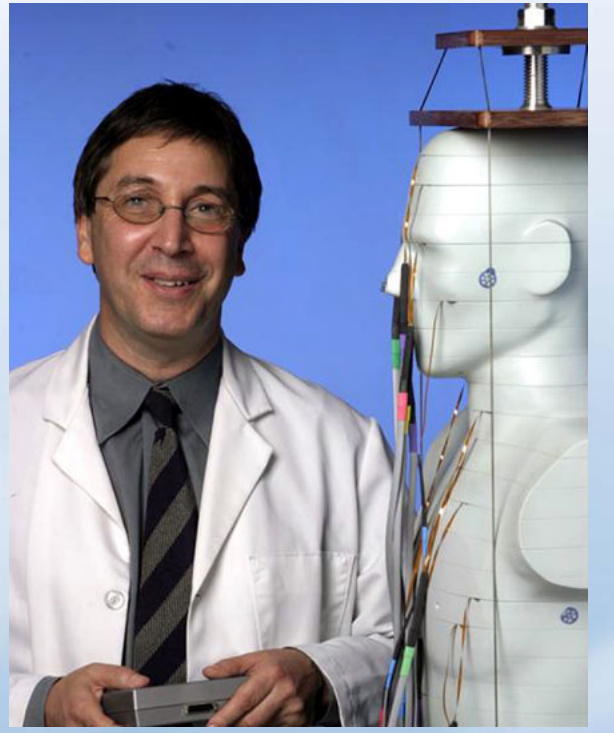
David J. Brenner, Ph.D., D.Sc.
Director, Center for Radiological Research,
Columbia University Medical Center

Essential radiobiology for radiation epidemiologists



Radiation Epidemiology & Dosimetry Course

National Cancer Institute

www.dceg.cancer.gov/RadEpiCourse

Essential Radiobiology for Radiation Epidemiologists

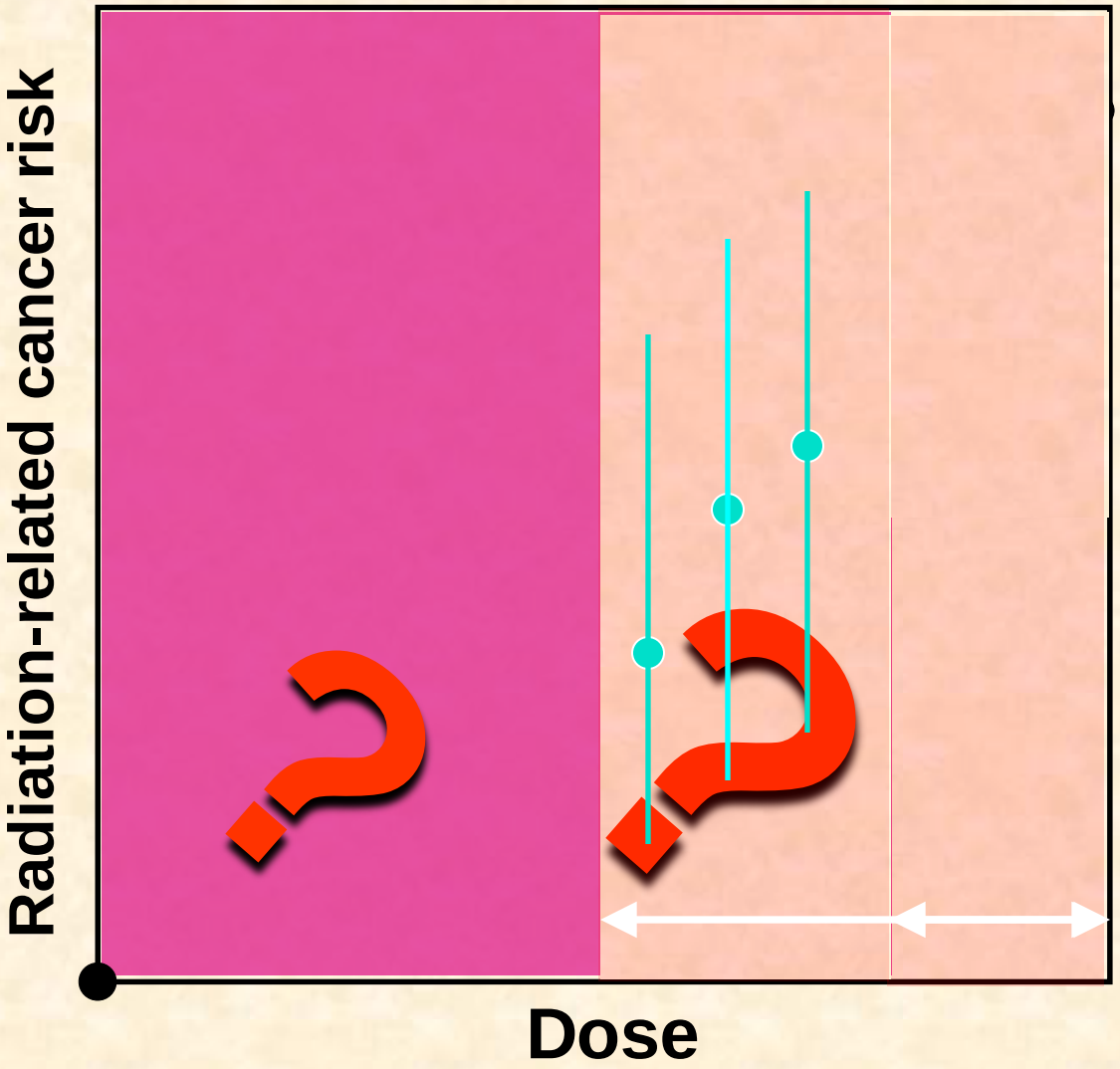
What every epidemiologist needs to know about radiobiology….

but was too deep in the bunker to ask

Do epidemiologists need radiobiology?

- **The exposure situations that we are interested in these days are generally not those that are amenable to quantitative radiation epidemiology**
- **Extrapolations:**
  - ***Dose***
  - ***Dose rate***
  - ***Radiation quality***
  - ***Age / genetics***

What is the problem?



About as low dose as epidemiology can go: Solid cancers in A-bomb survivors exposed to doses from 5-100 mSv

Small but statistically significant increase in risk

	Cancer incidence (1958-98)
Study population (5-100 mSv)	**27,789**
Total solid cancers observed	**4,406**
Solid cancers expected (controls)	**4,325**
Radiation-related excess solid cancers	**81**

Preston *et al* 2007

Low-dose trend tests for solid cancers in A-bomb survivors

Cancer Mortality

- 5 – 100 mGy P=0.04
- 5 – 150 mGy P=0.006

Cancer Incidence

- 5 – 100 mGy P=0.08
- 5 – 150 mGy P=0.01

Courtesy D.L. Preston (2011), based on RERF public dataset DS02can.csv (www.rerf.or.jp)

The 2012 UK CT Study

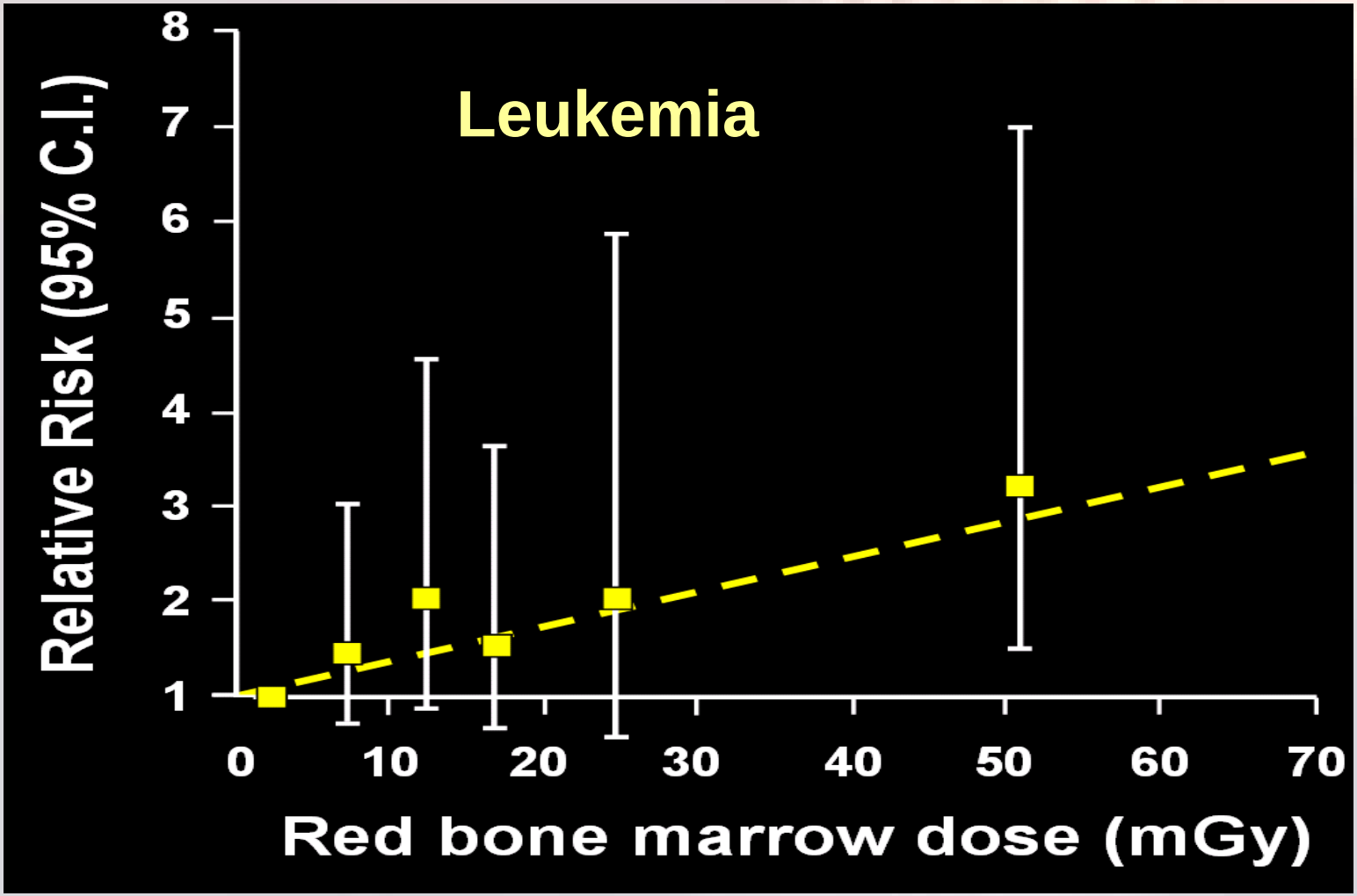
Radiation exposure from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a retrospective cohort study

Mark S Pearce, Jane A Salotti, Mark P Little, Kieran McHugh, Choonsik Lee, Kwang Pyo Kim, Nicola L Howe, Cecile M Ronckers, Preetha Rajaraman, Sir Alan W Craft, Louise Parker, Amy Berrington de González

www.thelancet.com **Published online June 7, 2012** **DOI:10.1016/S0140-6736(12)60815-0**

~10 year follow-up of 175,000 patients who received CT scans in the UK, age <22, between 1985 and 2002

- **Statistically significant linear associations seen between bone-marrow dose and leukemia risk (p=0.01) in the 5-50 mGy range**

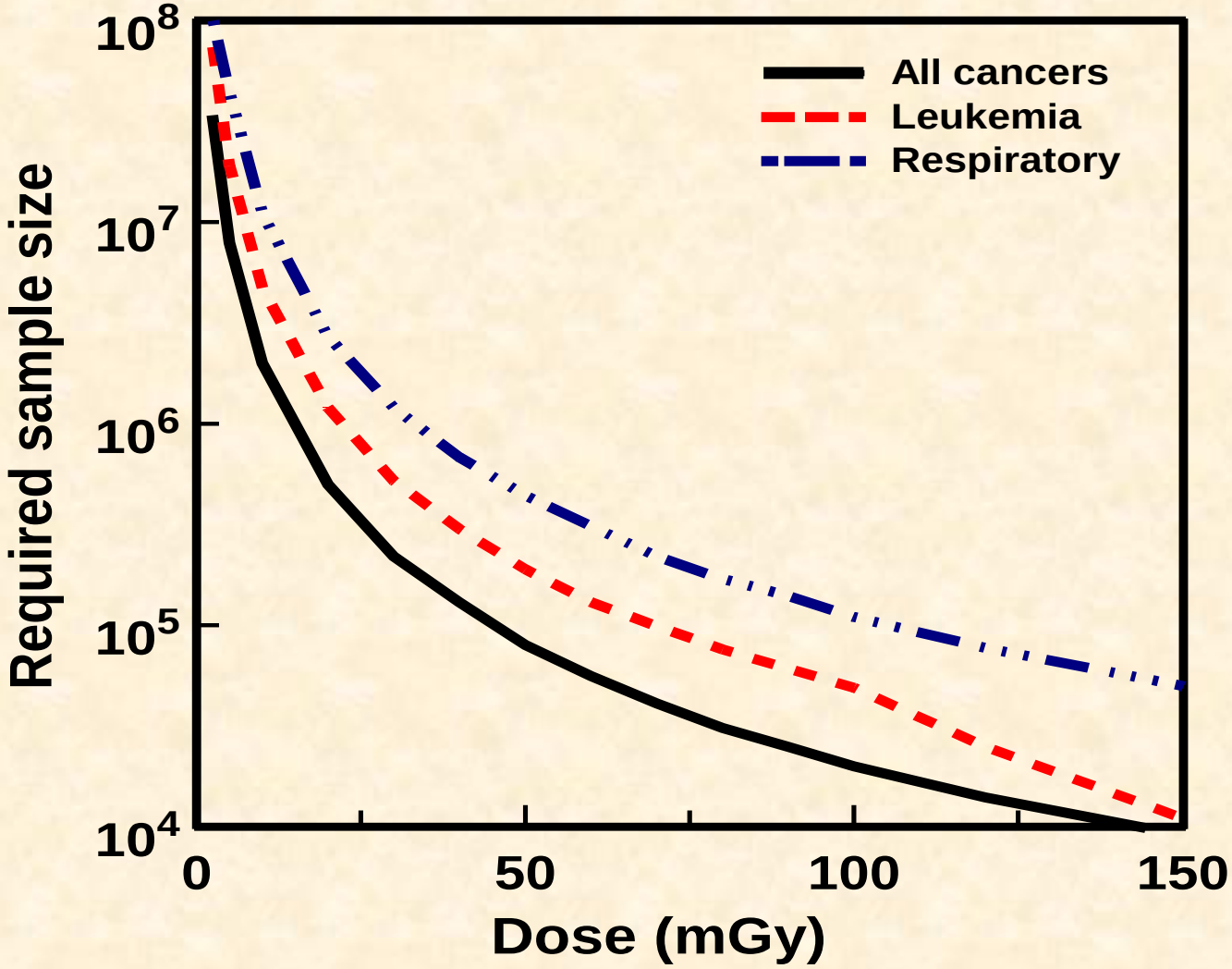


Why can't we get useful information from epidemiological studies at lower doses?

- **We don't have any "fingerprints" to uniquely identify a radiation-induced cancer**
  - **So epi studies currently involve looking for a radiation-associated increase in cancer rates relative to a background (unirradiated) population**

- **~40% of any study population will get cancer anyway**
  - **So looking for smaller and smaller excess risks due to lower and lower radiation doses requires bigger and bigger studies**

Size of cohort required to detect a significant increase in cancer mortality



From NRC 1995

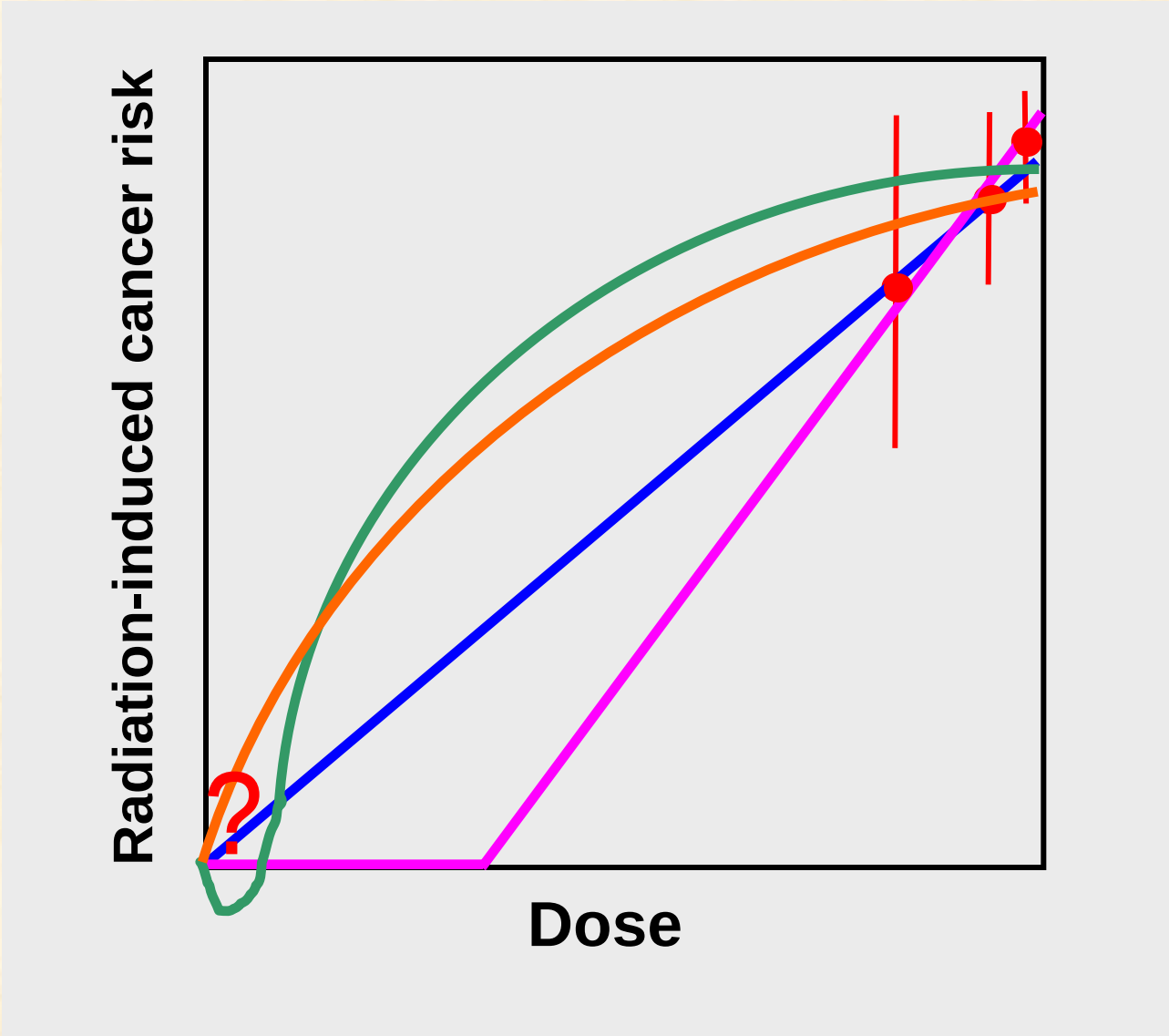
Three Studies of Mortality in Radiologists

STUDY	Relative Risk	
Matanowski (US)	1.2	Statistically significant increase
Berrington (UK)	0.68	Statistically significant decrease
Carpenter (UK)	1.03	No significant change

For the foreseeable future, we will continue to have to either scale or extrapolate the radiation-related cancer risks we need, based on higher dose epidemiological data

- To lower doses
- To different radiation qualities
- To different dose rates
- To populations with different background cancer risks
  - Different ages
  - Different genetic sensitivities

Estimating the risks associated with still lower doses of ionizing radiation



Can laboratory radiobiology studies help?

Not directly… ***we have no proven laboratory systems for quantifying radiation-induced cancer risks in man***

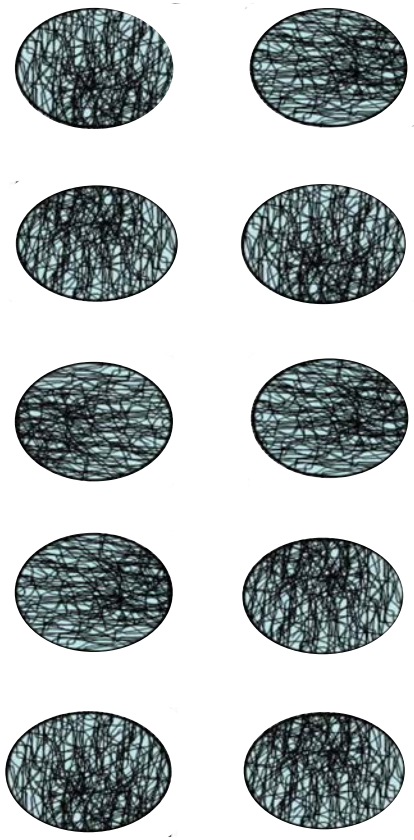
But indirectly…. ***they can help us understand how to extrapolate measured radiation-induced cancer risks at high doses to lower doses***

Can laboratory radiobiology studies help?

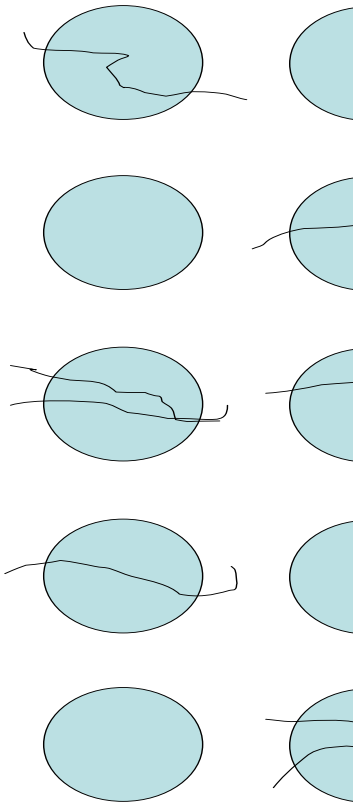
Radiation-induced cancer risks at different doses: The Biophysical argument

Radiation-induced cancer risks at different doses

1000 mGy

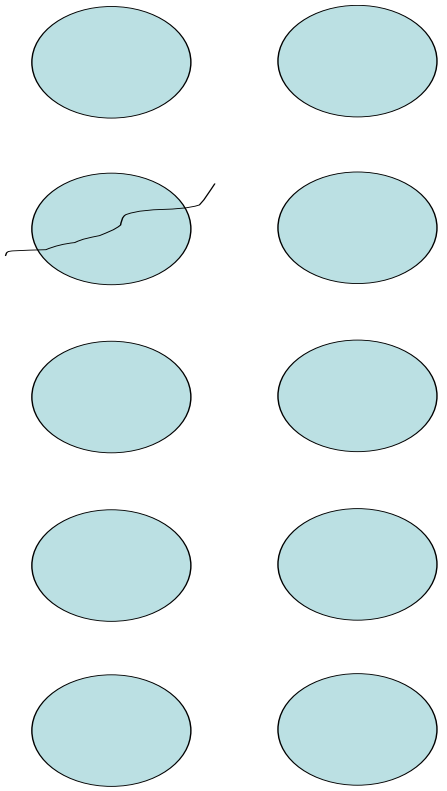


10 mGy



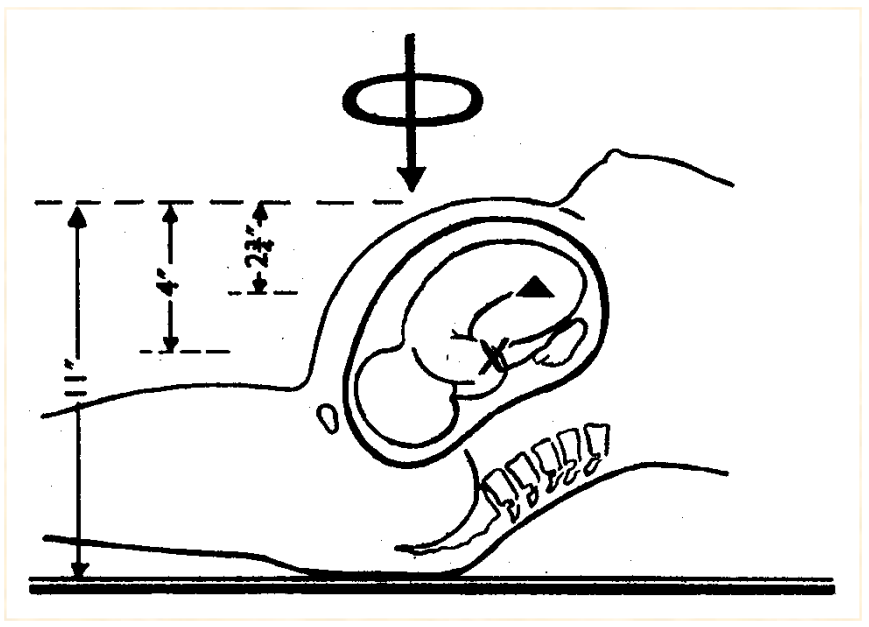
?

1 mGy



Childhood cancer after *in-utero* x-ray exposure

Pelvimetry or obstetric abdominal exam

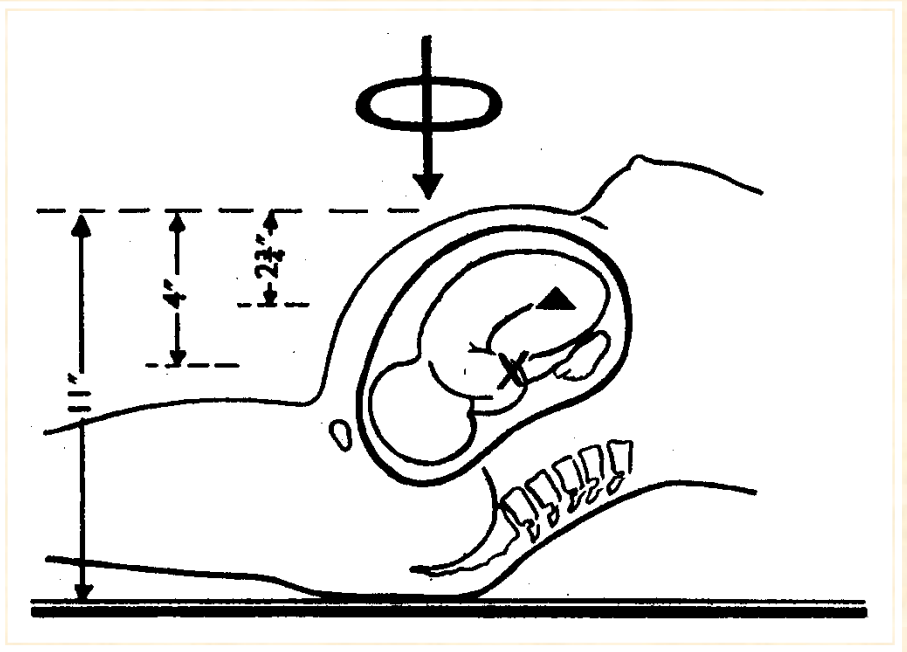


Mean dose ~6 mGy, 80 kVp x rays

Corresponds to a mean of ~1 photon / cell nucleus

The Oxford Survey of Childhood Cancers

- 15,000 case control pairs
- Mean dose ~ 6 mGy
- Significant increase in childhood cancer after *in-utero* x-ray exposure



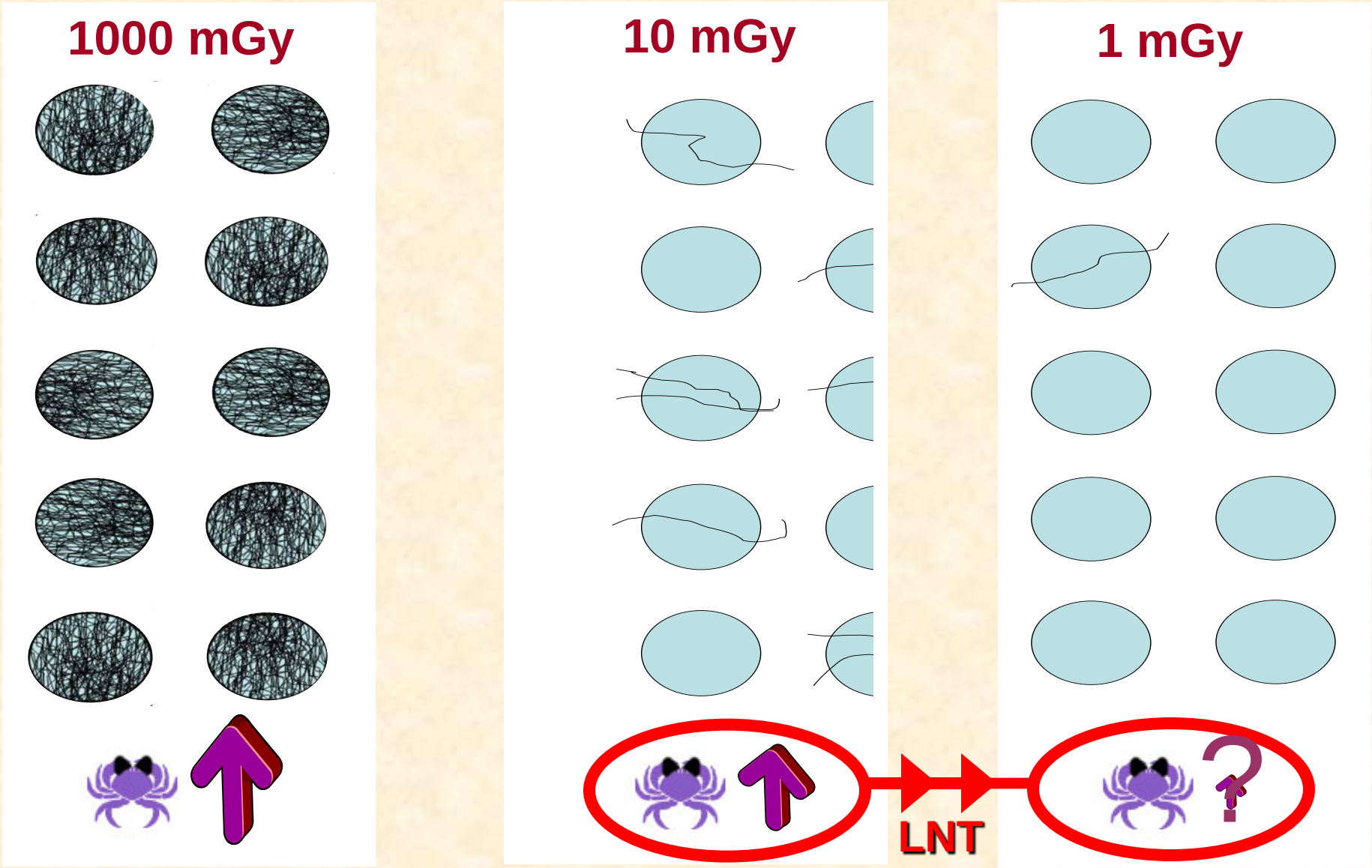
Doll and Wakeford 1997

Can *in-utero* x-ray exposure to ~6 mGy cause cancer?

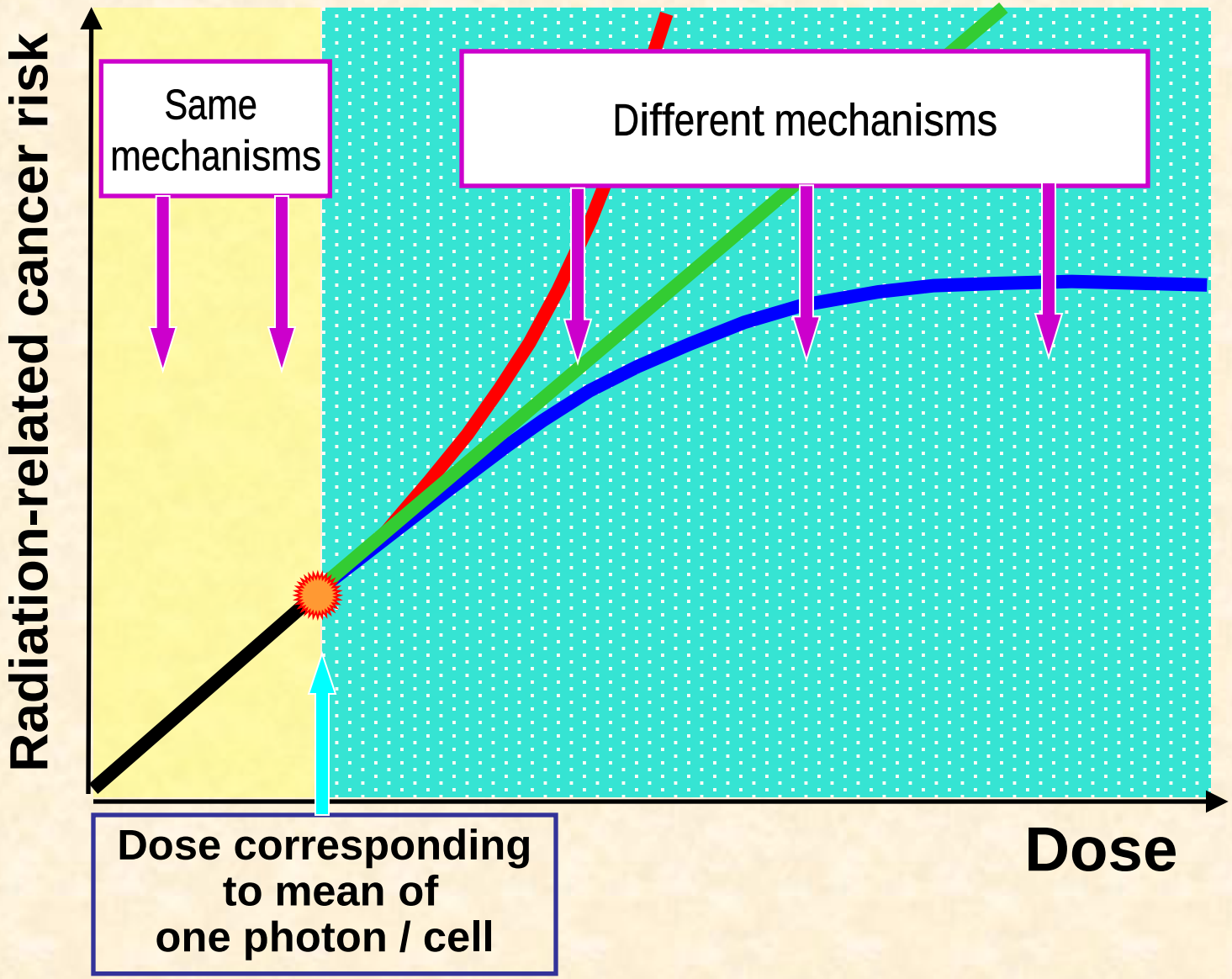
- **"It is concluded that radiation doses of the order of 10 mGy received by the fetus *in utero* produce a causal increase in the risk of childhood cancer".**

Doll and Wakeford 1997

Radiation-induced cancer risks at different doses



The biophysical argument

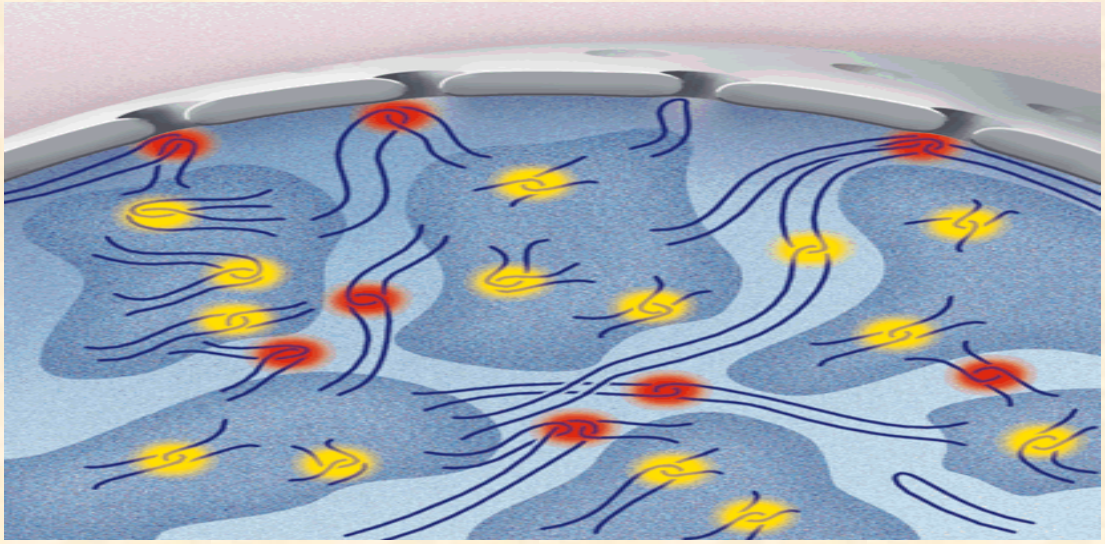


The biophysical argument makes a number of assumptions that can be questioned

- **Repair mechanisms: Can our very efficient DNA repair mechanisms always repair small amount of DNA damage?**
  - We have incredibly efficient DNA repair mechanisms, but occasionally they result in misrepair.
- **Immunosurveillance: Can immune systems "mop up" any small cluster of premalignant cells?**
  - Not so likely or we'd never get cancer
- **Assumes the development of tumors from a single damaged cell, independent of surrounding damaged cells**
  - But cells do talk to each other – the local microenvironment is important

DNA Repair

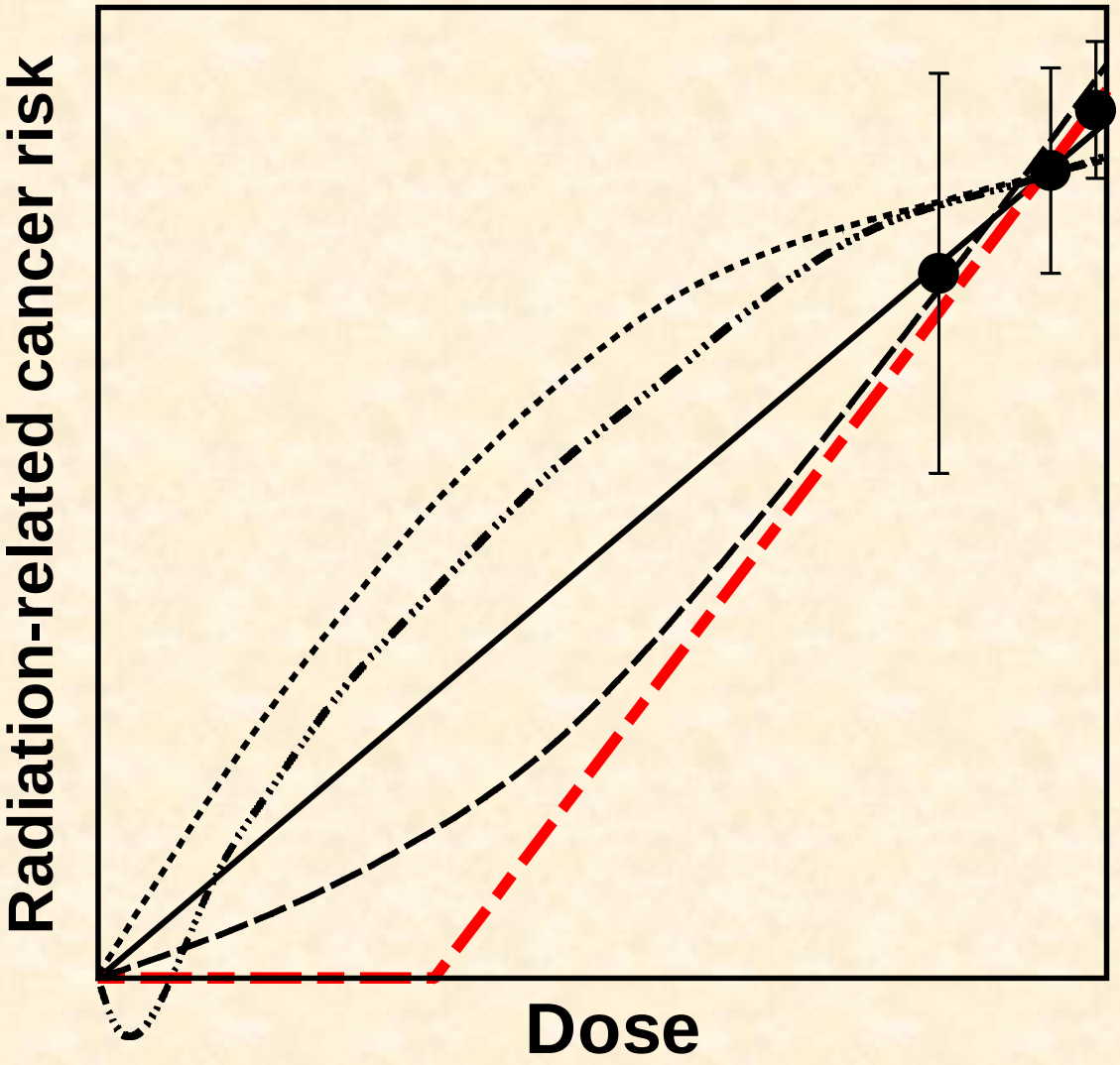
- We have been exposed to ionizing radiation for billions of years, and have developed exceedingly efficient DNA repair mechanisms
- But it is known that, along with DNA repair, there is always a small probability of DNA misrepair



Immuno-surveillance and the biophysical argument

- **If immuno-surveillance or other processes could always "mop up" small numbers of pre-malignant cells, the biophysical argument would not hold**

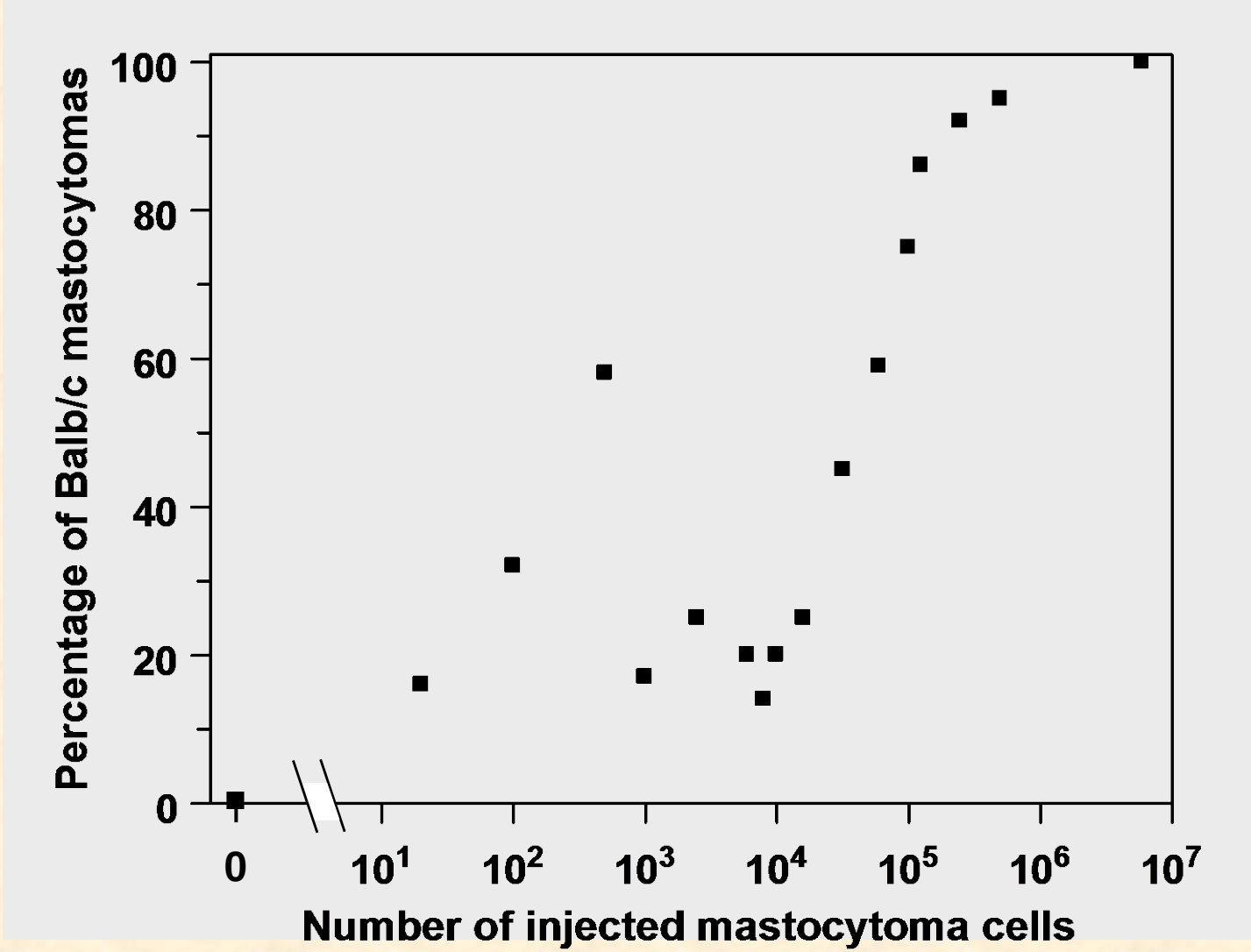
Immuno-surveillance and the biophysical argument



Immuno-surveillance and low-dose risks

- **If immuno-surveillance or other processes could indeed always “mop up” small numbers of pre-malignant cells, we would never get cancer!**

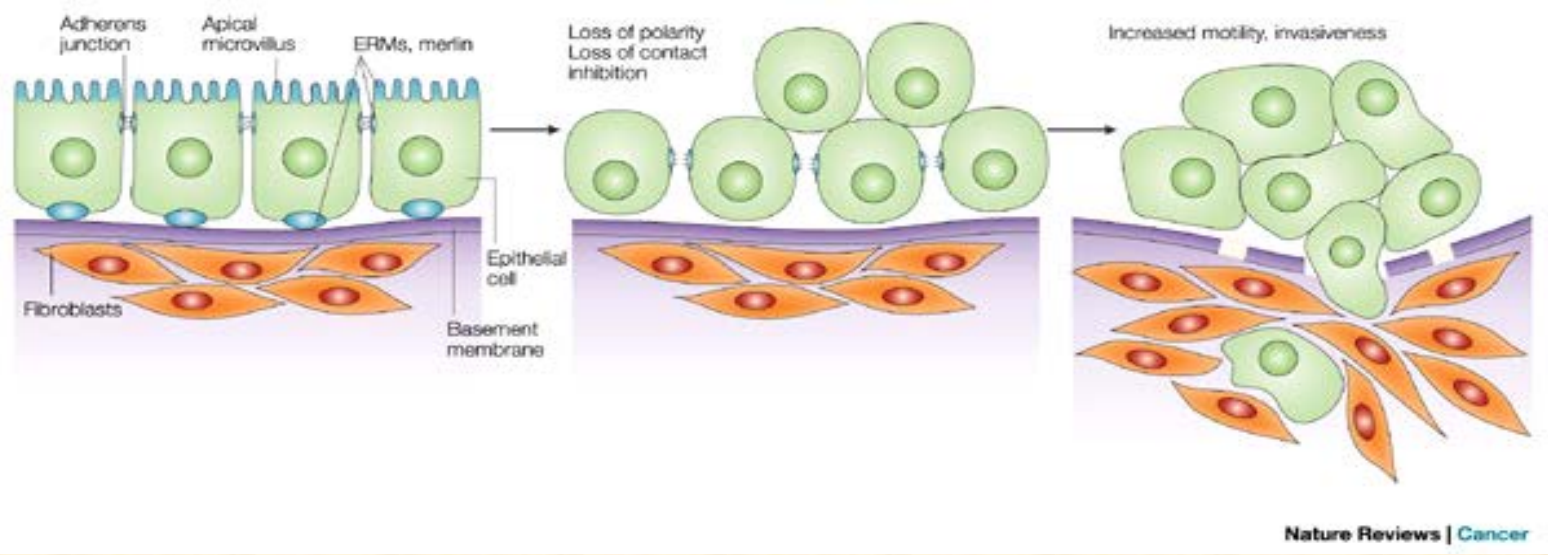
"Sneaking Through" immune surveillance



Kölsch *et al*. 1973

Weaknesses of the biophysical argument

- The argument refers to the development of monoclonal tumors by independently developing cells
- We know that cells talk to each other, and we know that the local microenvironment is important



The significance of inter-cellular communication for radiation-induced cancer

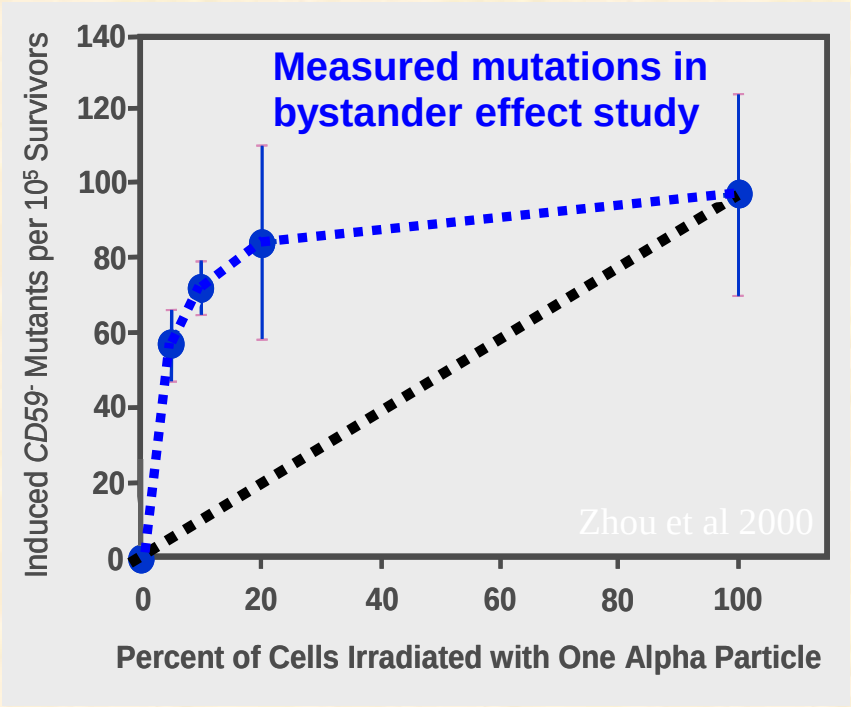
- **The biophysical argument refers to the development of monoclonal tumors by autonomous (independently developing) cells**

- **Are radiation-carcinogenic processes counteracted / amplified by mechanisms at the inter-cellular, tissue or organism level?**

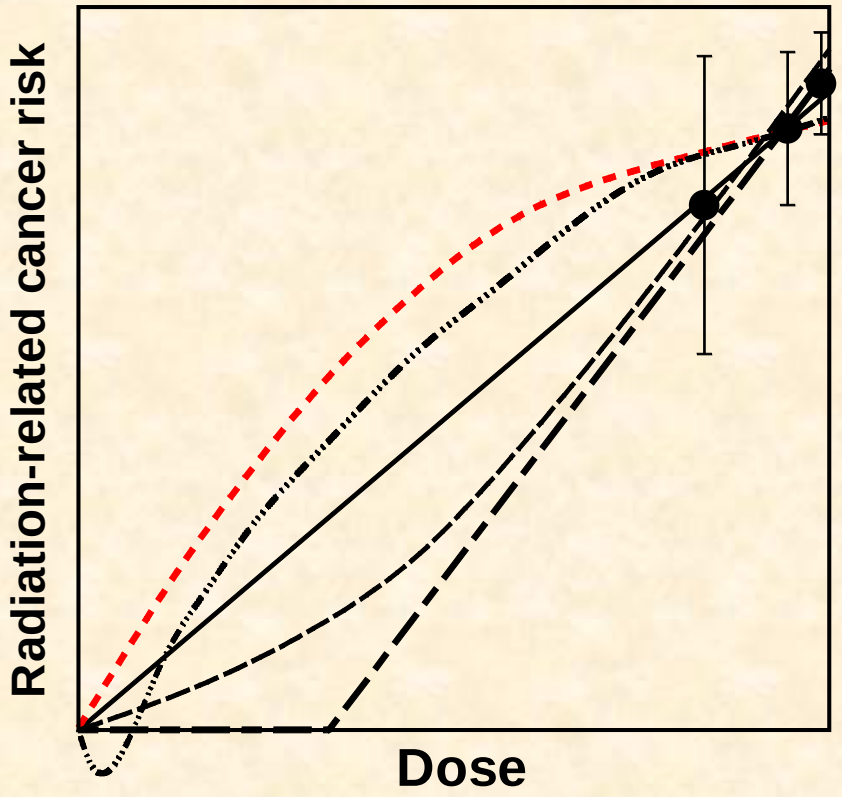
Cells in tissues do certainly talk to each other, but what are the implications for low-dose risks?

- **The most quantified radiation-related inter-cellular response is the bystander effect**

- **Where bystander responses have been quantitated, they have shown saturation**



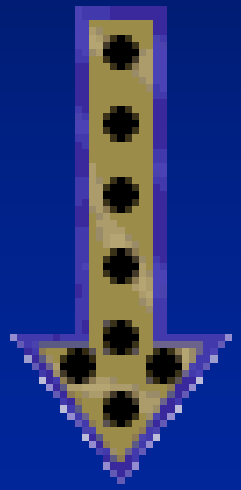
What we know of the effect inter-cellular communication suggests that it might modify the dose-response upwards at low doses



....but we don't know a lot, quantitatively

Dose Rate Effects

Shape of the acute dose-response curve at low doses

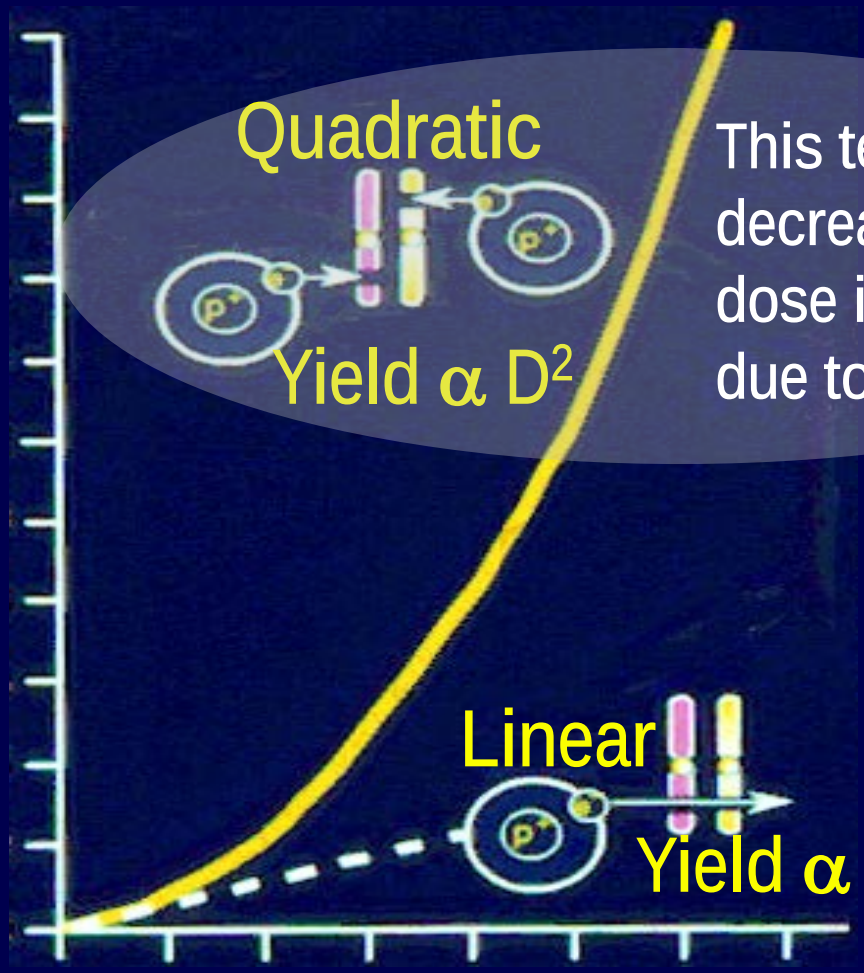


Dose rate effects

Splitting the Dose into Fractions

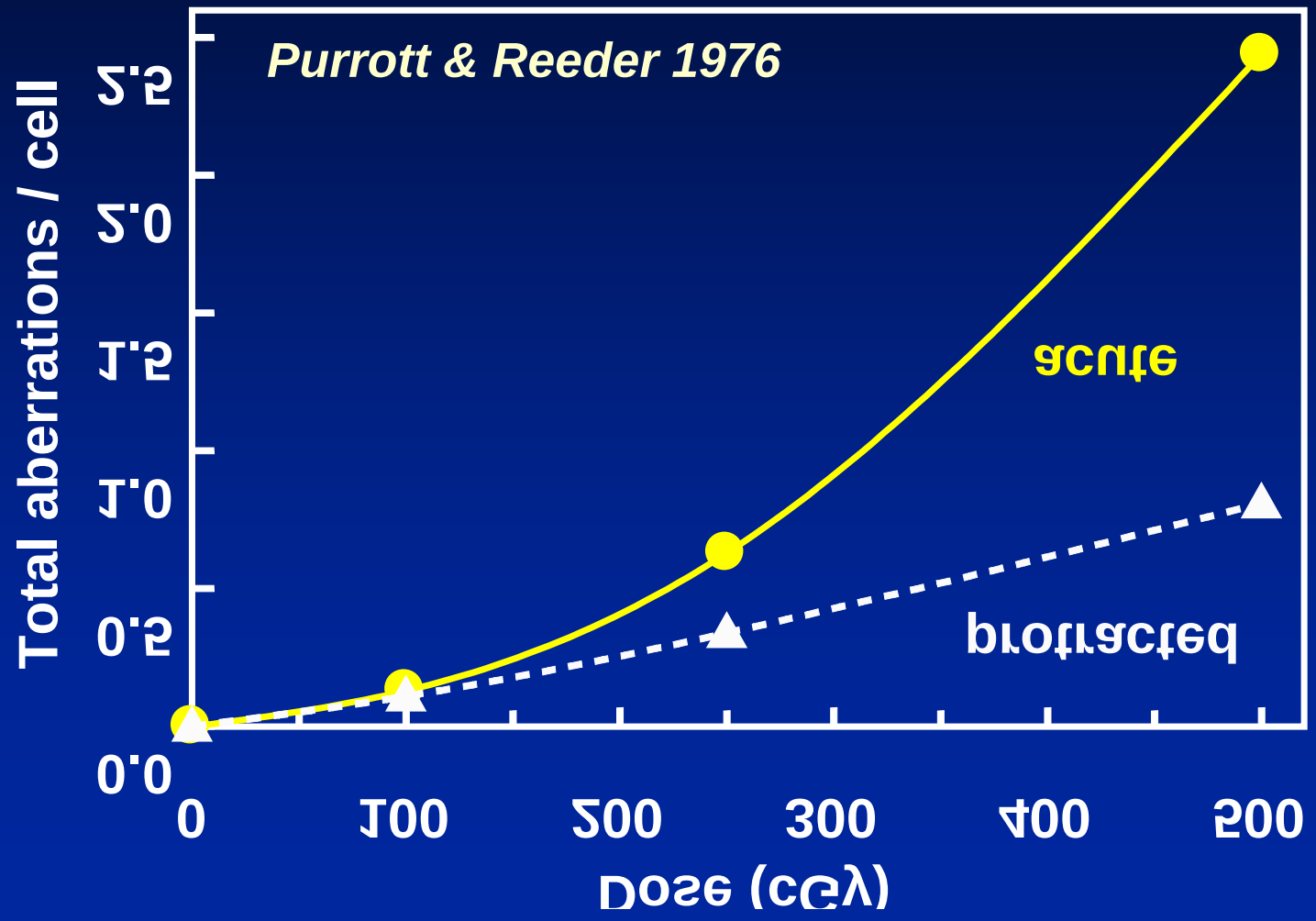
1 hit ➔ linear

2 independent hits ➔ quadratic

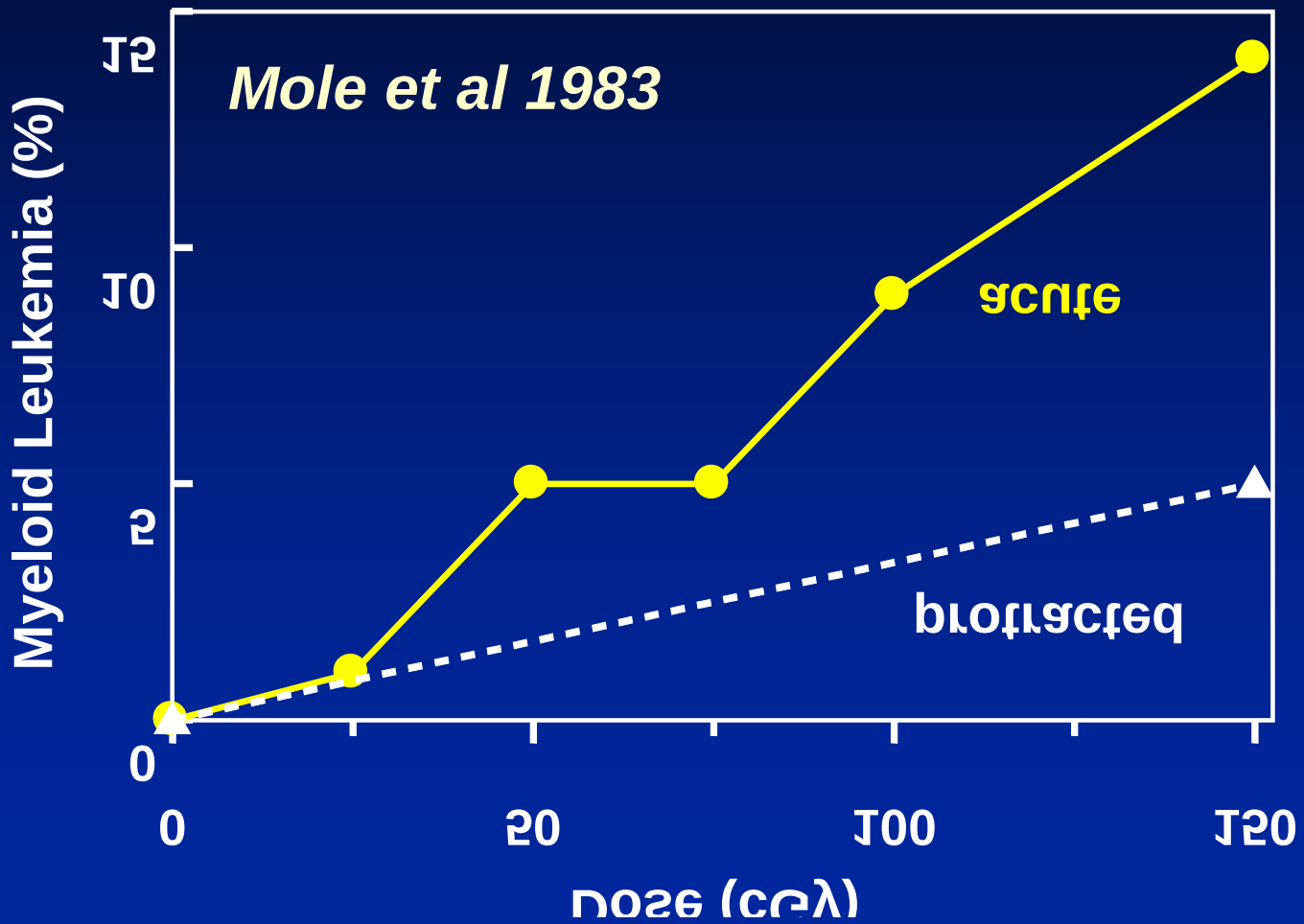


Yield = αD + βD²

Aberration induction in human lymphocytes
10 cGy/h vs 400 cGy/h



X-ray induction of myeloid leukemia in CBA/H mice

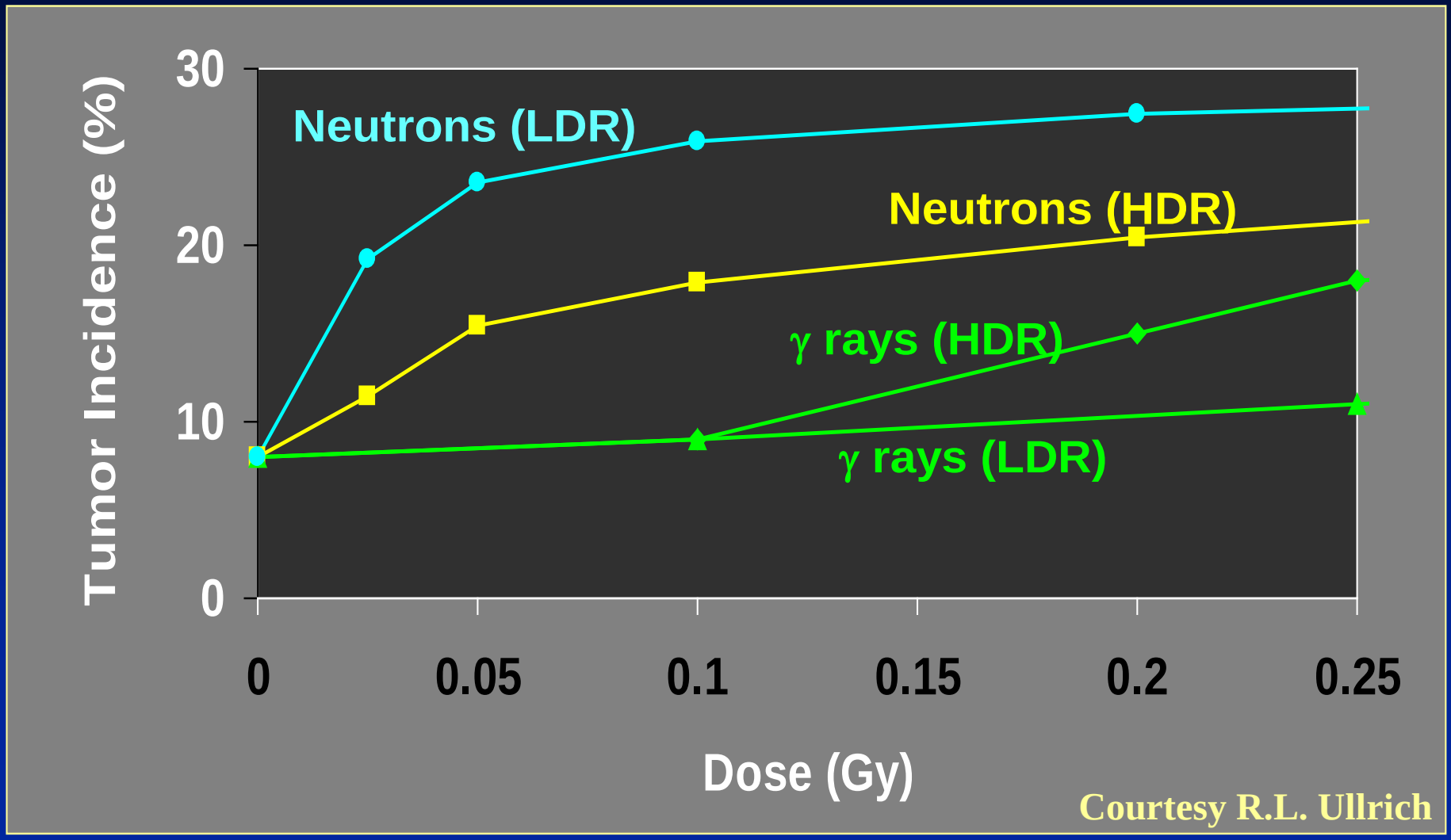


Excess leukemia in A-bomb survivors (Pierce *et al* 1996)

The inverse dose-rate effect…
for densely-ionizing exposures such as radon

For a given dose of densely-ionizing radiation, lowering the dose rate increases the cancer risk

Mammary tumors induced in BALB/c mice by low doses of γ rays and neutrons, HDR and LDR



Courtesy R.L. Ullrich

Relative Biological Effectiveness

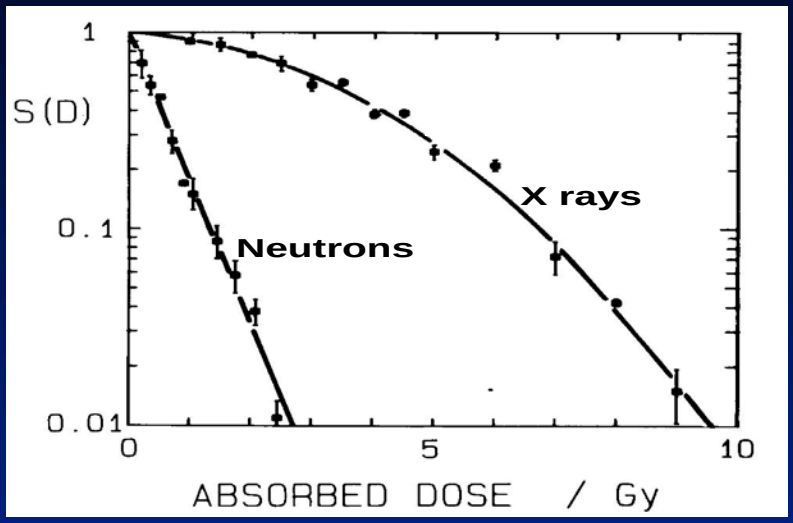
$$\text{RBE} = \frac{\text{Dose for given probability of effect by reference radiation}}{\text{Dose for given probability of effect by radiation of interest}}$$

Relevance of RBE

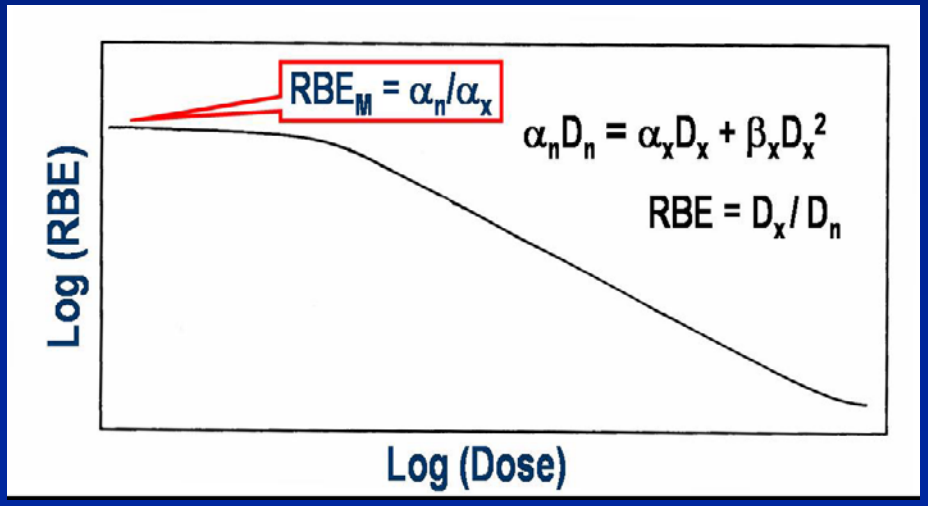
- Radon
- Mammography
- Neutrons
- I-131
- Space radiation
- Heavy ion radiotherapy

RBE is typically dose dependent

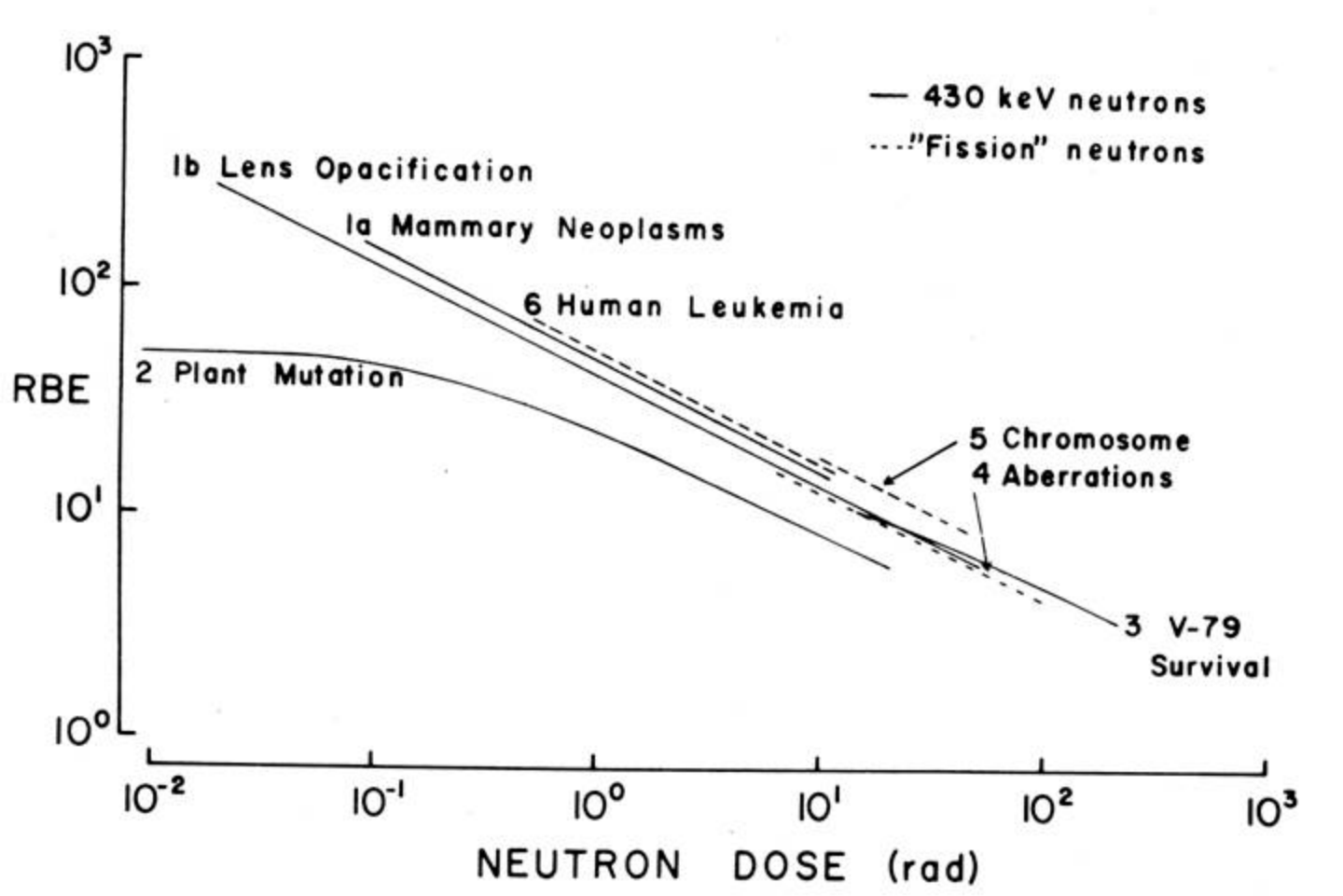
Photons have curved dose-response relations, while those for high-LET radiations are straighter



RBE is dose dependent, with a constant maximal value (RBE_M) at low doses

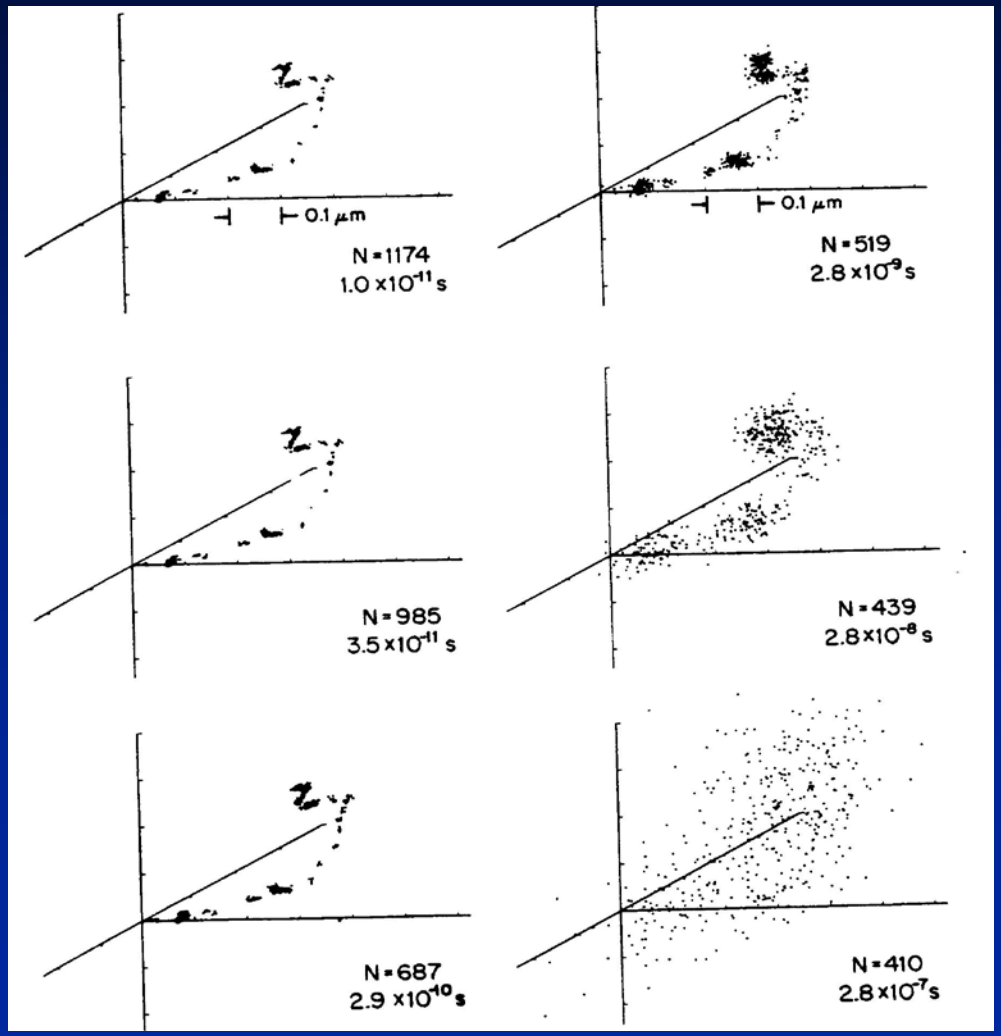


Neutron RBE vs dose for a variety of endpoints



Rossi 1980

RBE must be due to the initial track structure

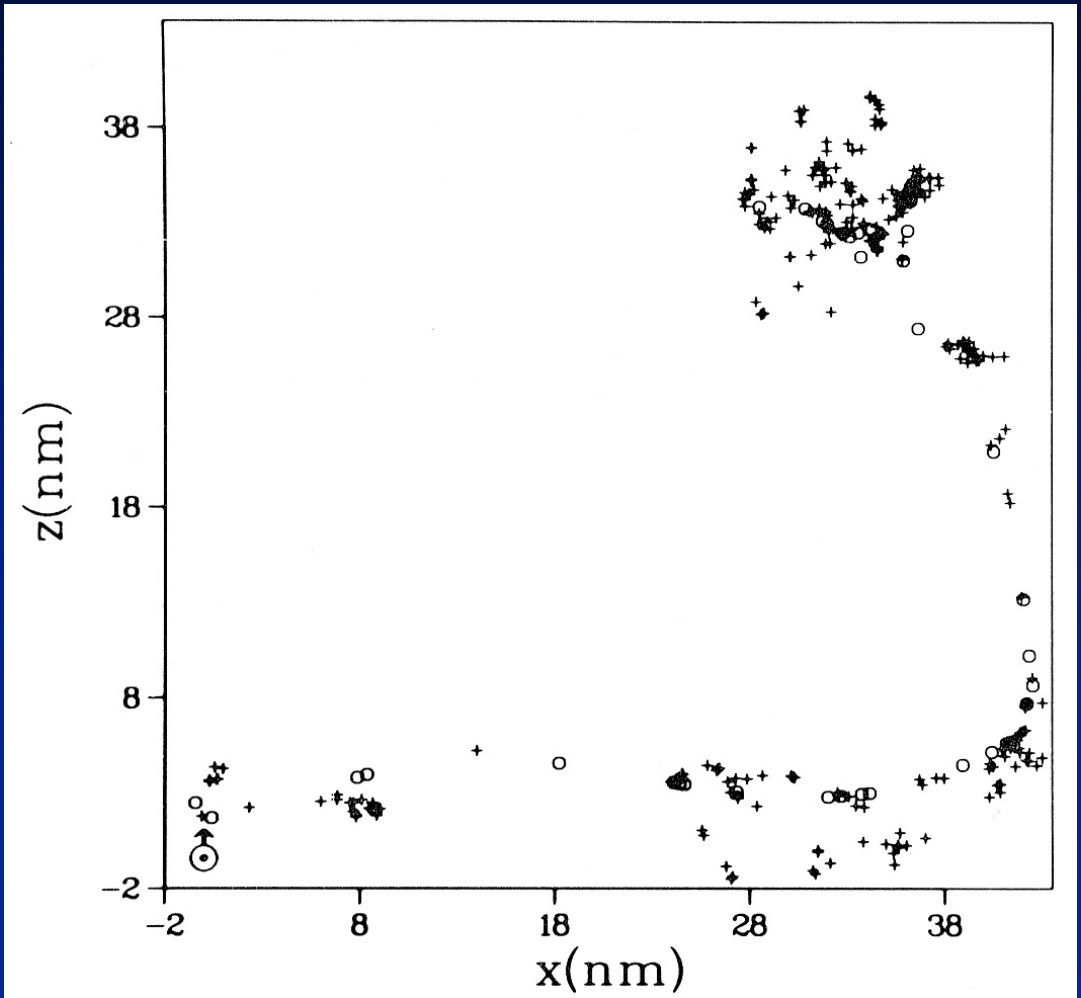


Wright et al 1982

Microdosimetry - The Study of Track Structure

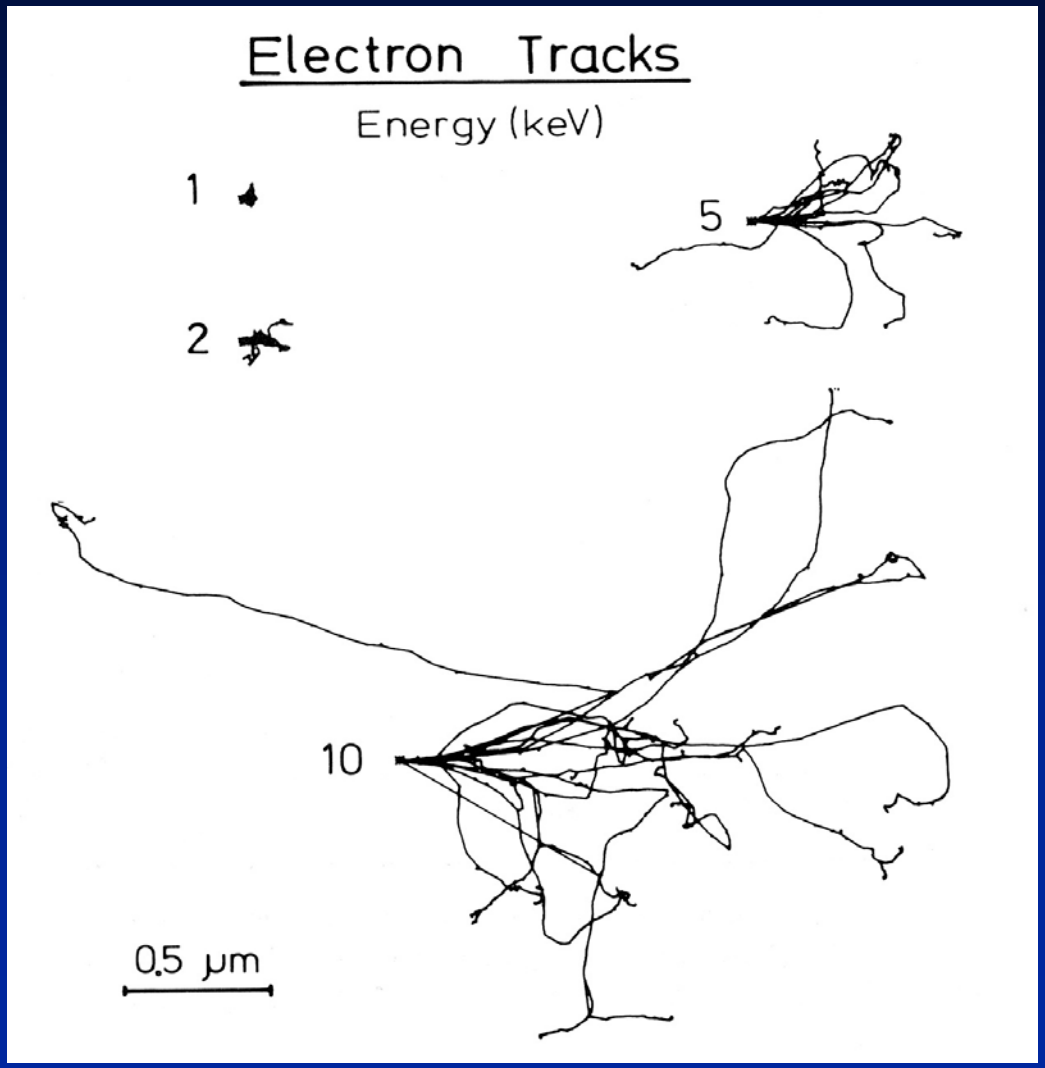
- Ionizing radiations deposit energy in a fundamentally different way from that of other mutagens or carcinogens
- The energy imparted, and the subsequent radiation products are not distributed in simple uniform patterns.
- The radiation track is structured, with energy depositions occurring in clusters along the trajectories of charged particles.
- The characterization of energy depositions on micrometer (and smaller) scales is the field of *microdosimetry*

Simulated track of 1 keV electron



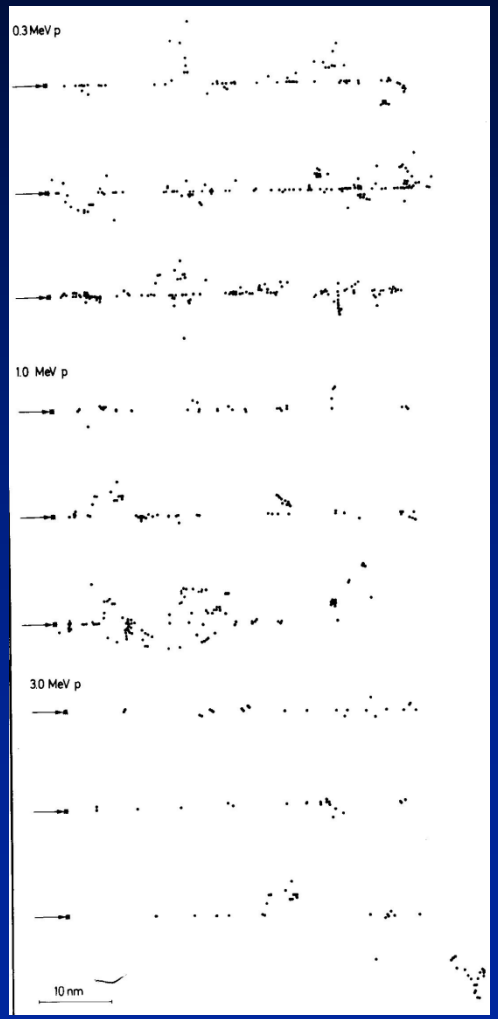
Zaider & Brenner 1983

Electron tracks of different energies

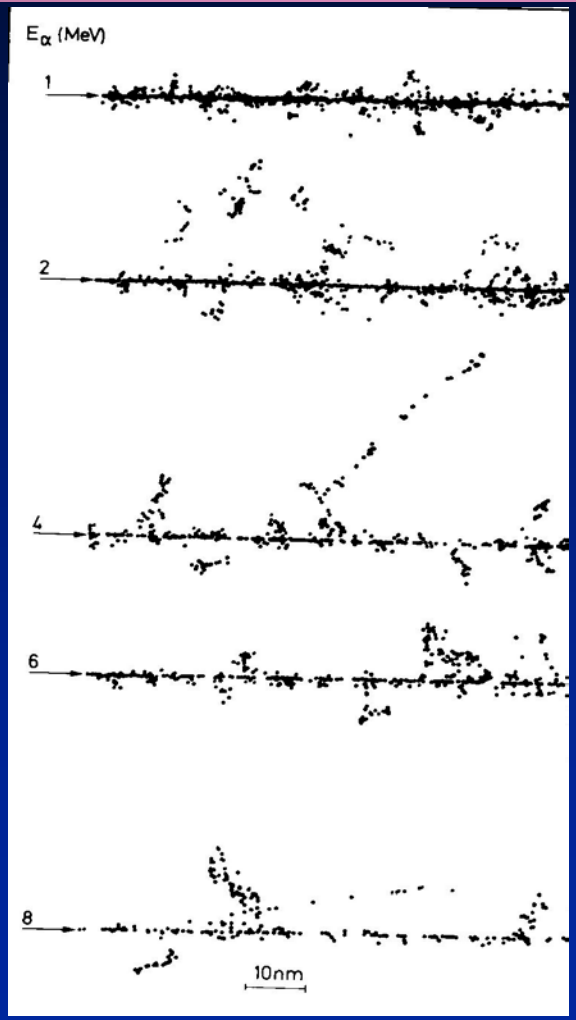


Paretzke 1987

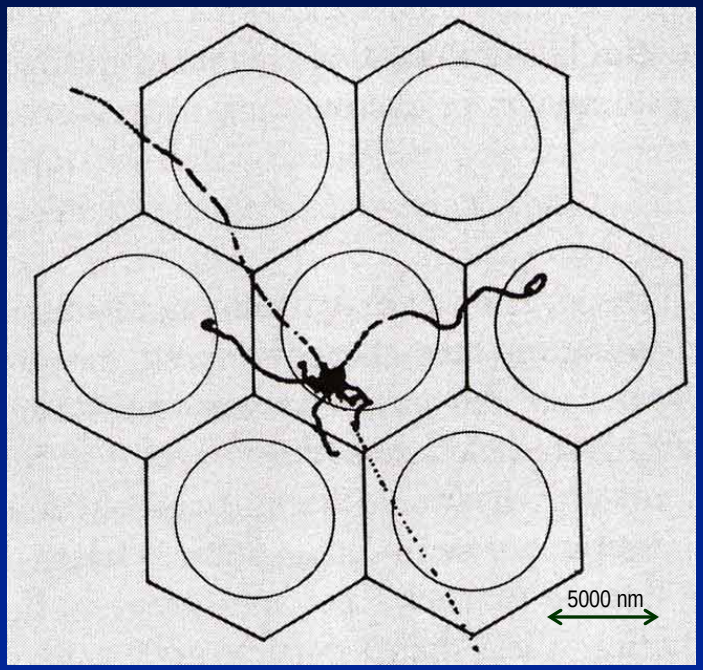
Simulated charged-particle tracks



protons



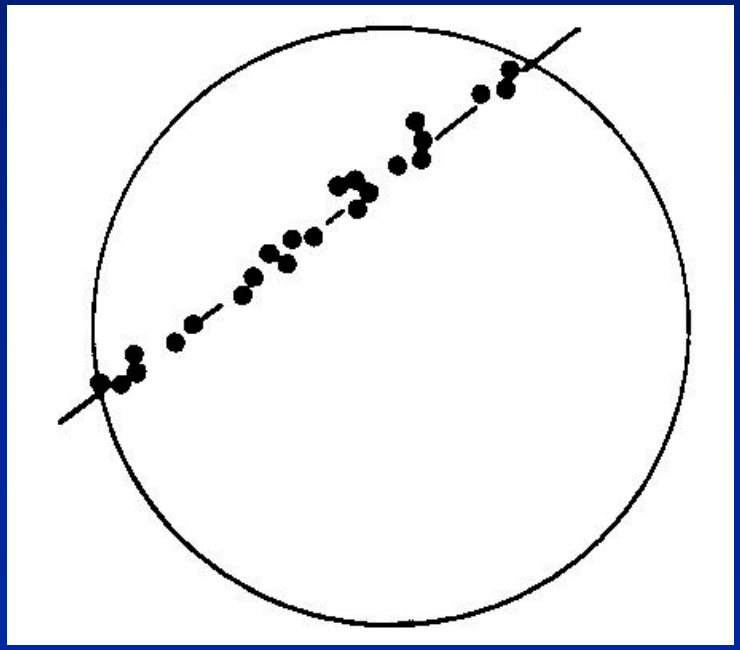
alpha particles



Cosmic-ray iron ion passing through lens of eye

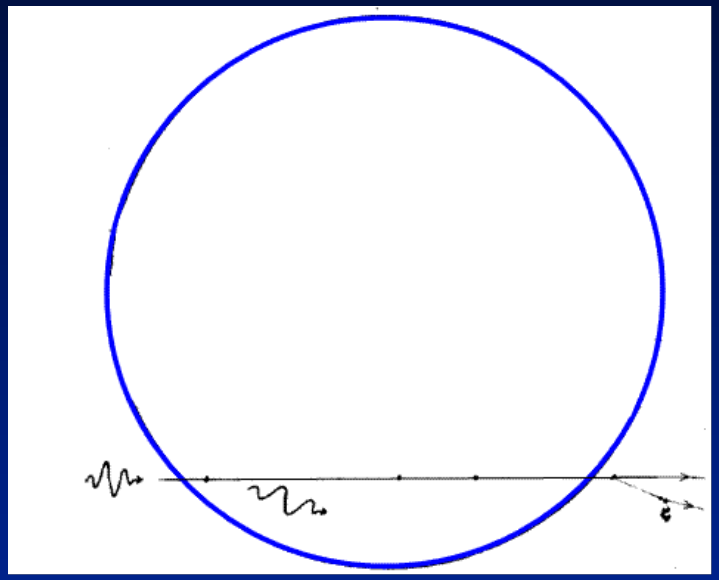
Microdosimetry: Lineal Energy (y)

Energy deposited in a target by a single radiation track, divided by the mean chord length of the target

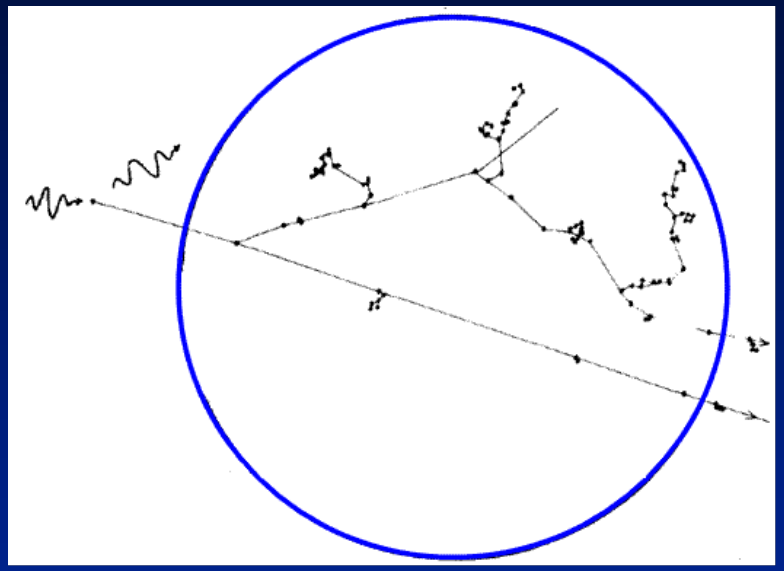


Microdosimetry:

Stochastics of ionizing radiation energy deposition



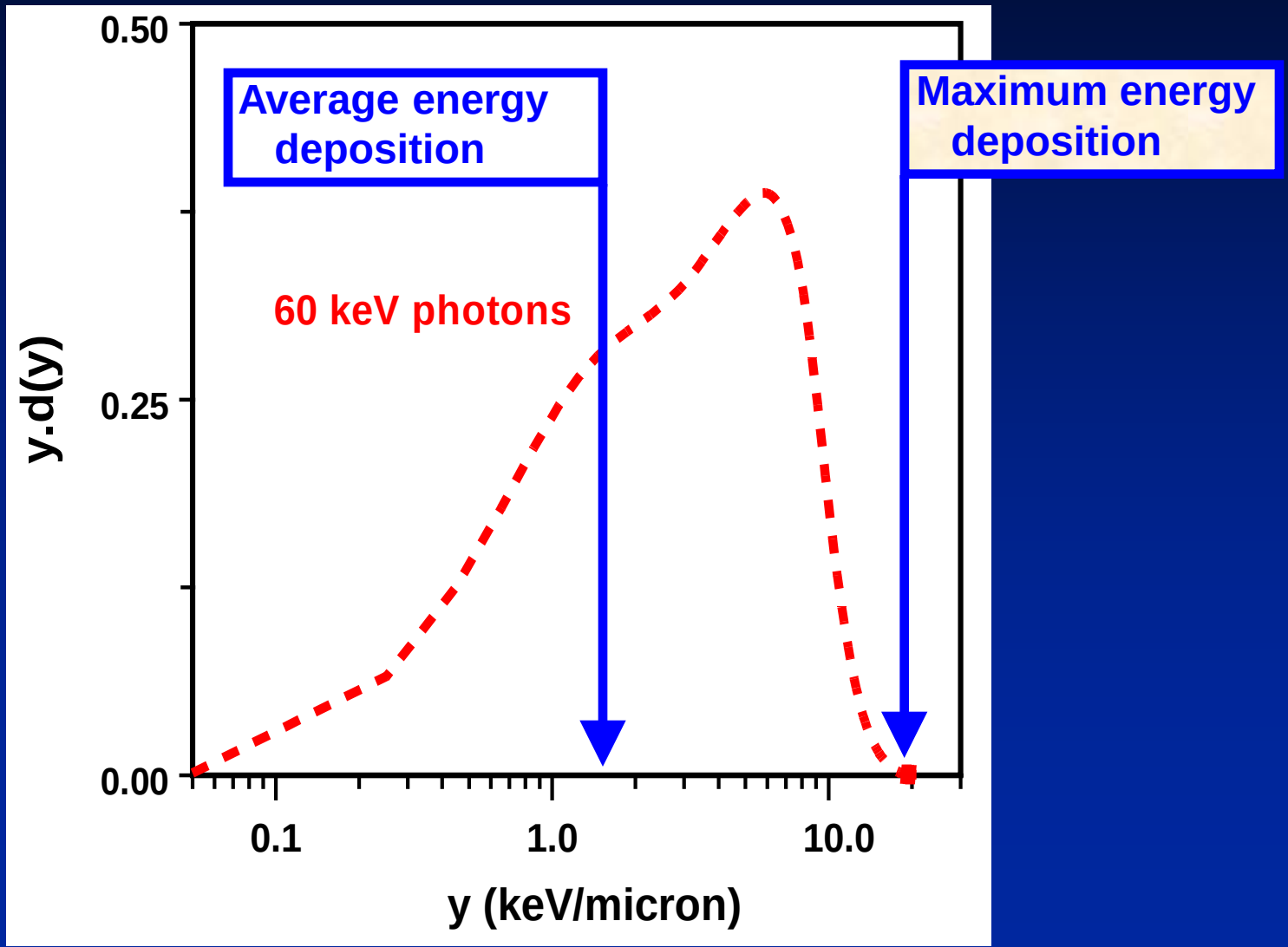
Simulation of single gamma ray passing through cell nucleus



Simulation of single gamma ray passing through cell nucleus

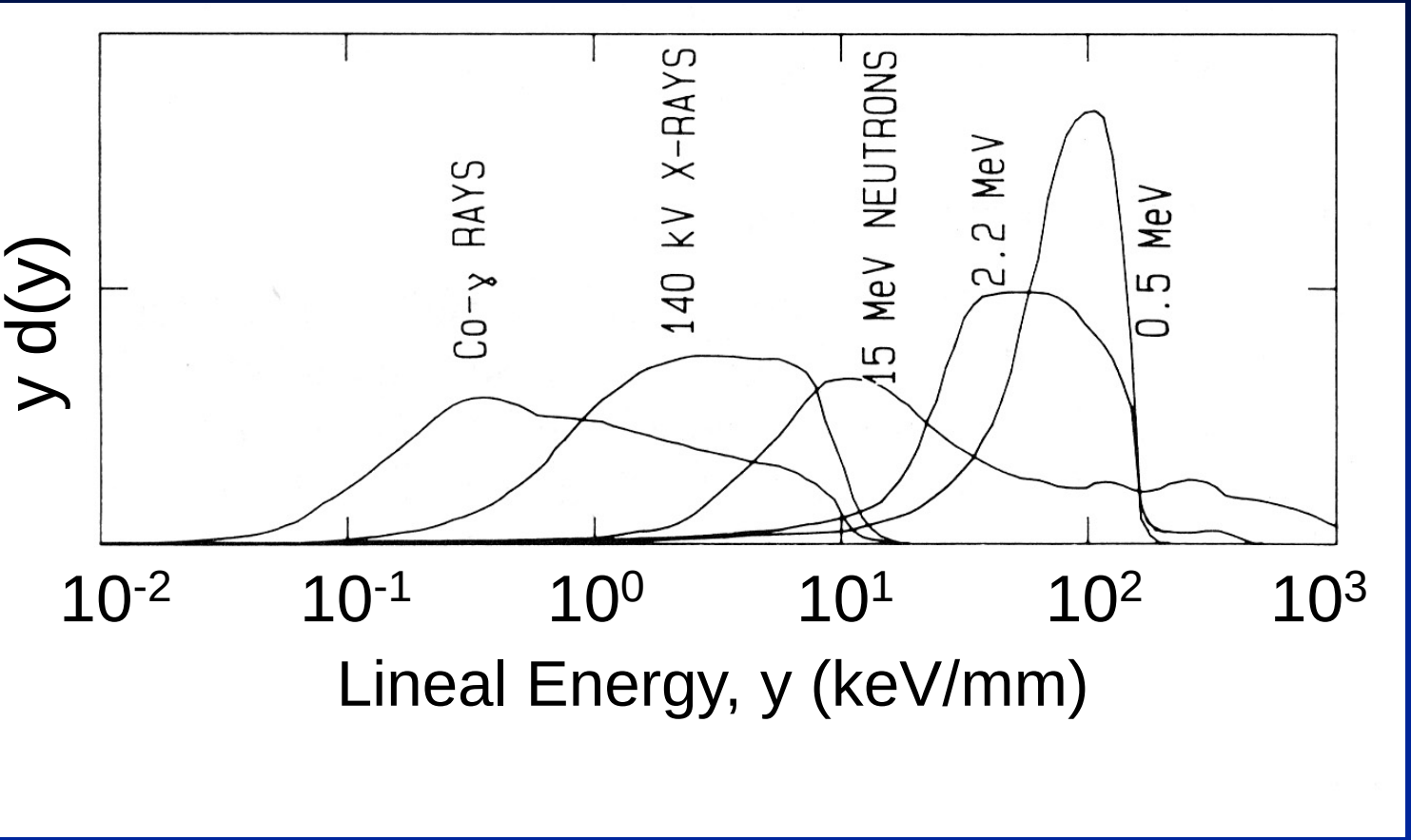
"Can a single photon really cause significant damage to the genome?"

The distribution of energy depositions in a cell nucleus by a single photon

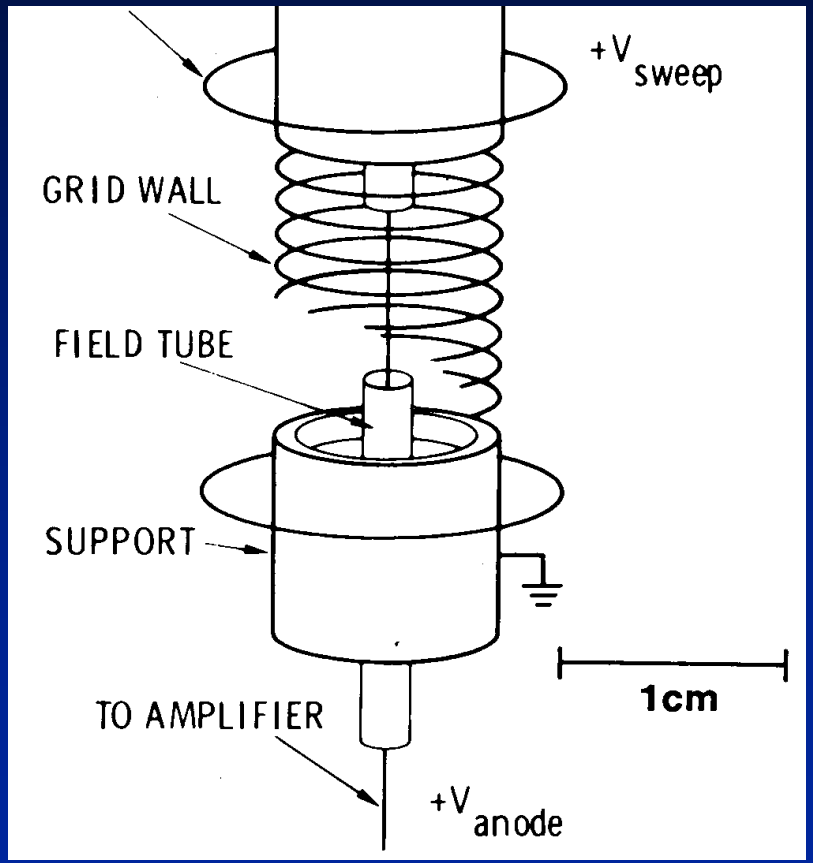


Microdosimetric Distributions:

Distributions of energy deposition in micron site sizes

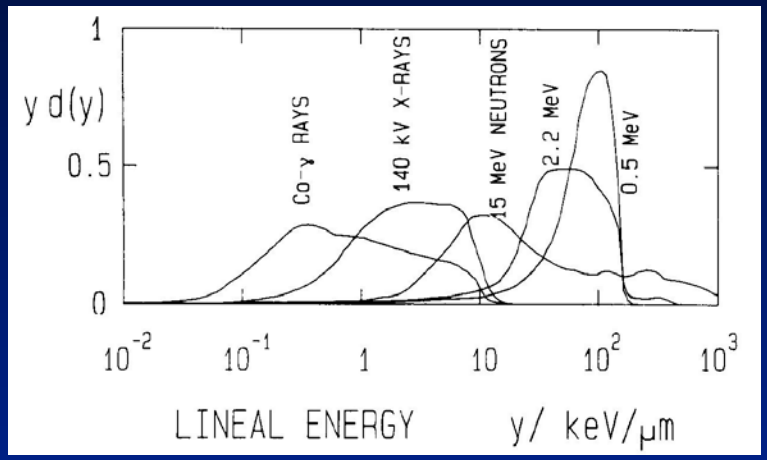


Microdosimetric spectra can be calculated or measured



From track structure to RBE_M

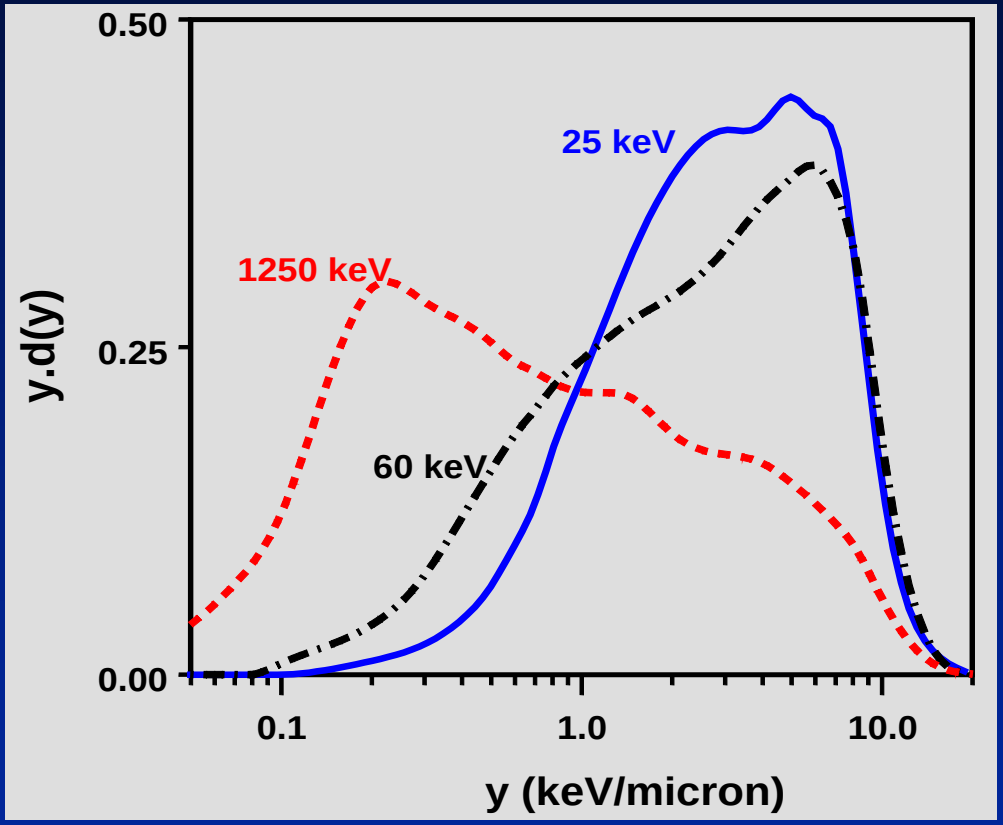
$$RBE_M = \int d(y)\, r(y)\, dy$$



Microdosimetric spectrum

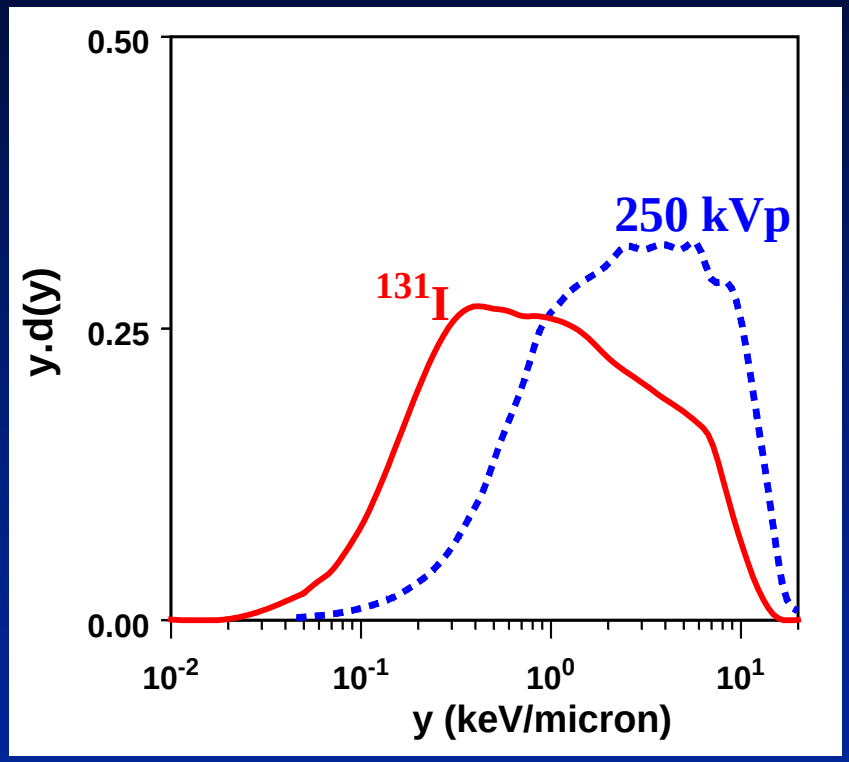
Biological response function

Different photon energies produce quite different microdosimetric spectra



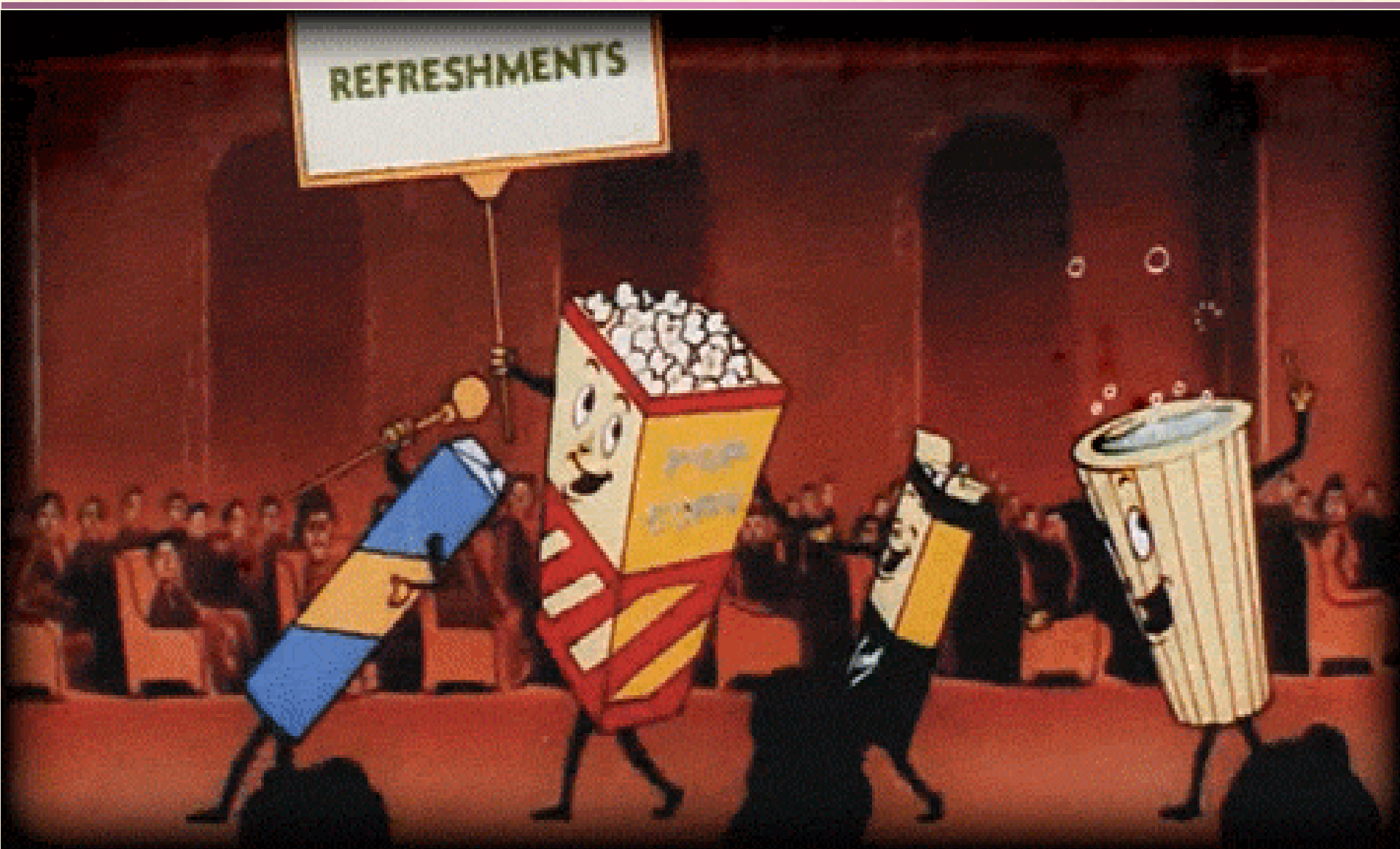
So, for example, mammographic x rays have an RBE of 2-3, compared to high energy photons

Low dose RBE of ^{131}I *vs.* 250 kVp x rays



- Based on microdosimetric spectra, RBE_M ~0.6

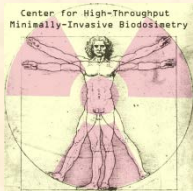
Half way through!!



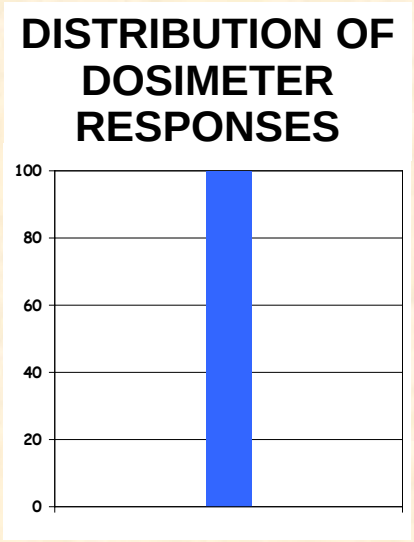
Biodosimetry

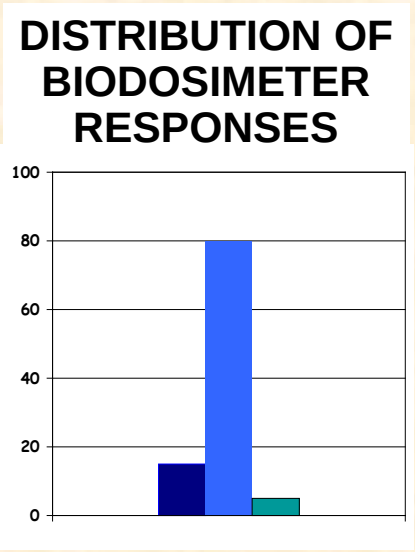
The use of biological markers to assess past radiation exposure

- **Advantages over physical dosimetry:**
  - ✓ **No need to be present during exposure**
  - ✓ ***Potentially more relevant medically***



Example: A hypothetical population all exposed to the same radiation dose





The need for high-throughput biodosimetry

1. Triage:

To prevent treatment locations from being overwhelmed

2. Treatment decisions:

Treatment options are dose dependent

3. Individualized prediction of radiation injury:

Triage "Beyond Dose"

4. In Support of Epidemiology:

Assessment of population long-term disease risks

5. Psycho-Social Considerations:

Active reassurance is an effective antidote to mass panic or mass skepticism

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The need for high-throughput biodosimetry

Triage

- 1987 radiation incident in Goiânia, Brazil, a city with about the same population as Manhattan.
- In the first few days after the incident became known, 130,000 people (10% of the population) came for screening, of whom 20 required treatment.

Center for High-Throughput Minimally-Invasive Biodosimetry

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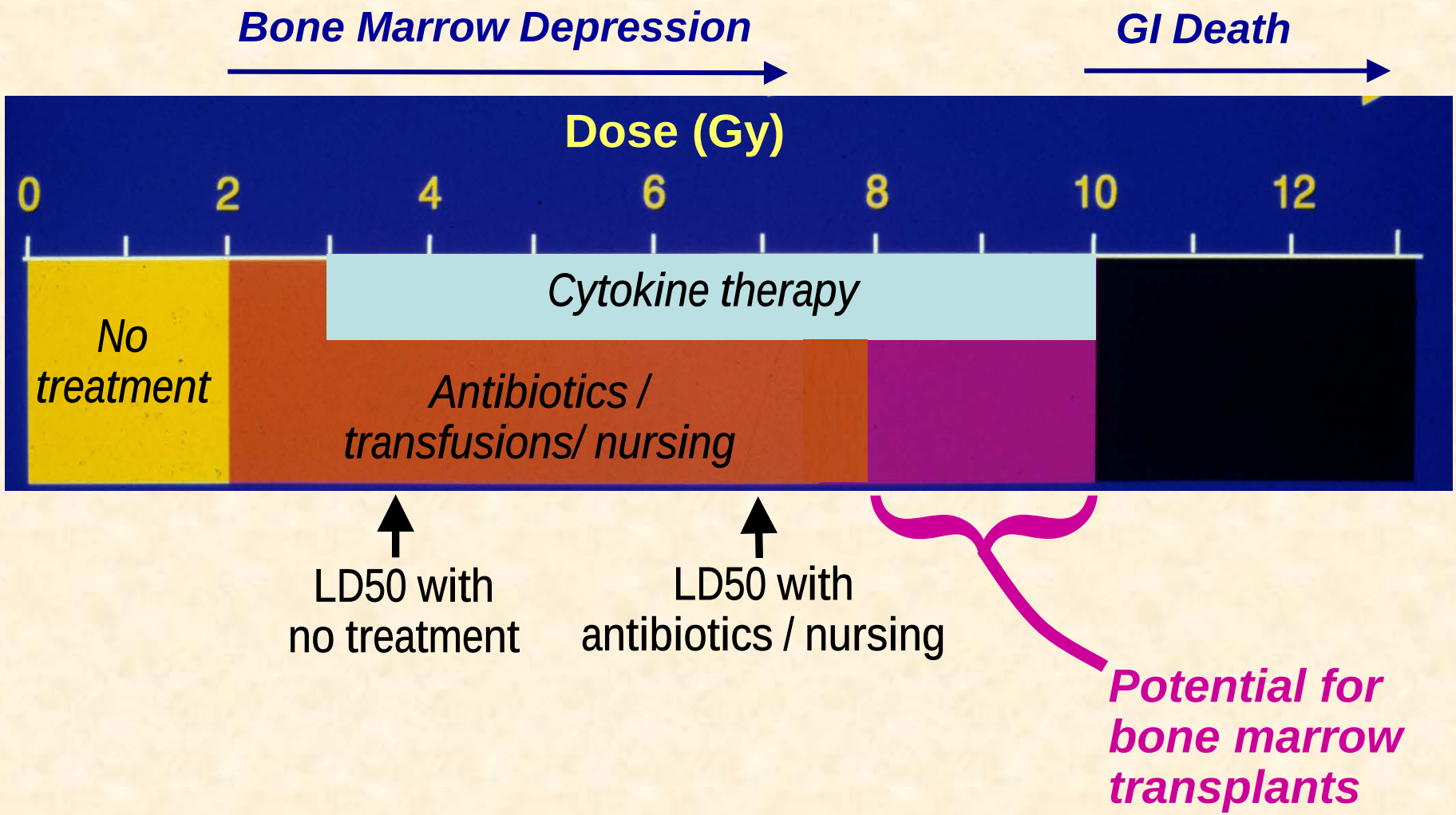
Biodosimetry is Essential to Optimize Treatment Decisions



Bone marrow transplants at Chernobyl

- **13 individuals were given bone marrow transplants, and three deaths can be directly attributed as the sequelae of the transplants given in individuals who received doses for which the transplants were not indicated**

Mettler et al 2007

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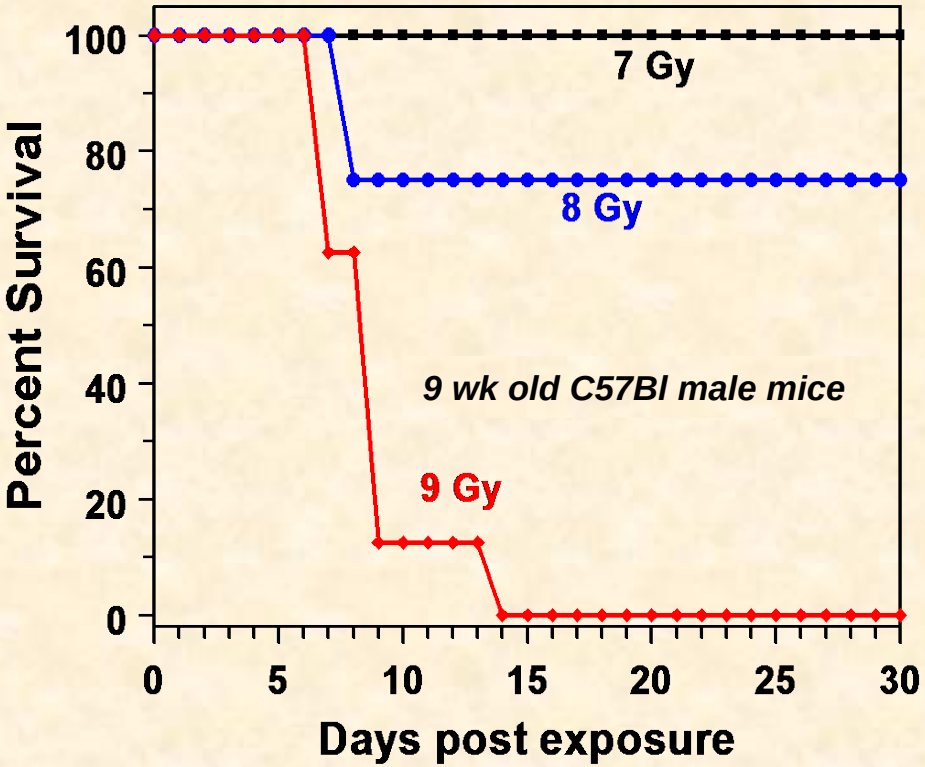
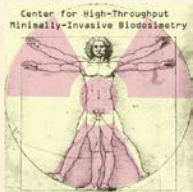
Triage "Beyond Dose"

High-throughput biomarkers for predicting individualized acute radiosensitivity



- After 8 Gy, 25% of the mice died within one week, but 75% of the mice survived long term
- Why?
- Can we provide a high-throughput methodology to predict which exposed individuals will suffer severe health effects and which will not?



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The need for high-throughput biodosimetry

Biodosimetry for Radiation Epidemiology

Health Phys. 2010 February ; 98(2): 109–117. doi:10.1097/HP.0b013e3181a86628.

CURRENT USE AND FUTURE NEEDS OF BIODOSIMETRY IN STUDIES OF LONG-TERM HEALTH RISK FOLLOWING RADIATION EXPOSURE

Steven L. Simon[*,†], **André Bouville**[*], and **Ruth Kleinerman**[*]

[*]Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, MD USA

Abstract

Biodosimetry measurements can potentially be an important and integral part of the dosimetric methods used in long-term studies of health risk following radiation exposure. Such studies rely on accurate estimation of doses to the whole body or to specific organs of individuals in order to derive reliable estimates of cancer risk. However, dose estimates based on analytical dose reconstruction (i.e., models) or personnel monitoring measurements, e.g., film-badges, can have substantial uncertainty. Biodosimetry can potentially reduce uncertainty in health risk studies by corroboration of model-based dose estimates or by using them to assess bias in dose models. While biodosimetry has begun to play a more significant role in long-term health risk studies, its use is still generally limited in that context due to one or more factors including, inadequate limits of detection, large inter-individual variability of the signal measured, high per-sample cost, and invasiveness. Presently, the most suitable biodosimetry methods for epidemiologic studies are chromosome aberration frequencies from fluorescence *in situ* hybridization (FISH) of peripheral blood lymphocytes and electron paramagnetic resonance (EPR) measurements made on tooth enamel. Both types of measurements, however, are usually invasive and require difficult to obtain biological samples. Moreover, doses derived from these methods are not always directly relevant to the tissues of interest. To increase the value of biodosimetry to epidemiologic studies, a number of issues need to be considered including limits of detection, effects of inhomogenous exposure of the body, how to extrapolate from the tissue sampled to the tissues of interest, and how to adjust dosimetry models applied to large populations based on sparse biodosimetry measurements. The requirements of health risk studies suggest a set of characteristics that, if satisfied by new biodosimetry methods, would increase the overall usefulness of biodosimetry to determining radiation health risks.

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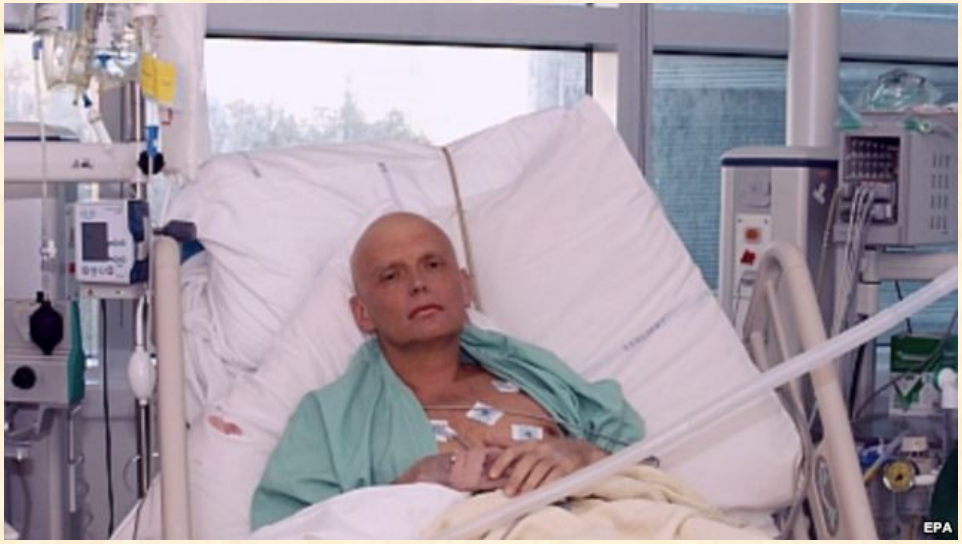
Lessons Learned from the 2006 Litvinenko Incident



Paper

THE LONDON POLONIUM INCIDENT: LESSONS IN RISK COMMUNICATIONS

G. James Rubin,* Richard Amlôt,† and Lisa Page*

Abstract—**Public responses to large-scale radiological incidents are often thought to be disproportionate to the objective risk and can involve widespread societal disruption. Recent experiences of the ^{210}Po incident in central London suggest that public responses depend heavily on the nature of the incident and the effectiveness of risk communication efforts. This paper describes the outcome of several studies done in the aftermath**

exposed to the radiation (Petterson 1988; Havenaar et al. 2003), mass spontaneous evacuation from affected areas (Ziegler et al. 1981), and avoidance of products, places, and people that are viewed as contaminated (Petterson 1988; Renn 1990; Tonnessen et al. 2002) have all previously been documented following radiological ac-

"More than anything else, the main lesson was that emphasis should be placed on providing accurate, up-to-date, and individually tailored information to affected people. Explicit attempts to reassure in the absence of information may be counterproductive"

Rubin et al 2011

The Japanese Public Response after Fukushima

IOP Publishing — Journal of Radiological Protection
J. Radiol. Prot. **32** (2012) 1–10 — doi:10.1088/0952-4746/32/1/1

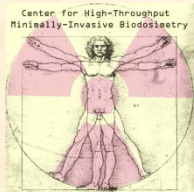
Epidemiological studies of Fukushima residents exposed to ionising radiation from the Fukushima Daiichi Nuclear Power Plant prefecture—a preliminary review of current plans

Suminori Akiba

Department of Epidemiology and Preventive Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Japan

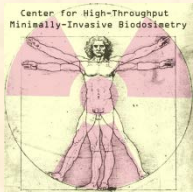
"Unfortunately, Japanese people, particularly the residents of Fukushima Prefecture, have begun to suspect that the Japanese government and local authorities are keeping important information from them"

Akiba, 2012



In future large-scale radiological events, worldwide, we should anticipate much skepticism regarding radiation information coming from the authorities

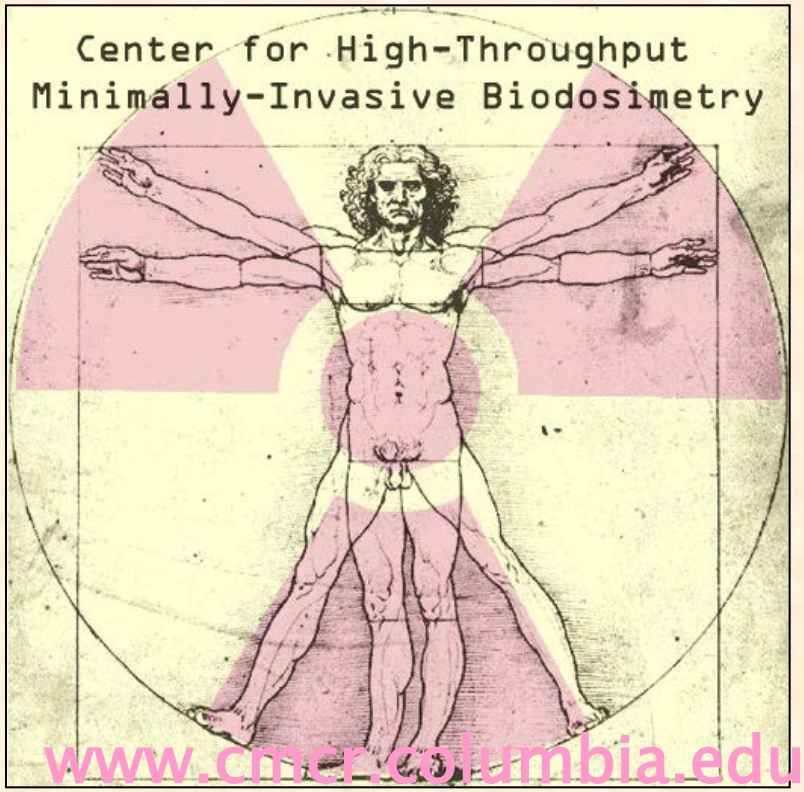
- One solution is to provide rapid and individualized measured radiation doses, for every person
  - To identify individuals who really got high doses
  - To reassure the great majority of people who got very low doses



What sort of sample numbers are needed for biodosimetry after an IND or RDD event?

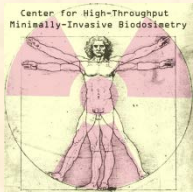
- **Some scenarios / approaches will require analysis of hundreds of samples**
  - Cytogenetic laboratory networks should be able to effectively cover this range
- **Other scenarios / approaches will require analysis of ~10^4 to 10^7 samples**

Columbia Center for High-Throughput Minimally-Invasive Radiation Biodosimetry



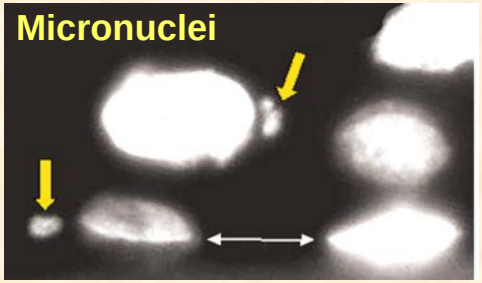
Issues for an Effective High-Throughput Radiation Biodosimetry System

- Processing throughput
- Sensitivity / specificity
- Processing time
- Signal stability
- Multi-use functionality
- Operational capability

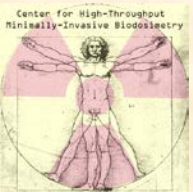


Program 1: Converting validated manually-based biodosimeters to ultra-high throughput (RABiT)

- ✓ **Current validated (manually-based) biomarkers**



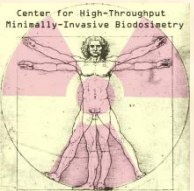
- ✓ **Fully automated robotically-based ultra-high-throughput system**



Program 2: Biodosimetry with a fully integrated biochip, using gene-expression signatures

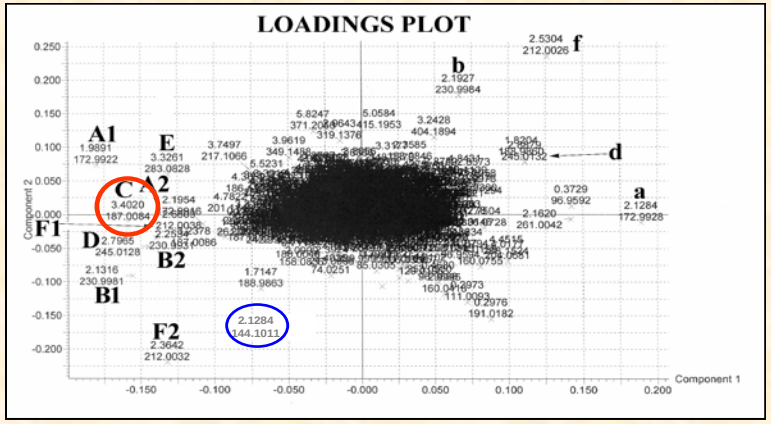
✓ **The genomic signature**

✓ **The cartridge detection device**

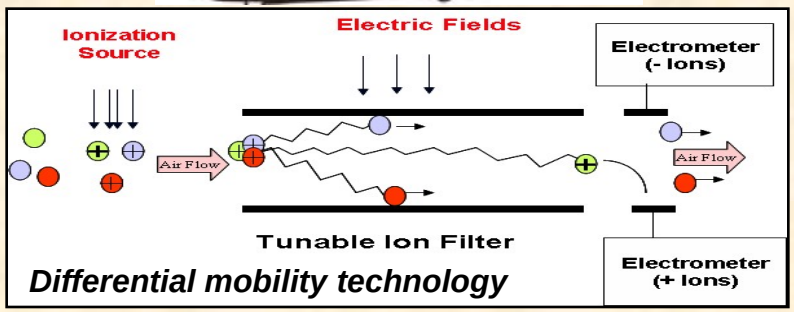


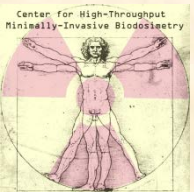
Program 3: Rapid non-invasive biodosimetry through metabolomics

- The metabolomic signature



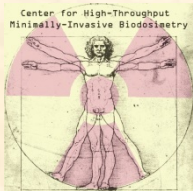
- The detection technology





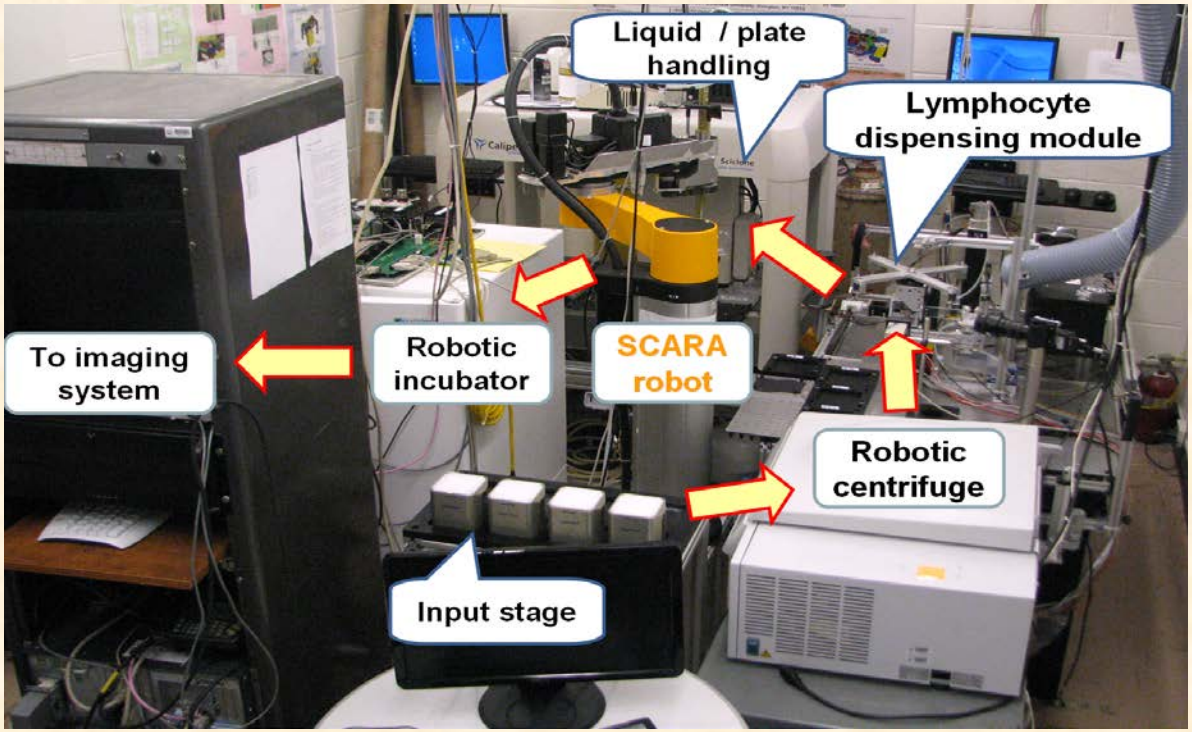
RABiT:
Rapid Automated Biodosimetry Tool

Converting manually-based radiation biodosimetry assays to high throughput, using fully automated robotically-based biodosimetry workstations



RABiT:
Rapid Automated Biodosimetry Tool

- Fully automates current manual biodosimetric assays
- Uses one fingerstick of blood per person
- *Analyzes up to 30,000 samples per day*



RABiT:
Rapid Automated Biodosimetry Tool

- **Fully-automated ultra high-speed robotic biodosimetry workstation**
- **One fingerstick of blood**
- **No further human intervention after blood samples put into the RABiT**
- **Automates well-established manual assays**
- **Can deal with partial-body exposure**

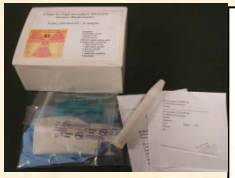
The main technical innovations are:

1) **Use of smaller samples – single drop of blood from a fingerstick**
2) **Complete full automation of biology, with *in-situ* imaging in multi-well plates**
3) **Innovations in high-speed imaging**
4) **Potential for use as a hospital-based multi-use routine diagnostic tool**

Collection Points: *Hospitals*

Capillary blood samples

RABiT Locations

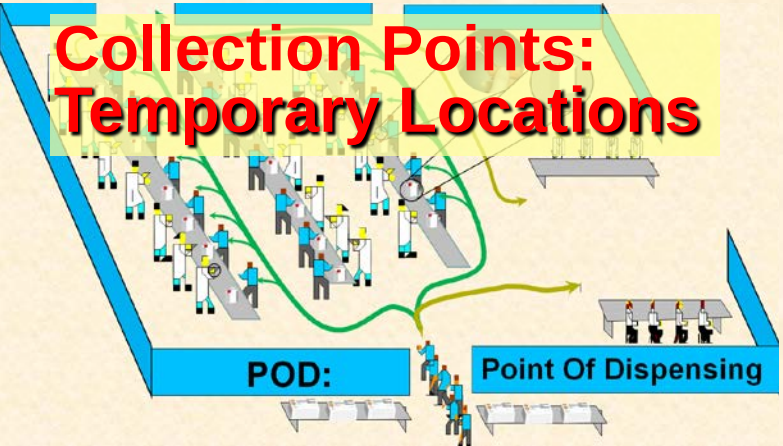


Capillary blood samples

Collection Points: Schools

Capillary blood samples

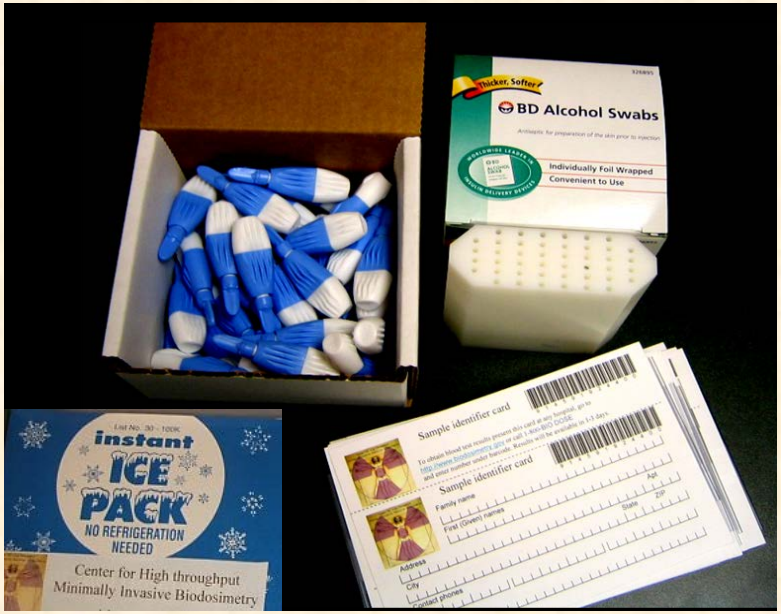
Collection Points: Temporary Locations



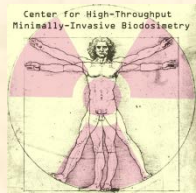
Capillary blood samples

Collection Points: Railway Stations

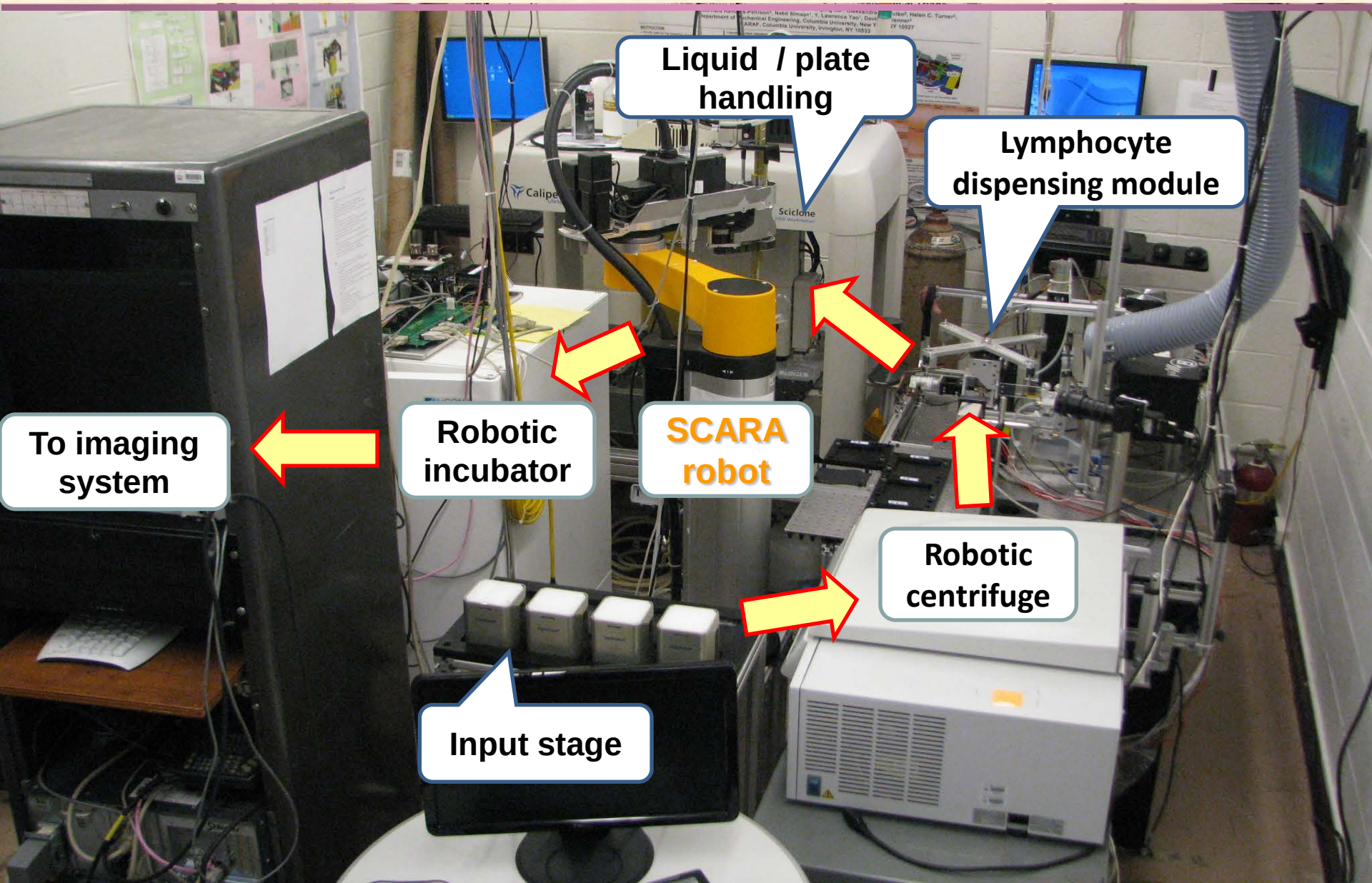
RABiT field collection kit



- Designed for 40 patients
- 2.5 hrs of sample collection for one minimally-trained field collector

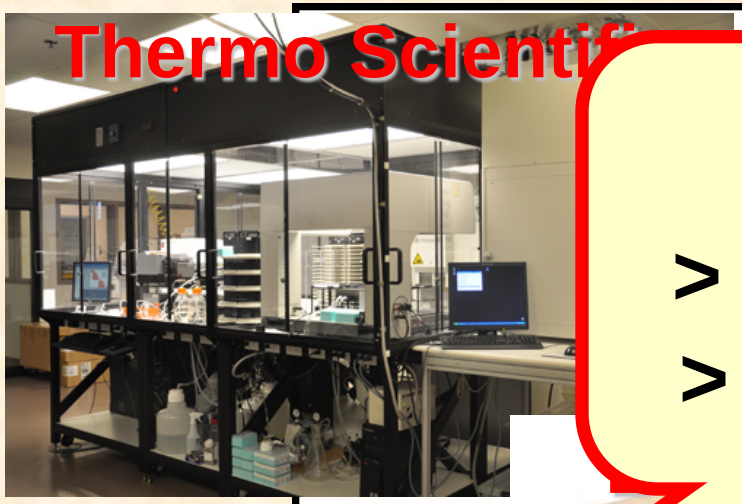


RABiT device overview



RABiT II

- In the past 5 years commercial high-content high-throughput cellular screening systems have become increasingly common



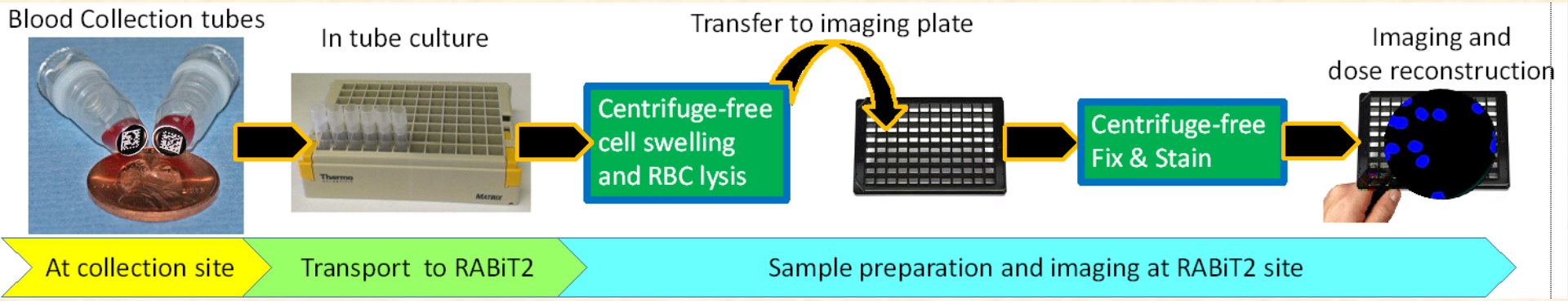
2014

> 25 in NYC

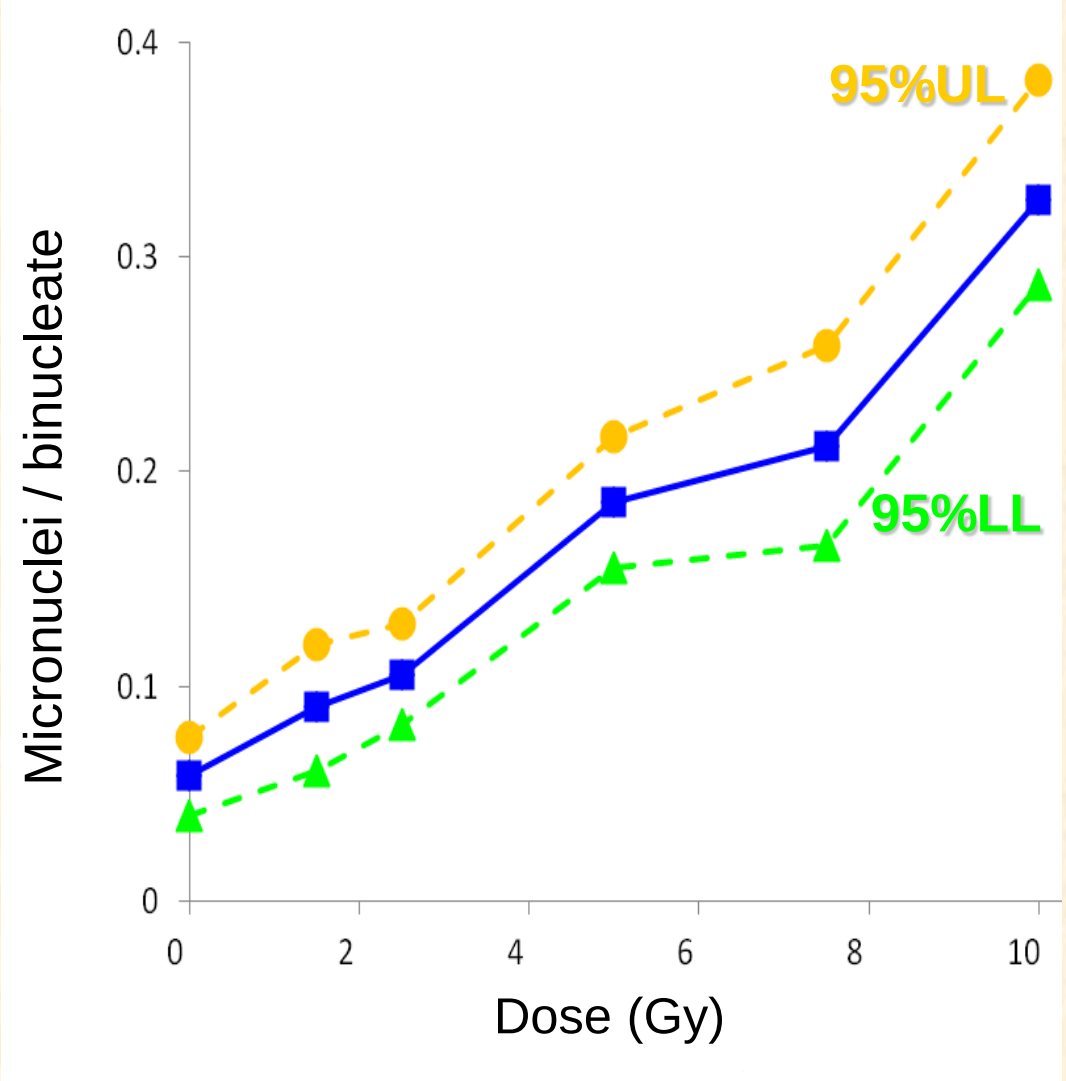
> 500 in US

tic arm for plate handling

We are taking advantage of these development to extend the RABiT approach to these commercial machines (RABiT II)

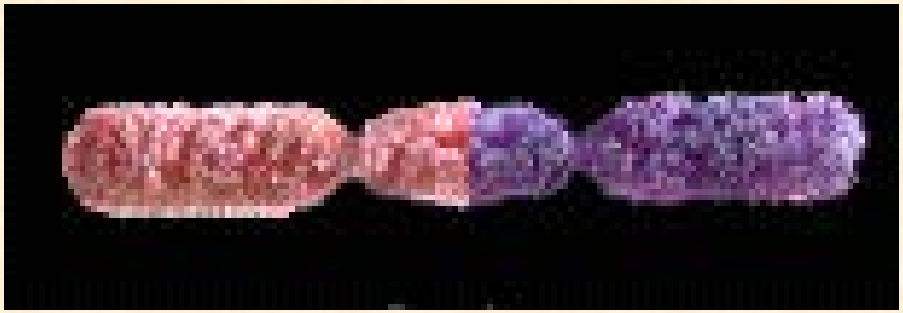


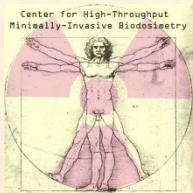
Micronuclei in ex-vivo irradiated human blood assayed with RABiT II protocol



The holy grail of high-throughput cytogenetics

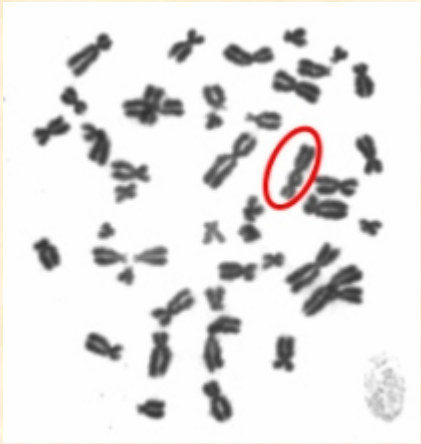
Fully Automated High-Throughput Dicentric Analysis





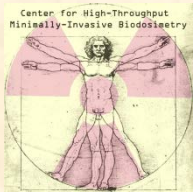
Automated High-Throughput Dicentric Analysis

People have been trying since the 1960's!



1. Make "good" metaphase spreads
2. Identify "good" metaphases

   Good = Metaphases in which all the chromosomes are well separated from each other
3. Identify dicentrics within good metaphases
4. Score dicentrics per metaphase

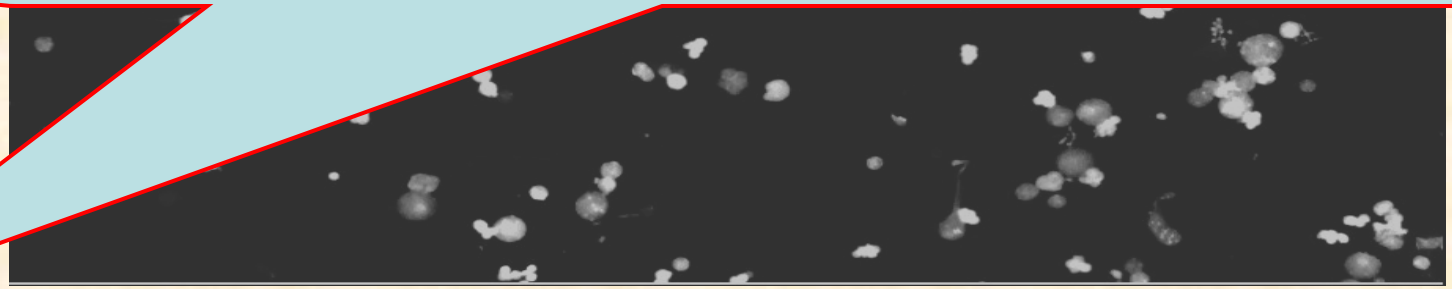


Chromosome Soup

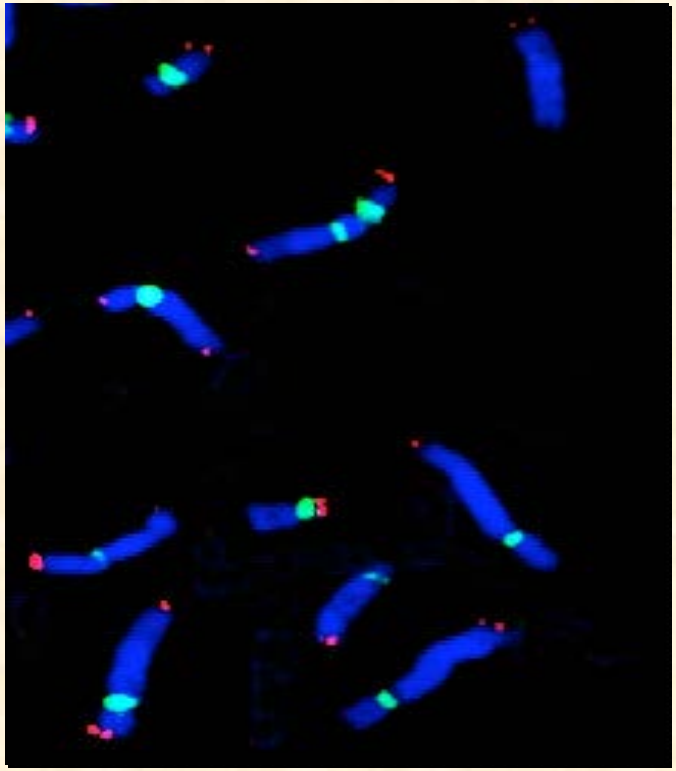
- **Don't make individual metaphase spreads**
- **Break up metaphase cells into a "chromosome soup"**

Chromosome Soup:

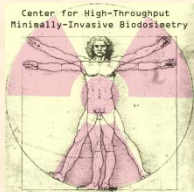
- ***Soup is a mix of chromosomes and nuclei, but easy to eliminate the nuclei with image analysis***
- ***Easy to control the separation between chromosomes, so avoids chromosome overlap***
- ***Technically easy to make soup: Automation friendly***
- ***Score dicentrics / chromosome (not dicentrics / cell)***



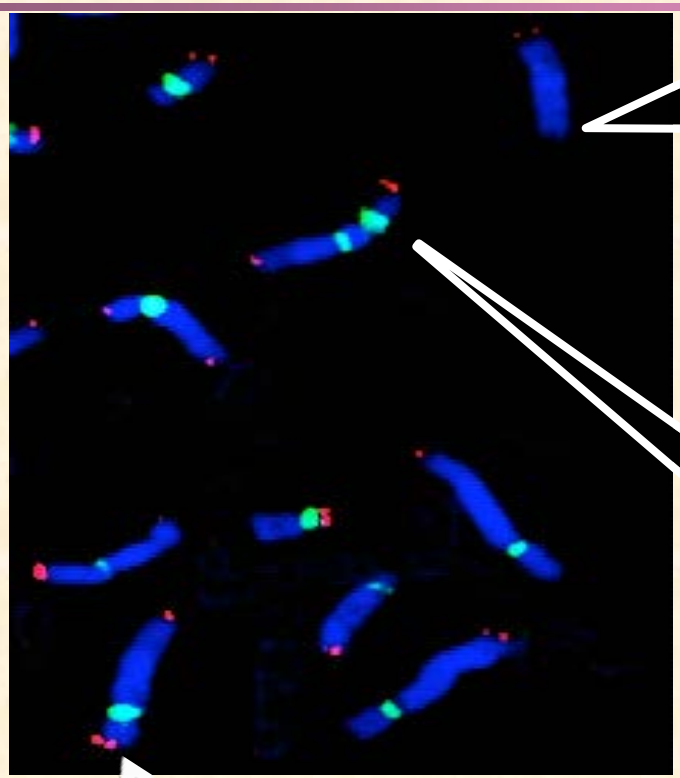
FISH Soup

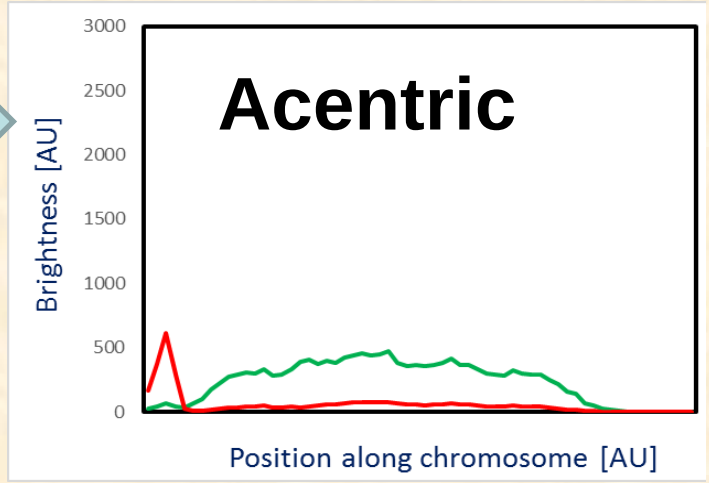


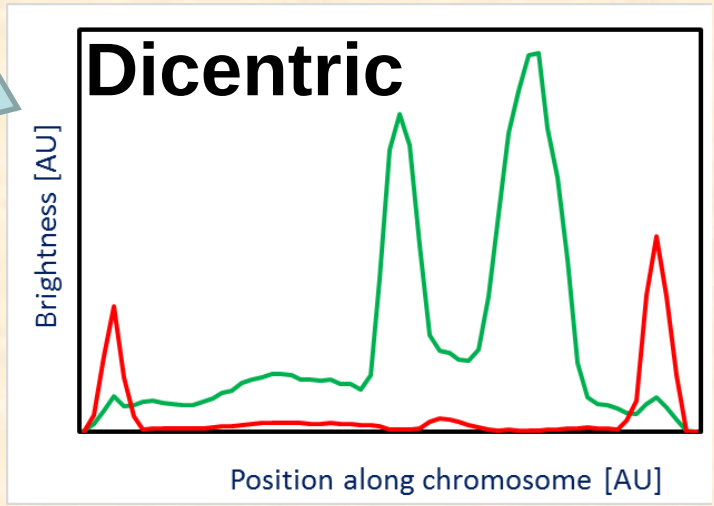
- We add centromeric and telomeric FISH stains to the Soup
- Because the chromosomes are well separated, they are amenable to rapid automated analysis

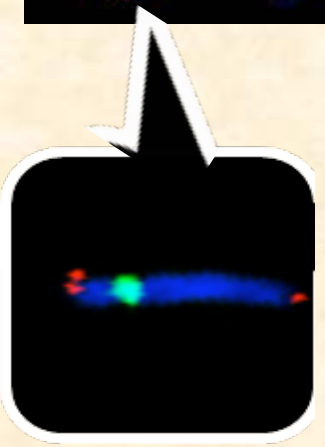


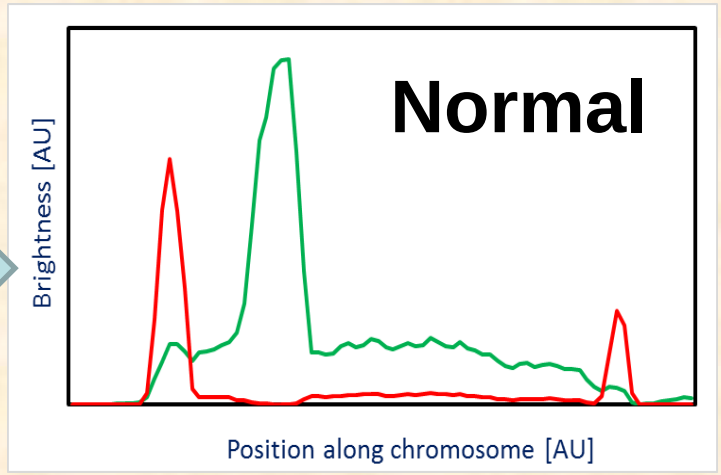
Finding dicentrics in chromosome FISH soup

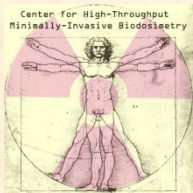








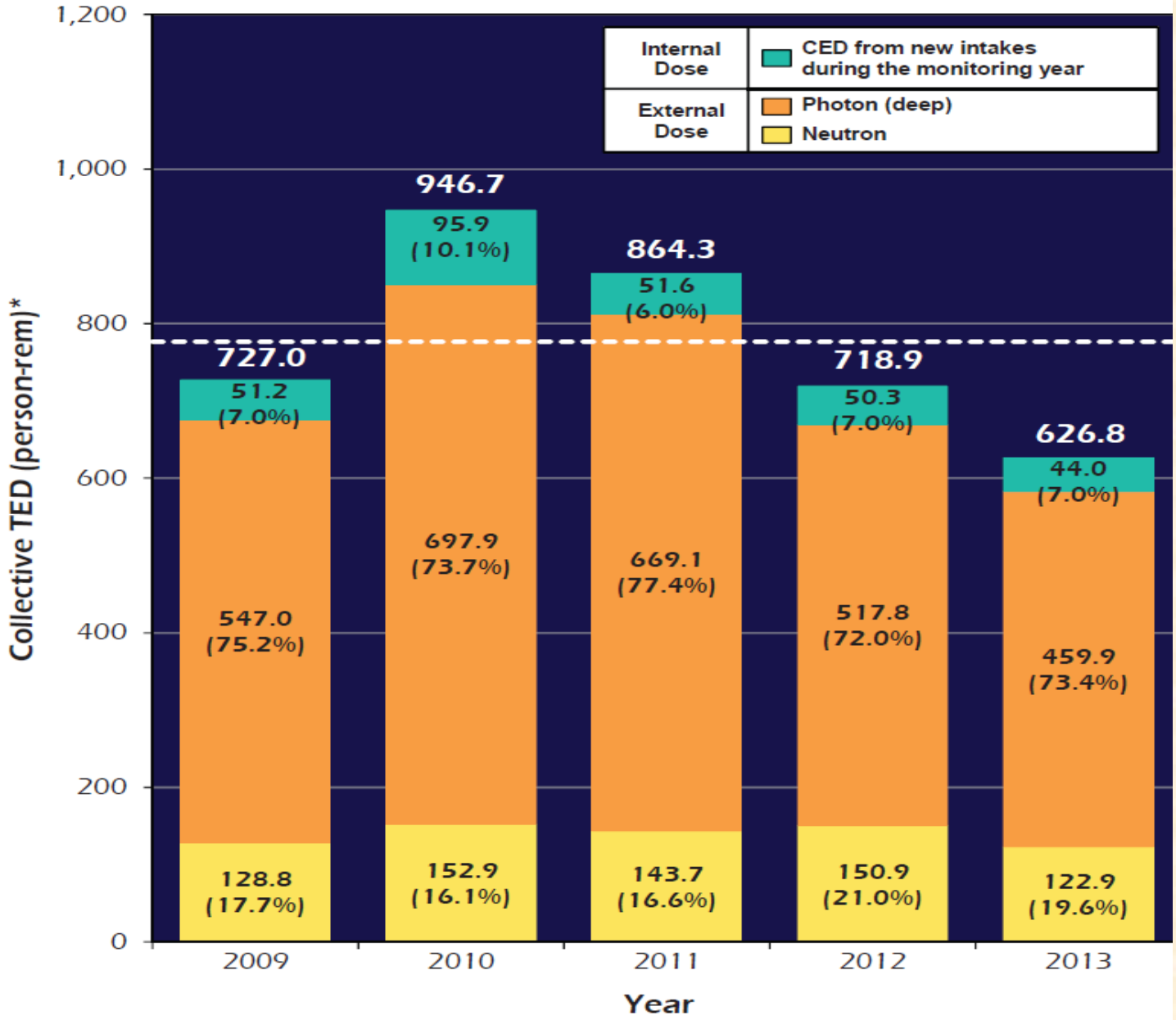




Another application of FISH: Retrospective biodosimetry in a mixed radiation environment

- What if individuals are exposed to a mixed low-LET / high-LET field?
  - X rays + neutrons (e.g. ground-burst IND)
  - Gamma rays + alpha particles (e.g. Mayak workers)
  - DOE Workers
- Can we develop a retrospective experimental biodosimetry system that distinguishes high-LET from low-LET exposure?
- Hard because in general high-LET produces the same biological endpoints as low-LET, just with higher efficiency / Gy

Collective Effective Dose to DOE Workers 2009-2013



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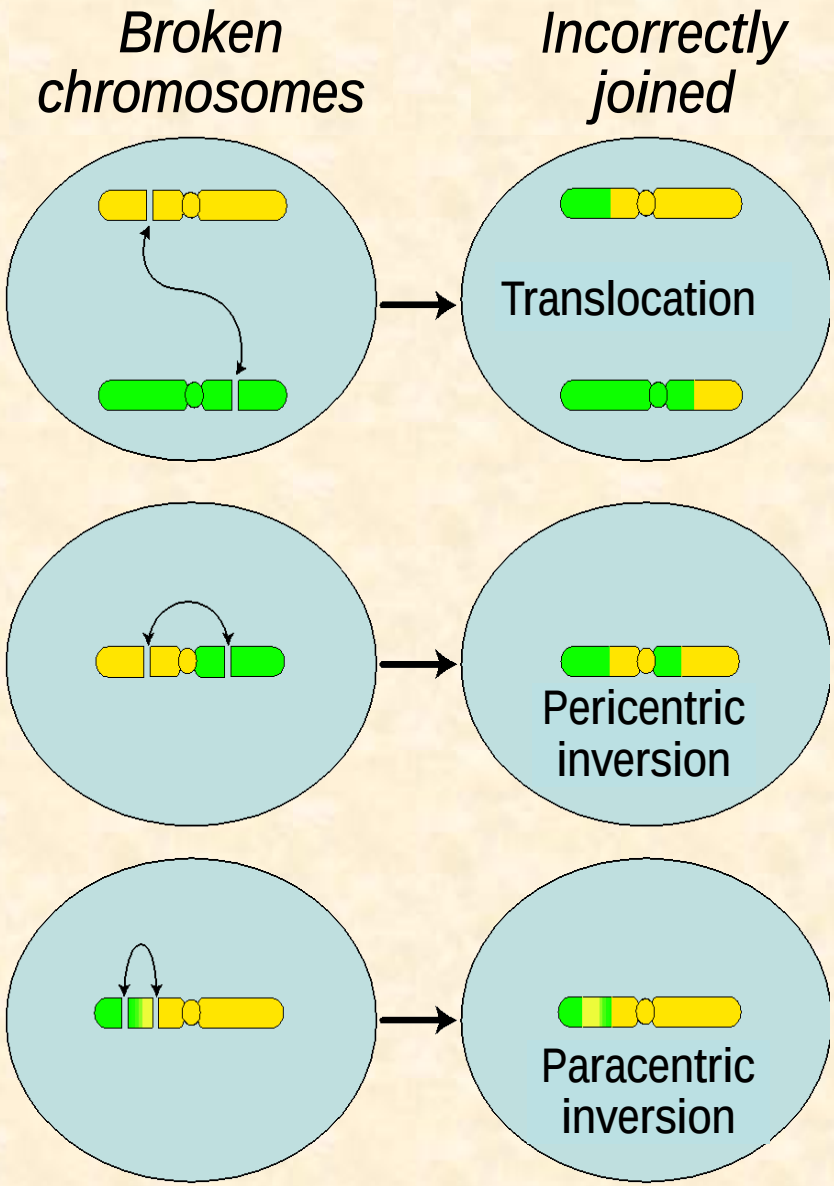
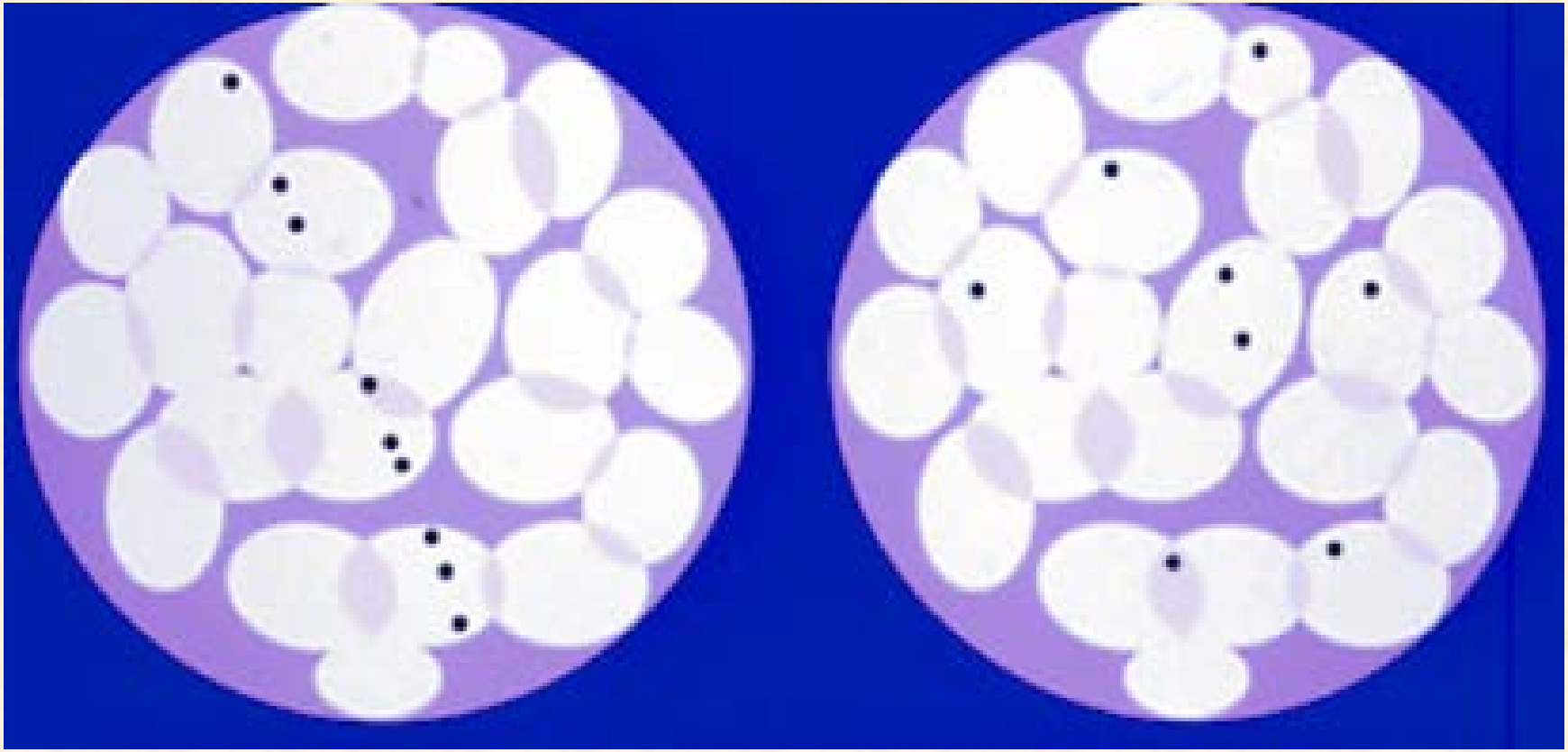
Stable exchange-type chromosomal aberrations

Intra-chromosomal aberrations (last 2 rows here) will be produced predominantly by densely-ionizing radiations, such as alpha particles or neutrons, relative to x rays



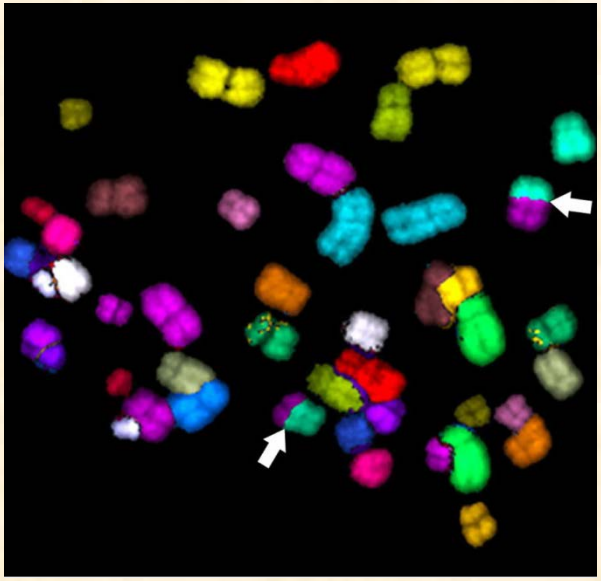
Densely-ionizing alpha particles and neutrons preferentially produce multiple chromosome breaks within single chromosomes, so there is a preference for them to produce intra-chromosomal aberrations within a single chromosome



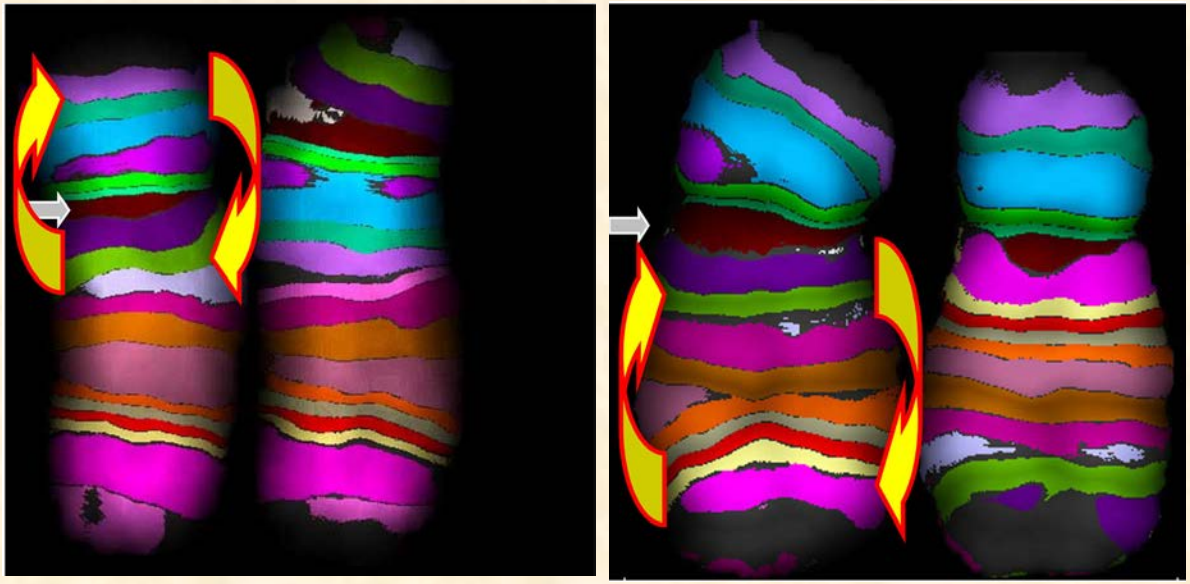
High-LET **Low-LET/Chemicals/Aging**

We can use FISH to measure inter- and intra-chromosomal aberrations in human lymphoctyes

mFISH:
Inter-chromosomal aberrations



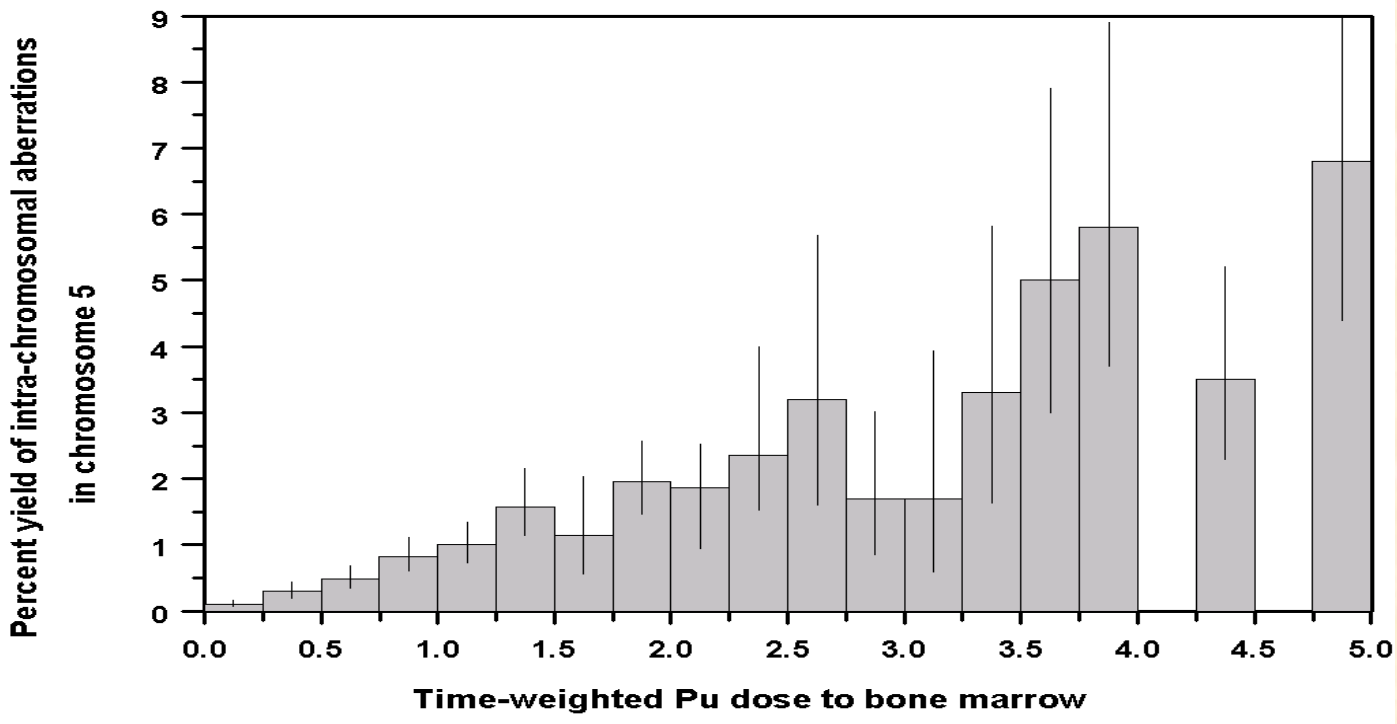
mBAND
Intra-chromosomal aberrations



Initial studies in Mayak workers

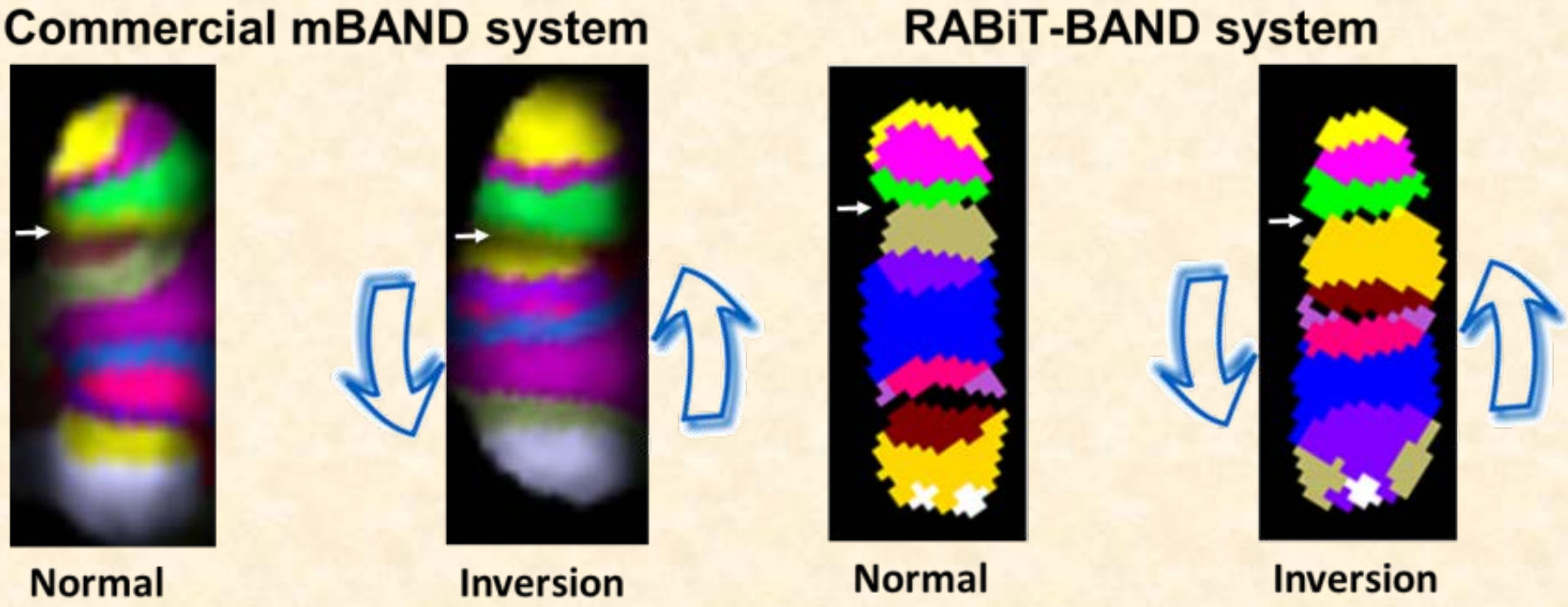
Hande *et al.*,
Am. J. Hum. Genet.
72, 1162-70 (2003)

Yield of intra-chromosomal aberrations in 350 Mayak workers, vs. Pu doses estimated with urine analysis



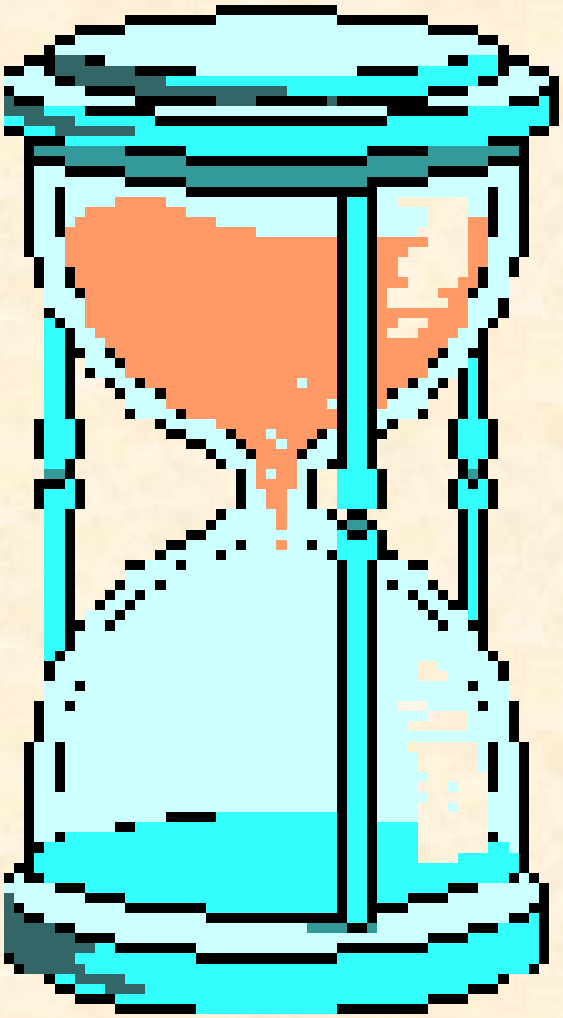
- Intra-chromosomal aberrations well correlated with estimated Pu doses
- very low in individuals only exposed to gamma rays
- very low in control individuals

RABiT-BAND: We have adapted the mBAND system for the high-throughput RABiT



RABiT-BAND:
another application of FISH Soup

My time is up!

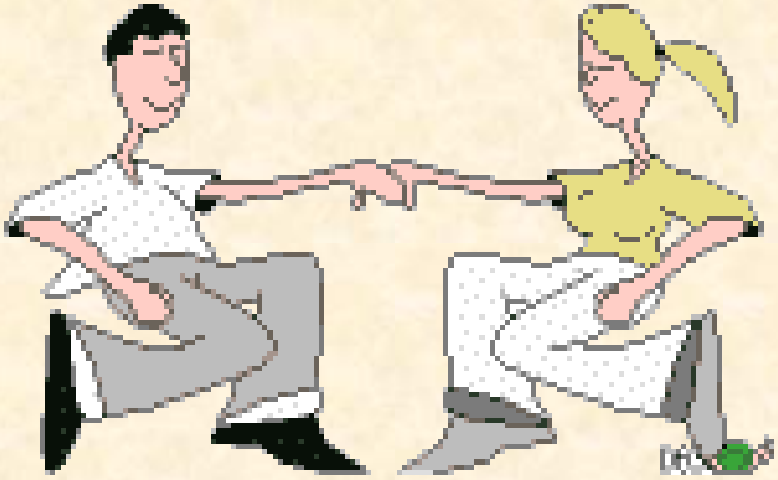


My Take-Home Message

Interactions between
radiation epidemiologists and radiation biologists
are going to become increasingly important,
as our field focuses more and more
on the effects of low radiation doses

NCI 2015

Radiation epidemiology



Radiation biology

In fond memory of Elaine Ron

Questions and Answers?

U.S. Department of Health and Human Services
National Institutes of Health | National Cancer Institute
www.dceg.cancer.gov/RadEpiCourse
1-800-4-CANCER

Produced May 2015