# The Utility of Cytogenetic Biodosimetry in Epidemiology and Occupational Medicine

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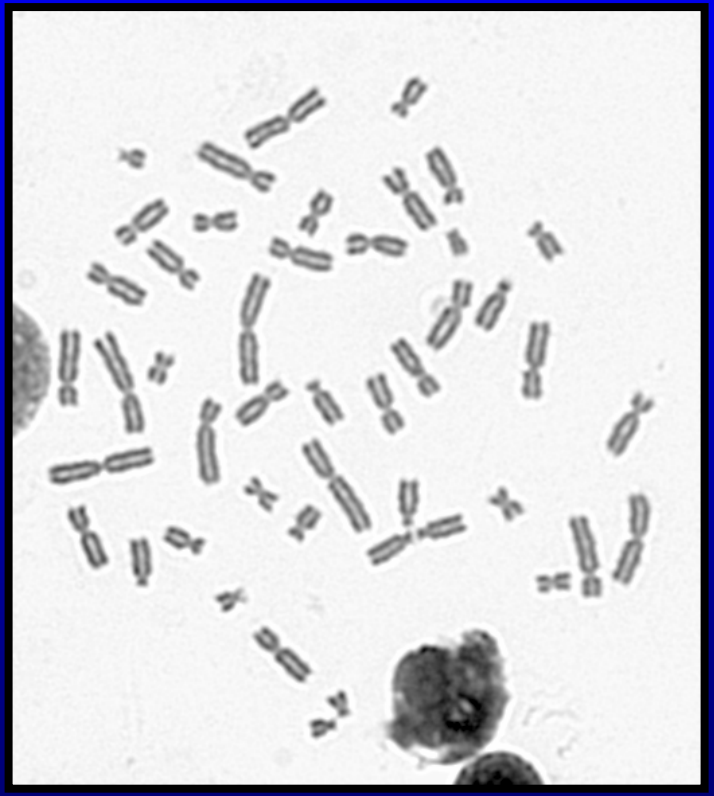


# CBL

The Cytogenetic Biodosimetry Laboratory (CBL) is an international emergency resource for radiation dose analysis. The CBL is operated as part of the Radiation Emergency Assistance Center / Training Site of the Oak Ridge Institute for Science and Education (ORISE). REAC/TS is an emergency response asset under DOE-NNSA.

http://orise.orau.gov/reacts/cytogenetics-lab.htm

# Where has Cytogenetic Biodosimetry been used?

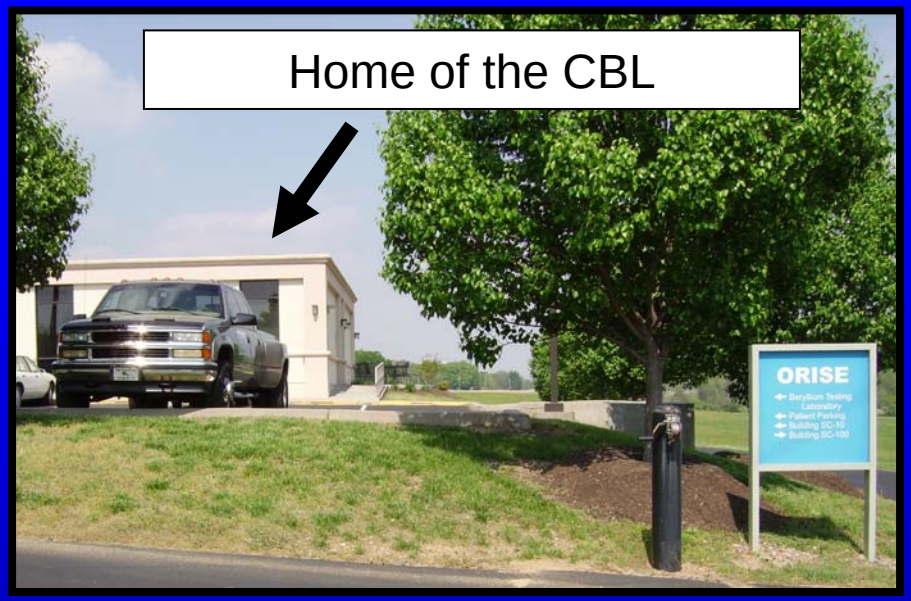
- Atomic Bomb Survivors
  - Dosimetry was a significant issue
    - Under-reporting
    - Over-reporting
  - Confirmation of dose by cytogenetic biodosimetry
- Chernobyl
- Radiation Accidents
  - Industrial, medical, public
- Epidemiology

# Cytogenetic Biodosimetry

## *Purpose*

The cytogenetic analysis of lymphocytes (scoring dicentrics) obtained from peripheral blood samples is a well established method to assess the absorbed dose for persons exposed to ionizing radiation when physical dosimetry is not available.

# Prior to Construction Beginning

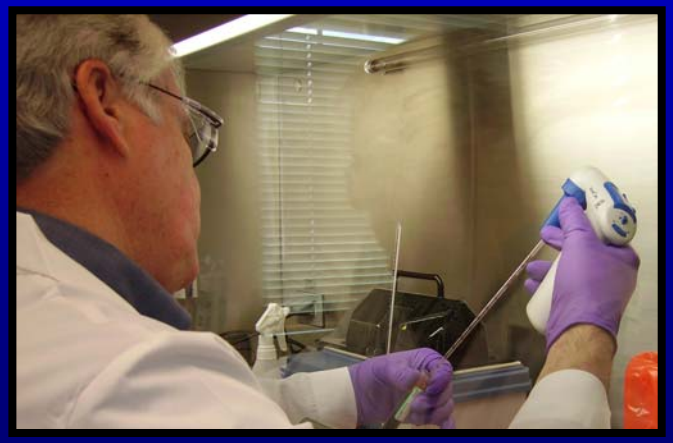



Laboratory Space
(early June 2006)

Moving in 10/20/06

Scientific Advisory Board Meeting 7/14/06

First Blood Culture 12/12/06



Dr. Gordon Livingston, CBL Technical Director; Admiral Joseph Krol, head of the National Nuclear Security Administration's Emergency Operations; Dr. Ron Townsend, ORISE Director; and Robert Brown, chief operating officer of the U.S. Department of Energy (DOE)—Oak Ridge Operations.

# CBL Status

- Calibration Curves Completed
  - Co-60
  - X-ray (150kVP)
  - Neutron (Cf-252)
- Inter-laboratory comparison study completed with AFRRI, Canada, Japan, Germany
- State of Tennessee Medical Laboratory License obtained
- CLIA Application submitted
- Two analyses completed for clients
- Participation in TOPOFF 4
  - 12 triage samples

# Recent vs. Retrospective Exposures

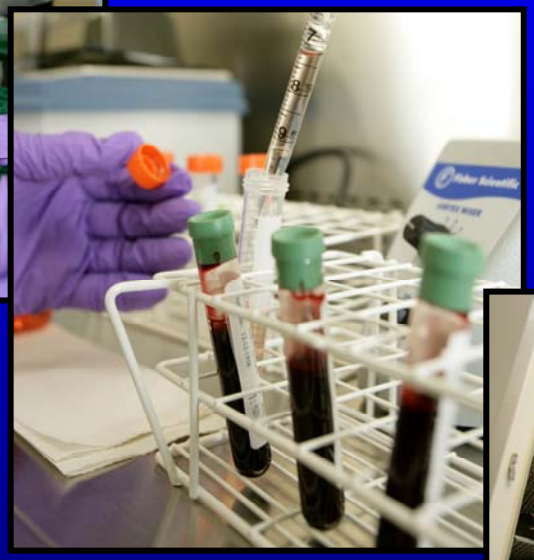
- Recent Exposure (often acute)
  - Dicentric Assay (Giemsa)
    - Unstable Aberrations
  - REAC/TS Mission
    - Emergency Radiation Medicine Response and Training
  - No dosimeters
  - External (X-rays, gamma rays, neutrons)

# Recent vs. Retrospective Exposures

- Retrospective Exposure (chronic)
  - Translocations (Karyotyping and FISH)
    - Stable Aberrations
  - Medical Surveillance and Epidemiology
  - Occupational exposures (dosimeters)
  - Internal and external (alpha, beta, x-rays, gamma rays, neutrons)

Blood samples are collected and shipped at room temperature to the Cytogenetic Biodosimetry Laboratory in Oak Ridge, Tennessee.

Blood samples arrive at the CBL.



The whole blood samples are aliquoted into 15 ml centrifuge tubes with complete media for culture.

# Dicentric Assay

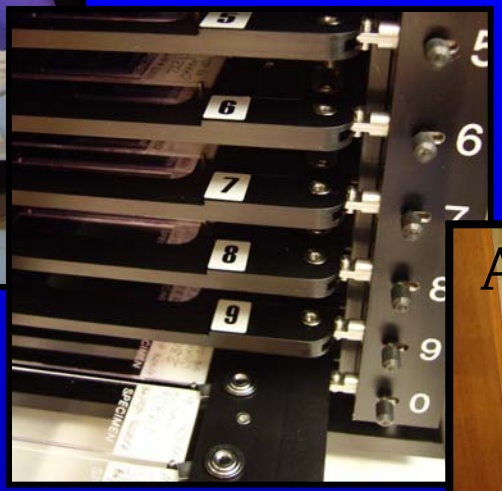
White blood cells, lymphocytes, are cultured under sterile conditions in an incubator for 48 hours.

Culture tubes are centrifuged and cells are re-suspended in a weak salt solution, which allows the chromosomes to separate and spread evenly on slides.

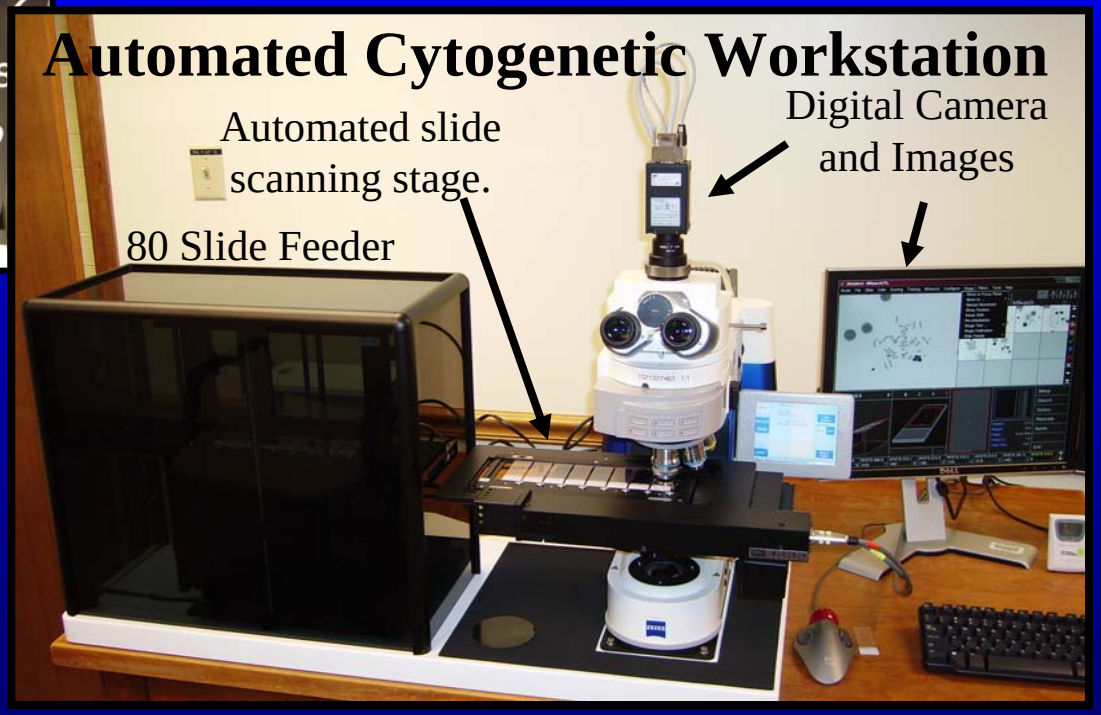
Further centrifugations and re-suspensions of the cells in fixative are performed to preserve the cells.

The fixed cell suspension is then dispensed on slides and allowed to dry in a controlled atmosphere.

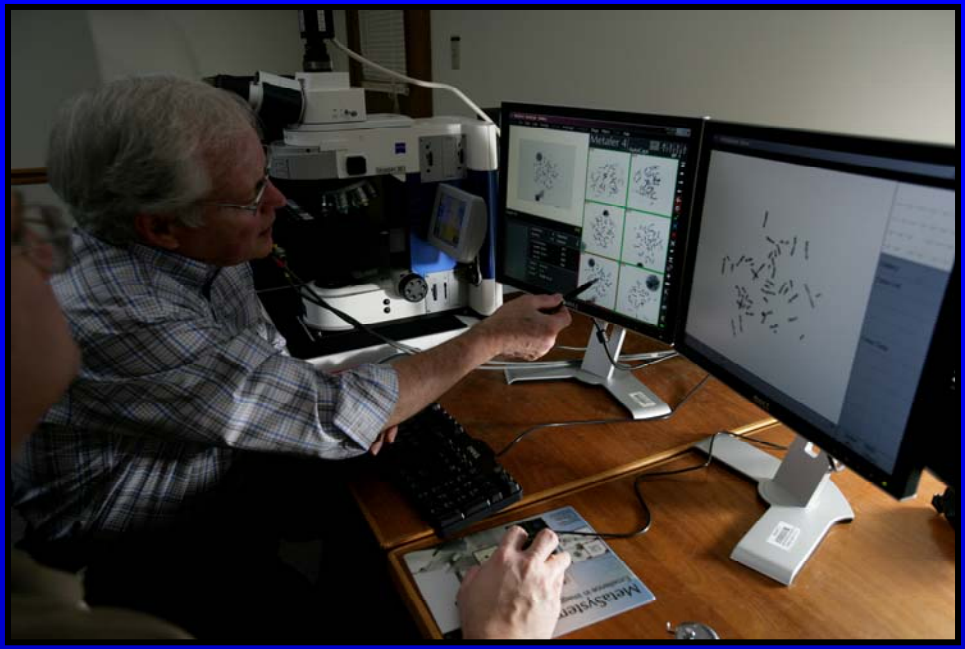
Chromosomes are stained and the slides are then mounted using glass cover slips, which protect and preserve the cells.



The slides are loaded onto frames and placed in the slide feeder (10 frames with 8 slides each).

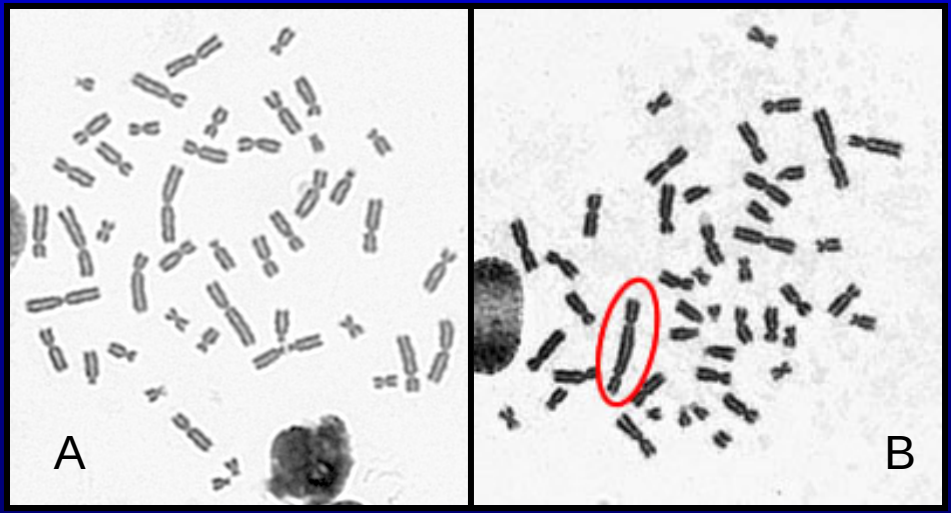



The Metafer automated cytogenetic workstation, a motorized Zeiss microscope coupled with a slide changer, automatically locates metaphase spreads on the slide and can scan slides unattended overnight.

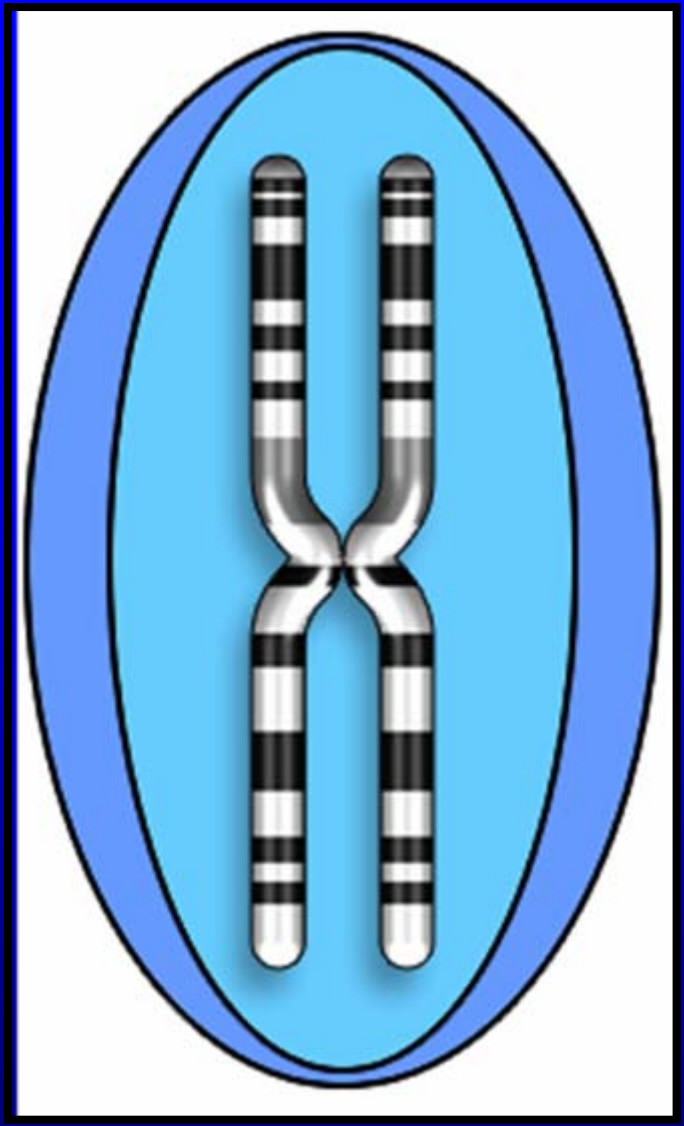


A digital photo of the metaphase spread is taken at 63x magnification. The capture of the images can also be performed automatically by the automated cytogenetic workstation. The cytogeneticist then analyzes the cells, counts the number of dicentrics and compares the result to calibration curves to estimate radiation dose.

Image A - normal metaphase spread
Image B - dicentric chromosome



# Architecture of the Chromosome

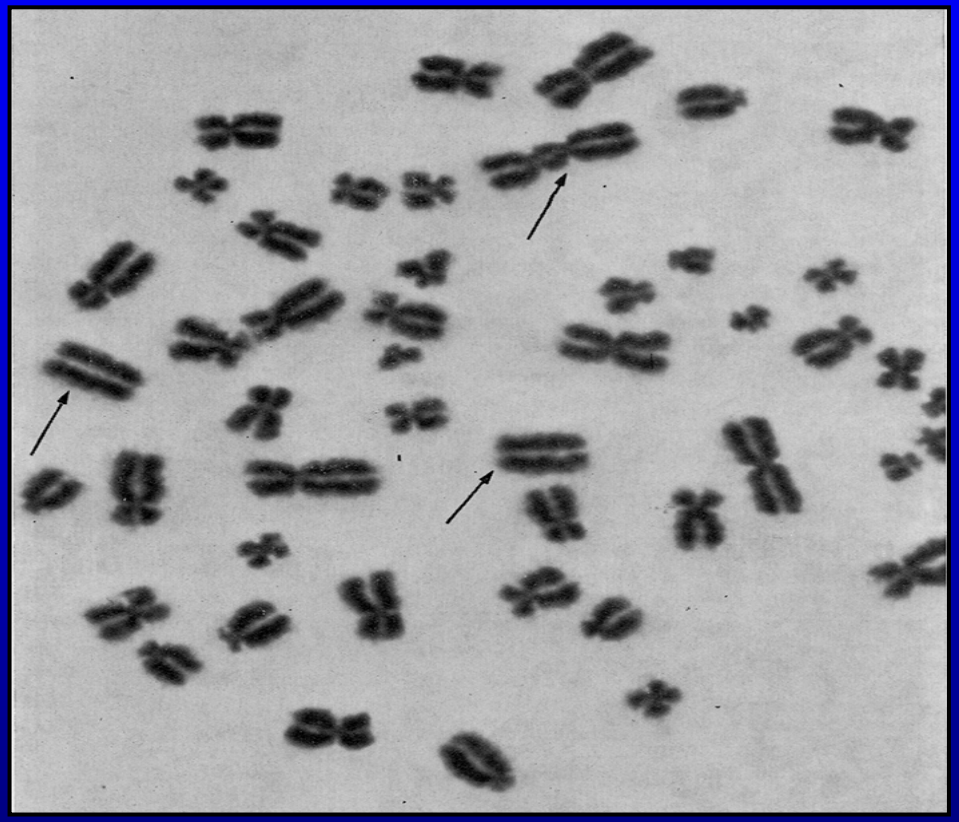


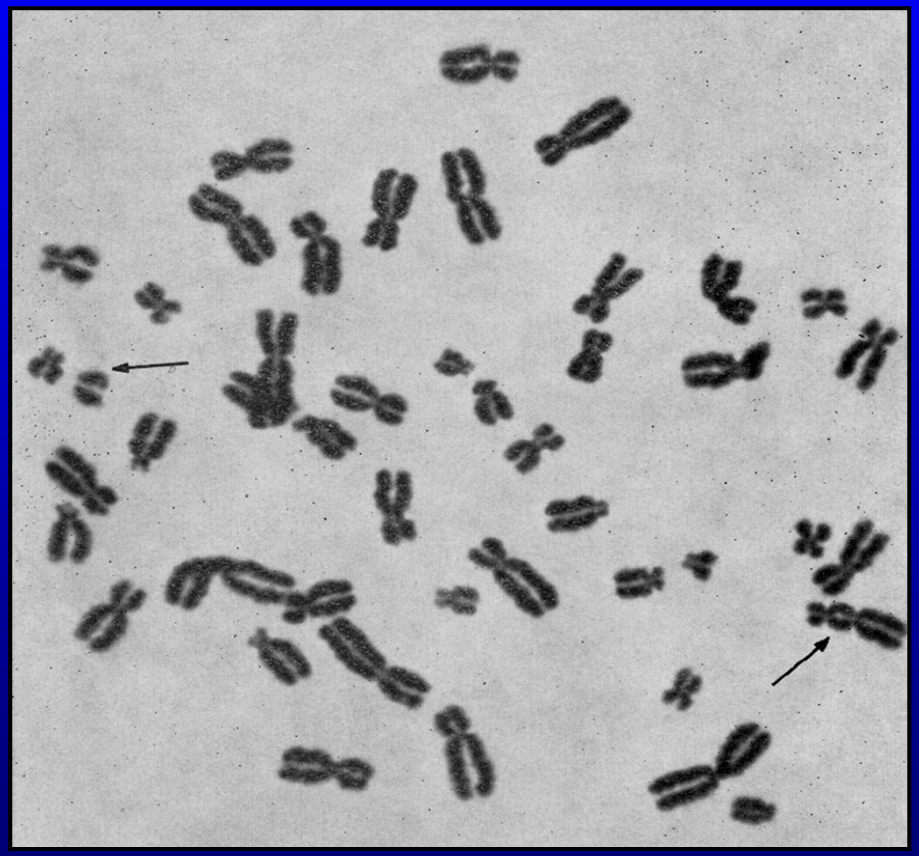
# The International System for Human Cytogenetic Nomenclature (ISCN 2005)

## 7 Chromosome Groups (A - G) Distinguished by Solid Giemsa Staining

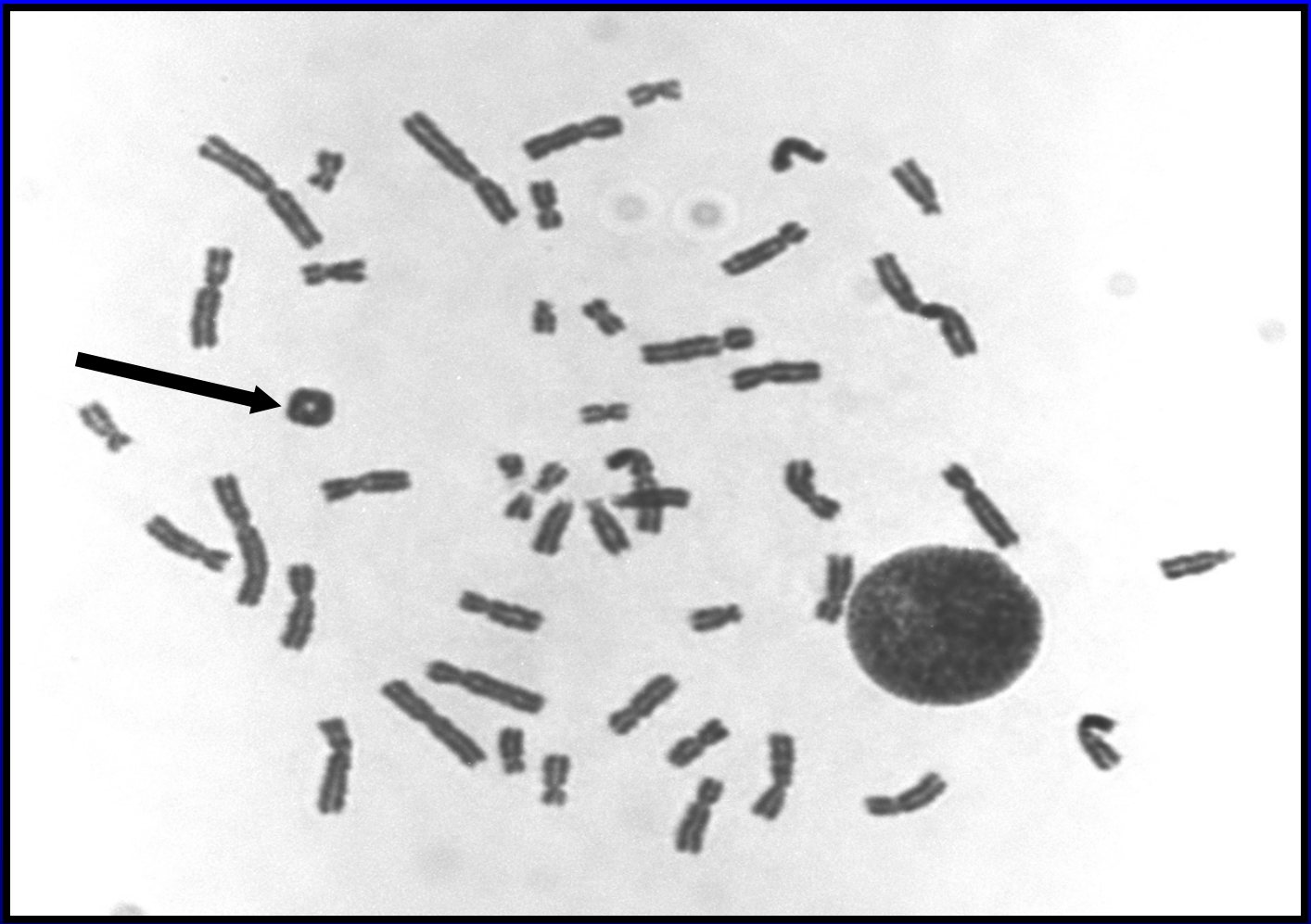
| | |
|---|---|
| Group A (1-3) | Large metacentric chromosomes readily distinguished by size (length) and centromere position. |
| Group B (4-5) | Large submetacentric chromosomes. |
| Group C (6-12 & X) | Medium-sized metacentric or submetacentric chromosomes. |
| Group D (13-15) | Medium-sized acrocentric chromosomes with satellites. |
| Group E (16-18) | Relatively short metacentric or submetacentric chromosomes. |
| Group F (19-20) | Short metacentric chromosomes. |
| Group G (21-22 & Y) | Short acrocentric chromosomes with satellites. The Y chromosome bears no satellite. |

# Recent Exposures
## (Unstable Aberrations - Dicentrics and Acentrics)

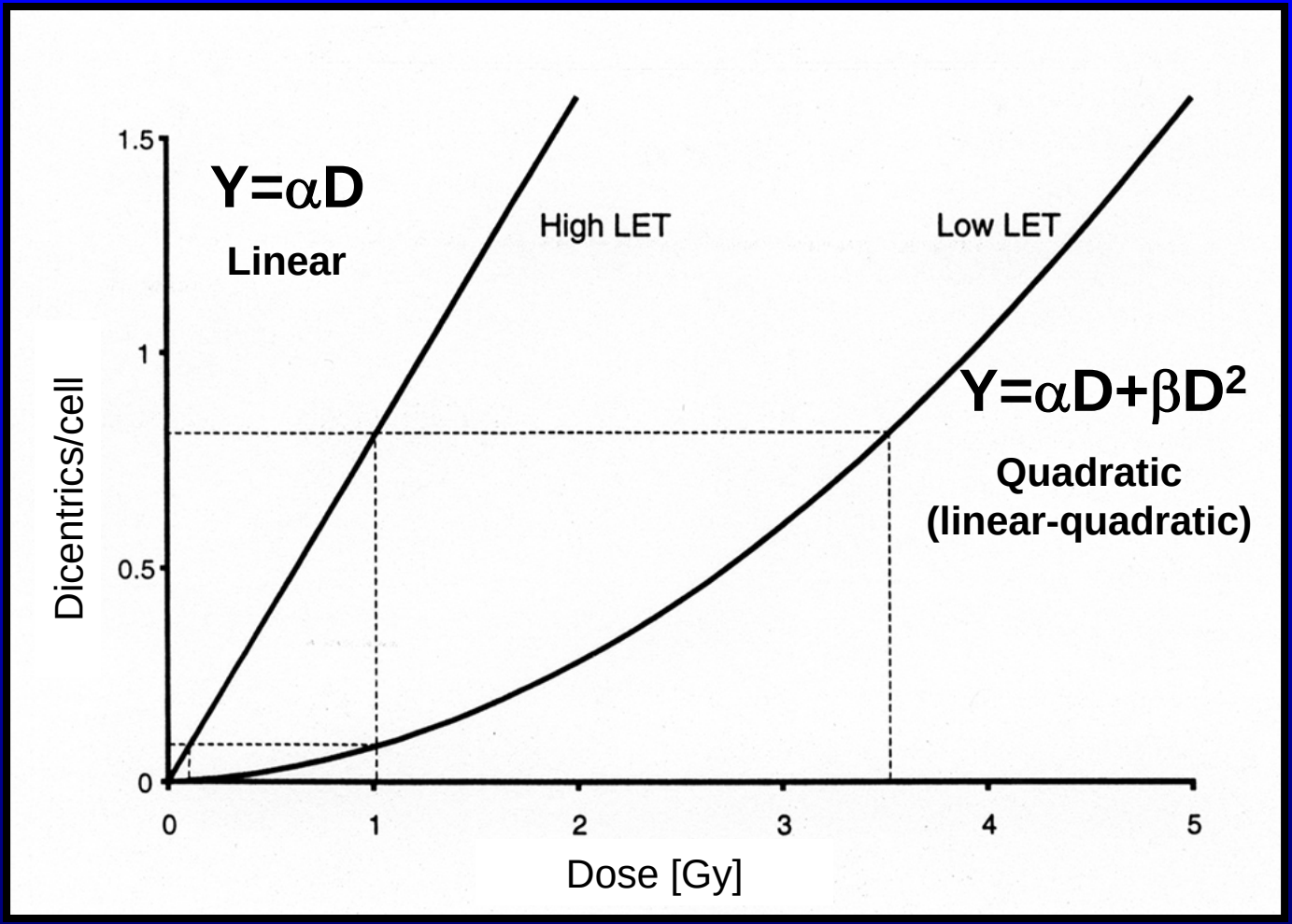




# Ring Chromosome



# Typical Dose Response Curves

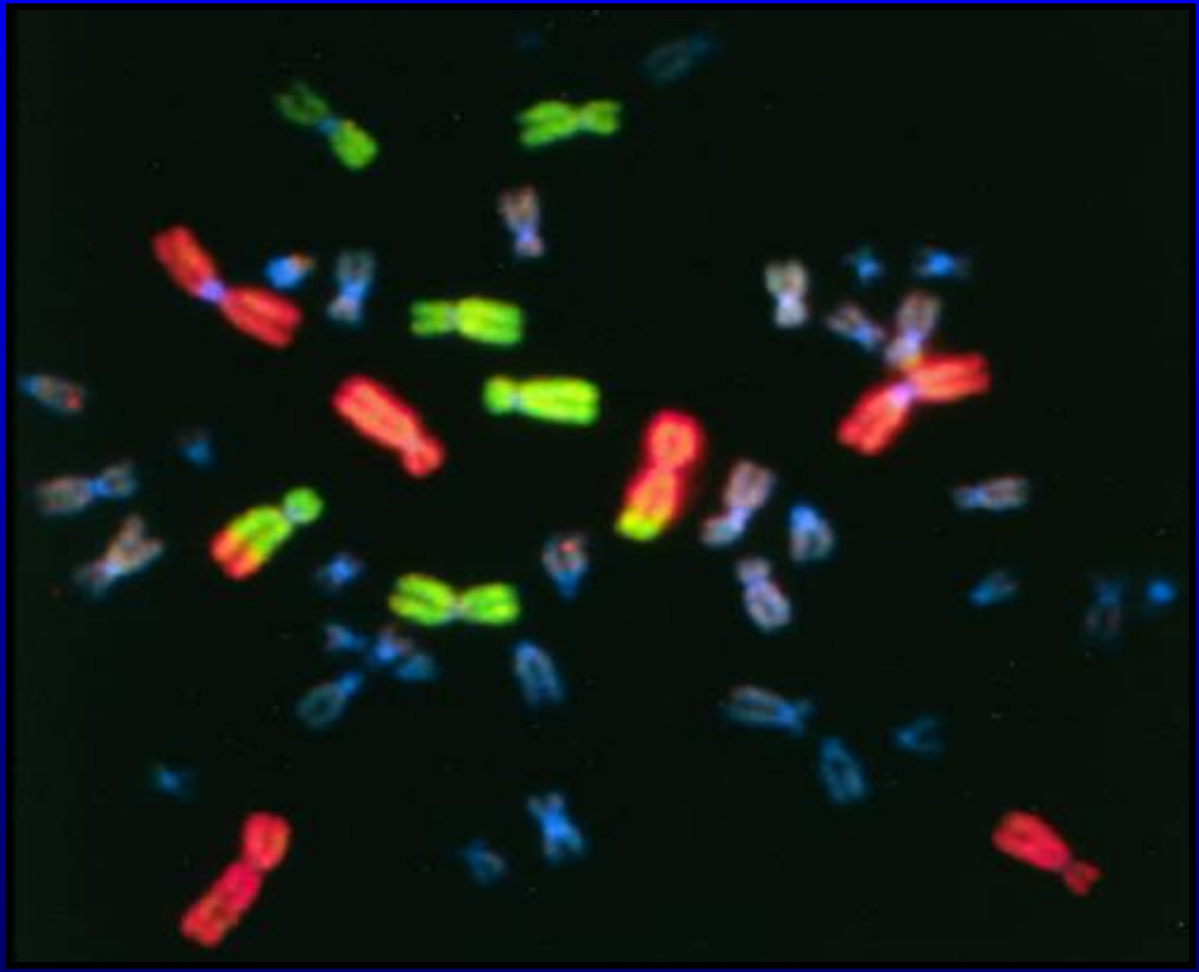


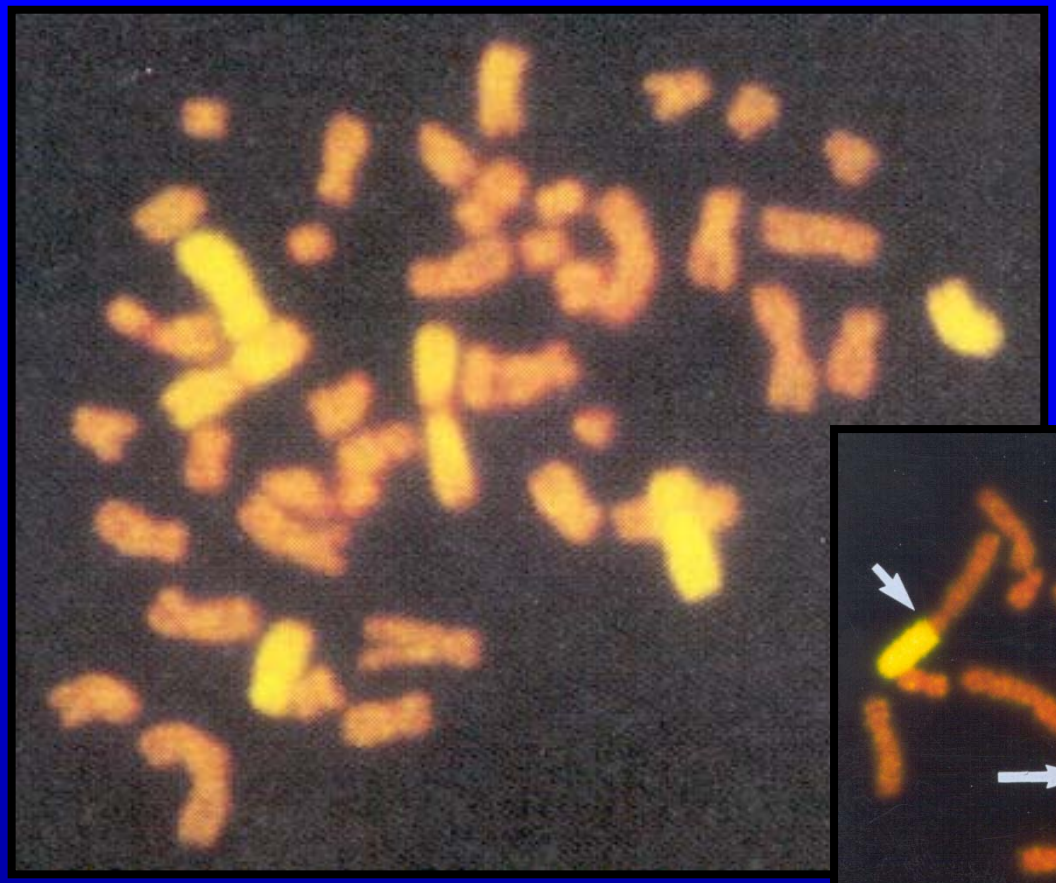
# Retrospective Exposures
## (Stable Aberrations – Translocations)

Plutonium processing in a glovebox

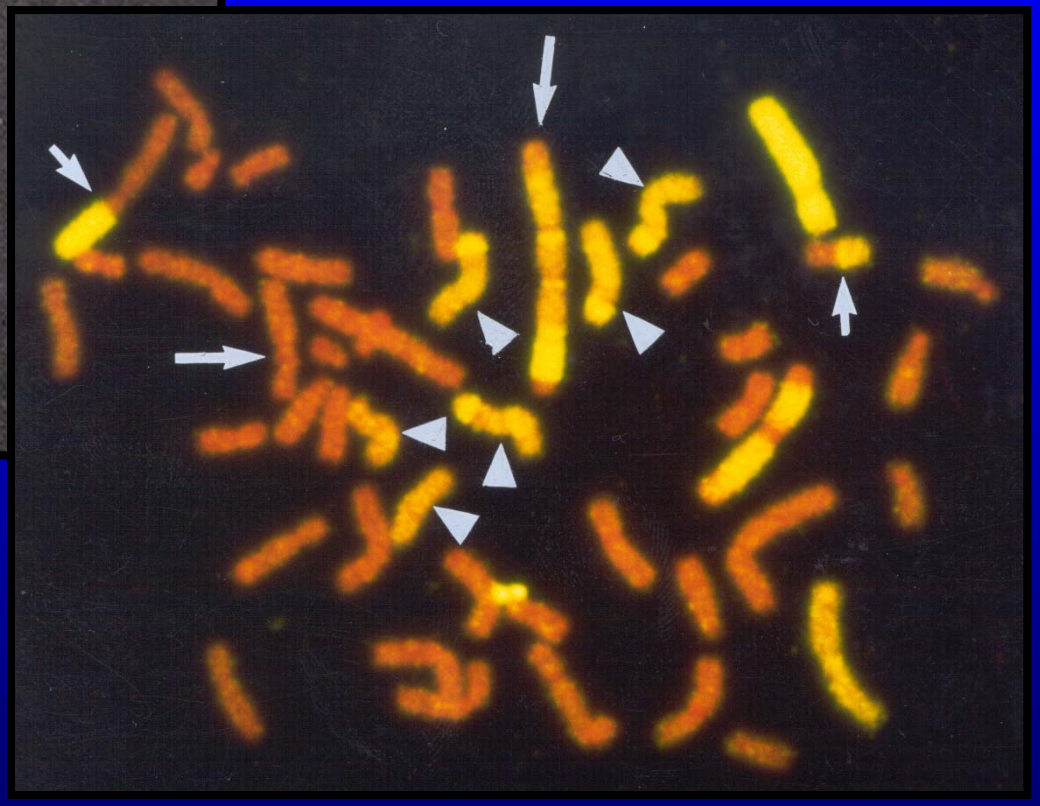
Brushing oxide from a "button" of plutonium

# Two-Way Translocation & Two Color Junctions



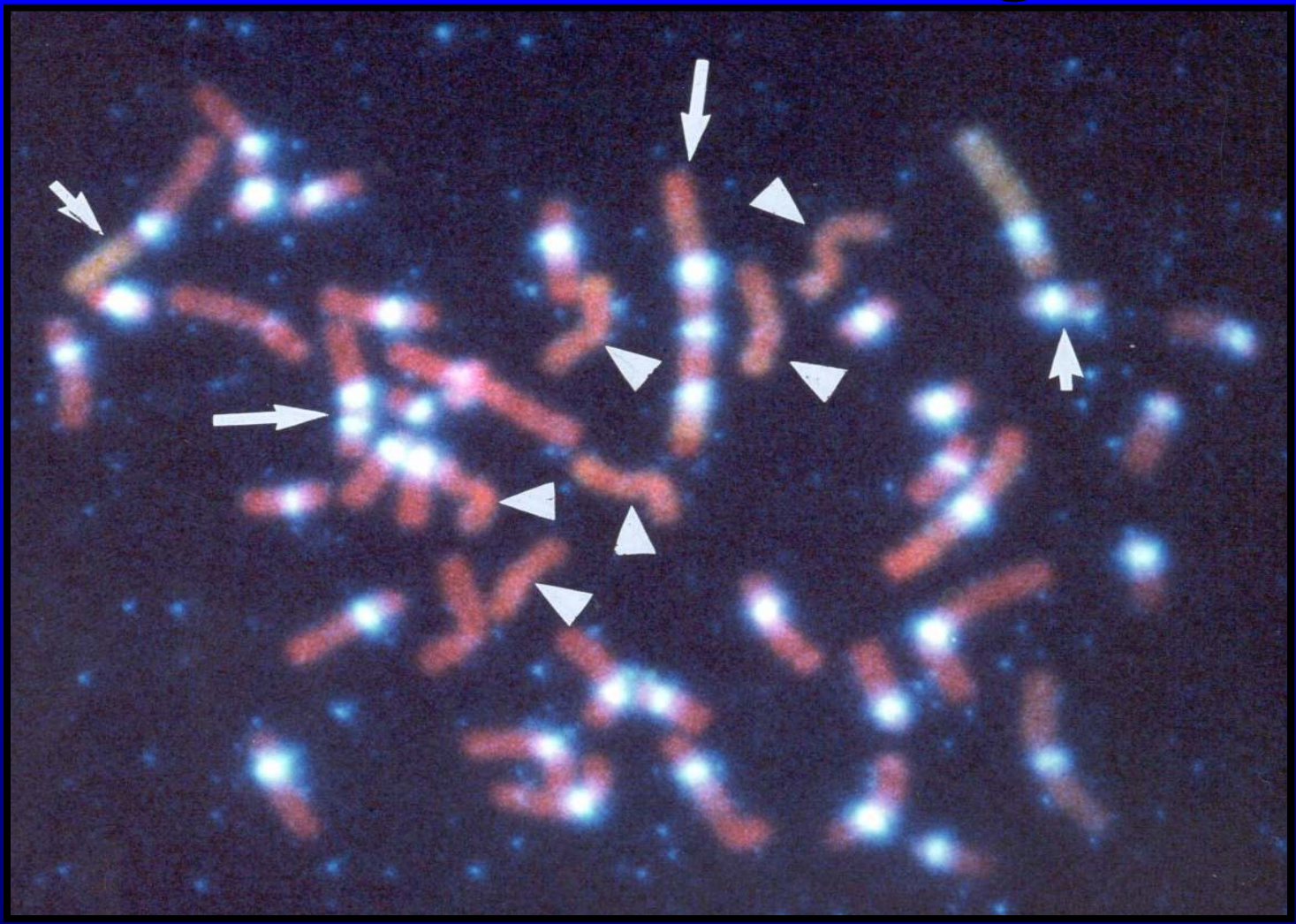


Normal FISH
Chromosomes 1, 4, and 12

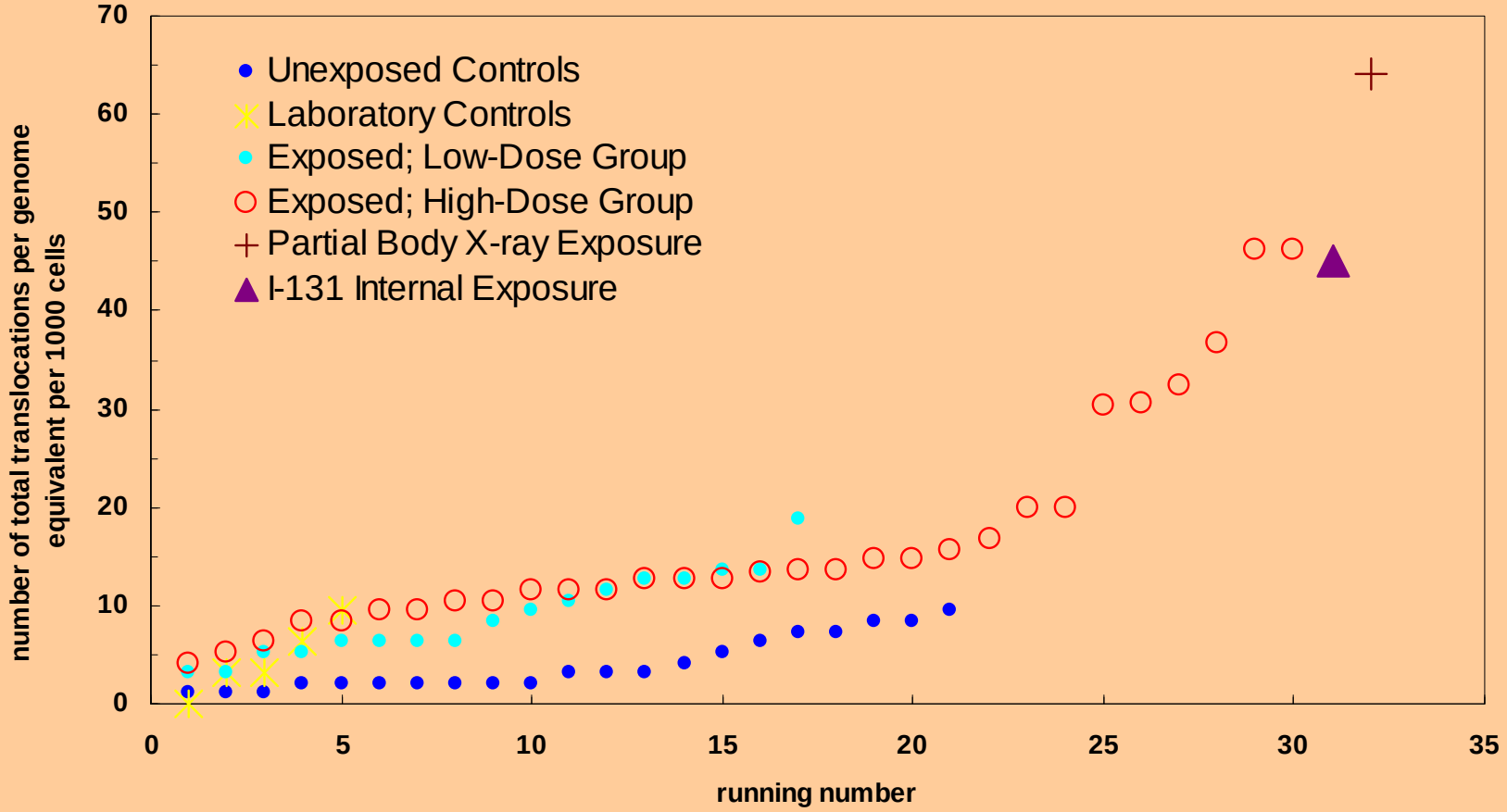


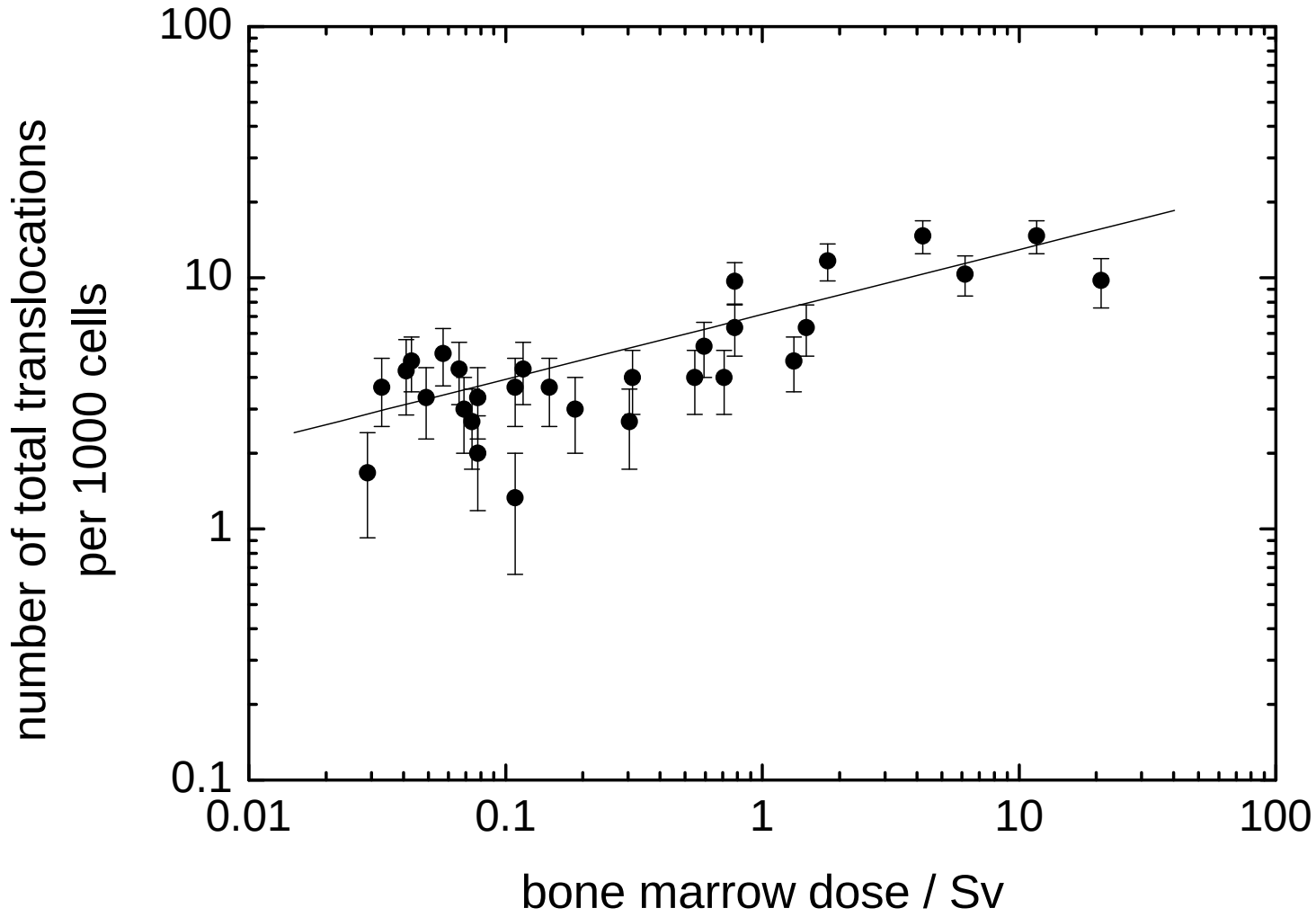
Two-Way
Translocation
& Two Color Junctions

# Centromere Painting



# Plutonium Worker Study




number of total translocations
per 1000 cells
100
10
1
0.1
0.01
0.1
1
10
100
bone marrow dose / Sv

# Study Design, Dosimetry Data, and Total Translocations by Study Group

| Parameter | Original FISH Study | | FISH Subset | |
|---|---|---|---|---|
| | *Unexposed Controls* | *High - Dose Workers* | *Unexposed Controls* | *High - Dose Workers* |
| No. Individuals | 21 | 30 | 15 | 15 |
| Mean Age (yr) | 60.1 | 70.7 | 59 | 72 |
| Median Bone Marrow Dose (mSv) (range) | 0 | 168 (29 - 20,904) | 0 | 710 (29 – 11,700) |
| No. Cells Analyzed | 62,230 | 88,162 | 45,000 | 45,000 |
| No. Genome Equivalents (GE) | 19,789 | 28,036 | 14,310 | 14,310 |
| Total Translocations (TT) | 80 | 473 | 61 | 316 |
| **Mean TT per GE x $10^{-3}$ ± SE** | **4.00 ± 0.6** | **16.98 ± 2.05** | **4.26 ± 0.72** | **22.08 ± 3.34** |

Image Post Sharpening

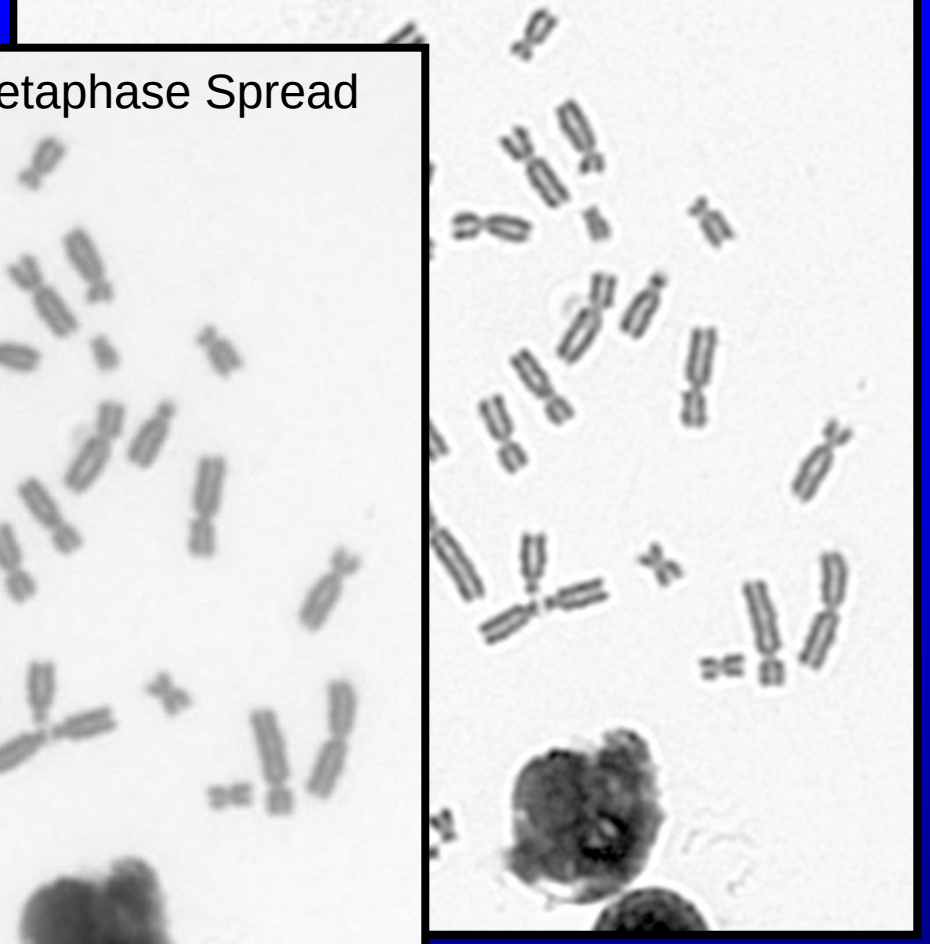
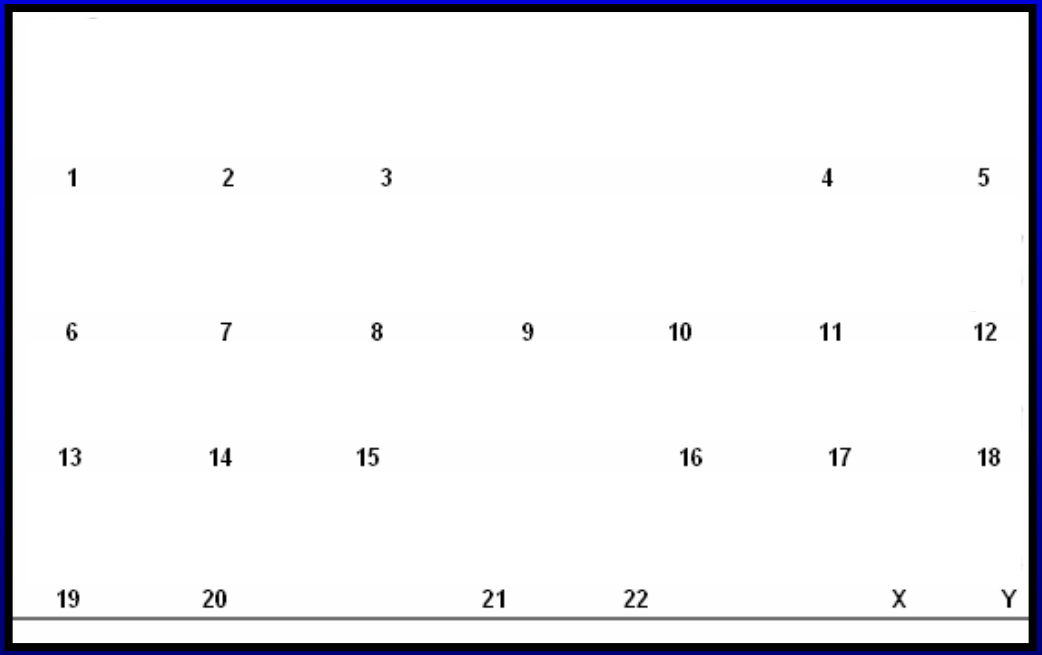
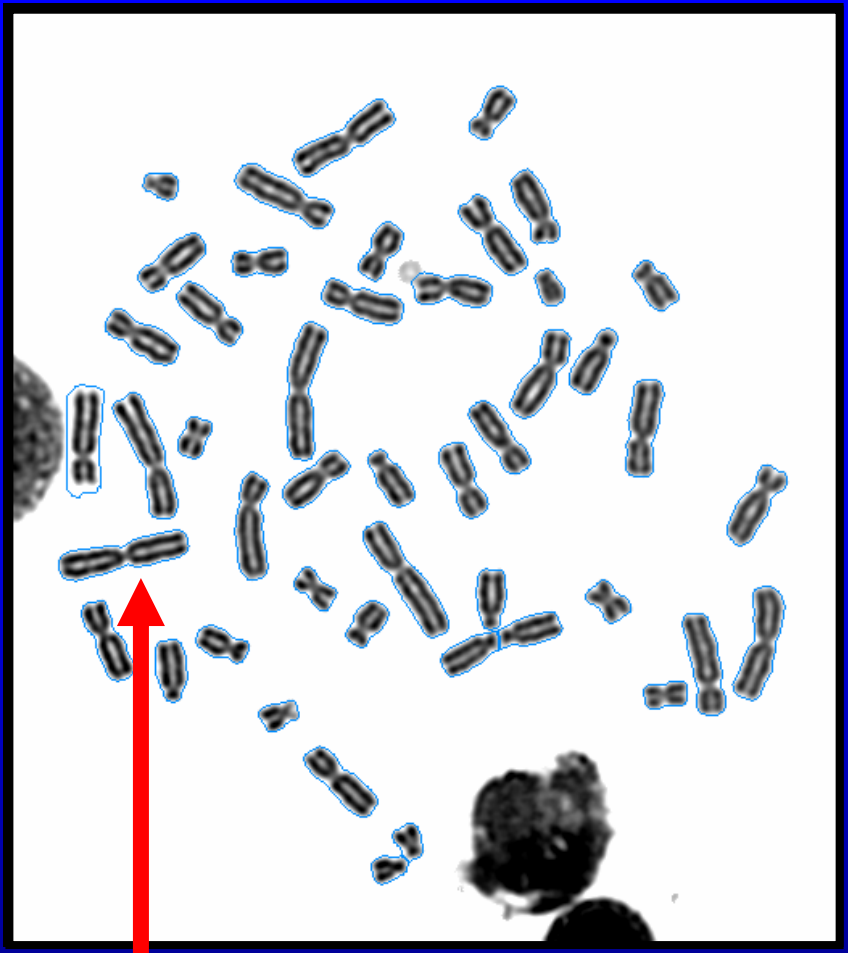


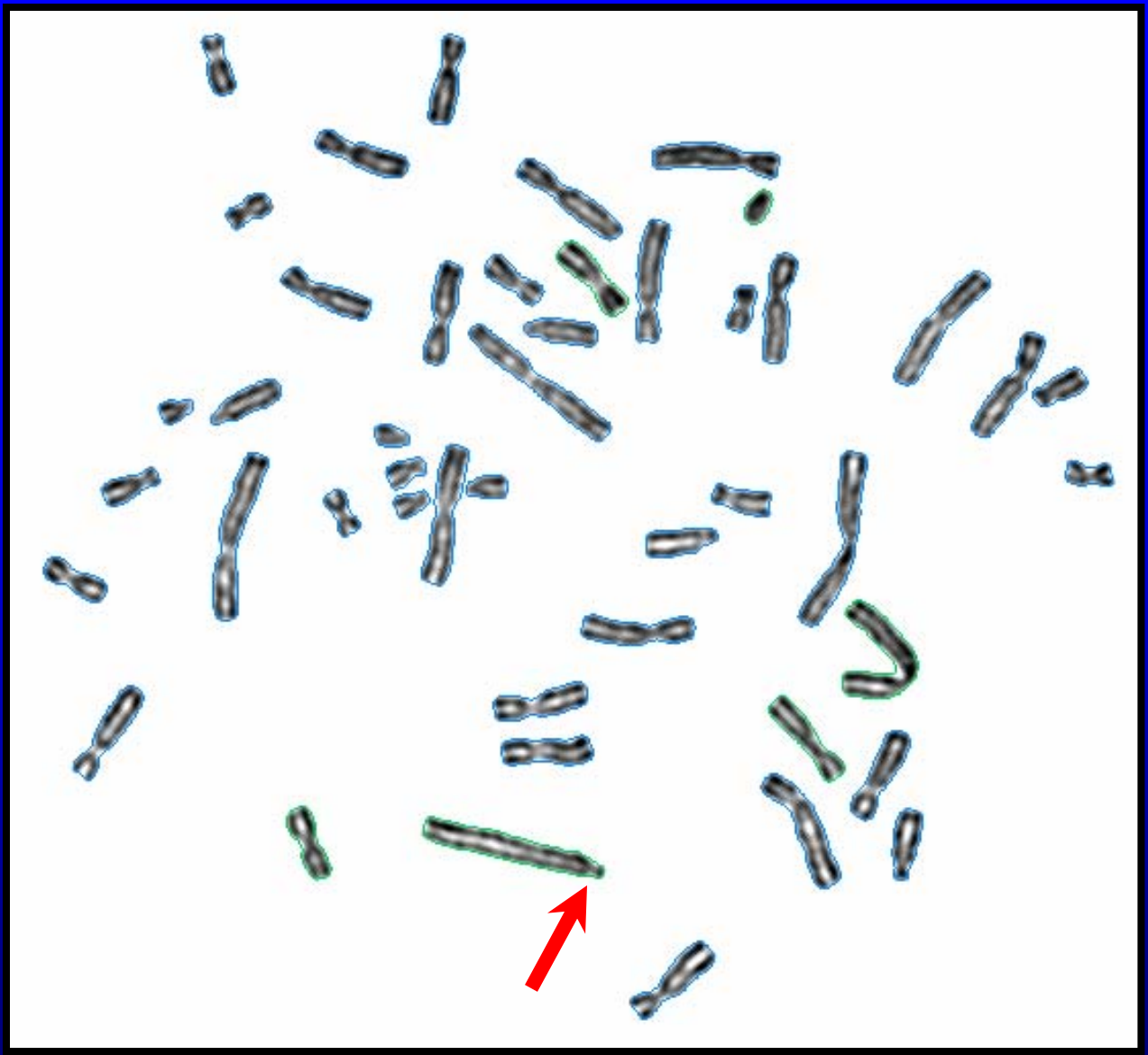
Image of Normal Metaphase Spread

# Automated Karyotyping

| Group | Chromosomes |
|---|---|
| A | 1-3 |
| B | 4-5 |
| C | 6-12, X |
| D | 13-15 |
| E | 16-18 |
| F | 19-20 |
| G | 21-22, Y |



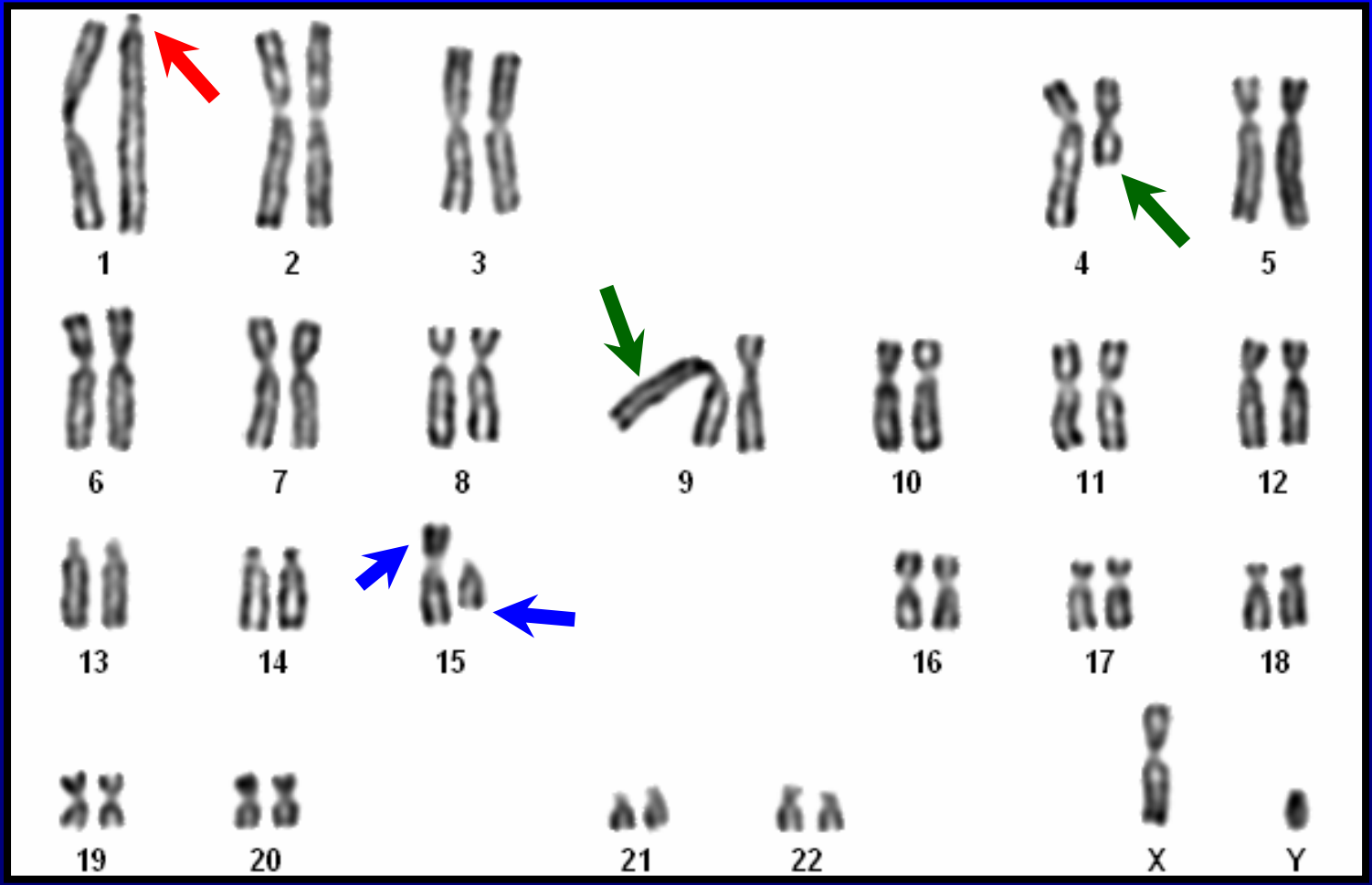


Inversion, Chromosome 1

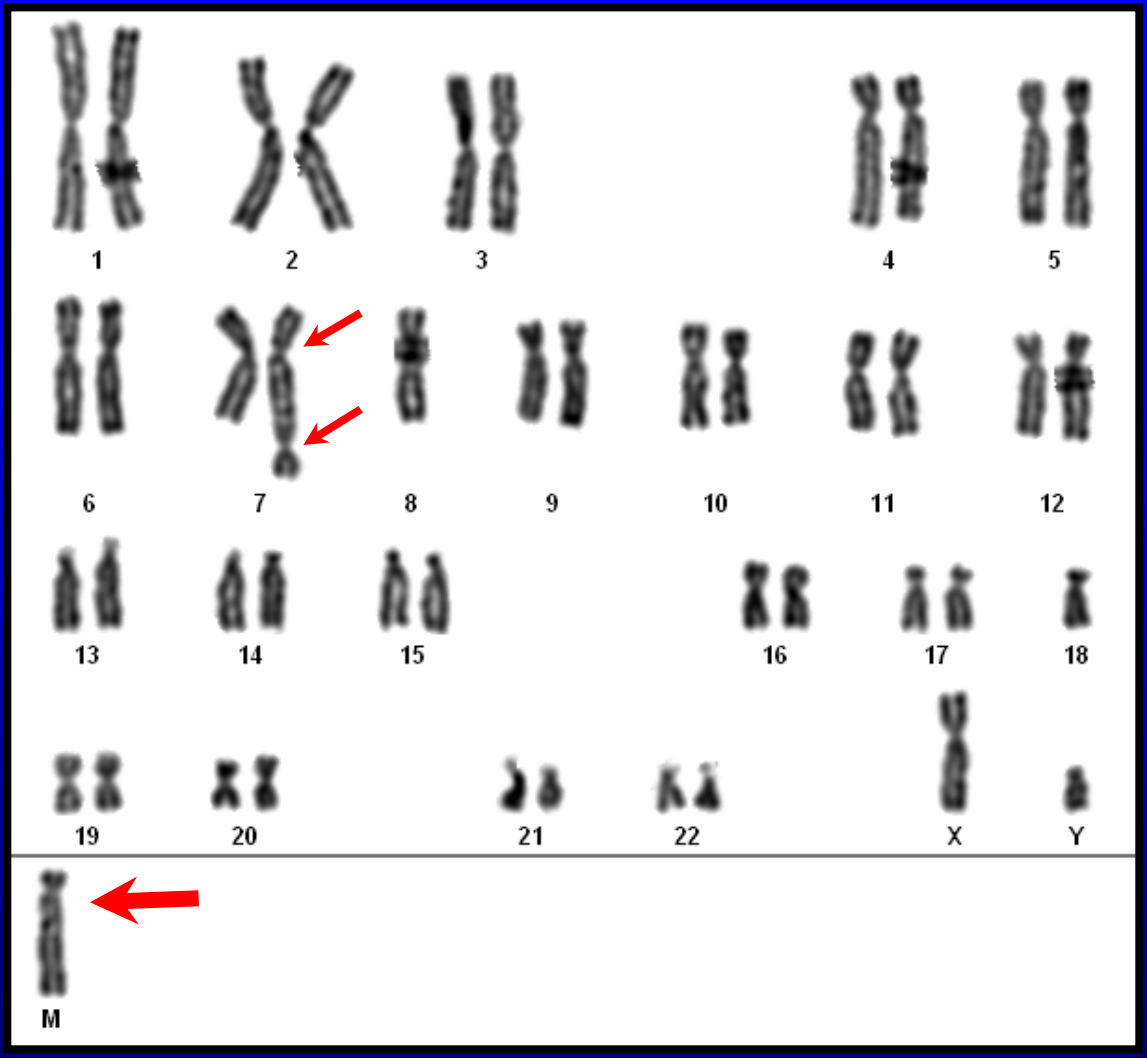
Translocations?

# Karyotype Screening

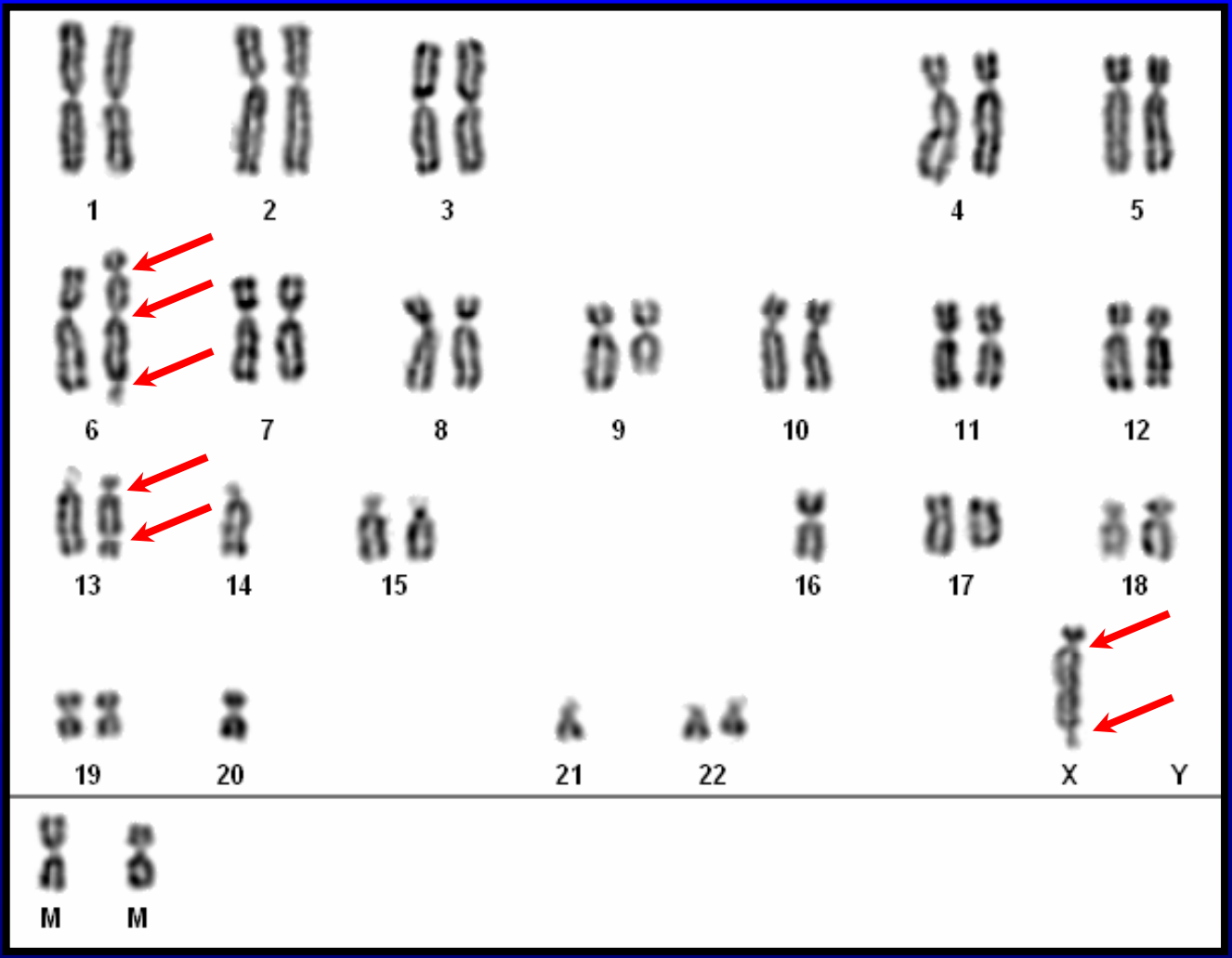
## Inversion (1) / Translocations (4, 9) & (15, 15)



# Dicentric Chromosome 7

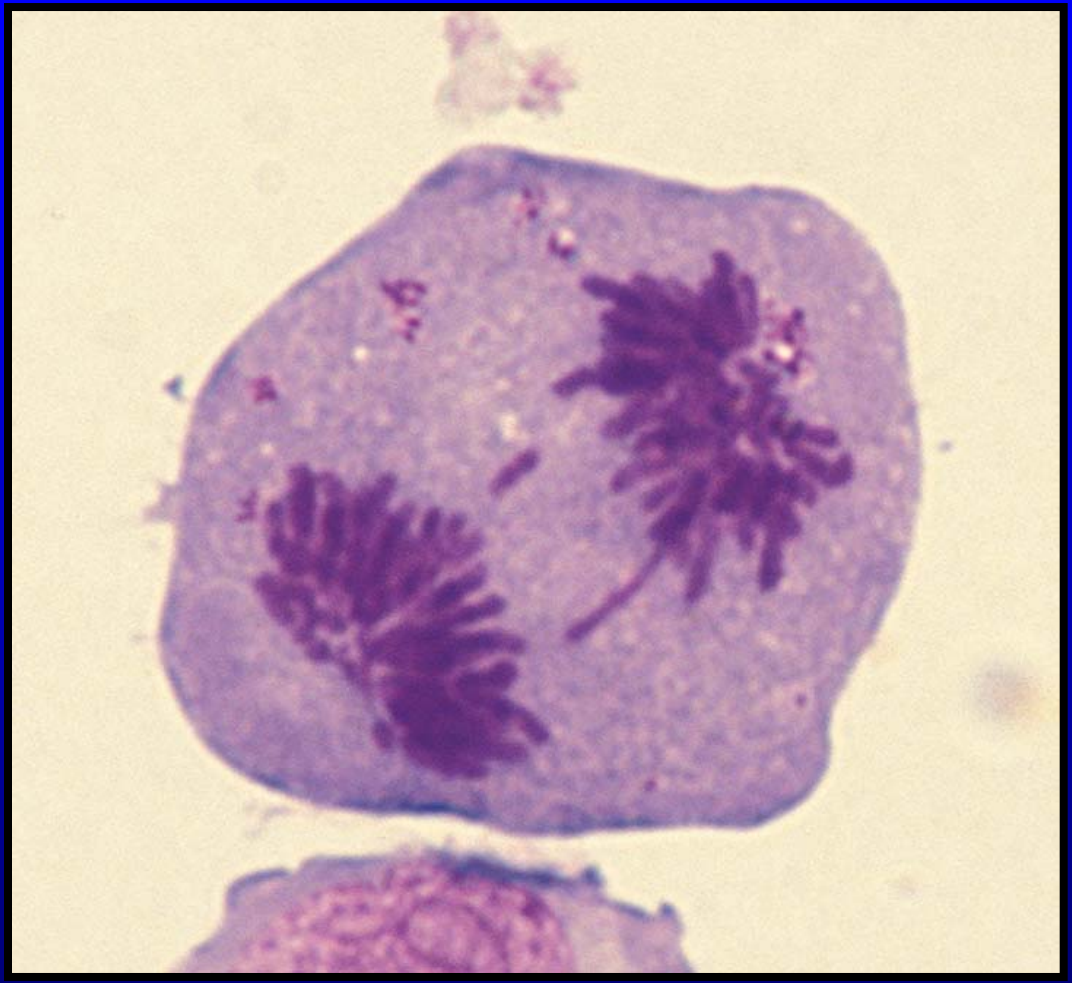


# Tricentric & Dicentrics

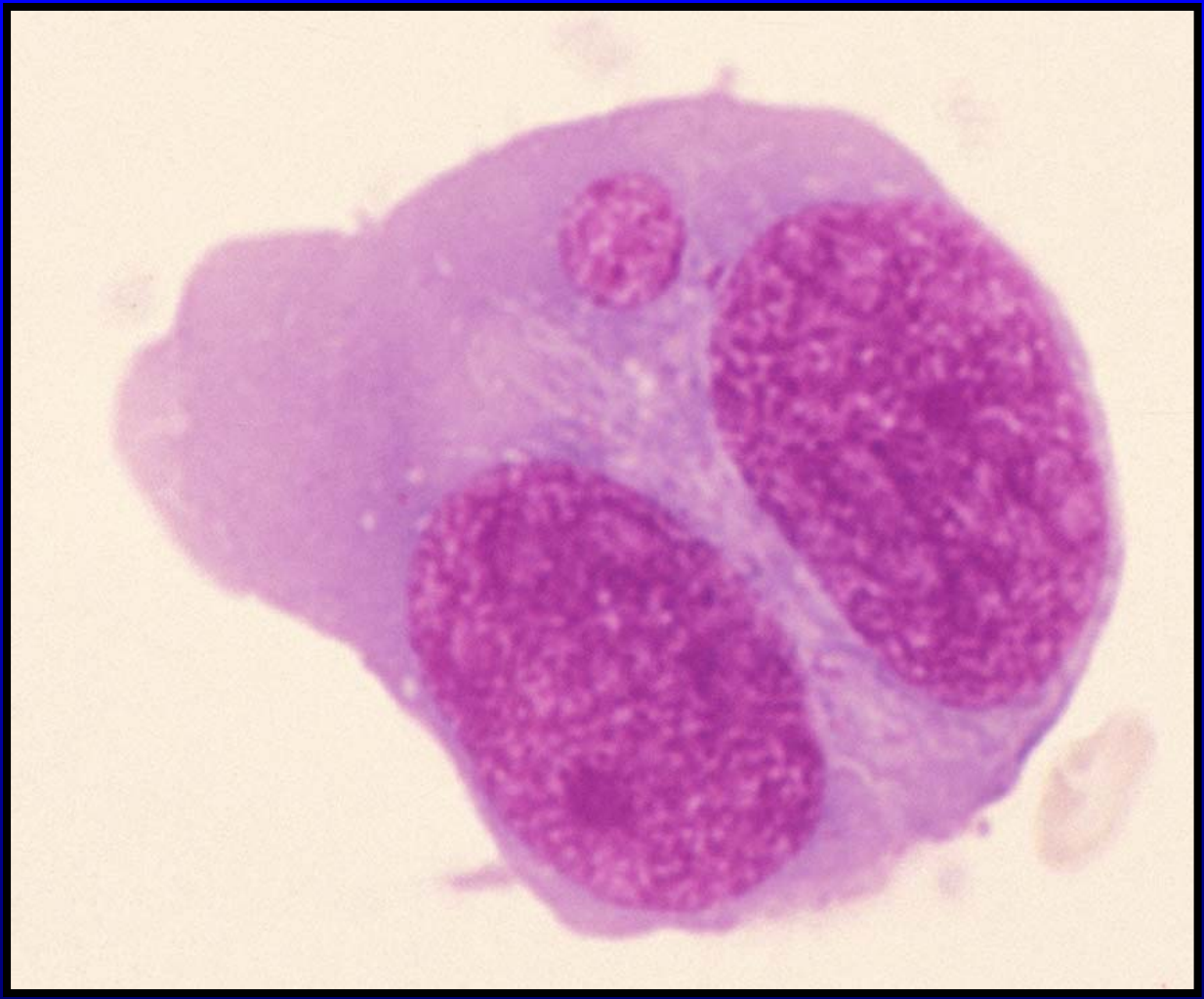


# Lymphocyte in Normal Anaphase

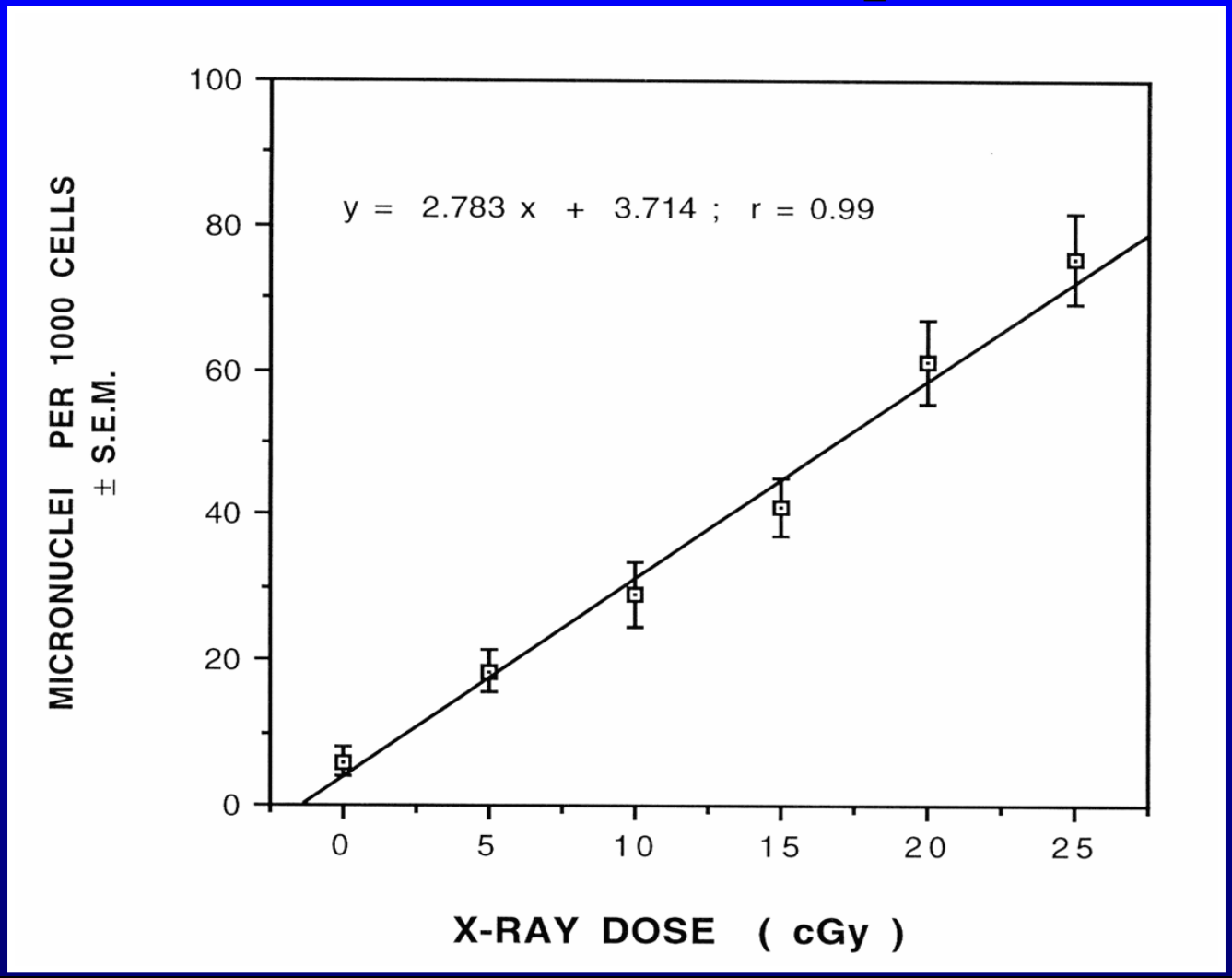
# Lymphocyte with Abnormal Anaphase



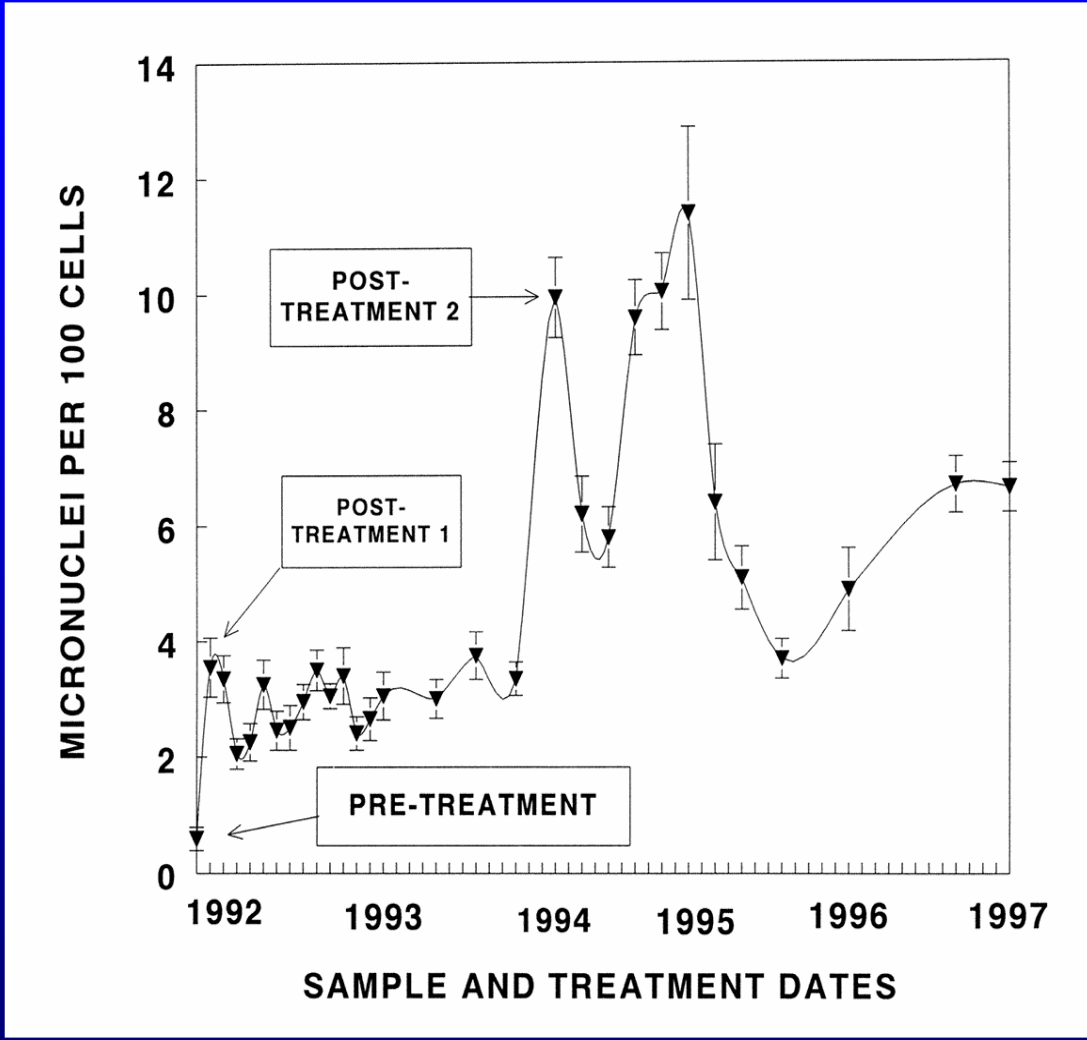
# Bi-nucleated Cell with Micronucleus



# In Vitro Dose Response



# I-131 Time Course Study

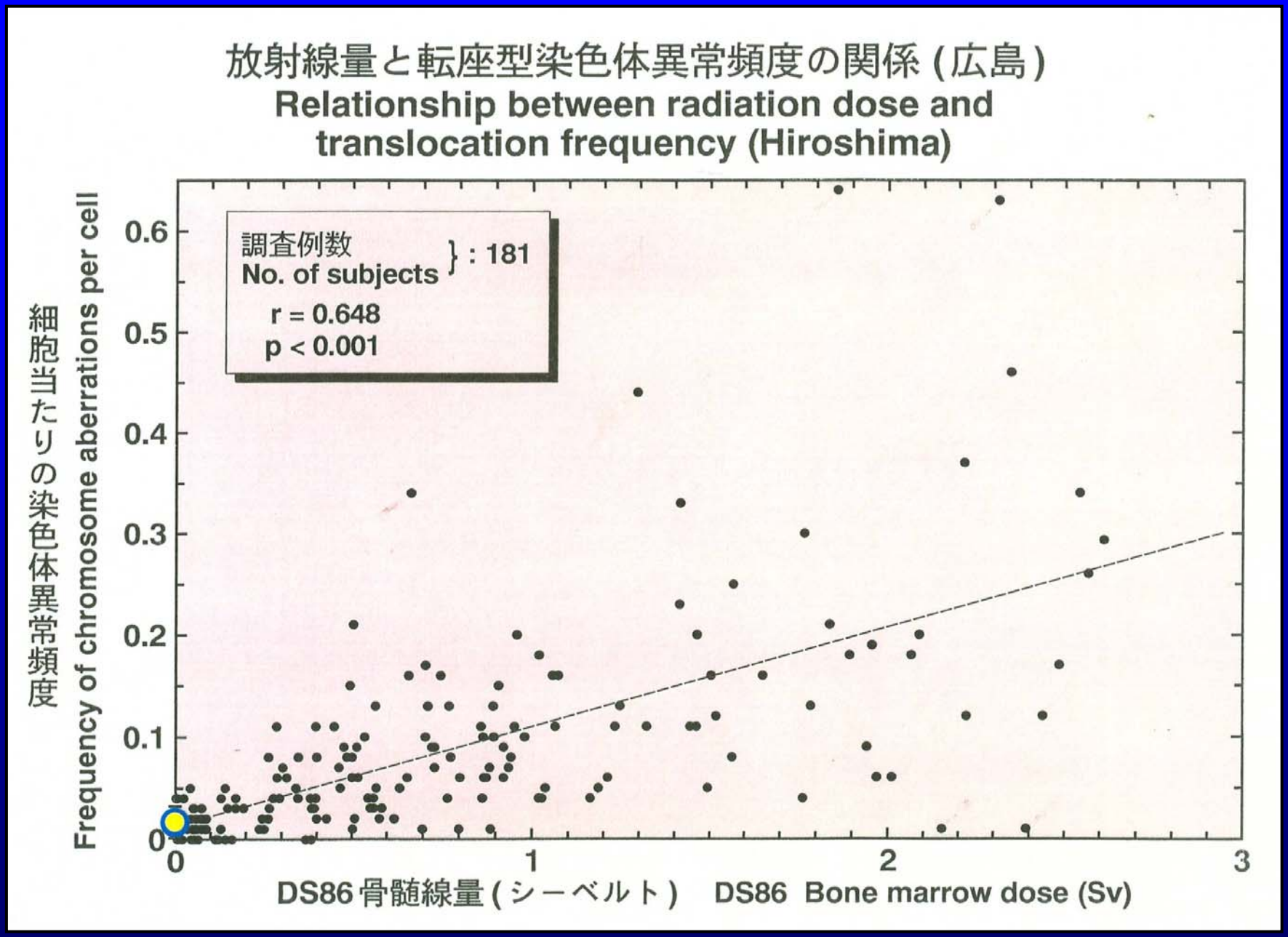


# Cytogenetic Biodosimetry Applications

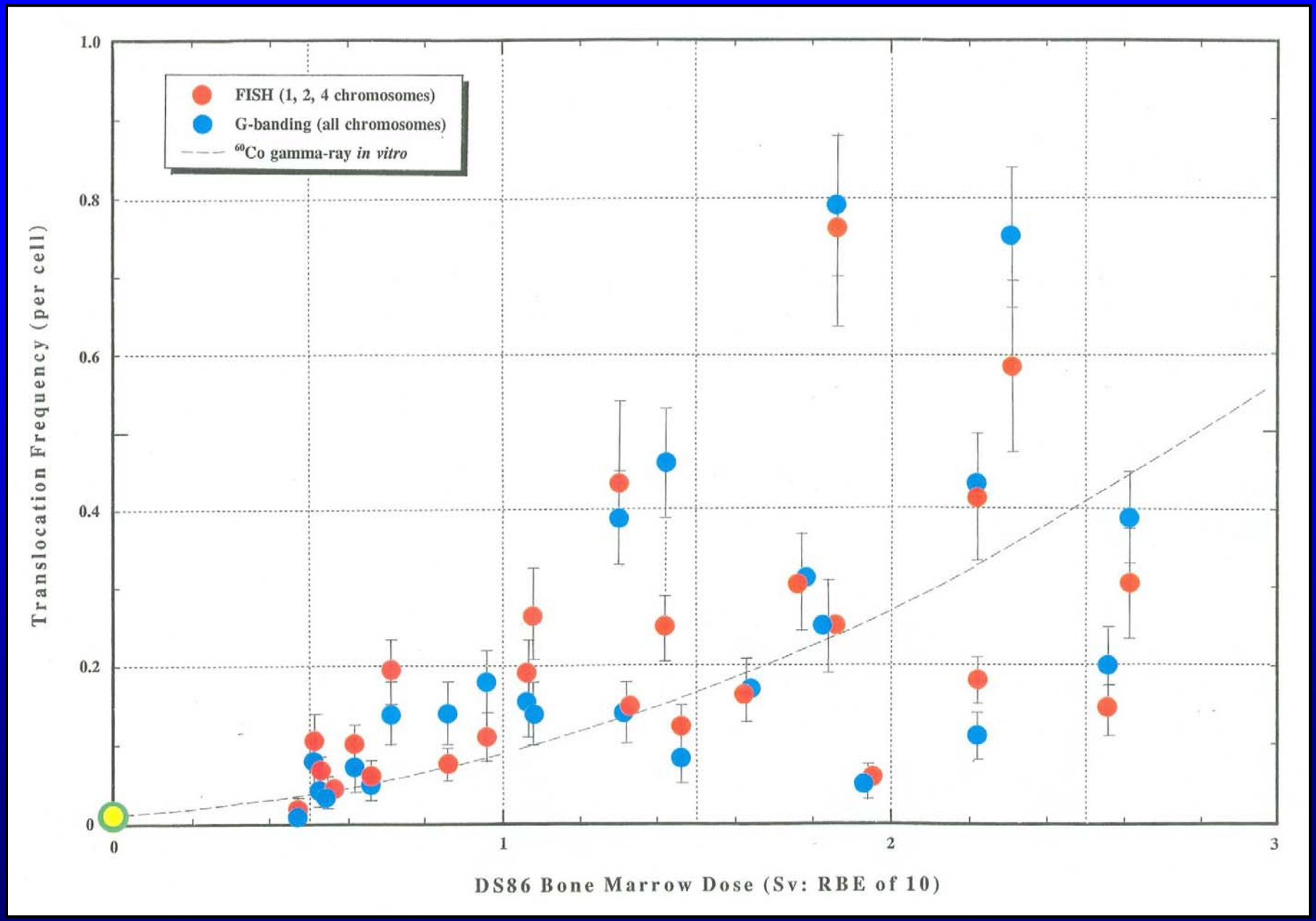
- Events - dosimetry may be in question or non-existent (recent or historical)
  - Tokai-Mura
  - Goiania
  - Emergency response to a radiation exposure event without dosimetry
    - Suspected high dose groups
    - Reassurance of no dose

# Cytogenetic Biodosimetry Applications (cont.)

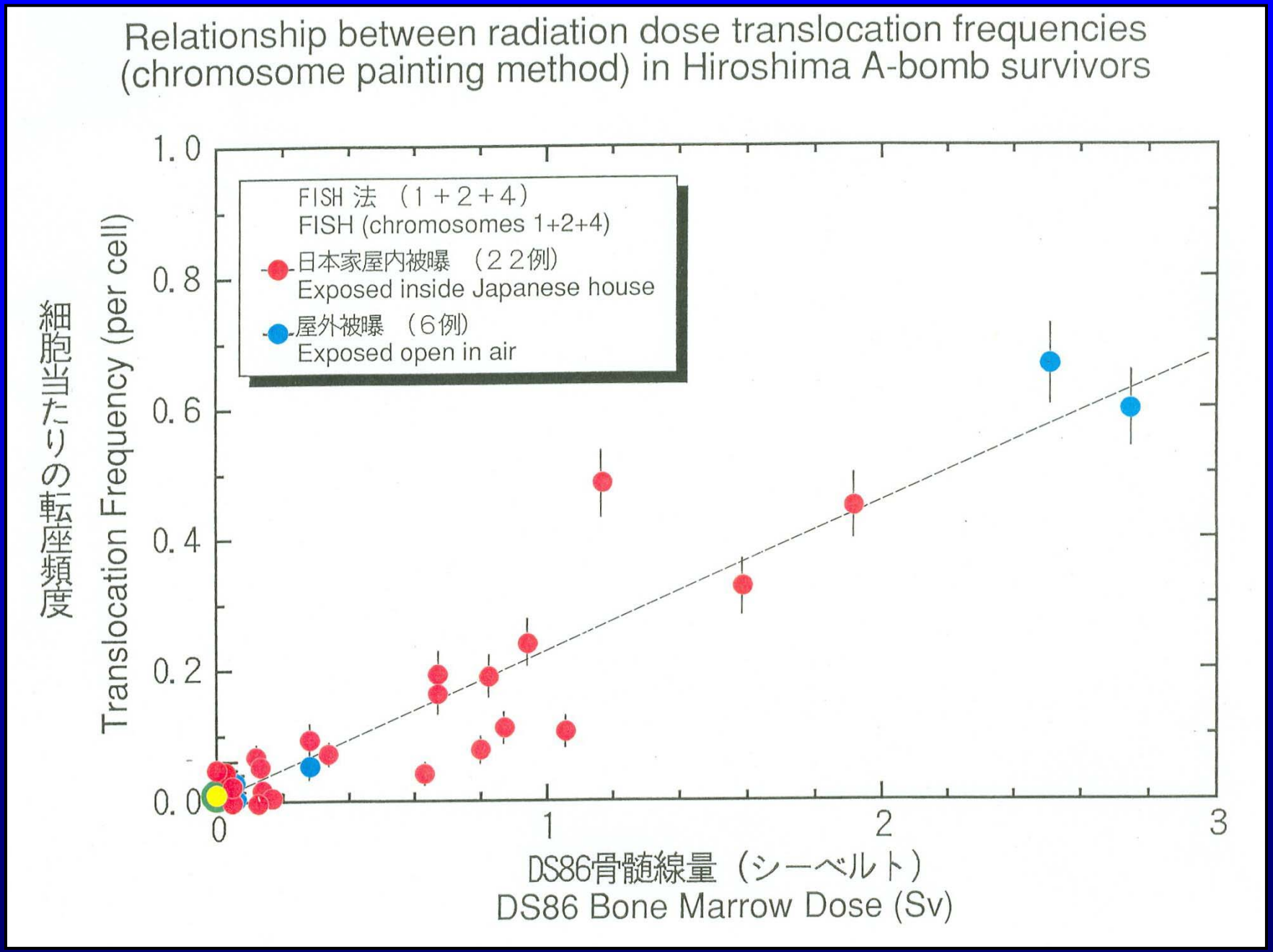
- Occupational
  - Physical dosimetry and routine bioassay, but the dose recorded may be in question.
  - Detection Limits
- Epidemiology for two of the largest radiation exposure populations.
  - Japan (Hiroshima and Nagasaki)
  - Chernobyl
  - Pu workers
    - Internally deposited 239Pu leads to persistent chromosomal aberrations (both stable and unstable)
    - Sellafield, England
    - Rocky Flats, CO




Dr. Akio Awa, RERF (retired)




**Dr. Akio Awa, RERF (retired)**




**Dr. Akio Awa, RERF (retired)**

Original Pu-Worker Study funded by:

- U.S. Department of Energy (DOE) and
- German Federal Office of Radiation Protection

Follow-up study funded by:

- National Research Council,
- NIOSH, and
- DOE.

The re-establishment of the REAC/TS Cytogenetic Biodosimetry Laboratory was supported by:

- DOE-NNSA,
- DOE-HSS, and
- Nuclear Regulatory Commission.

# *Questions*