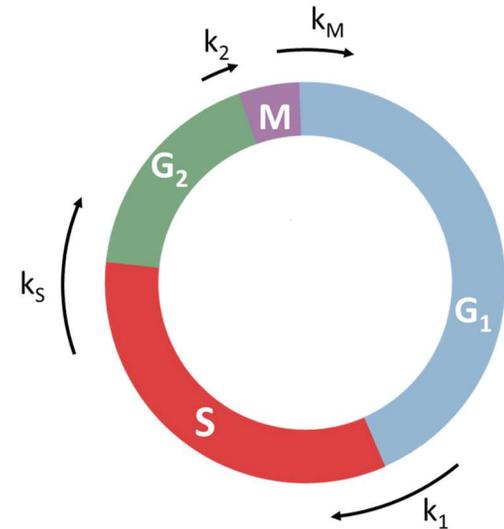


# Modello compartimentale ODEs

$$\begin{cases} \frac{dN_{G_1}(t)}{dt} = +2k_M N_M(t) - k_1 N_{G_1}(t), \\ \frac{dN_S(t)}{dt} = +k_1 N_{G_1}(t) - k_S N_S(t), \\ \frac{dN_{G_2}(t)}{dt} = +k_S N_S(t) - k_2 N_{G_2}(t), \\ \frac{dN_M(t)}{dt} = +k_2 N_{G_2}(t) - k_M N_M(t) \end{cases}$$

$$N(t) = N_{G_1}(t) + N_S(t) + N_{G_2}(t) + N_M(t)$$

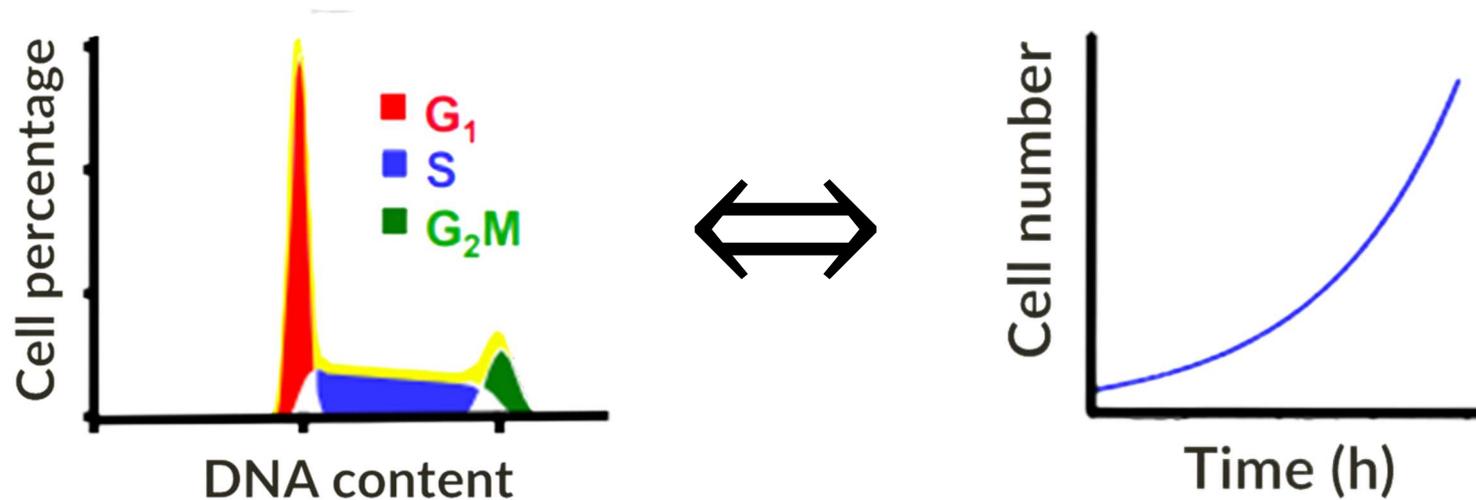
$k_p$  sono ratei di transizione  $p \in [G_1, S, G_2, M]$



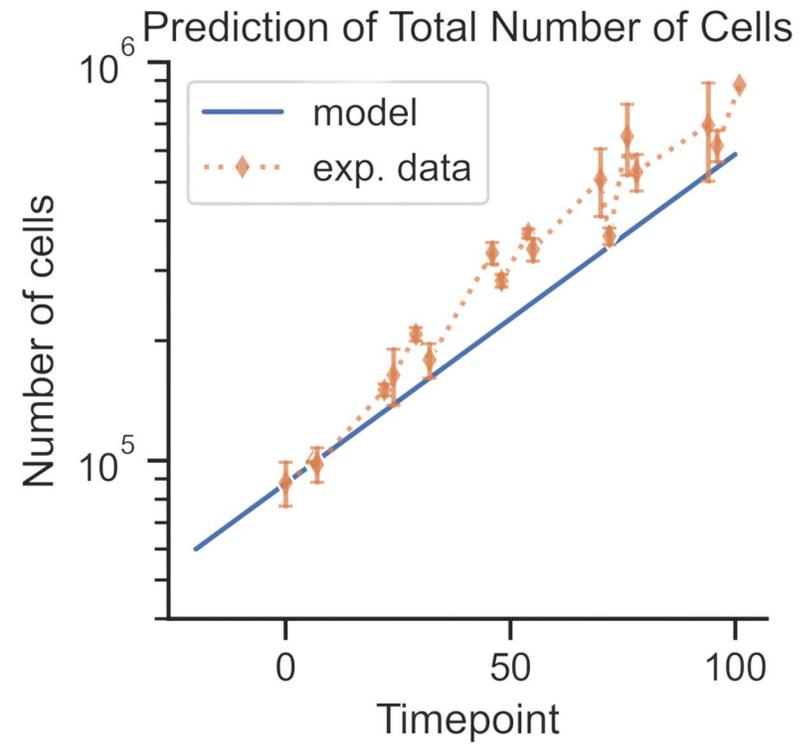
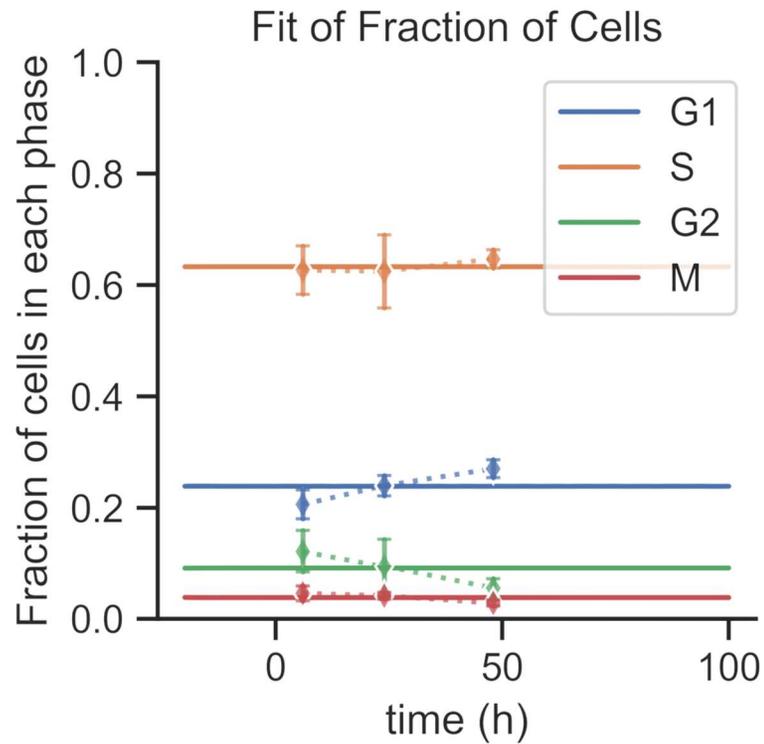
# Stato stazionario

The ODE system is in Steady State (SS) when: proportions of cells between phases is constant and unchanging in time.

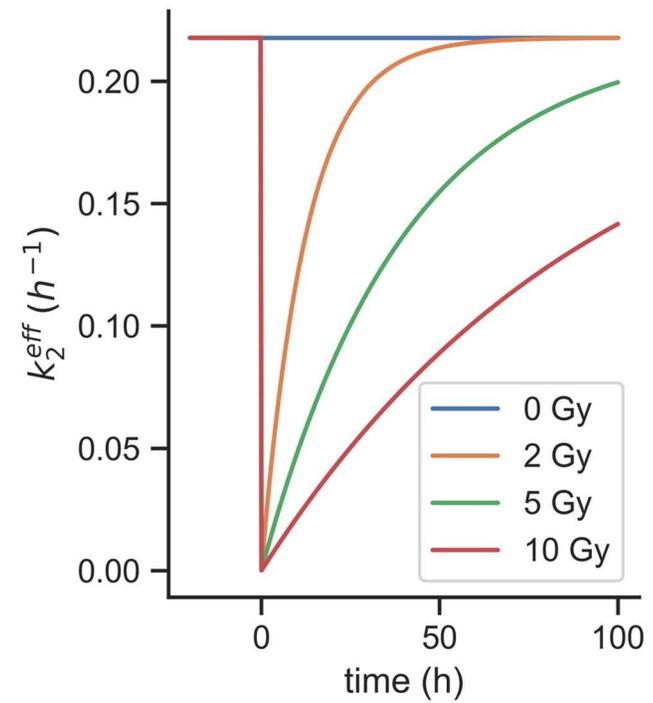
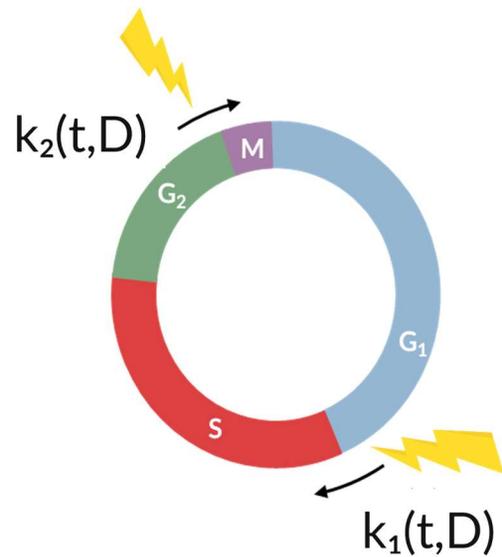
Crescita asincrona di popolazione cellulare.



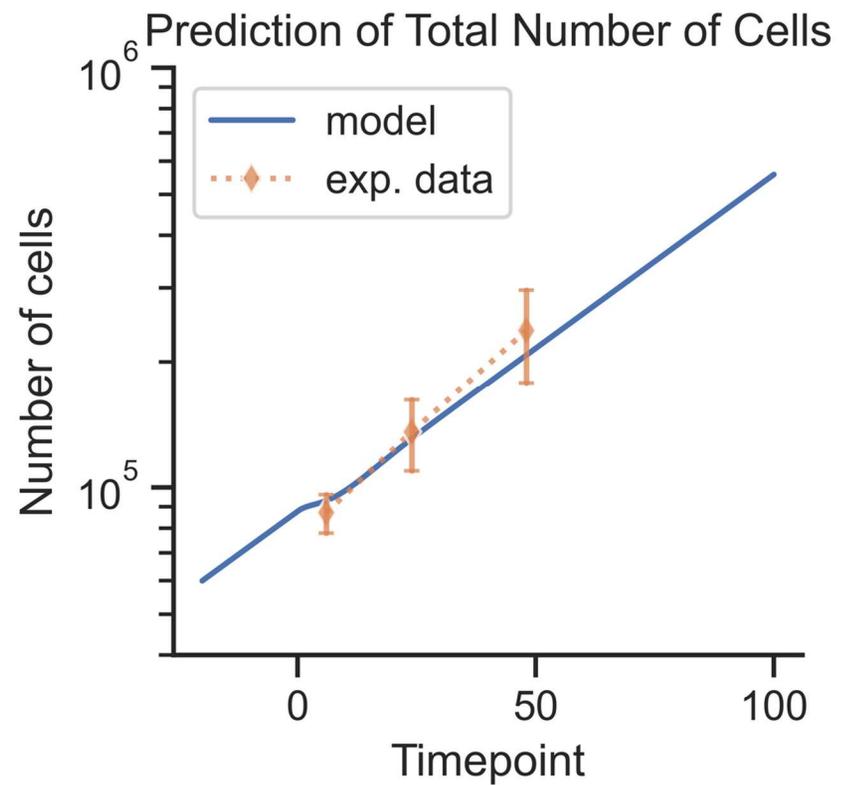
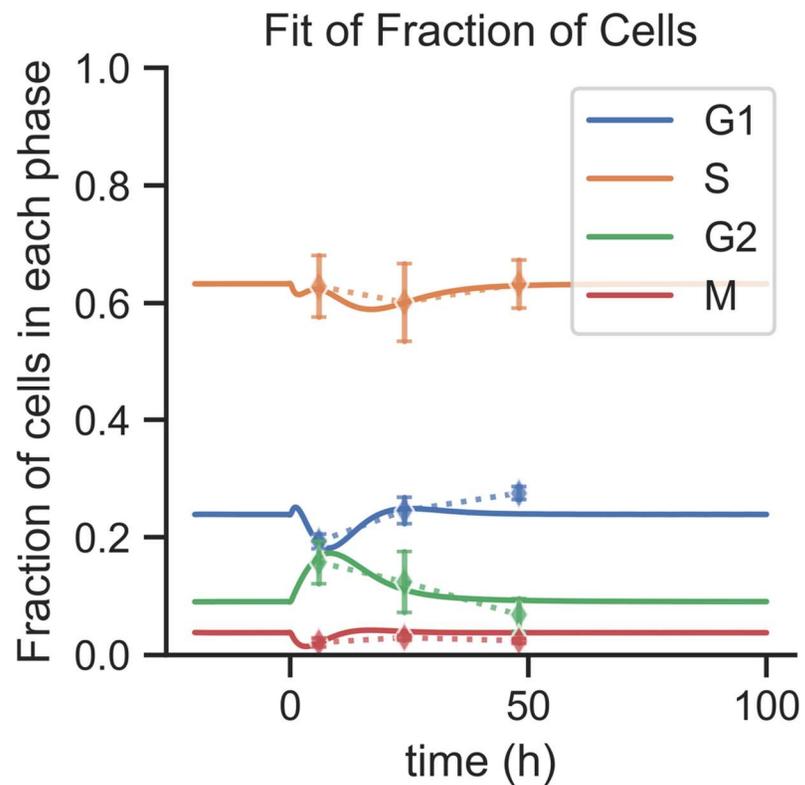
# Stato stazionario



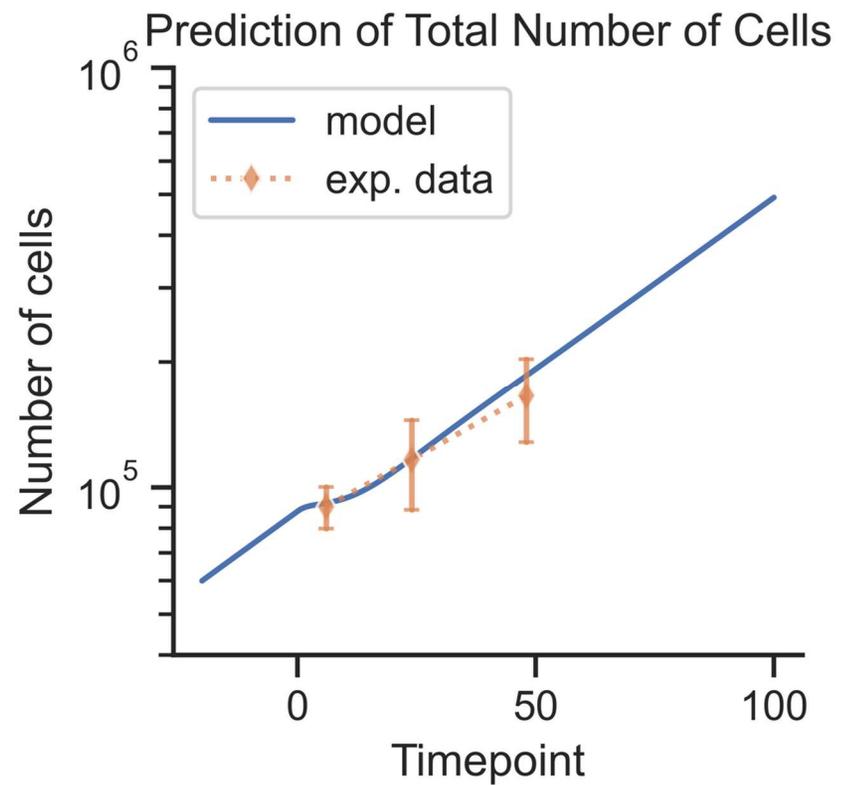
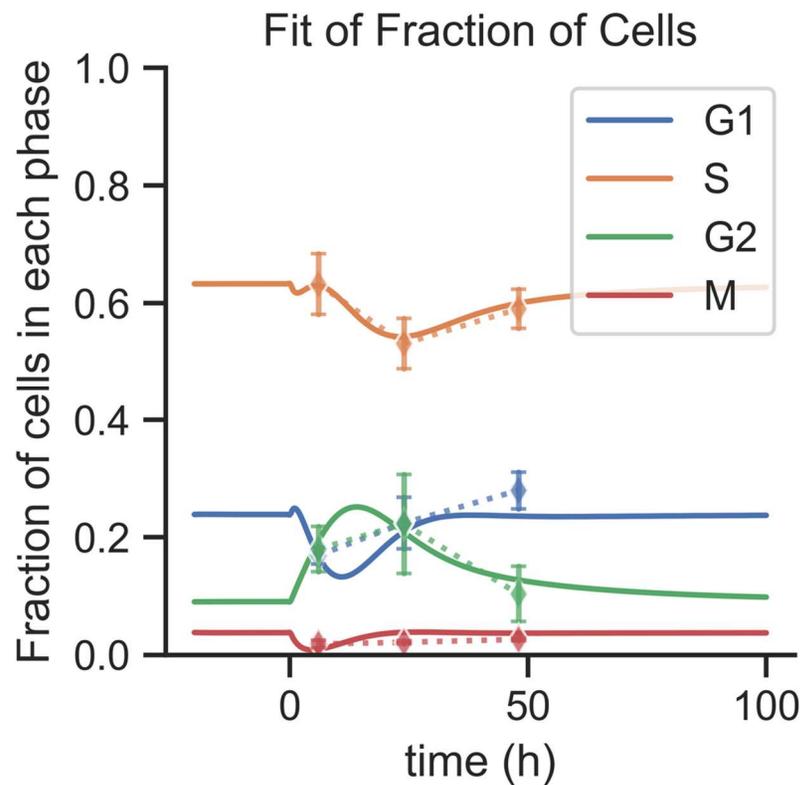
# IR-perturbed model



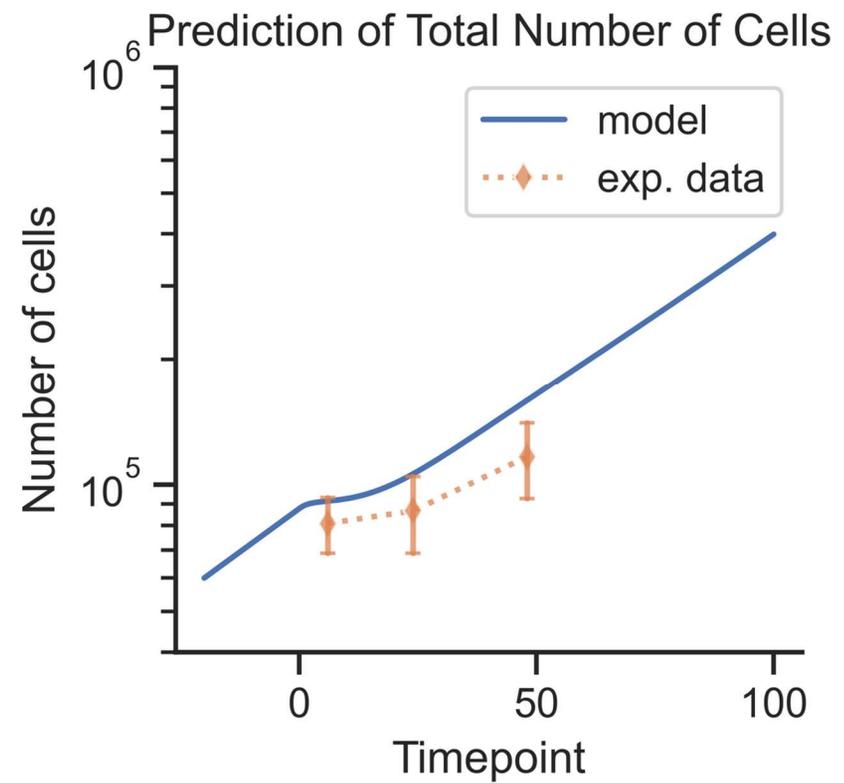
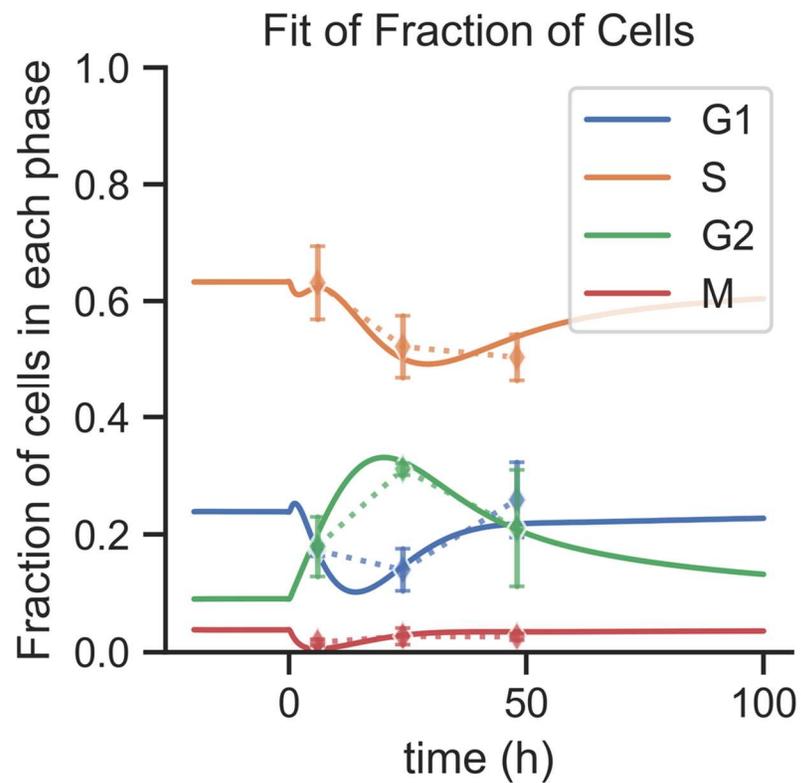
# 2 Gy model: fit and prediction



# 5 Gy model: fit and prediction



# 10 Gy model: fit and prediction



# Modello ibrido multi-scala per risposta tumorale a RT

Osservabile del modello: dimensione finale di popolazione cellulare tumorale

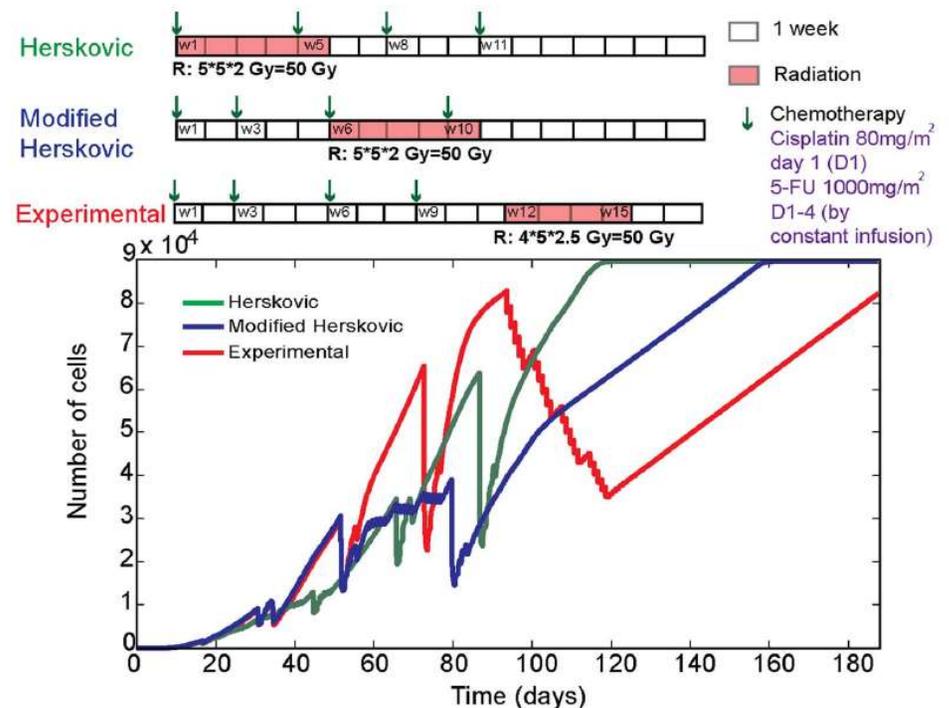
Cell-cycle phase-based radiation therapy

July 2013 | Volume 9 | Issue 7 | e1003120



Towards Predicting the Response of a Solid Tumour to Chemotherapy and Radiotherapy Treatments: Clinical Insights from a Computational Model

Gibin G. Powathil<sup>1\*</sup>, Douglas J. A. Adamson<sup>2</sup>, Mark A. J. Chaplain<sup>1</sup>



# TRANSAT (TRANSversal Actions for Tritium) + TITANS (Tritium Impact and Transfer in Advanced Nuclear reactorS)

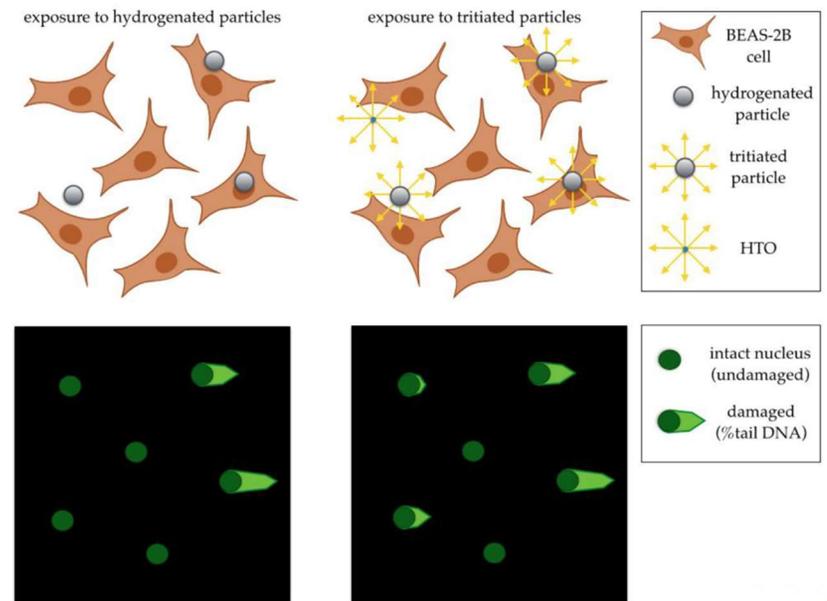
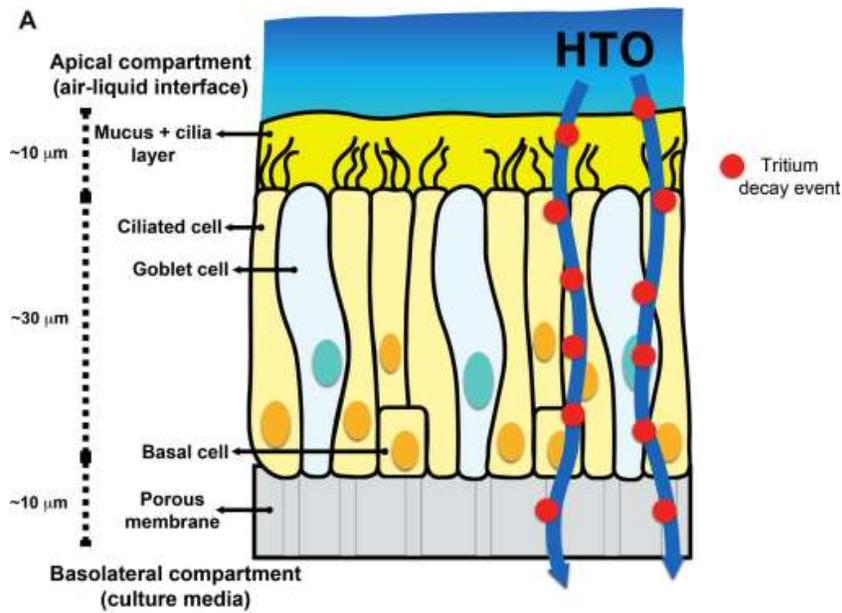
Studio e miglioramento di smaltimento di  $^3\text{H}$  in impianti di fissione e fusione nucleare

Studio potenziale tossicità da inalazione di prodotti radioattivi in condizioni fisiologiche controllate.

**Modello biologico:** modello 3D di cellule epiteliali delle vie aeree umane, esposto ad acqua triziata (HTO).

Endpoint: stima di dose, assorbimento di trizio, viabilità cellulare, integrità epiteliale, risposta proinfiammatoria.

# TRANSAT (TRANSversal Actions for Tritium) + TITANS (Tritium Impact and Transfer in Advanced Nuclear reactorS)



# Alterazioni sensoriali radioindotte

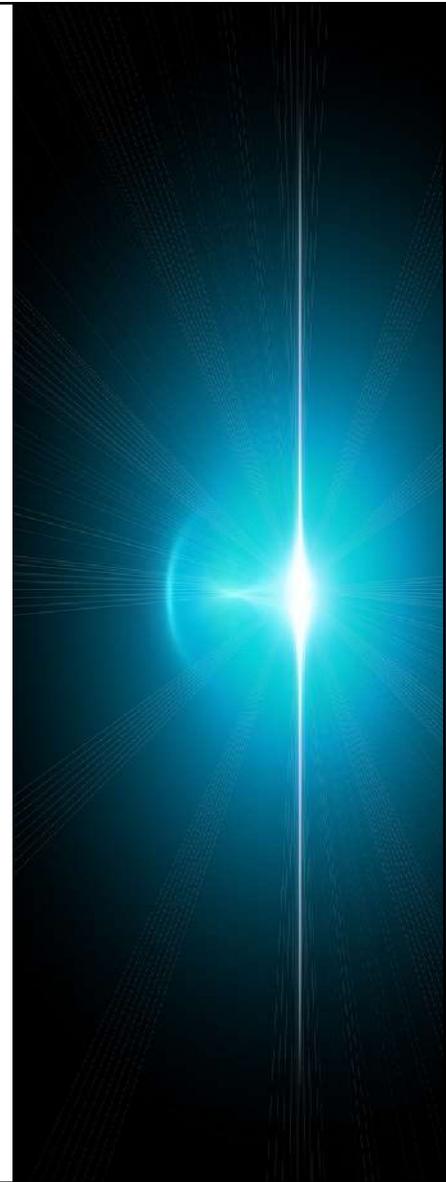
Evidenze sperimentali di attivazione sensoriali da IR, quali: flash di luce (LF), suoni, sapori

In:

- Astronauti (sin da missioni Apollo)
- Pazienti RT
- In animali *in vivo*

Life Sci.in Space. Res. Vol 26-2020 [doi.org/10.1016/j.lssr.2020.04.006](https://doi.org/10.1016/j.lssr.2020.04.006)

Front. Cell. Neurosci., Sec. Cellular Neurophysiology Vol 16-2022  
[10.3389/fncel.2022.917273](https://doi.org/10.3389/fncel.2022.917273)



# VISAIR: (*Visual System Activation by Ionizing Radiation*)

Studio *ex vivo* di occhi interi di conigli

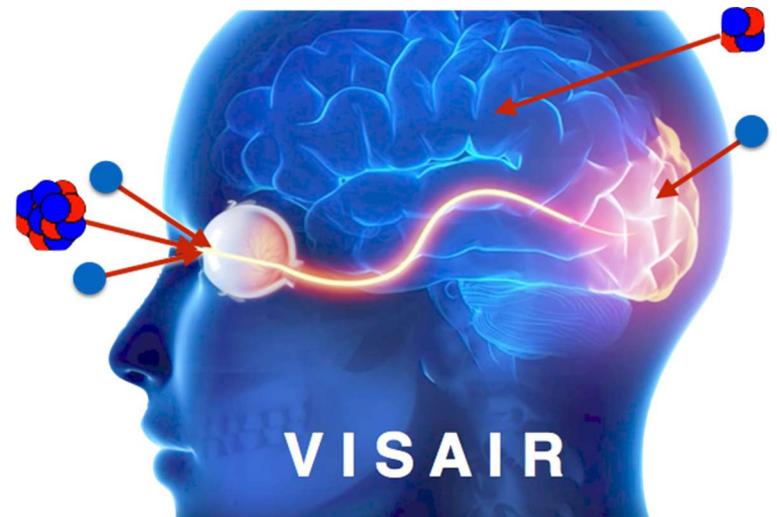
Simulazioni (PHITS) per caratterizzare campo di radiazione spaziale che arriva all'organo in termini di dose spaziale e distribuzione microdosimetrica.

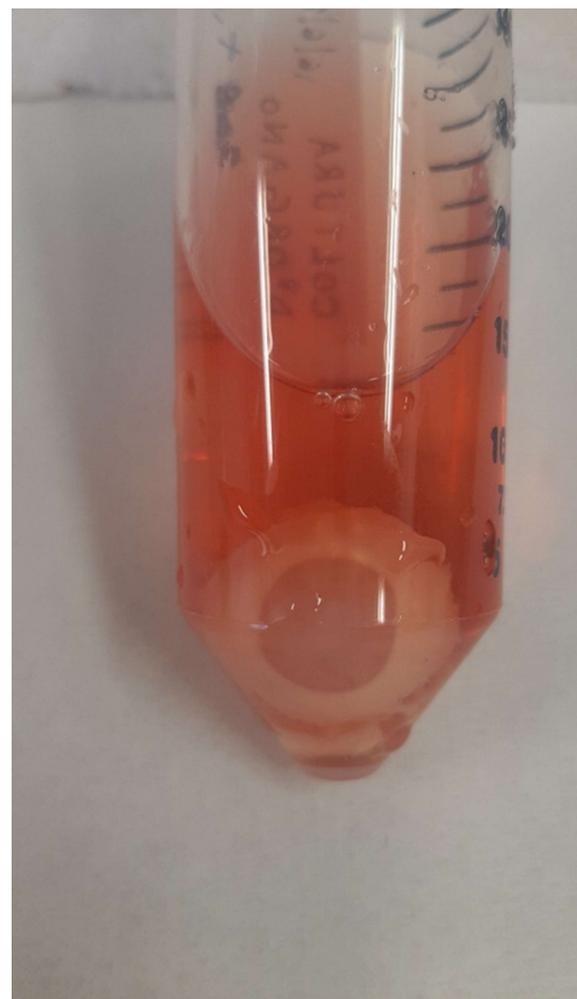
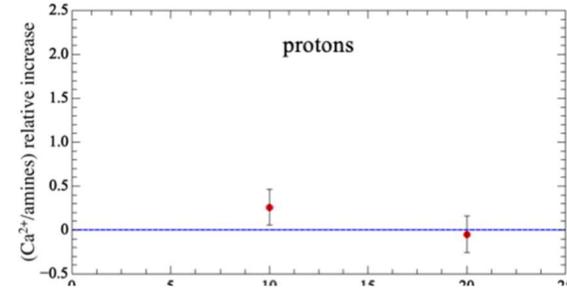
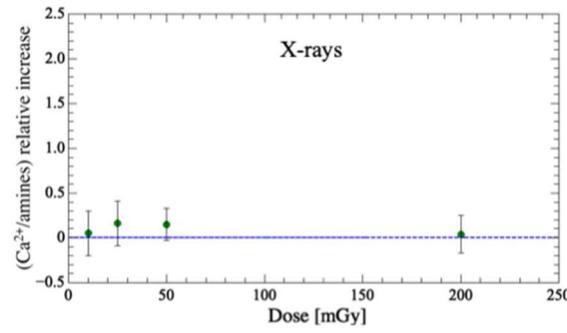
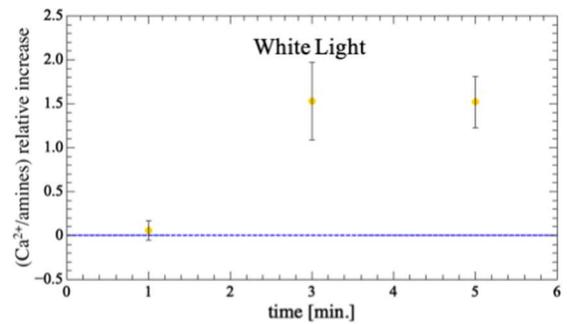
**Ipotesi:** IR attivano le vie di segnalazione

Università di Roma



Università degli Studi  
di Roma Tor Vergata





# Impianti di irraggiamento



Istituti  
Clinici  
Scientifici  
Maugeri



Fondazione IRCCS  
Policlinico San Matteo

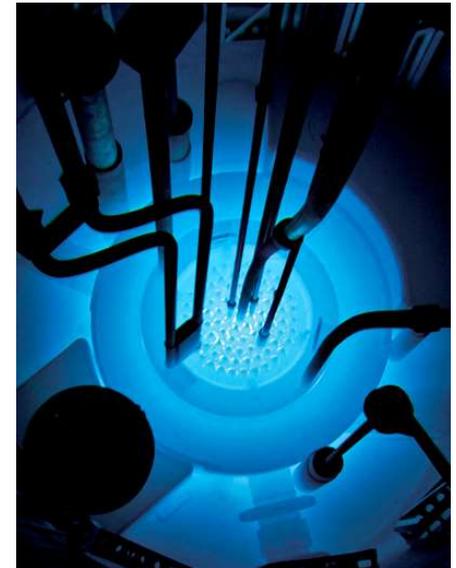
**CNAO**  
Centro Nazionale di Adroterapia Oncologica



Fotoni

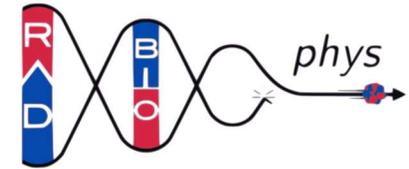


Protoni e ioni carbonio



Neutroni

# Laboratorio di Radiobiologia e Biofisica delle Radiazioni - RadBioPhys



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<http://radbiophys.unipv.eu>



## Membri del gruppo:

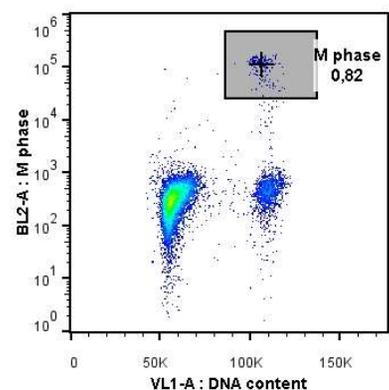
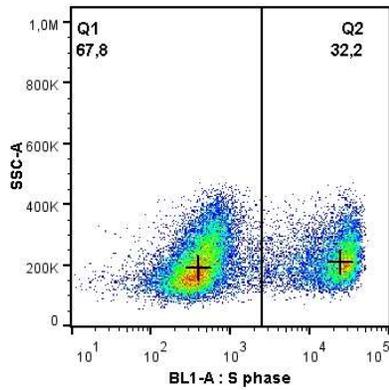
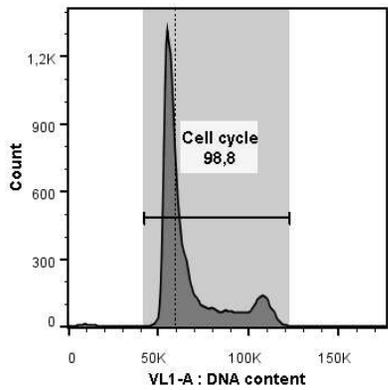
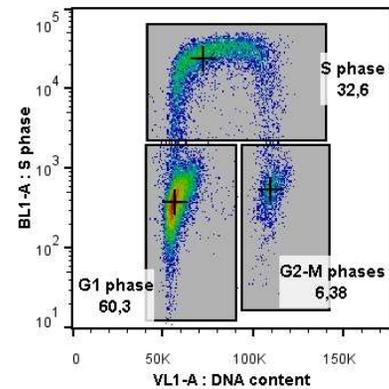
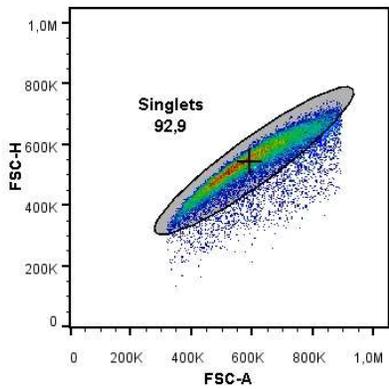
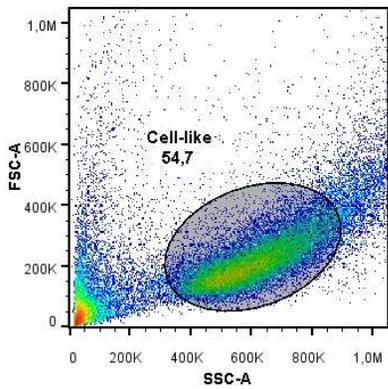
Prof. Giorgio Baiocco  
Isabella Guardamagna  
Alice Mentana  
Leonardo Lonati  
Ombretta Iaria  
Andrea Previtali

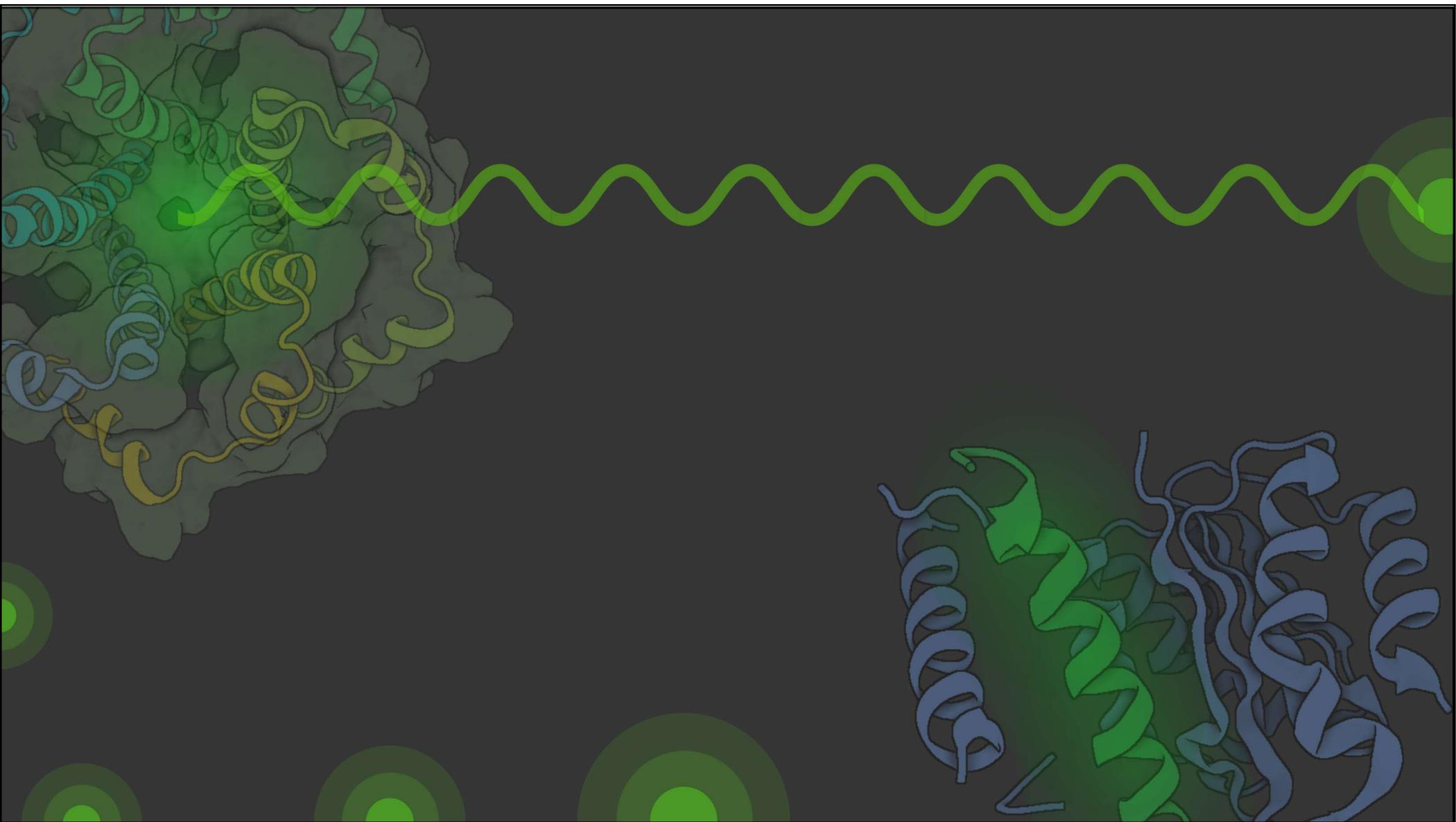


Studi teorici e sperimentali di effetti di radiazioni ionizzanti su strutture biologiche: applicate a radioterapia, diagnostica, radioprotezione.

**EXTRA**

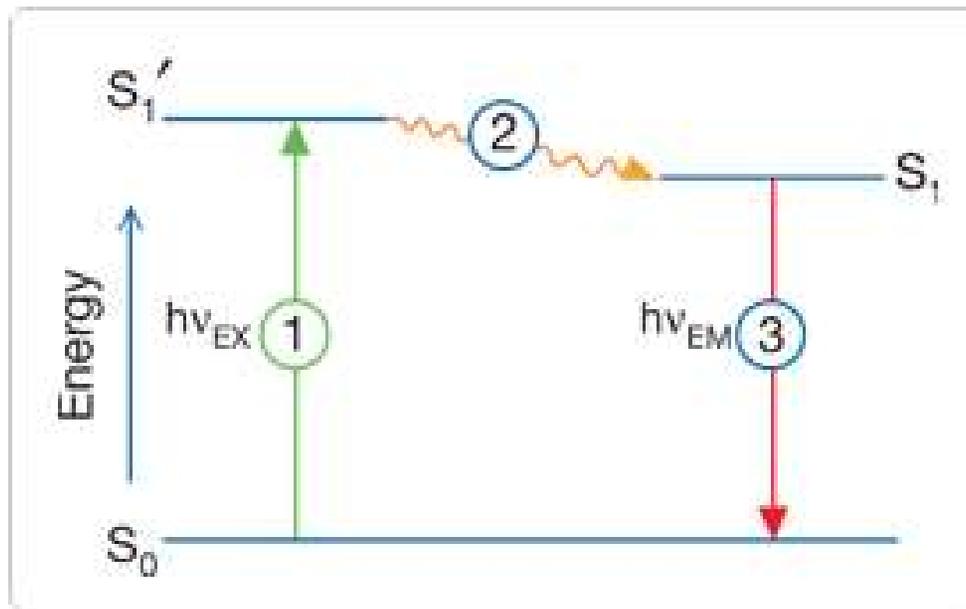


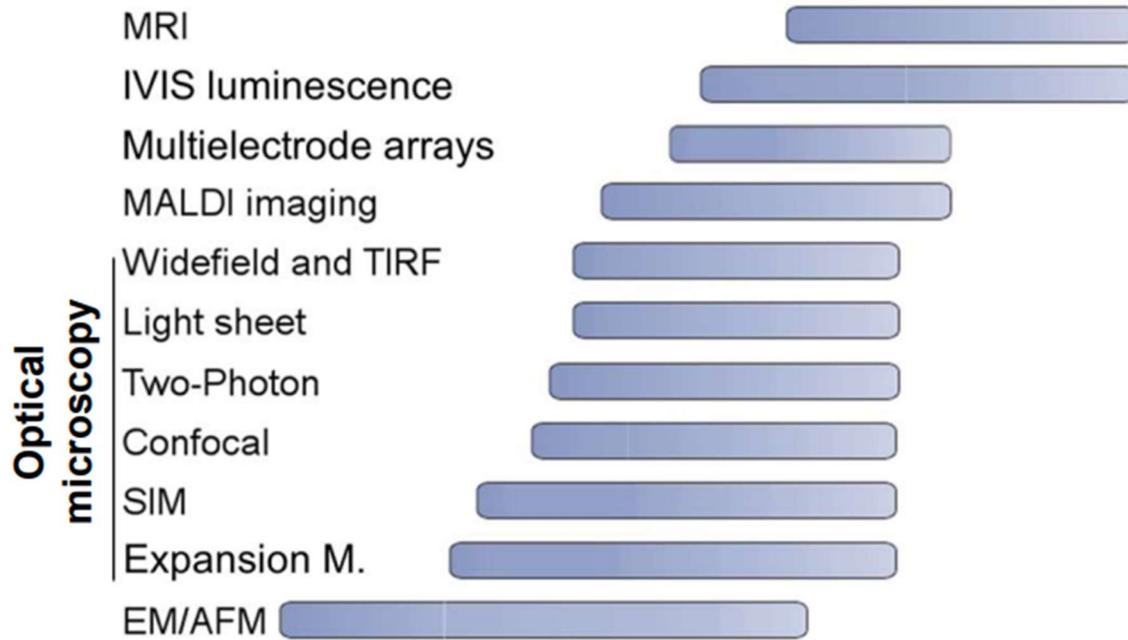
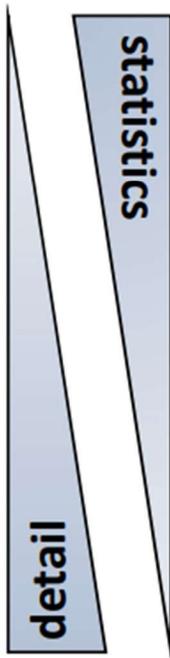
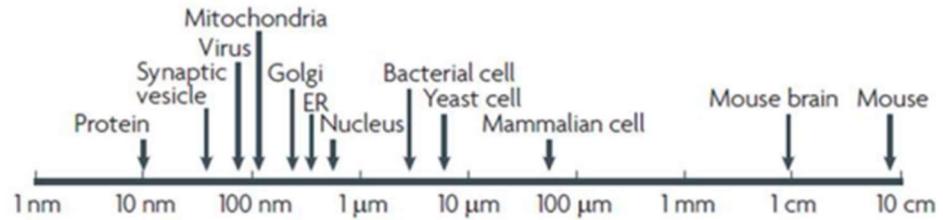




# Fluorescence

Fluorescence is the result of a three-stage process in particular molecules called fluorophores





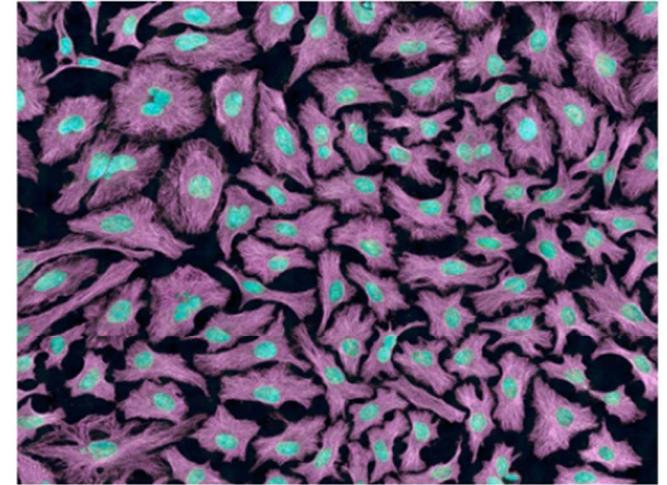
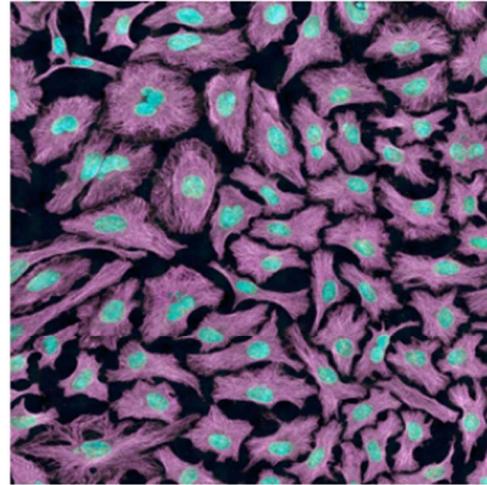
Modified from Fernandez-Suarez, Nat Rev 2008

*in vitro*  
in vī·tro/

EXPERIMENTAL  
MODEL SYSTEM

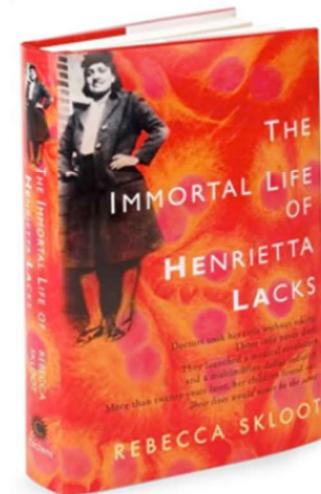
### Established cell lines

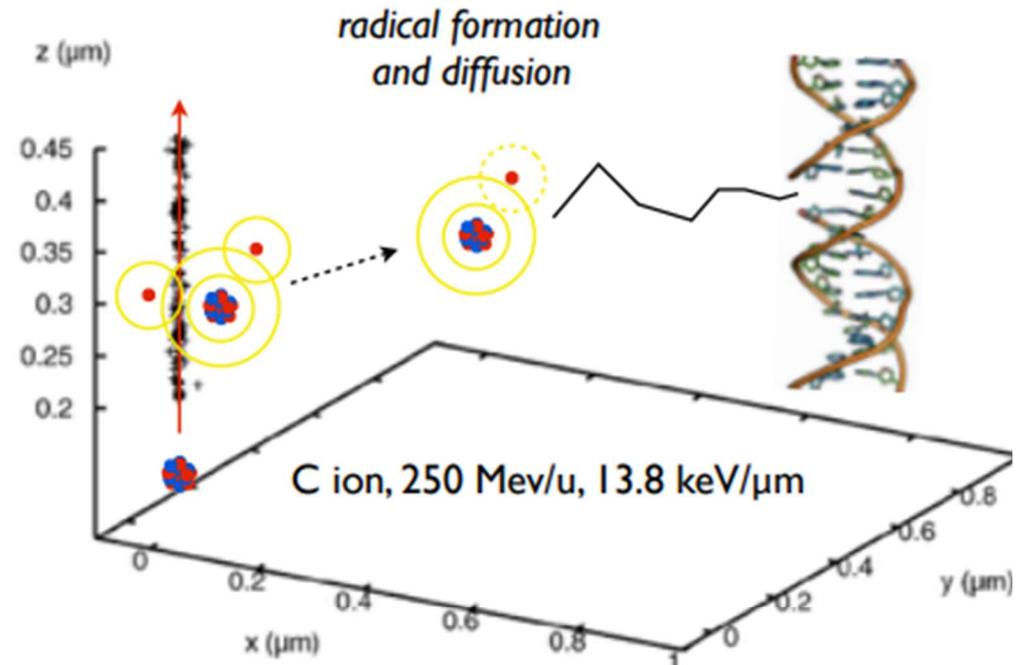
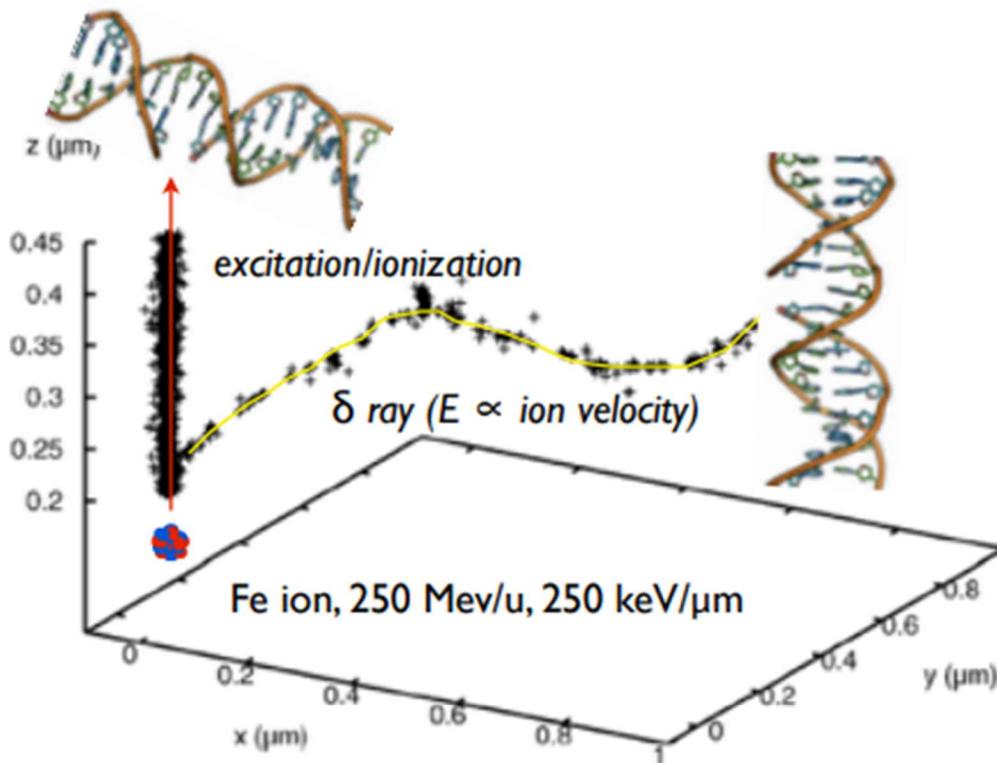
- commercial supplying
- homogeneity of the model system
- reproducibility in data generated



HeLa cells - the oldest immortalized h HeLa cells - the oldest immortalized human cell line

*In 1951, Henrietta Lacks came into John Hopkins Hospital in Baltimore, worried about a lump in her abdomen, where she was diagnosed and treated for cervical cancer (adenocarcinoma of the cervix, a particularly aggressive type of cancer). She eventually died of her cancer later that year, without knowing what her cells would help accomplish. The surgeon treating Henrietta's adenocarcinoma had been collecting cancerous tissue samples from patients for research lead by Dr. George Gey, Director of the Tissue Culture Laboratory at John Hopkins. His objective was to cure cancer by creating an immortalized cell line for research, in order to develop therapies and medicines. For years, Dr. Gey and his wife Margaret (a trained surgical nurse) had been trying to cultivate human cells in vitro. All their previous attempts at growing human cells in a laboratory lead to the death of the cell cultures within a few generations. That is, until Henrietta's tumour sample: HeLa, named after the two first letters of Henrietta and Lacks.*





PARTRAC developed at Helmholtz Zentrum München, in collaboration with East Carolina University & UniPv

Alloni D et al (2012) Int. J. of Rad. Biol.88 (1-2) 77-86

Friedland W. et al (2011) Mutation Research 711, 28-40

Friedland W. et al (2017) Sci. Rep. 7 45161

# Microscopia ottica 2D

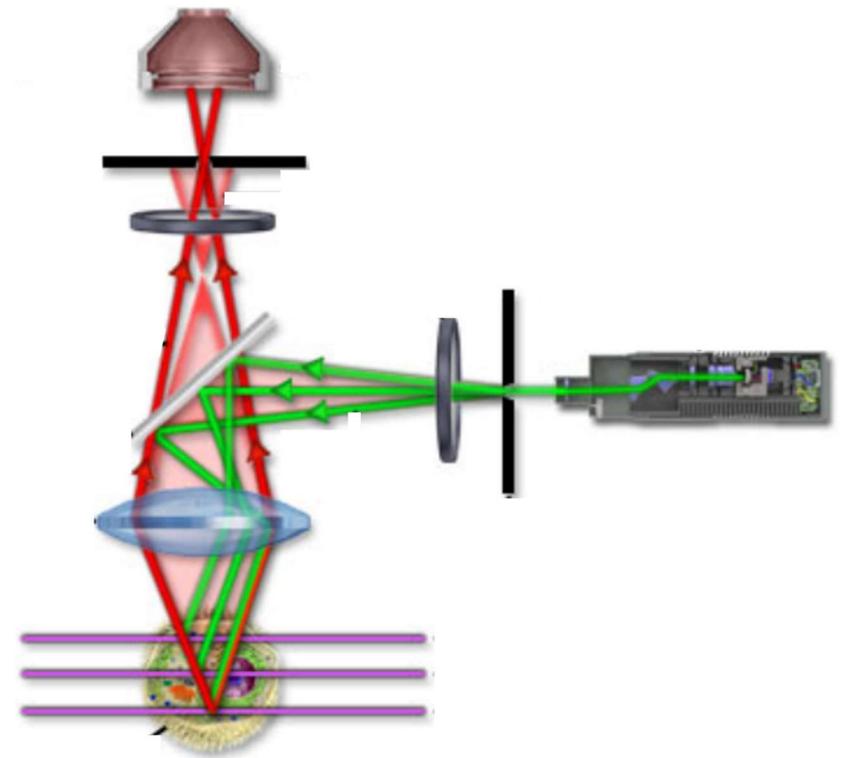
Approccio Biologia Tradizionale  
immagini 2D

1 solo piano focale a fuoco per  
acquisizione

Foci visibili non rappresentano  
solo foci singoli, ma anche  
artefatti derivati da super-  
imposizione di foci lungo  $Z$

**BASSA  
RISOLUZIONE**

# Microscopia confocale

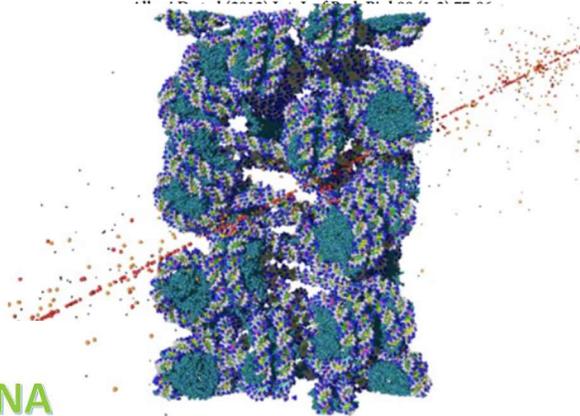


# Simulazioni Monte Carlo di danno radio-indotto

## Codici di struttura di traccia

Struttura di traccia: usa eventi singoli prodotti dalla radiazione, considerando ogni tipo di interazione (ionizzazione o eccitazione), l'energia depositata e la distribuzione spaziale degli eventi (**evento-per-evento**)

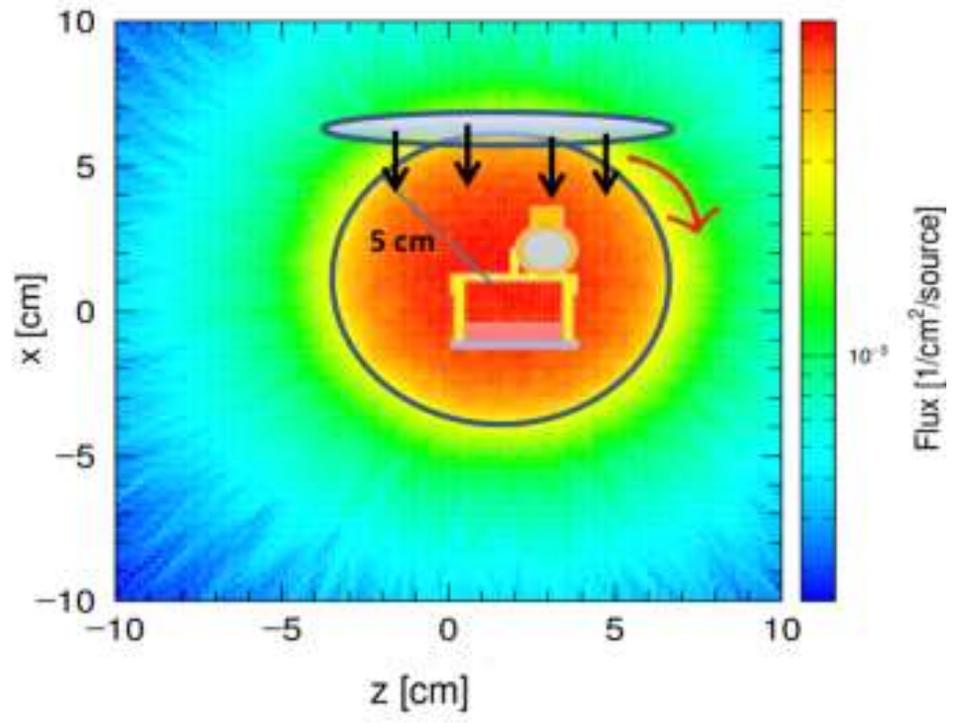
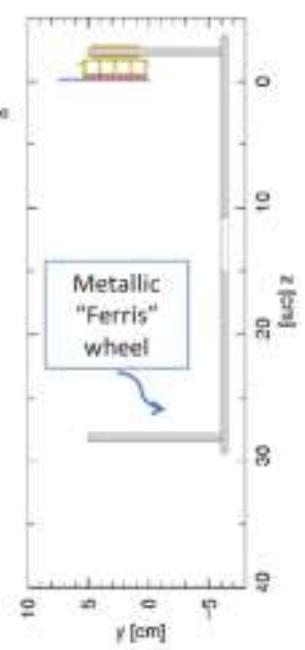
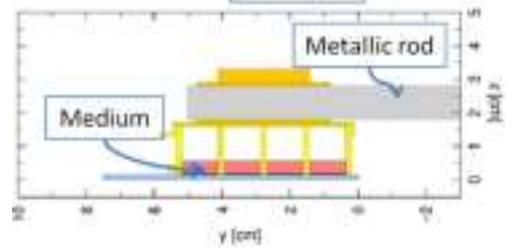
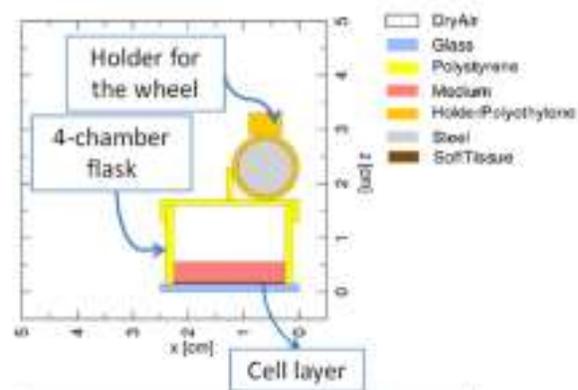
PARTRAC developed at Helmholtz Zentrum München, in collaboration with East Carolina University & UniPv



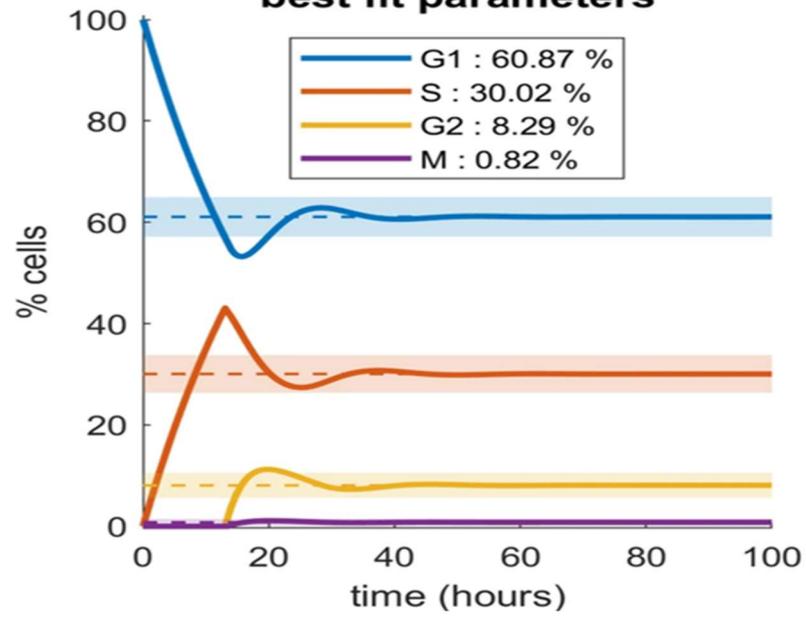
## Codici di trasporto delle radiazioni

Per particelle cariche, codici di trasporto usano valori medi di deposizioni multiple di energia (e.g. inelastic collision with target electrons), in segmenti di traccia (**storia-condensata**)





**(b) Model for sham condition with best fit parameters**

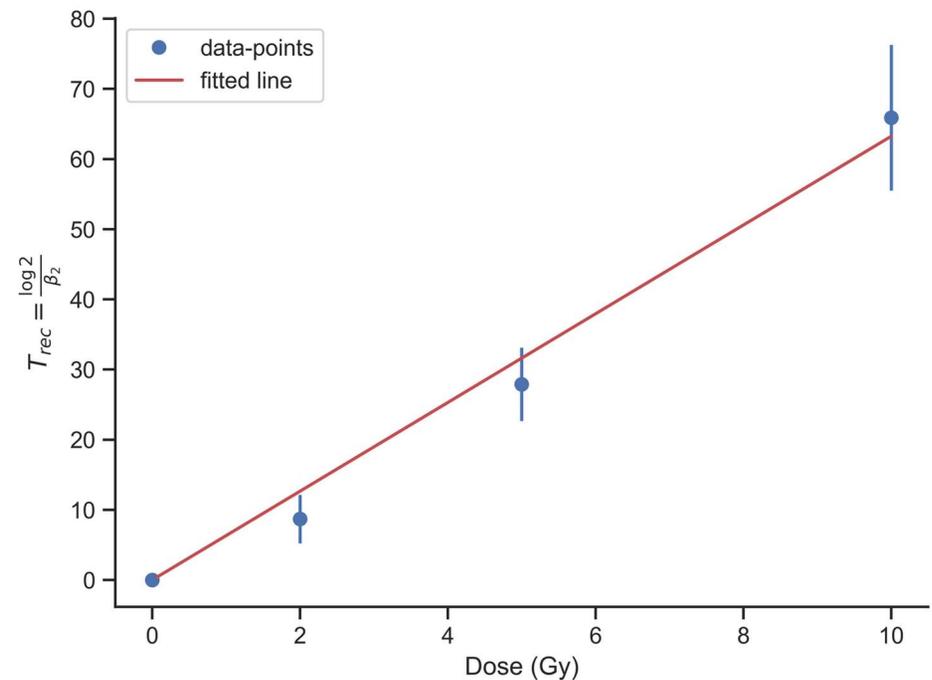


# IR-perturbation: dose-dependent parameters

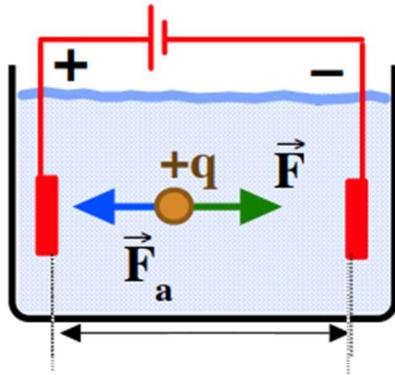
$$\overline{\beta_1} = \mathbb{E} \langle \hat{\beta}_1(d) \rangle = 0.785(489) h^{-1}$$

$\beta_1$  as a constant hyperparameter

$$\beta_2 = \frac{\ln 2}{T_{rec}} = \frac{\ln 2}{\rho D} \implies \text{Fit } T_{rec} \text{ vs dose}$$



## Gel Electrophoresis



Basic principle:

a charged molecule will migrate in the E field generated by the electrodes, winning frictional forces of viscous nature

$$\begin{aligned}\vec{F} &= +q \vec{E} \\ \vec{F}_a &= -f \vec{v}\end{aligned} \Rightarrow v_s = \frac{qE}{f} = \mu E$$

$$\mu \propto q, 1/\text{size}$$

electrophoretic mobility  $\propto$  charge/mass ratio

use of a dense porous gel - e.g. agarose or polyacrilamide

→ migration velocity strongly determined by the size!

→ shorter distances travelled by heavier molecules

## Single-cell electrophoresis

COMET assay

