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Homeland Defense & Security Information Analysis Center

HDIAC Medical Webinar Briefing:
Current High Throughput Cytogenetic Techniques for Radiation Biodosimetry
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DoDIACs

Department of Defense Information Analysis Centers

To understand the cellular basis of chronic and acute ionizing radiation injuries and their impact on human health.

Sources of Radiation Exposure

Natural sources

- Cosmic rays
- Radon – radioactive gas resulting from the decay of Uranium
- Potassium-40 and Carbon-14 from food
- Ingesting contaminated air and water

Man-Made

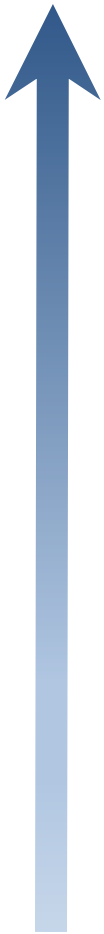
- Diagnostic X-rays
- Radioisotopes
- Occupational exposure (Radium/Uranium)
- Nuclear accidents
- TVs, computers & mobile phones
- Luminous markers
- Radioactive watch dials

Biological Effects Depend On

- Total dose
- Dose rate
- Volume of tissue or anatomical body part irradiated
- Type of radiation (Low and high LET)
- Pre-existing physical conditions, trauma, illness, burns
- Genetic predisposition - inherent radio sensitivity due to genetic mutations

Human Sensitivity to Ionizing Radiation

Most Radiosensitive



Least Radiosensitive

Embryo

Fetus

Child

Adult

How can you measure the absorbed radiation dose in humans?

Three Methods of Dosimetry

Physical dosimeter

Personal dosimeters

Biological Dosimetry

Use of biological markers (Cytogenics, Proteomics Transcriptomics, Metabolomics)

Most relevant for predicting genetic effects

Clinical

Nausea
Vomiting
Leukopenia
Skin reddening

Situations that Require High Throughput Radiation Biodosimetry

- Nuclear Accidents (Chernobyl, Goiania and Fukushima)
- Nuclear Device Detonation
- Dirty Bomb
- Radiological Terrorism

In the above scenarios, several hundreds of people may be exposed to radiation.

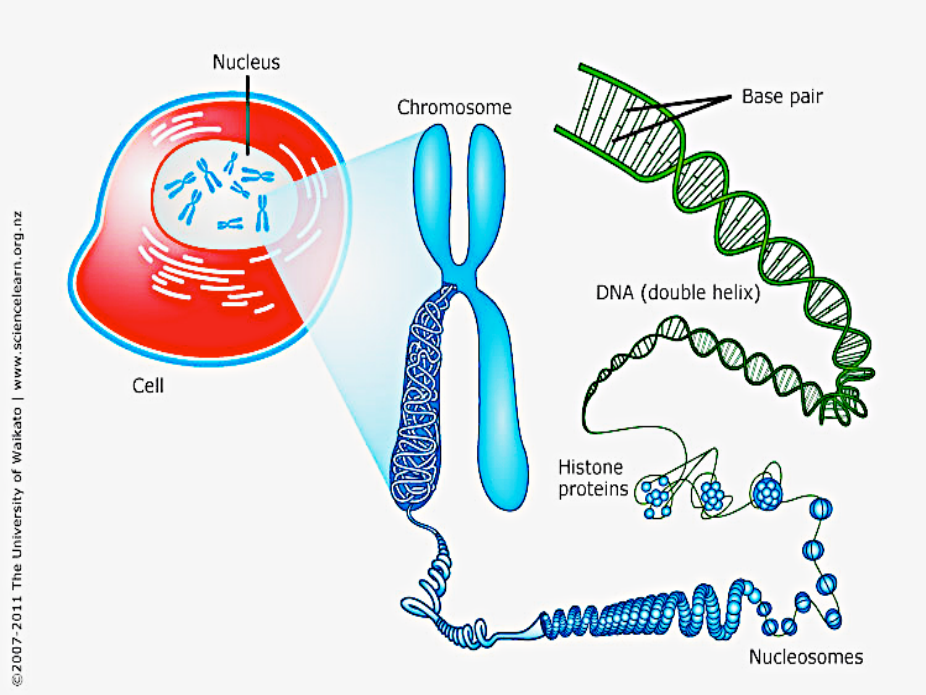
Advantages of High Throughput Radiation Biodosimetry

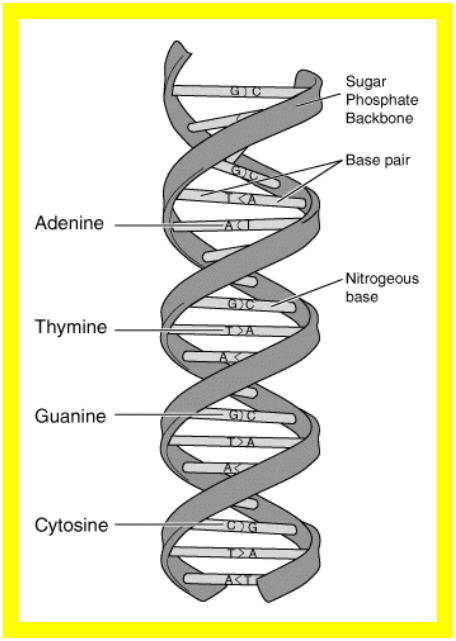
- Individualized dose assessment will help in identifying the degree of exposure (low vs high)
- Predict the long–term health effects including tissue degeneration and cancer risks
- To avoid psycho-social effects in low dose exposed human population
  - Reassurance that the effects are not lethal

Prerequisites for High Throughput Biodosimetry

- Biological samples need to be minimally invasive
- Sensitive and specific for radiation dose assessment
- Rapid processing and analysis of large scale samples
- Stability of biomarkers over time
- Reliable and reproducible

What is the Biological Target for Ionizing Radiation?





Chromosomal DNA is susceptible to ionizing radiation induced DNA double strand breaks.

Most Commonly Used Biodosimeters: Cytogenetic Endpoints

- Cytogenetics is the study of chromosome structure and function
- Each human cell nucleus has 46 chromosomes (22 pairs of autosomes and 1 pair of sex chromosomes)
- Chromosomes are made up of genes and genes are made up of DNA complexed with histone proteins
- Genes are crucial for our disease-free survival
- Ionizing radiation exposure produces breaks in the DNA thereby disrupting the chromosome structure and function
- Misrejoining of broken chromosomes result in dicentrics, rings and translocations

Preparatory Steps for Chromosome Analysis

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Human blood collection in tubes

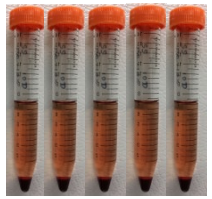
Transfer 0.5 mL blood+ 9.5 mL of growth medium+ 2% Phytohaemagglutinin (PHA).

(Growth medium: RPMI1640, 10%FBS and antibiotics)

Transfer to an incubator with 5% CO_2 at 37 °C for 44 h and 4 h with Colcemid (0.05 µg/mL).

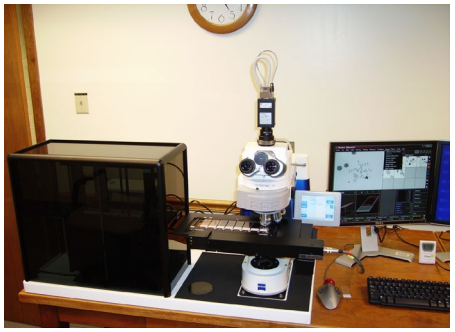
Centrifuge for 10 min at 800 rpm.

Remove the supernatant. Add 10 ml of 0.56% KCl. Incubate at 37 °C for 14-16 min.



Centrifuge for 10 min at 800 rpm.

3 times



Imaging and analysis in Metafer

Stain with 5% Giemsa, rinse in water & air dry.

Drop 50 µL of cell suspension on slide.

Remove supernatant. Suspend the pellet in 500 µL of fixative.

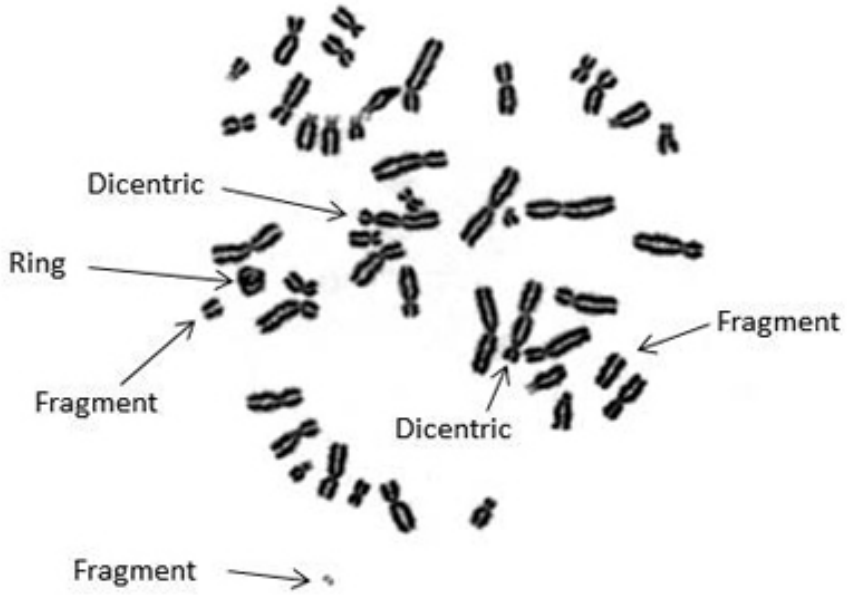
Centrifuge for 10 min at 800 rpm.

Remove supernatant. Add 10 mL of 1:3 acetic acid:methanol Fixative.

Chromosome Aberrations

Normal

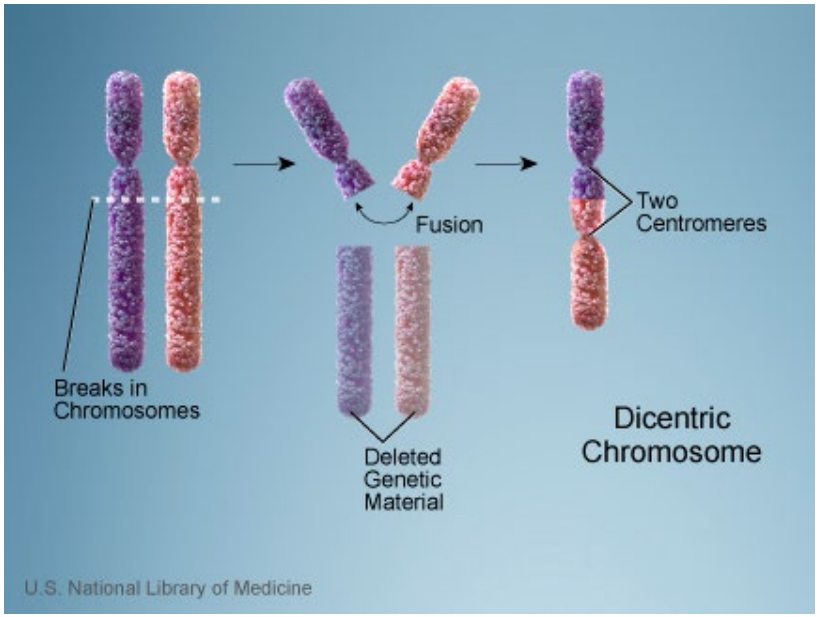
Aberrant Metaphase

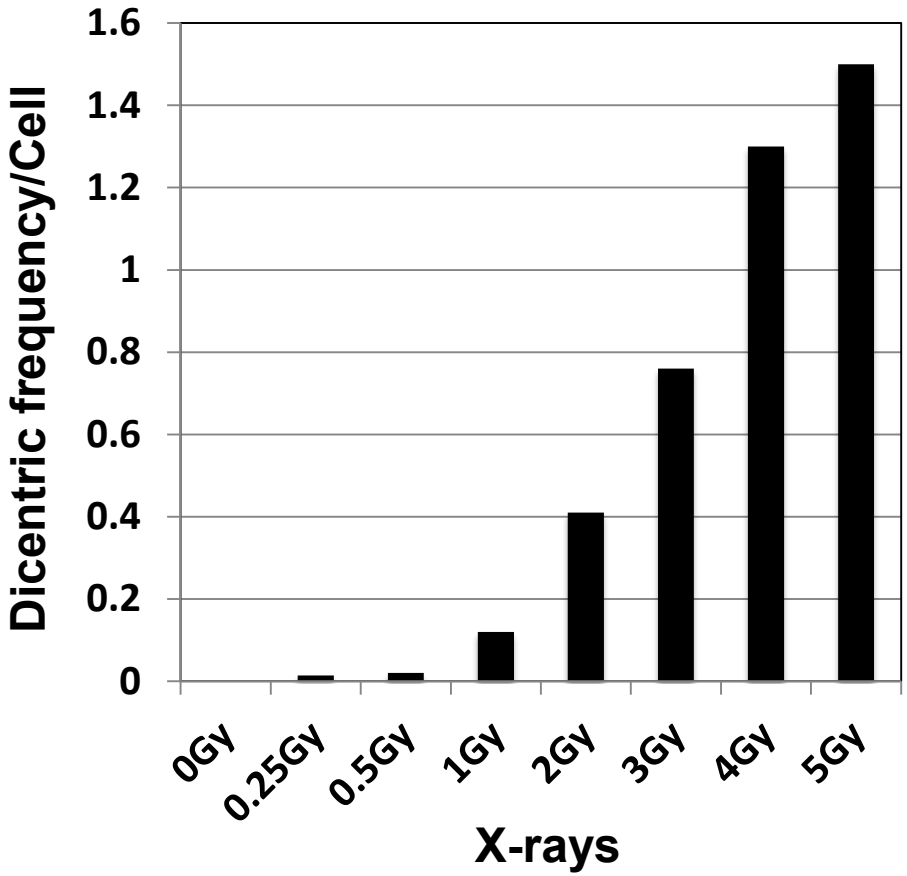


- Ionizing radiation induces chromosome aberrations.
- Radiation causes the formation of dicentric chromosomes, ring fragments and translocations due to misrejoining of strand breaks.

Types of Cytogenetic Biodosimeters

1. Dicentric Chromosome





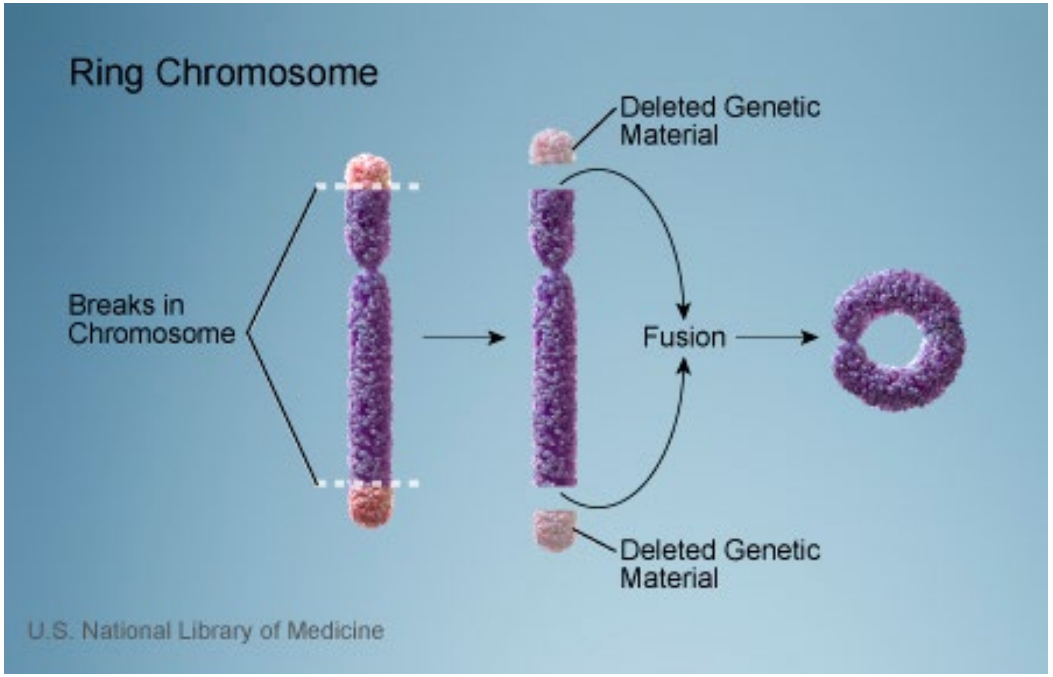
Dicentric frequency is radiation dose dependent.

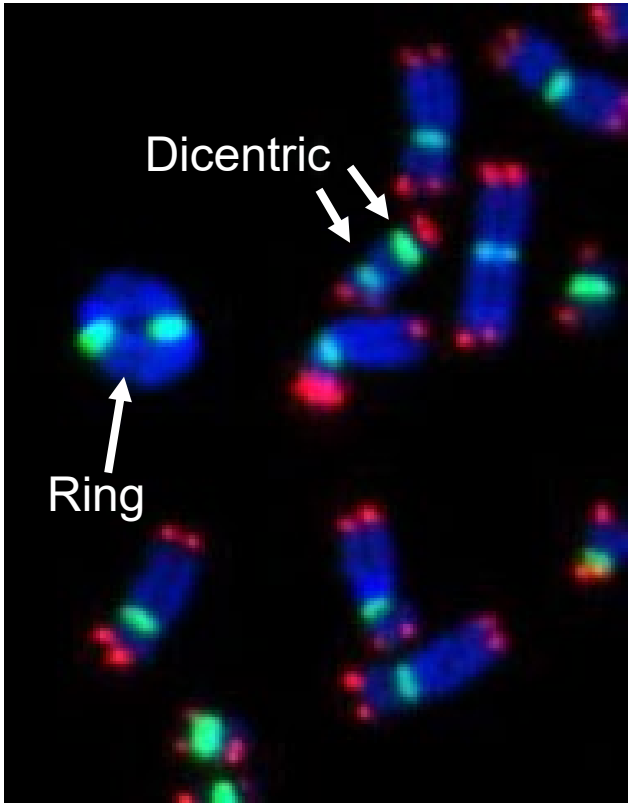
Dicentric Assay

Dicentric Assay is the “gold standard” for dose assessment.

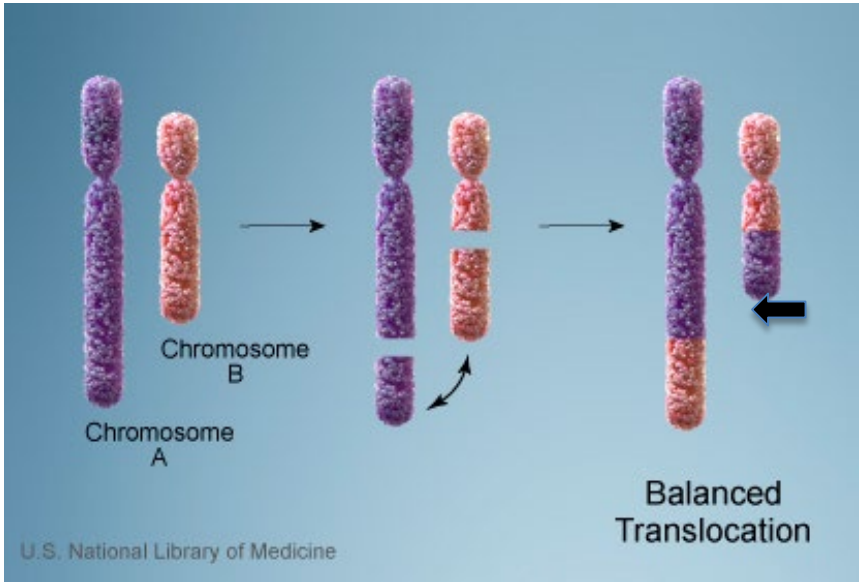
- Low background rate (1 per 1,000 cells)
- Independent of age and gender
- Ease of detection
- Sensitivity range is 0.20 to 5.0 Gy
- Reproducible dose response
- Proven in accidents over four decades

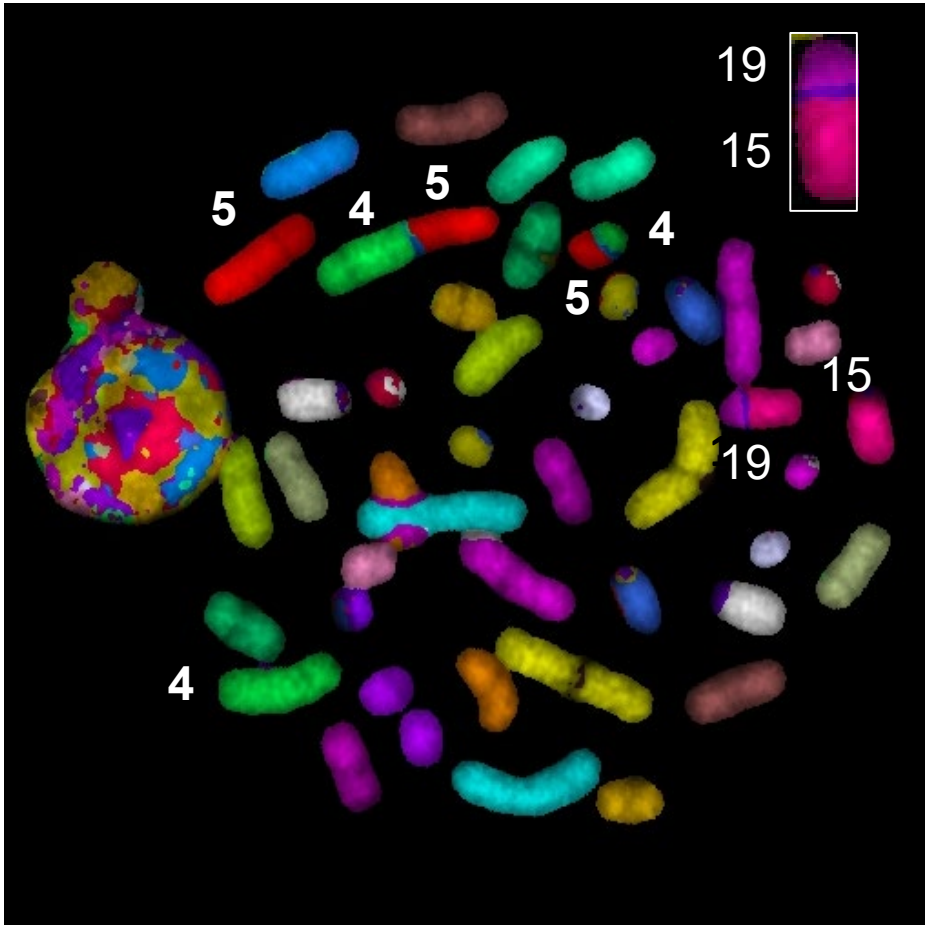
Rings


Ring Chromosome
Deleted Genetic Material
Breaks in Chromosome
Fusion
Deleted Genetic Material
U.S. National Library of Medicine


Dicentric
Ring

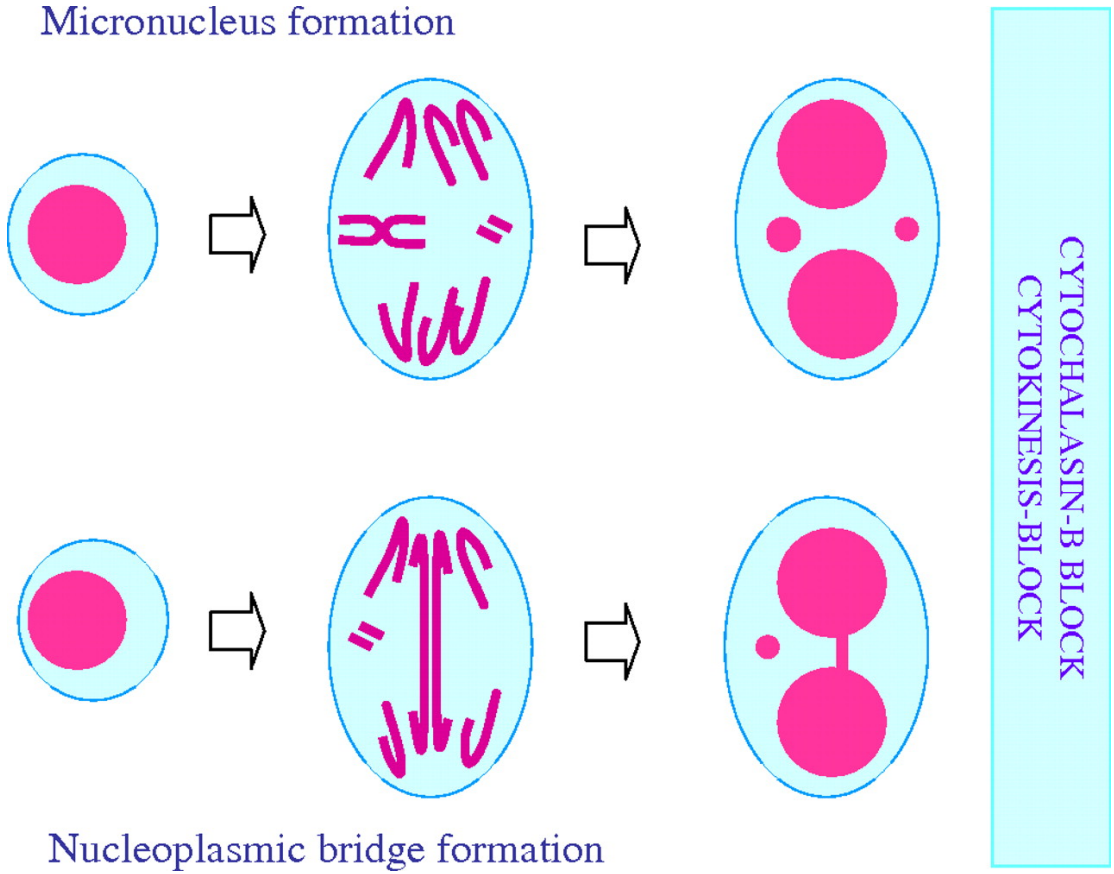
Translocation





Micronuclei

Micronuclei is a useful biomarker for radiation exposure.



M. Fenech et al. Mutagenesis 2011; 26:125-132

Limitations of Cytogenetic Biodosimeters

Difficult to increase high throughput sampling because cytogenetic assays are usually done in 15 ml conical tubes

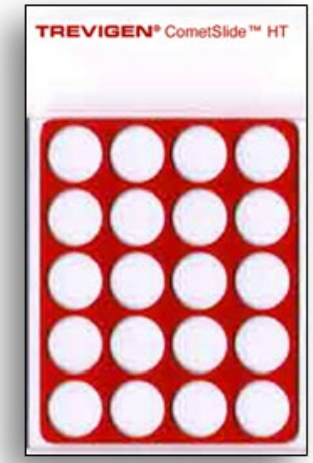
Processing time is long (48-72hr) and labor intensive

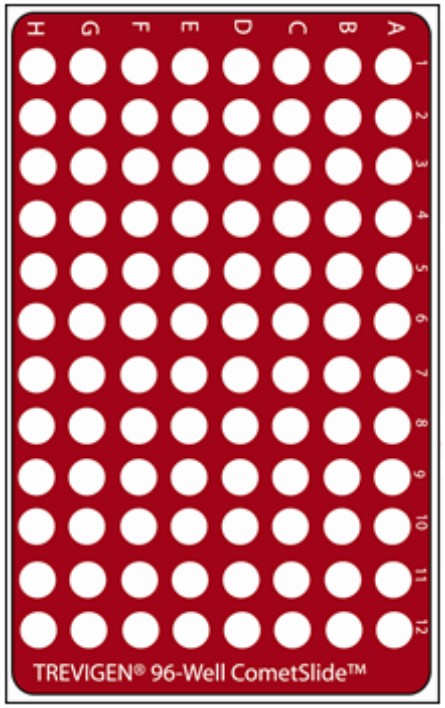
Manual analysis is time consuming

Automation of high throughput techniques for sample processing, imaging and analysis is critical for biodosimetry in case of radiological/nuclear incident(s)

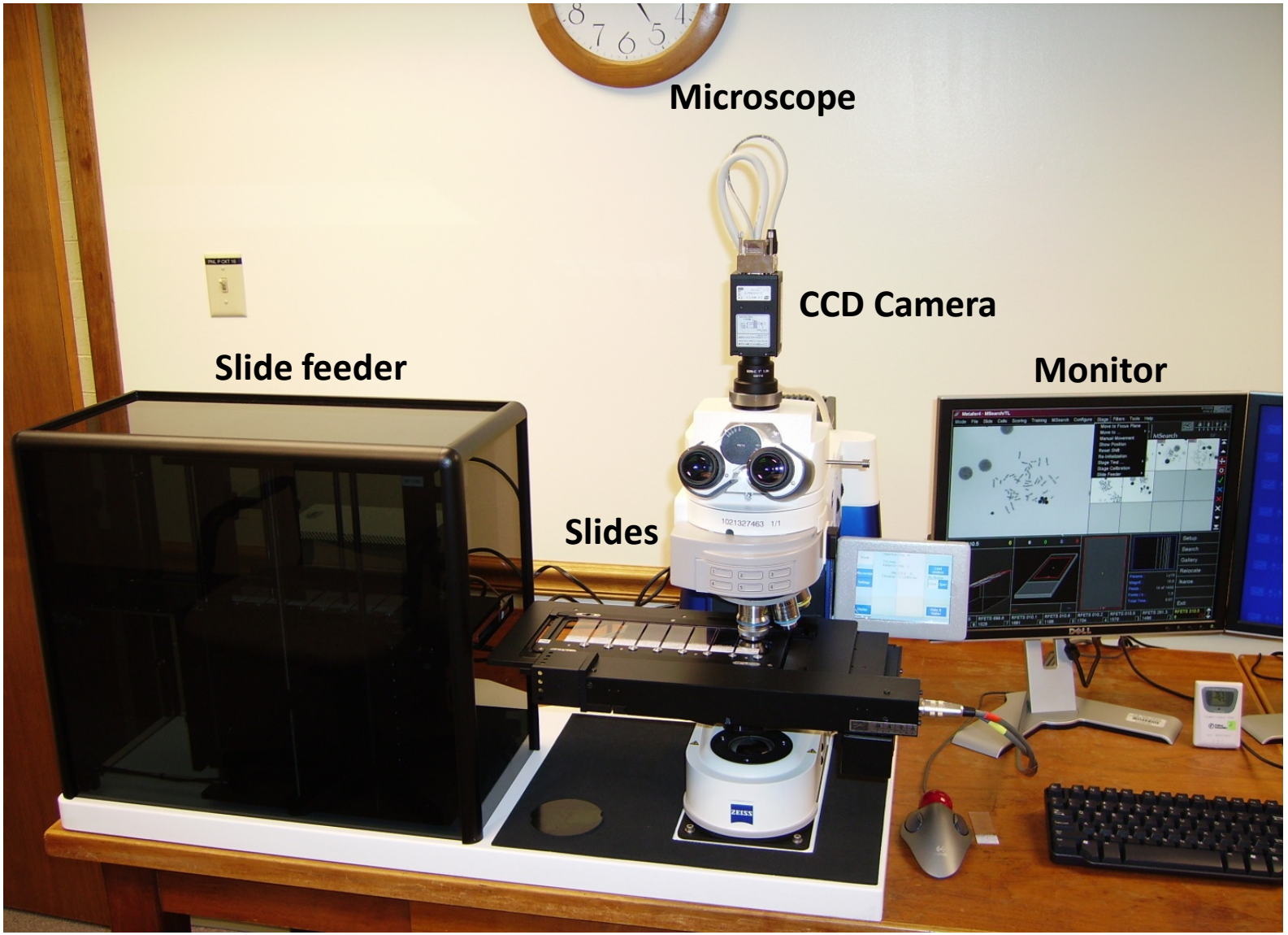
Maximizing the Sample Processing

Use of bar coded 1.2 mL tubes can be used for high throughput sample processing for cytogenetic analysis using as little as 50 µL of blood

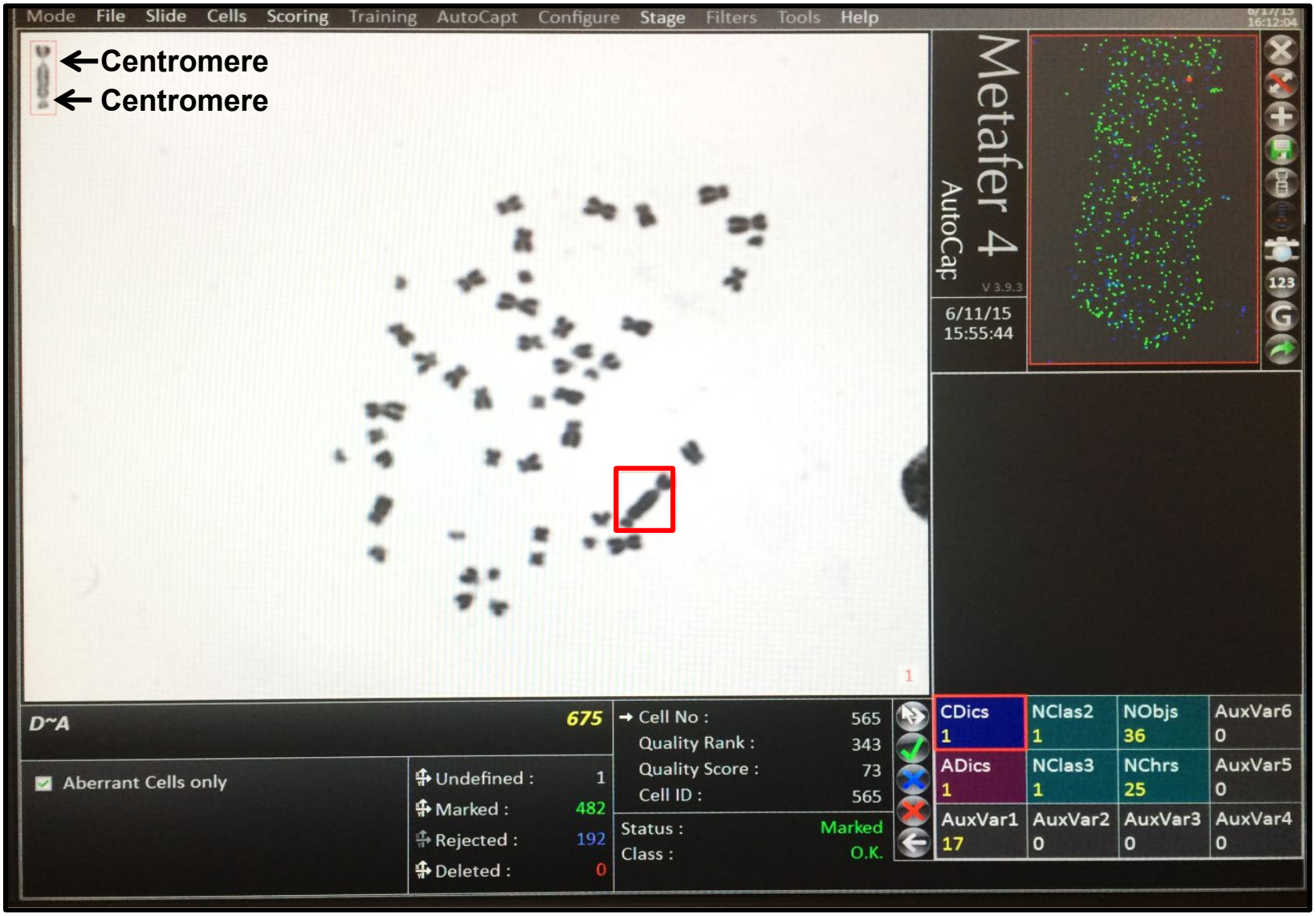




Samples can be spotted on multi-chambered glass slides for high throughput imaging and analysis


Microscope
CCD Camera
Slide feeder
Monitor
Slides

Automated Detection of Human Dicentric Chromosomes Using Metafer



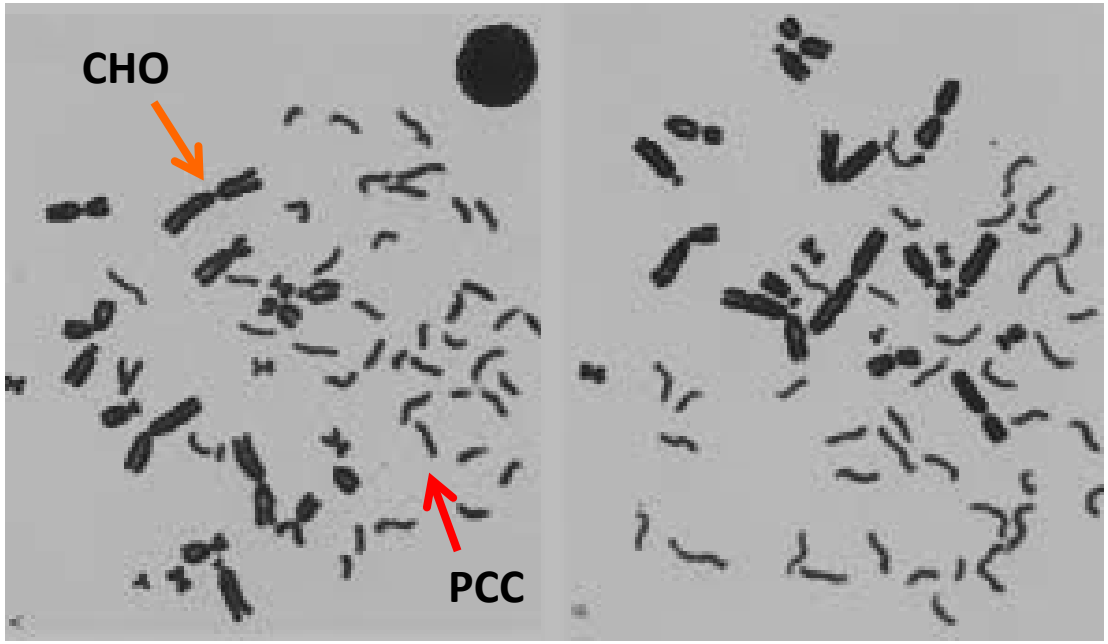
Timeline

Time Line for Single Metaphase Image Scanning , Capture and Dicentric Analysis

	Anonymous scorer (Manual)	Metafer (Automated)
Spotting a metaphase spread at 10X Objective	10-15 sec	~0.4 sec
Analysis at 63X Objective		Capture/analysis time
Cell with 0 dicentrics	30 sec	8.5 ± 0.1 sec
Cell with 1-2 dicentrics	35 sec	8.5 ± 0.1 sec
Cell with 2-4 dicentrics	45 sec	8.5 ± 0.1 sec
Cell with 6-8 dicentrics	1 min	8.5 ± 0.1 sec
Cell with > 8 dicentrics	1 min 15 sec	8.5 ± 0.1 sec
Average time for a metaphase	49 sec	8.5 sec
Average time for 1000 metaphases	13.61 h	2.47 h

Premature Chromosome Condensation (PCC) Technique

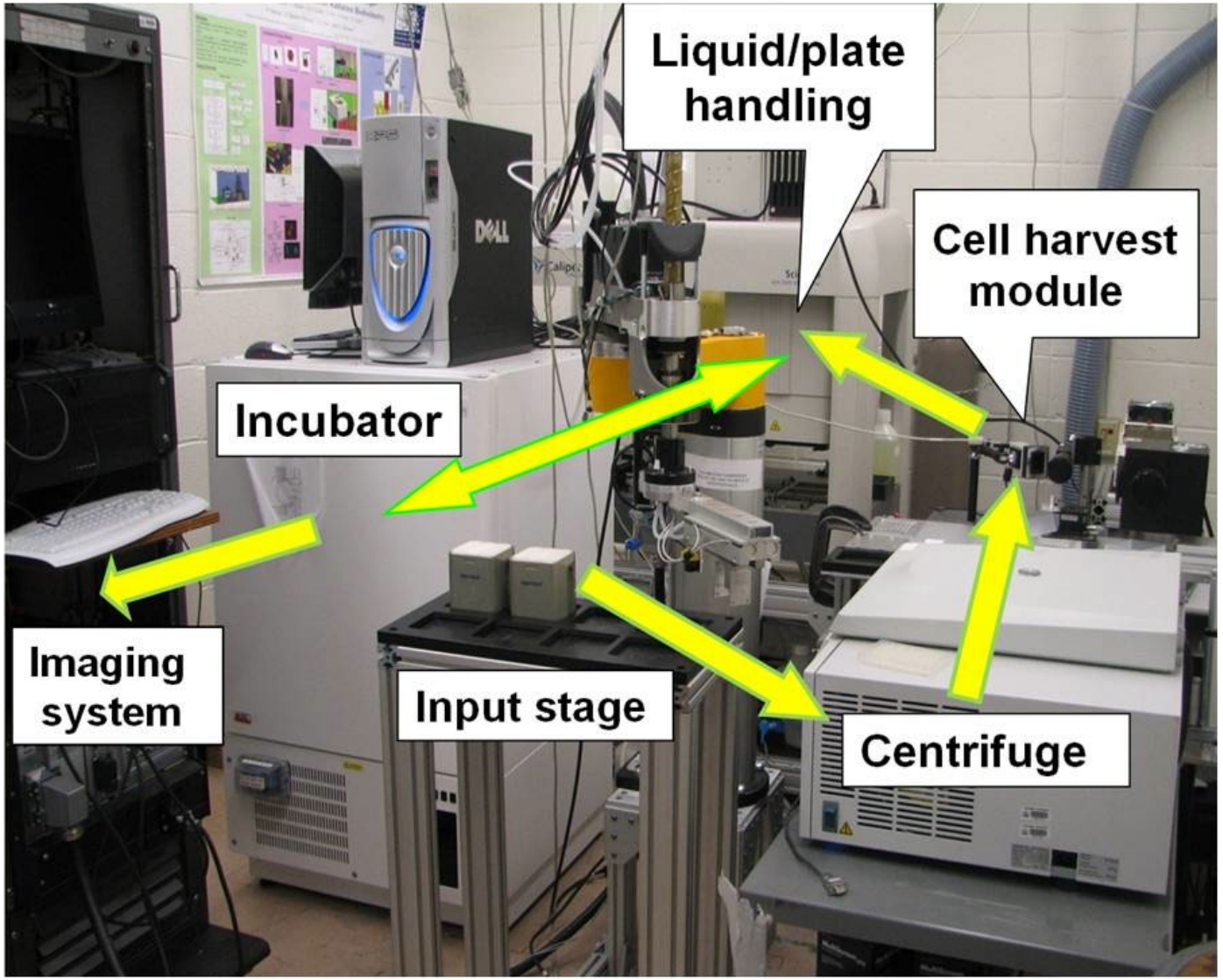
Fusion of mitotic CHO cells with human G0 lymphocytes



Dicentrics, rings and translocations can be detected on PCC by FISH

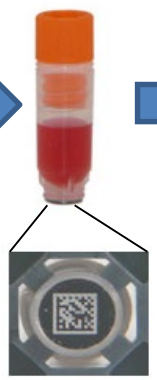
Dicentric assay time can be cut short to 6h after blood collection

Rapid Automated Biodosimetry Tool (RABiT)



CUMC NY

https://www.youtube.com/watch?feature=player_embedded&v=ESeT63F3YQY#t=0



Matrix plate

Each tube individually barcoded

To RABiT

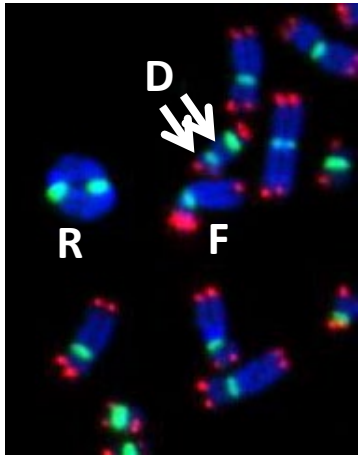
- Add medium
- Culture
- Fix cells

Clear bottom plate

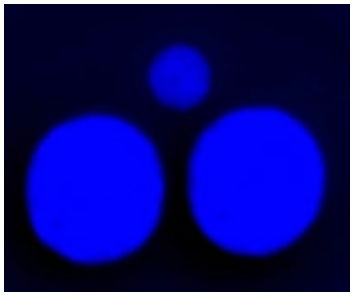
- Add dye
- Image analysis

Multiple High Throughput Radio Biodosimetry Assays

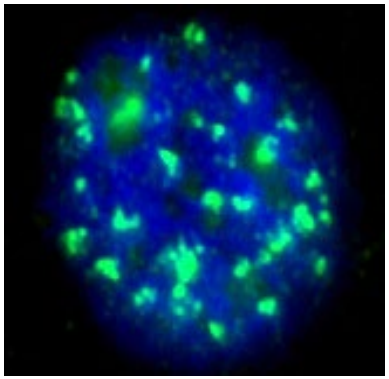
Dicentric Assay



Micronucleus Assay

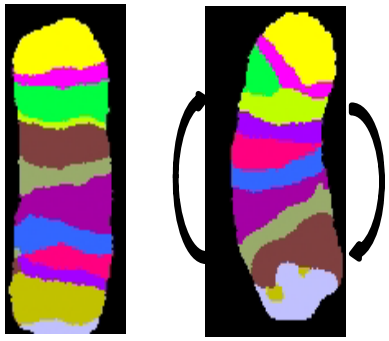


RABiT with robust imaging system



Y-H2AX Assay

Inversion in Chr.5



M-band FISH Assay

An automated imaging system for radiation biodosimetry.

Microsc Res Tech. 2015 Jul;78(7):587-98. doi: 10.1002/jemt.22512. Epub 2015 May 4.

Biomarkers

Biomarkers for multiple organ dysfunction syndrome (MODS) after radiation exposure *in vivo.*

1. Hematopoietic system/Bone marrow aplasi - Flt3-L
2. Gastrointestinal tract - Citrulline
3. Liver and Cardiovascular - Oxysterols
4. Salivary gland - Amylase

Flt3-L has been successively used to distinguish between total and partial body irradiation in mice.

Conclusions

- Recent years have seen a tremendous improvements in developing multiple genomic, proteomic, metabolomics and transcriptomic biomarkers for assessing radiation exposure and dose prediction.
- All these diverse biomarkers will be further defined, validated and available for future use in case of nuclear accidents!
- Thanks to all of those radiobiology scientists who tirelessly work towards achieving the goals for human health and welfare.

Acknowledgements

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