Back to Basics: Biological Dosimetry

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BIOLOGICAL DOSIMETRY

- is the use of any biological change in an irradiated person that can be sufficiently quantified to indicate their radiation dose

- is very valuable for dose assessment in radiation protection (occupational, medical or accidental exposures) in addition to physical dose reconstruction and clinical evaluation.

PHYSICAL DOSIMETRY

↓

DOSE RECONSTRUCTION
personal dosimeters

BIOLOGICAL DOSIMETRY

↓

BIOLOGICAL CHANGES
dicentrics,
micronuclei,
DNA repair foci, …

CLINICAL DOSIMETRY

↓

CLINICAL SIGNS AND SYMPTOMS:
nausea, vomiting,
blood cell counts,
skin reactions...

Additive value of biological dosimetry to clinical signs and symptoms?

- biodosimetry can detect false positives /negatives
- biodosimetry can alarm for later clinical developments
- dose information can inform doctors in the dose range where medical intervention is needed
  - doses > 1 Gy → clinical follow-up/treament
  - doses <1 Gy → no treatment, only counselling
  - false alarms, no dose → reassurance
- biodosimetry can detect partial body exposure

Doctors treat symptoms, not doses!

Additive value of biological dosimetry to physical monitoring?

- physical dosimeters do not necessarily reflect the person's dose
- members of the public, who are not wearing a physical dosimeter, can be involved in radiation events
- physical dosimeters do not take into account individual radiosensitivity

The ideal specifications for a biological dosemeter

- specific to radiation
- low background
- sensitive at low doses
- low donor variability
- dose response calibration
- persistent effect
- easy sampling
- rapid result
- reasonable cost

Possible methods for biological dosimetry

Table 4. Research Areas in the Field of Radiation Biodosimetry Based on Capability, Level of Study, and Development Phase

Phase	*Assay Type*	*Dose Range(Gy)*[a]	*Level of Study*	*Throughput Capacity*	*Current Capability or in Development*
Investigative phase	Nascent Methodologies	n/a	Proof of concept	n/a	Basic research phase
Early Development Phase	Transcriptomics	0.5-12	Murine *in vivo* study[b]	n/a	Basic research phase
	Genomics	0.1-10	Human clinical study	Automated high throughput	In development
	Proteomics	1-14	Human Accidental Exposure Case Studies	Automated high throughput	In development
Late Development Phase	EPR	1-40	Human Accidental Exposure Case Studies	Surge capacity	In development
	Metabolomics	0.5-10	Human Accidental Exposure Case Studies	Automated high throughput	Current capability[c]
Deployment Phase	Lymphocyte kinetics/ clinical exam	1-14	Human Accidental Exposure Case Studies	Automated high throughput	Current capability[d]
	Cytogenetics	0.1-5	FDA Approved	Automated high throughput/ laboratory surge capacity	Current capability/ in development[e]

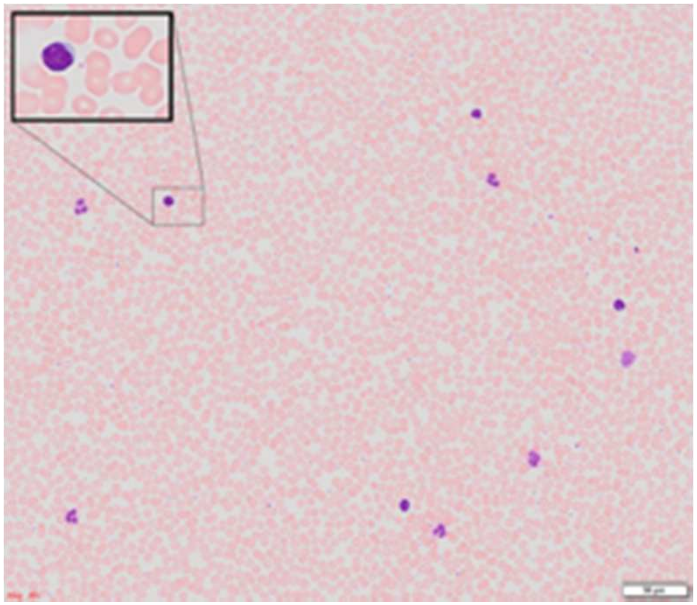
[a]Dose ranges are based on dose response relationships reported in research findings not on consensus operational guidelines.
[b]Predominant studies in this field use murine models, though at least one study using clinical samples has been reported.
[c]Metabolomics current capability is limited to clinical laboratory assessment of plasma citrulline.
[d]Lymphocyte kinetics is considered a current capability through access of existing clinical laboratory networks and available clinical support.
[e]Cytogenetic biodosimetry assessment is currently available but not at high-throughput or surge capacity levels.
Note: Rankings are based on complexity of the model studied (in vitro, murine, non-human primate, or human clinical study), their current level of technological deployment capability, and level of advancement in the basic research.[59]

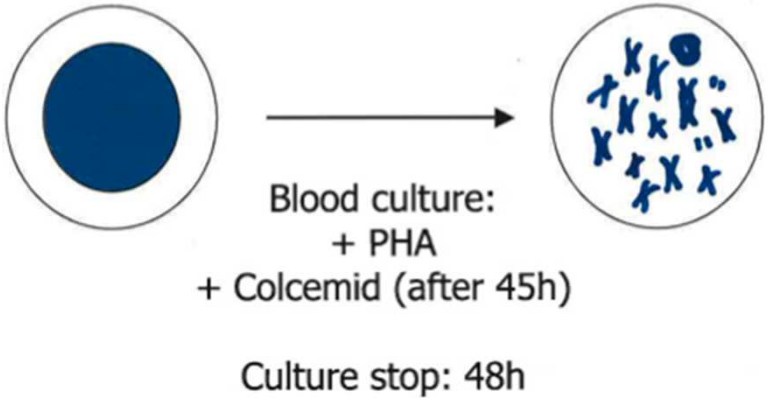
Biodosimetry: A Future Tool for Medical Management of Radiological Emergencies. M. T. Sproull et al., Health Security, Volume 15, number 6, 2017

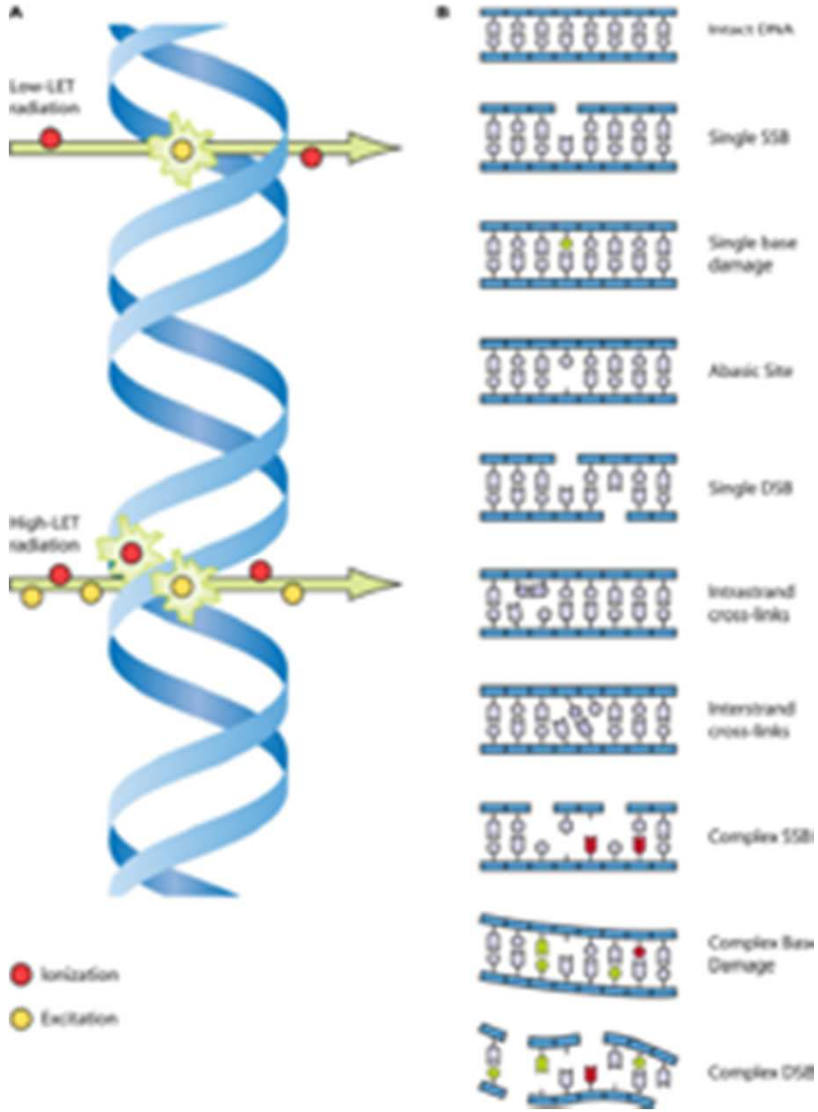
method of choice (IAEA): cytogenetics

Cytogenetic dosimetry

- analysis of chromosome abberations
- material: lymphocytes that can be easily obtained in large quantities from peripheral blood
- vast majority of peripheral lymphocytes reside in G_o phase of the cell cycle
- phytohaemagglutinin (PHA) converts resting lymphocytes into dividing cells allowing visualization of DNA lesions in methaphase chromosomes = chromosome abberations







DNA damage

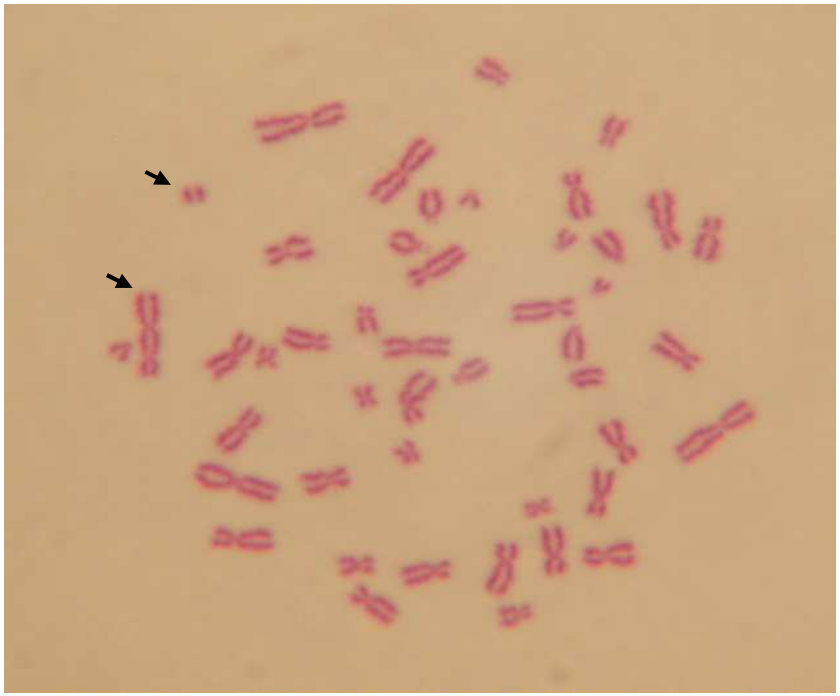
chromosome aberrations

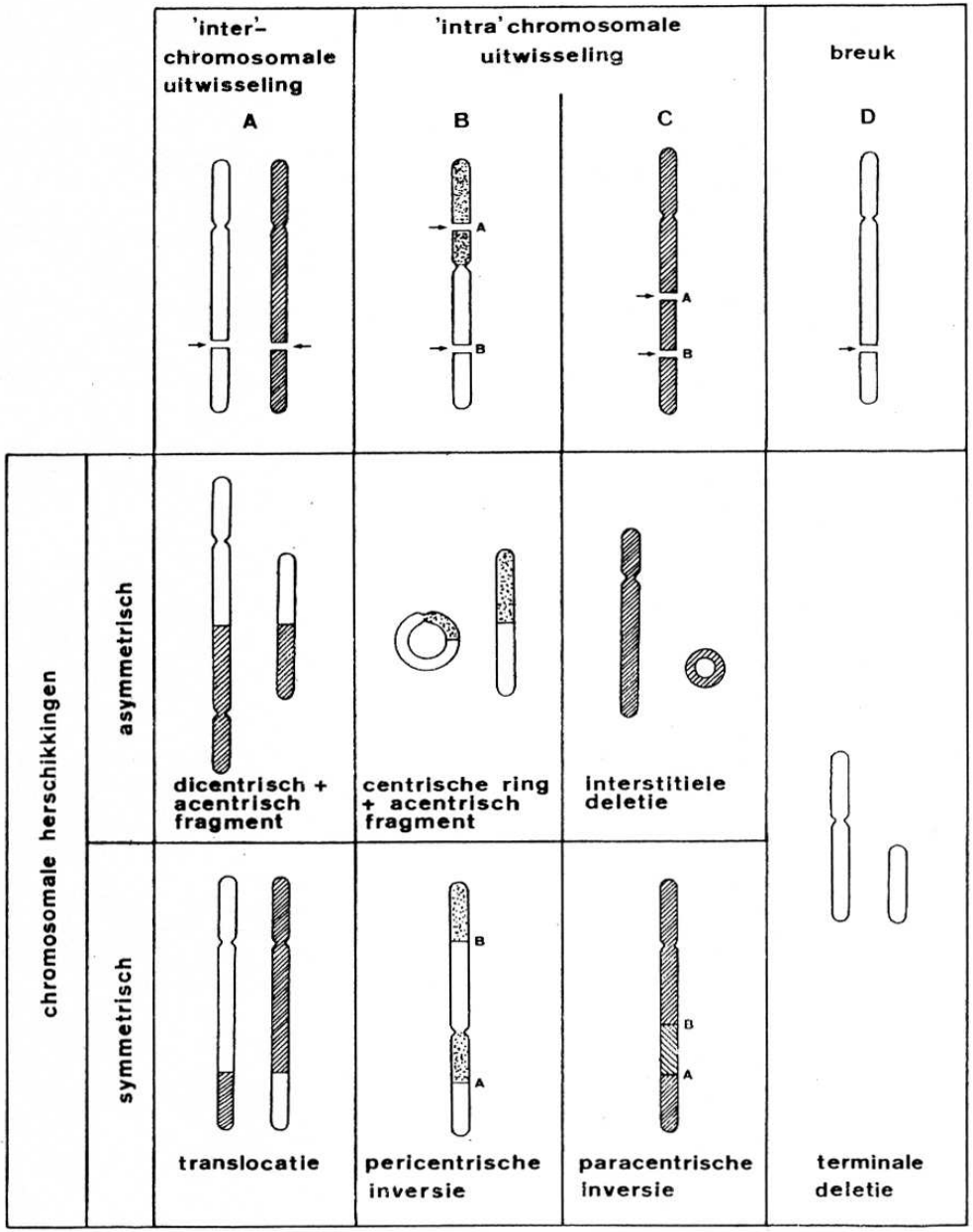
misrepaired or non-repaired DNA double strand breaks

*dsb repair mechanisms:

NHEJ: non-homologous endjoining

HR: homologous recombination





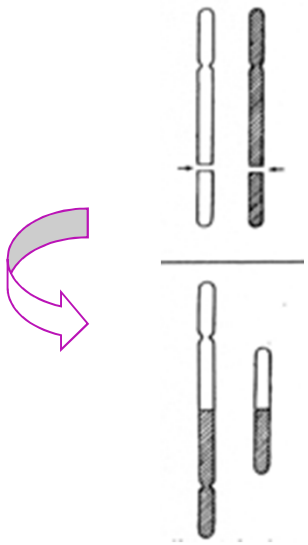
Mechanismen betrokken bij de vorming van chromosoom aberraties (uitleg zie tekst). (gemodifieerd naar Savage, 1983)

Figuur 24

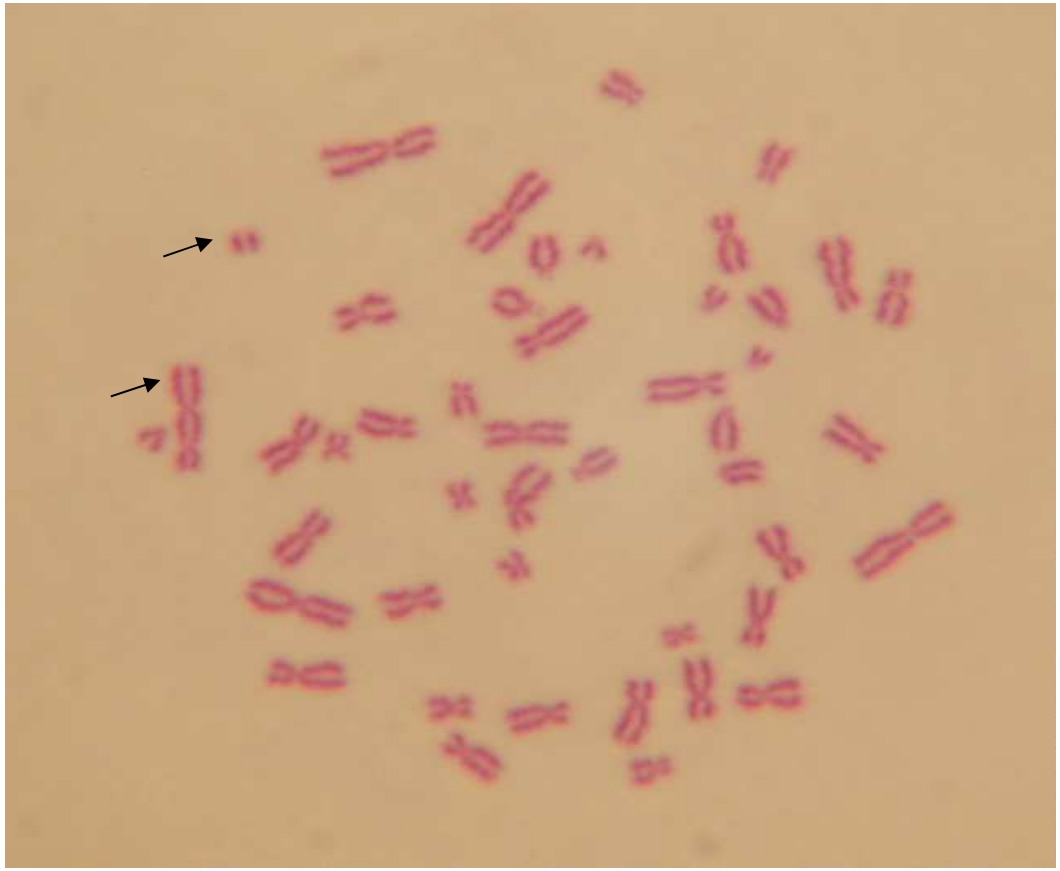
Different types of chromosome aberrations

(chromosomale uitwisseling = chromosomal exchange; breuk = break; chromosomale herschikkingen = chromosomal rearrangements)

Dicentric assay = gold standard (IAEA)

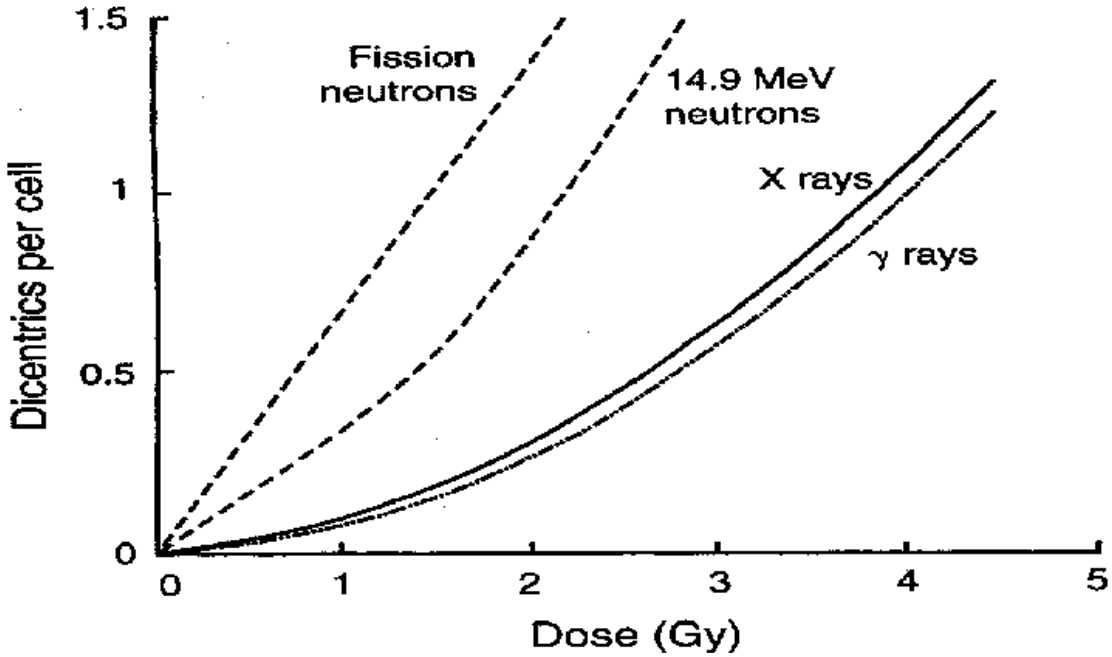


unstable aberration



Picture taken from IAEA Manual 2011 Cytogenetic Dosimetry: Applications in Radiation Emergencies

Calibration curves

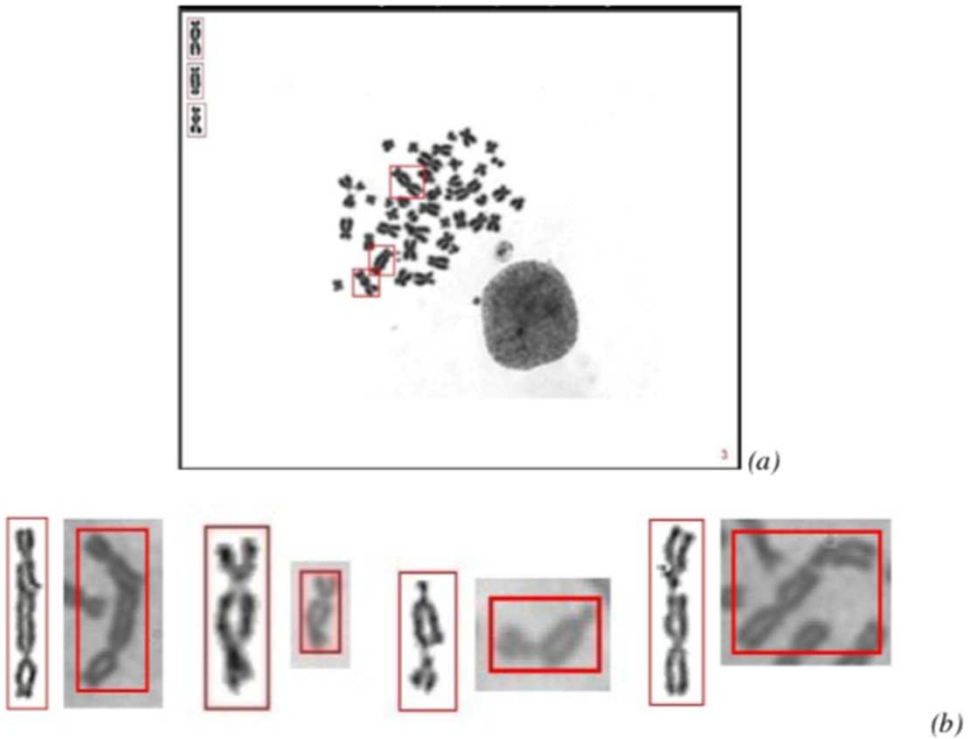


Application of the dicentric assay for dose estimation requires knowledge of the radiation type during exposure. Dose estimation is then based on the specific dose-effect relationship of the particular radiation source.

Dicentric assay

Background frequency	very low: 1 dicentric/1000 metaphases → most accurate and specific method!
Typical radiation scenario applications	Acute, protracted, recent exposures
Photon equivalent, acute dose range (Gy) for whole body dose assessment	0.1 - 5Gy
Useful for partial body exposure applications	Yes, but limited
Useful for triage dose assessment	Yes, but time consuming * development of automated scoring

Automated scoring of dicentrics



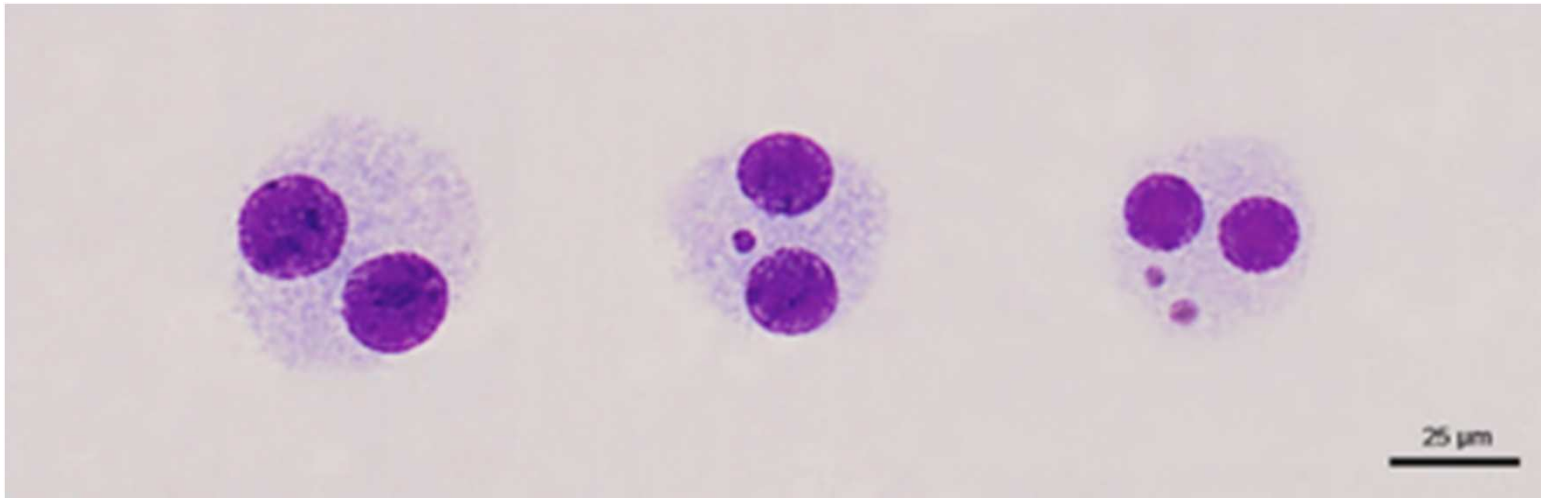
(a)

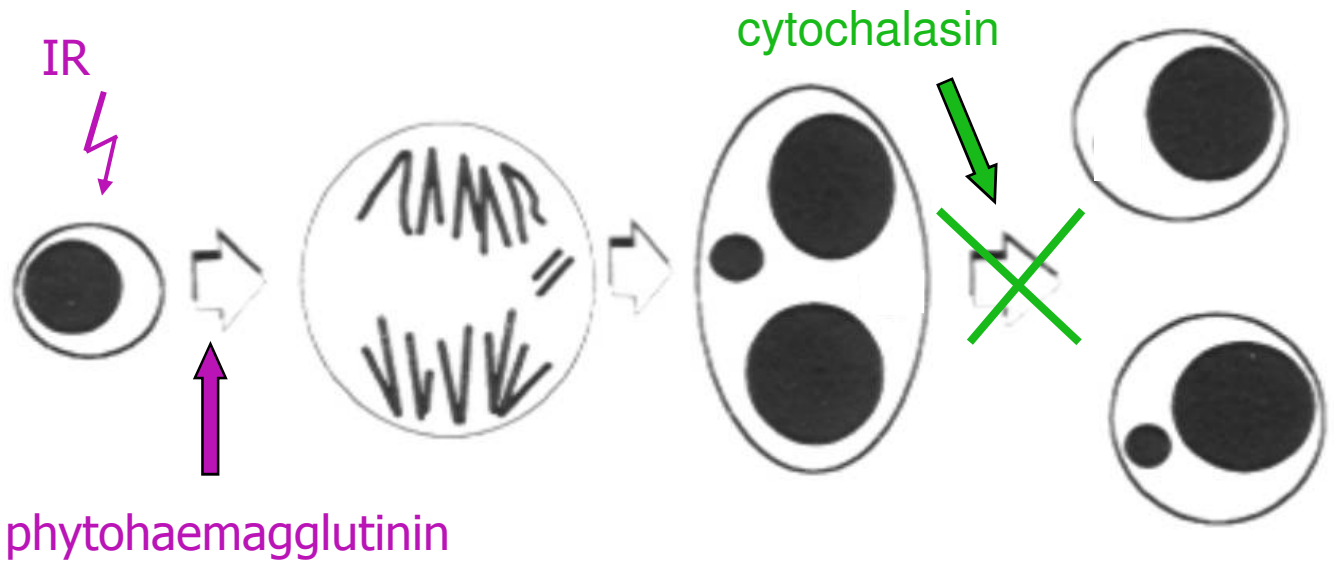
(b)

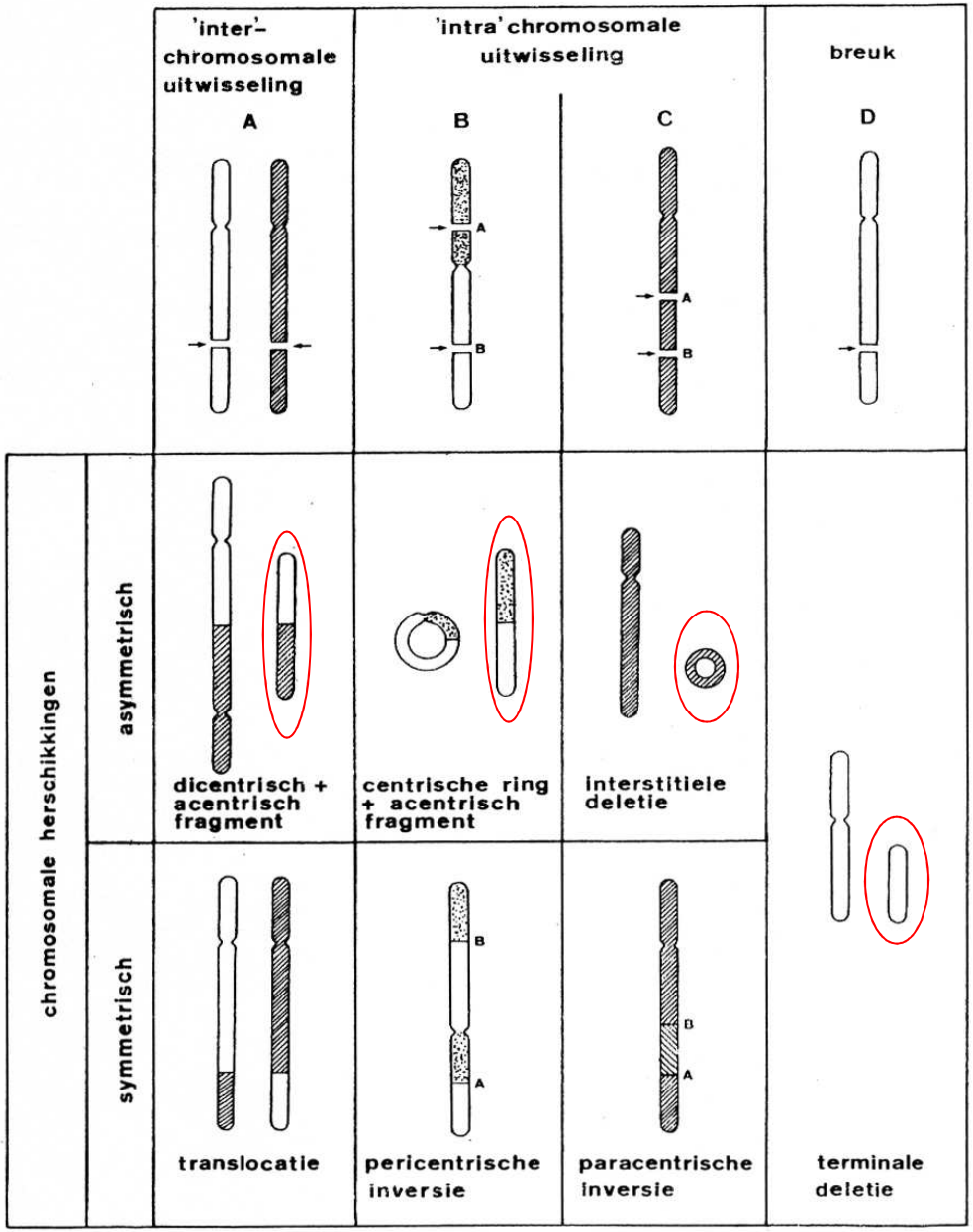
FIG. 44. a) Automatically detected dicentric candidates are identified, which makes evaluation easier and faster. b) False positive dicentric candidates can easily be recognized (i.e. overlapping chromosomes, twisted chromatids or not segmentated objects) and rejected.

Picture taken from IAEA Manual 2011 Cytogenetic Dosimetry: Applications in Radiation Emergencies

Cytokinesis-Block (CB) Micronucleus assay



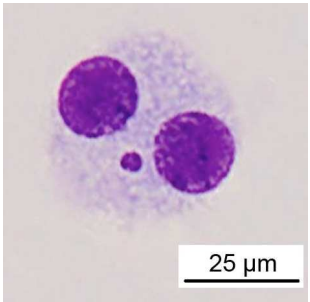




Mechanismen betrokken bij de vorming van chromosoom aberraties (uitleg zie tekst). (gemodifieerd naar Savage, 1983)

Figuur 24

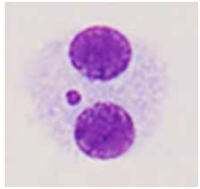
Micronuclei

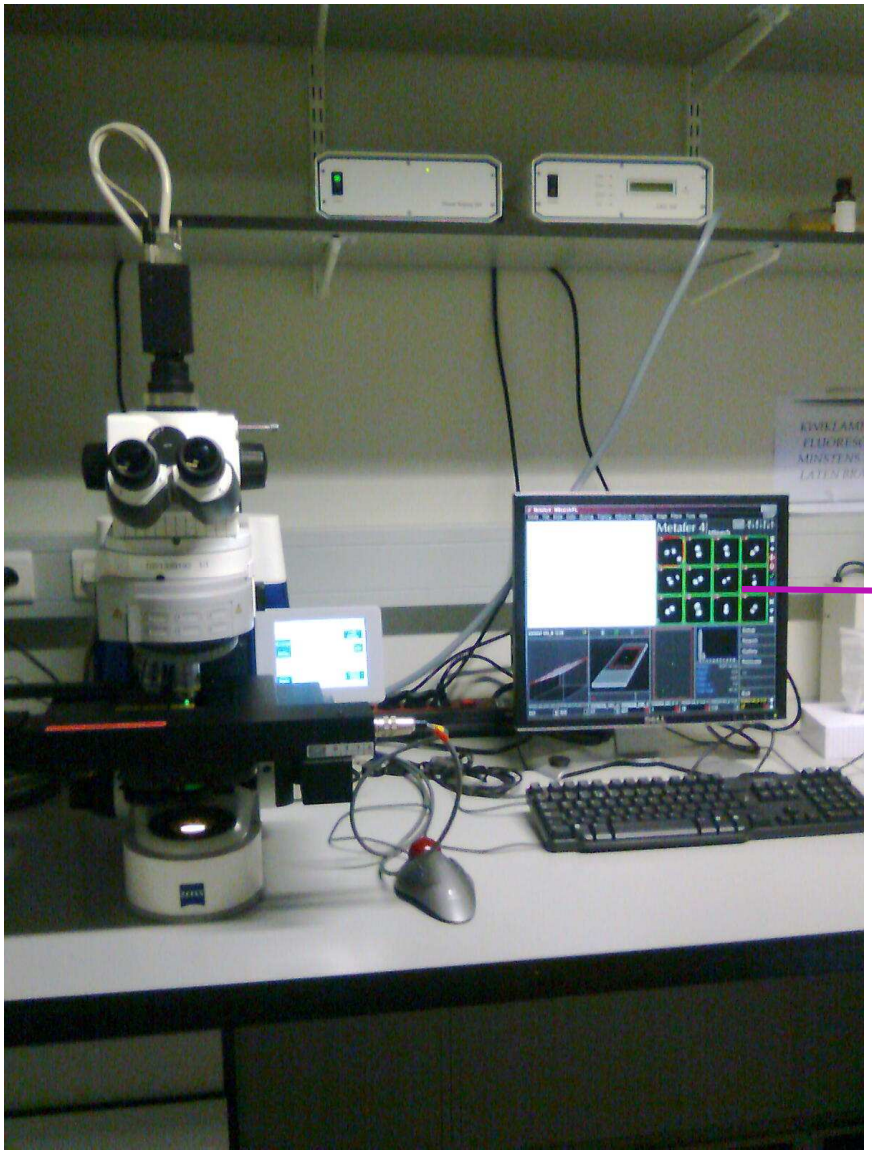


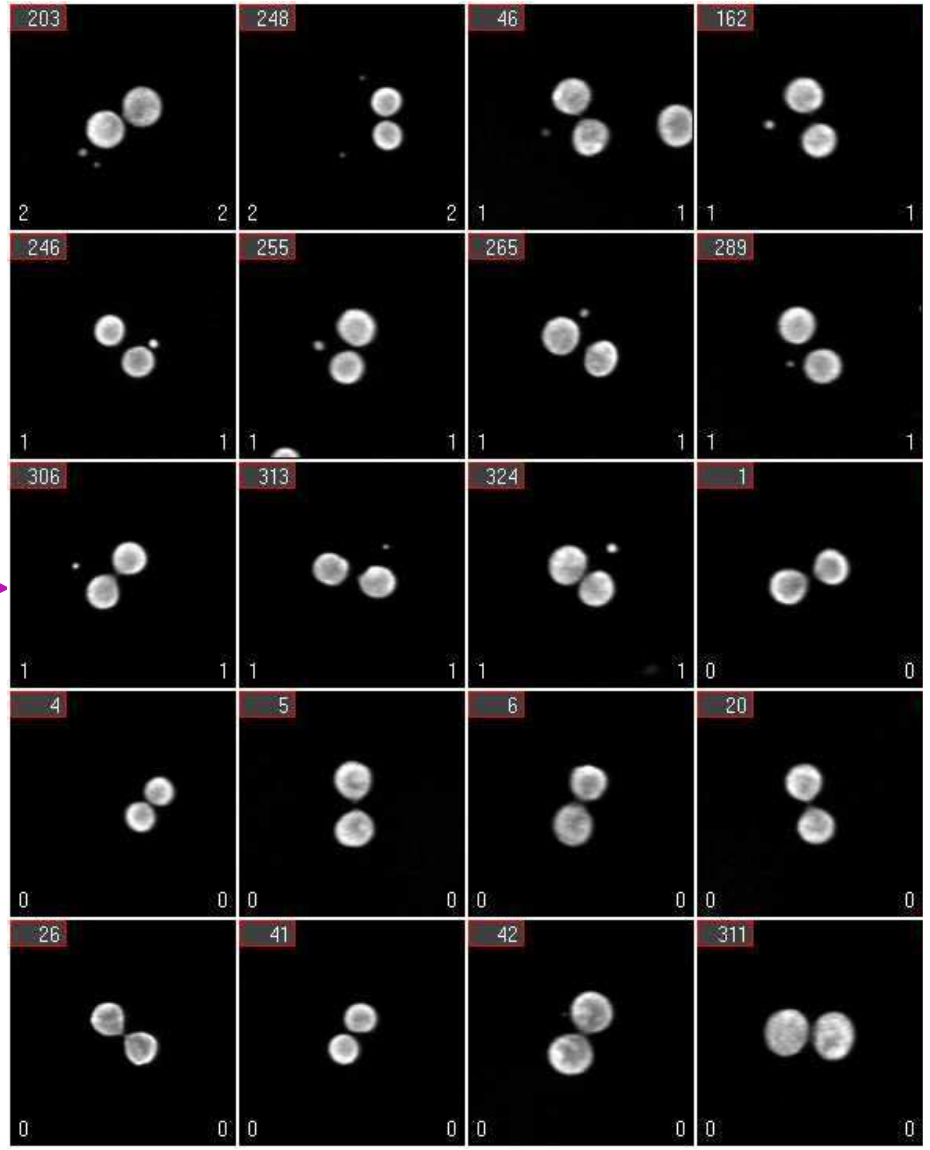
CB Micronucleus assay

▪ Background frequency	▪ Rather high and variable (2-35 MN/1000 BN cells), age dependent
▪ Typical radiation scenario applications	▪ Acute, protracted, recent exposures
▪ Photon equivalent, acute dose range (Gy) for whole body dose assessment	▪ 0.3 – 5 Gy * 0,1 Gy: MN-centromere assay
▪ Useful for partial body exposure applications	▪ Yes, but limited
▪ Useful for triage dose assessment	▪ **Yes !** easy and relative quick scoring method compared to other assays * development of automated scoring

Development of an automated MN scoring method

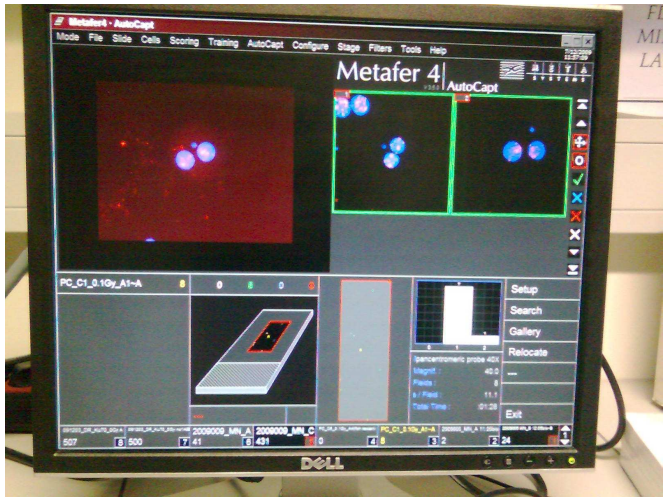




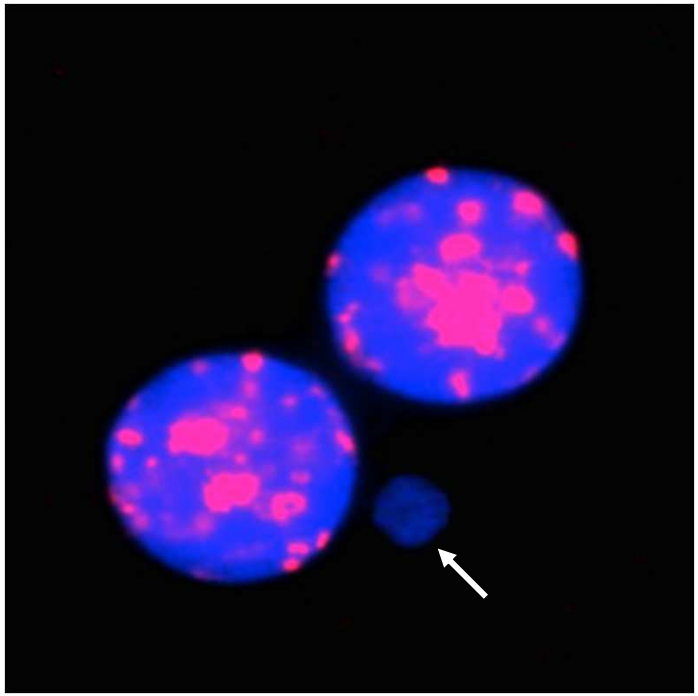


Example of gallery images obtained by automated analysis

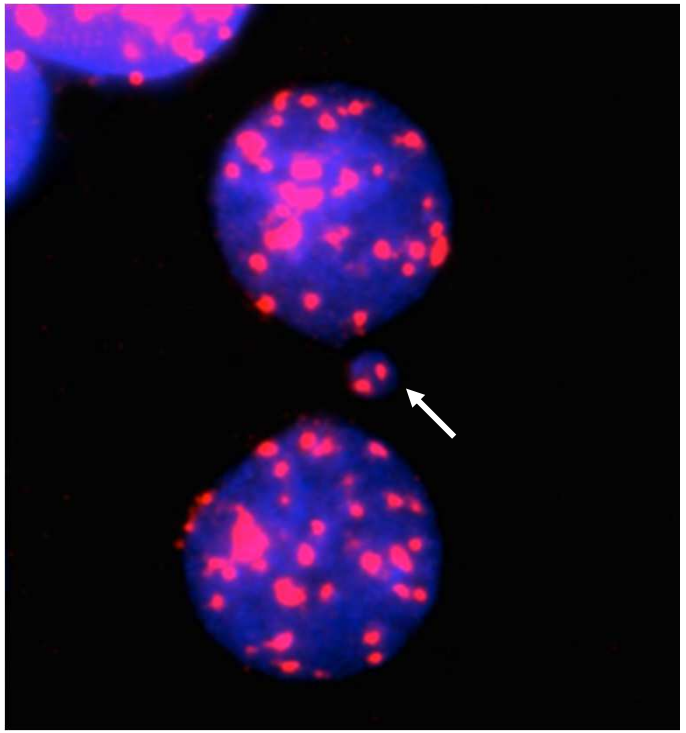
Development of a combined CBMN centromere assay for automated scoring



semi-automated analysis of centromere-positive (MN CM+) and centromere-negative MN (MN CM-) by the Metafer (autocapt software)

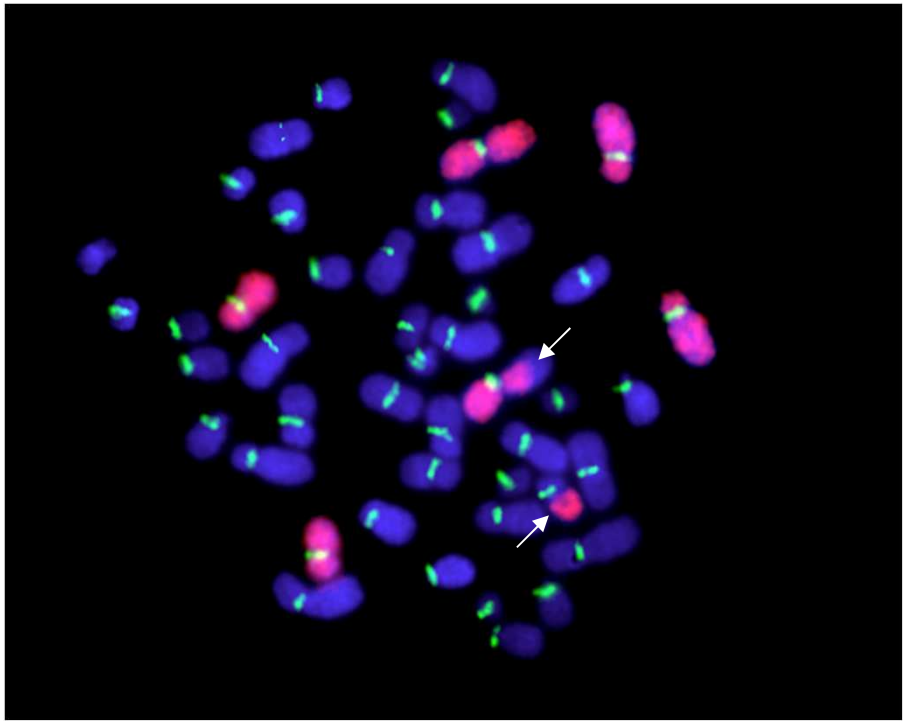


MN CM negative

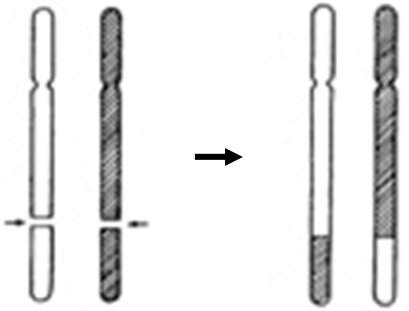


MN CM positive

FISH translocation assay



Translocations detected by FISH technique



stable aberrations

Pictures taken from IAEA Manual 2010 :
Cytogenetic Dosimetry: Applications in Radiation Emergencies

FISH Translocation assay

▪ Background frequency	▪ higher then for dicentrics, age dependent
▪ Typical radiation scenario applications	▪ acute, protracted, old exposures ! (retrospective dosimetry)
▪ Photon equivalent, acute dose range (Gy) for whole body dose assessment	▪ 0.3 - 5 Gy
▪ Useful for partial body exposure applications	▪ NA
▪ Useful for triage dose assessment	▪ NA

Premature Chromosome Condensation assay
PCC assay

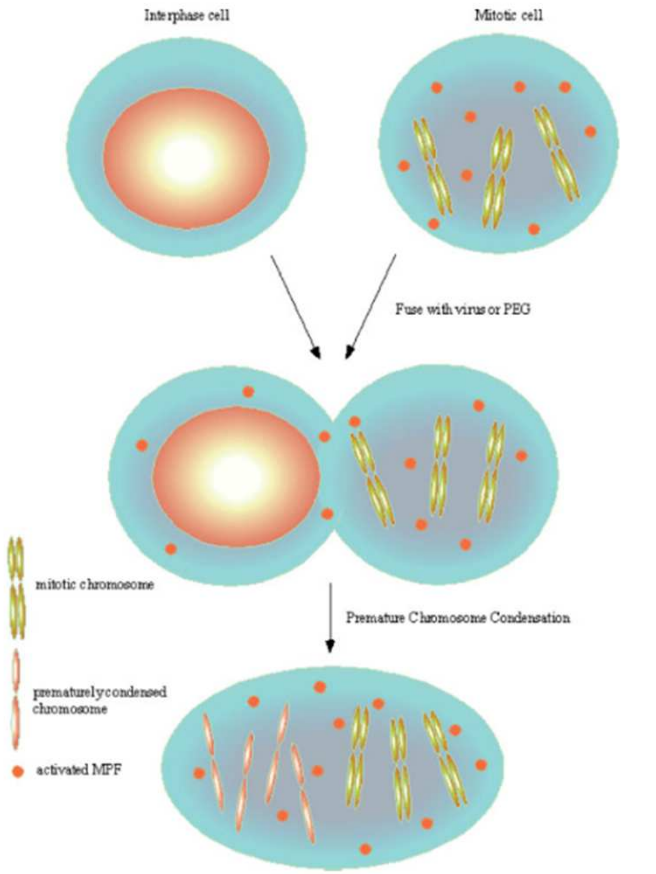
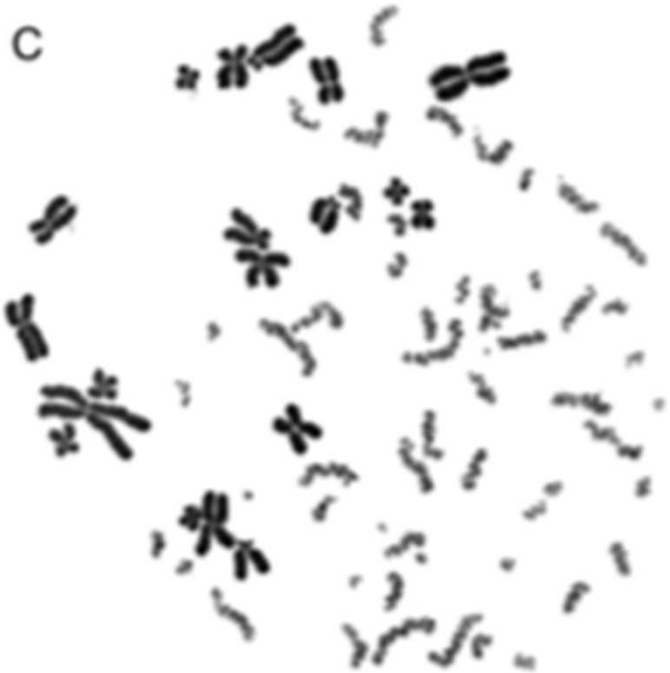


Fig. 1. Mechanism of fusion-induced PCC. Fusion is generally achieved either by virus or chemicals (PEG).

Gotoh & Durante, Journal of cellular physiology 209: 297-304 2006



Pantelias & Terzoudi, Mutat Res Gen Tox EN 836 (2018) 65-71

PCC induced by mitotic fusion - giemsa staining

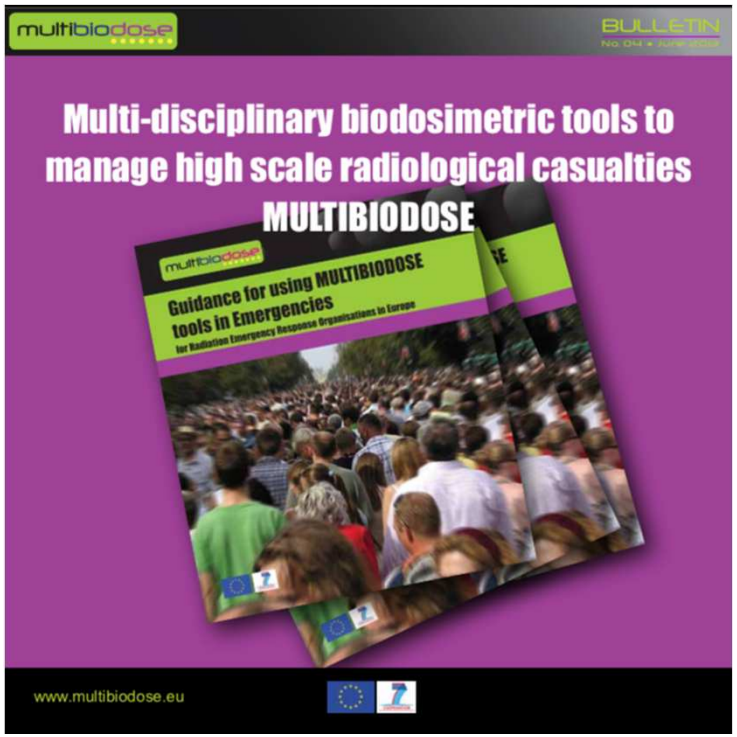
PCC assay

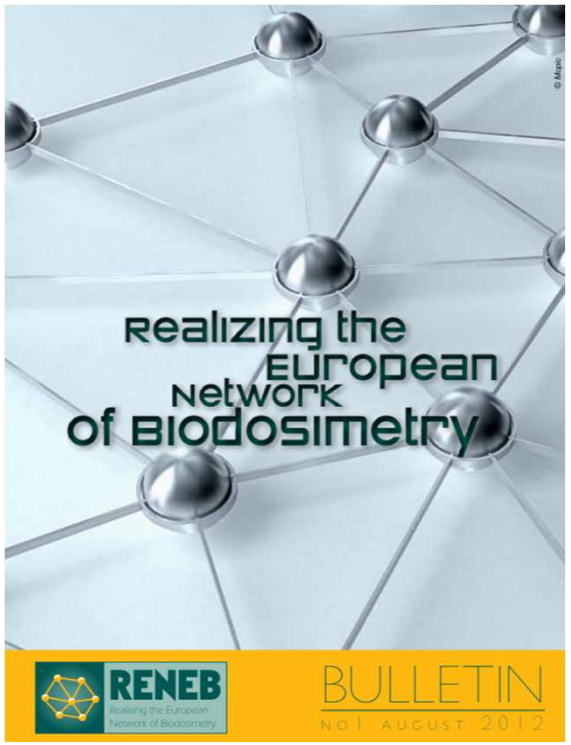
Background frequency	NA
Typical radiation scenario applications	acute, recent exposure
Photon equivalent, acute dose range (Gy) for whole body dose assessment	0.3 - > 10 Gy
Useful for partial body exposure applications	Yes
Useful for triage dose assessment	Yes, quick dose estimate possible

Other methods for biological dosimetry

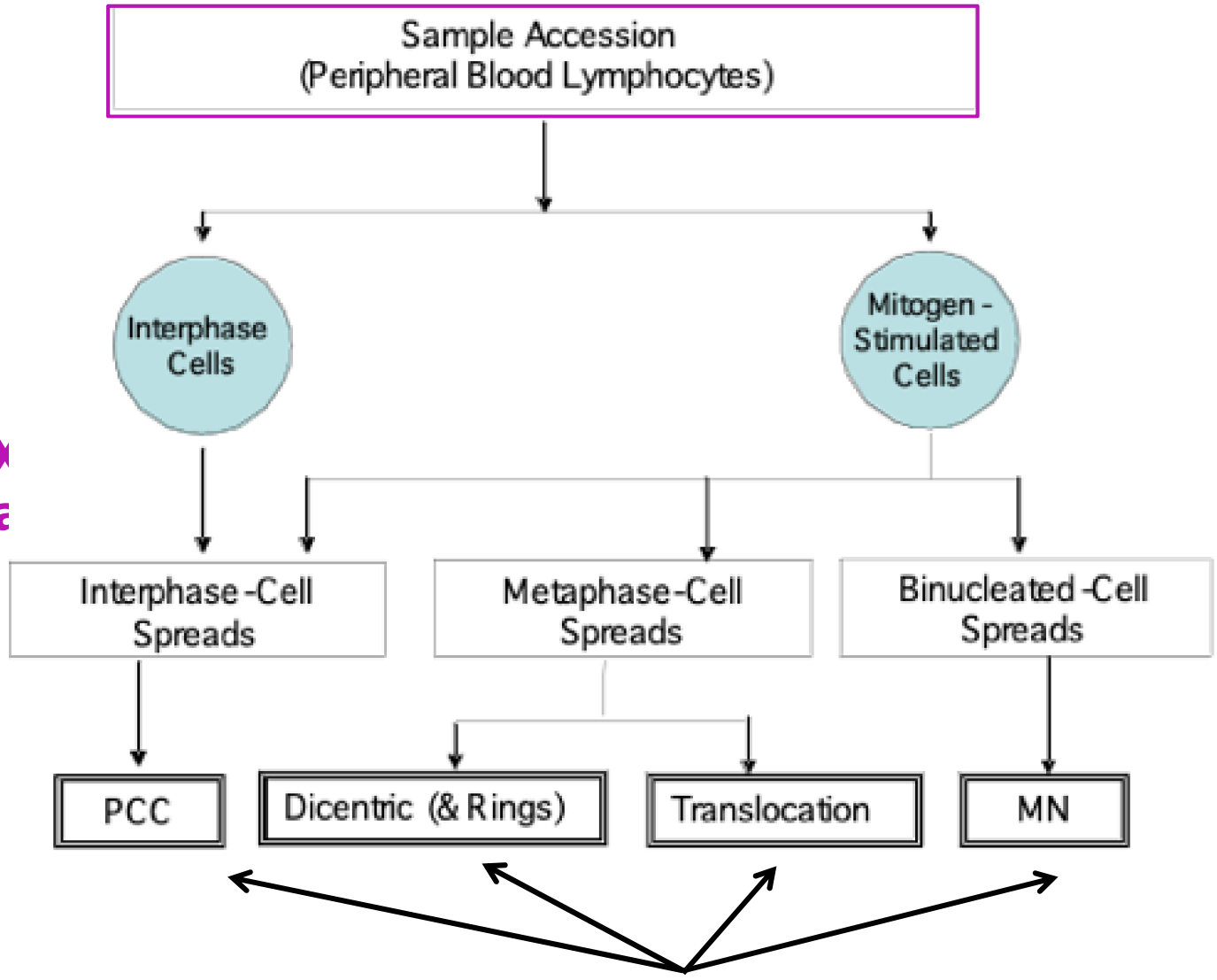
→ have esp. been developed to improve the emergency response in the case of large scaled radiological events

- γ-H2AX foci assay, gene expression analysis, biophysical assays: EPR and OSL





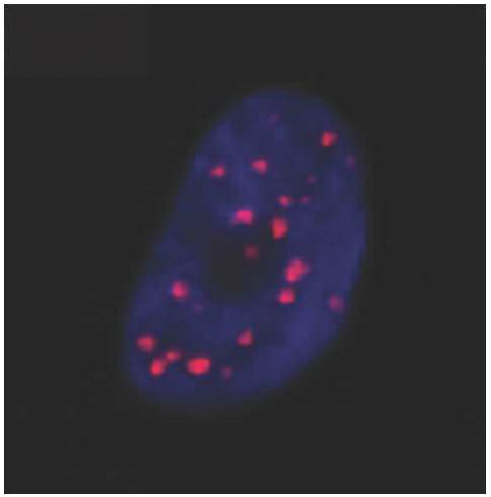
γ-H2AX
assa



Cytogenetic assays

γ-H2AX foci assay

- γ-H2AX is a protein biomarker for radiation exposure that is specific for DNA double strand breaks (dsb)
- exposure to IR triggers the phosphorylation of H2AX histons surrounding a dsb
- 1 foci (± 2000 phosphorylated H2AX proteins) ≈ 1dsb
- doses as low as a few mGy can be detected
- no need of a cell division
- foci disappearance ≈ dsb rejoining



γ-H2AX foci assay

Background frequency	NA
Typical radiation scenario applications	acute, recent exposure
Photon equivalent, acute dose range (Gy) for whole body dose assessment	0.2 – 5 Gy
Useful for partial body exposure applications	No
Useful for triage dose assessment	Yes, quick dose estimate possible * development of automated scoring

Physico-chemical methods

OSL: optically stimulated luminiscence
EPR: Electron paramagnetic resonance spectroscopy

The RENEB operational basis: complement of established biodosimetric assays

Andrzej Wojcik, Ursula Oestreicher, Lleonard Barrios, Anne Vral, Georgia Terzoudi,Elizabeth Ainsbury, Kai Rothkamm, Francois Trompier & Ulrike Kulka

INTERNATIONAL JOURNAL OF RADIATION BIOLOGY, 2017 VOL. 93, NO. 1, 15–19

Table 1. General characteristics of the biodosimetric assays used in RENEB. Sensitivity is given for low LET radiation. See text for explanation of assay acronyms.

	Time span after exposure during which the assay can yield usable results				Exposure scenario that can be detected by each method alone				
Assay	Days	Weeks	Months	Years	Acute	Pro-tracted	Partial body	Specific for ionising radiation	Sensitivity of the assay (dose range in Gy)
DIC	√	√	√		√	√	√	√	0.1–5
FISH	√	√	√	√	√	√			0.3–5
MN	√	√	√		√	√	√		0.3–5
PCC	√				√		√	√	0.3–>10
gH2AX	√				√		√		0.2–5
EPR	√	√	√	√	√	√		√	1–>10
OSL	√	√			√	√		√	0.01–>10

Gene expression (qRT-PCR and microarrays): early and precise dose estimates can be performed, in particular at doses < 2Gy (further development needed)

Information form to request biodosimetry

UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

Biologische dosimetrie

Biologische dosimetrie is de detectie en de kwantificatie van een eventuele blootstelling aan ioniserende straling. Het is een belangrijke en onafhankelijke methode in de radioprotectie supplementair aan de fysische dosimetrie.
Als eerste screening, scoren we het aantal micronuclei, daarna voeren we een scoring uit van dicentrische chromosoomabberaties, de meest gevoelige en betrouwbare biologische indicator voor stralingsschade.

Meer info ivm biologische dosimetrie vindt u in de IAEA manual 'EPR-biodosimetry 2011'die men kan downloaden via volgende link:
http://www-pub.iaea.org/MTCD/publications/PDF/EPR-Biodosimetry%202011_web.pdf
prijs: 350 euro per test

Wat hebben wij nodig voor het uitvoeren van de analyse:

- 5 ml bloed afgenomen in Lithium-heparine buizen (groene dop, zonder gel), de persoon hoeft niet nuchter te zijn
- ontvangst van het bloed op de dag van de afname (indien mogelijk voor 14 uur)
- bewaren en transport van het bloed bij kamertemperatuur
- op voorhand verwittigen wanneer bloedstaal zal afgenomen worden

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