

Testing the role of oxygen in mediating the FLASH effect

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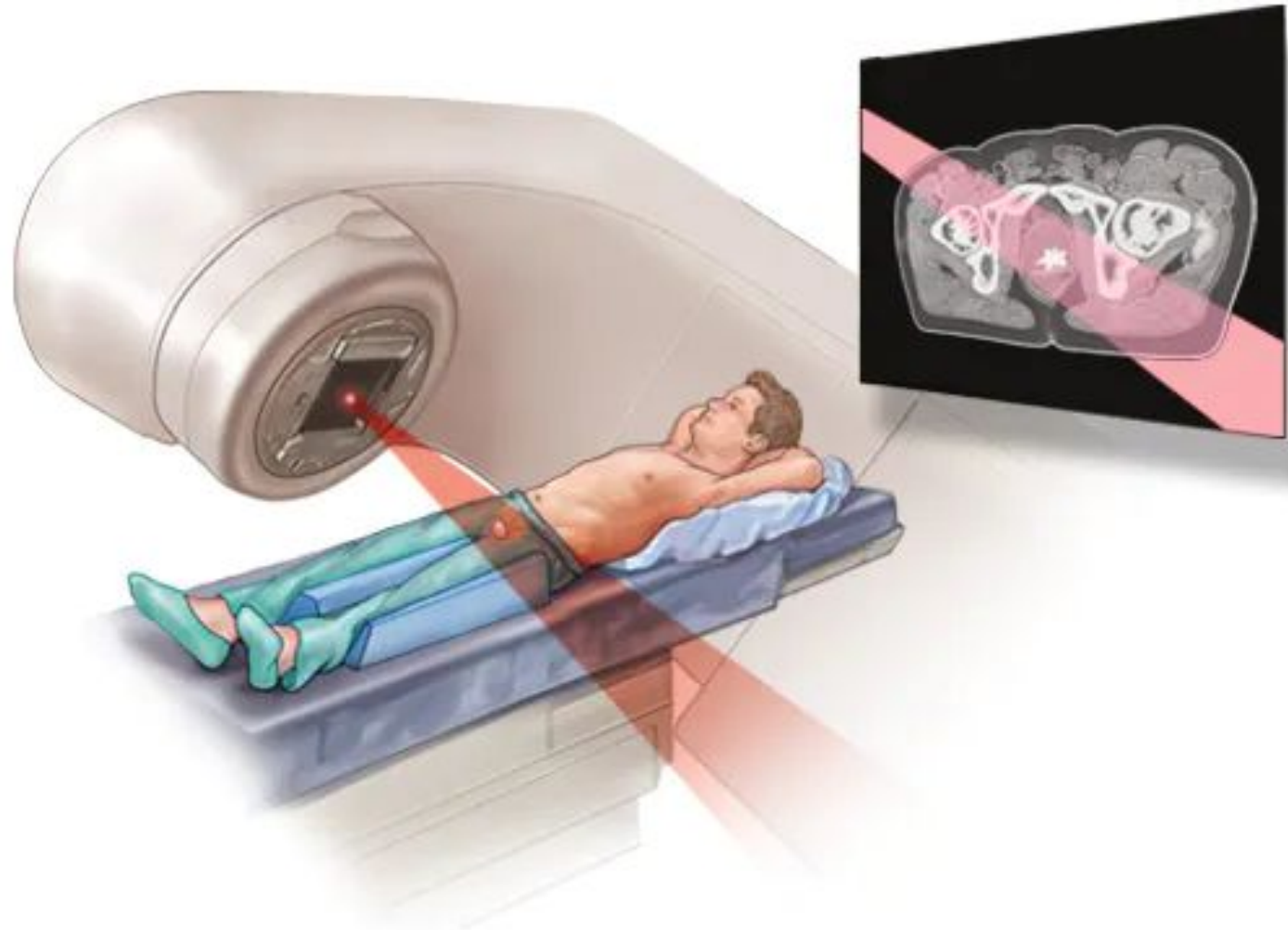
August 1, 2024



Radiotherapy in Cancer Treatment

Background

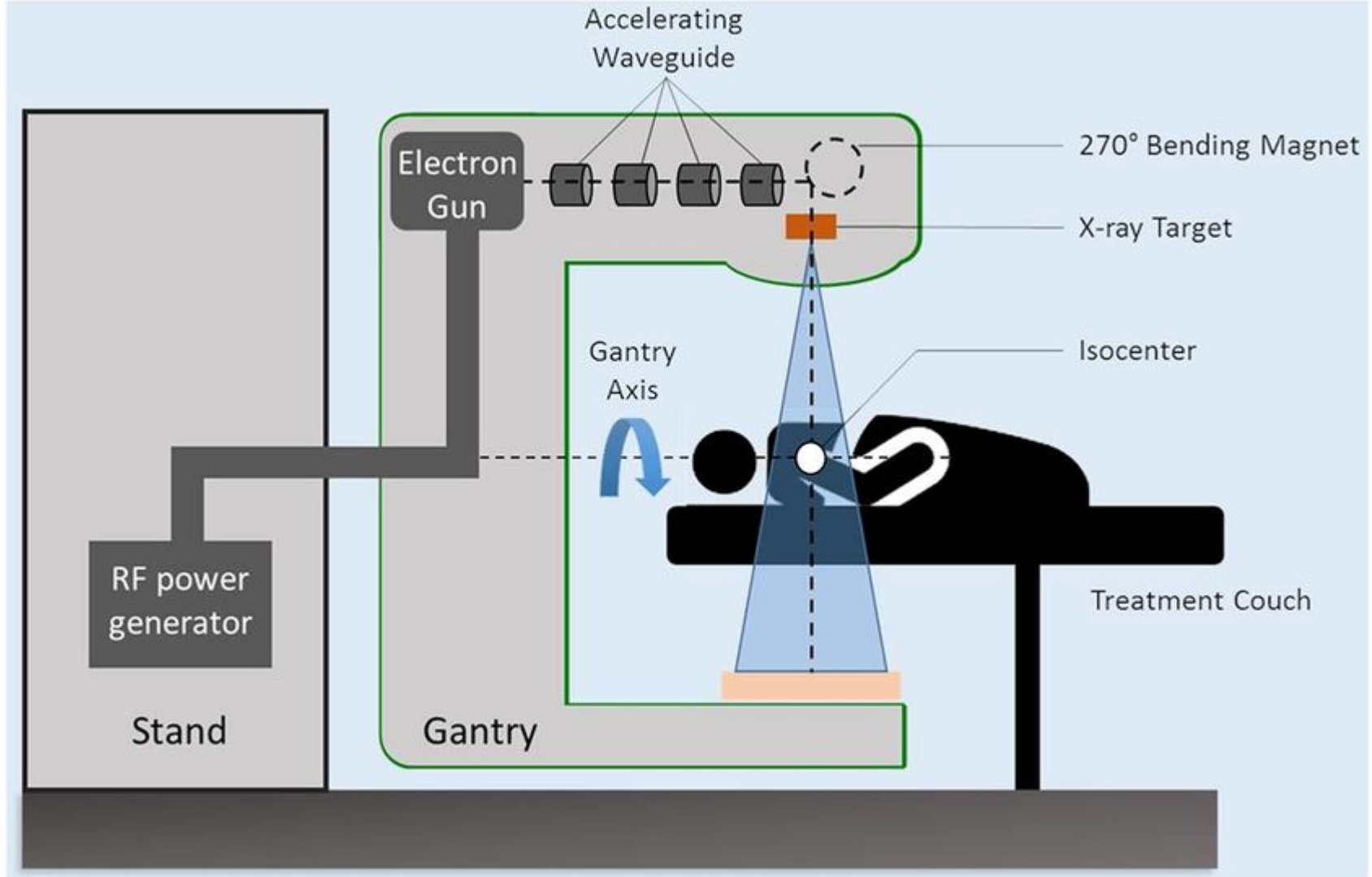
Radiotherapy is used to treat more than 50% of cancer patients



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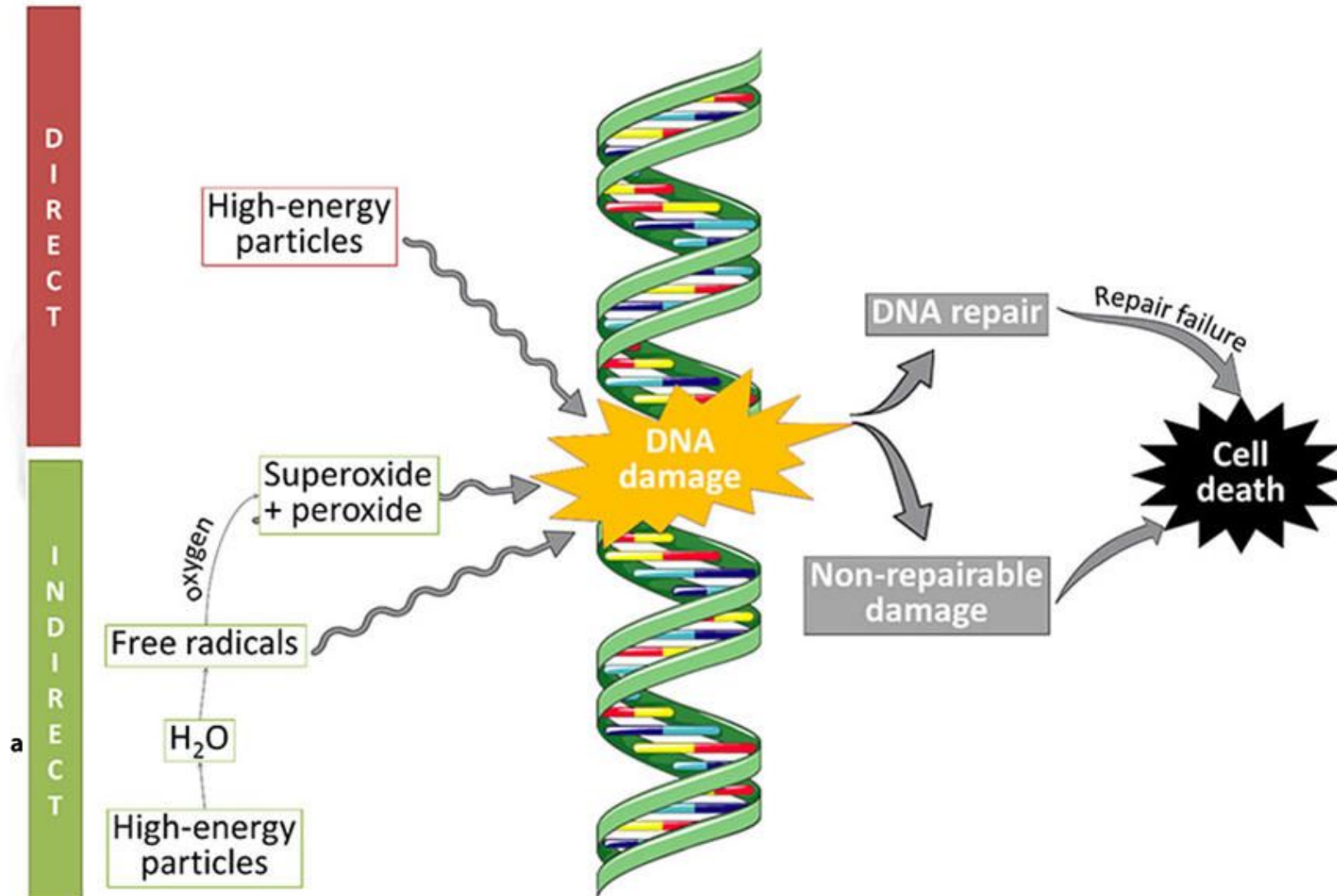
Linear Accelerators for Radiotherapy

Background



How does radiotherapy impact cancer cells?

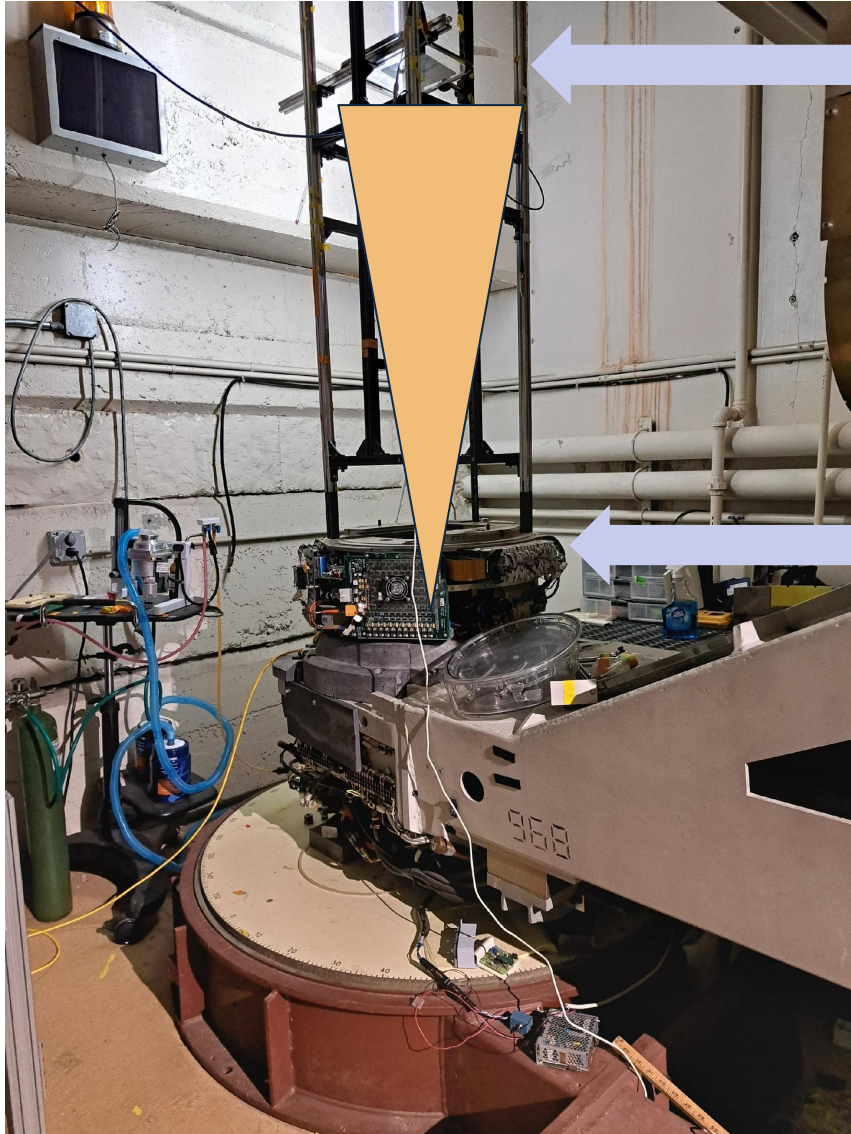
Background



How do we avoid adverse effects to healthy cells?

FLASH RT and the FLASH effect

Background



Conventional Dose Rate (1 Gy/s - 1 Gy/min)

cancer cell killing
healthy cell killing

FLASH Dose Rate (>40 Gy/s)

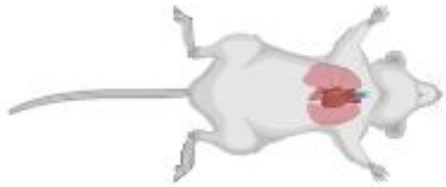
cancer cell killing
healthy cell sparing

Flash Effect

Dose: 1 Gy = 1 J/kg

FLASH Effect in Action

Background



lung fibrosis with CONV but not FLASH
(Favaudon et al 2014)



suppressed tumor growth with human
tumors (Favaudon et al 2014)



neurocognitive effects of radiation worse
with CONV (Montay-Gruel et al 2019)



increased cell survival in 7 different tumor
and healthy cell lines (Adrian et al 2021)

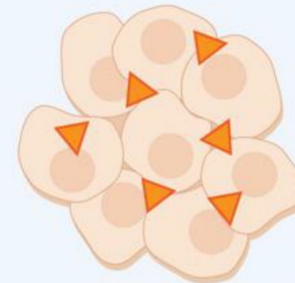
Possible Mechanisms for FLASH effect

Background

Inter-track Interactions

Transient Oxygen Depletion Hypothesis

CONV



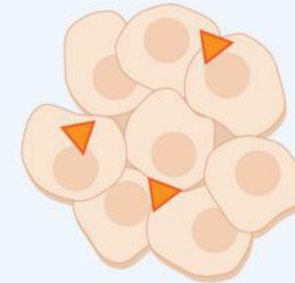
Healthy tissue



Vasculature

Toxicity

FLASH



Healthy tissue



Vasculature

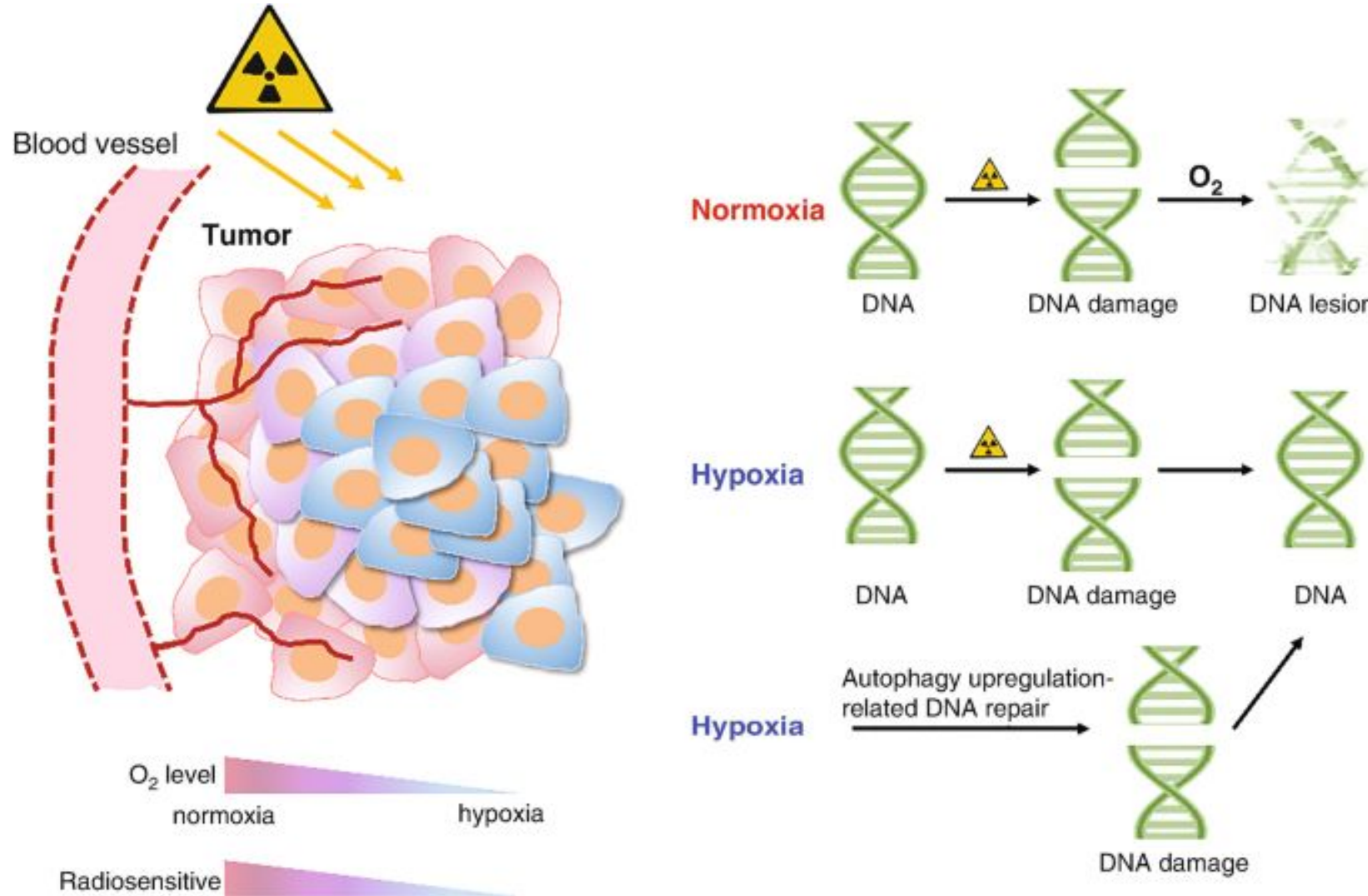
Less toxicity

▲ = ROS

● = O₂

Radioresistance with hypoxia

Background



How do oxygen conditions mediate the FLASH effect?

- Does FLASH irradiation decrease the concentration of reactive oxygen species in water?

water irradiation and measuring hydrogen peroxide concentrations

- In a hypoxic environment, does FLASH irradiation contribute to the normal cell sparing effect?

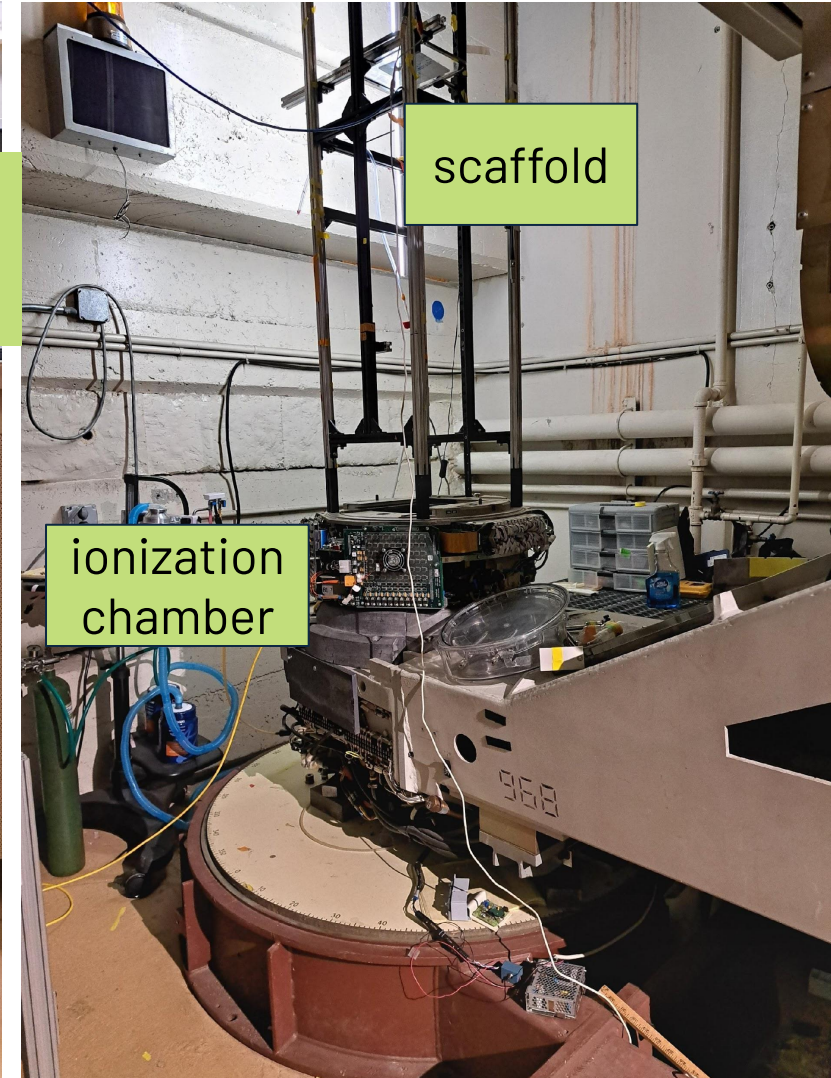
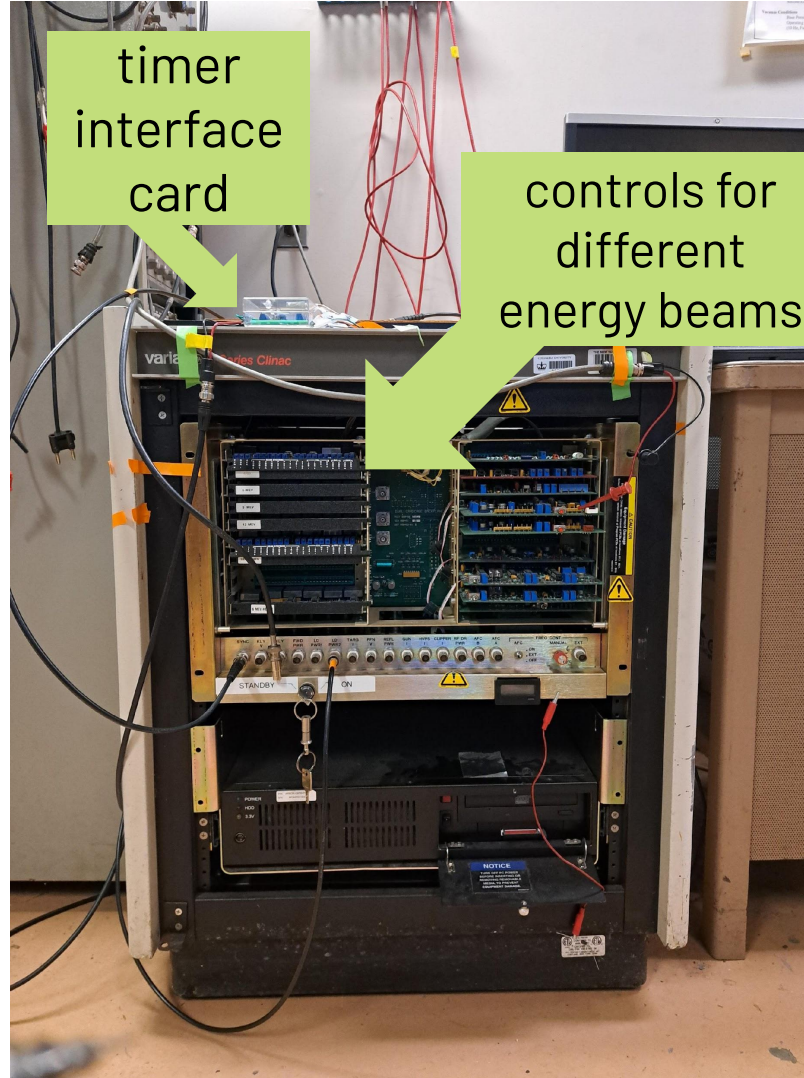
cell irradiations with normoxic (20% O₂) and hypoxic (~4-15% O₂) conditions

Modified Clinac

Methods

changes to the Clinac for FLASH irradiations

tunability and optimization of the machine



Dosimetry

Methods

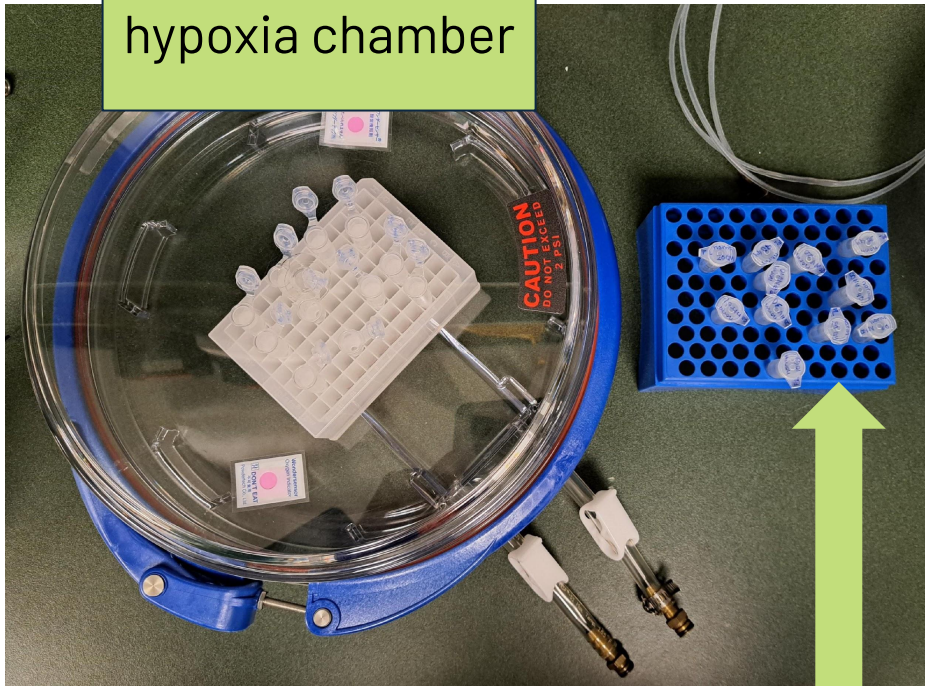
films by
increasing dose



Water Radiolysis

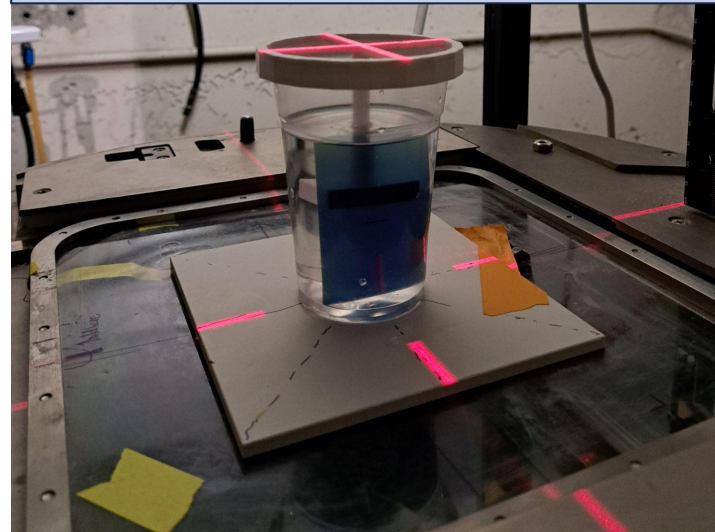
Water Irradiation

hypoxia chamber

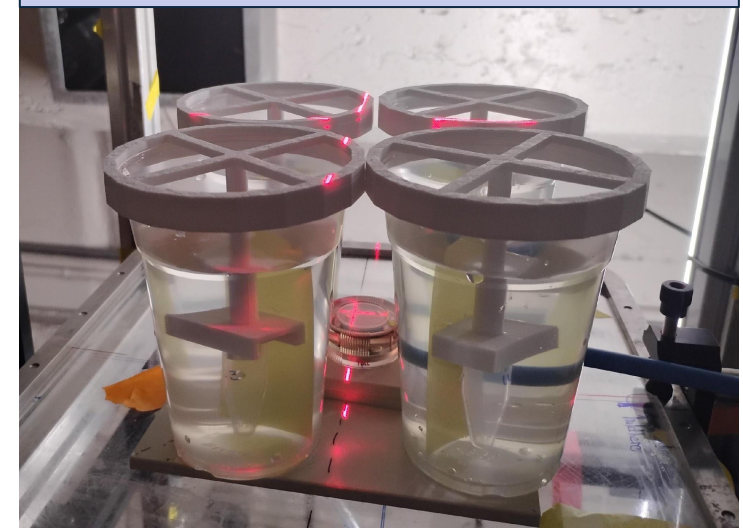


normoxia tubes

FLASH Irradiation Setup

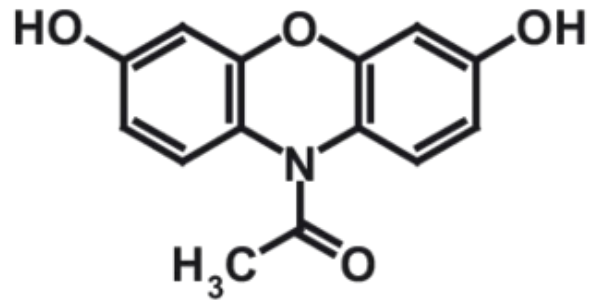


CONV Irradiation Setup



Measuring Fluorescence Intensity

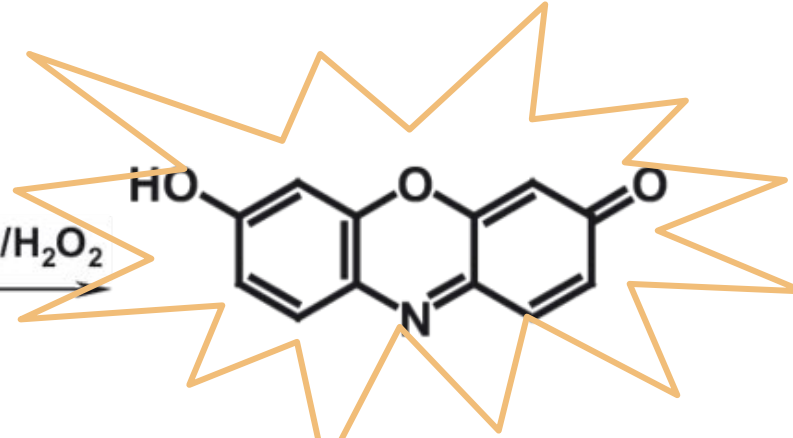
Water Irradiation



Amplex Red

10-acetyl-3,7-dihydroxyphenoxazine
(Non-fluorescent)

Peroxidase/H₂O₂



Resorufin

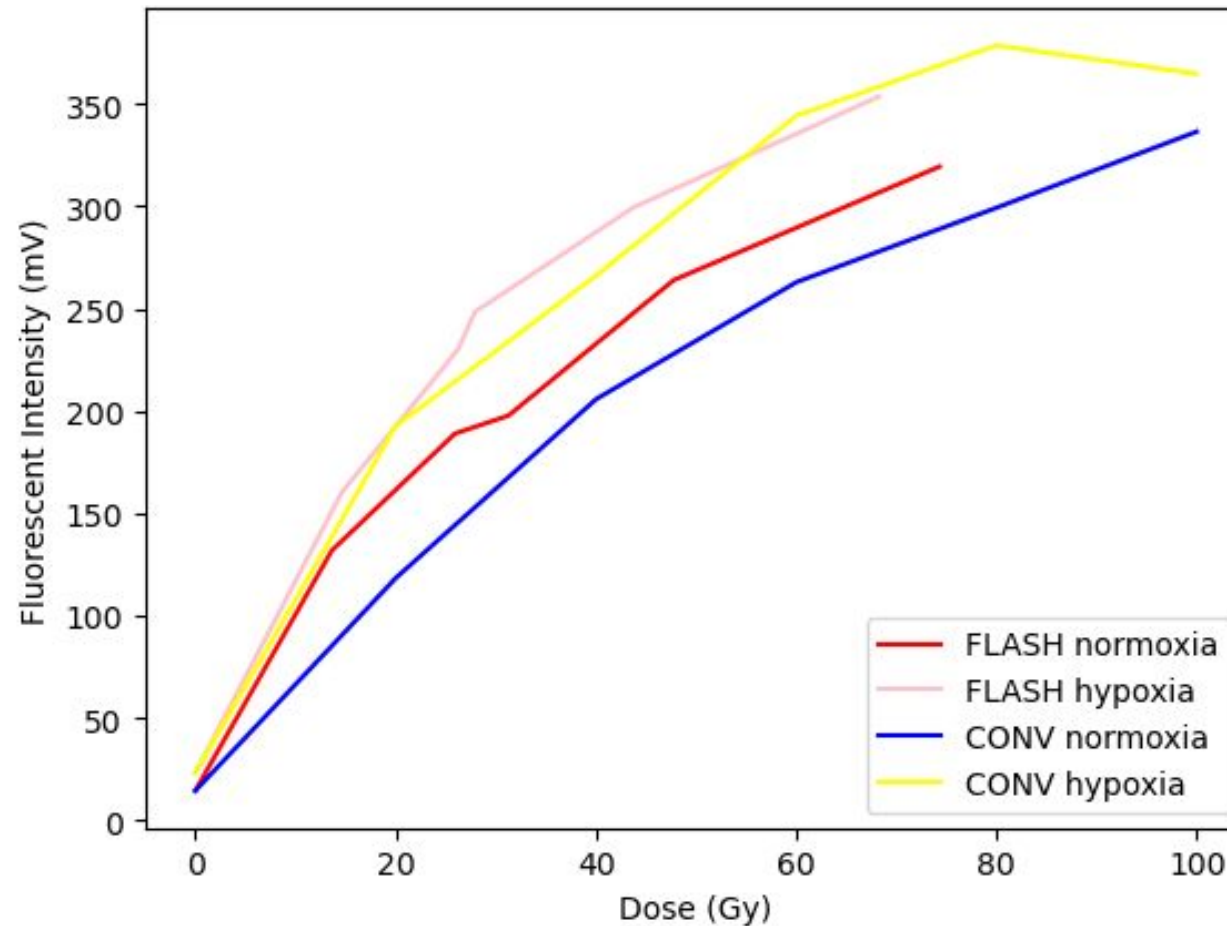
7-hydroxy-3H-phenoxazine-3-one
(Fluorescent)



<https://www.degruyter.com/document/doi/10.2478/s11535-008-0021-x/html>

Hydrogen peroxide concentrations post-irradiation

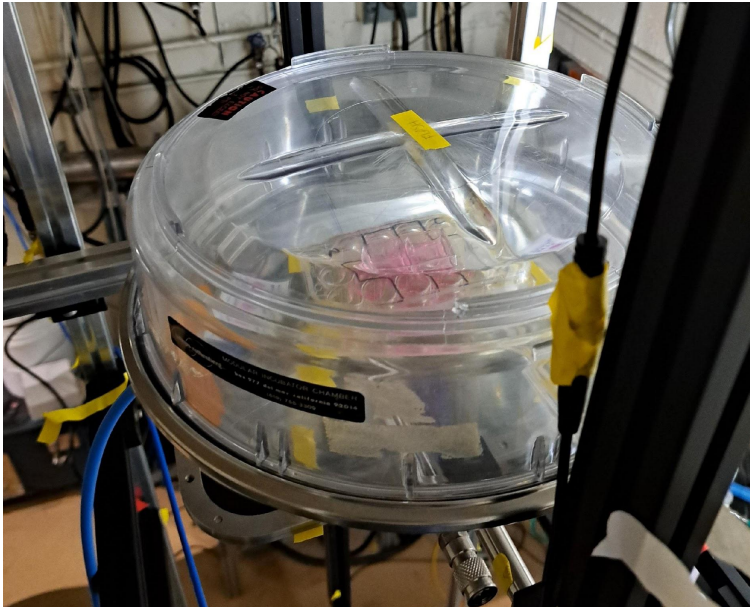
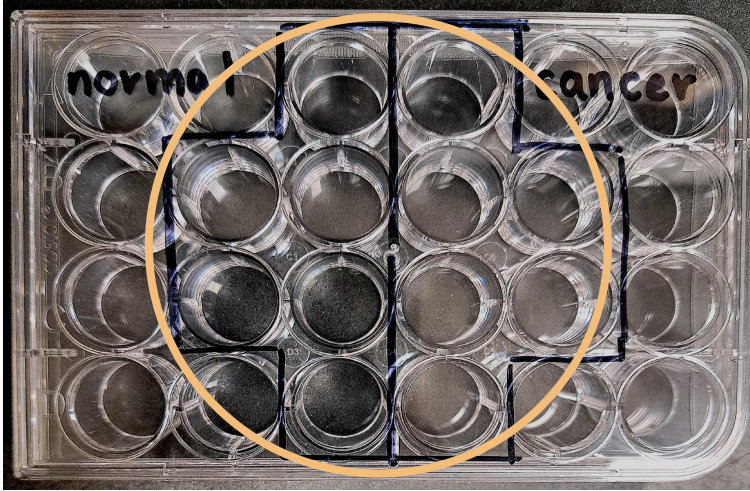
Water Irradiation



CONV samples had lower hydrogen peroxide concentrations compared to FLASH samples

Clonogenic Assay

Cell Irradiation



0 Gy

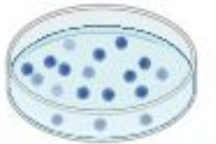


count and
plate cells

10 Gy



incubate for
1 week

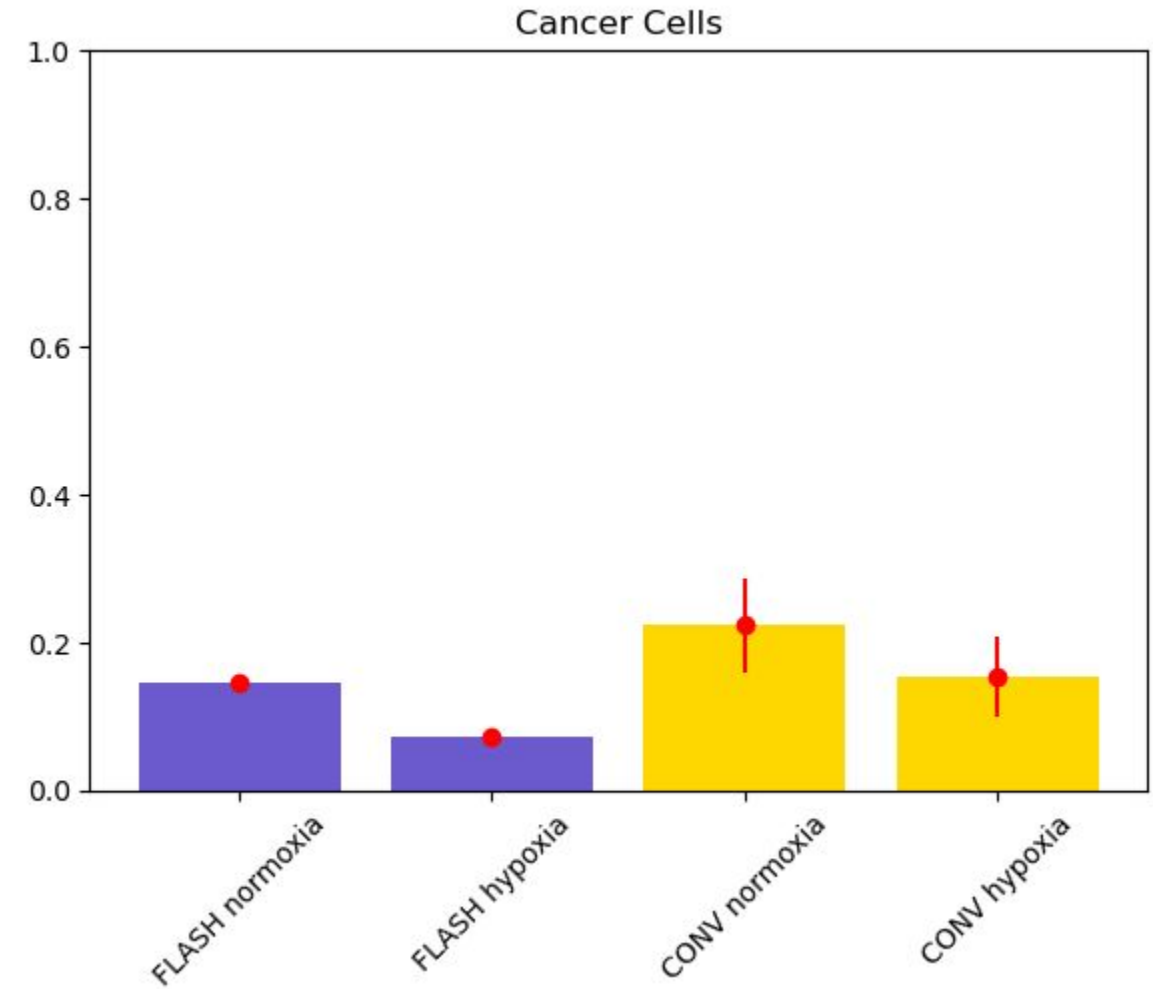
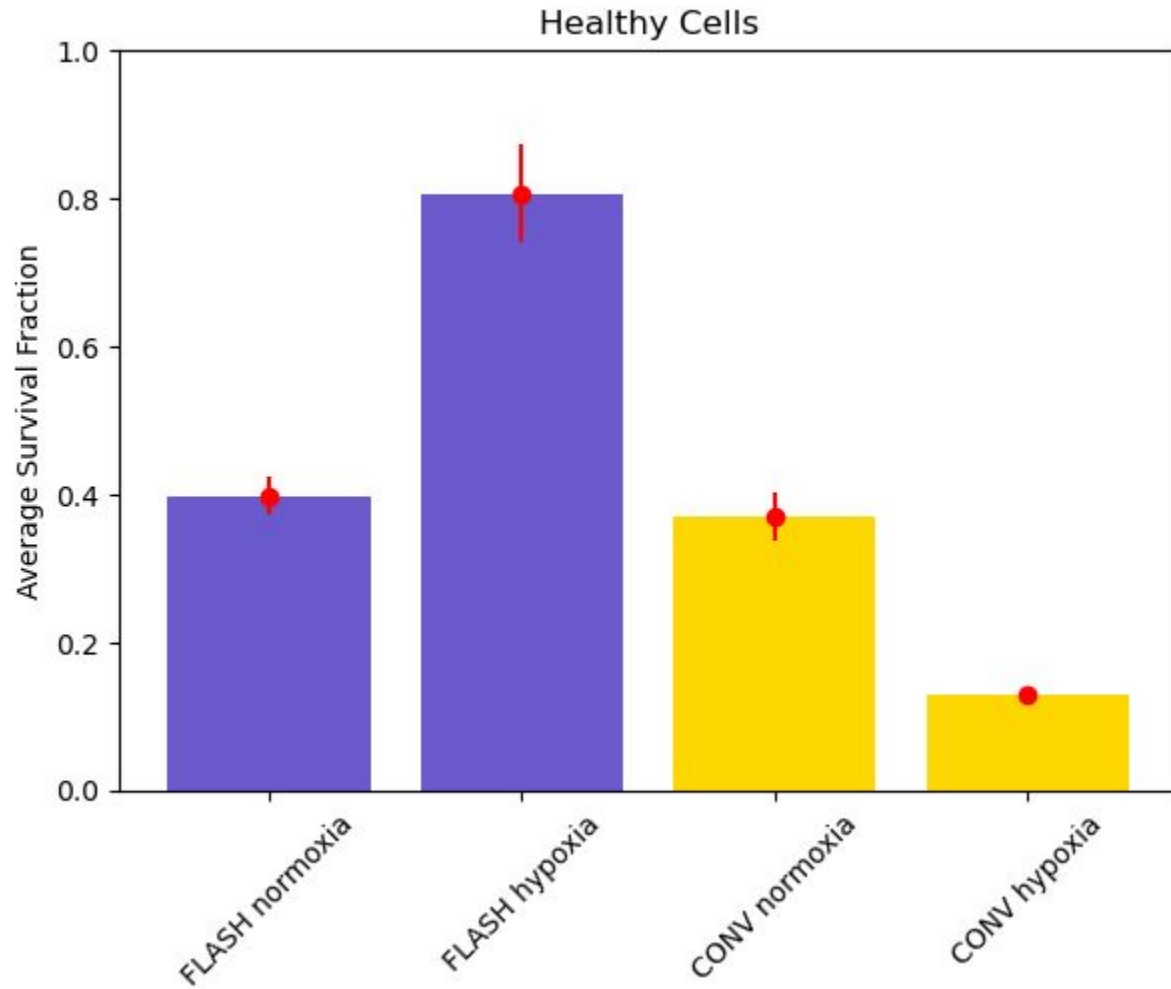


stain and count
colonies

Created in BioRender.com 

Survival fraction 1 week post-irradiation

Cell Irradiation



Hypoxia conditions induced a sparing effect in FLASH irradiated healthy cells

Oxygen conditions and the FLASH effect

Conclusions

Why are these preliminary results important?

- testing protocol for creating hypoxia conditions
- testing assay for measuring hydrogen peroxide
- determining plating efficiency of cell lines under hypoxia

water irradiation

- define hypoxia chamber setup
- observe reactive oxygen species production with FLASH

cell irradiation

- test cell viability in hypoxia condition
- observe FLASH effect in healthy and cancer cells

organoid irradiation

- use patient derived healthy and tumor tissue
- observe FLASH effect in 3D model

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