BIODOSIMETRY IN THE 21st CENTURY
Training Meeting
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# FISH and PNA-FISH

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## FISH and PNA-FISH

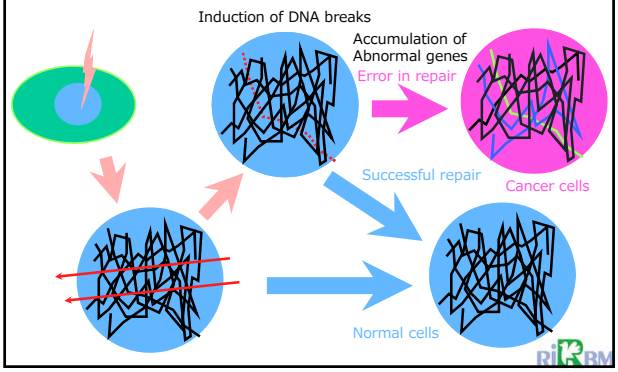
1) chromosome

2) FISH

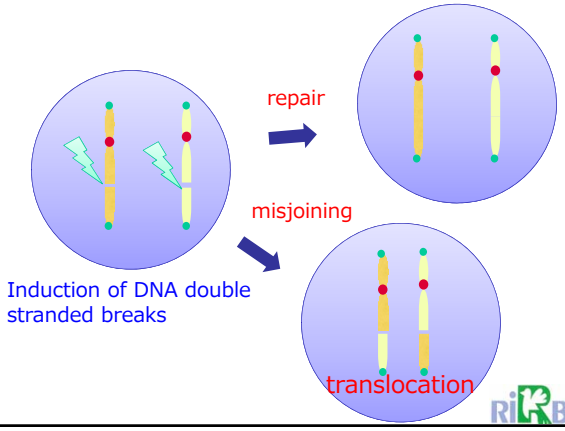
3) PNA-FISH

## Stochastic effect

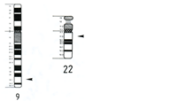
- Chromosome DNA is broken by ionizing radiation



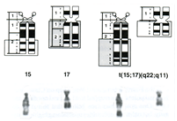

## Chromosome translocations



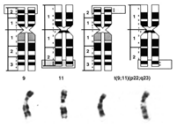

## Therapy-related leukemia and chromosome translocations



- t(9;22) ; Chronic myelocytic leukemia
  - ABL - BCR
  - Alu in the BCR gene
  - A-Bomb survivors (Hiroshima)



- t(15;17) : Acute promyelocytic leukemia
  - PML – RARA
  - 3 BCRs in the PML gene
  - 2nd intro of the RARA gene
  - Mitoxantrone, radiation



- 11q23 abnormalities; Therapy-related and infantile leukemias
  - MLL - more than 50 partner genes
  - BCR in the MLL gene
  - Topo II inhibitors (etoposide)

"Cancer Cytogenetics" Heim, S., Mitelman, F., 1995

## Radiation and cancer

radiation

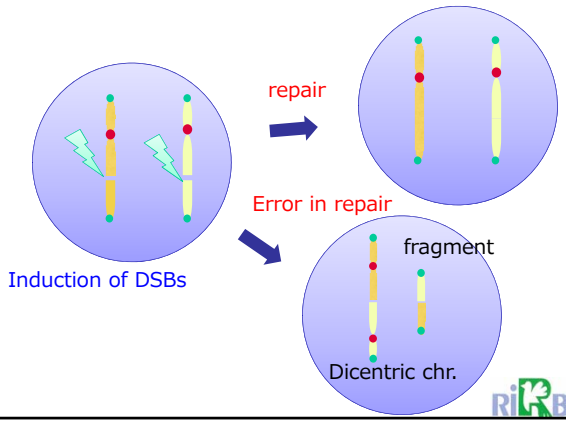
↓

DNA damage and error in repair

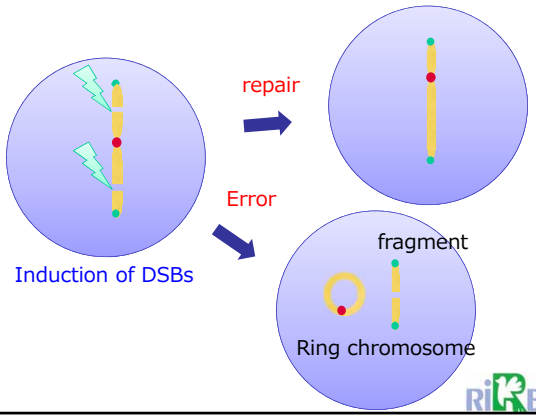
↓

cancer

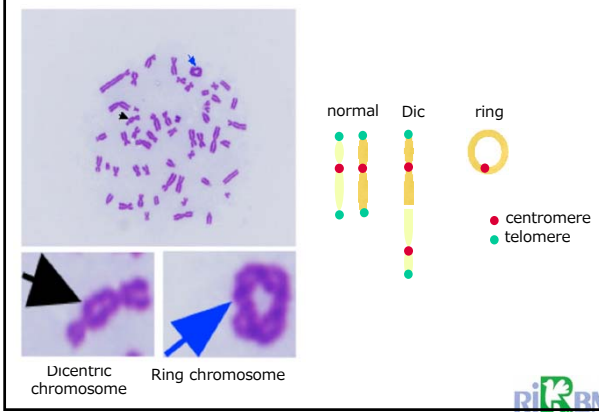
## Dicentric chromsome



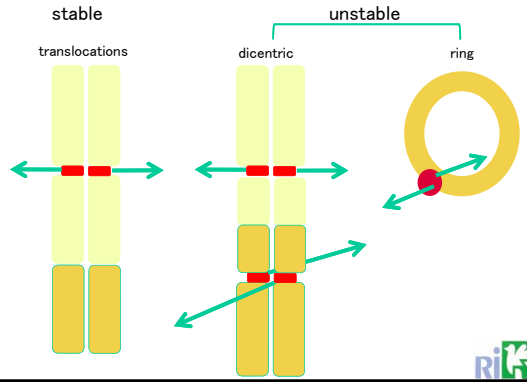

## Ring chromosome



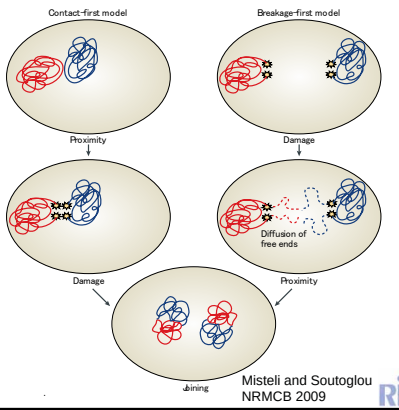

## "Dic and Ring" induced by ionizing radiation



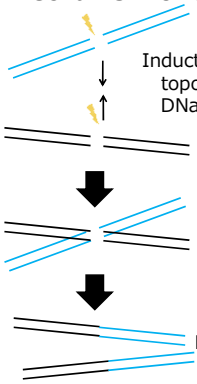

## Stable and unstable abnormalities




## Two models for chromosome translocations




## Mechanism of chromosome translocation (1)



Induction of DNA double stranded breaks (DSBs)
topoisomerase II concensus seq., Alu
DNase I hypersentivity sites?

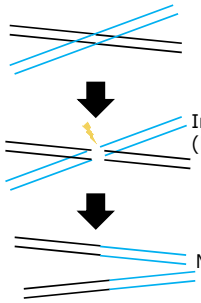
encounter of two DSBs?

Break first

Misjoining of DSBs

movement of DSBs (g-H2AX foci) induced by alpha particle (Aten et al, 2005)

## Mechanism of chromosome translocation (2)



encounter of two specific loci
gene positioning

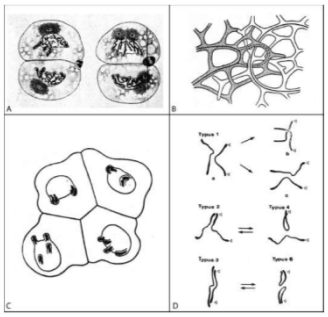
Contact first

Induction of DNA double stranded breaks (DSBs)
topoisomerase II concensus seq., Alu
DNase I hypersentivity sites?

Misjoining of DSBs

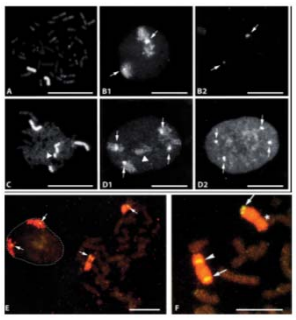
no movement of DSBs induced by I*Sce*1 (Misteli et al, 2007)

## Chromosome territories



Theodor Boveri, 1888, 1909

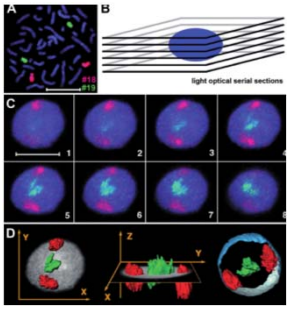
## non-radioactive in situ hybridization with chromosome specific DNA probes



specific detection of more than one hybridization sites in both metaphase spreads and interphase nuclei.

Lichter et al., 1988

## Light optical serial sectioning and 3D reconstruction of chromosome 18 and 19 in a human lymphocyte



specific detection of more than one hybridization sites in both metaphase spreads and interphase nuclei.

Cremer C and Cemer T, 2006

## FISH and PNA-FISH

1) chromosome

2) FISH

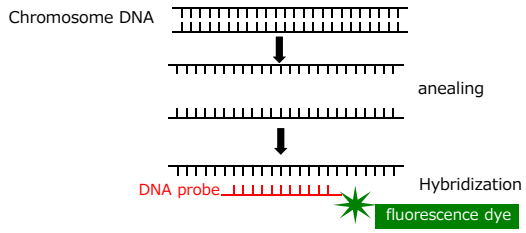
3) PNA-FISH

## Fluorescence in situ hybridization (FISH)

- DNA; DNA-FISH
- RNA; RNA-FISH

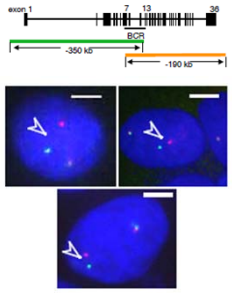
- Multicolor FISH
- 3D FISH

## Fluorescence in situ hybridization (FISH)



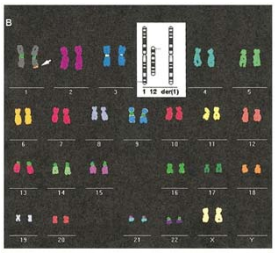

## Fluorescence in situ hybridization (FISH)

Gene locus



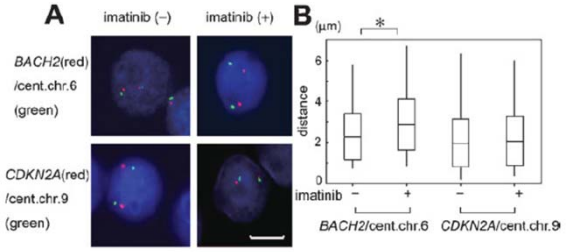

Sun J., et al; PLoS one2010

Chromosome painting



Uhrig S., et al; Am. J. Hum. Genet 1999

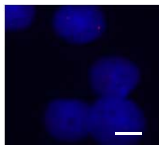
## Topological association of the Bach2 gene with the centromere of chromosome 6 DNA-FISH



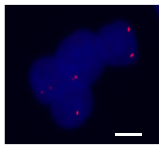

Ono A., et al; Genes, chomosomes & cancer.2006

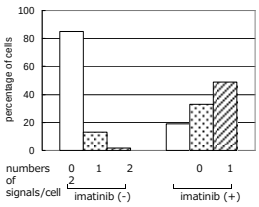
## Induction of BACH2 gene by imatinib RNA-FISH

before



After Imatinib treatment

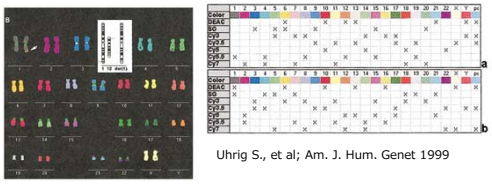





Ono A., et al; Genes, chomosomes & cancer.2006

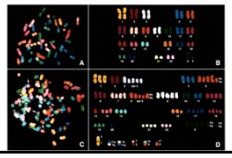
## Multicolor FISH

- M-FISH



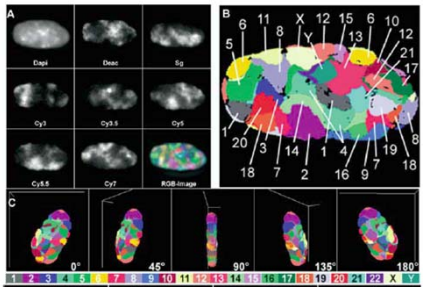
Uhrig S., et al; Am. J. Hum. Genet 1999

- Spectral Karyotypying (SKY) FISH



Garini Y., et al; Bioimaging 1996

## 24-color 3D FISH



Bolzer A., et al; PLoS Biol 3(5): e157.2005

## Biological dosimetry with chromosome analysis

Gold Standard!!

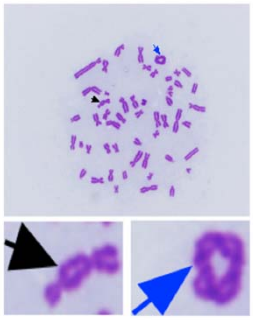
1)Dicentrics and/or rings analysis (unstable)

2)Chromosome translocation analysis (stable)

1)Premature chromosome condensation (PCC) analysis

1)The Cytokinesis-block micronucleus (CBMN) assay

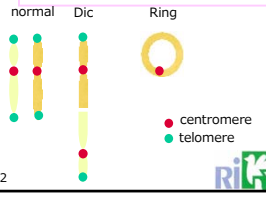
## Chromosome analysis with Giemsa stained samples



Dicentric chromosome Ring chromosome

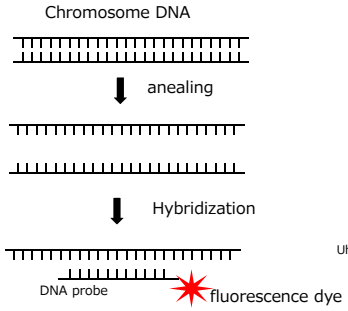
1) Culture PBLs with PHA for 48 hrs
2) Fixed with Carnoy
3) Preparation of metaphase spreads
4) Giemsa staining

Good; fast, inexpensive
Not good; difficult




Shi L., et al; Rad Res 2012

## Fluorescence in situ hybridization (FISH) analysis




Uhrig S., et al; Am. J. Hum. Genet 1999

Good; easy
Not good; time consuming, expensive

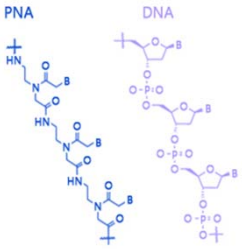
## FISH and PNA-FISH

1) chromosome

2) FISH

3) PNA-FISH

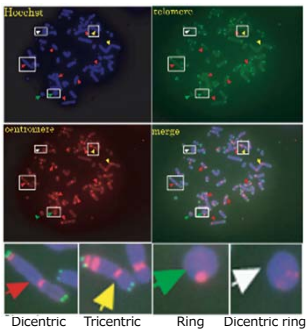
## PNA probe



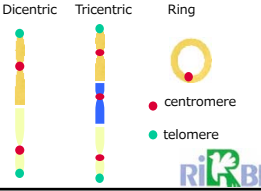

•High binding affinity to its complementary DNA or RNA
•Differentiation of sing-base mismatch by high destabilizing
•stability to nuclease and protease
•Salt independence during hybridization with DNA sequence
•Triplex formation with continuous homopurine DNA

PANAGENE

## FISH analysis with centromere and telomere PNA probes




1) Culture PBLs with PHA for 48 hrs
2) Fixed with Carnoy
3) Preparation of metaphase spreads
4) Hybridization with centromere and telomere PNA probes



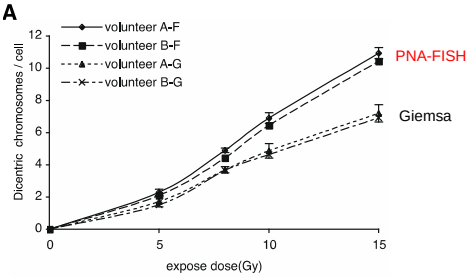

Shi L., et al; Rad Res 2012

## Times required for chromosome analysis

| | Giemsa | FISH | PNA-FISH |
|---|---|---|---|
| Cell culture | 48 hrs | 48 hrs | 48 hrs |
| aging | 0 | > 48 hrs | 0 |
| Staining/Hybridization | 0.5 hrs | > O/N | 1 hr |

## Dicentric chromosome analysis

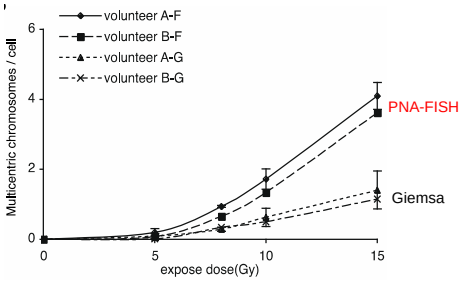
Dicentric chromosome analysis using FISH with telomere and centromere PNA probes could be useful for biological dosimetry.




The incidence of dicentric chromosomes in the metaphase spreads detected by FISH correlated very well with those by Giemsa analysis except the samples irradiated at 15 Gy. Squareroot of mean squared error in estimated dose using inverse spline regression using FISH analysis with dicentric chromosomes was lower than that using Giemsa analysis (0.391 vs 0.791, respectively). Shi L., et al; Rad Res 2012

## Multi-centromeric chromosomes formed after ionizing irradiation

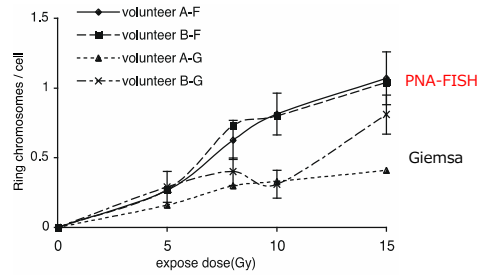
Multicentromeric chromosomes could be more frequently missed in Giemsa than FISH analysis.




The incidence of multi-centromere chromosomes detected by Giemsa analysis failed to show linear correlation with the irradiation dose.
Shi L., et al; Rad Res 2012

## Ring chromosomes formed after ionizing irradiation

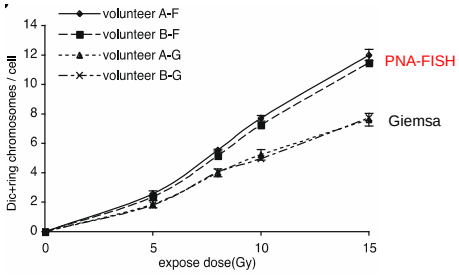
Ring chromosomes could be more frequently missed in Giemsa than FISH analysis.




The incidence of ring chromosomes detected by Giemsa analysis failed to show linear correlation with the irradiation dose.
Shi L., et al; Rad Res 2012

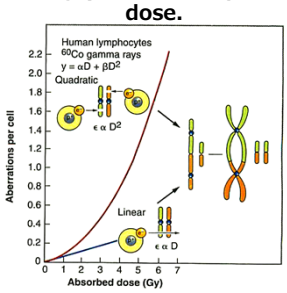
## Dic + Ring chromosomes after ionizing irradiation

Since the mean squared error of the estimated irradiation doses is smallest using dic + ring in both FISH and Giemsa analysis, dic + ring could be a good index for the estimation of radiation doses.




The mean squared error of the estimated irradiation doses of samples irradiated at higher dose with dic + ring (0.337 and 0.590 by FISH and Giemsa, respectively) were smaller compared to those of dicentric (0.391 and 0.791 by FISH and Giemsa, respectively). Shi L., et al; Rad Res 2012

## The frequency of chromosomal aberrations (dicentrics and rings) is a linear-quadratic function of dose.




At low doses, both breaks may be caused by the same electron; the probability of an exchange aberration is proportional to dose (D). At higher doses, the two breaks are more likely to be caused by separate electrons. The probability of an exchange aberration is proportional to the square of the dose ($D^2$).

From Radiobiology for the Radiologist 7th ed, Eric J. Hall, Amato J. Giaccia

## Uncertainty of dose estimation

If 25/500 cells carry one dicentric chromosome,

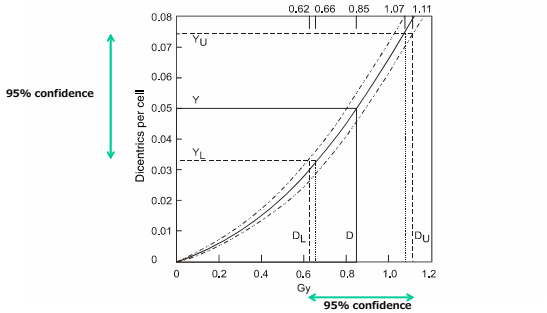



*FIG. 13. A dose-response calibration curve with its 95% confidence limits, used to estimate uncertainties on dose by approach C.*

(Modified from Cytogenetic analysis for Radiation Dose Assessment, A Manual, IAEA 2001)

## Factors affecting chromosome abnormalities

Numbers of Double strand breaks
(associated with radiation dose)

Misjoining of DNA ends
(Accuracy of DNA repair) → Difference among individual?

More resolution and more speed!