Cytogenetic methods in Radiation Biology using normal and tumor cell lines

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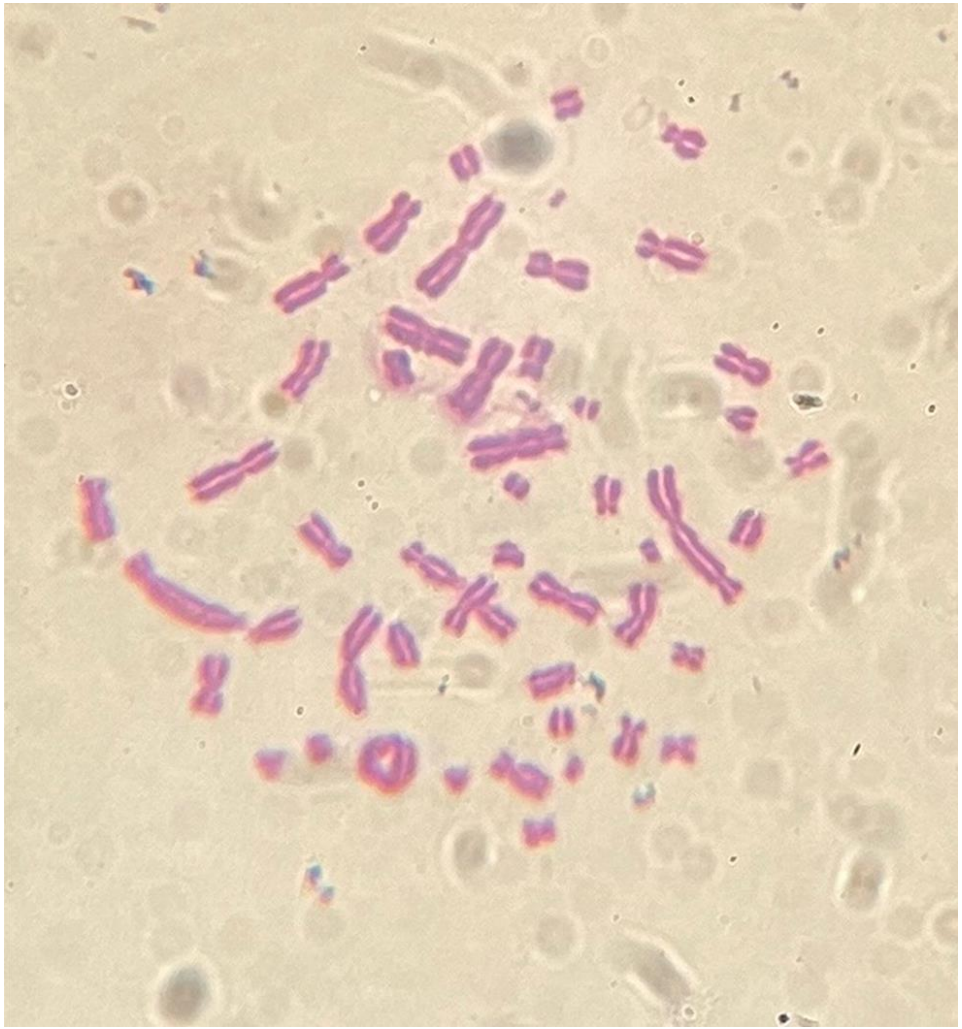
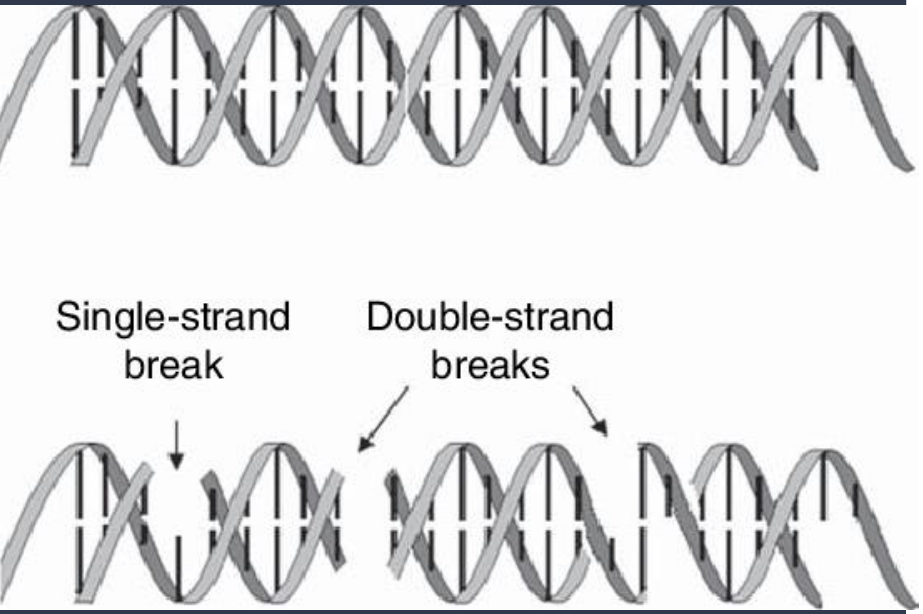
Outline

- Background
- Aim and Objectives
- Methods
  - PCC
  - Metaphase
- Analysis
  - PCC
  - Metaphase
  - Results
- Conclusion
- References
- Acknowledgements



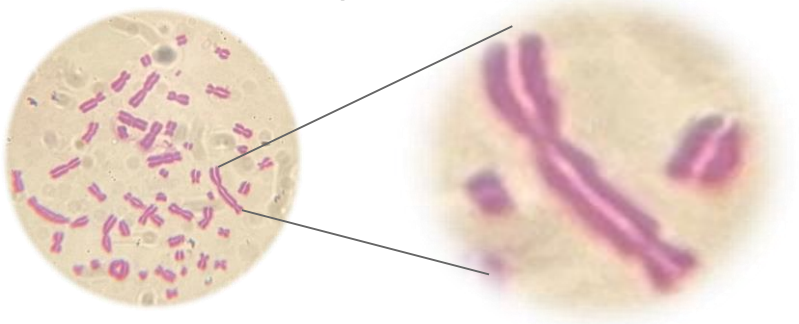
Background



•Radiation biology investigates how ionizing radiation (e.g., X-rays, gamma rays) causes DNA damage and structural changes in living cells (Al-Qabandi and Alshammary, 2022).

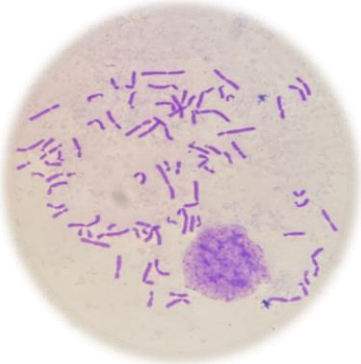
•Such damage can lead to chromosomal aberrations, which are a serious form of damage as well as an important and sensitive indicator used in cancer therapy and radiation protection.

•Cytogenetic methods allow direct visualization and analysis of chromosomal damage, helping to assess radiation exposure and its biological effects (Belli & Indovina, 2020).

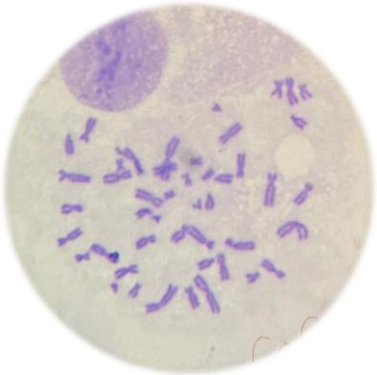


Purpose

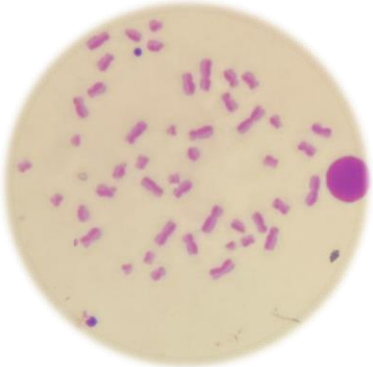
To investigate the effect of ionizing radiation on various normal cells and cancer cells: human glioblastoma (U87), human breast carcinoma (Cal51), and normal human blood lymphocytes using cytogenetic methods



U87



Cal51

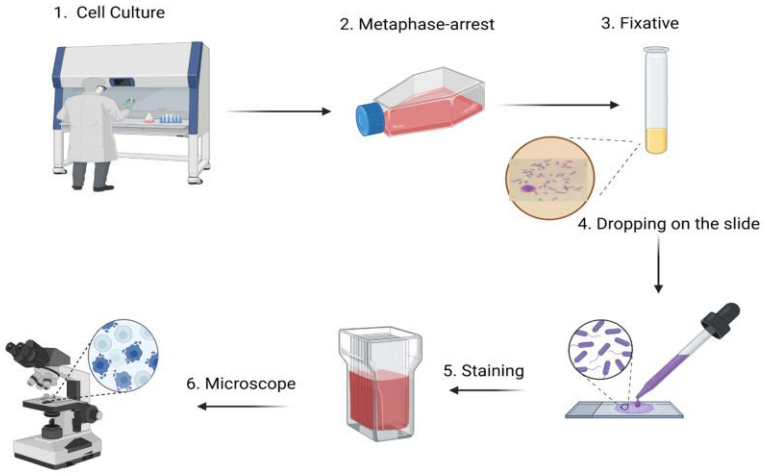


lymphocytes

Objectives

- To culture and prepare metaphase and PCC spreads from U87, CAL51, and healthy donor lymphocytes
- To apply Giemsa and mFISH staining for visualization of chromosome aberrations
- To classify and compare simple and complex chromosomal aberrations detected by mFISH method.
- To evaluate the relative sensitivity of each cytogenetic technique and staining method across different cell types.

Materials and Method—Metaphase



Cal51 cells

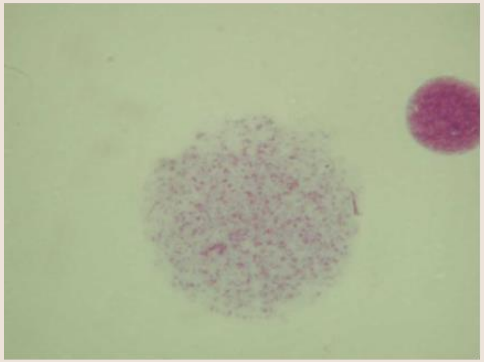
U87 cells

Optimization of standard protocol for different cell lines culture

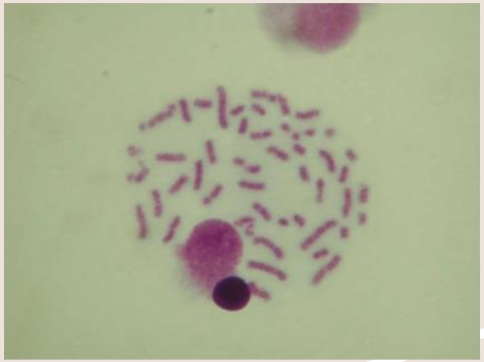
Cell line	Medium	FCS	AB *	Col*	Trypsin	0.075 M KCl	Fixative 3:1
Human breast carcinoma (Cal 51)	DMEM (low glucose)	15%	+	1 hour 10µL/mL	0.25% + EDTA	10 mins + s***	X2
Human glioblastoma (U87)	DMEM (high glucose)	10%	+	1 hour 10µL/mL	0.05% + EDTA	8 mins + s***	X2
Normal human blood lymphocytes	RPMI 1640	20%	+	2 hour 15µL/mL	In suspension	20 mins	X3

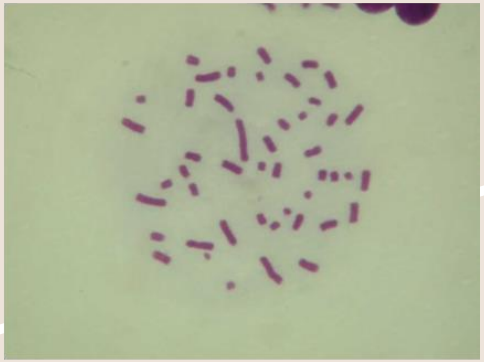
Classification & Analysis - Premature Chromosome Condensation (PCC)

(a) S-phase

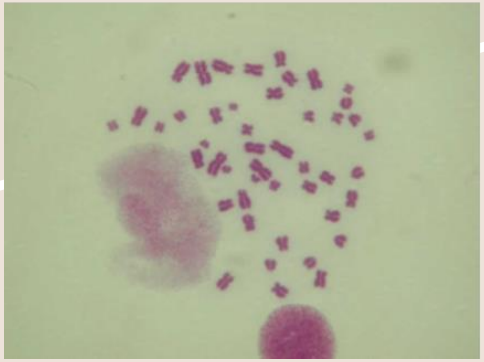


(b) Early G_2-phase (c) Late G_2-phase

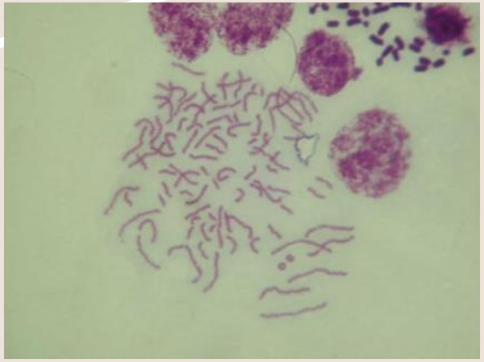




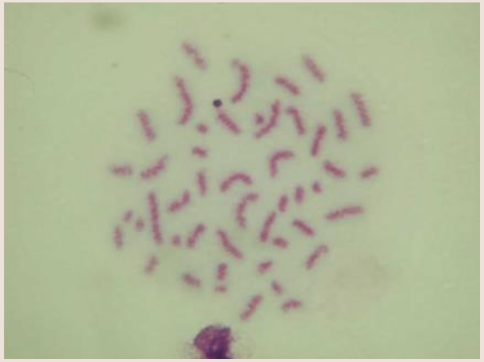
(d) Metaphase



(e) Late metaphase



(f) G_1-phase



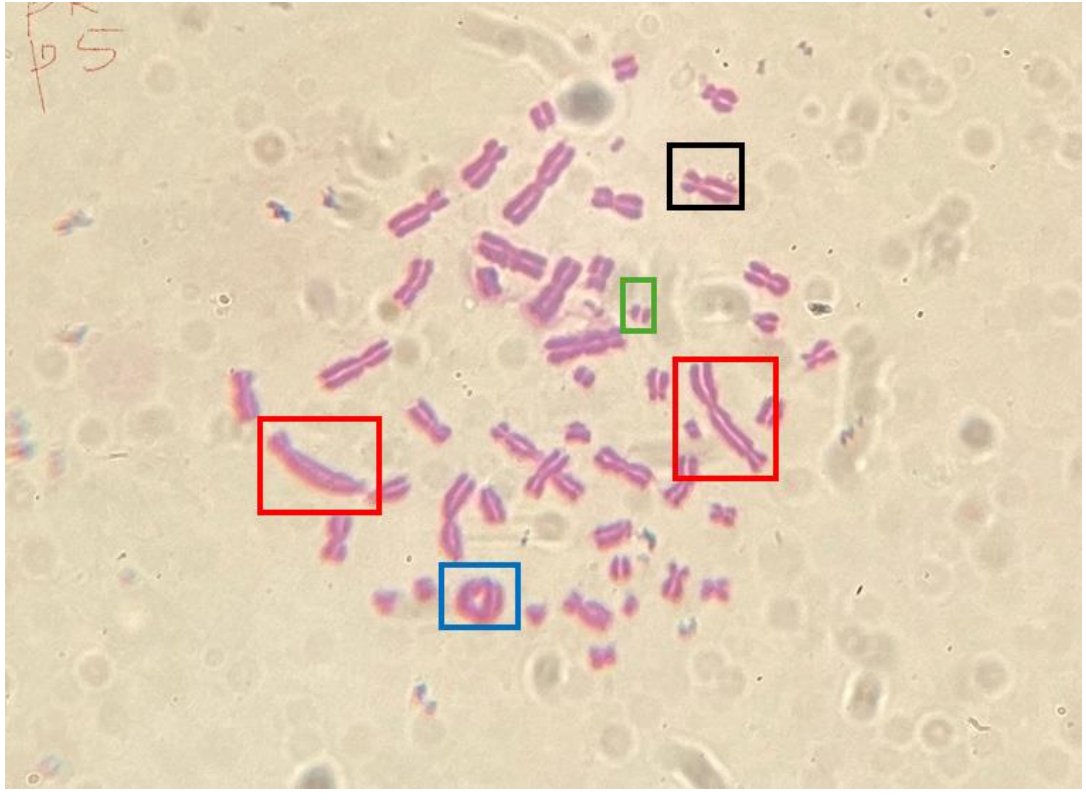
Classification & Analysis - Metaphase

BLACK: Normal chromosome

RED: Dicentric chromosome

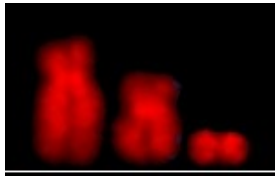
BLUE: Ring chromosome

GREEN: Acentric fragment



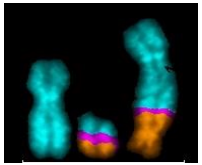
Classification & Analysis – mFISH

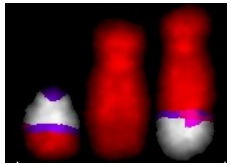
Simple aberrations:

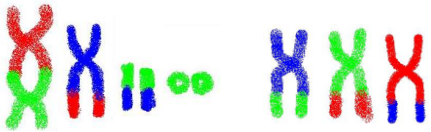


one break aberrations: *acentrics*

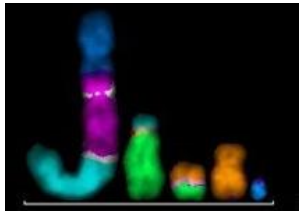
two-break aberrations: simple exchanges (*dicentrics, rings, translocations*)

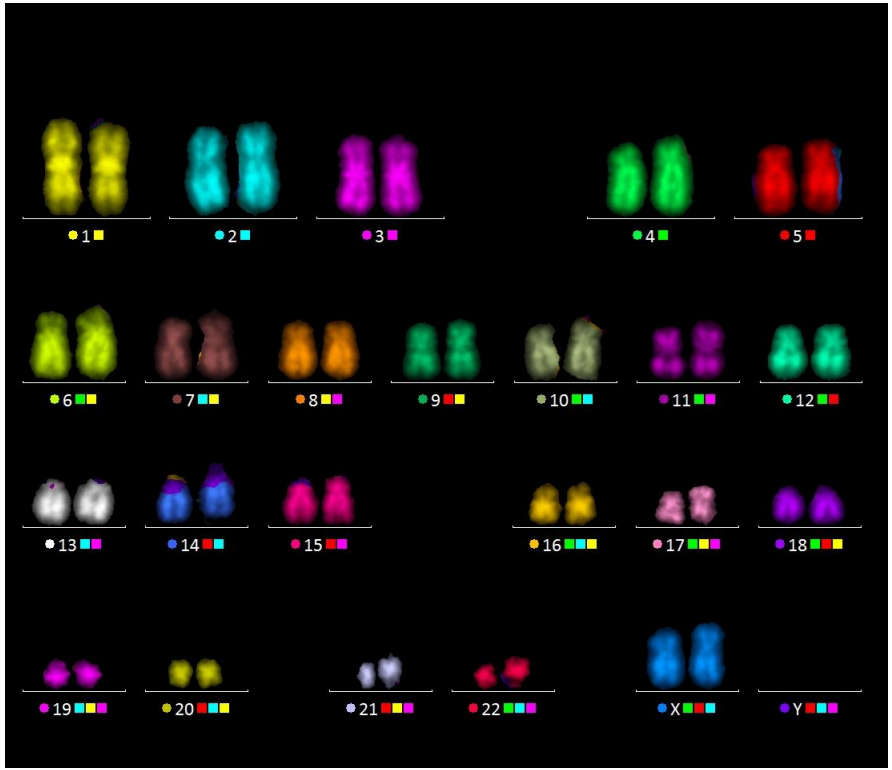




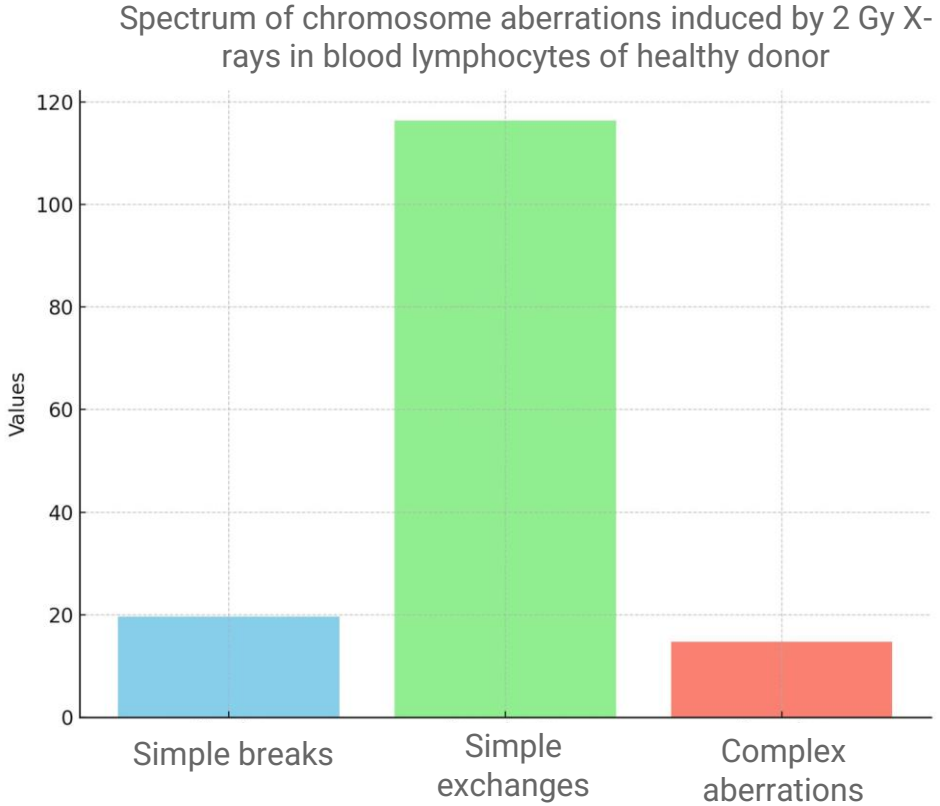


Complex aberrations: 3 or more breaks in 2 or more chromosomes.





Spectrum of radiation-induced aberrations



Conclusion

- Cytogenetic methods (PCC, metaphase, mFISH) effectively detect radiation-induced chromosome aberrations
- The study successfully identified both simple and complex chromosome damage in U87, Cal51, and lymphocyte cells.
- These techniques are valuable tools for applications in radiation therapy monitoring, biodosimetry, and radiation biology research.

ACKNOWLEDGMENTS

NRF National Research Foundation | iThemba LABS Laboratory for Accelerator Based Sciences

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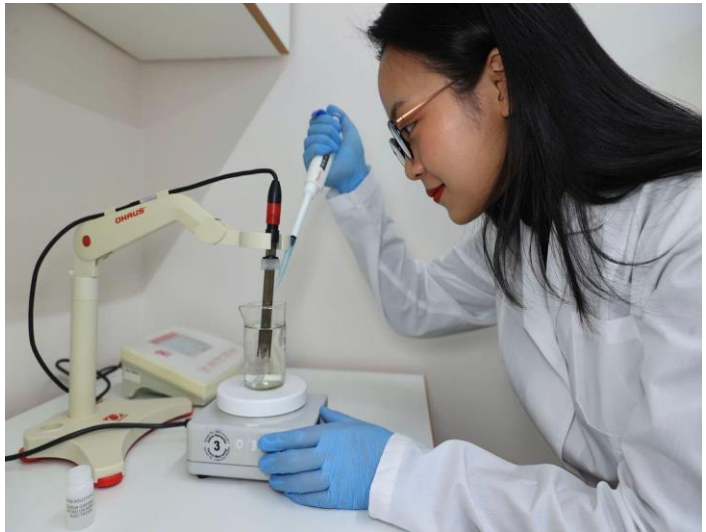

Thank you for your attention!