UK Health Security Agency

Biological and physical retrospective dosimetry

16^{th} EURADOS School: Contribution of dosimetry in the field of nuclear emergency preparedness and radiological accident management,

June 2023

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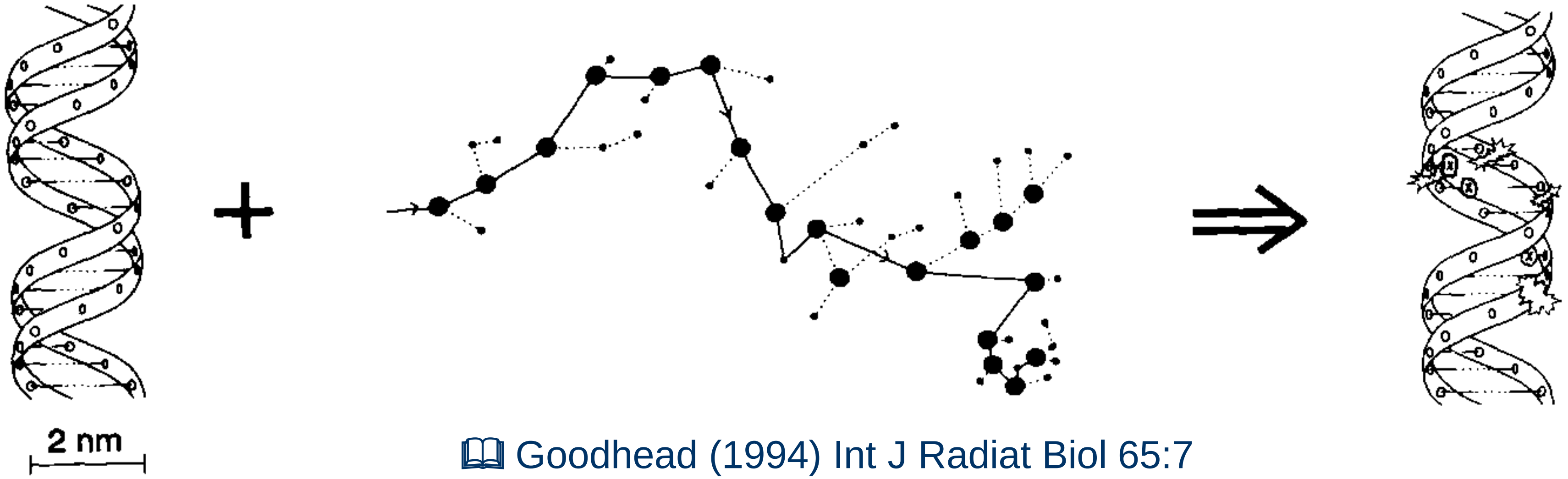
Dose assessment approaches

PHYSICAL DOSIMETRY	BIOLOGICAL DOSIMETRY	CLINICAL DOSIMETRY
Dosimetry badge Fortuitous: Luminescence Electron spin resonance Bioassays	Cytogenetics (Dicentrics, FISH, PCC, MNA)# Biochemical markers Somatic mutations Gene/miRNA expression	Vomiting Diarrhoea Blood cell count Skin reactions...

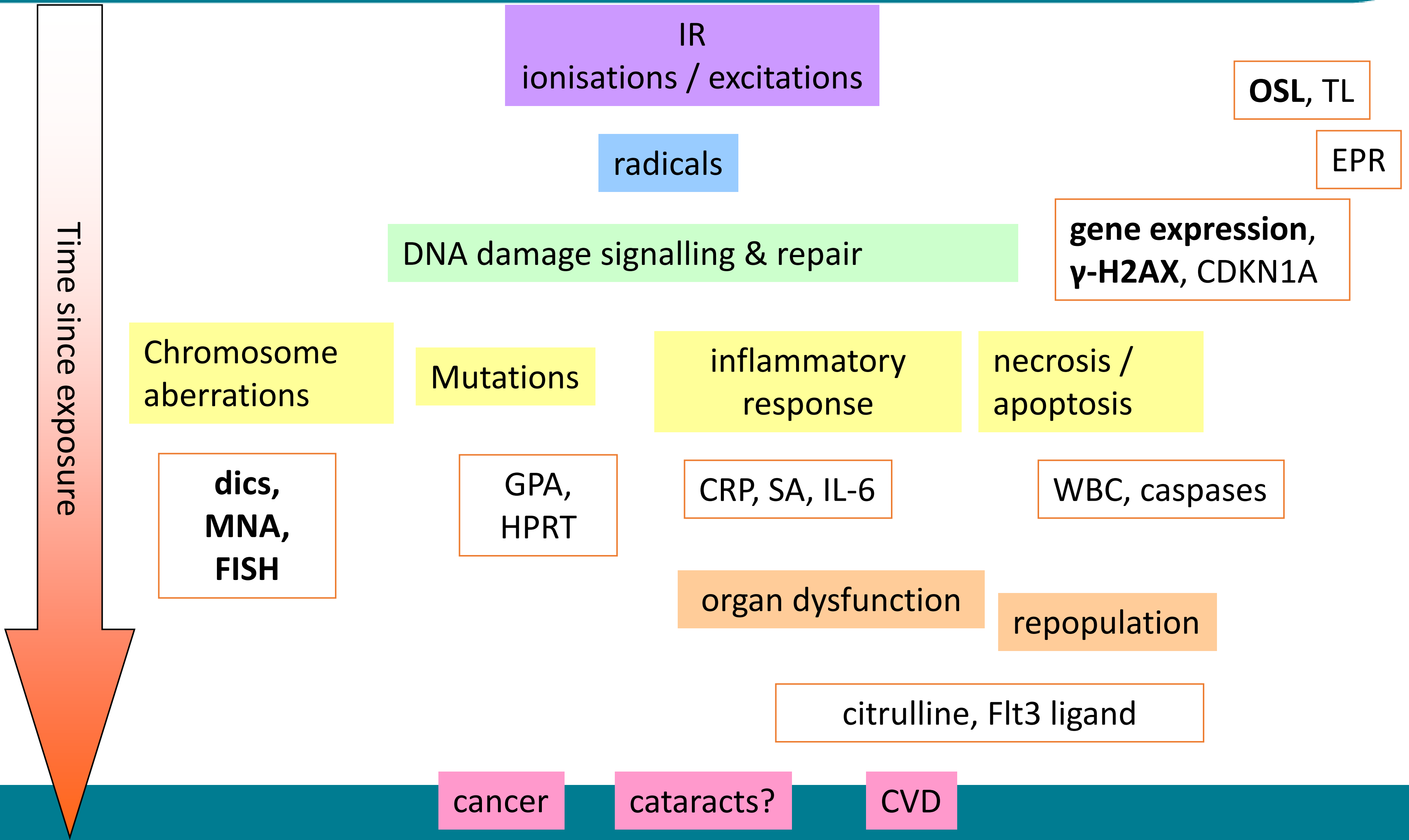
Spectrum of damage induced by IR

Damage induced by 1 Gy X-rays in a human cell:	**physical**	• 100,000 ionisations in the cell nucleus • 2,000 direct ionisations in the DNA
	biochemical	• 1,000 single-strand breaks • 1,000 damaged bases • 150 DNA protein crosslinks • **35 double-strand breaks**
	cellular	• **0.1 dicentrics / micronuclei** • 0.3 lethal events • 10^{-5} *hprt* mutations



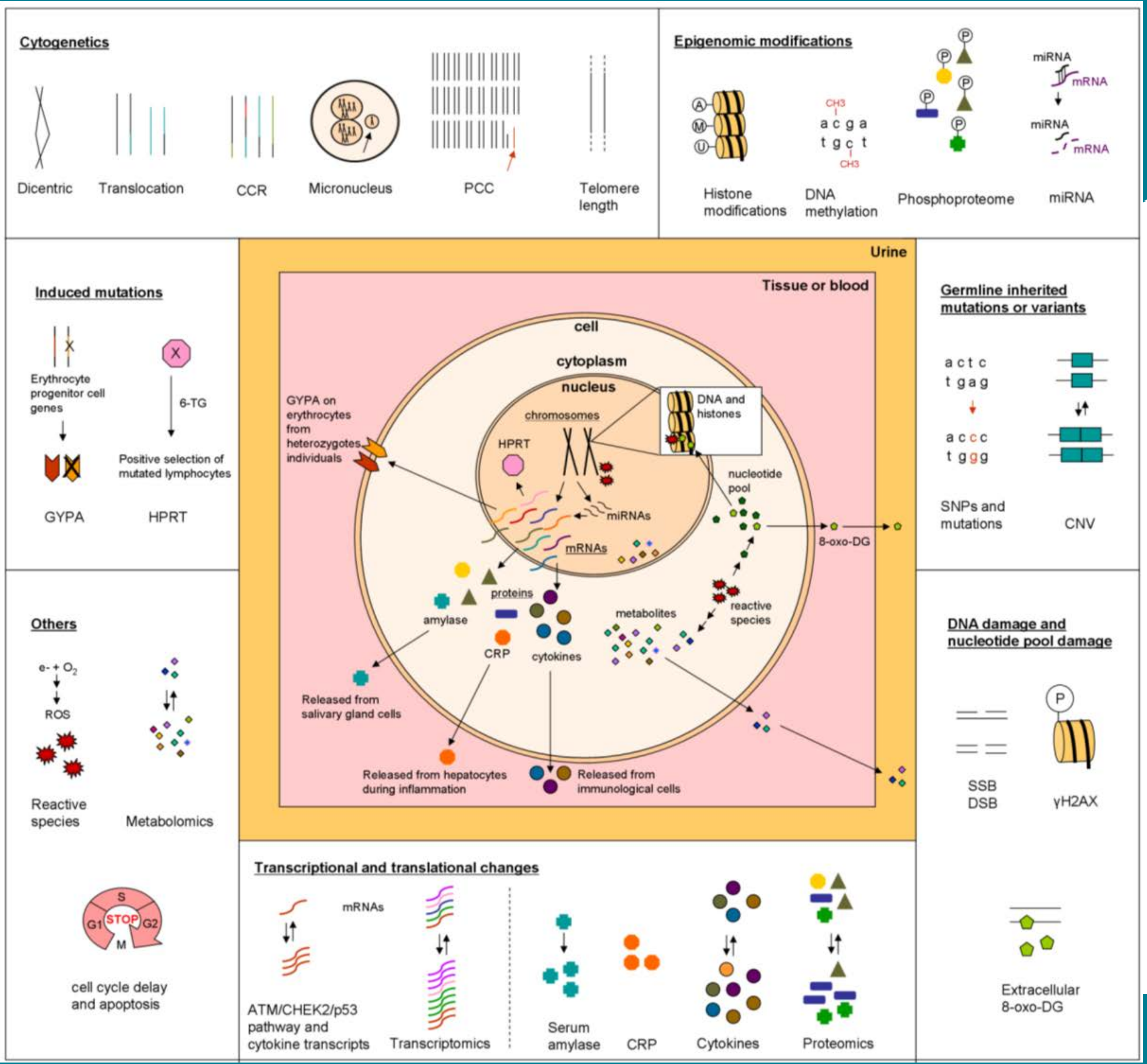
📖 Goodhead (1994) Int J Radiat Biol 65:7

Biological effects of IR -> Biodosimetry



Exposure biomarkers

Pernot et al, Mutat Res 751: 258-286



The ideal dosimeter

Specific to radiation

Low background

Low donor variability

Low doubling dose

Dose response calibration

Persistent effect

Ease of sampling

Rapid analysis

Low cost

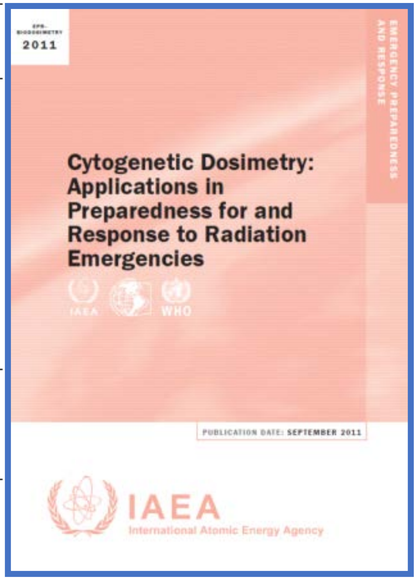
‘Risk meter’

Characteristics of biodosimetry methods

📖 Ainsbury et al (2011) Radiat Prot Dosimetry, 147:573-92; Rothkamm & Lloyd (2013) Comprehensive Medical Biophysics Vol. 8, Ch.14; Wojcik et al, 2017, IJRB.

Method	Radiation-responsive tissue	Time since exposure: **d**ays **m**onths **y**ears	Exposure: **P**rotracted **N**on-uniform	Time (h) from sample receipt to dose estimate	Agent specificity	Dose range (Gy) for photon equivalent acute whole body exposure 24 h ago[%]	Triage use	Automated analysis	Standardisation
OSL/EPR	PED[£]	d	P	<1	IR	>0.01	✓	Yes/No	
OSL	Teeth / nails	d		<1	IR	>3	✓	Yes	
EPR	Teeth / bones	d – m – y	P	1-48	IR	>0.05/>1		No	ISO in preparation
γ-H2AX	Lymphocytes	d	N	3	Genotoxins	>0.2	✓	Yes	
Gene expression	Peripheral blood	d	N	4 / 36[&]	Genotoxins	>0.1	✓	Yes	
Dicentrics – full	Lymphocytes	d – m	P; N	55	IR	0.1 to 5		Semi	ISO 19238
Dicentrics - triage	Lymphocytes	d – m	P	52	IR	0.5 to 5	✓	Semi	ISO 21243
PCC fragments	Lymphocytes	d	N	2[$]	IR	0.2 to 20	✓	No	
PCC rings	Lymphocytes	d – m	P	40[*]	IR	1 to >20	✓	No	
Micronuclei	Lymphocytes	d – m	P	75	Genotoxins	0.2 to 4	✓	Yes	ISO in preparation
MN centromere	Lymphocytes	d – m	P	80	Genotoxins	0.1 to 4		Semi	
FISH	Lymphocytes & their stem cells	d – m – y	P	120	IR	0.25 to 4		No	
HPRT	Lymphocytes	d – m		400	Mutagens	>1		No	
GPA	Erythroblasts	m – y		3	Mutagens	>1		Yes	
TCR	Lymphocytes	d - m	P	180	Mutagens	>0.5		Yes	
CRP	Hepatocytes	d		1	Wide range	>1	✓	Yes	Routine diagnostics
SA	Salivary gland	d		1	Wide range	>1	✓	Yes	Routine diagnostics
Flt3 ligand	Bone marrow	d		1	Wide range	>1	✓	Yes	Routine diagnostics
Citrulline	Enterocytes	d		1	Wide range	>1	✓	Yes	Routine diagnostics
Metabolomics	Range of organs	d		<1	IR	>1	✓	No	

[£]Personal electronic devices
[$]PCC fusion method
[*]PCC chemically induced
[&]PCR / array analysis
[%]Longer post-exposure time required for GPA to allow for mutant erythroblasts to mature to erythrocytes



Principles of retrospective dosimetry

1. **Identify** potentially exposed individuals

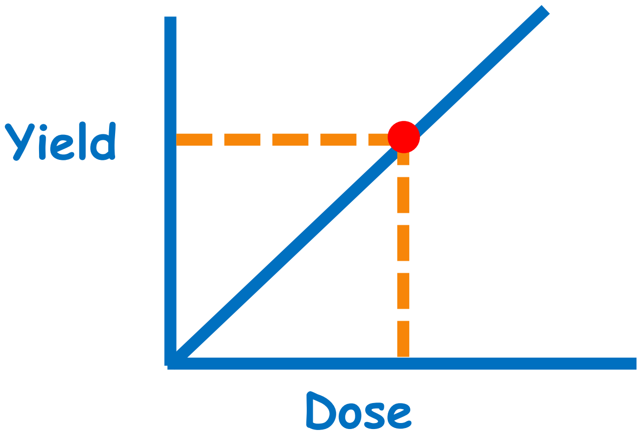
2. Take a **sample**

    A. Blood for biodosimetry

    B. Personal belongings for OSL/EPR

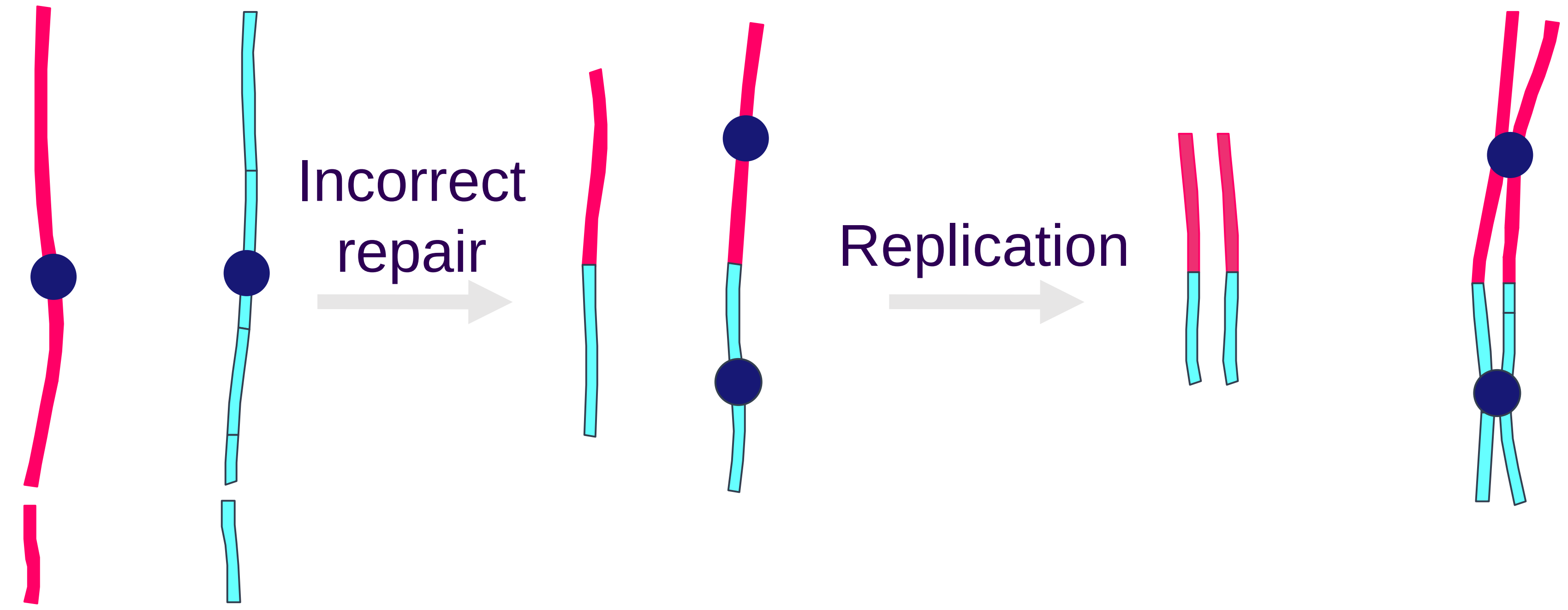
3. **Measure** radiation-induced signal

4. **Interpret**: Compare the test result with a calibration curve for dose response; estimate uncertainties



5. Inform **clinical treatment** decisions

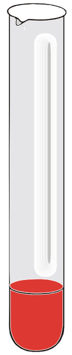
Dicentric formation



Chromosome dosimetry

- Blood lymphocyte culture

Phytohemagglutinin



Cytochalasin B

culture time = 72h

Colcemid

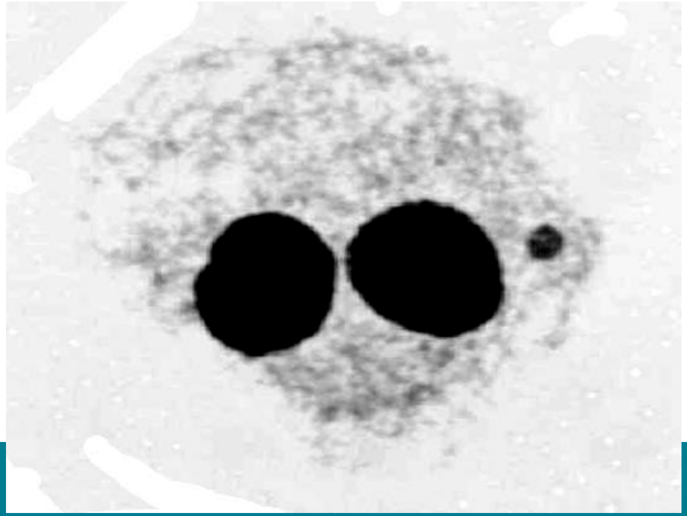
culture time = 48h

harvest
slide preparation
staining

Metaphase – Dicentrics/ translocations

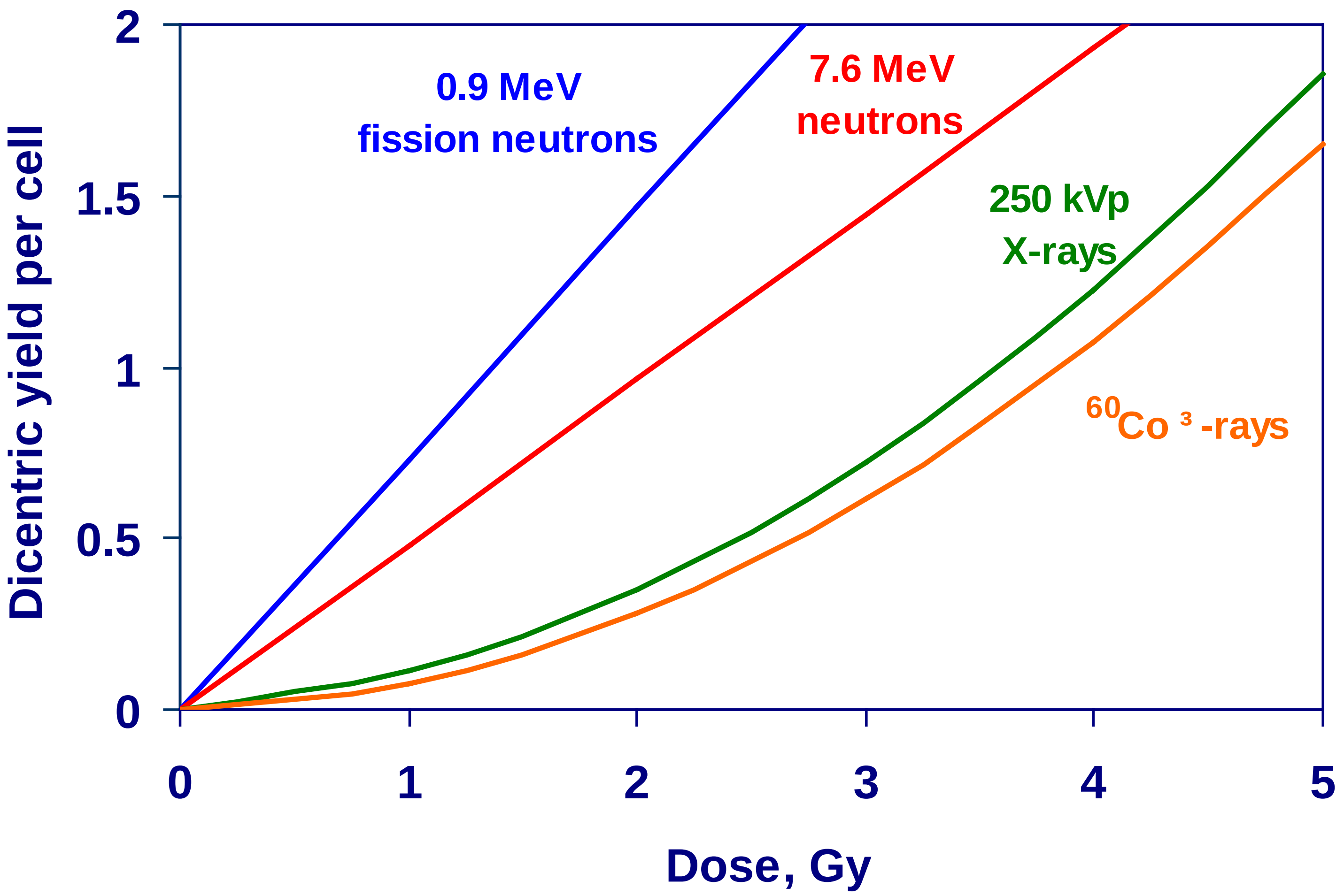
📖 IAEA (2001) TRS 405; IAEA (2011) Cytogenetic Dosimetry

Cytokinesis - Micronuclei



Dicentrics - Dose response relationship

Human lymphocytes:



Automatic dicentric hunting

📖 Vaurijoux et al (2009) Radiat Res 171:541

Manual scoring:

~ 50 cells in 1 hr

~ 500 cells in 10 hr

Automatic scoring:

1. Rapid response mode:

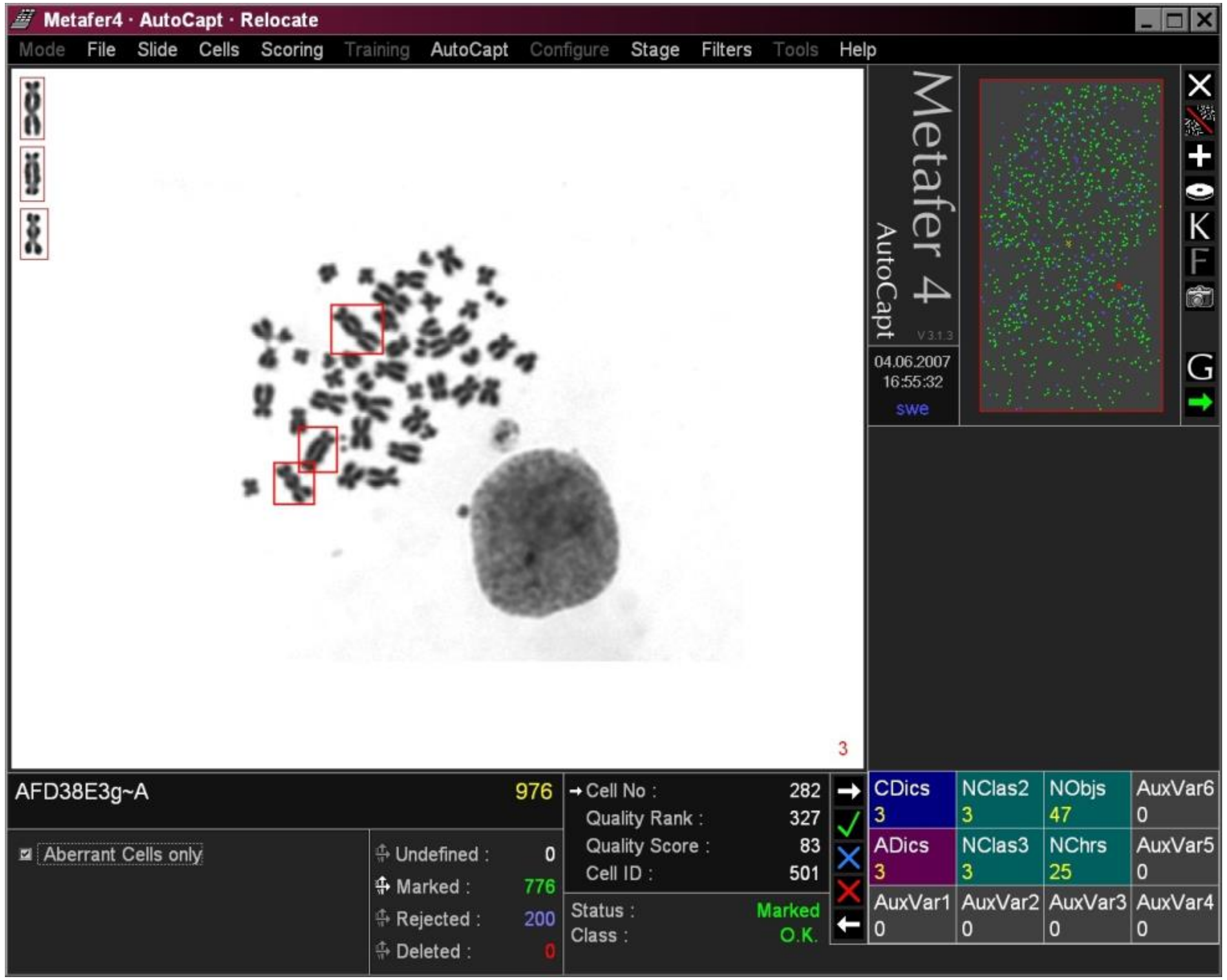
- 150 cells in 30 mins; ~ 2 min staff effort;
- +/- 0.5 Gy

2. Full mode:

3000 cells in 10 h;

~ 40 min staff effort;

+/- 0.15 Gy

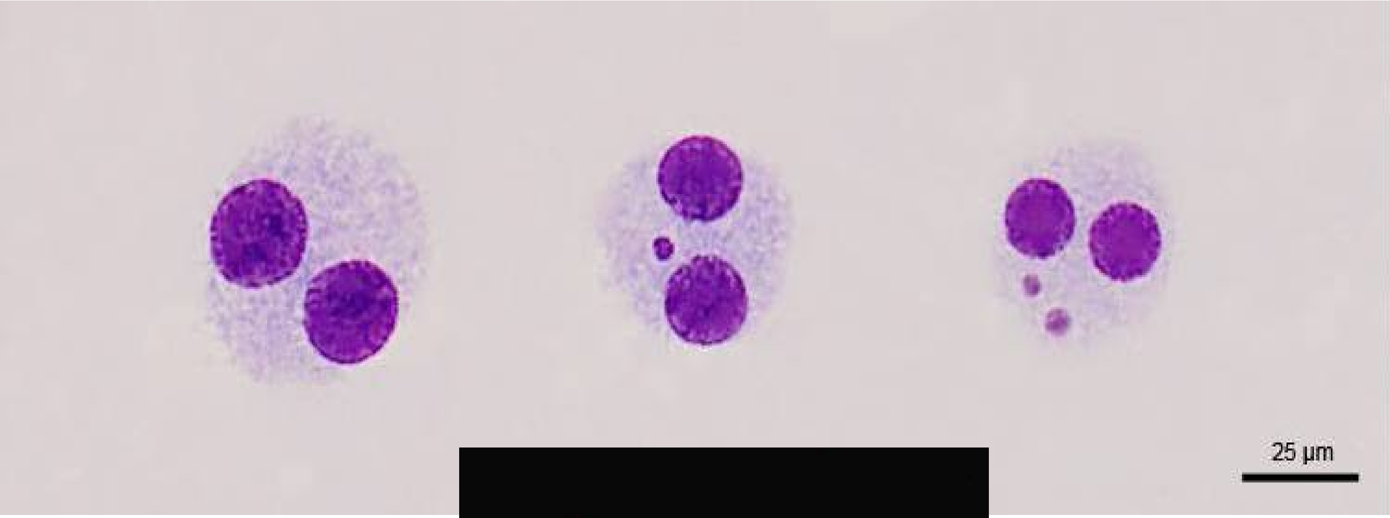


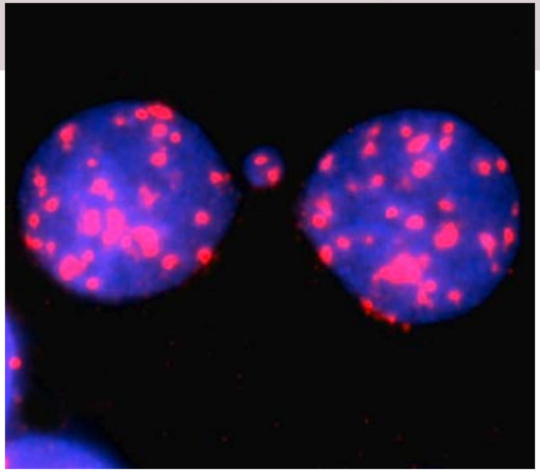
The micronucleus assay

Vral et al (2011) Mutagenesis 26:11

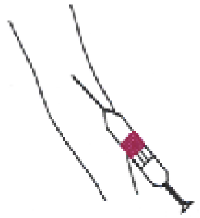
Comparison to dicentric assay

- MN arise mainly from acentric fragments
- Scoring easier & faster
- Not specific to radiation
- More background noise (especially for women…)
- Less sensitive (dose limit >0.2 Gy)
- Unstable signal fades like the dicentric
- Cannot easily discriminate between total and partial body exposure

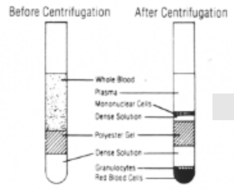




Premature chromosome condensation



PERIPHERAL BLOOD



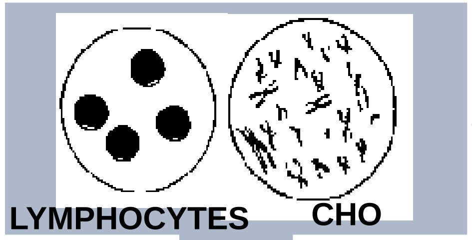
FICOLL SEPARATION

CHINESE HAMSTER OVARY (CHO) CELLS (Grown in BrdU)#

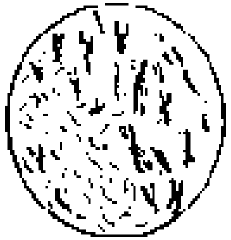
COLCEMID

MITOTIC SHAKE OFF (METAPHASE CELLS)#

FUSE IN PEG



Incubate 1 h
(Medium+PHA+Colcemid)#



PCC

Some limitations with current cytogenetic analysis methods

📖 Vinnikov et al (2010) Radiat Res 174:403

Ideal scenario: Acute, whole body, homogeneous, external irradiation of a known low LET source, with dose in the region of > 0.1 - ~ 5 Gy…

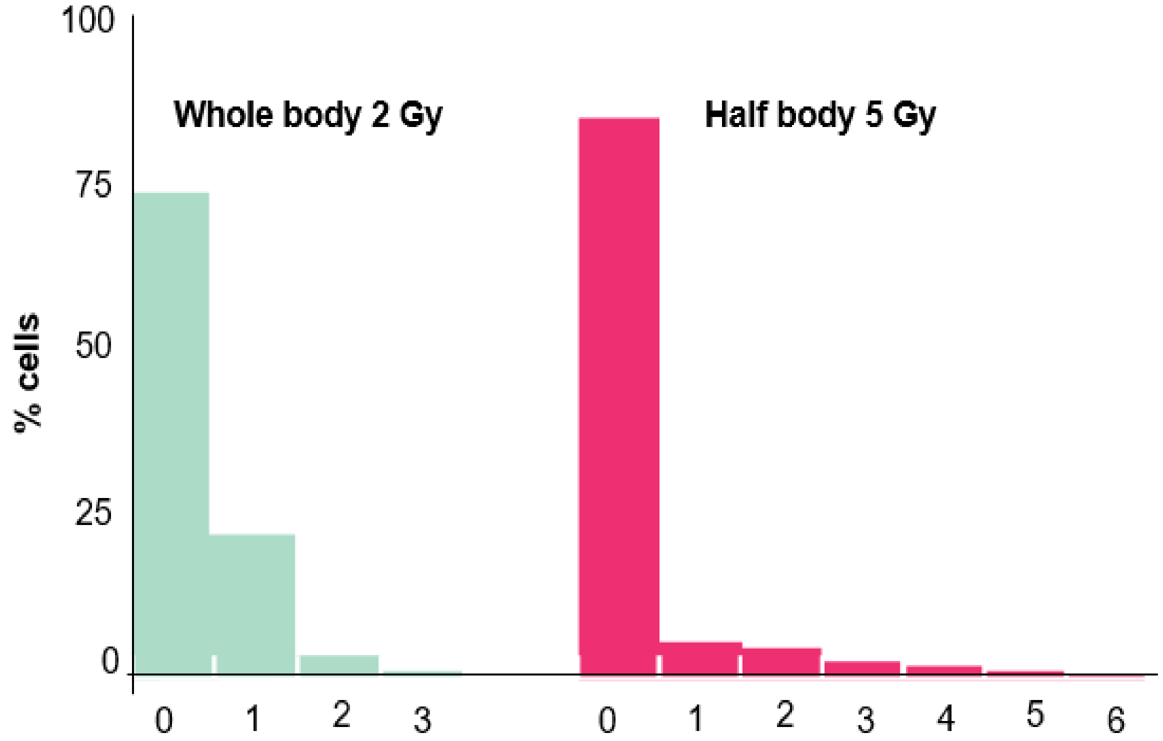
Real life exposures:

- Delayed blood sampling
- Protraction or fractionation, chronic exposures
- Inhomogeneity
- Very high or very low doses
- High LET radiation
- Internally deposited radionuclides
- Mass casualty scenarios
- Inter-scorer, -lab, -assay variation and other confounders

Non-uniform exposure

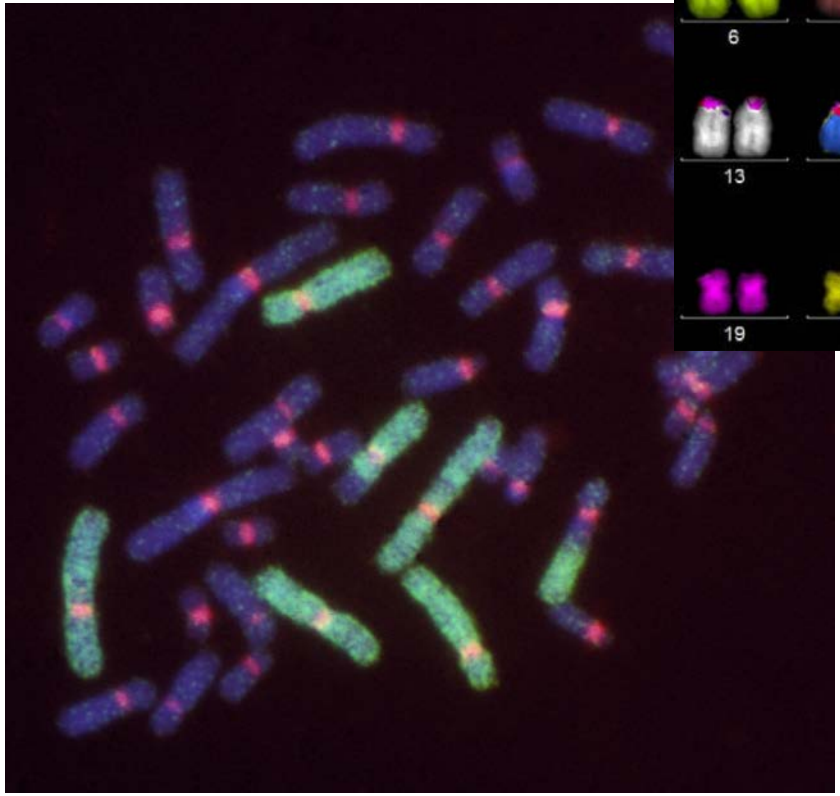
- Practically all accidents involve inhomogeneous / part-body exposure
- Dicentrics in lymphocytes indicate averaged whole body dose
- Uniform exposure (e.g. in test tube) – Poisson distribution
- Non-uniform – Overdispersion
- Dolphin et al.: ‘Contaminated Poisson’ method

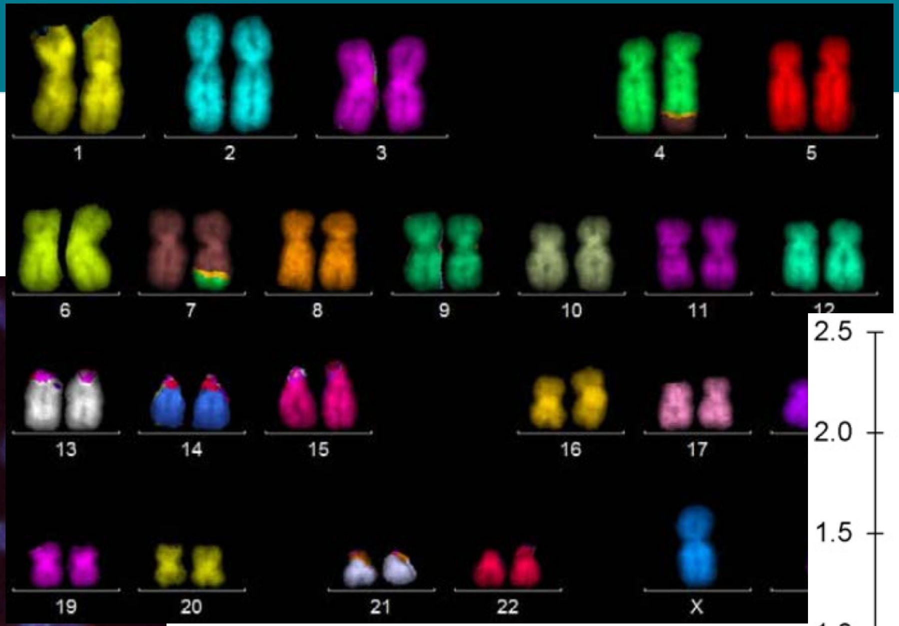
<u>0.3 dicentrics per cell:</u>

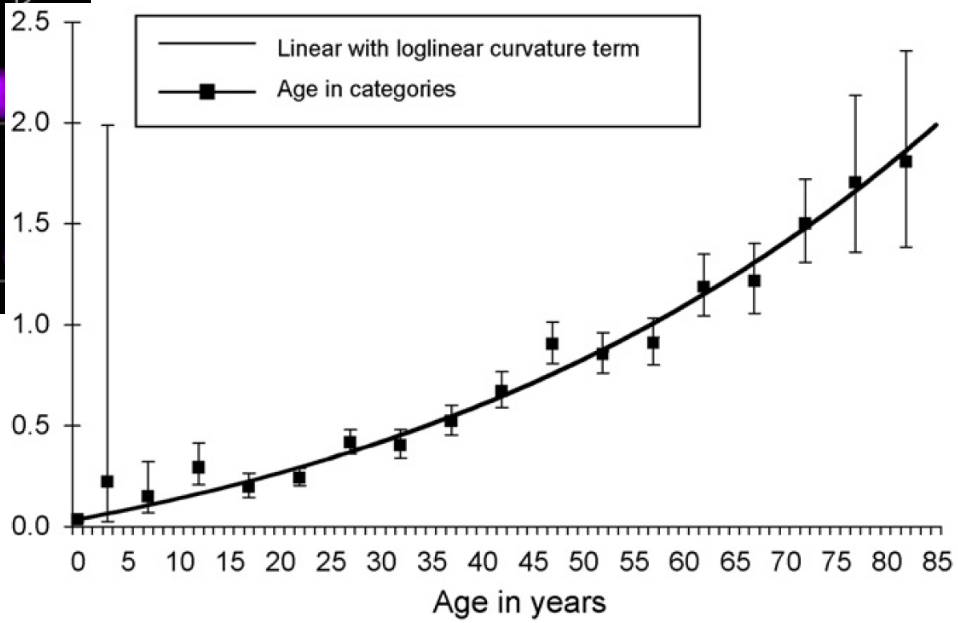


Dicentric distribution, dicentrics per cell

Fluorescence *in situ* hybridisation for stable translocations







📖 Edwards et al (2005); Edwards et al (2007); Hartel et al; Sigurdson et al (2008)

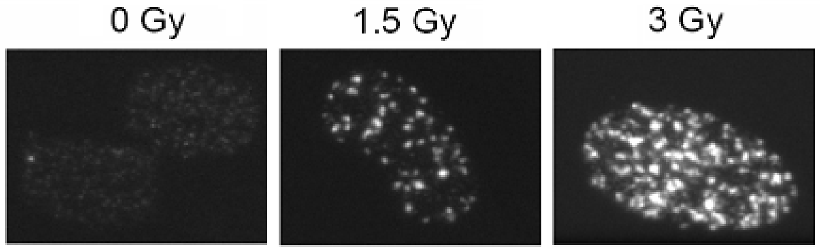
DNA Damage - Gamma-H2AX as a marker for DSB

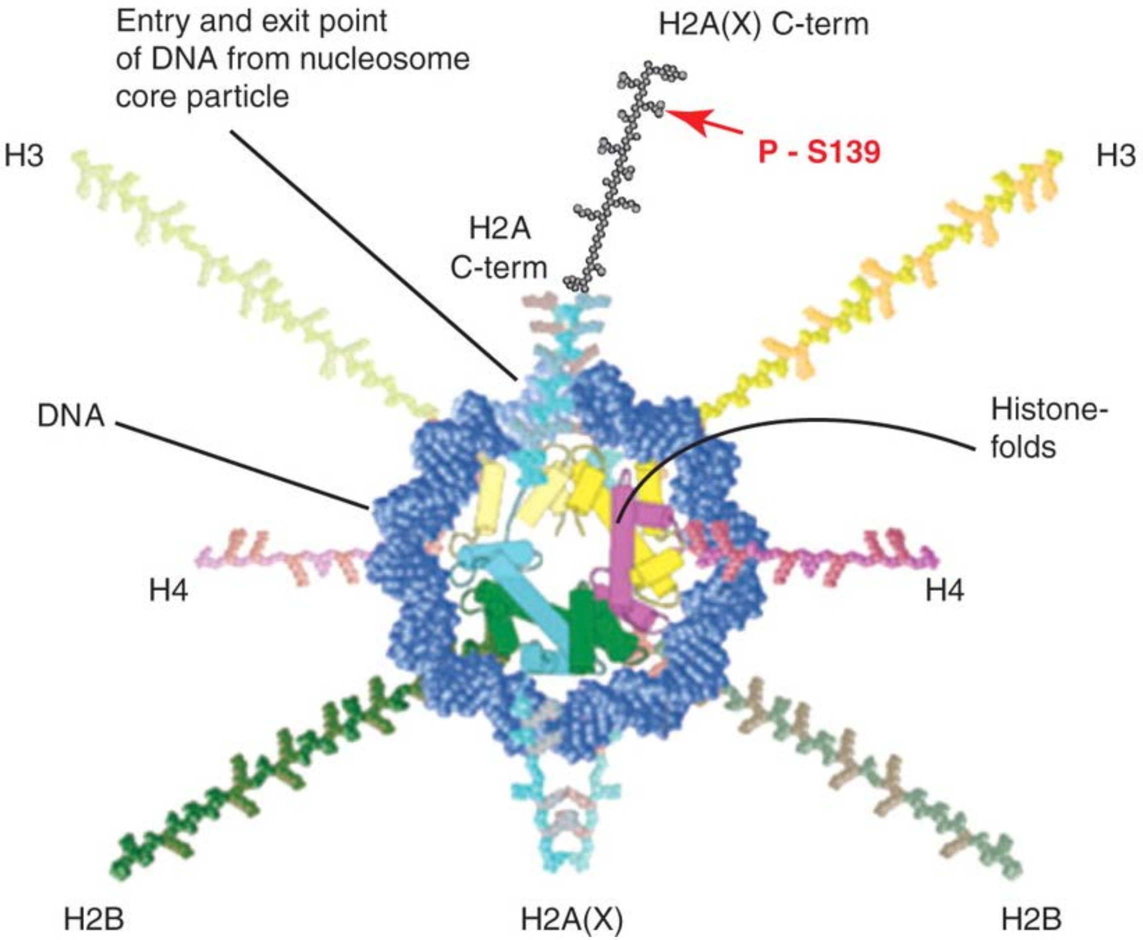
📖 Rothkamm & Horn (2009), Ann Ist Super Sanita 45:265-71; Barnard et al (2013), Genome Integr 4:1

- H2AX is a component of the DNA packaging scaffold (chromatin) in the cell

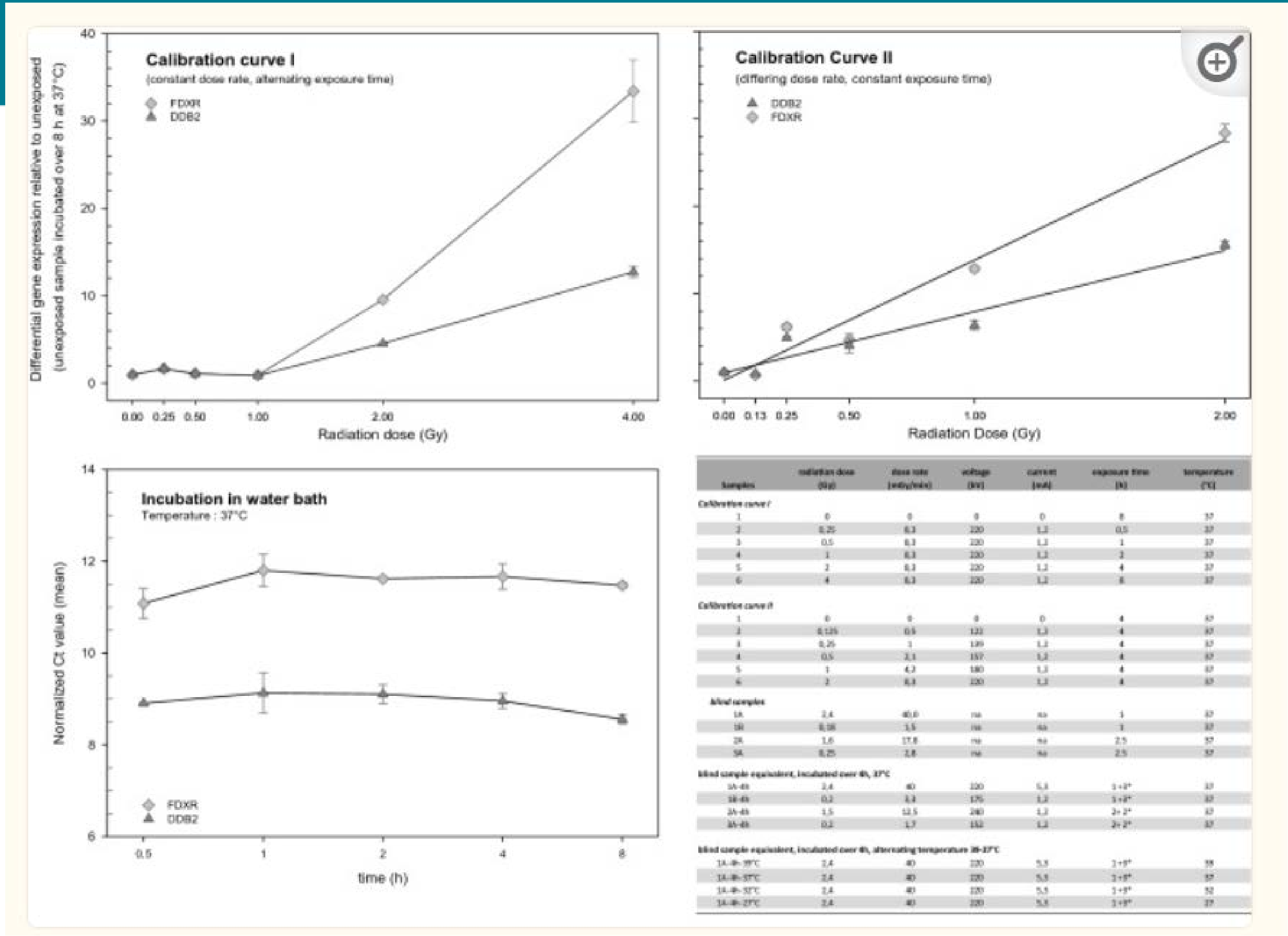
- Each radiation-induced DSB induces hundreds of H2AX phosphorylations.

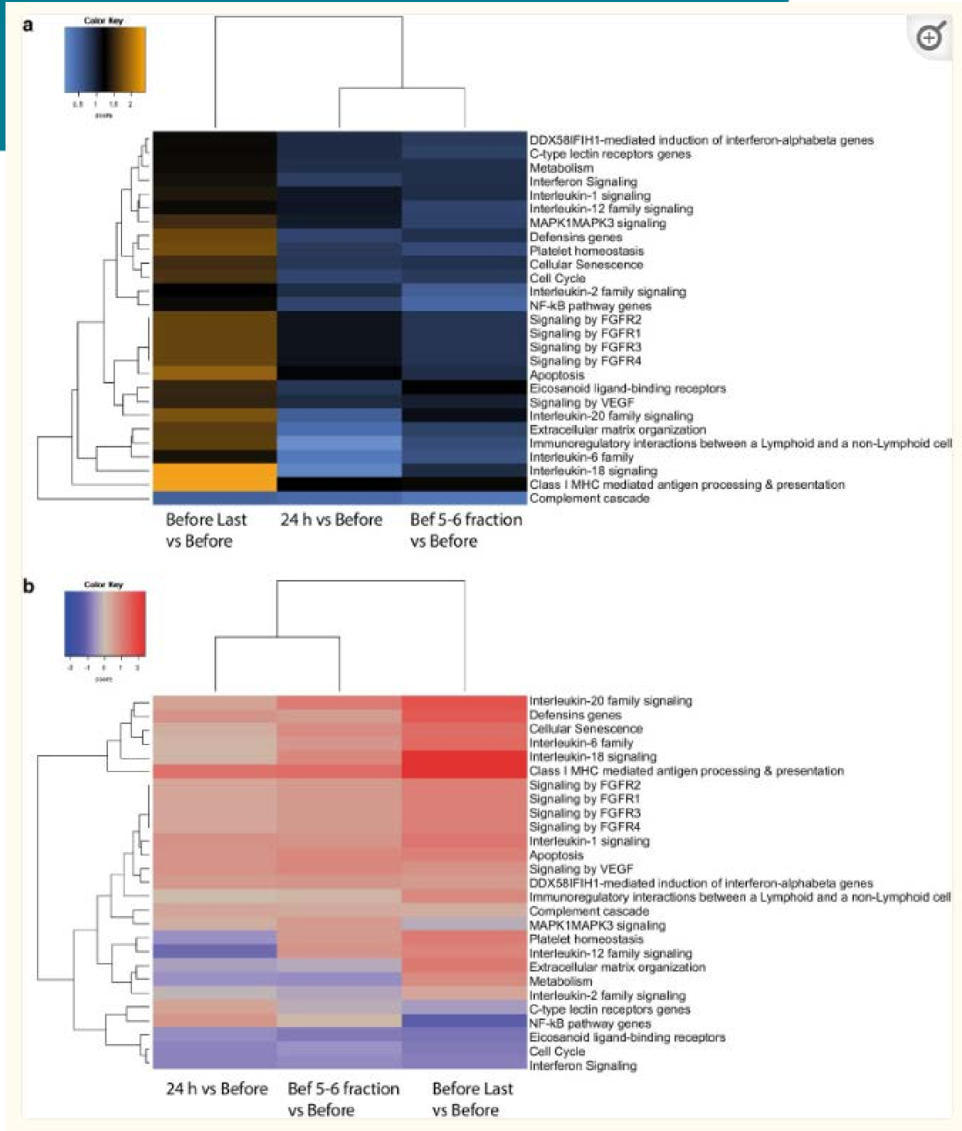
- γ-H2AX antibody visualises and quantifies individual DSBs at very low doses.





Gene expression, transcriptional responses

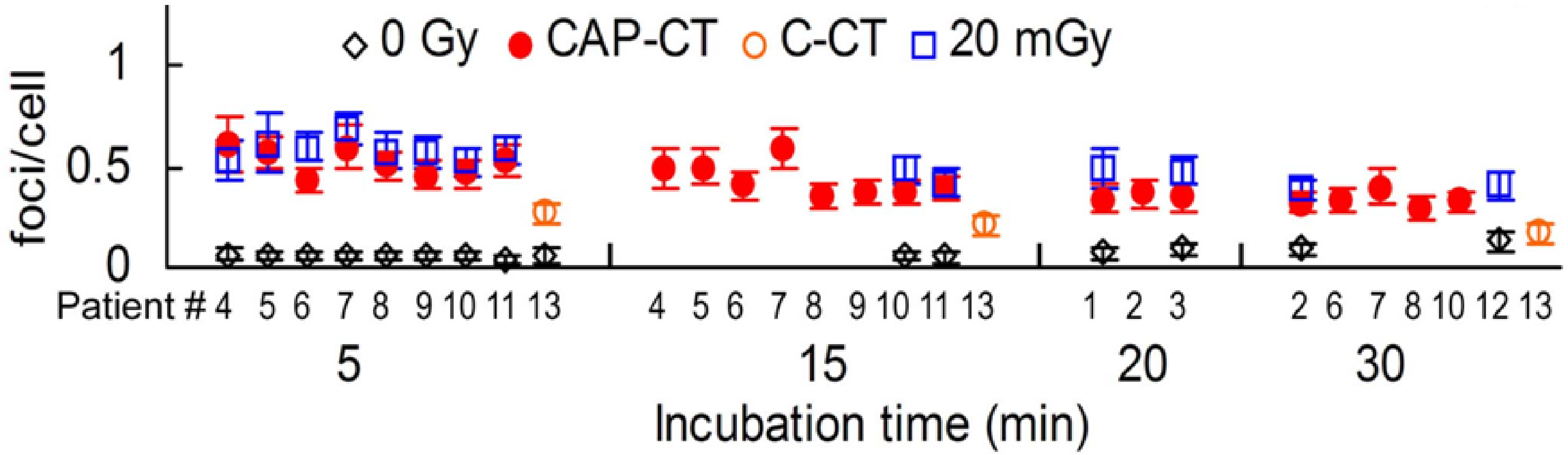


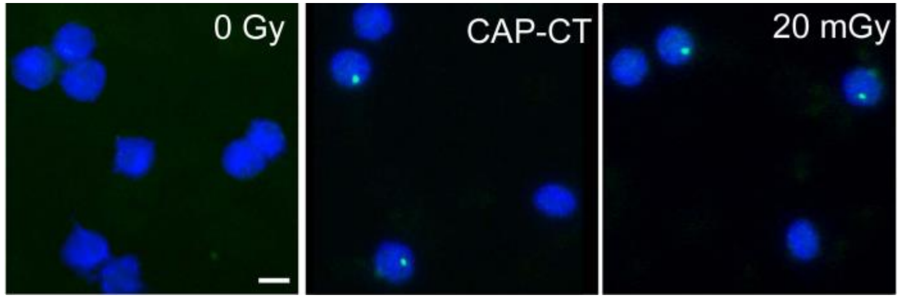


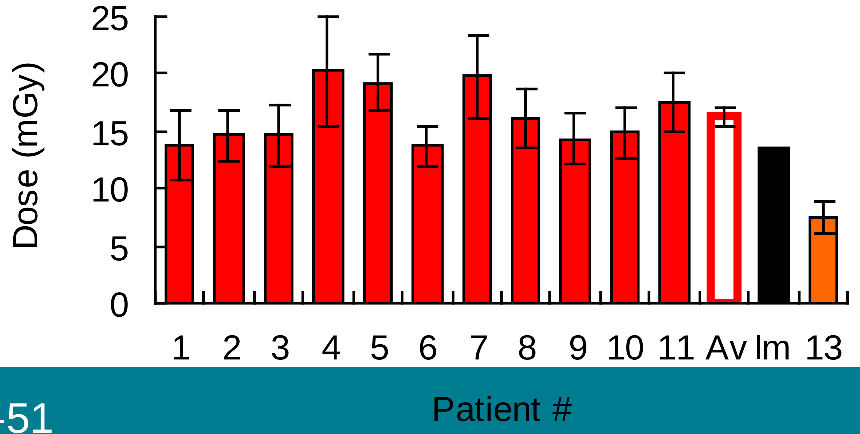
Abend et al., 2021: 10.1038/s41598-021-88403-4; Cruz-Garcia et al, 2022; 10.1186/s13014-021-01807-4

³ -H2AX assay for biological dosimetry

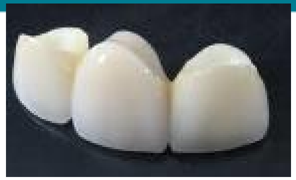
Blood samples taken after CT scans:

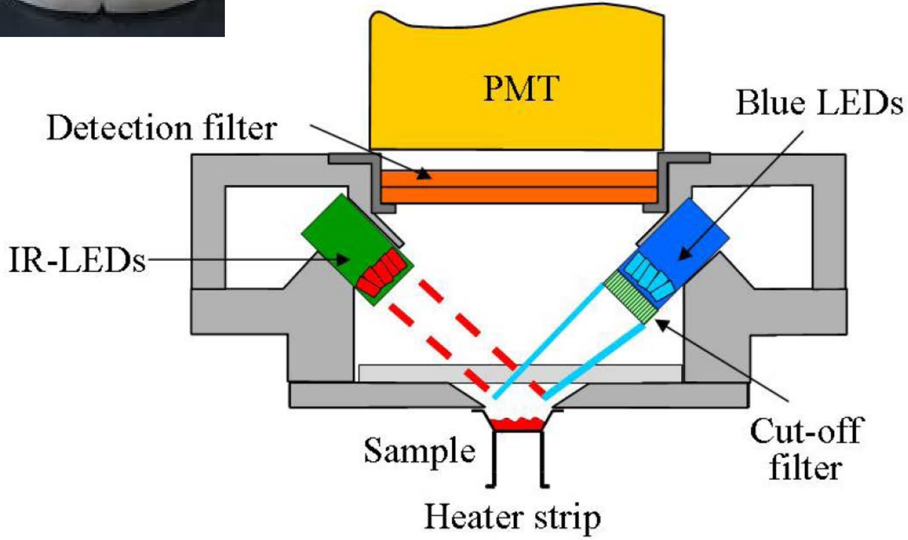






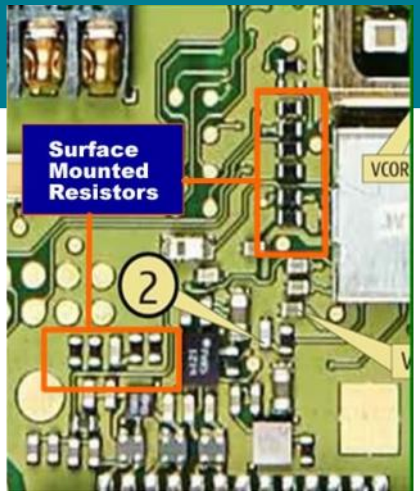
Thermo-/optically- stimulated luminescence

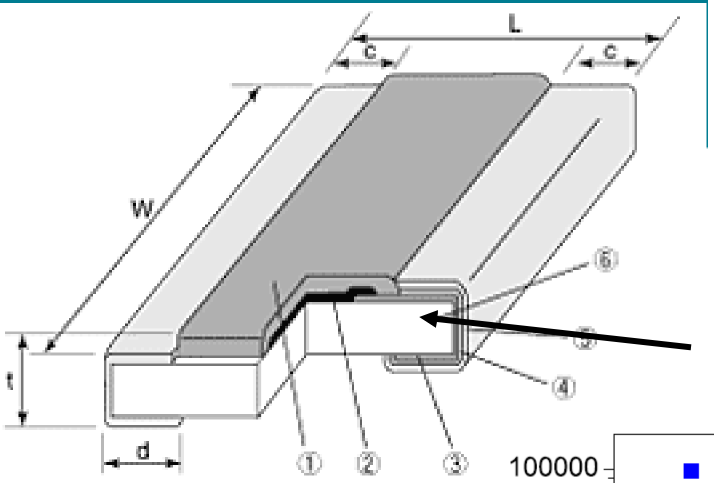




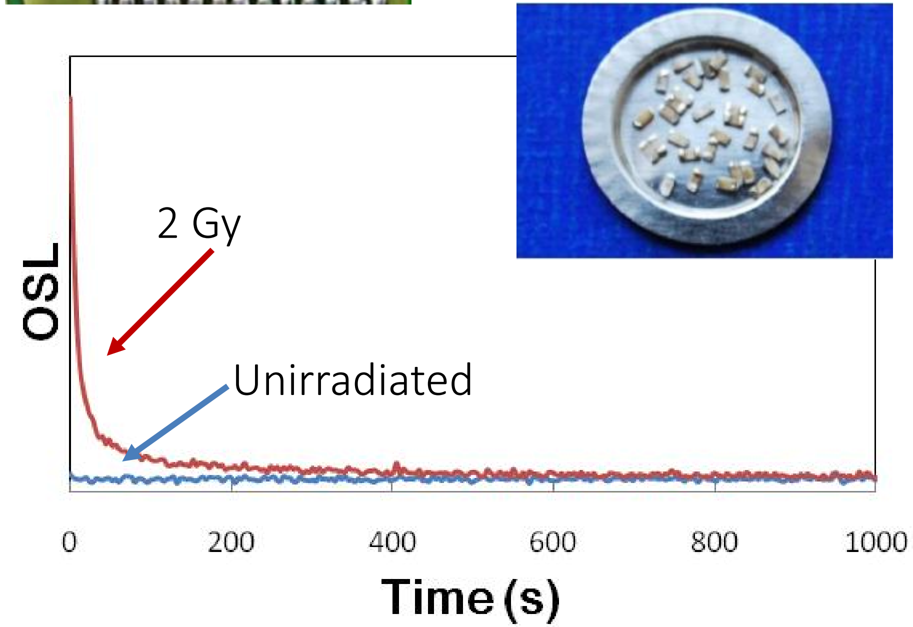
📖 Woda et al (2009) Ann Ist Super Sanita 45:297; Göksu & Bailiff (2006) Radiat Prot Dosimetry 119:413

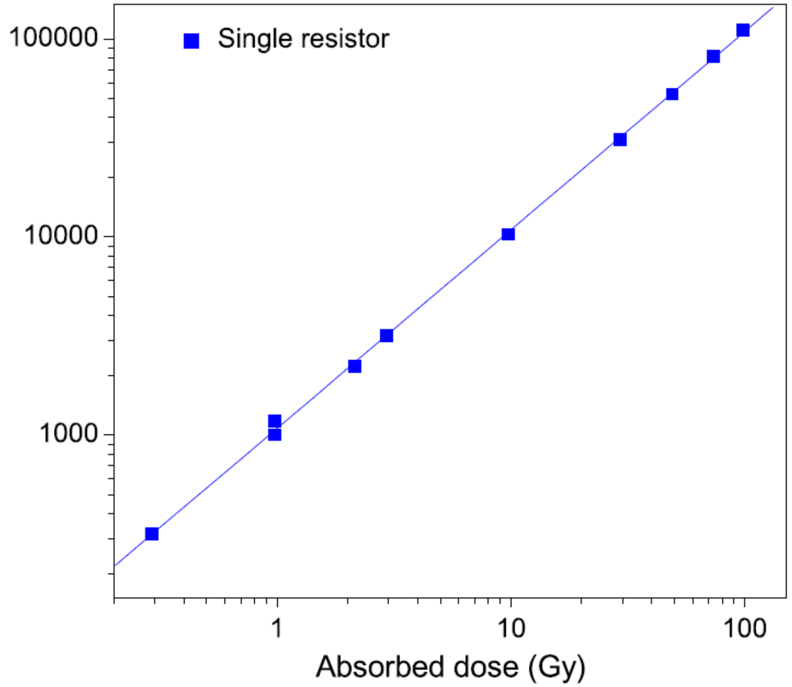
OSL - resistors





(1) Protective coating
(2) Resistive film
(3) Inner electrode
(4) Ni plating
(5) Sn plating
(6) Ceramic substrate





~10-20 mGy detectable but 50% fading in 10 days

Electron paramagnetic resonance

📖 Fattibene & Callens (2010) Appl Radiat Isot 68:2033;
Trompier et al (2009) Ann Ist Super Sanita 45:287

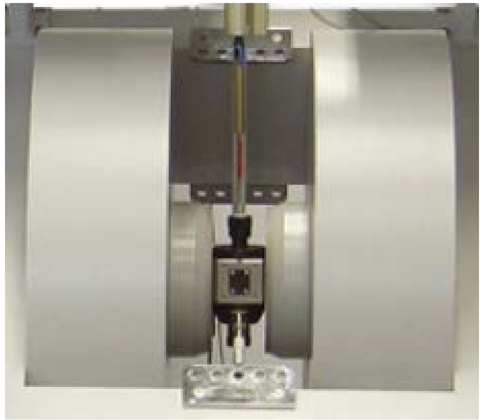
EPR dosimetry measures the concentration of radicals with unpaired electrons in a sample.

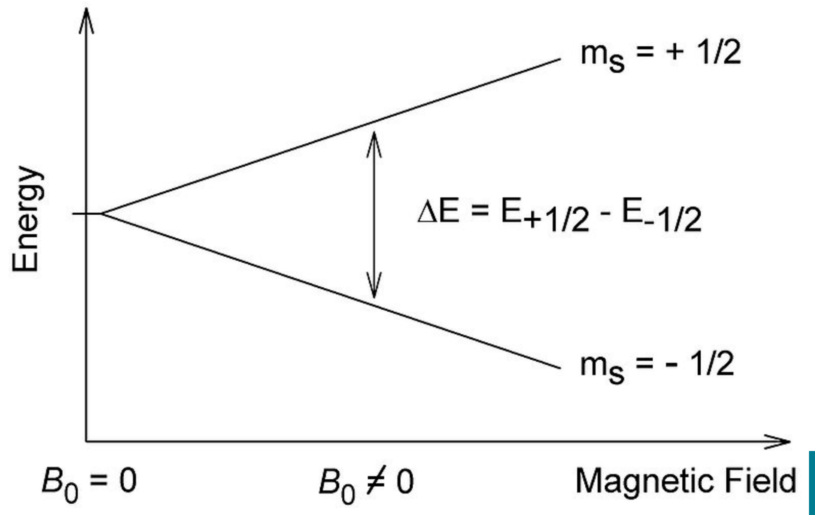
[carried out by measuring how much microwave energy is absorbed due to 'flipping' of electron spins in a magnetic field]

Radicals persist sufficiently long only in very few 'solid' biological materials:

• Calcified tissues (teeth, bones)
• Finger/toe nails
• Hair

...but also detectable in personal belongings, e.g. glass (watch, glasses, phone…)





EPR *in vivo*: teeth

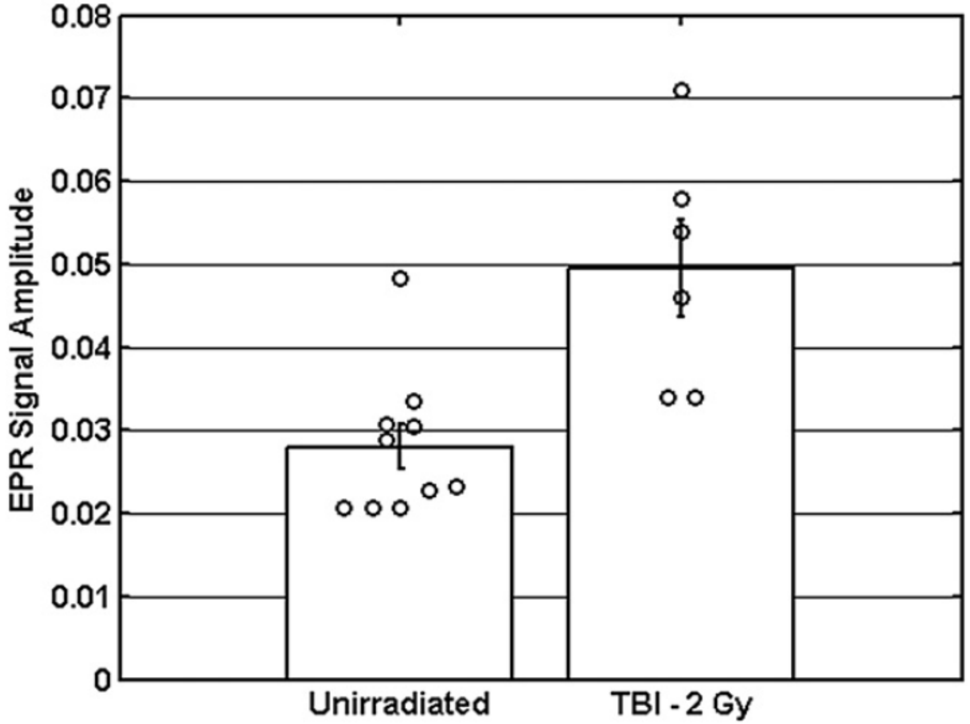
📖Swartz et al (2006) Radiat Prot Dosimetry 120:163

Williams et al (2011) Radiat Meas 46:772-7

Field deployable *in vivo* EPR tooth dosimeter being developed…

- 17 cm dipole magnet
- SE ~0.9 Gy
- Smaller versions planned

http://www.dartmouth.edu/~eprctr/research/tooth_dosimetry.shtml

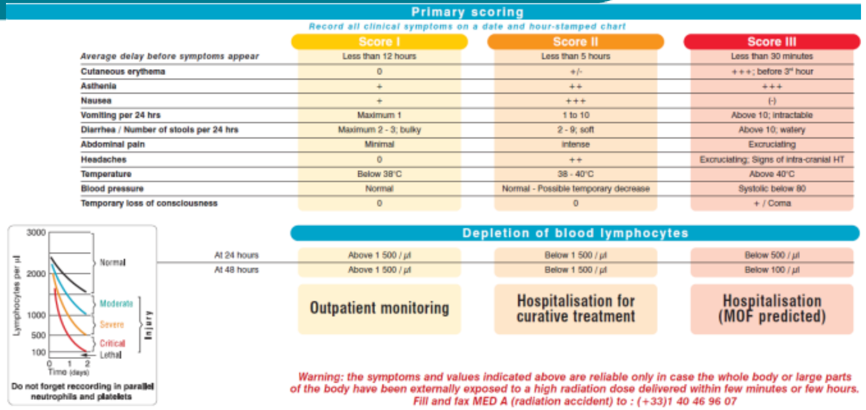


Future work: Improve sensitivity further, develop smaller field-deployable systems suitable for triage

Mass casualty radiation accidents

1) Initial clinical triage - **vomiting, etc**

2) Secondary triage - **retrospective dosimetry**



Primary scoring

Record all clinical symptoms on a date and hour-stamped chart

	Score I	Score II	Score III
Average delay before symptoms appear	Less than 12 hours	Less than 5 hours	Less than 30 minutes
Cutaneous erythema	0	+/-	+++; before 3rd hour
Asthenia	+	++	+++
Nausea	+	+++	(-)
Vomiting per 24 hrs	Maximum 1	1 to 10	Above 10; intractable
Diarrhea / Number of stools per 24 hrs	Maximum 2 - 3; bulky	2 - 9; soft	Above 10; watery
Abdominal pain	Minimal	Intense	Excruciating
Headaches	0	++	Excruciating; Signs of intra-cranial HT
Temperature	Below 38°C	38 - 40°C	Above 40°C
Blood pressure	Normal	Normal - Possible temporary decrease	Systolic below 80
Temporary loss of consciousness	0	0	+ / Coma
Depletion of blood lymphocytes			
At 24 hours	Above 1 500 / µl	Below 1 500 / µl	Below 500 / µl
At 48 hours	Above 1 500 / µl	Below 1 500 / µl	Below 100 / µl
	Outpatient monitoring	Hospitalisation for curative treatment	Hospitalisation (MOF predicted)

Warning: the symptoms and values indicated above are reliable only in case the whole body or large parts of the body have been externally exposed to a high radiation dose delivered within few minutes or few hours.
Fill and fax MED A (radiation accident) to : (+33)1 40 46 96 07

Problem

Small very specialised laboratories with limited surge capacity

Solutions

- Score fewer cells at first (e.g. 50 instead of 500) (ISO 21243)
- ‘Quickscan’ (Flegal et al (2010) Health Phys 98:276)
- Automation
- New methods / combination of methods
- Assistance networks

Retrospective dosimetry assistance networks

Regional:

EU: RENEB (Kulka et al, Int J Radiat Biol. 2017 Jan;93(1):2-14); based on recent research projects

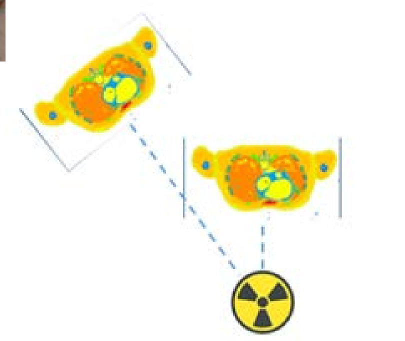
Latin America, Japan, Canada, …

Worldwide:

International Atomic Energy Agency – Response Assistance Network (RANET)

World Health Organization – BioDoseNet

EURADOS WG 10



Venue

IRSN, Bâtiment 33
12 rue de la redoute
Fontenay-Aux-Roses (close to Paris)
France

- By metro Line 13 station Châtillon/Montrouge then Tramway T6 stop Division Leclerc
- By train RER B (Charles de Gaulle/Robinson) station Fontenay-Aux-Roses then Bus 394 stop Division Leclerc

Further information & Registration

dosicourse@irsn.fr
http://www.concert-h2020.eu/

Organizing committee

Sophie Ancelet (IRSN, France, Chair)
Liz Ainsbury (PHE, UK)
Clemens Woda (HMGU/EURADOS, Germany)
Augusto Giussani (BfS, Germany)

This course is funded by the Europ… Joint Programme for the Integrati… Radiation Protection Research (CONCERT) 2018 Education & Trai… call

Uncertainty in biological, physical and internal dosimetry following a single exposure

April 15-19, 2019
IRSN, Paris, France

SATELLITE EVENT

Training Course on "How to Measure and Analyze Luminescence Signals for Potential Applications in Radiation Dosimetry: Theory and Computational Procedures" (WG10)

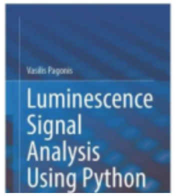
The training course takes place on Monday, June 12th, 08:00-12:30 and consists of two parts:

PART I: *Steve McKeever, Emeritus Regents Professor of Physics, Oklahoma State University, USA*

"Garbage in: Garbage Out". Understanding What You Measure is Critical

The discussion will cover understanding kinetics, what the standard equations mean and don't mean, how to collect the data, essential basic analytical tools, rudimentary and novel peak fitting approaches.

PART II: *Vasilis Pagonis, Emeritus Professor of Physics, McDaniel College, USA and Associate Editor, Radiation Measurements*



Luminescence signal analysis with open access software in Python and R

Practical examples will be demonstrated using software codes in Python and R for TL/OSL analysis, using actual experimental data files from dosimetric materials. Codes will be made available at the workshop in the form of Jupyter notebooks, which participants will be able to download freely from the web. Using their own laptops, participants can log into their Google Drive account and can run the codes immediately in …

…laim a 20 % …opic. A flyer

Joint EURADOS WG7 (Internal dosimetry) and WG10 (Retrospective dosimetry) workshop: "Biological Dosimetry techniques and EPR applied to accidental intakes of radionuclides"- open to all

9th October 2022, 13:30 – 18:00

Aim: To discuss the key open research questions and the potential to address these through collaborative research within and external to EURADOS.

Presentations will include:

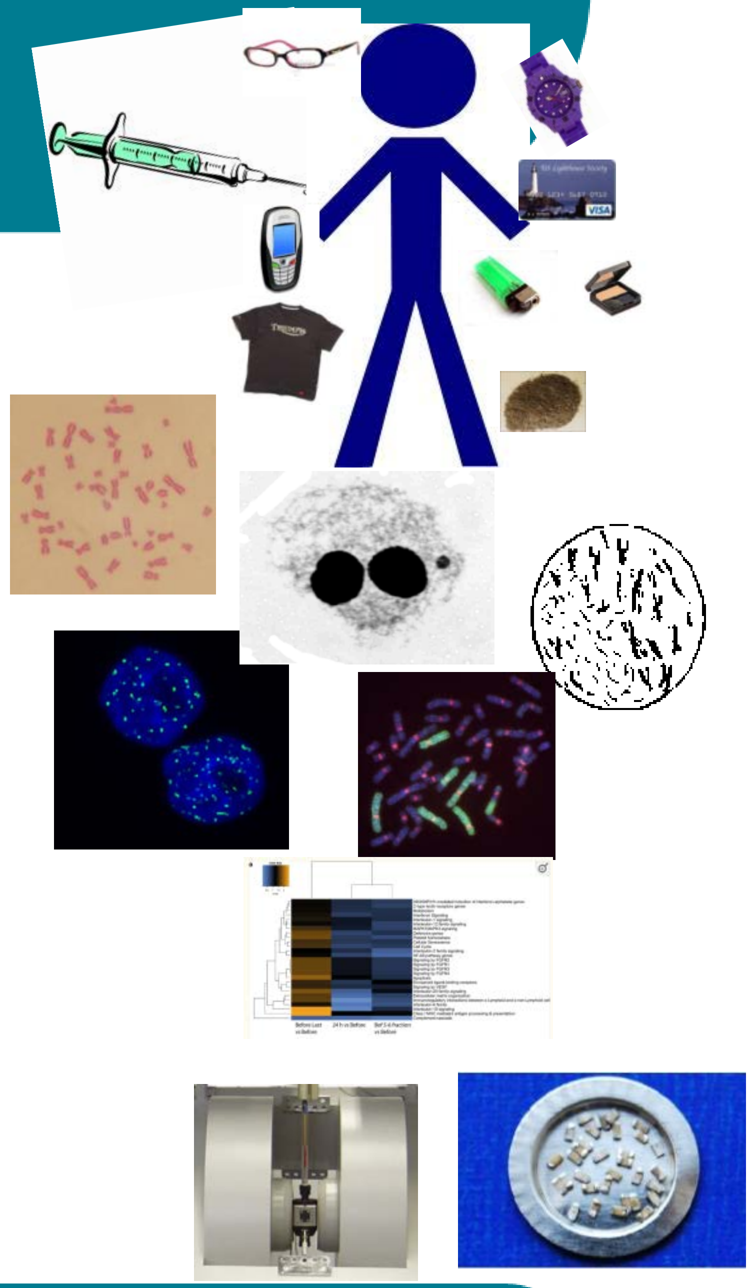
•Topic 1: **Biological and internal dosimetry for radiation medicine: current state and prospects for the future**, Satoshi Tashiro (Hiroshima University)

•Topic 2: **Establishment of calibration curves and intercomparison on calibration**, Liz Ainsbury (UKHSA)

•Topic 3: **Uncertainties in internal dosimetry/biodosimetry**, Augusto Giussani (Federal Office for Radiation Protection BfS) and Paco Barquinero (Universitat Autonoma de Barcelona)

The presentations will be followed by round table discussions on how to move forward with these topics.

Summary



- Retrospective dosimetry quantifies radiation-induced changes to biological or certain physical ‘fortuitous’ materials carried by suspected exposed individuals
- Development started in the 1960s; today we have a robust set of tools and techniques to estimate dose and in some circumstances say something about circumstances of exposure
- Development continues!
- For large scale emergency response, networking in development and validation is absolutely key to ensuring we are able to respond with the state of the art for retrospective dose estimation
- EURADOS WG 10, RENEB and other networks remain essential – this has been and must remain an international effort

Thank you for listening!

All work/images not referenced are my own or presented on behalf of WG 10 – thank you to all colleagues

Questions or comments?

Interested to join WG10?

Do get in touch:

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