

# **EPR-dosimetry, harmonization of techniques**

## ***Biological dosimetry in Atomic bomb survivors***

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# Biological dosimetry in A-bomb survivors

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- **Chromosome study**
  - **Conventional Giemsa staining study (1968-1993)**
  - **Fluorescence in situ hybridization (FISH) study (1994- )**
- **Electron paramagnetic resonance (EPR) study (1992- )**

**Purpose:** to provide information to confirm or improve our current estimates of individual doses, which are used to evaluate both cancer and non-cancer risks of A-bomb radiation exposures.



## Conventional Giemsa staining

- Most basic staining method
- Homogeneous staining  
Appropriate for the analysis of number and shape of the chromosome



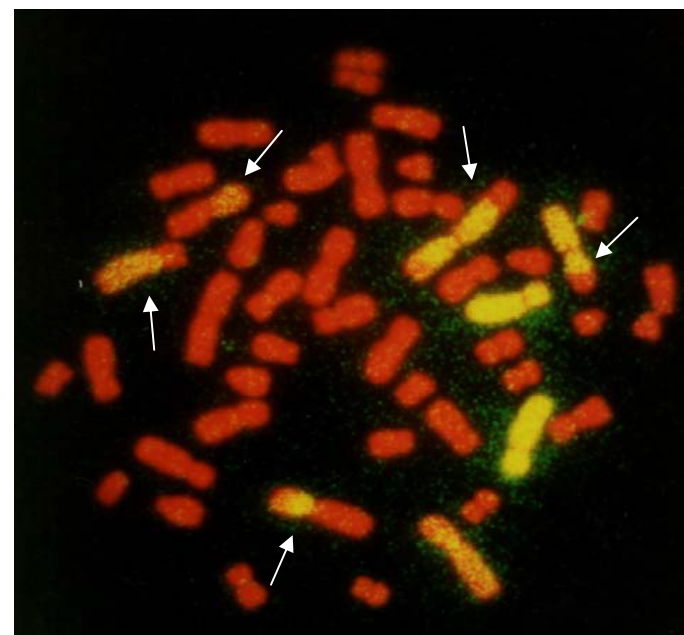
Biodosimetric study  
(detection of **dic** chromosome)

## FISH technique

- Chromosome specific DNA probes
- Quick and accurate detection of translocations



Retrospective biodosimetry  
(detection of **t** chromosome)



t(1), t(1), t(2)

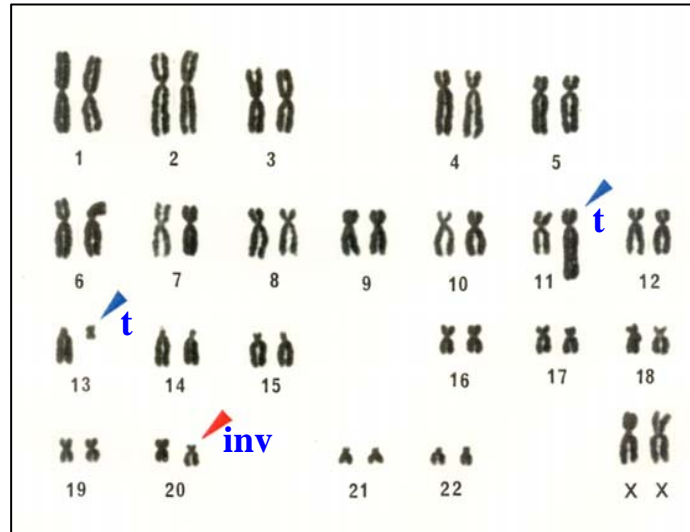
Exchanges		Remarks
Intra-chromosomal	Inter-chromosomal	
<p>Ring+fragment ( r + ace)</p> <p>Pericentric inversion (inv)</p>	<p>Dicentric+fragment ( dic + ace)</p> <p>Reciprocal translocation ( t)</p>	<p><b><u>Unstable type</u></b></p> <p>Easy to detect but <b>unstable</b> Over time</p> <hr/> <p><b><u>Stable type</u></b></p> <p>Difficult to detect but <b>stable</b> over time</p>

- Chromosome study of A-bomb survivors had initiated at 1968.
- Most of **unstable type** aberrations disappeared from the lymphocytes of survivors and only **stable type** aberrations remained.

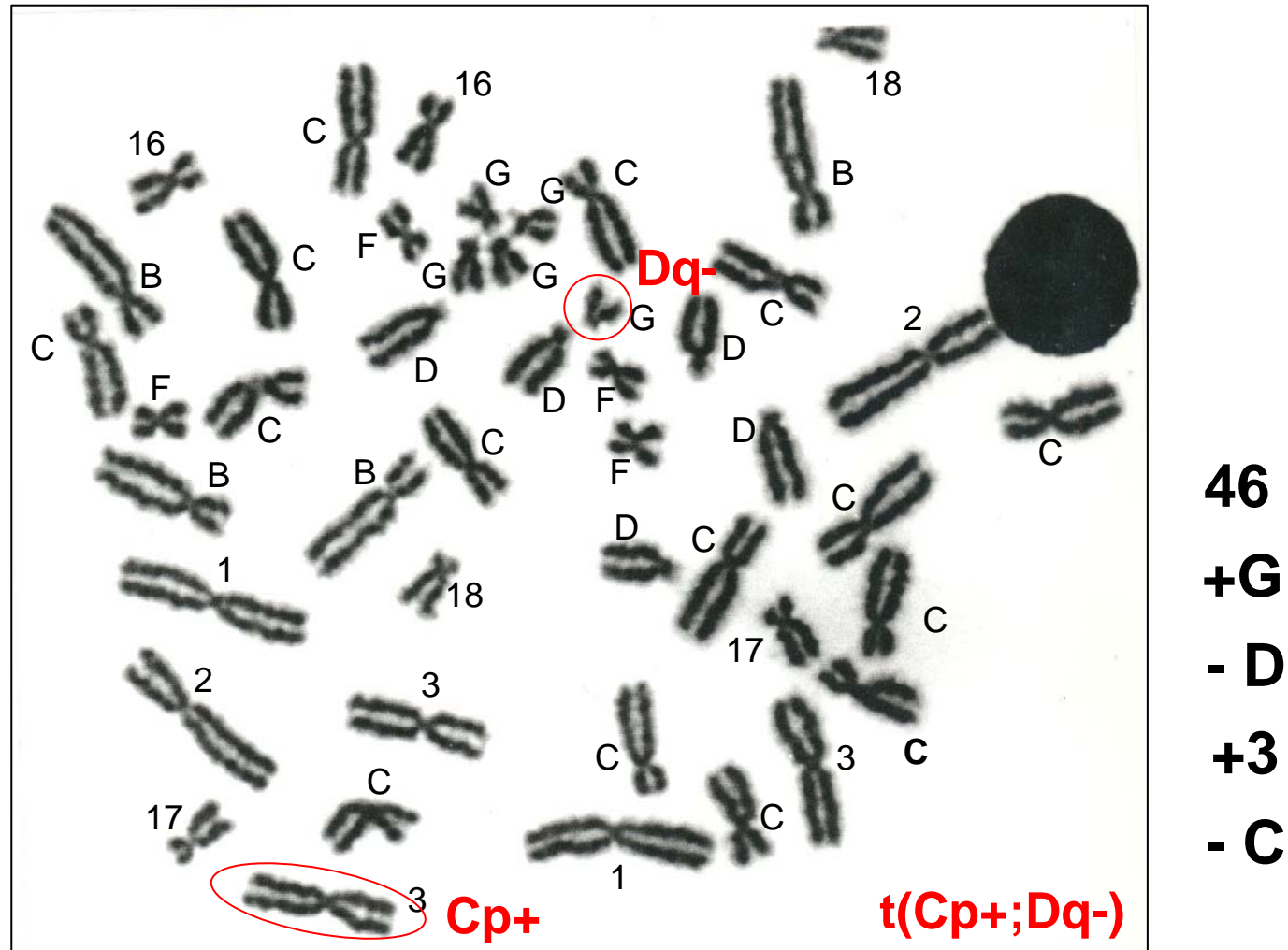
Unstable type aberrations (dic, r)



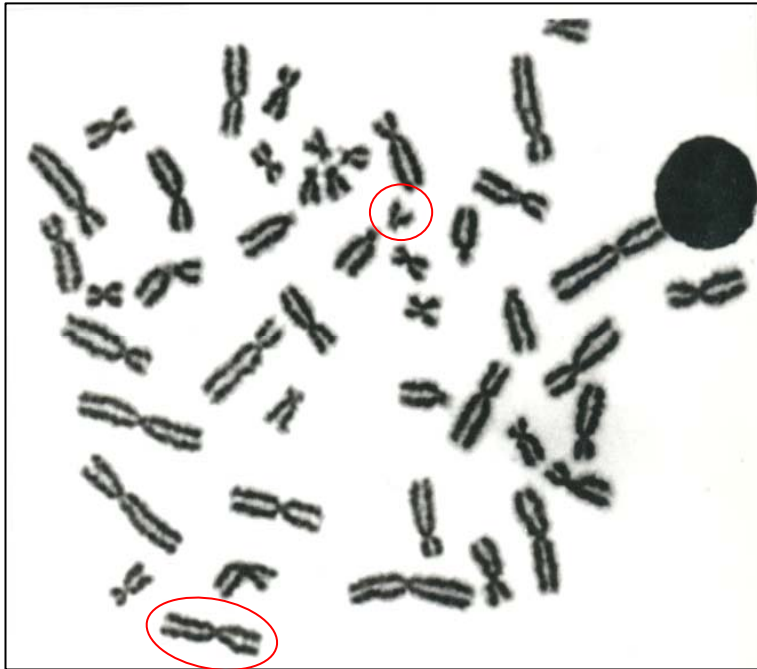
Stable type aberrations (t, inv)



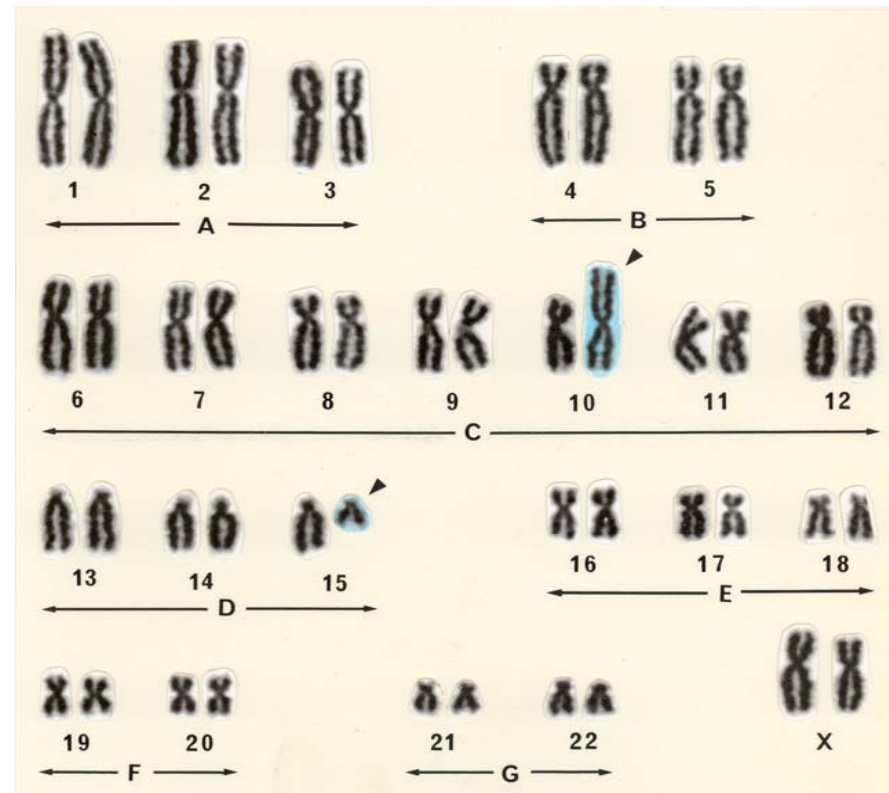
# How to detect stable chromosome aberrations by conventional Giemsa staining method under microscope



# How to detect stable chromosome aberrations by conventional Giemsa staining method under microscope



t(Cp+;Dq-)



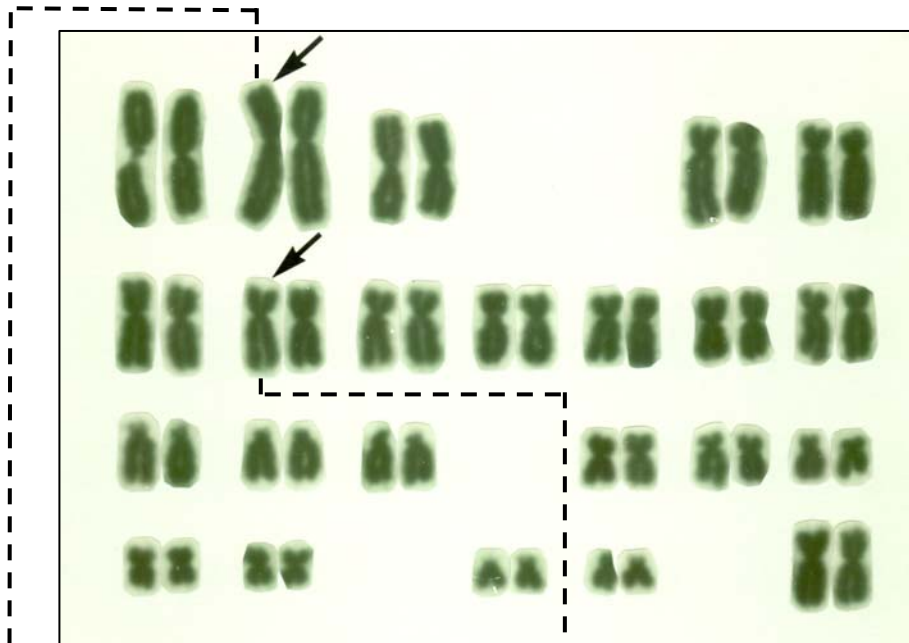
\* <http://www.rerf.or.jp/Gene/eng/giemsa.htm>

“It has already been stressed that Cs cells can only be detected with very low efficiency” *UNSCEAR Report (1969)*

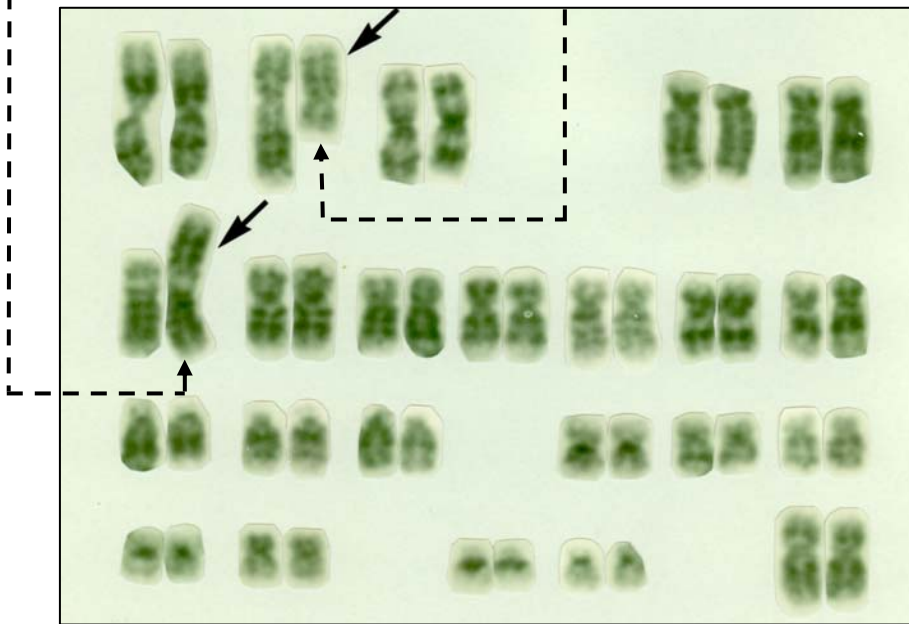
“Obvious symmetrical interchange can be recorded but the analysis is time consuming and it not recommended.”

“Reciprocal translocations are particularly difficult to observe in conventionally stained preparations..... There is somewhat increase in resolution when banded.... but even then the efficiency .... is around 50%....” *IAEA Technical Report No. 260 (1986)*





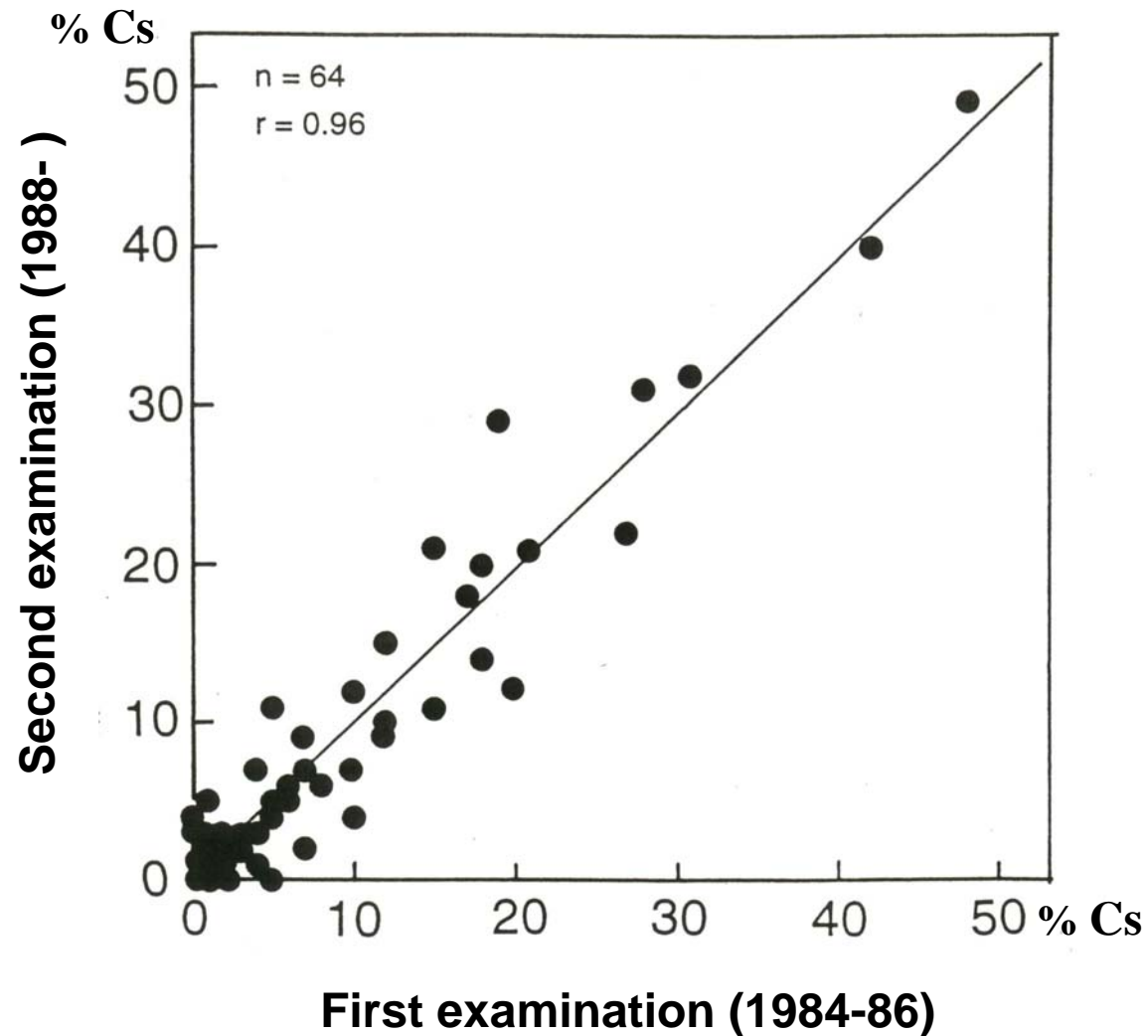
Conventional method;  
46,XX, **Normal**



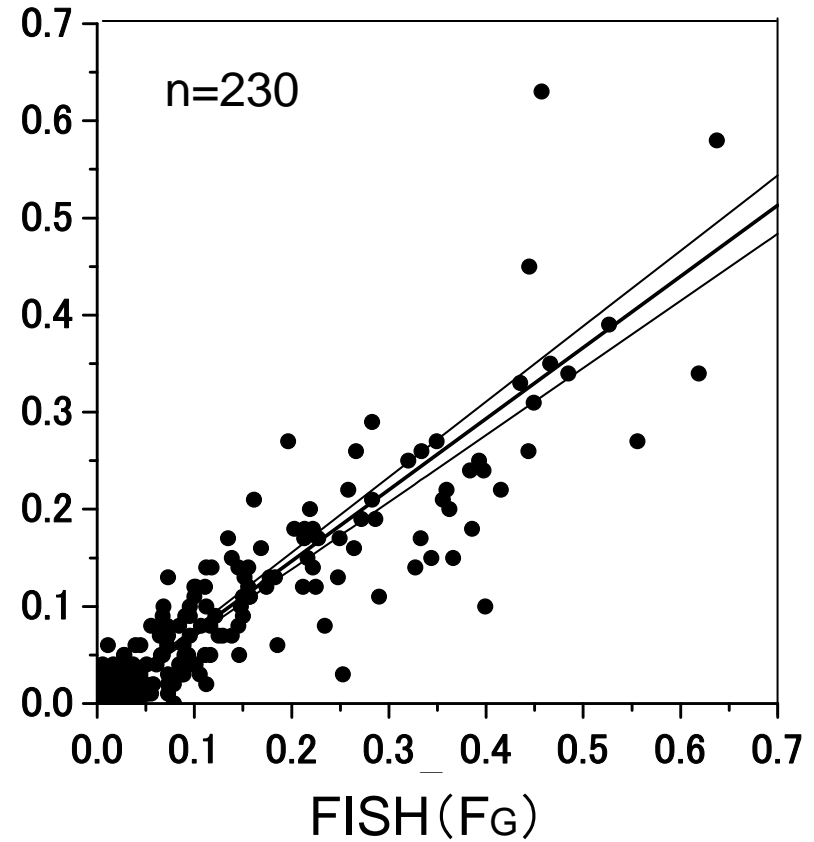
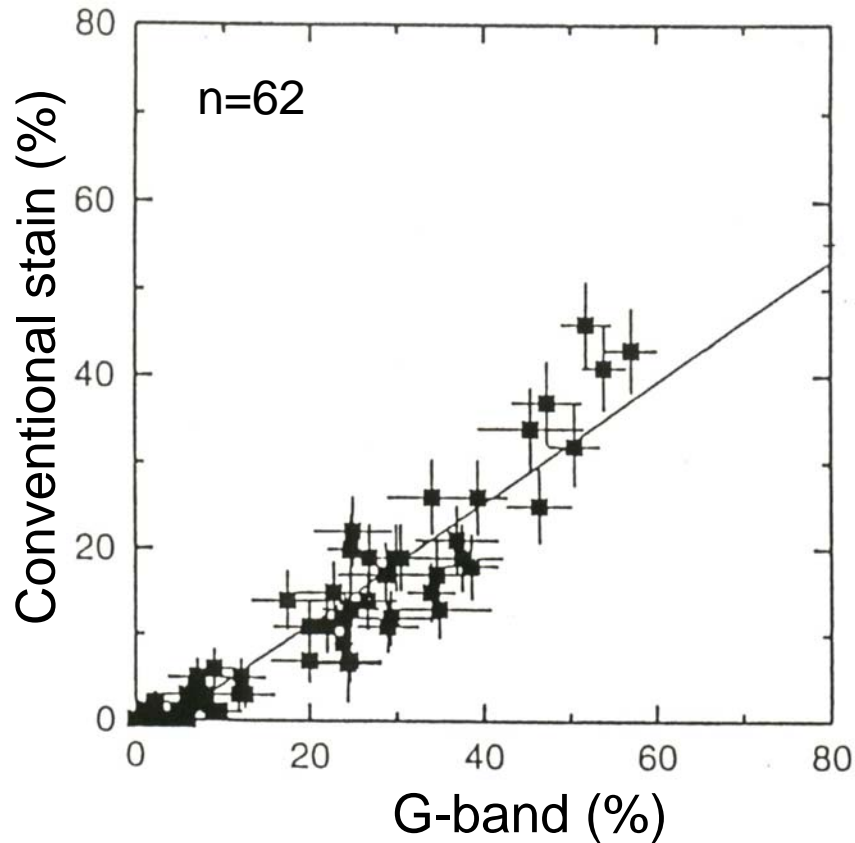
G-banding method;  
46,XX, **t(2q-;6p+)**

# Reproducibility of conventional chromosome analysis for stable-type aberrations (Cs cells)

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# Comparison of translocation frequencies

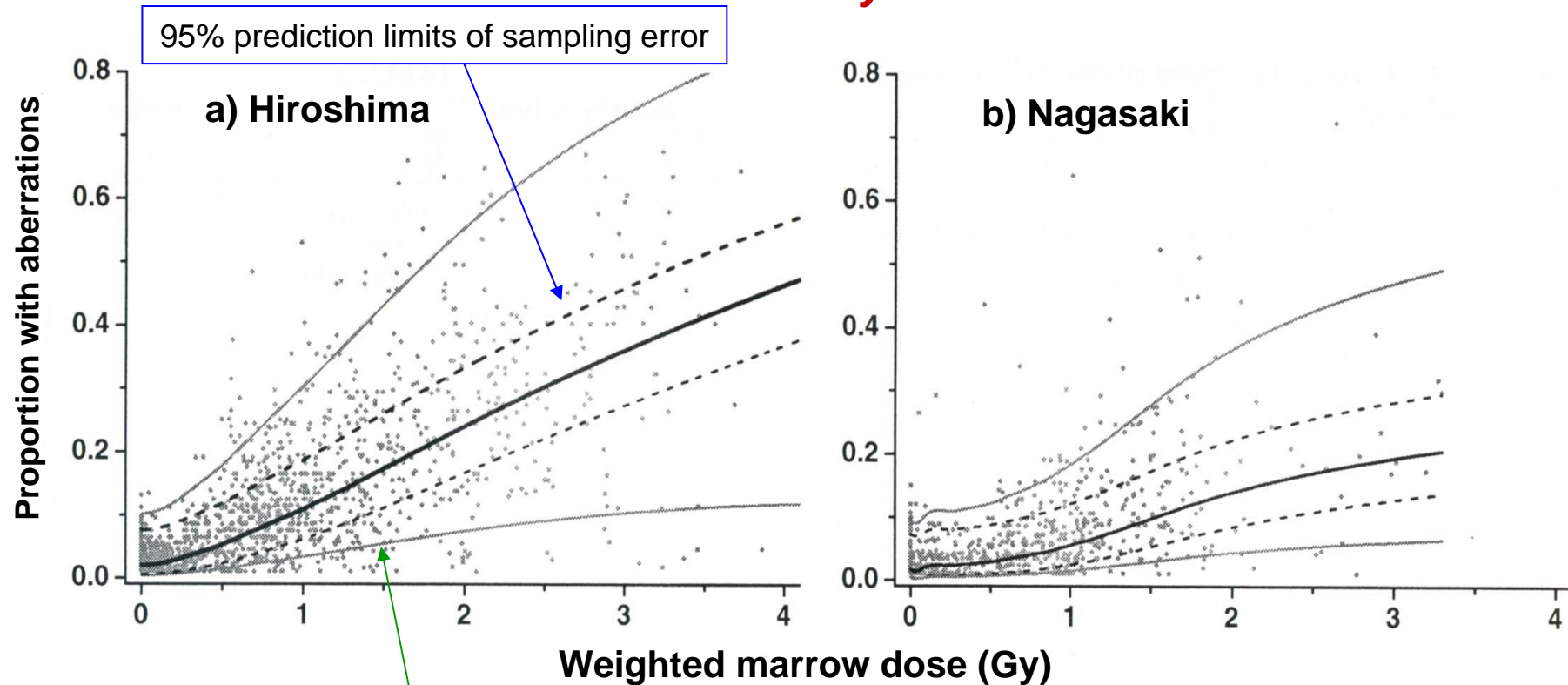


Conventional Giemsa staining method  
can detect about 70% of translocations

Nakano et al, Int J Radiat Biol, 2001

# Chromosome study by Giemsa staining

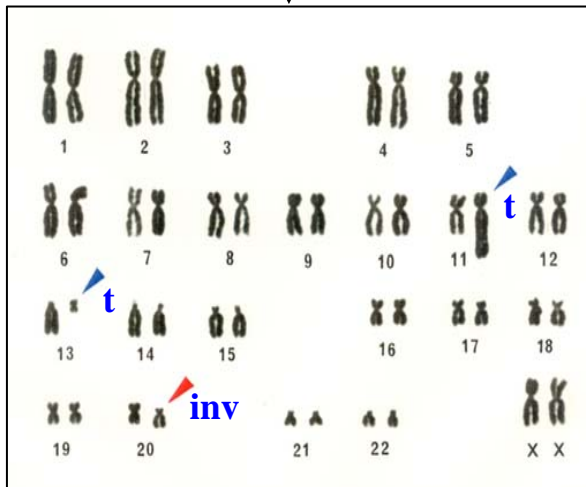
- Why the distribution is so wide?
- City difference is real?



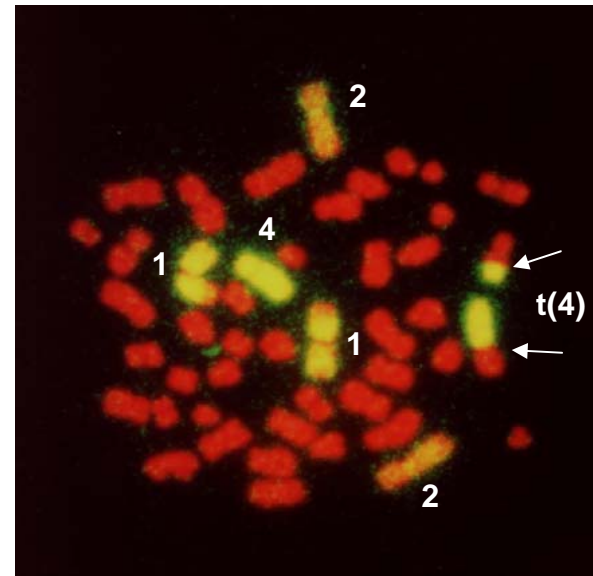
Kodama et al, *Radiat Res* 2001

# Cytogenetic techniques

## Translocation analysis by Giemsa staining method (1968-1993)



## FISH can detect translocation rapidly and objectively (1994-)



Painted chromosomes: #1, #2, #4

No of cells scored: 500\*

**\*All measurements were done in Hiroshima laboratory.**

## Rapid translocation frequency analysis in humans decades after exposure to ionizing radiation

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(Received 22 October 1991 ; revised 27 January 1992 ; accepted 2 February 1992)

**Abstract.** This paper presents an analysis of the utility of fluorescence *in situ* hybridization (FISH) with whole chromosome probes for measurement of the genomic frequency of translocations found in the peripheral blood of individuals exposed to ionizing radiation. First, we derive the equation:  $F_p = 2.05fp(1-fp)F_G$ , relating the translocation frequency,  $F_p$ , measured using FISH to the genomic translocation frequency,  $F_G$ , where  $fp$ , is the fraction of the genome covered by the composite probe. We demonstrate the validity of this equation by showing that: (a) translocation detection efficiency predicted by the equation is consistent with experimental data as  $fp$  is changed; (b) translocation frequency dose-response curves measured *in vitro* using FISH agree well with dicentric frequency dose-response curves measured *in vitro* using conventional cytogenetic procedures; and (c) the genomic translocation frequencies estimated from FISH measurements for 20 Hiroshima A bomb survivors .....

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<sup>1</sup>Lawrence Livermore National Laboratory, <sup>2</sup>Radiation Effect Research Foundation,

<sup>3</sup>University of California, San Francisco, <sup>4</sup>Oak Ridge Associated Universities

# Estimation of translocation frequency by FISH (1)

$$F_G = F_P / 2.05 f_p (1 - f_p)$$

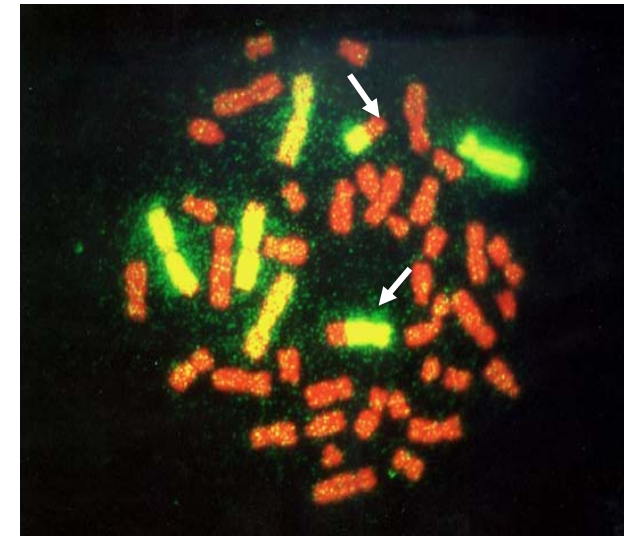
$F_G$ : full genome aberration frequency

$F_P$ : translocation frequency measured by FISH

$f_p$ : fraction of genome painted

2.05: coefficient excluding exchanges within the same chromosome

(Lucas et al, IJRB 62:53-63, 1992)



Chromosomes 1+2+4

Chromosomes 1+2+4 = 22%  $\longrightarrow$   $F_G = 2.81 \times F_P$  (female)

$2.77 \times F_P$  (male)

500 cells = 178 cell equivalent

*\*Cytogenetic dosimetry: Applications in preparedness for and response to radiation emergencies, pp87-89, IAEA, 2011*



# Estimation of translocation frequency by FISH (2)

## 3 color FISH

#1:red、 #2:green、 #4:white (DAPI:blue)

$$F_G = F_P(1+2+4) / 2.05 [f_1(1-f_1)+f_2(1-f_2)+f_4(1-f_4) - (f_1f_2+f_1f_4+f_2f_4)]$$

(Lucas et al, Cytogenet Cell Genet 62:11-12, 1993)

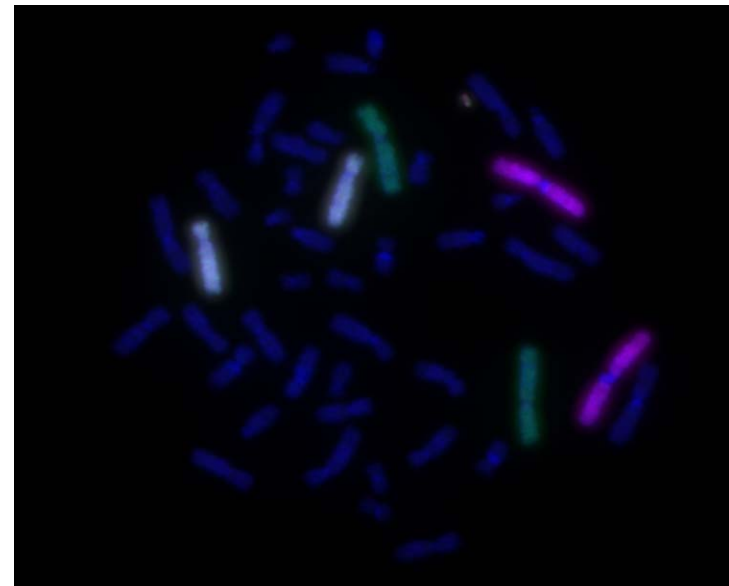
(IAEA manual 2011, Cytogenetic Dosimetry, pp 87-)

$$F_G = F_P \times 2.567 \text{ (female, } f_p=0.2234^*)$$

$$F_G = F_P \times 2.533 \text{ (male, } f_p=0.2271^*)$$

(\*IAEA manual 2011, Table 2)

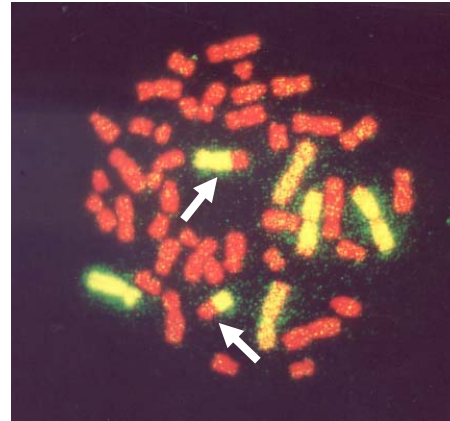
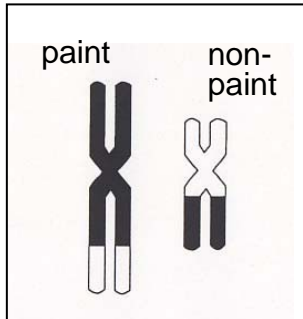
500 cells = 196 cell equivalent





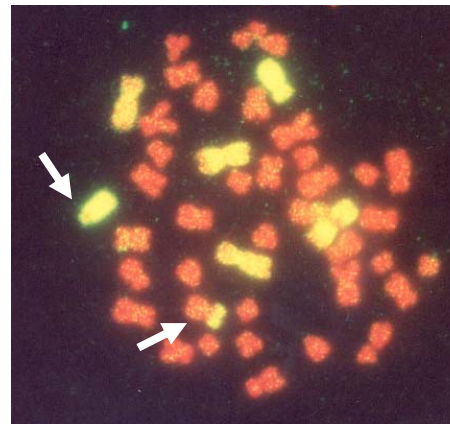
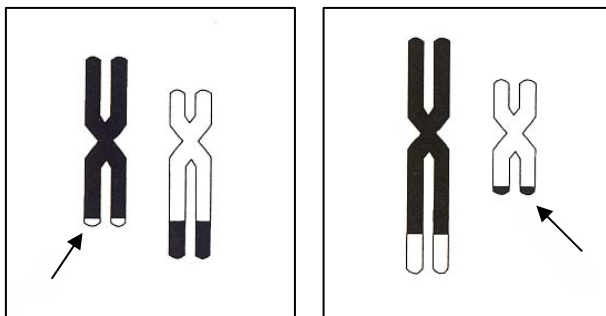
# Type of translocations by FISH

Two way

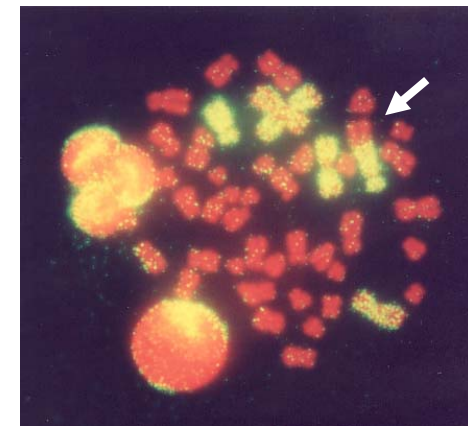


Type 1 (70%~)

One way



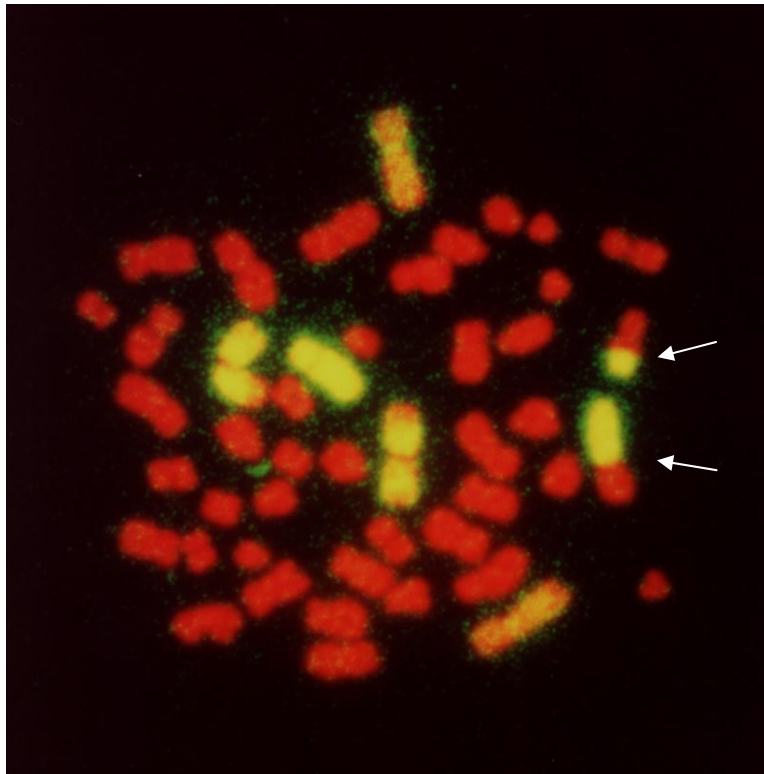
Type 2 (20%~)



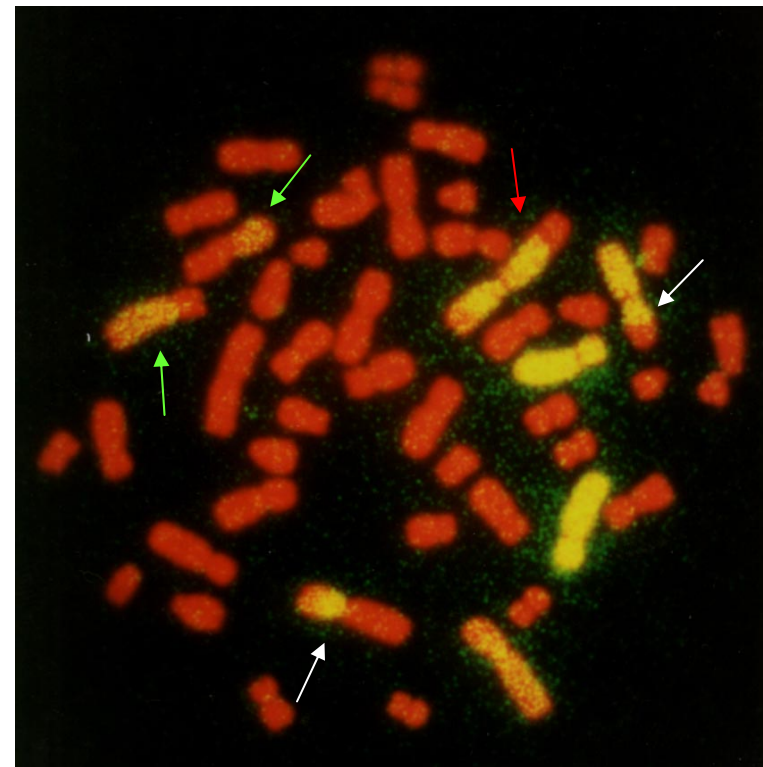
Type 3 (~5%)

Translocated segment  
is too small to detect

# Detection of translocations by FISH



t(4p+;Cq+) : Type 1



t(1p-;Bp+): Type 1  
t(1q+): Type 3  
t(2p-;Cp+): Type 1

or

Number of color  
Junctions= 5

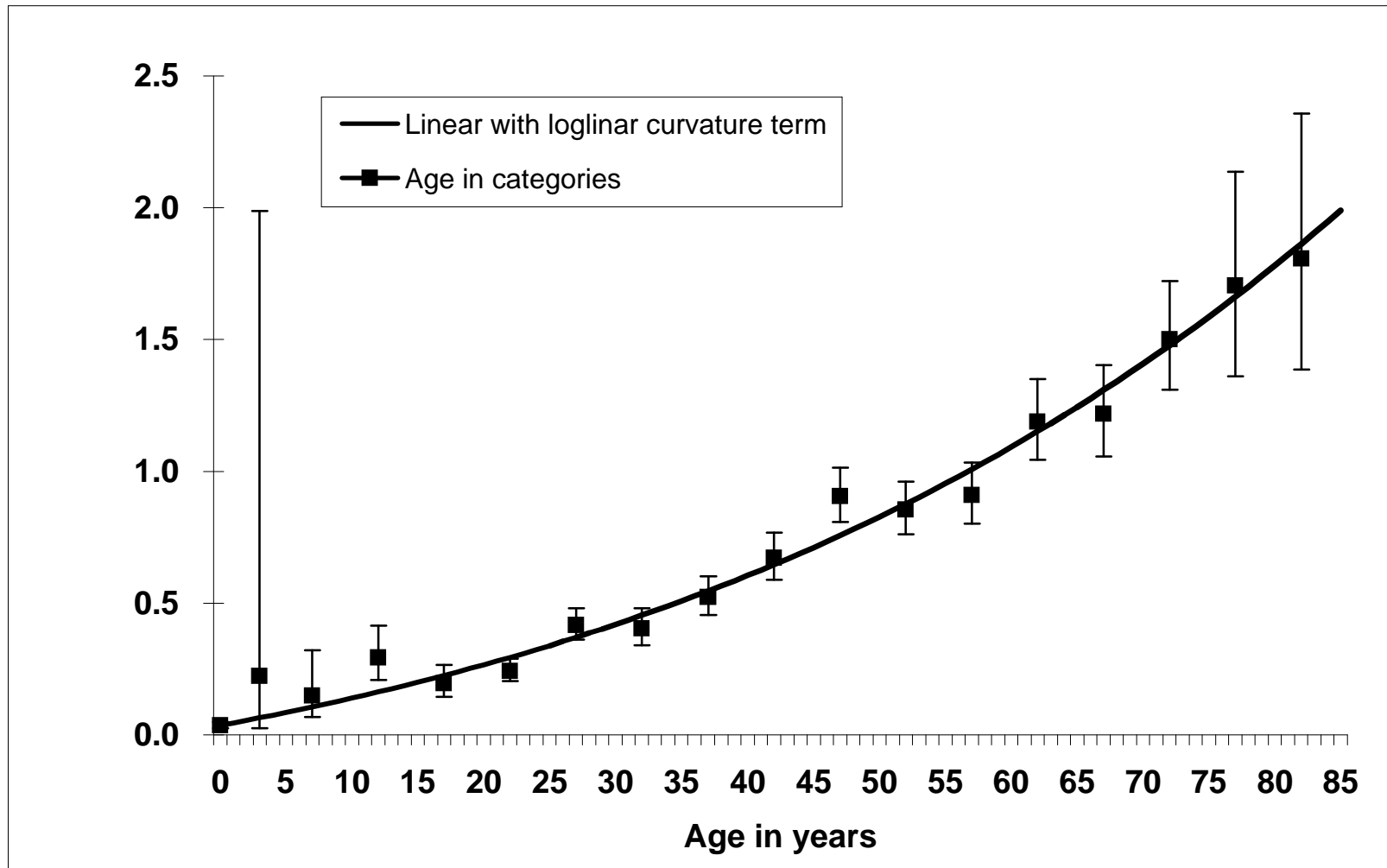


3 translocations\*

\**PAINT* system  
(Tucker et al, Cytogenet Cell Genet 68:211-221,1995)

# Translocation frequencies in control population by FISH

Sigurdson et al, Mutat Res, 652:112-121, 2008, Fig. 2.



# Translocation frequencies in control population by FISH

Sigurdson et al, Mutat Res, 652:112-121, 2008, Fig. 4.

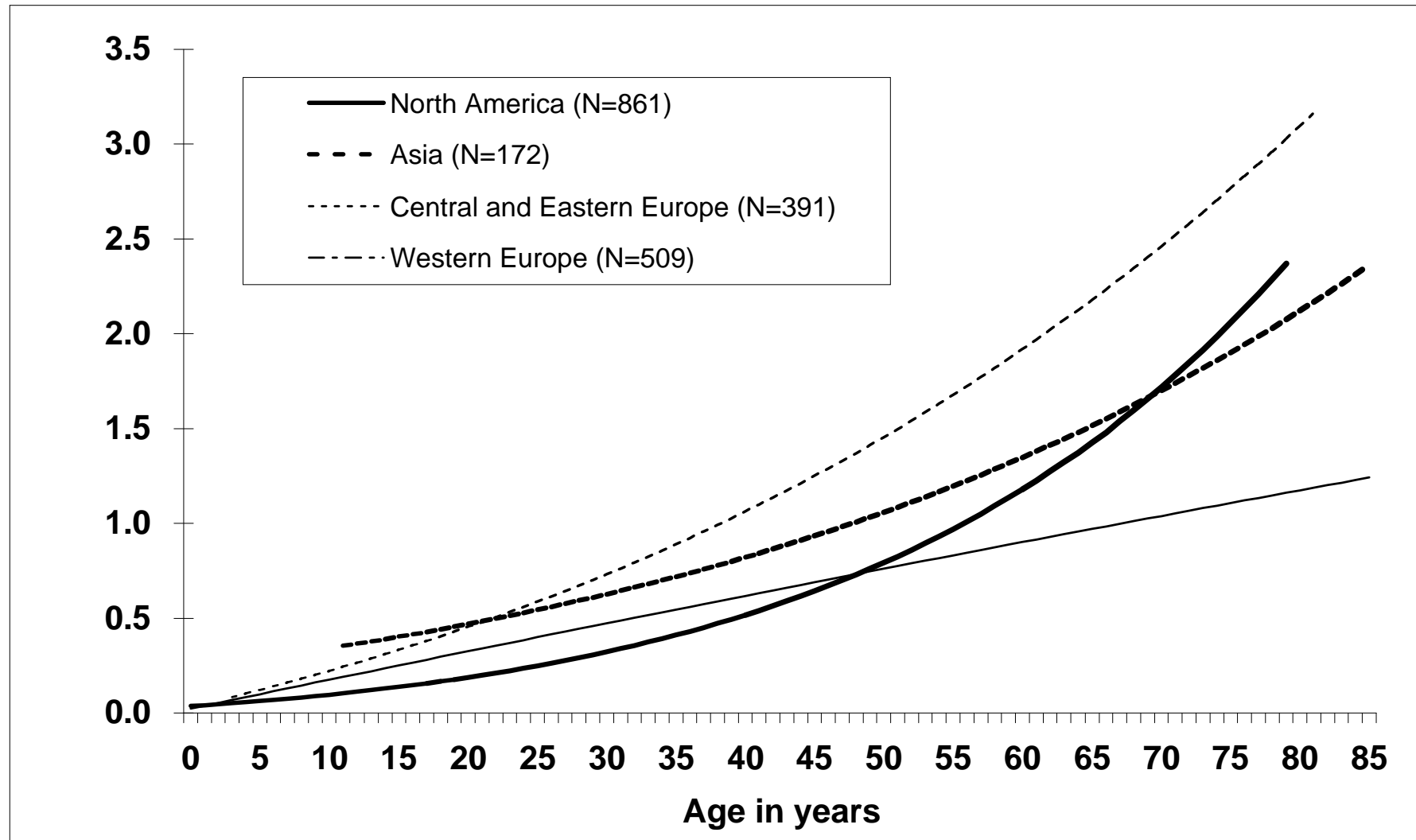
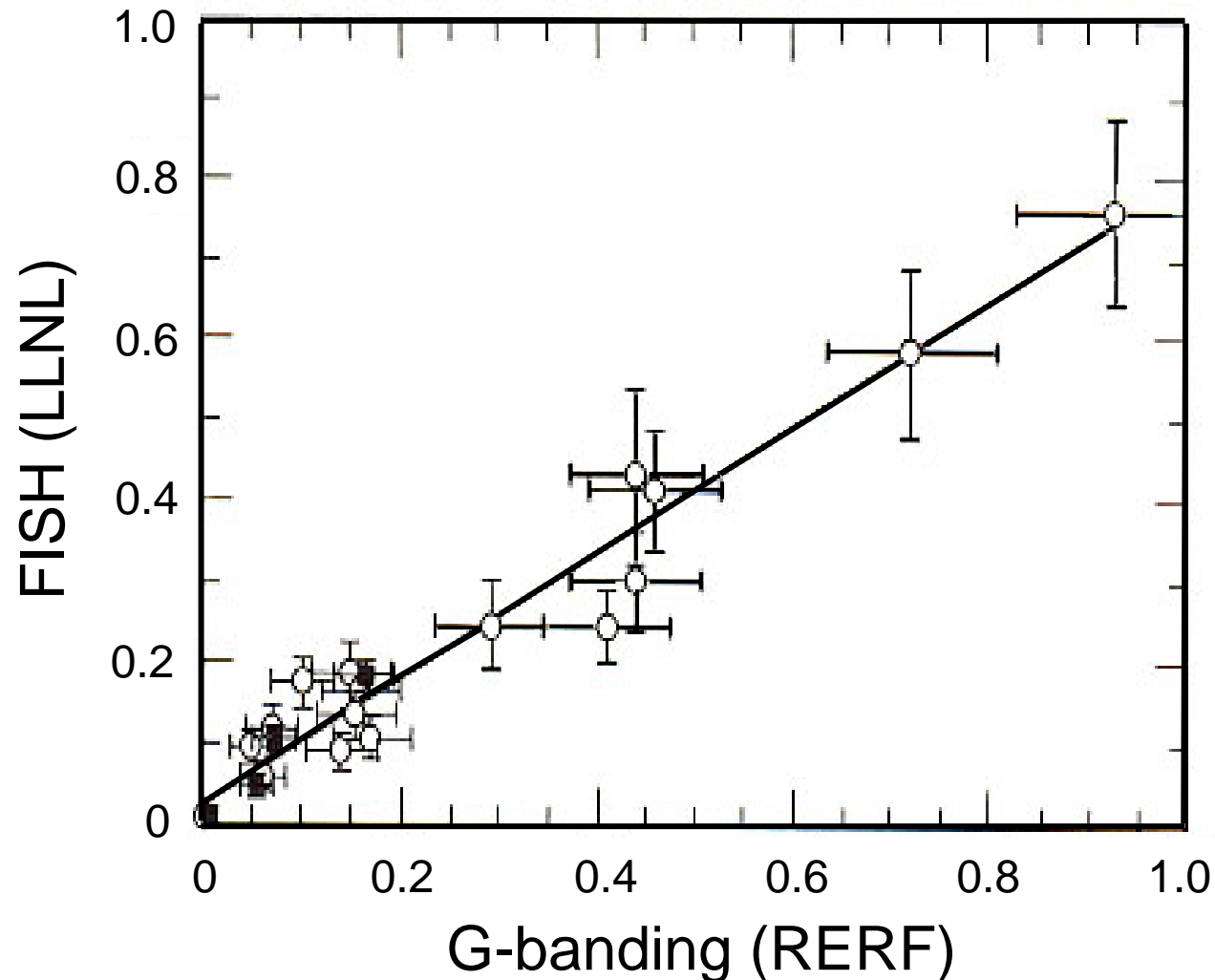


Table 1a. Translocation frequencies in A-bomb survivors measured by FISH for chromosomes 1, 2 and 4 and by G-banding (Lucas et al., 1992)

Subjects	Dose* (Sv)	FISH (LLNL)			G-banding (RERF)		
		Cells	Translocations total(#1,#2,#4)	Trans/cell (genomic)	Cells	Translocations	Trans/cell
H6549	2.22	490	31 (10,7,14)	0.179	100	10	0.10
H6550	0	2027	5 (4,0,1)	0.007	100	0	0.00
H6551	1.63	824	49 (20,14,15)	0.168	100	16	0.16
H6770	0.62	455	16 (8,2,6)	0.099	100	5	0.05
H6579	1.06	284	19 (4,6,9)	0.189	100	15	0.15
H6580	2.61	178	19 (4,8,7)	0.302	100	37	0.37
H6584	0.66	687	15 (6,4,5)	0.062	100	6	0.06
H6585	1.32	343	17 (7,6,4)	0.140	97	15	0.15
H6615	2.31	141	29 (11,8,10)	0.582	100	72	0.72
H6616	1.94	743	16 (6,7,3)	0.061	100	6	0.06
H6617	4.82	216	23 (8,10,5)	0.301	100	44	0.44
H6688	1.46	375	16 (4,9,3)	0.121	100	7	0.07
H6690	1.86	146	39 (14,11,14)	0.756	97	90	0.93
H6708	0	813	4 (2,1,1)	0.014	100	1	0.01
H6718	2.22	178	26 (6,12,8)	0.413	100	46	0.46
H6722	1.84	205	18 (9,4,5)	0.248	100	29	0.29
H6723	0.96	416	16 (9,1,6)	0.109	100	17	0.17
H6725	1.42	378	33 (10,13,10)	0.247	100	41	0.41
H6728	0.86	530	14 (8,4,2)	0.075	100	14	0.14
H6731	1.30	105	16 (10,3,3)	0.431	100	44	0.44

\*Dose to bone marrow assuming DS86 dosimetry and a neutron RBE of 10.

# Comparison of genomic translocation frequencies



FISH results fit well with G-banding results

# Purpose of FISH study in RERF

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- To examine the difference in translocation dose responses between Hiroshima and Nagasaki survivors under different shielding categories

# Summary

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- Highly significant and nonlinear dose responses were observed in both Hiroshima and Nagasaki.
- A wide scatter of individual translocation frequencies against physical dose was observed as seen in the previous Giemsa staining study. This suggests the dose errors in DS02 dose estimates in some survivors.
- Difference between Hiroshima and Nagasaki was much reduced suggesting the large city difference in the past study was mainly due to different aberration detection rates between Hiroshima and Nagasaki laboratories.
- Both people exposed outside but shielded by houses and Nagasaki factory workers had significantly lower dose responses than people who were exposed inside Japanese houses.



# Why physical dose does not fit well with chromosome data?

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- ◆ Observer biases in chromosome study?
- ◆ Different radiation sensitivity?
- ◆ Dosimetry errors?
- ◆ Errors in interview records?



Estimate the radiation dose by a method totally independent from cytogenetic measurement.

# Biological dosimetry

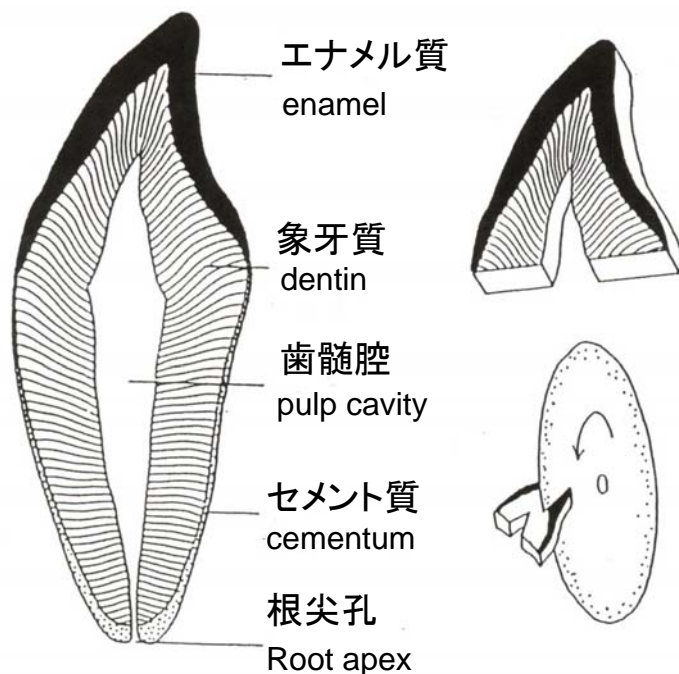
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- **Chromosome study**
  - **Conventional Giemsa staining study (1968-1993)**
  - **Fluorescence in situ hybridization (FISH) study (1994- )**
- **Electron paramagnetic resonance (EPR) study (1992- )**

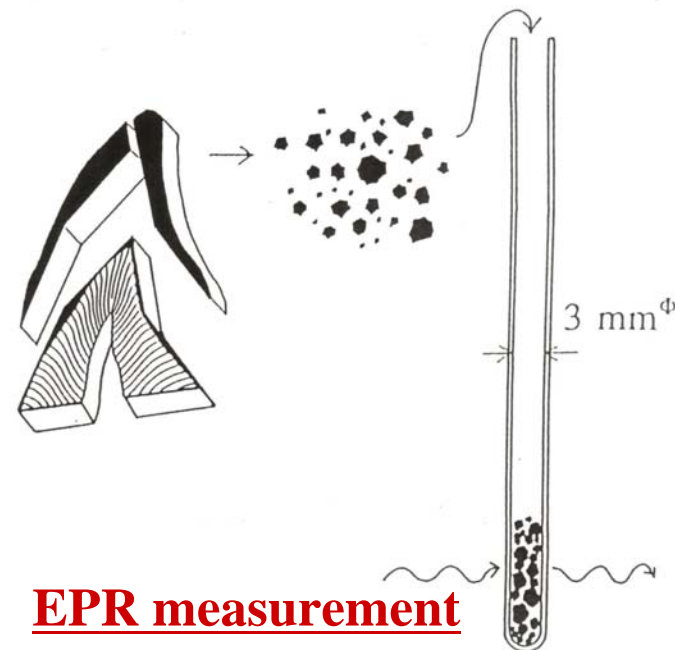
**Purpose:** to clarify the variation of cytogenetic data against physical dose

# Electron paramagnetic resonance (EPR) study

## Structure of a tooth



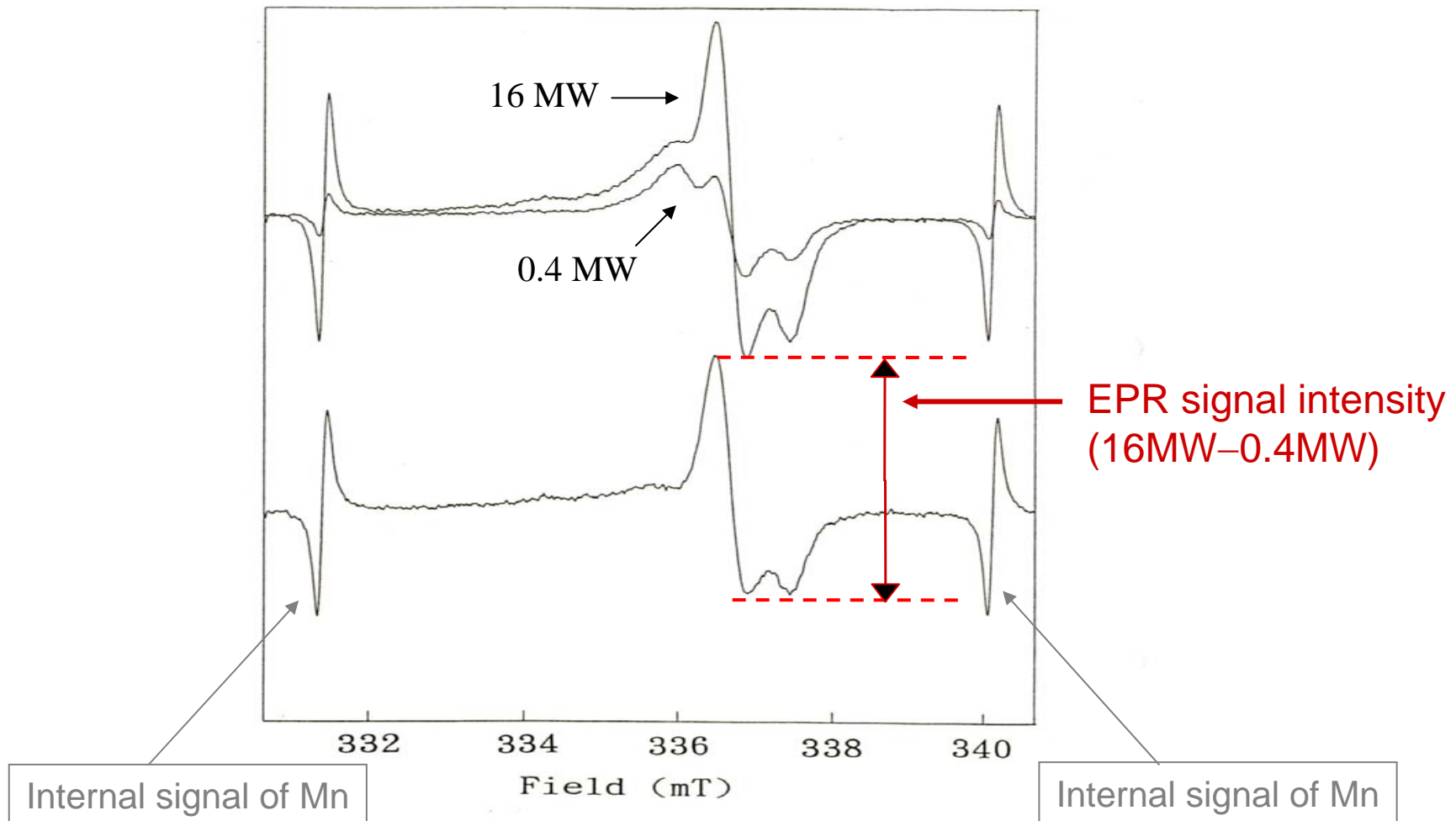
## Separation of enamel



The major component of enamel is hydroxyapatite. After irradiation,  $\text{CO}_2^-$  radicals are formed, which can be measured by EPR.

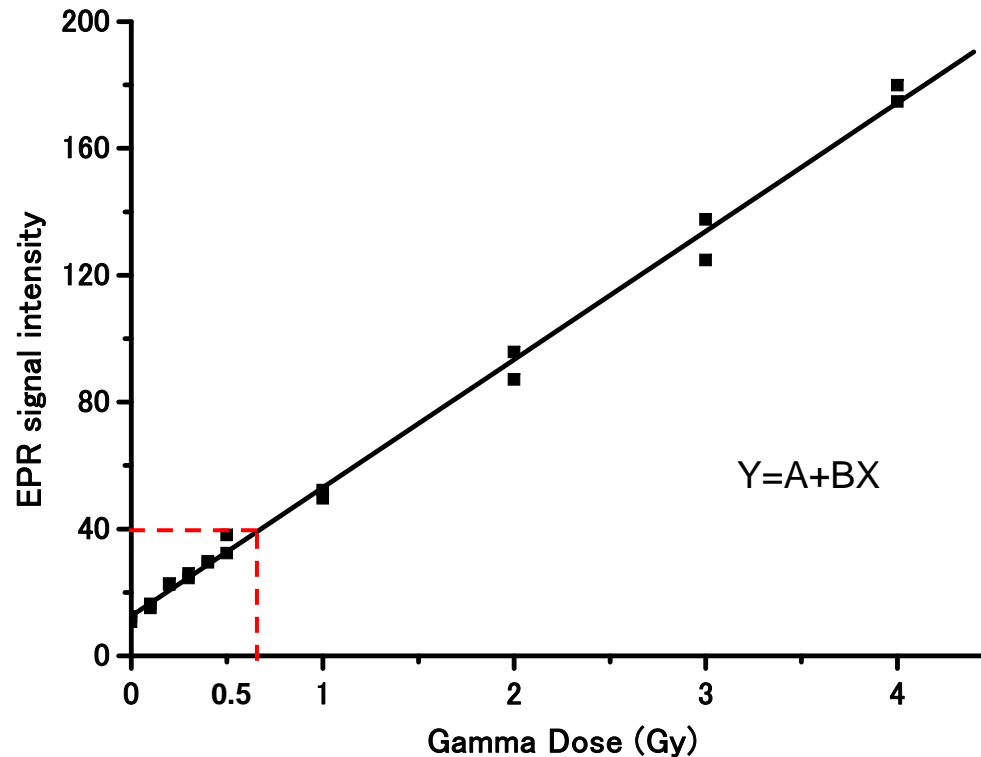
# EPR signal looks like this

**Selective saturation method** is used for subtraction of the background signal from EPR spectrum of tooth enamel.



# Calibration curve

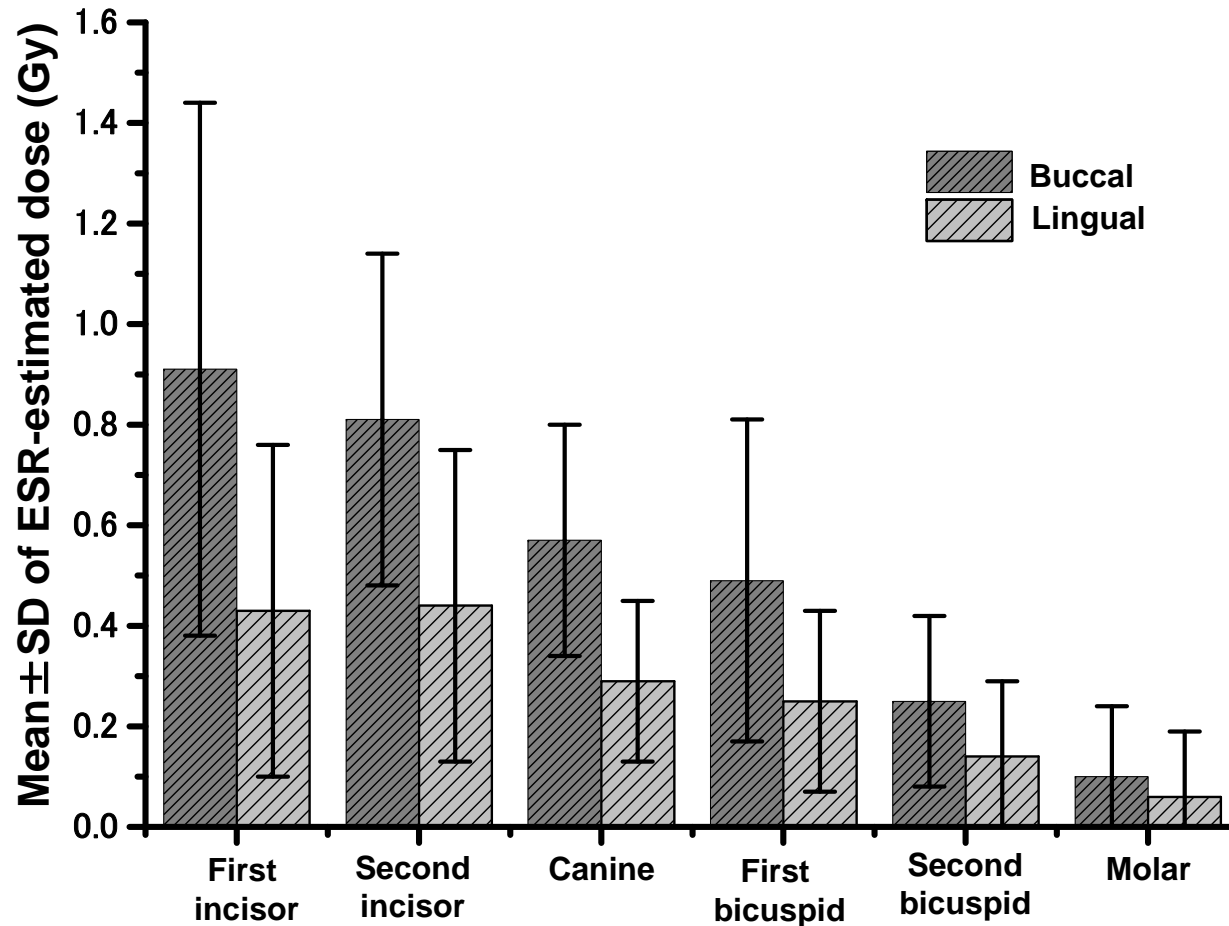
A calibration curve was done by preparing pooled enamel from 20 molars donated by residents of Fukushima prefecture (located in northern Japan). This pooled sample was then divided into 20 aliquots, and each aliquot was irradiated with a defined dose of  $^{60}\text{Co}$  gamma radiation to construct a calibration curve.



# EPR- estimated doses in types of teeth

n= 96 teeth from 53 survivors (control group with doses <5 mGy)

(N. Nakamura, Radiat. Res., 2006)



# Preparation of enamel

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- Donor age at the time of bomb were  $\geq 10$ .
- Donors who received radiotherapy were excluded.
- Molars were used.
- Each tooth was divided in two halves (buccal and lingual portions).
- Enamel from two sites was separated independently.
- Enamel was ground (about 500mm), and measured by EPR.

EPR machine



Evaluation of individual radiation doses  
of tooth enamel from Hiroshima atomic  
bomb survivors by EPR



# Summary

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Comparison of the EPR dose with Chromosome dose of the same survivors confirmed their close association.

The results turned out to validate the chromosome aberration data to be useful for individual dose estimation.

Wide distribution of individual chromosome dose against DS02 dose seems to be related dose errors rather than individual difference in radiosensitivity of lymphocytes.

Both EPR and chromosome doses deviated substantially from individual DS02 doses. This suggests the dose errors from physical estimates in a fraction of survivors.