Radiation Biodosimetry II.
FISH / Cytogenetics

Jim Tucker, Ph.D.
Department of Biological Sciences
Wayne State University

jtucker@biology.biosci.wayne.edu

May 10, 2004

WAYNE STATE UNIVERSITY

Outline of this Talk

- Cell and chromosome biology
- Chromosome aberrations
  - unbanded
  - banded - karyotyping
  - painting
- Lessons learned human studies
  - importance of age, smoking, genotype
- Biodosimetry / translocation persistence
- Risk Analysis
- Controls
- New methods
- Summary

Cell and Chromosome Biology

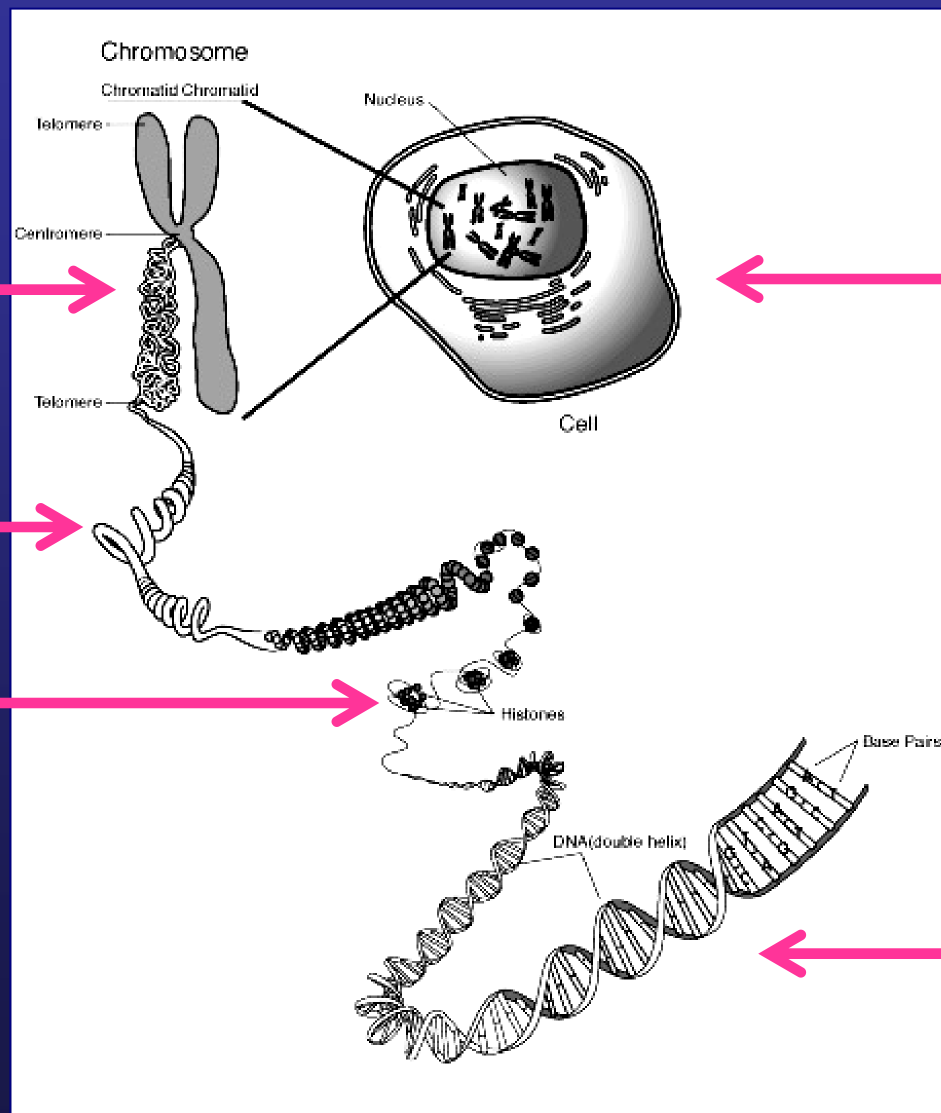
Chromosome

Genes

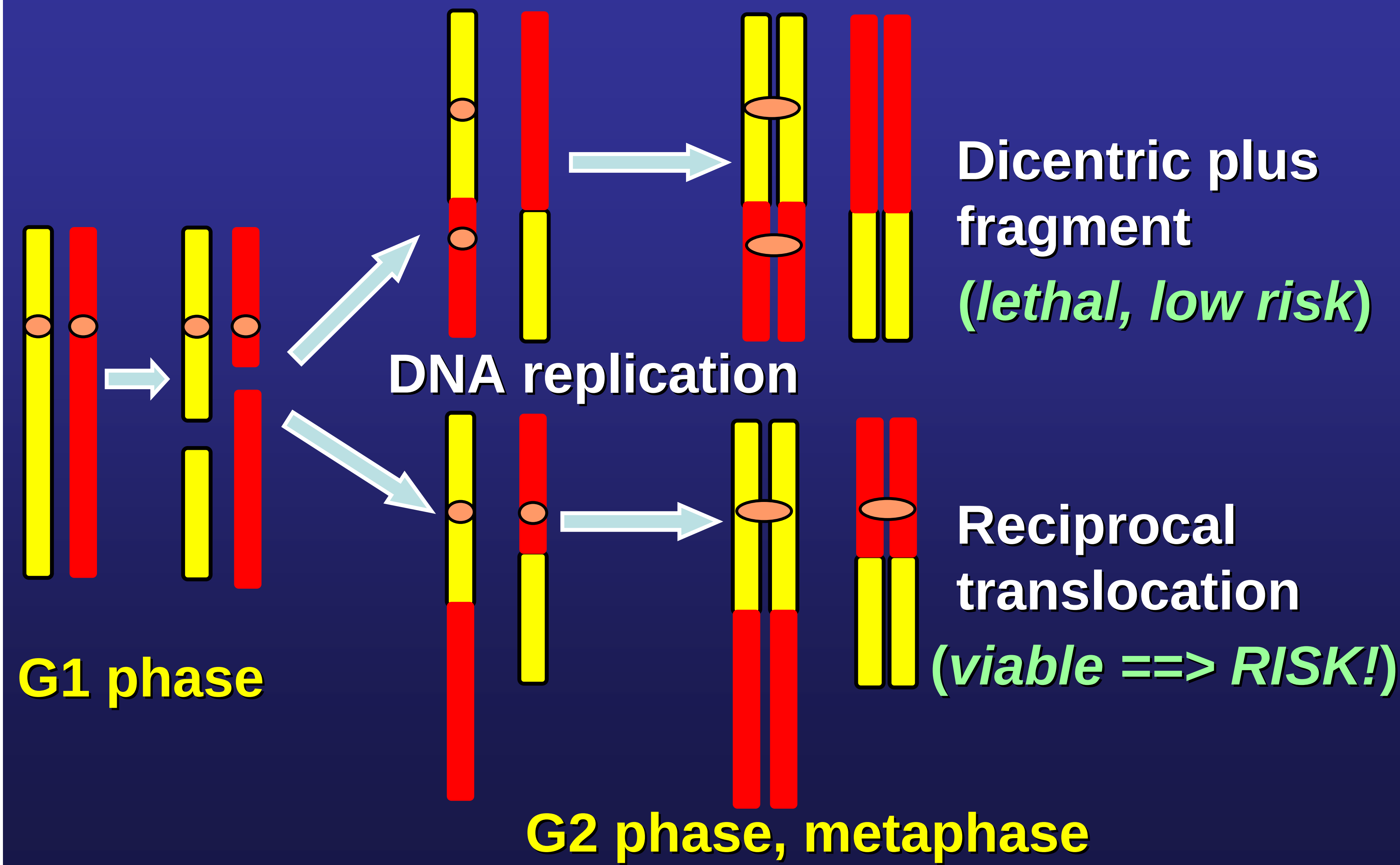
Histones

Cell

DNA



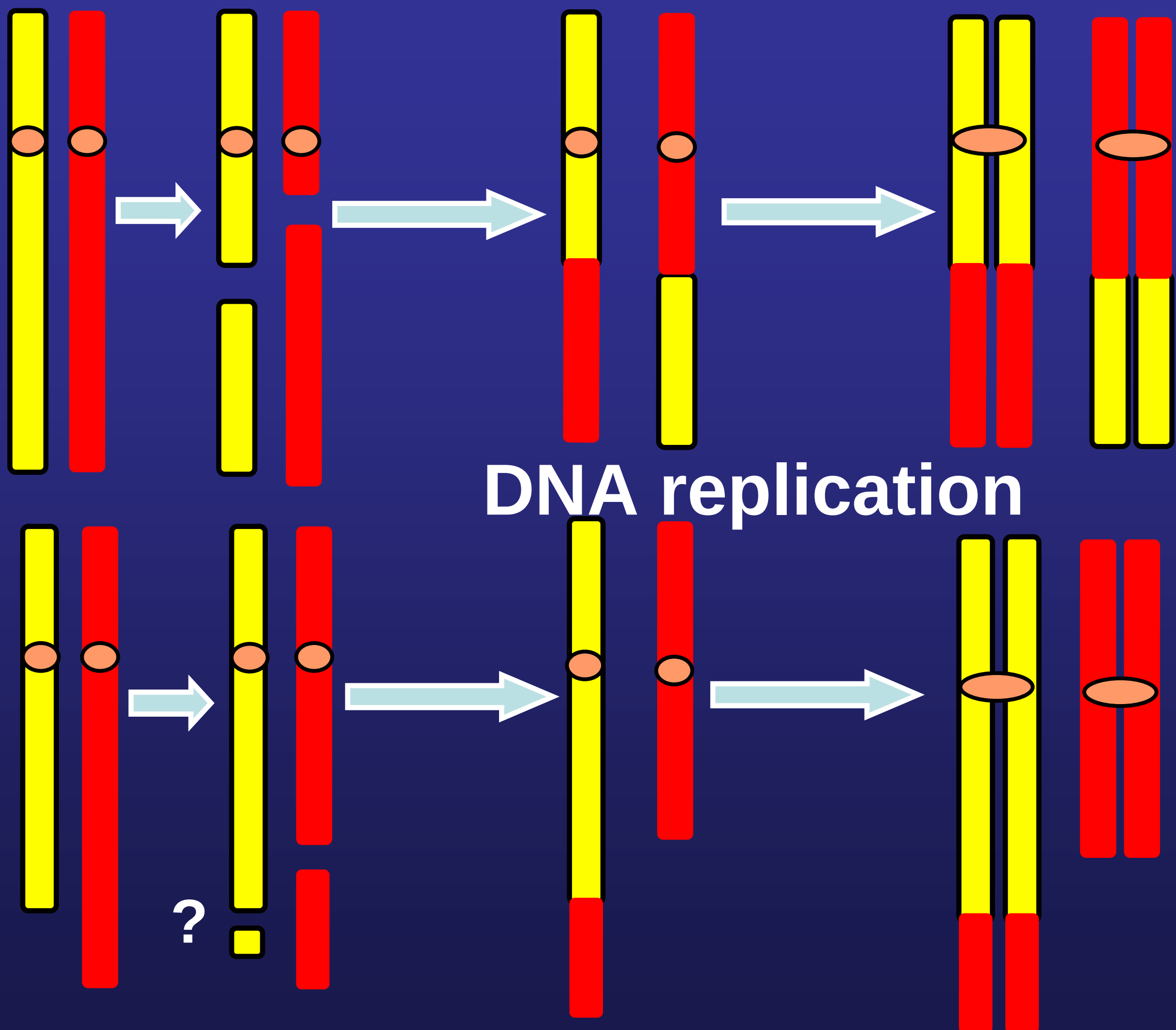
Chromosome Aberrations: Translocations and Dicentrics



Characteristics of Translocations

- Induced at frequencies equal to dicentrics
- Stable through cell division
  persist in vivo for many years
  (whereas dicentrics disappear rapidly)
- Dosimetry for acute exposure is known
- Accumulate with chronic exposure
- Ideal for biodosimetry ("Gold Standard")

Reciprocal and Non-Reciprocal Translocations



Reciprocal translocation

fully viable?

Non-reciprocal translocation

not fully viable?

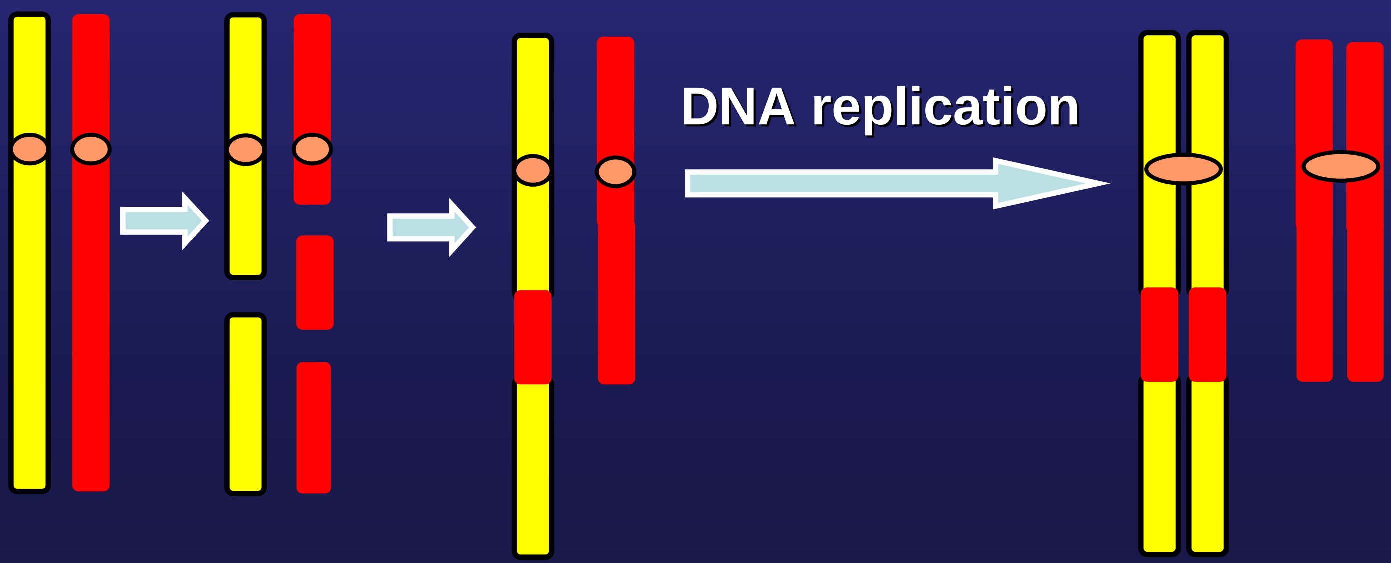
G1 phase

G2 phase, metaphase

Chromosome Aberrations: Rings and Insertions

Ring plus fragment

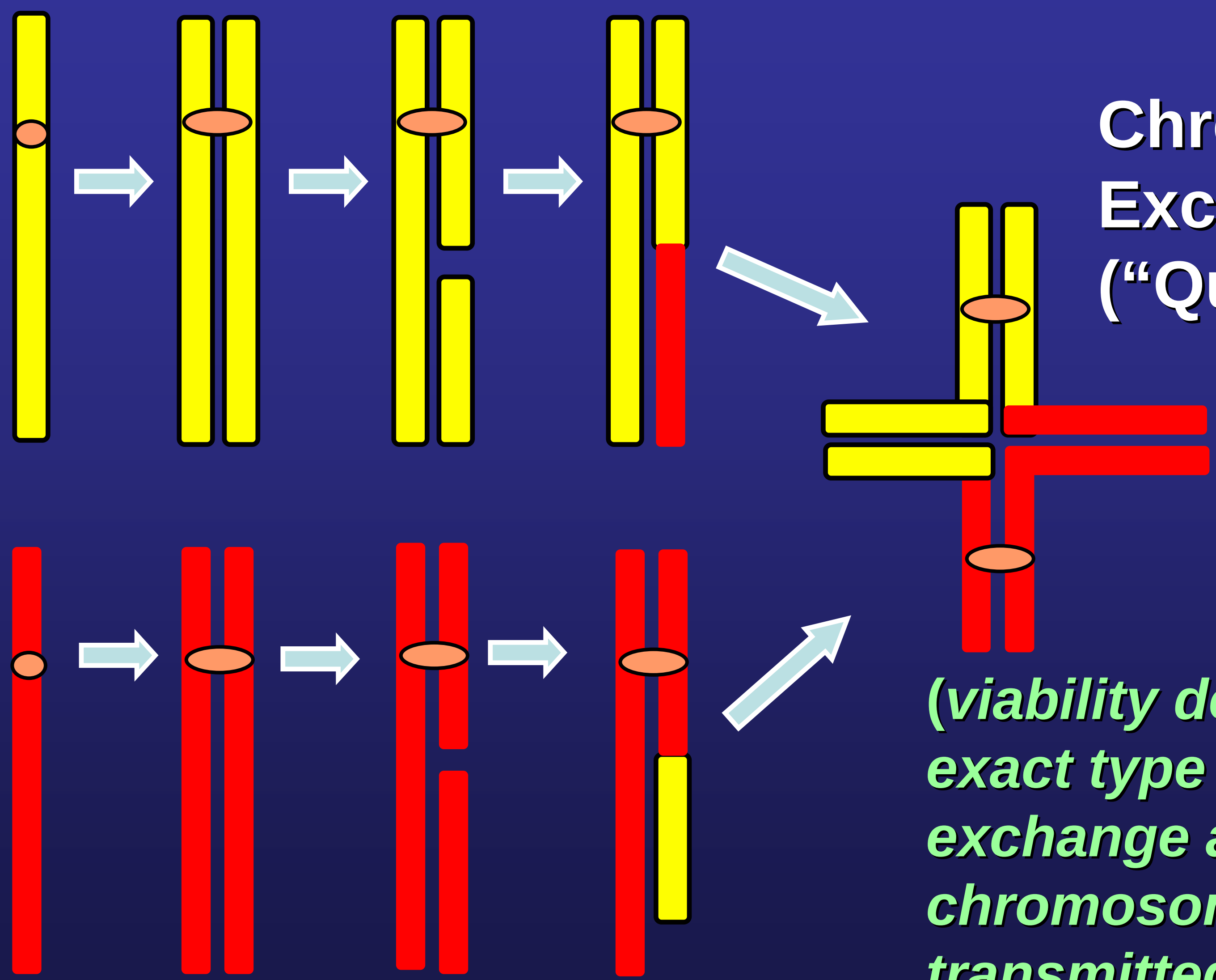
(lethal)



Insertion

(viable)

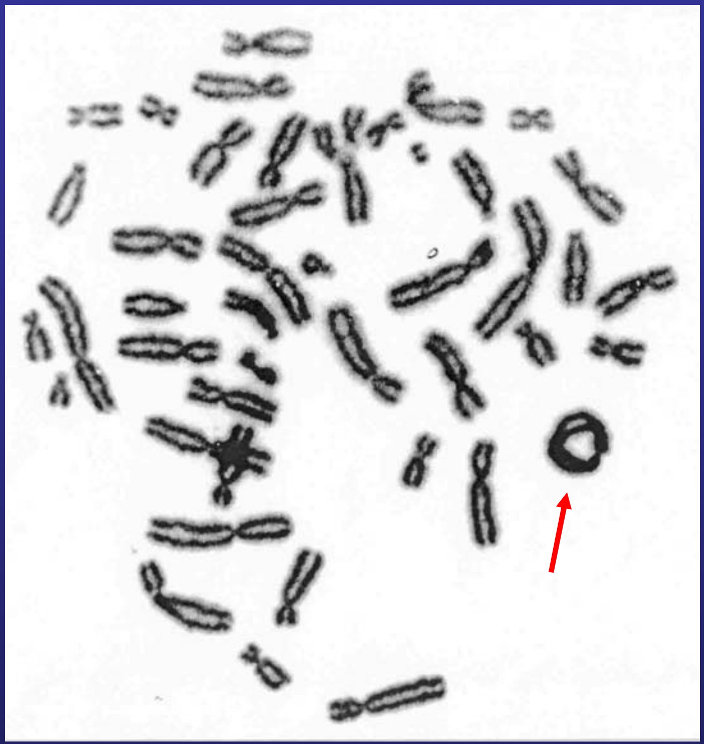
Chromatid Aberrations - One Example



Chromatid Exchange ("Quadriradial")

(viability depends on exact type of exchange and which chromosomes are transmitted together)

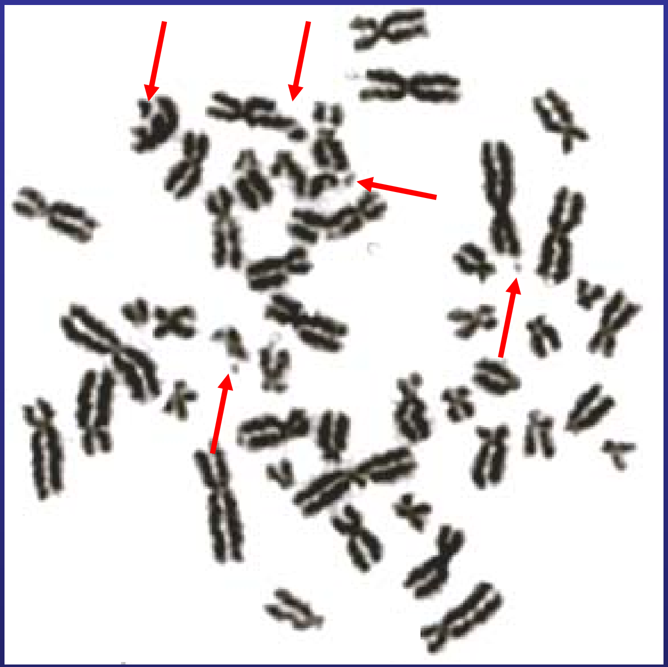
Unbanded Chromosomes



- Detects "unstable" events
- Used widely in research
- Moderate analysis speed
- Inexpensive reagents

Giemsa Stained Metaphase

Chromosome Aberrations - Unbanded



Human cell in metaphase stained with Giemsa

With conventional stains, some categories of chromosome aberrations cannot be seen.

Fragments - yes

Dicentrics - yes

Chromatid damage - yes

Translocations - generally no

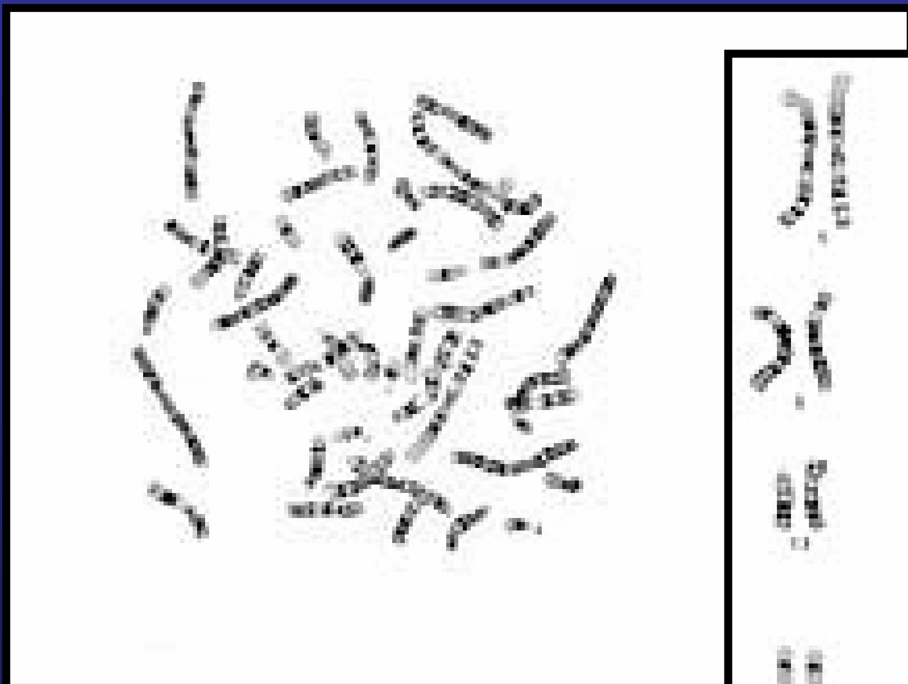
Insertions - no

Complex rearrangements - no

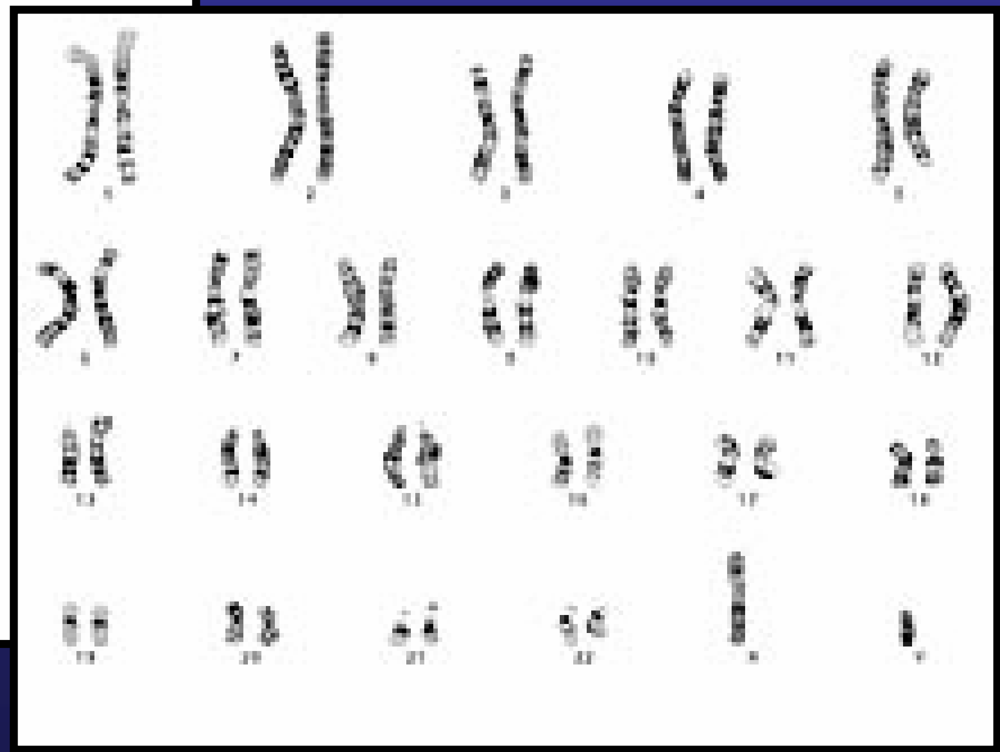
Resolution is limited!

Karyotyping

Giemsa Banded Cell

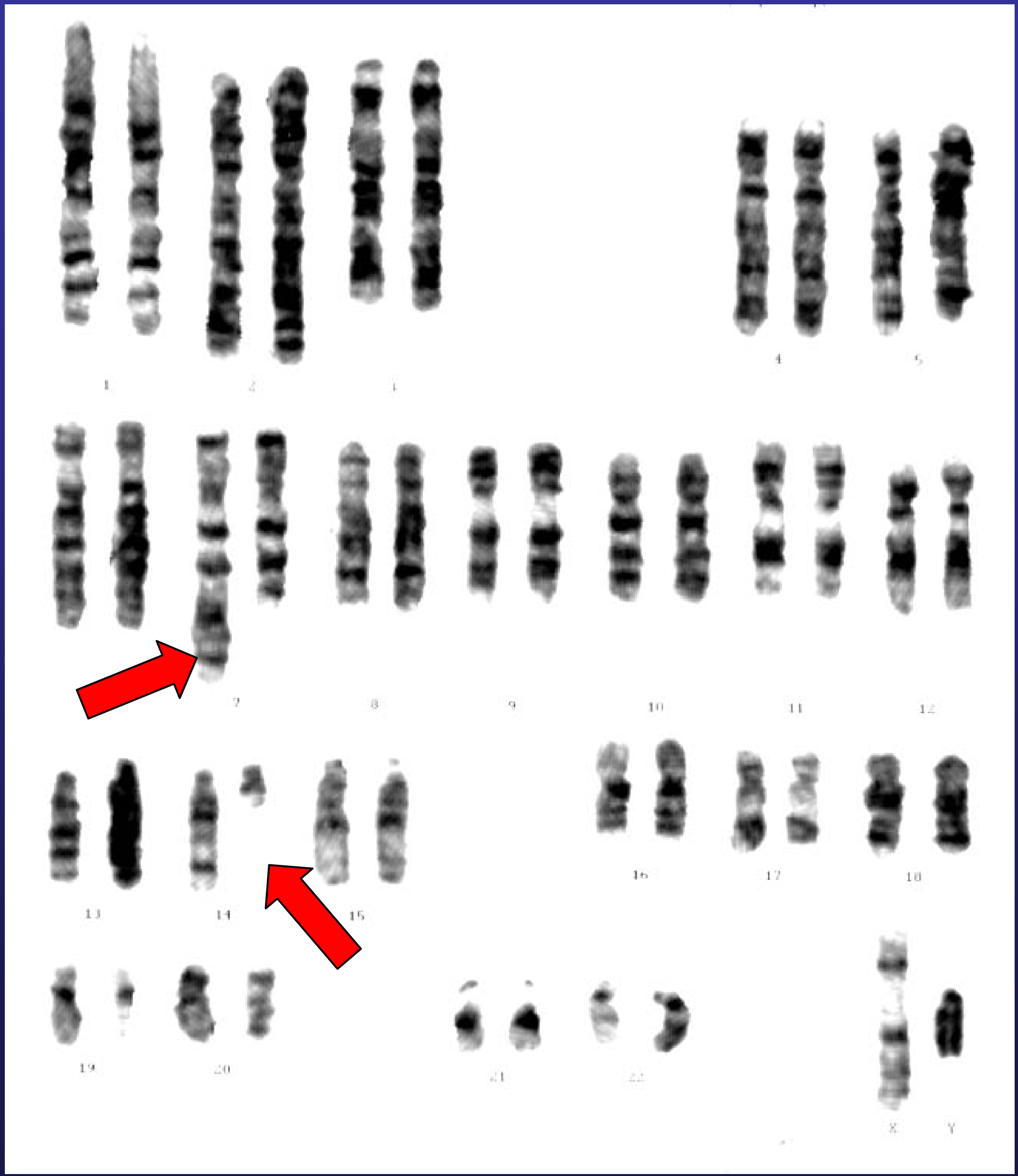


Metaphase



Karyotype

Karyotype with a translocation



Slow and expensive!

Karyotyping

With chromosome banding, all categories of chromosome aberrations can be seen.

Fragments - yes

Dicentrics - yes

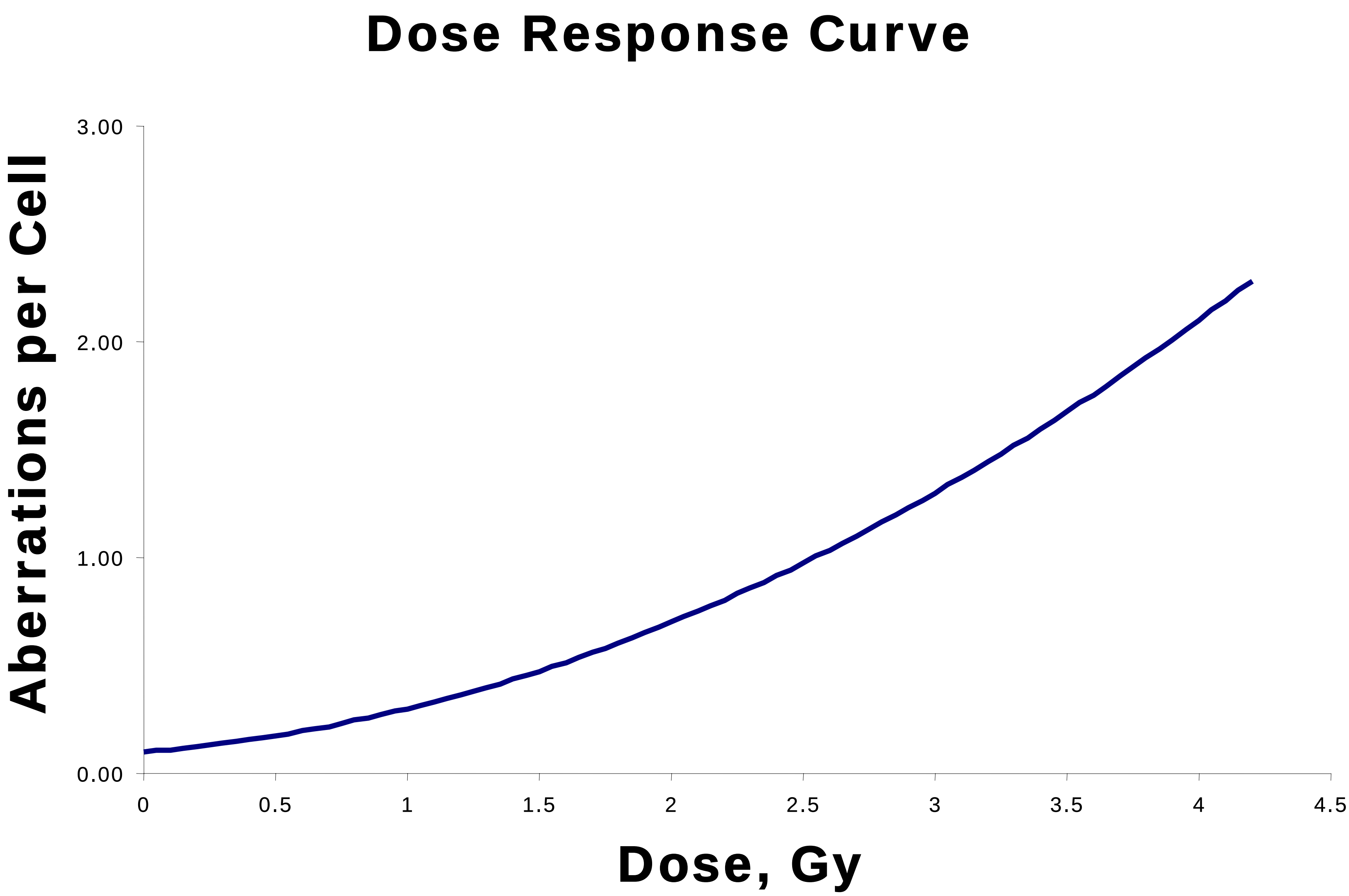
Chromatid damage - yes

Translocations - yes

Insertions - yes

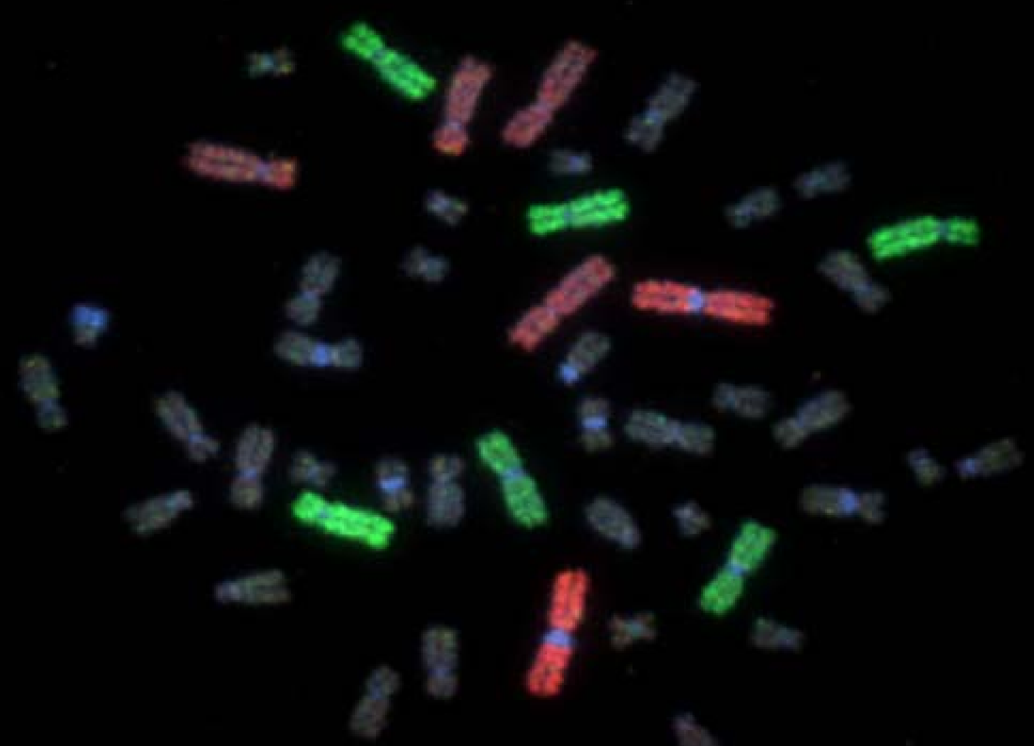
Complex rearrangements - yes

Speed is limited!


Dose Response Curve
Aberrations per Cell
3.00
2.00
1.00
0.00
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
Dose, Gy

Human Chromosome Painting

Normal chromosomes



Chromosomes 1, 2, and 4 are painted red; 3, 5, and 6 are painted green.

What is the difference between FISH and Chromosome Painting?

FISH: fluorescence in situ hybridization

Chromosome painting: one of many applications of FISH

Not all chromosome painting is done by FISH

Not all FISH is chromosome painting

Chromosome Painting

Chromosome painting is accomplished by using a cocktail containing many thousands of DNA sequences.

Each sequence in the cocktail is unique to a single chromosome type.

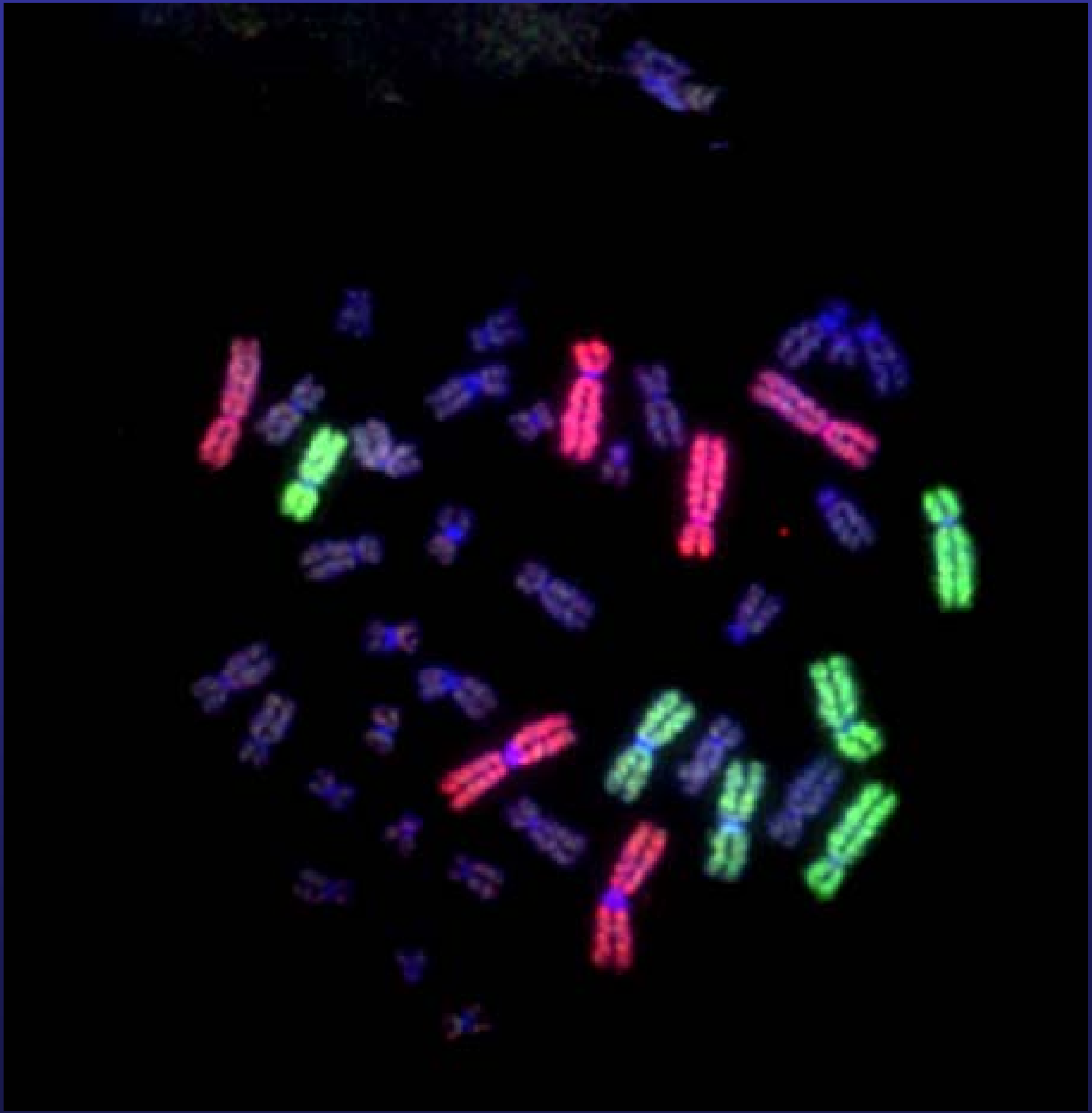
Chromosome Painting

The cocktail of DNA is made into a probe by labeling that DNA with nucleotides modified with a covalently attached fluorochrome.

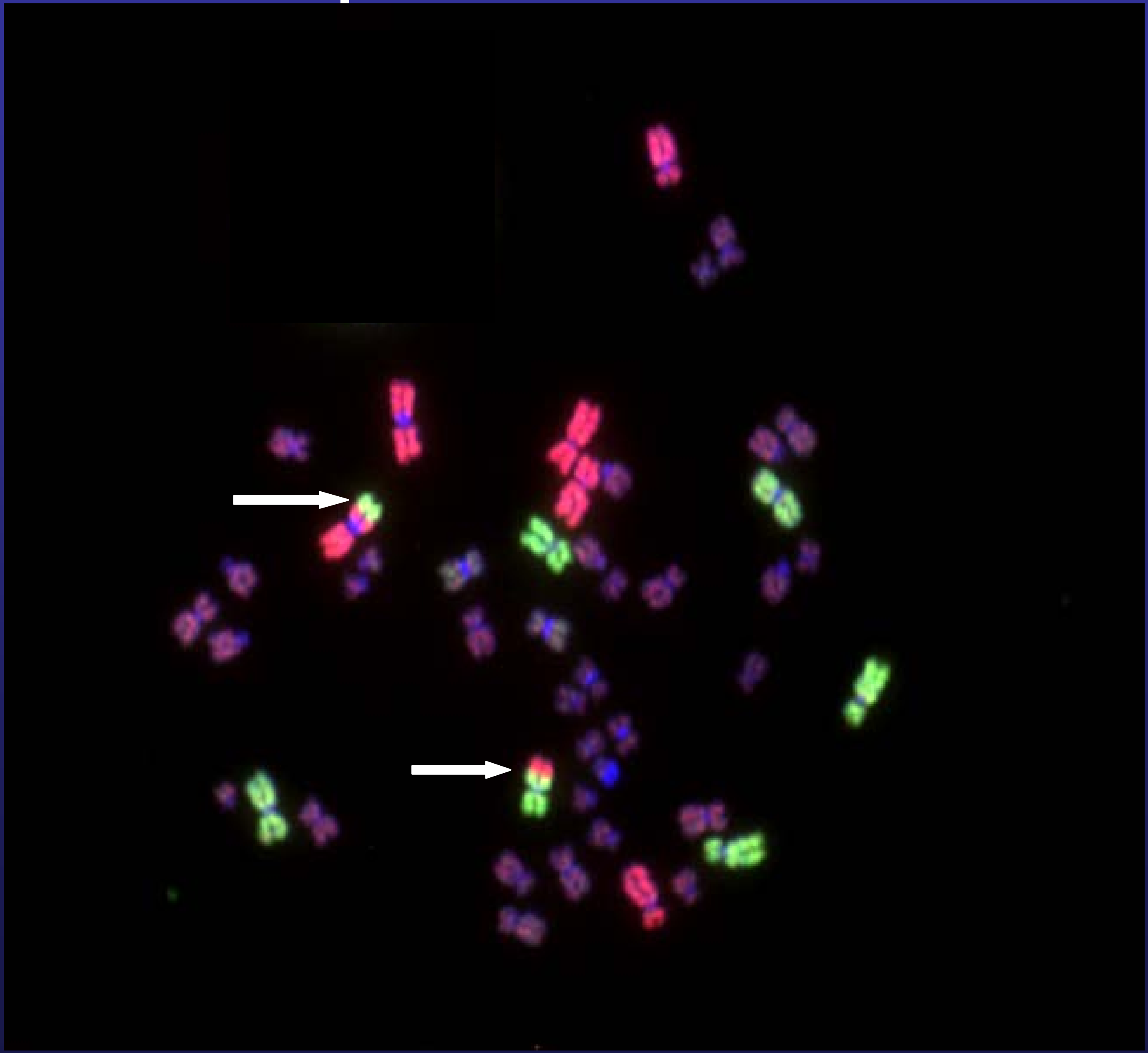
The probe is then hybridized to the slide containing the chromosomes to be studied.

Multiple colors are achieved by using a different fluorochrome for each probe cocktail.

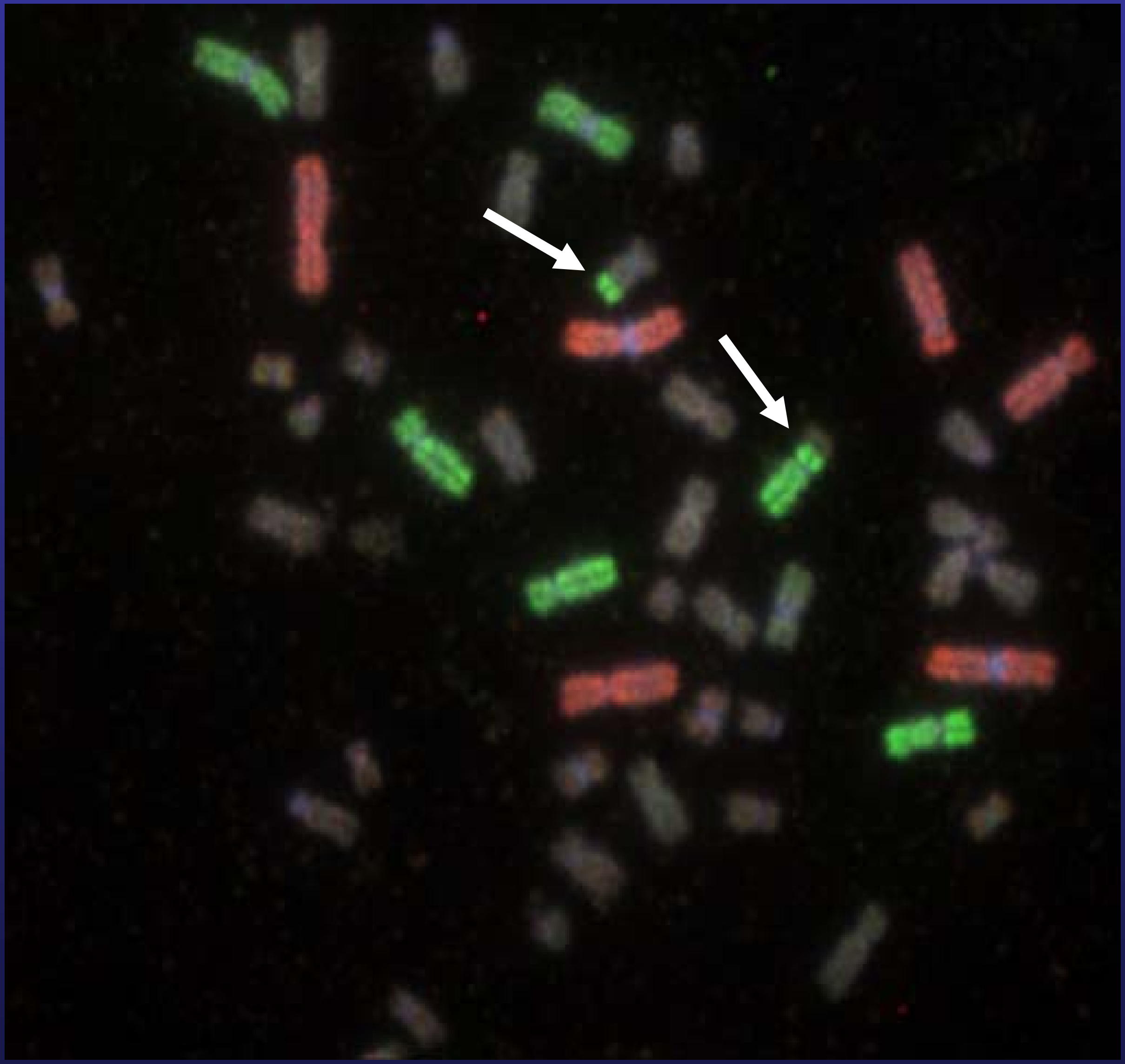
Normal chromosomes



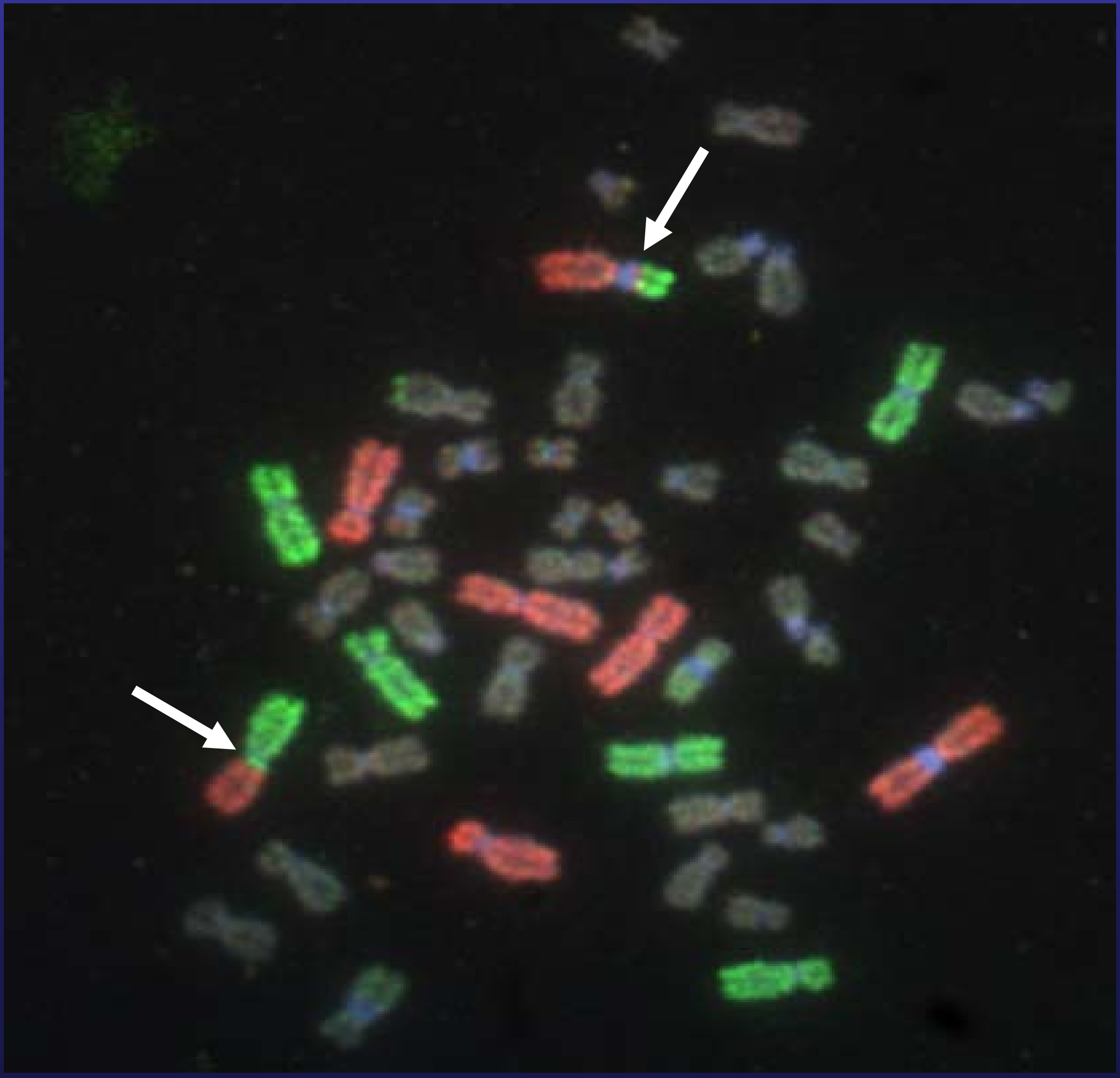
Reciprocal translocation



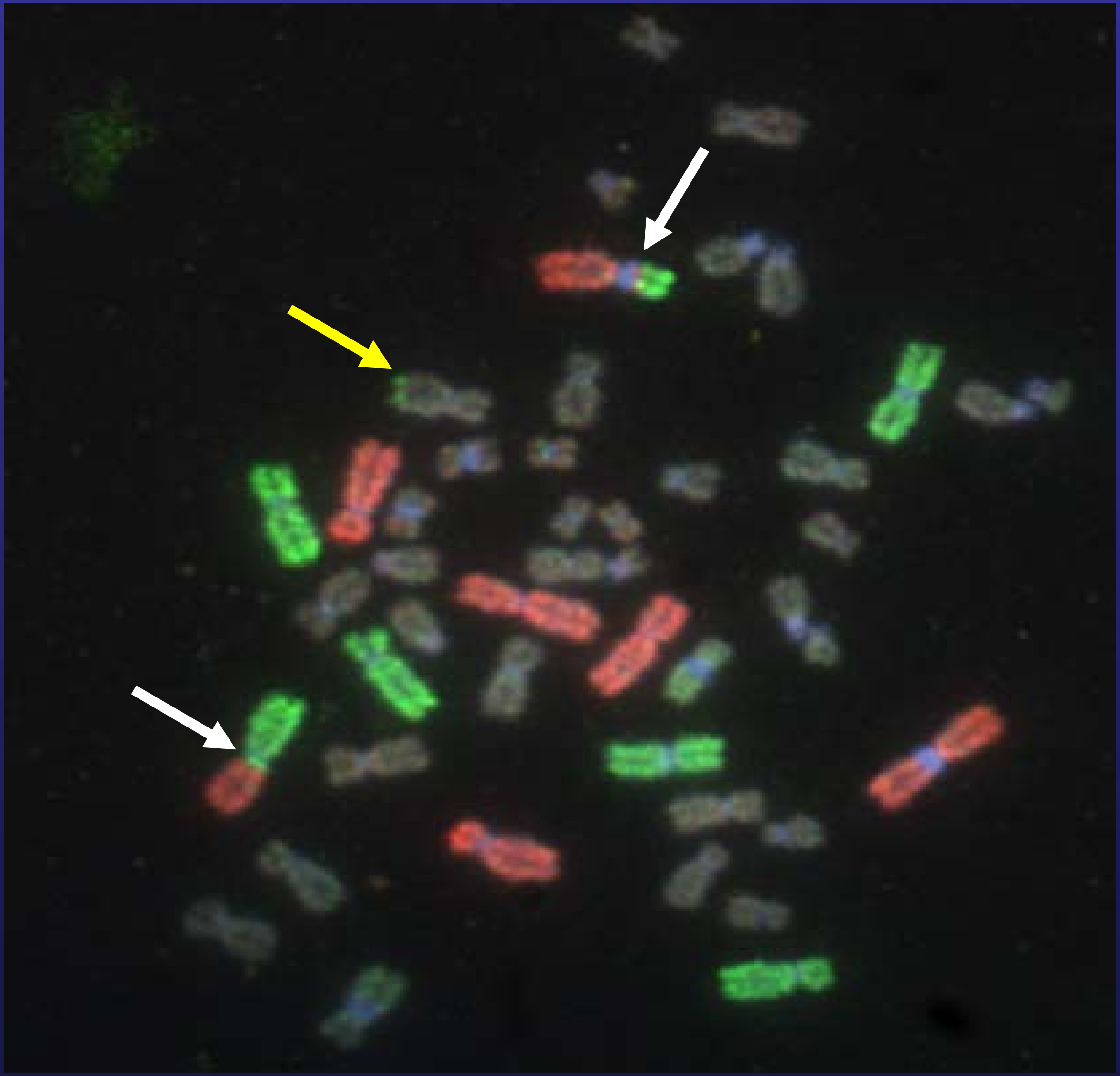
Reciprocal translocation



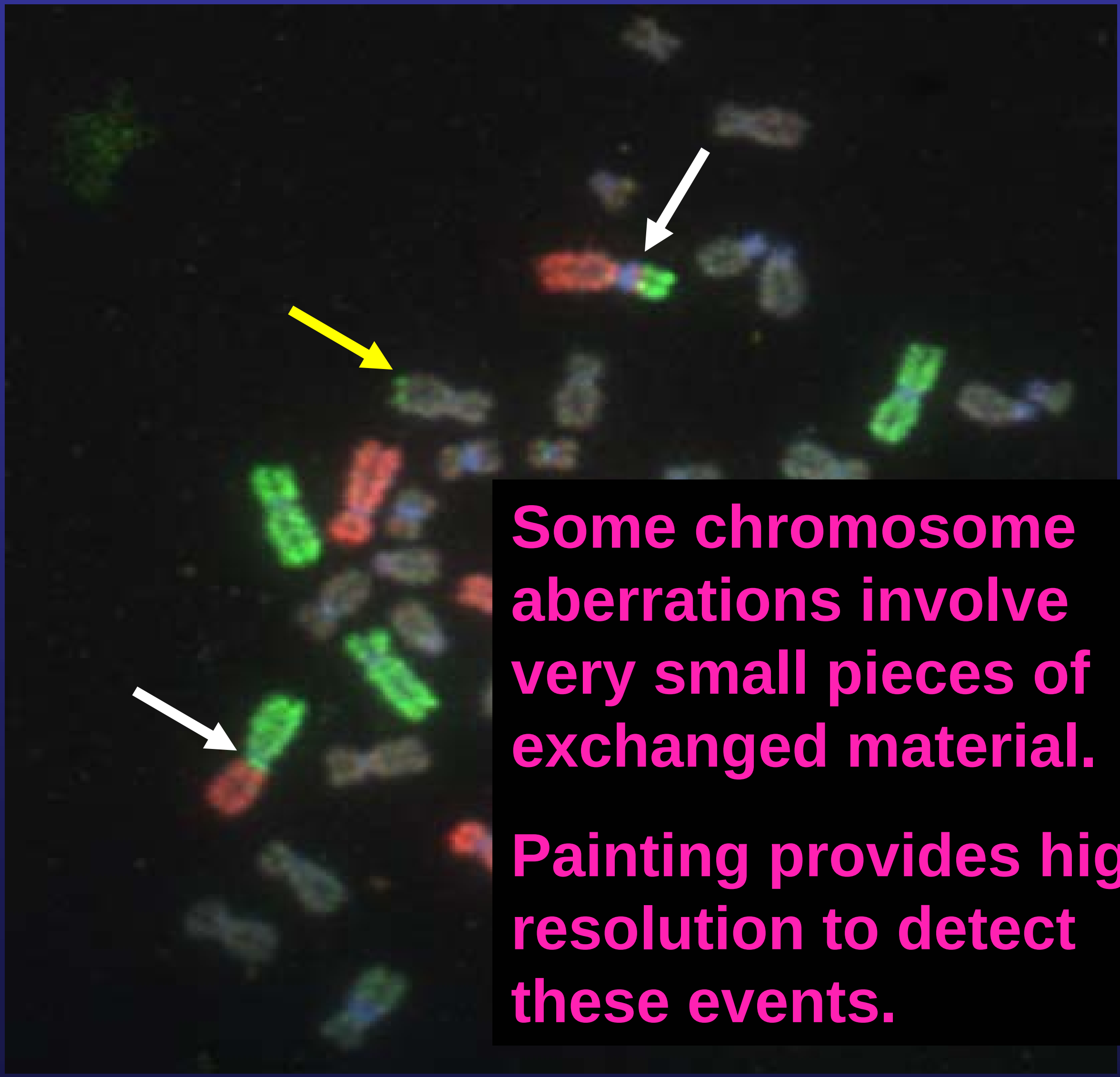
Reciprocal translocation, and . . . ?



Reciprocal Translocation, and a non-reciprocal translocation



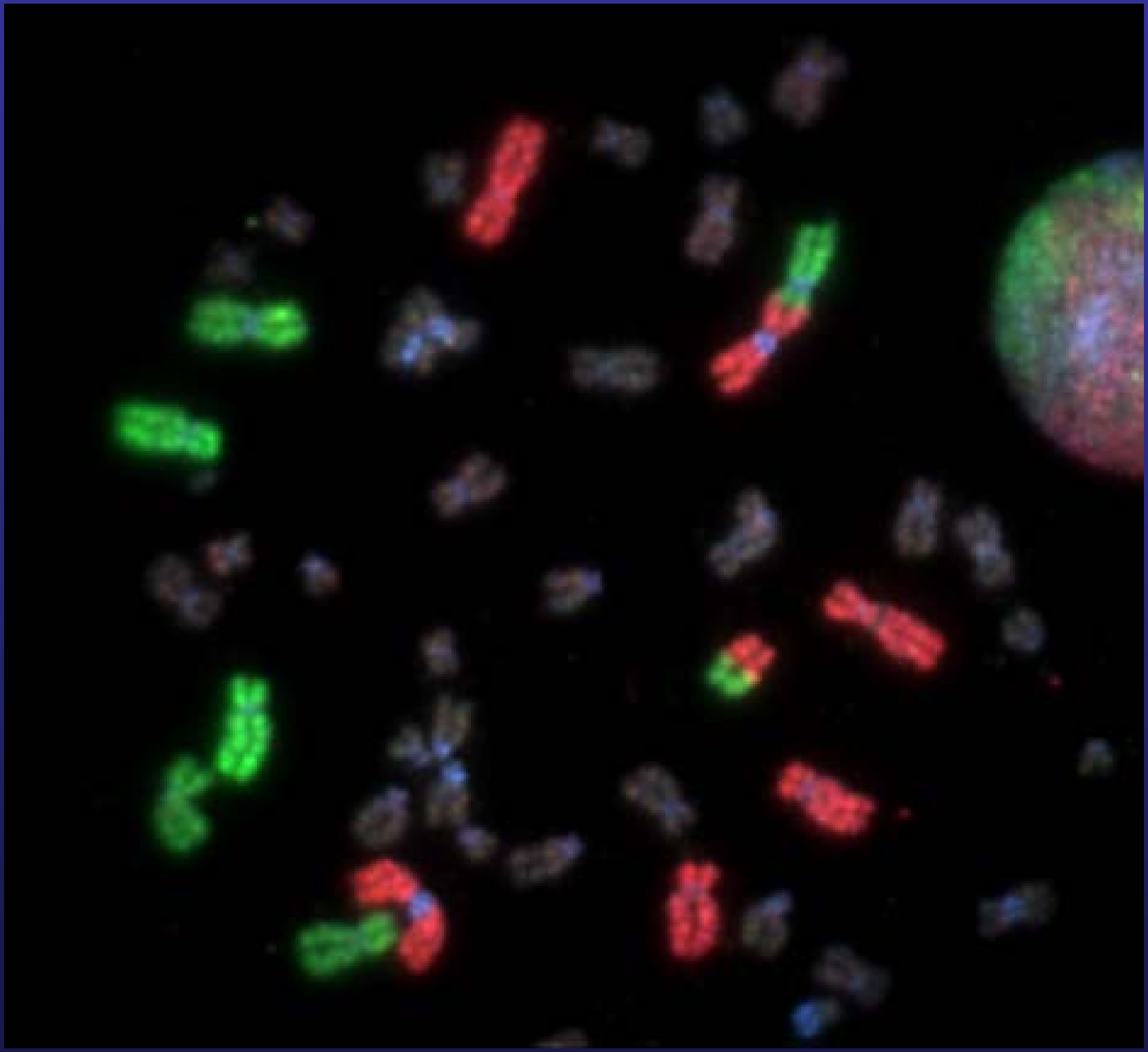
Reciprocal Translocation, and a non-reciprocal translocation



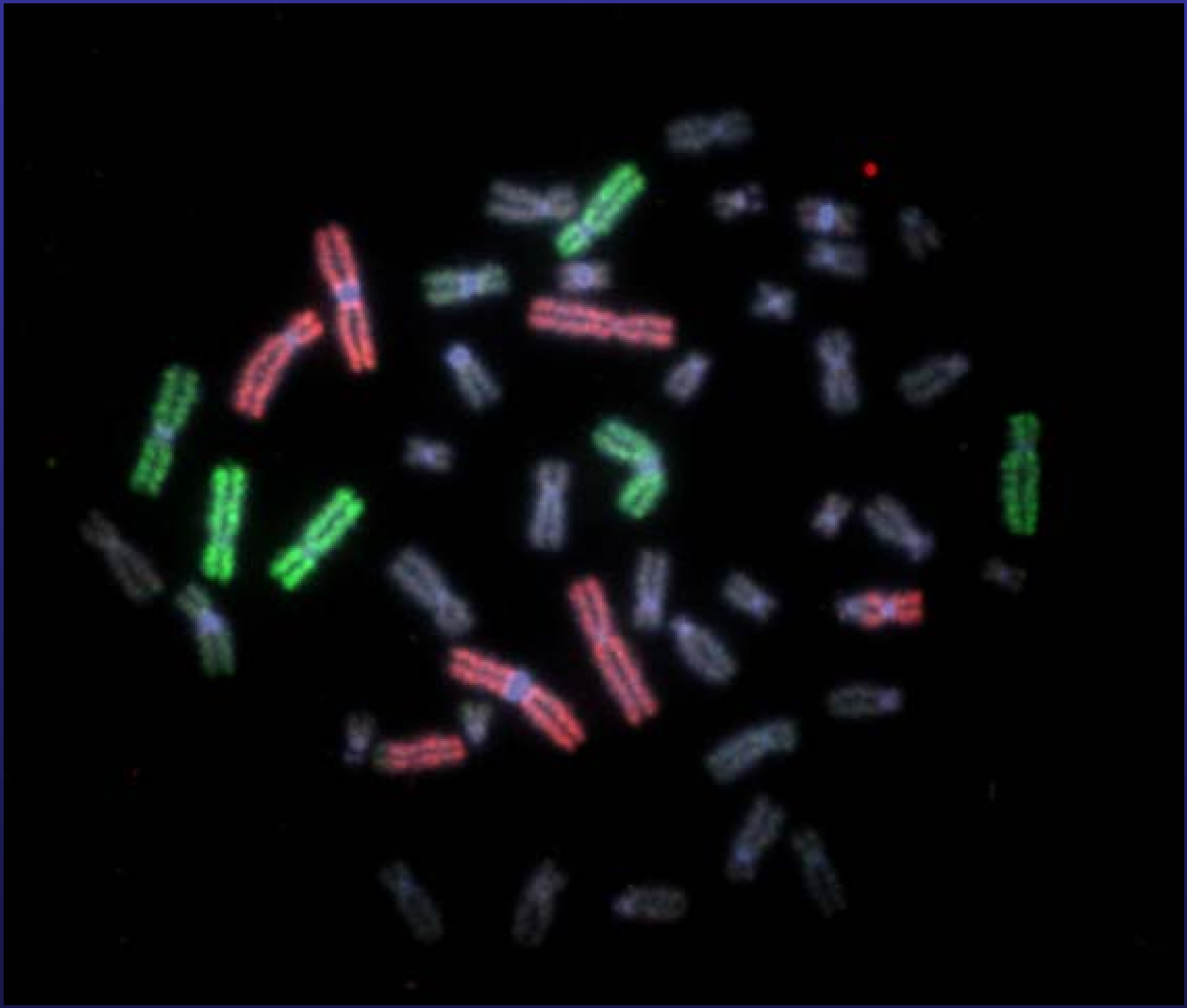
Some chromosome aberrations involve very small pieces of exchanged material.

Painting provides high resolution to detect these events.

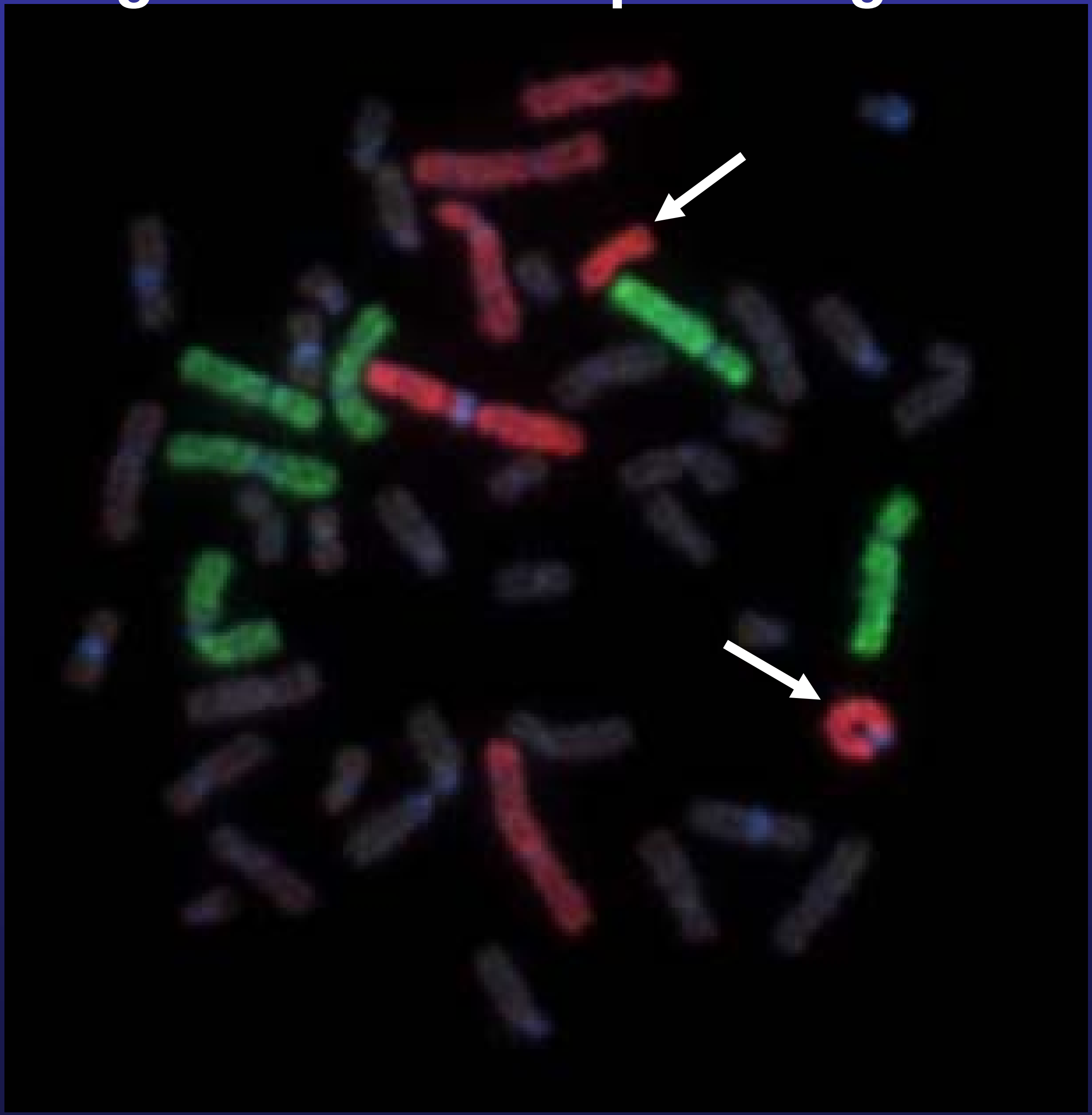
Dicentric plus Acentric Fragment



Dicentric plus Acentric Fragment

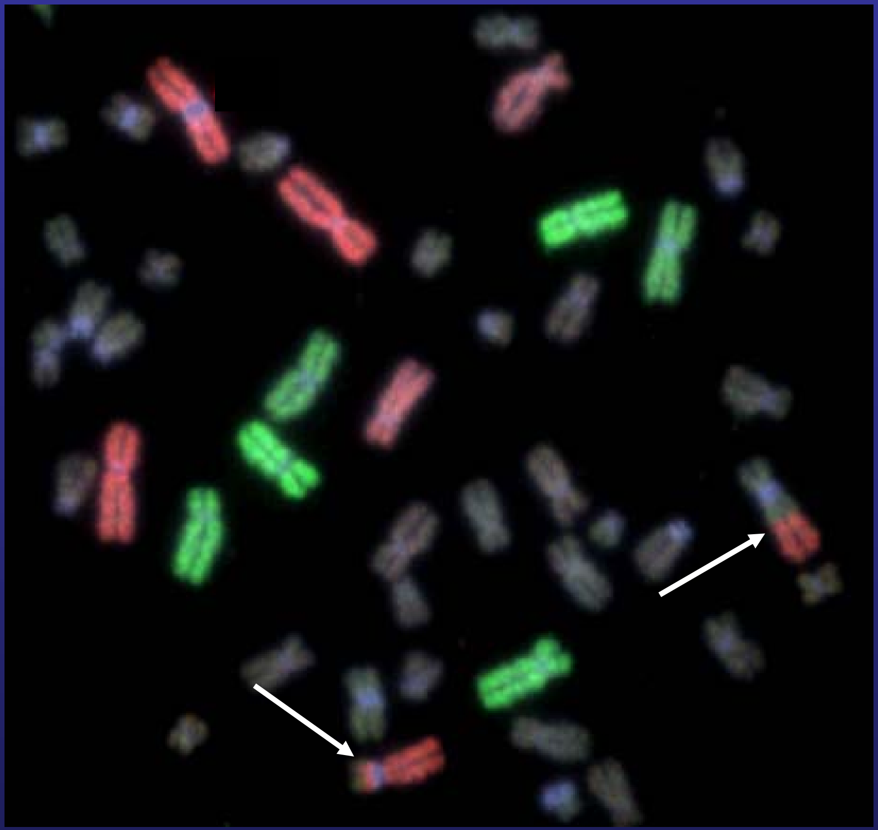


Ring chromosome plus fragment



May 10, 2004

Translocations by Painting



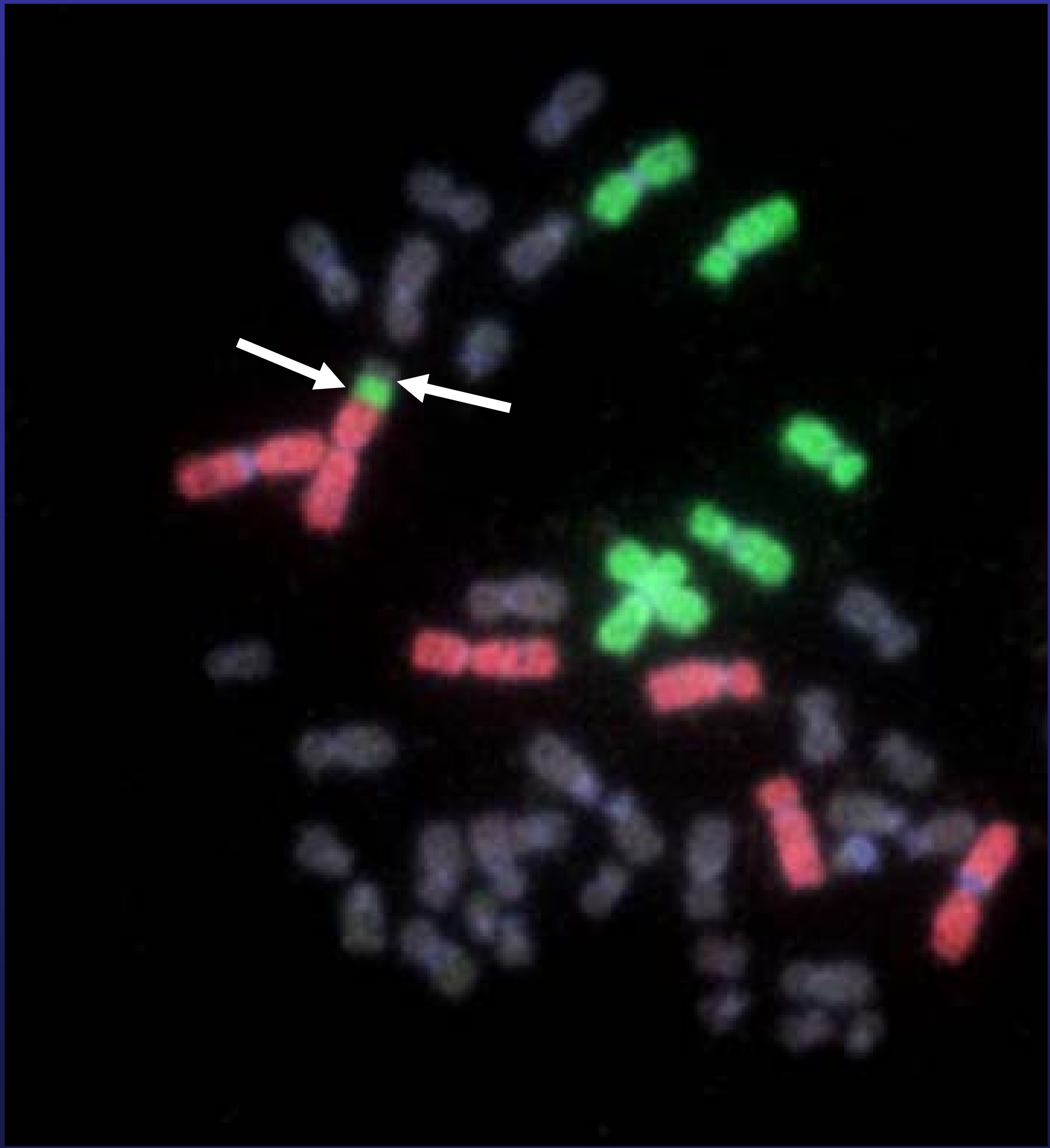
Advantages for biodosimetry:
- **speed (color junctions)**
- **sensitivity**
- **reliability**
- **relevance**

Painting detects:
- **color "junctions"**
- **breaks / fragments**

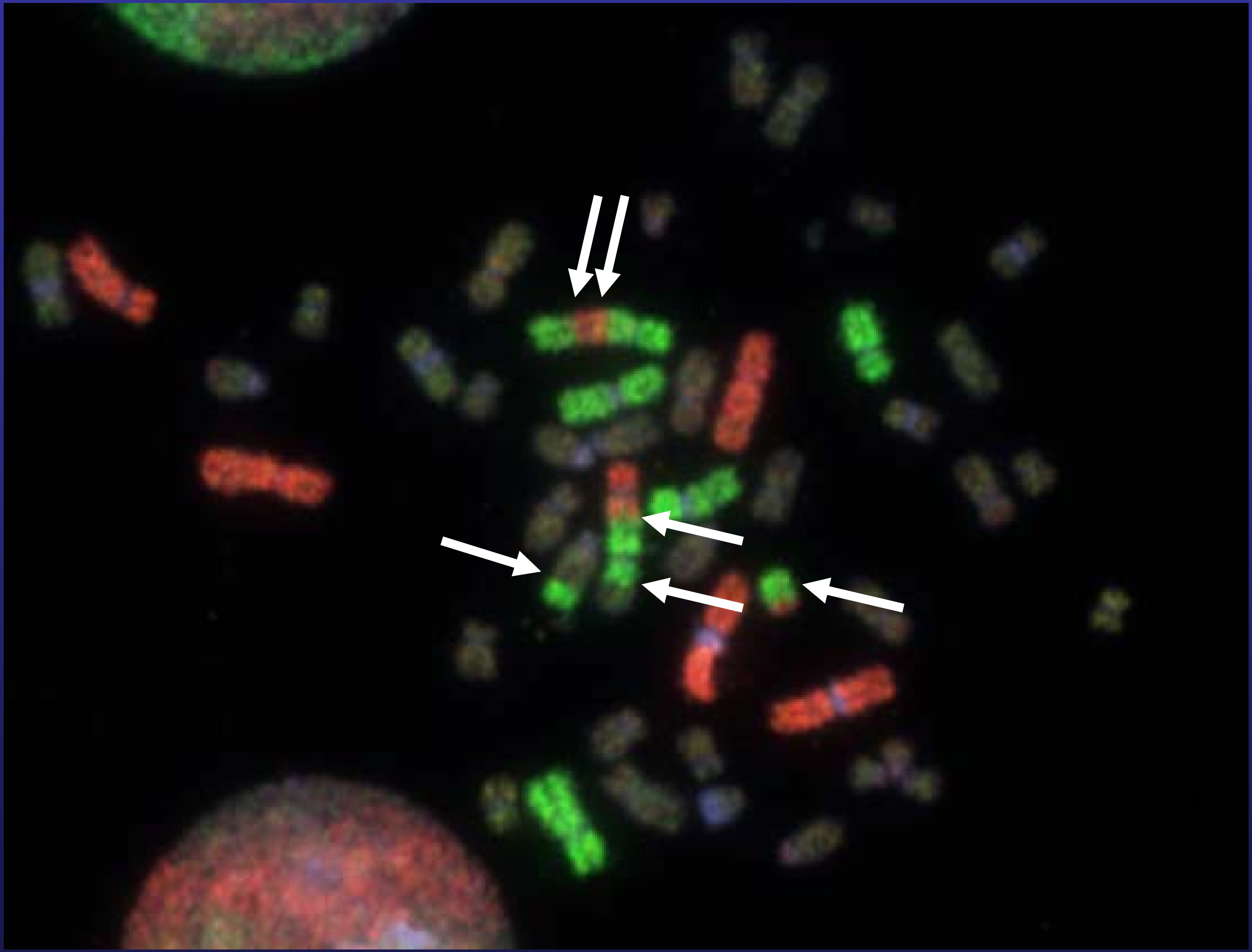
Reciprocal translocations are the hallmark of ionizing radiation exposure

BUT: Not every aberration is detected!

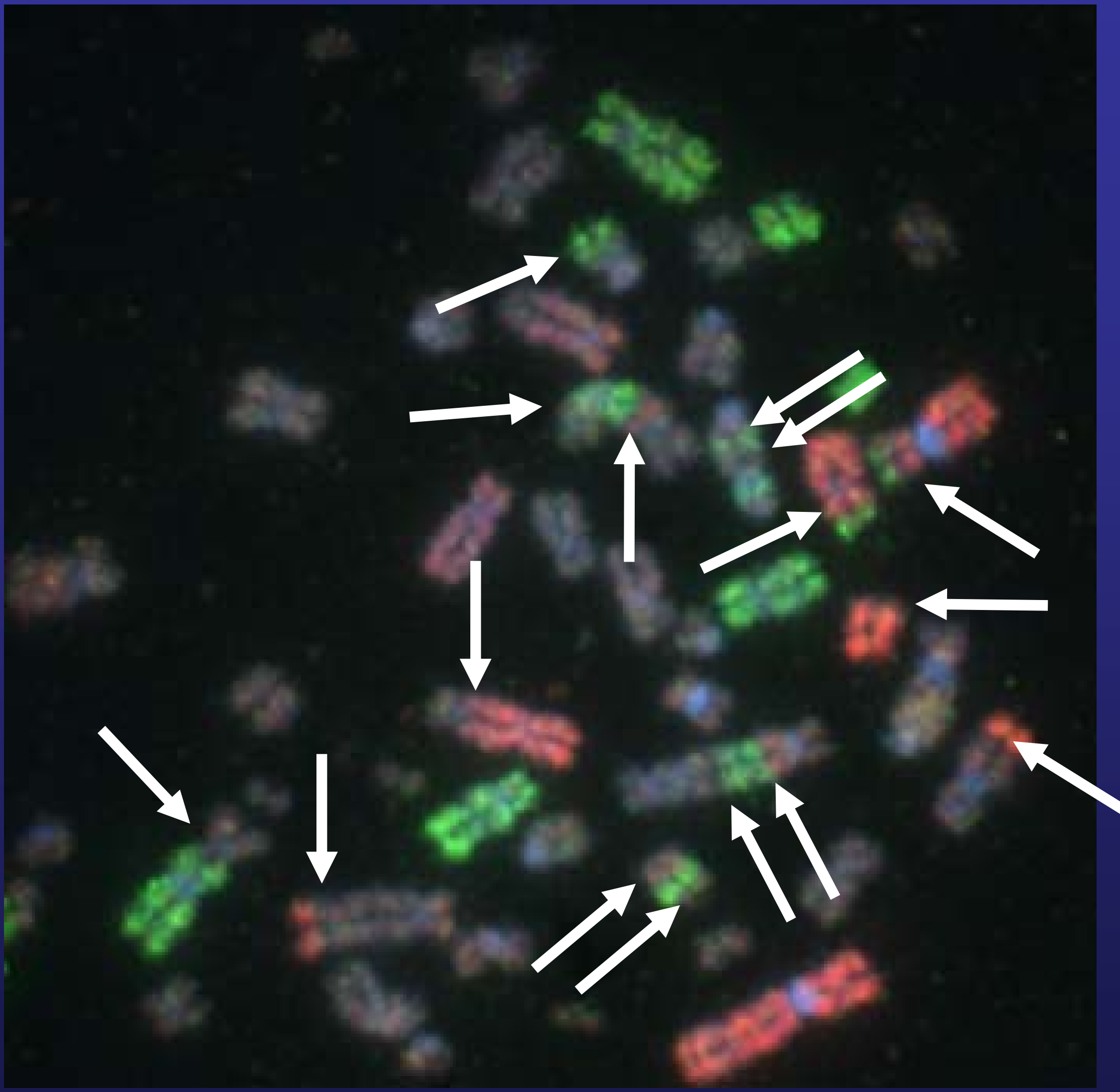
Chromosome damage can be complex



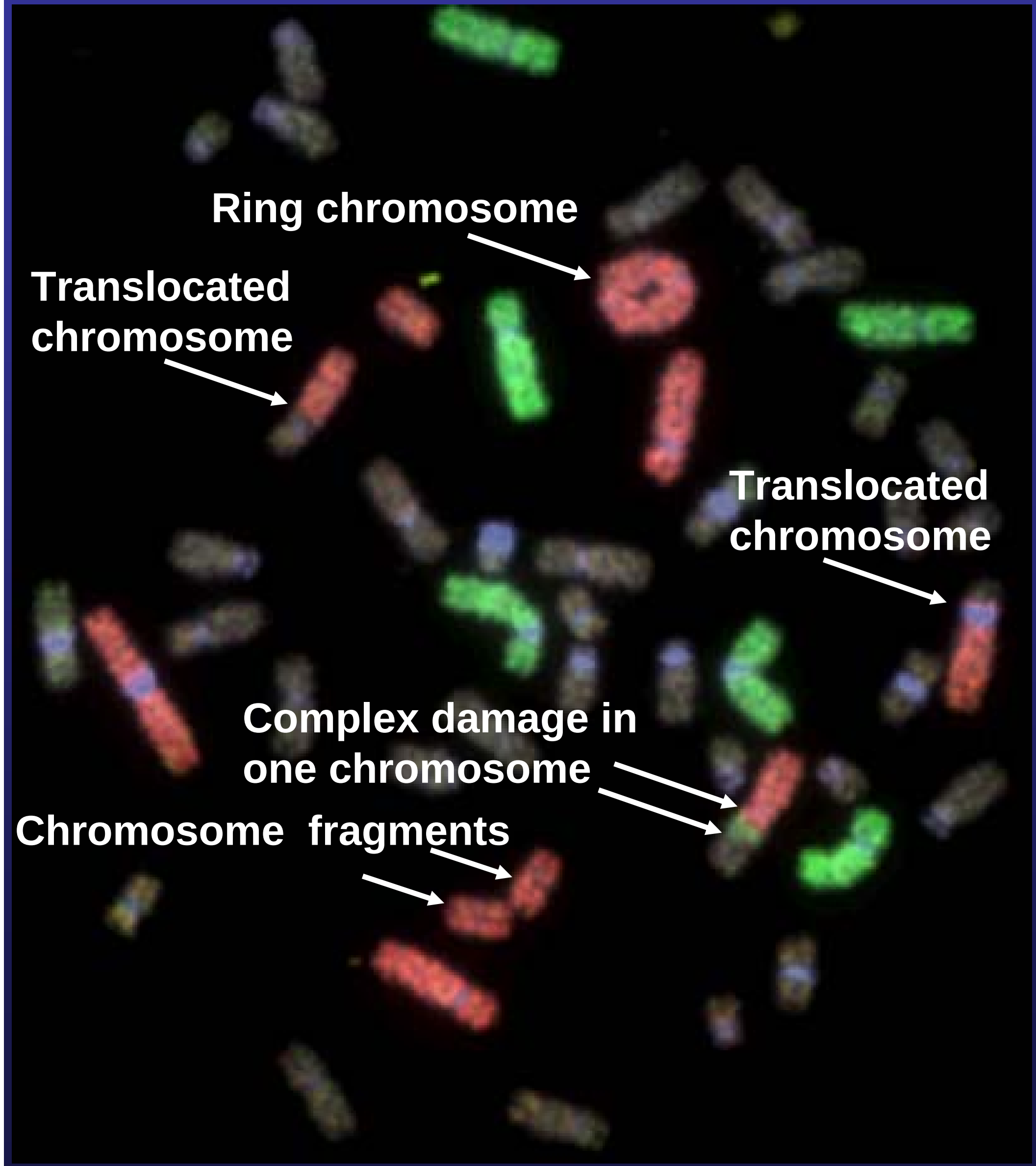
Chromosome damage can be complex



Chromosome damage can be very complex



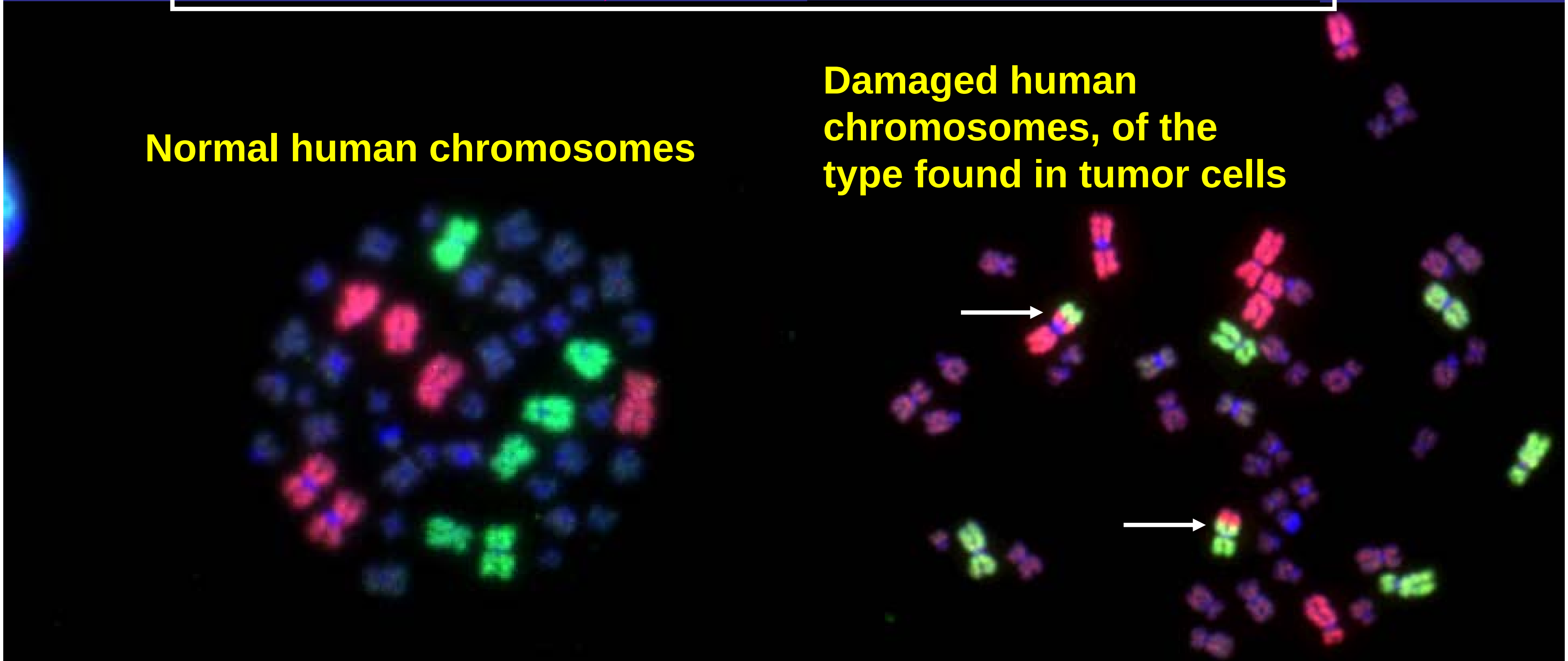
Complex Chromosome Aberrations



- Common in tumors
- Seen with some types of exposure
- Presumed high risk if cell survives
- Distribution of aberrations per cell may be important for risk assessment
- May be a marker for high-LET exposure

Relationships Between Environmental Exposure and Adverse Health Outcomes

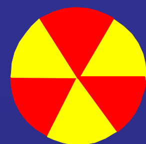
- Environmental Exposure → Genetic Damage → Cancer
- Environmental Exposure → Birth Defects → Cancer

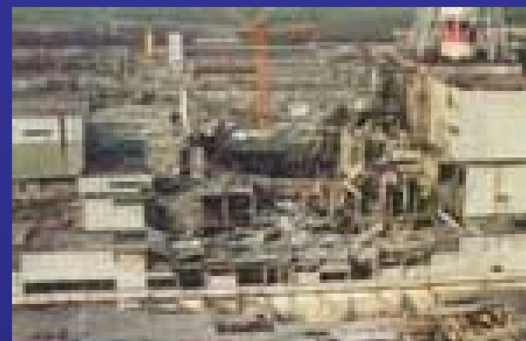


Chromosome Damage Can Cause Cancer

Why Living is Hazardous to our Health

Radiation





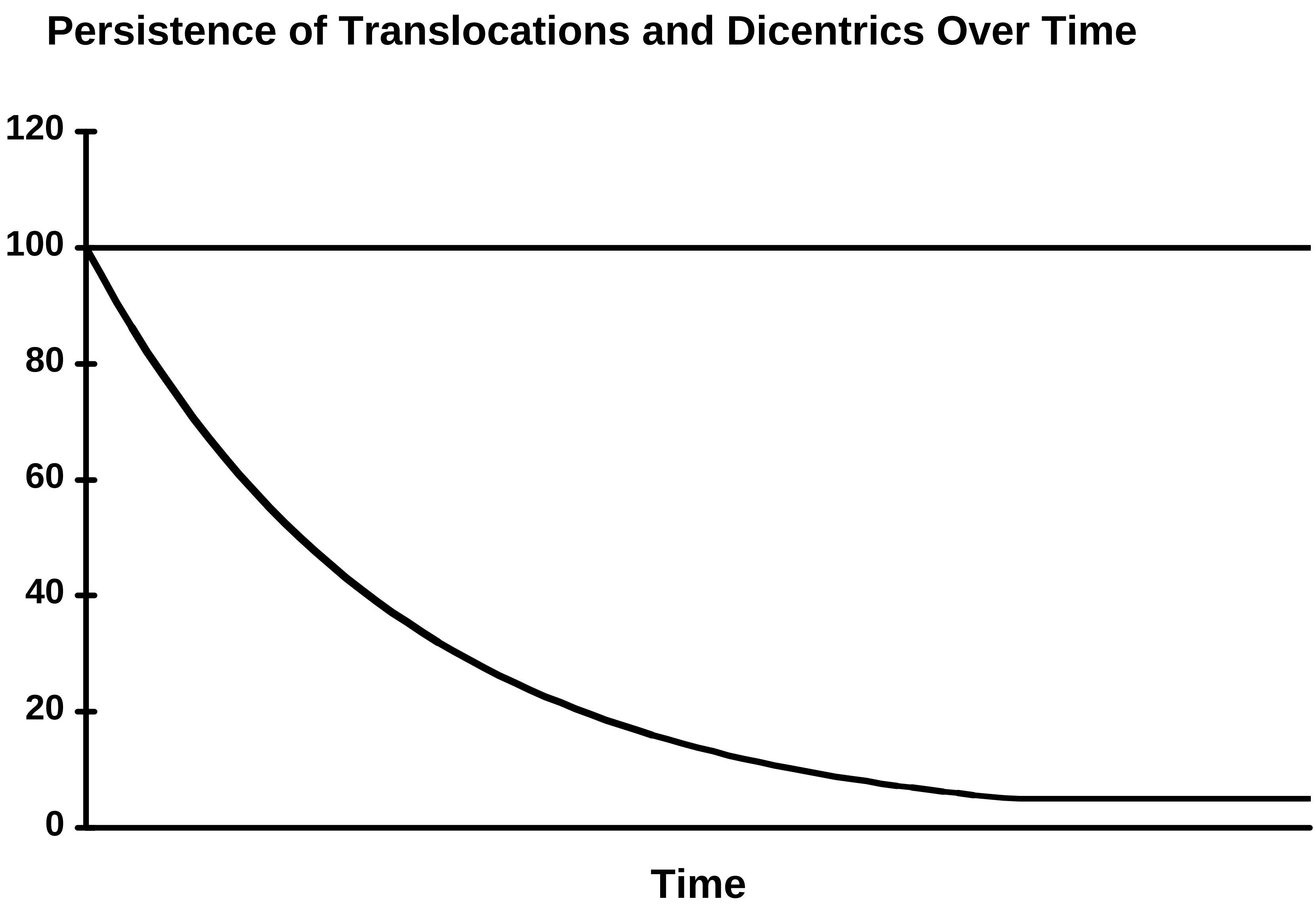
Cigarette smoke

Food

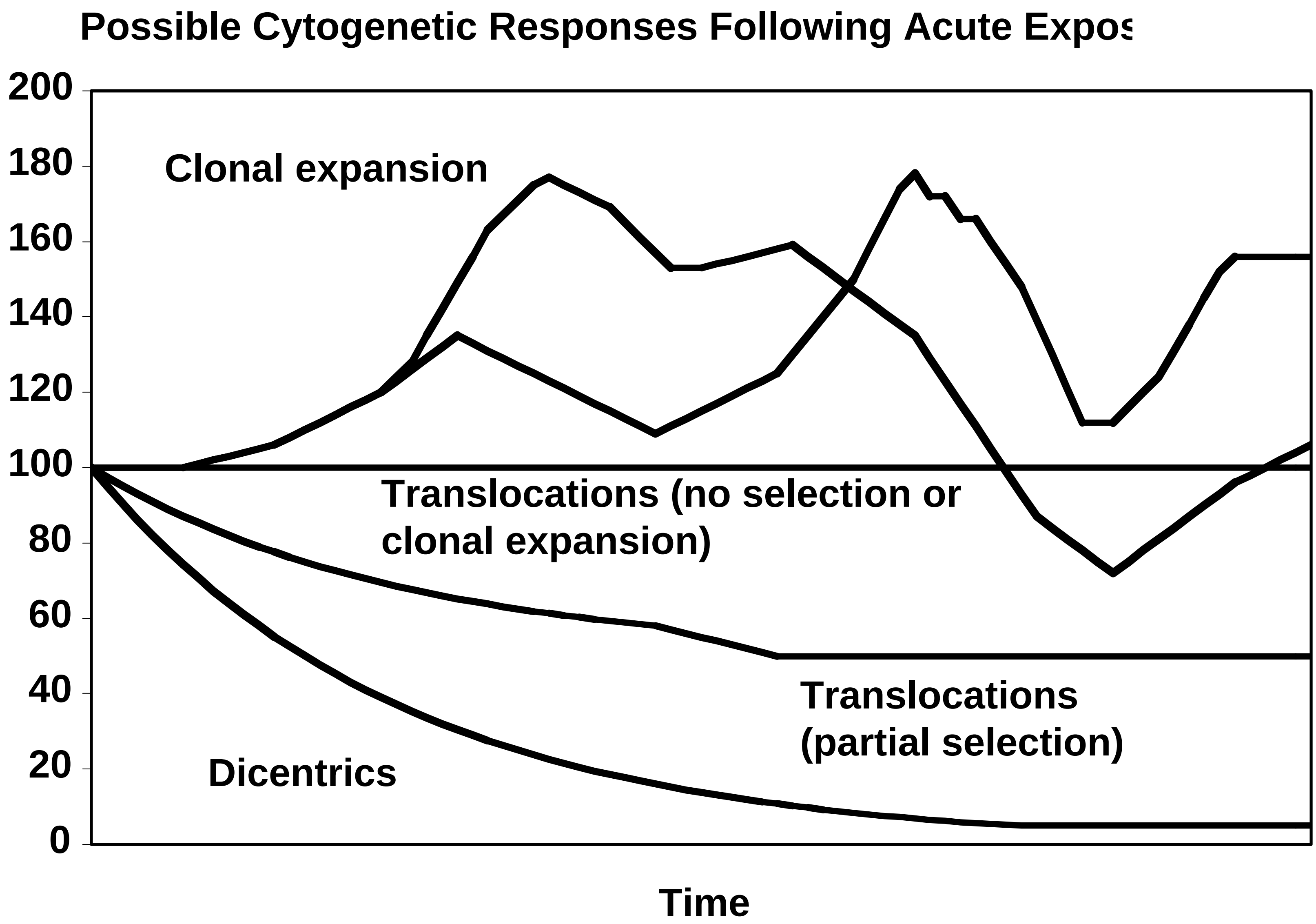
Oxygen

The key assumptions for retrospective dosimetry are that translocations persist and accumulate.

This makes translocations ideal candidates for assessing acute and chronic exposures.

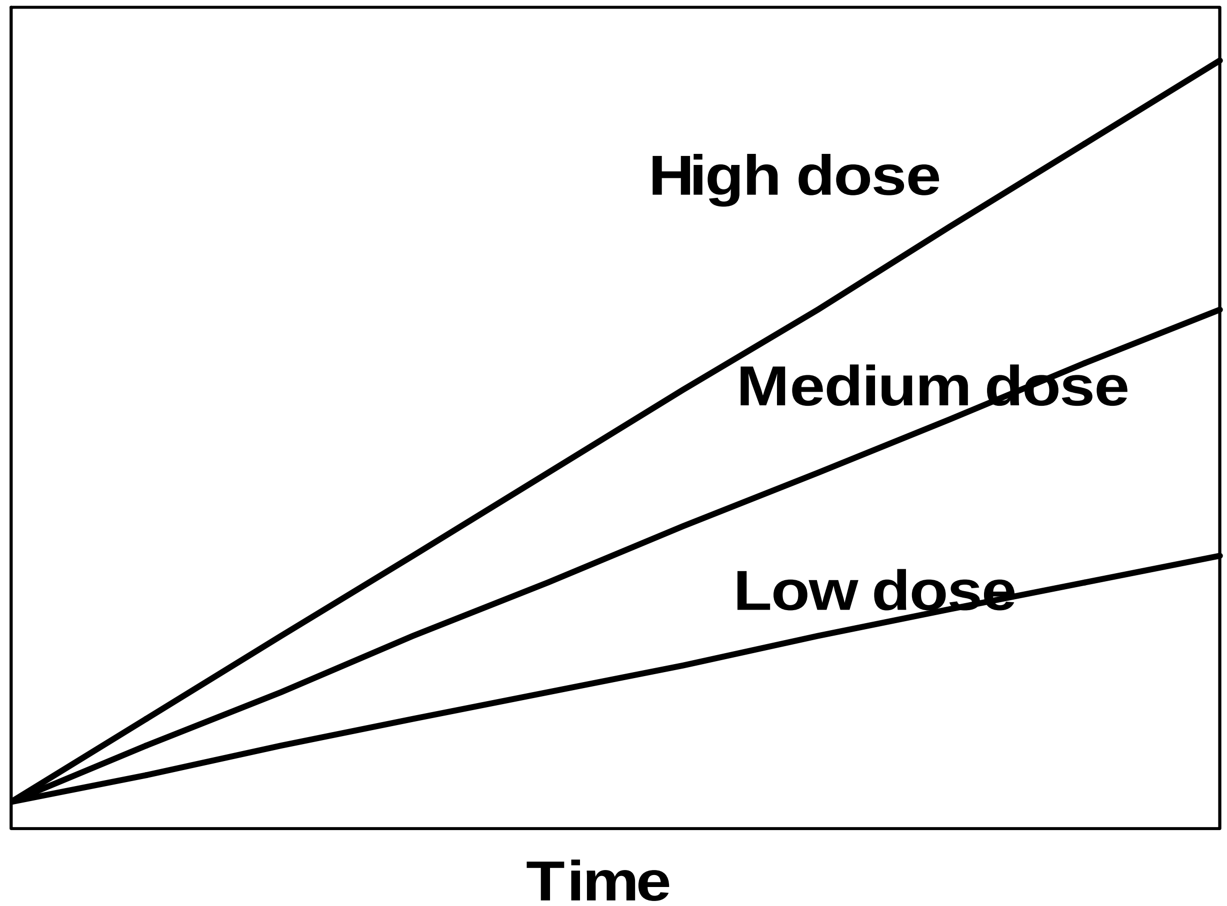


Persistence of Translocations and Dicentrics Over Time
120
100
80
60
40
20
0
Time

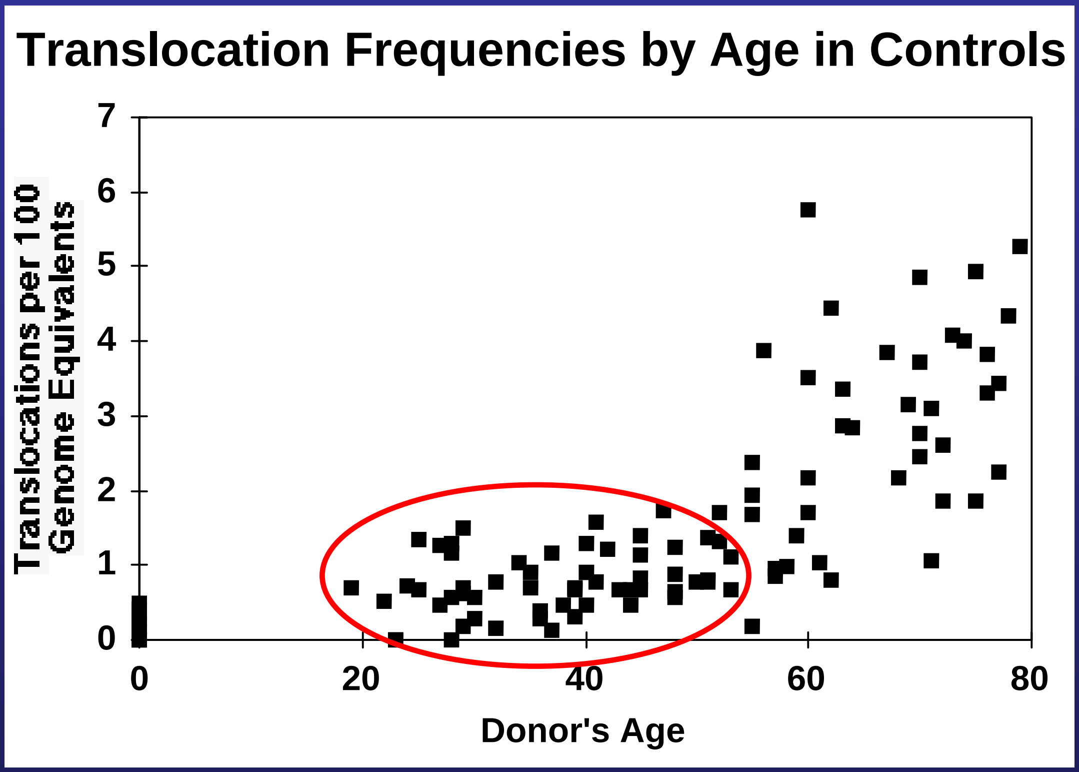

Possible Cytogenetic Responses Following Acute Expos
200
180
160
140
120
100
80
60
40
20
0
Clonal expansion
Translocations (no selection or
clonal expansion)
Translocations
(partial selection)
Dicentrics
Time

Theoretical Accumulation of Translocations with Time

[redacted]



Source of Variation: Aging

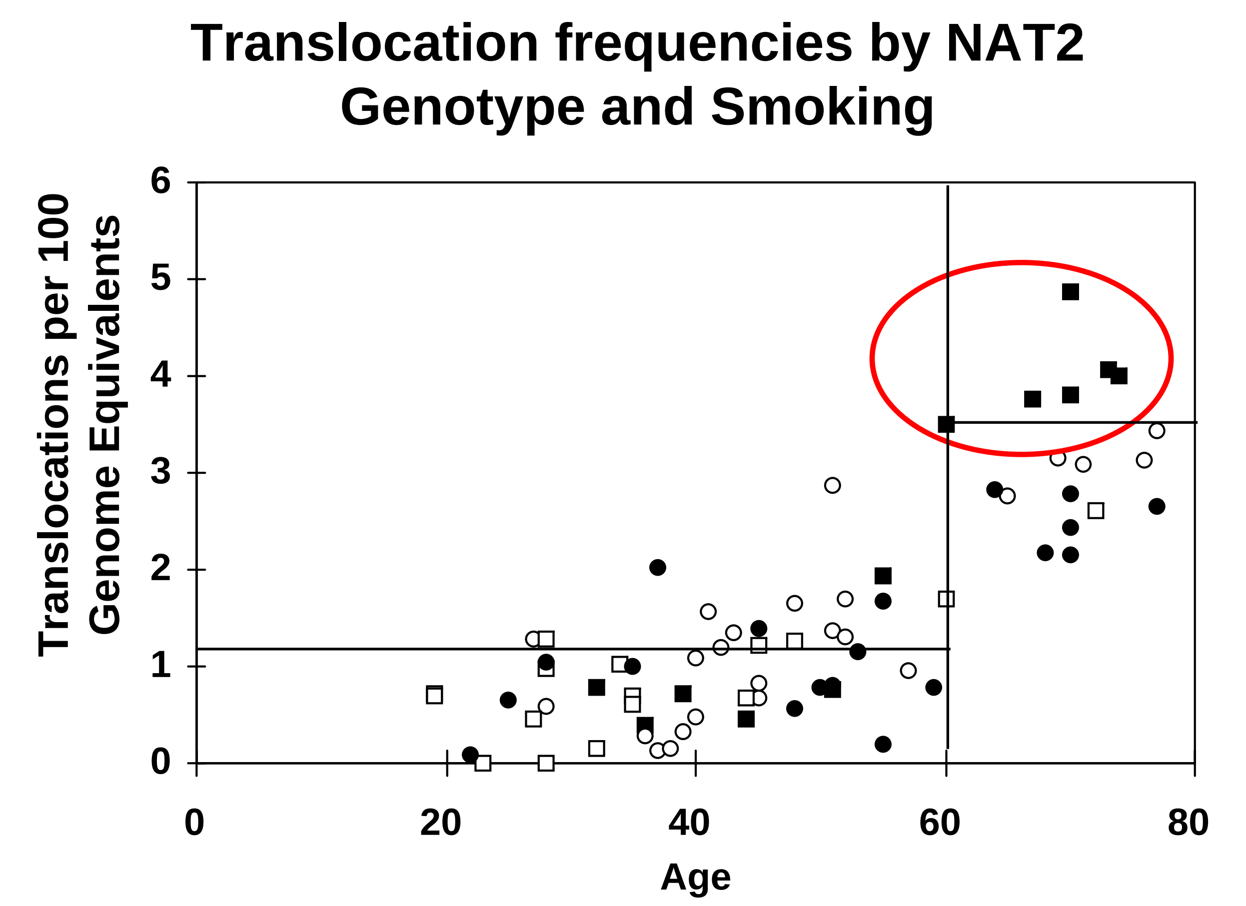


Data from Ramsey et al. (1995) Mutation Research 338:95-106.

Translocation frequencies:

- Show little change between ages of 20 and 50
- Increase rapidly after age 50
- Appear similar to cancer aging data

Source of Variation: Individual Genotypes

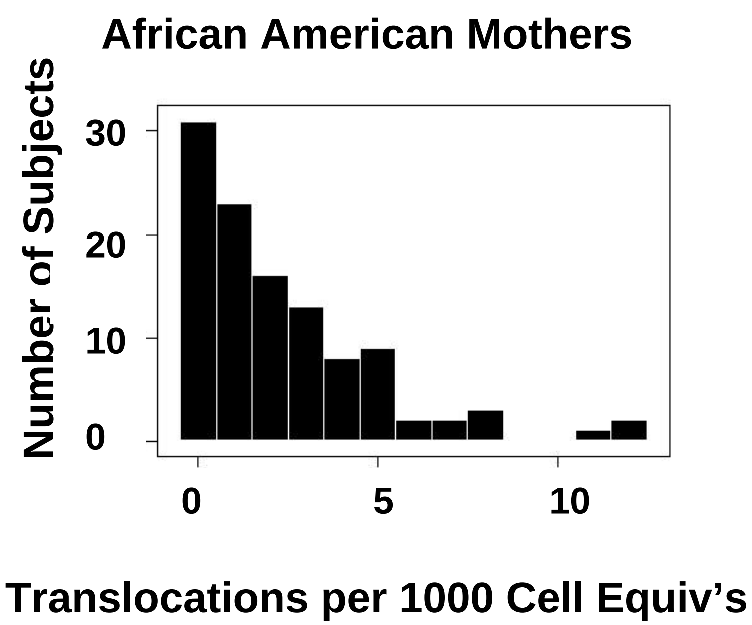


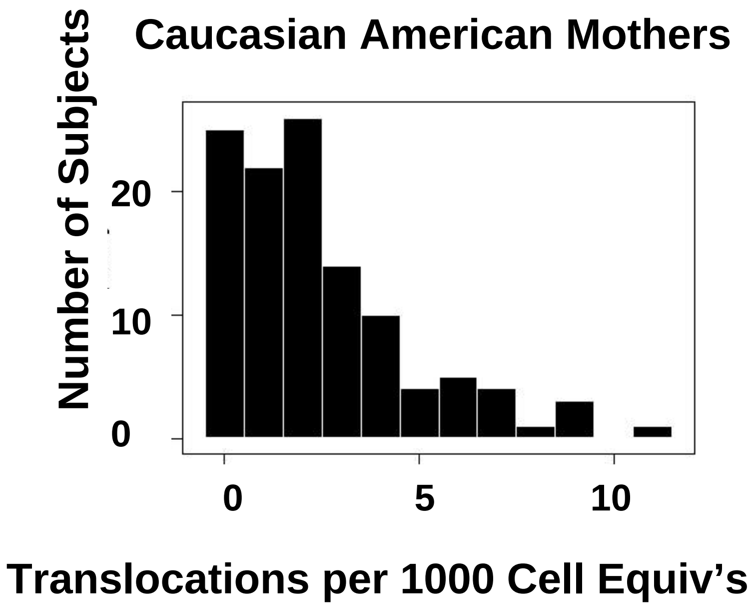
Genotype is important for estimating risk

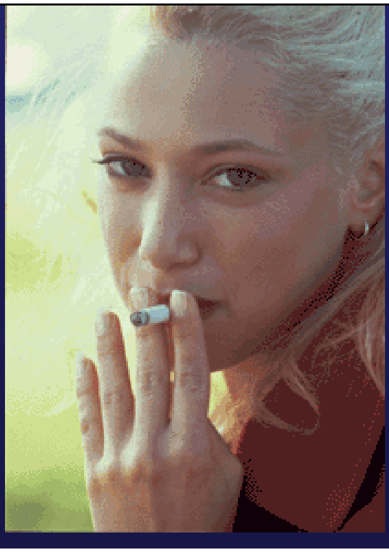
Smokers who are NAT2 “rapid” acetylators have significantly more translocations than everyone else.

Data from Pluth et al., (2000) Pharmacogenetics 10:311-319.

Chromosome Translocations and Race







No significant difference between African American and Caucasian American women at time of delivery.

Radiation Genotoxicity from the Chernobyl Accident

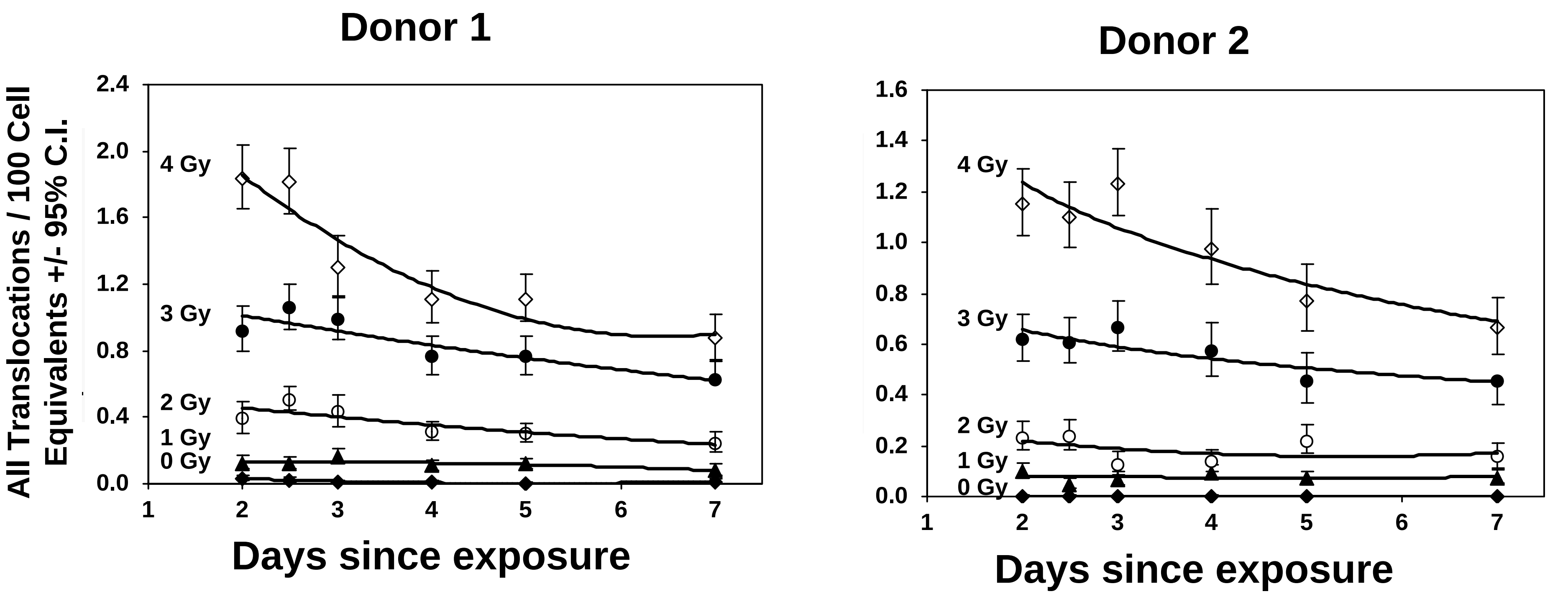
Results:

1. The average dose to the clean-up workers was ~9.5 +/- 2.2 cGy, which is half the anticipated dose.

2. Translocation frequencies increase significantly with age and smoking.

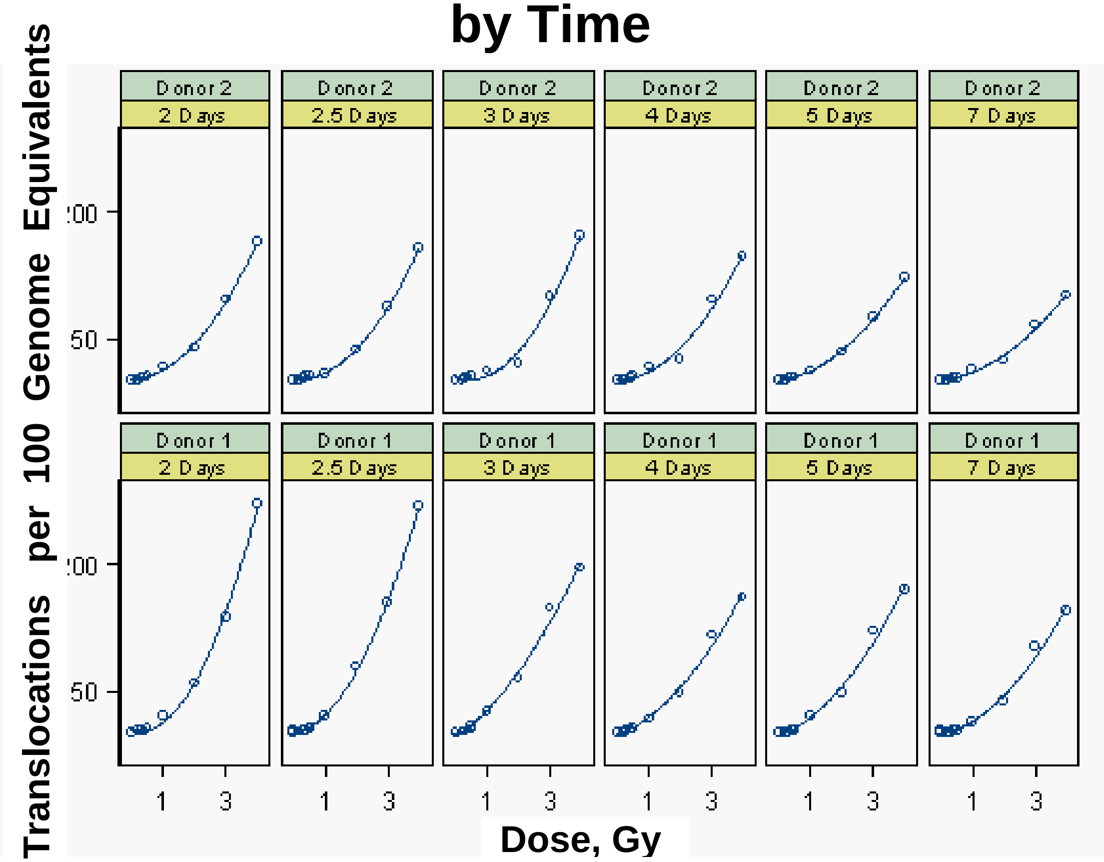
3. Cytogenetic analyses have the power to detect a radiation exposure effect in the presence of confounding factors.

Translocation Persistence (in vitro)



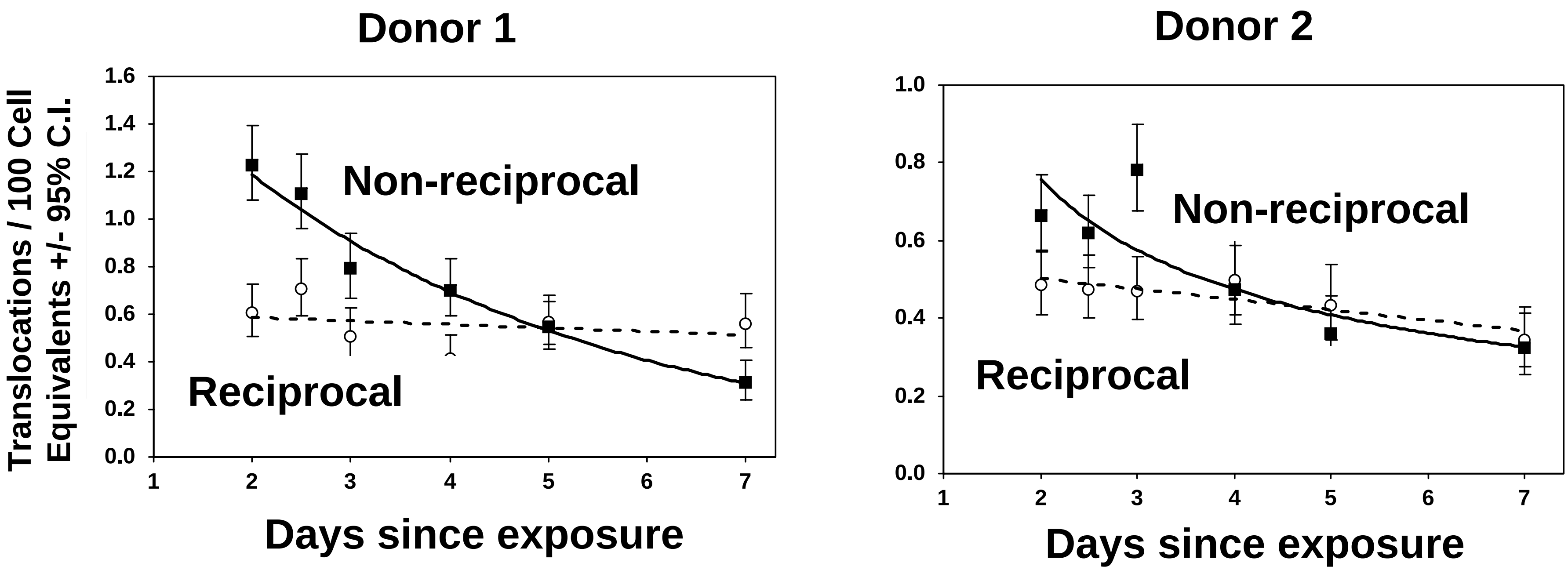
Time Since Exposure

Translocation Dose Response Curves by Time



- **Translocation dose responses decline ~40% with time.**
- **Dose-dependency is retained.**

Reciprocal Translocations Show Greater Persistence than Non-reciprocal Translocations



Why is Translocation Persistence So Important?

Translocations are lost with time

Ability to perform accurate dosimetry diminishes, but not completely

Understanding the kinetics of translocation loss is important

Low Dose Biodosimetry:
What is the lowest detectable dose?

There is no single answer. Major issues are:

- **age of subjects**
- **smoking status**
- **control sample matching**
- **dose rate**
- **time since exposure**
- **radiation quality**
- **level of effort expended (counting statistics)**

Can chromosome painting detect a doubling of the background translocation frequency?

Yes, rather easily

Number of cell equivalents which need to be scored to detect a doubling of translocations

	Non-smokers		Smokers (2 packs/day)	
age	Power = 0.9	Power = 0.8	Power = 0.9	Power = 0.8
0	11,872	8,279	11,872	8,279
10	11,656	8,128	11,656	8,128
20	10,341	7,211	10,341	7,211
30	7,917	5,521	6,104	4,256
40	5,435	3,790	3,861	2,692
50	3,584	2,499	2,554	1,781
60	2,376	1,657	1,751	1,221
70	1,616	1,127	1,240	865

This analysis assumes that smoking begins at age 20 and continues throughout the lifetime.

Risk Assessment

Two Generalizable Sources of Risk:

1. Exposure
   - radiation, chemicals
2. Genotype
   - metabolism, repair genes

Our understanding of the interactions between genotype and exposure is growing.

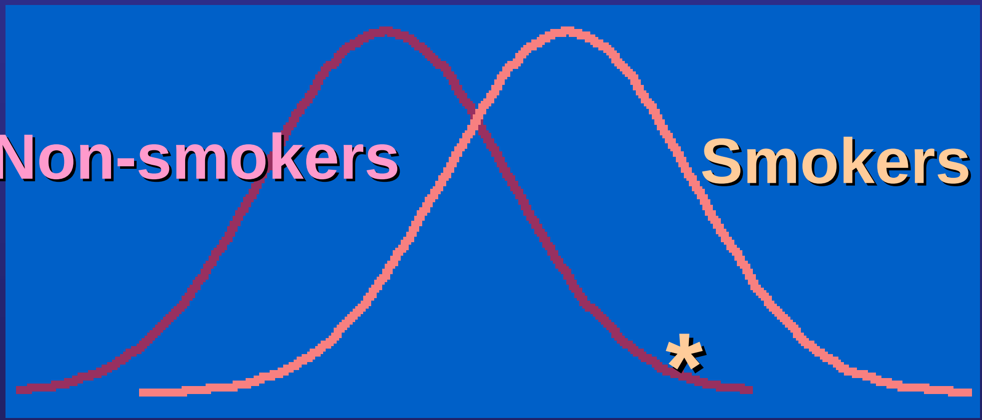
Normal Control Values from Unexposed Subjects

Important to have "normal" curves, by

- age
- cigarette smoking status
- genotype

And perhaps by

- gender
- ethnicity



<u>Need answers to the questions:</u>

1. "What is the chance that a given person was exposed?"
2. "Is this exposed person at risk?"
3. "If there is a risk, what are its characteristics?"

Low Dose Biodosimetry: Controls

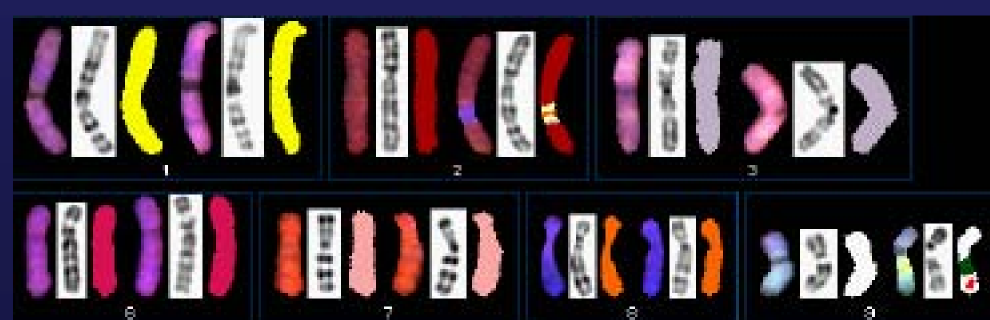
When estimating exposure, accurate control translocation counts are essential

- **same donor:** ***ideal, usually not possible***
- **matched control:** ***practical, but limits detection power***
- **population reference:** ***requires control population***

Newer FISH painting methods . . .

Multi-Color Karyotyping (SKY, M-FISH)

- **Identifies aberrations in <u>all</u> chromosomes, but this is not essential for biodosimetry**
- **Trade-offs between speed and completeness of analysis**
- **Ideal for tumors**
- **<u>Very</u> labor-intensive**

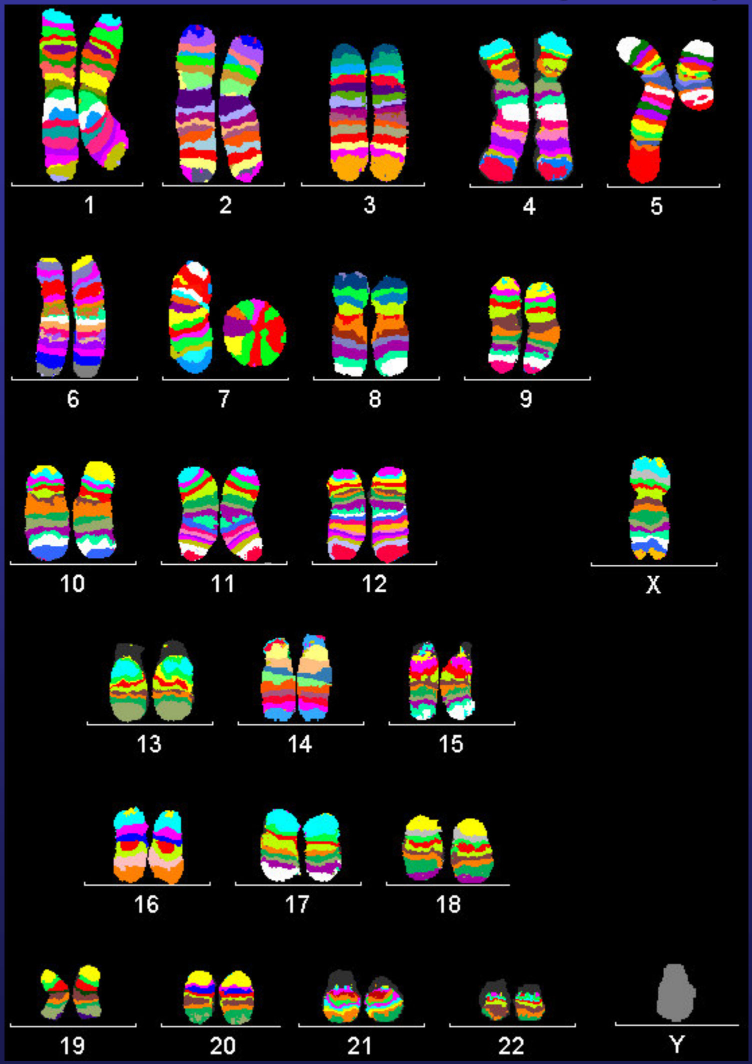


Images courtesy of Dr. Alison Director, Armed Forces Institute of Pathology

Multi-Color Karyotyping of a Tumor Cell

Image courtesy of Dr. Alison Director, Armed Forces Institute of Pathology

mBANDs: multi-color bands by FISH



www.metasystems.de

- Identifies all aberrations, including inversions, but this is not essential for biodosimetry
- Trade-offs between speed and completeness of analysis
- Clinically useful
- Very labor-intensive

What do all these studies tell us?

What have we really learned?

Parameters for Radiation Exposure Assessment

Induction - is a measure of the relationship between exposure (dose) and some type of genetic response.

Persistence - is a measure of the longevity of induced damage.

Accumulation - is a measure of the total amount of damage in a cell, tissue, animal or person. Combines induction and persistence.

Principles for Retrospective Exposure Analysis I.

1. Selection against cells damaged by exposure does not occur or can be taken into account.
2. Translocation frequencies pre-existing in the exposed individuals should be known or be estimated from appropriate controls.
3. Clones of cytologically abnormal cells are recognizable, and their number and prevalence can be accurately measured.

Principles for Retrospective Exposure Analysis II.

4. **Breaks are distributed among chromosomes in a manner that is proportional to their size.**
5. **The rate of exposure is known, and the effects of dose rate upon translocation frequencies are understood.**
6. **The influence of other confounding exposures, which may fluctuate with time, are negligible.**
7. **The importance of recent exposure history for determining subsequent biological responses, *i.e.*, "adaptation," is known.**

Principles for Retrospective Exposure Analysis III.

8. Tumor cells are not present in the tissue being analyzed.
9. Differences between individuals with respect to the above considerations are negligible, or can be adjusted for.
10. Changes in the frequency of genetic damage with age must be well characterized.

To the extent these principles hold true, dosimetry using translocations can be achieved many years after exposure.

Summary of FISH Radiation Biodosimetry

Translocations are the preferred endpoint

- FISH painting still the best method
- fast and reliable
- sensitive enough to detect low-dose radiation exposure in a population

Major confounders:

- age
- cigarette smoking
- possibly genotype

Summary of this Talk

- Cell and chromosome biology
- Chromosome aberrations
  - unbanded
  - banded - karyotyping
  - painting
- Lessons learned human studies
  - importance of age, smoking, genotype
- Biodosimetry / translocation persistence
- Risk Analysis
- Controls
- New methods