Mays Cancer Center
UT Health San Antonio MD Anderson Cancer Center

A DNA Double-Strand Break Dosimeter

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Acknowledgments

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- N. Kirby, E. Y. Shim, and S. E. Lee, “Devices and methods for a DNA double-strand-break dosimeter,” USPTO 62/290,985.

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The Measurement Problem

UNITS: Gray

$$\frac{Energy\ Deposited}{Mass}$$

The Measurement Problem

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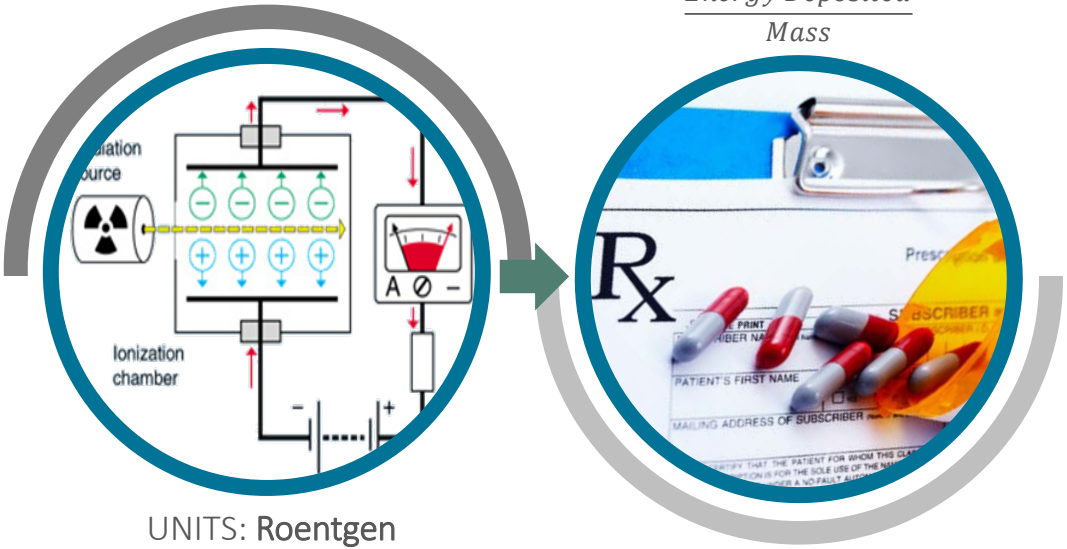
$$\frac{Energy\ Deposited}{Mass}$$



UNITS: **Roentgen**

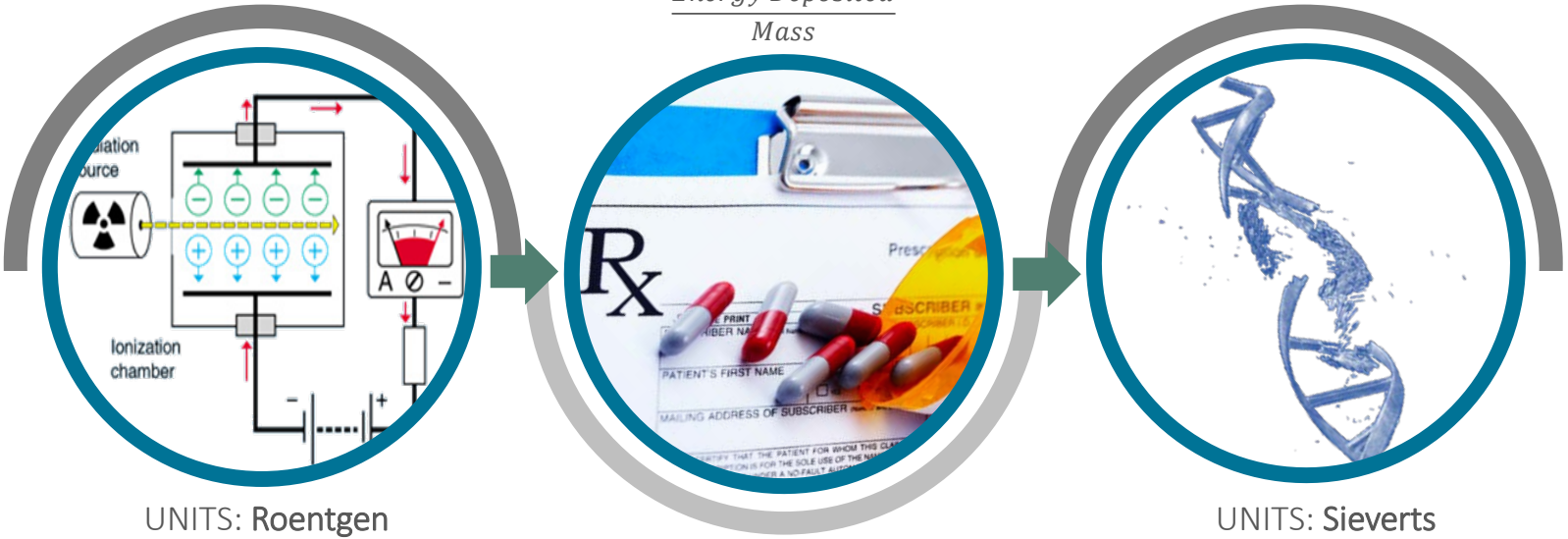
$$\frac{Charge\ Ionized}{Mass}$$

Exposure is connected to absorbed dose using calibration protocols for specific scenarios.

The Measurement Problem

UNITS: **Gray**

$$\frac{Energy\ Deposited}{Mass}$$



UNITS: **Roentgen**

$$\frac{Charge\ Ionized}{Mass}$$

UNITS: **Sieverts**

$$Biological\ Damage$$

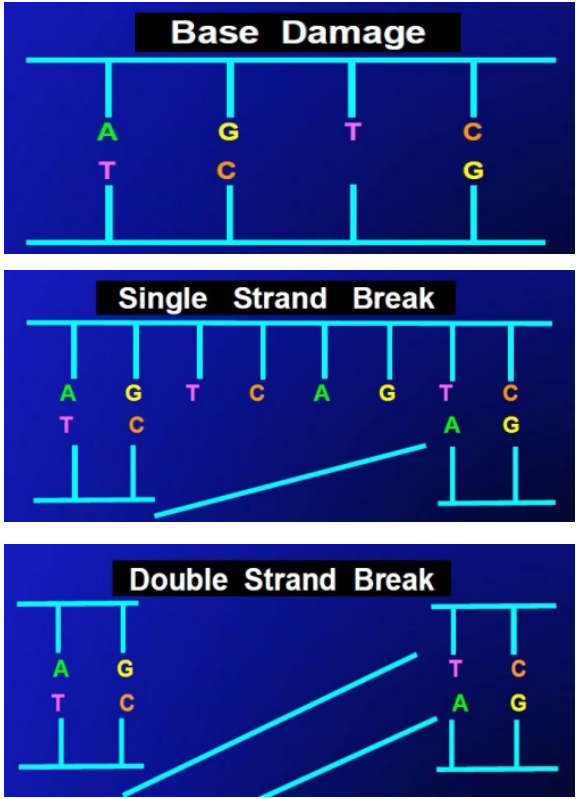
Exposure is connected to absorbed dose using calibration protocols for specific scenarios.

Absorbed dose is connected to biological damage using weighting factors.

Radiation Induced DNA Damage

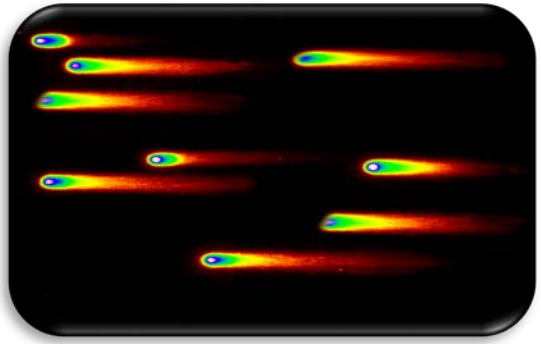
- Radiation causes different types of damages to the DNA in cells.

- DNA double strand break (DSB) is the dominant factor for radiation-induced cell damage (difficult to repair).



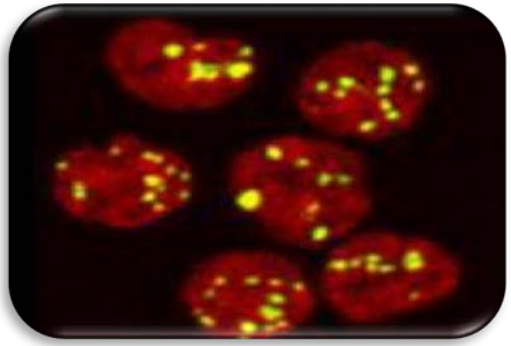
Methods Used to Detect DNA Damage

Comet Assay



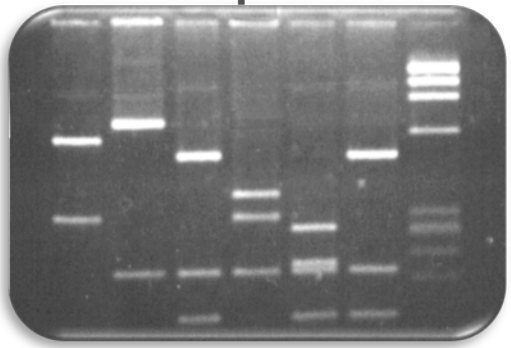
Cells embedded in agarose on a microscope slide are lysed with detergent

γ-H2AX Assay



Phosphorylation of γ-H2AX histone if cells have DSB

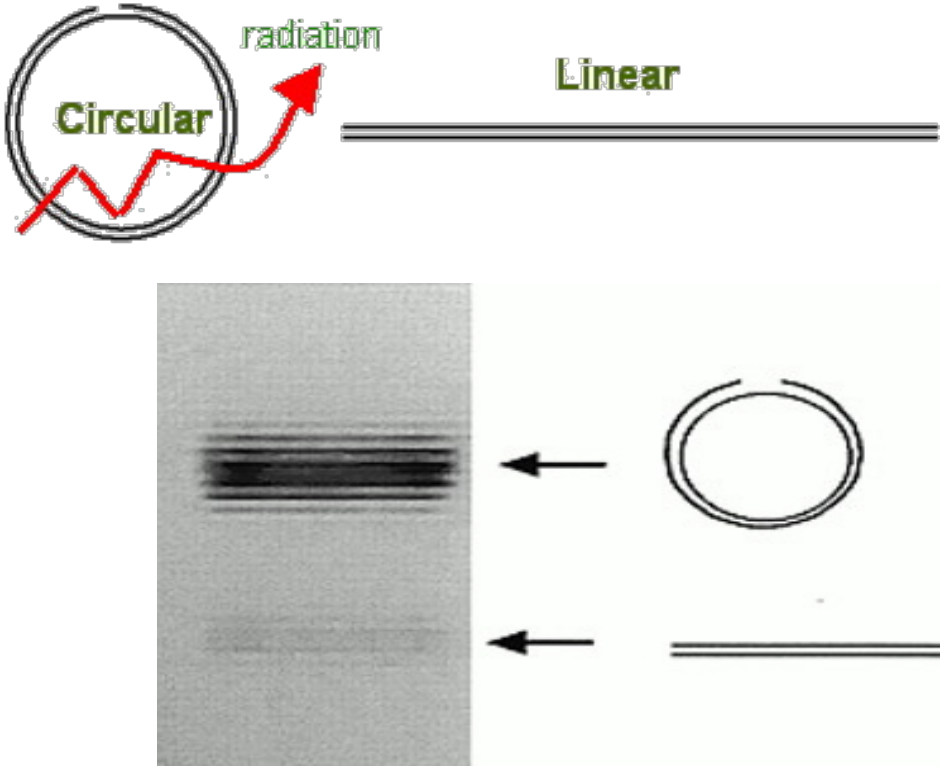
Gel Electrophoresis



Separate DNA and its fragments according to size and charge.

Time consuming & not practical to be used in a daily basis at clinic

Previous Work – Chen et al., 1995



W. Chen, E. Blazek, and I. Rosenberg. "The relaxation of supercoiled DNA molecules as a biophysical dosimeter for ionizing radiations: a feasibility study." Medical Physics 22.9 (1995): 1369-1375.

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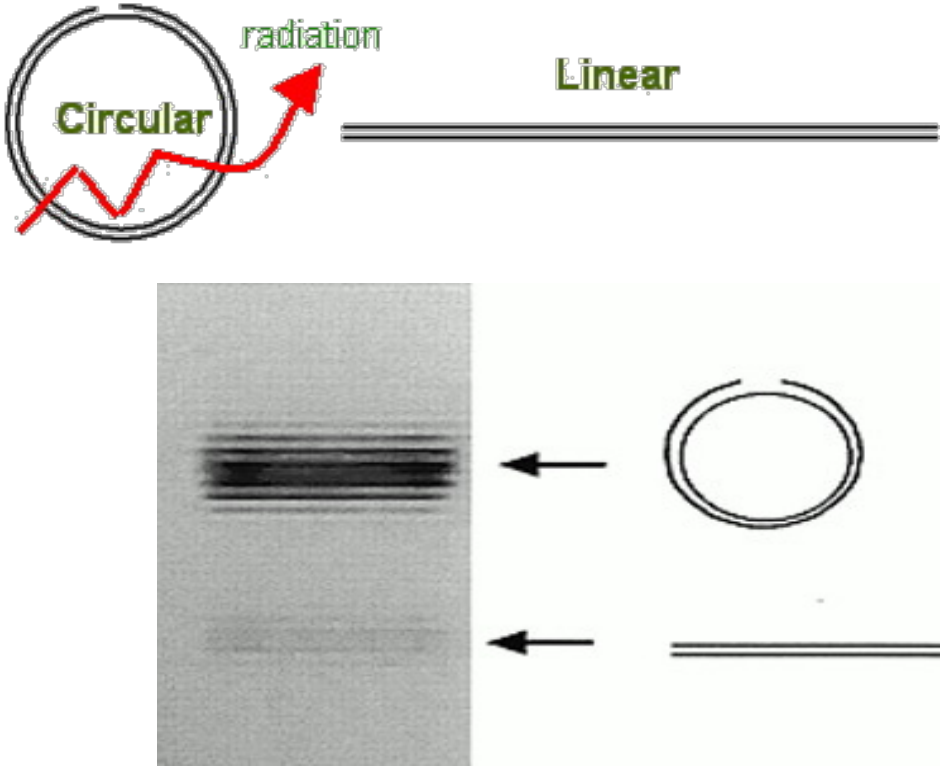


Usability Issues

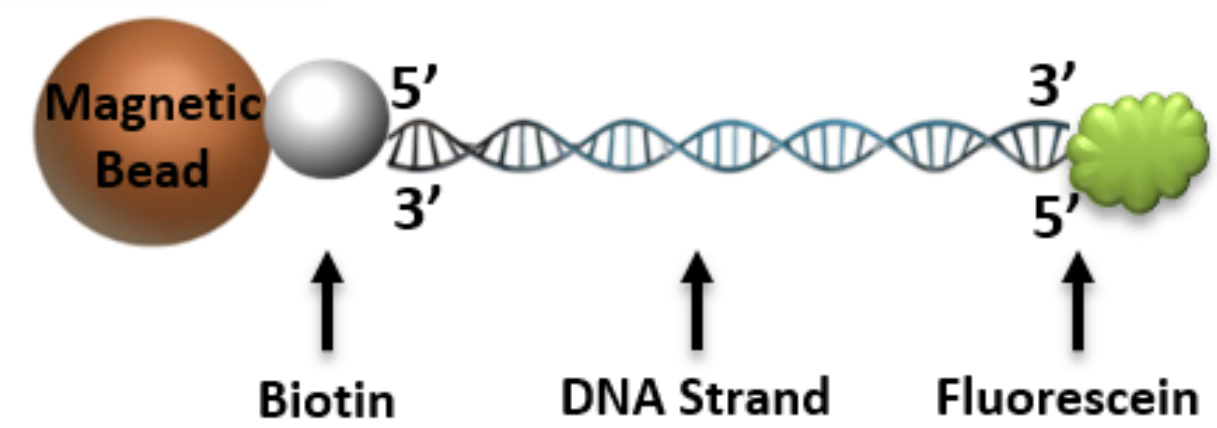
1. Need to irradiate and then transfer to gel.
2. Need to wait for gel separation.

W. Chen, E. Blazek, and I. Rosenberg. "The relaxation of supercoiled DNA molecules as a biophysical dosimeter for ionizing radiations: a feasibility study." Medical Physics 22.9 (1995): 1369-1375.

Purpose of This Work

- Create a more user-friendly DNA DSB dosimeter.
- Want to irradiate and separate in the same solution, faster than running a gel.

The Dosimeter Design



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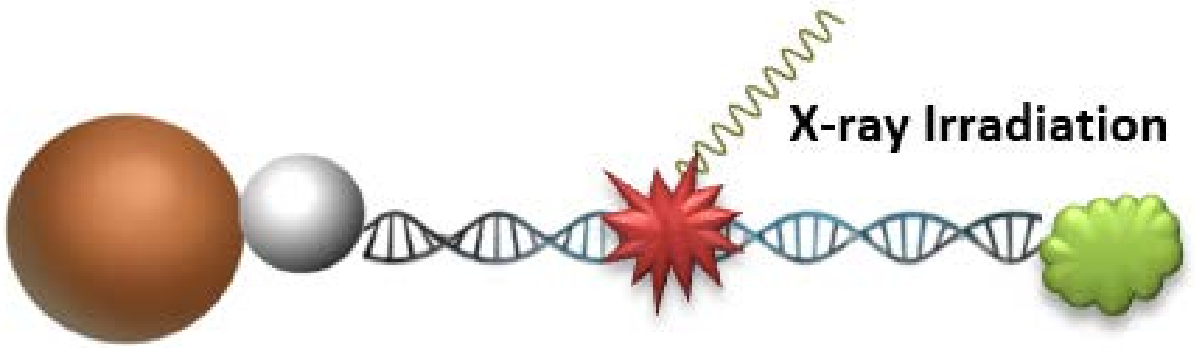

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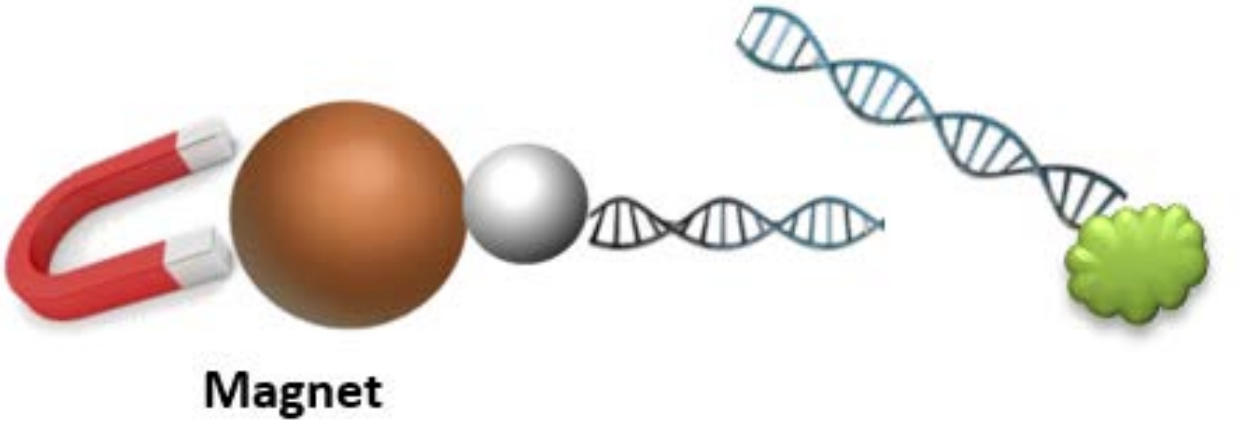
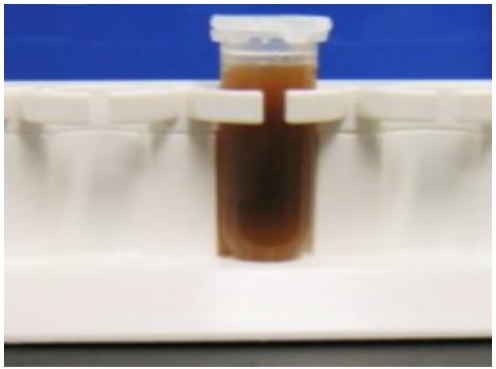
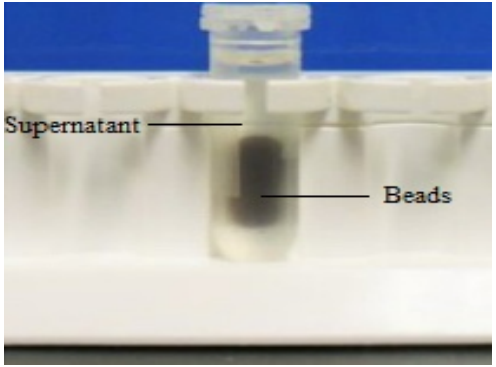
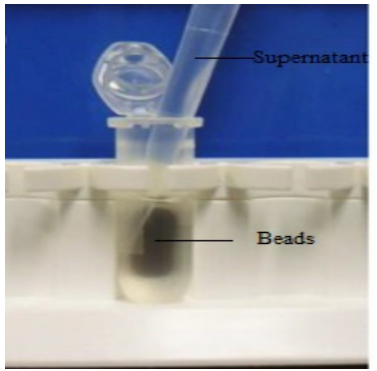


Measurement of DSB Probability

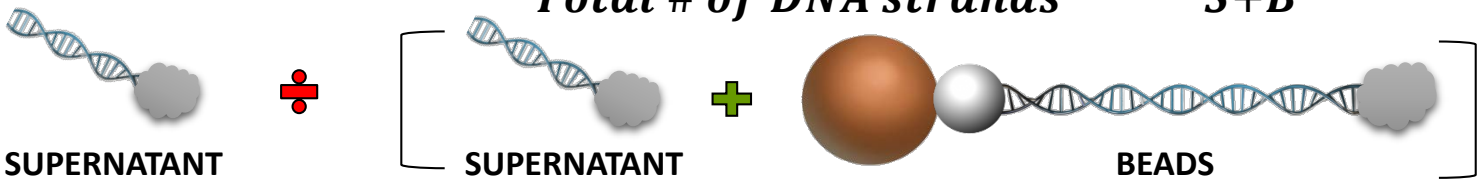
A magnet is used to separate broken from unbroken DNA



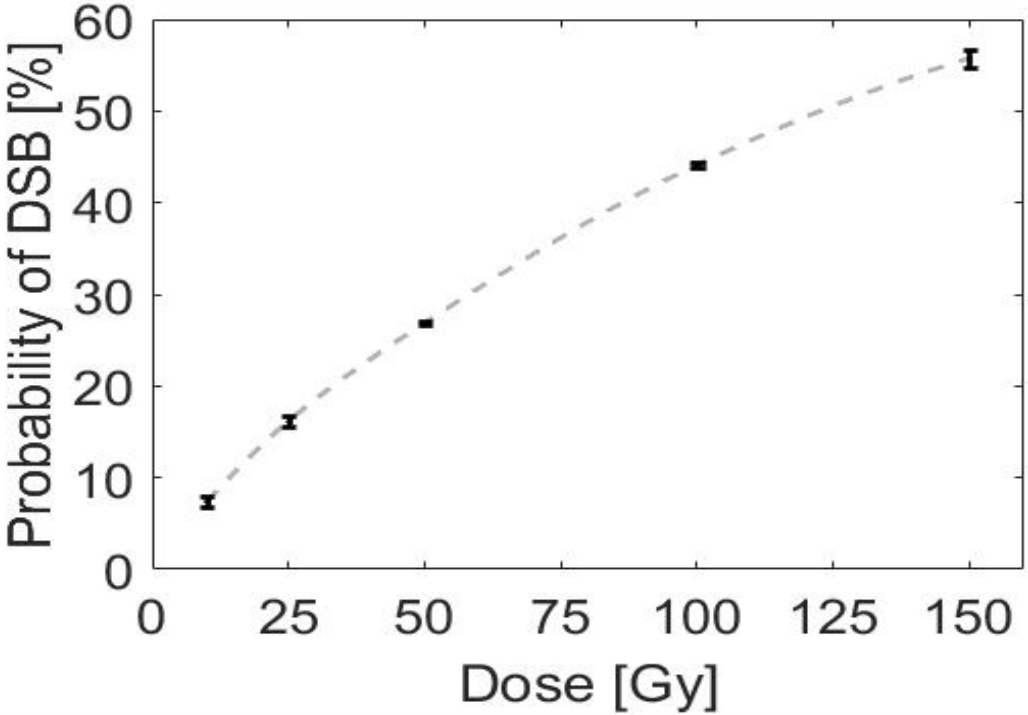




$$P\,(DSB) = \frac{\#\ of\ DNA\ strands\ with\ DSB}{Total\ \#\ of\ DNA\ strands} = \left(\frac{S}{S+B}\right)$$

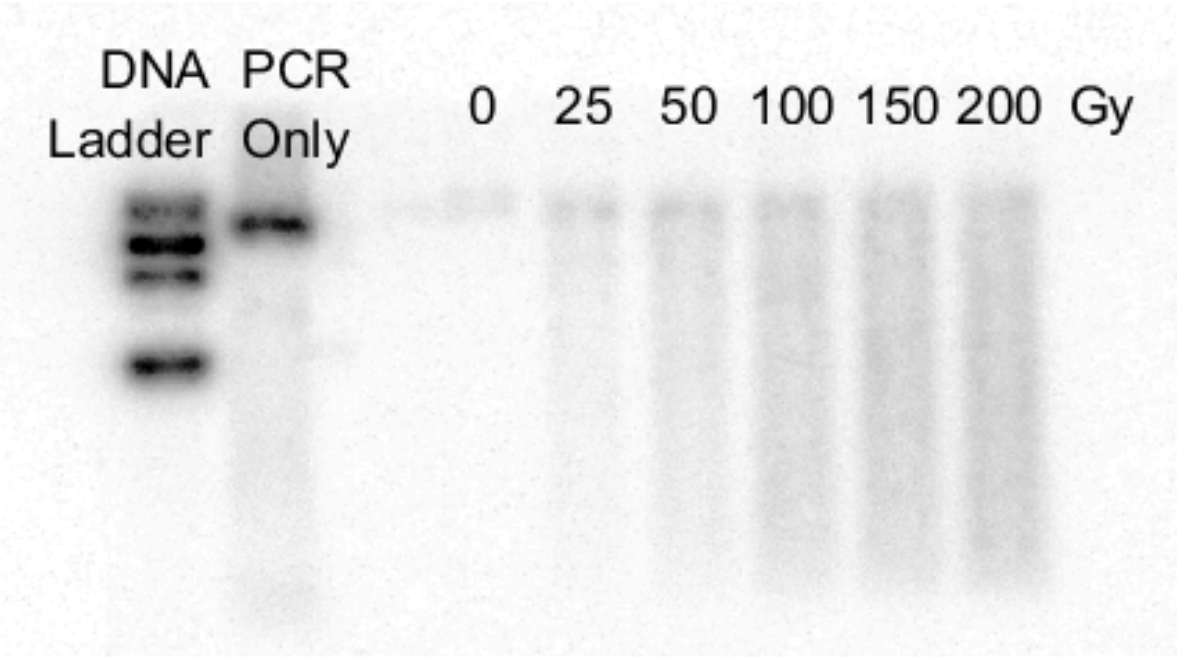


Initial Results



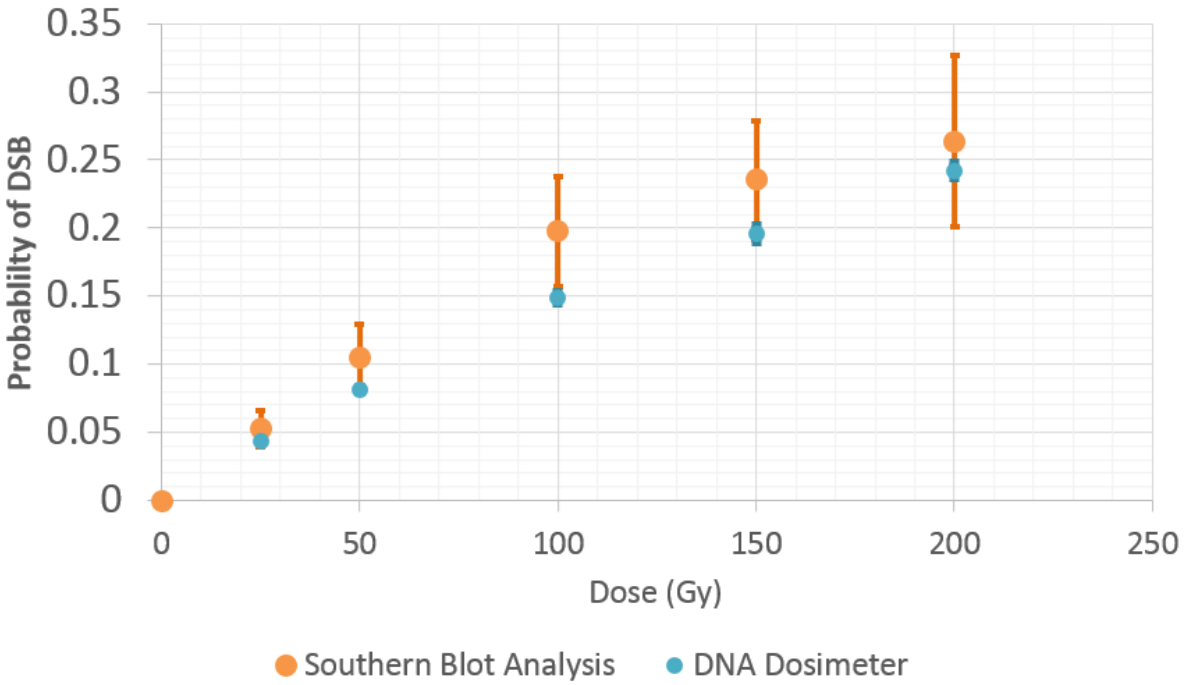
Benchmarking with Southern Blot

- Irradiated dosimeters.
- Ran gel electrophoresis on supernatant.
- Performed radiolabeling to enable quantitative determination of DNA length



Benchmarking with Southern Blot

- Results for composite of 3 separate experiments with 6 MV photons.
- The DNA dosimeter agrees with the Southern Blot analysis.

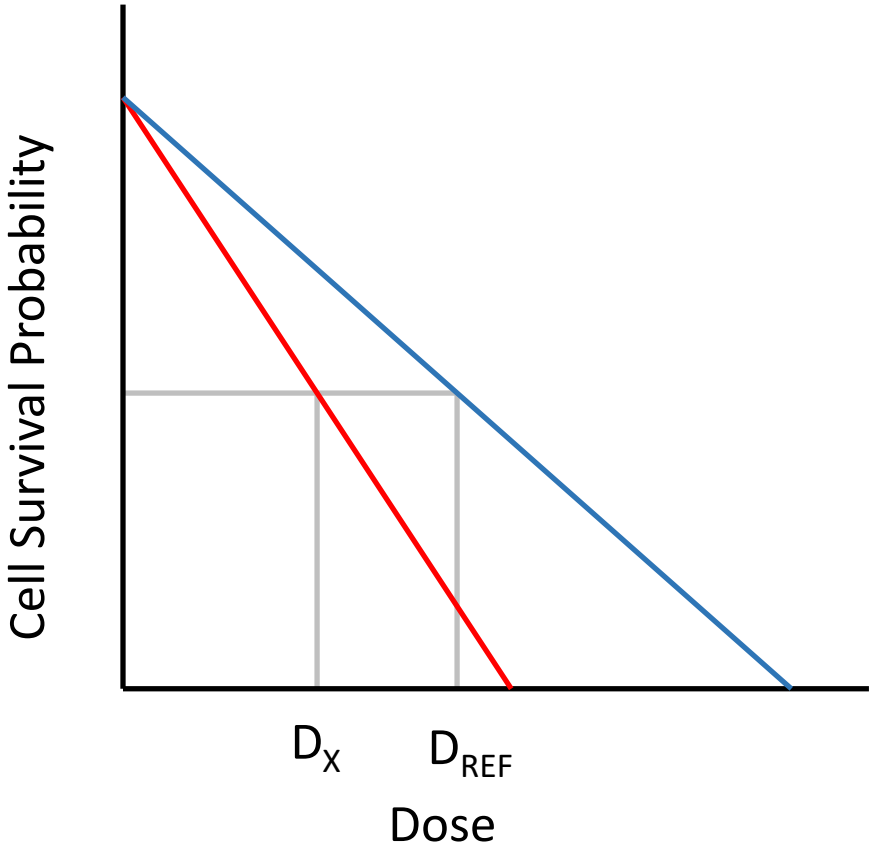


Dosimeter Properties

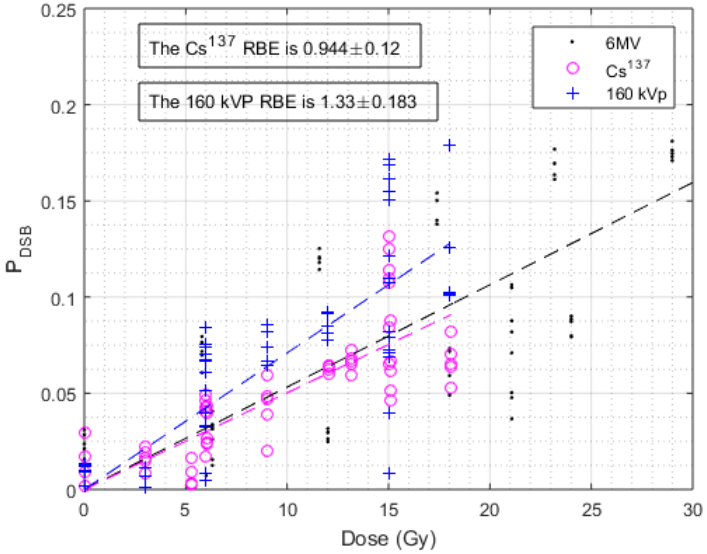
- Linearity – Linear below 50 Gy, but non-linear above.
- Range – Limited by precision. At 5, 10, and 50 Gy, we have COV of 14, 7, and 1%, respectively.
- Energy dependency – No apparent dependency for MV-scale photons, but an increase for lower energy photons.
- Directional dependence – Exhibited a directional dependence if the dosimeters settle to the bottom of encapsulation tubes.
- Dose-rate dependency – Have exhibited an increase in response to lower dose rates (30% increase at 0.6 Gy/min compared to 12.4 Gy/min).
- Cost - ~$6 per dosimeter.

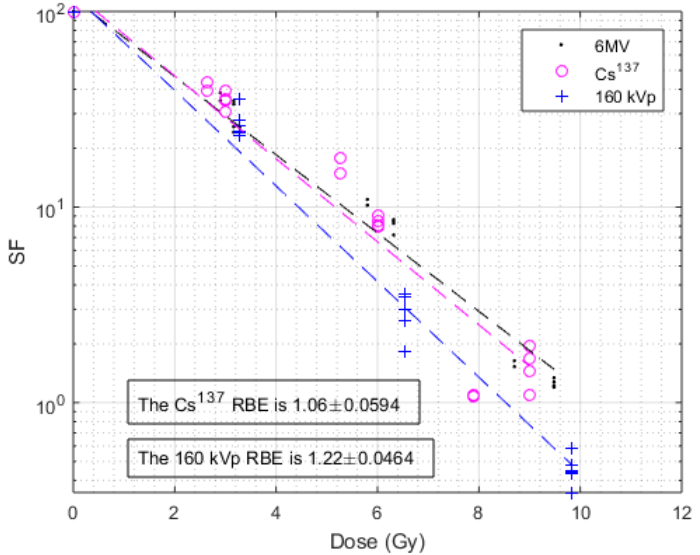
Relative Biological Effectiveness

$$RBE = \frac{D_{REF}}{D_X}$$



Relative Biological Effectiveness





	Linac	^{137}Cs	160 kVp
DNA Dosimeters	1	0.944±0.12	1.33±0.12
Murine Neural Stem Cells (mNs-5)	1	1.06±0.06	1.22±0.05

Future Work

- Extending RBE measurements to other cell lines.
- Proof-of-principle testing with neutron irradiation.
- Further refining of the technique.
- Benchmarking with Monte Carlo.

Final Remarks

- DNA dosimeters can measure the probability of DSB for a length of DNA.
- Initial experiments suggest these are biologically meaningful.
- Could you make a radiation standard for the number of DSB per base pair? Maybe, but we also know the results cannot be universally meaningful.

Thank you!