Novel high-throughput irradiators for in vitro radiation sensitivity bioassays

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The Biological Frontier

Physics/engineering concepts are ubiquitous in biology and have been for a very long time.

Gel electrophoresis is all about “charge collection” and “charge mobility”.

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Robotics and automation have paved the way for high-throughput biology.

**137Cs Irradiators Shortcomings**
- Non-tunable dose rate
- Decay Corrections
- Dose Calibration
- Regulatory & Security Issues

**X-ray Irradiators Shortcomings**:
- "Heel" effect & non-uniformity
- Output drift
- Dose Calibration
- Blanket Dose
The days of stationary fold-changes in biology are long gone.
Motivation

There is a compelling need for an irradiator with precise dosimetry that is compatible with current high-throughput bioassay equipment.

High-throughput Irradiator

We developed a novel fully automated high-throughput irradiator for in vitro radiation sensitivity investigations.

Design Goals:

1. Performance
   - Variable dose and dose rate and high radiation field uniformity

2. Engineering
   - Fully automated and high-throughput

3. Precision
   - NIST traceable dosimetry

High-throughput Irradiator

1. Cal-Lab 3-axis precision positioning system
2. Source control rack
3. On-board ionization chamber
4. Irradiator translation sample stage
5. "Beam-eye" video camera
6. Xoft Axxent® miniature x-ray source eBx™
   Electronic Brachytherapy Source
7. Surface Applicator Collimator
1. 96-well cell culture plate
2. "Beam-eye" video camera
3. Surface Applicator Collimator

Source operates stably between 30-300 μA allowing dose rate modulation.

A special film phantom using the 96-well form factor was machined to test field characteristics.
Using the 20 mm applicator, groups of 4 wells are irradiated at a time to increase throughput and the number of replicates.

- TLD verification of irradiation dose formalism
  - ^{60}\text{Co} dose calibration curve (3-point: 50, 100, 200 cGy)
  - Irradiator measurements
- TLDs nominally irradiated to 100 cGy
- Correction factors applied for intrinsic energy dependence & finite detector size
- Mean dose delivered: 99.8 ± 2.7 cGy

The dosimetry formulism originally developed by Fulkerson et al for the Xoft source and surface applicators was modified for this work.

\[
\bar{D}_{\text{measured}} = \bar{M} \cdot N_K \cdot P_{\text{elec}} \cdot P_{\text{TP}} \cdot P_{\text{cham}} \cdot P_{\text{POM}} \cdot P_{\text{Geo}}
\]

Modification to \(P_{\text{cham}}\) (measured) and \(P_{\text{Geo}}\) (modeled using MCNPS)

Fulkerson et al., Dosimetric characterization and output verification for interstitial brachytherapy surface applicators. Part I. Electronic brachytherapy source, Medical Physics 41, (2014)
Irradiator Dose Formulism

The dosimetry formulism originally developed by Fulkerson et al for the Xoft source and surface applicators was modified for this work.

Monte Carlo Correction Factor for Dose to Cellular Plane

~40% dose lost to 1.3 mm of polystyrene.


Biovalidation of Irradiator

We also sought to biovalidate our system with known radiation-induced biological effects to ensure that the irradiator system environment does not perturb the biological response of cells.

Known linear effects associated with DNA double strand breaks:
1. Intercellular reactive oxygen species production
2. Physical DNA double strand breaks
3. Cellular DNA DSB repair pathway activation via promoter γH2AX

Intracellular ROS detection with Chloromethyl-H₂DCFDA staining

\[ R^2 = 0.9694 \]
$\text{OTM} = \frac{\text{Tail DNA}}{\text{Tail % DNA}} \times 100$

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</table>

"Comet-Assay" was used to measure DNA double strand breaks.

$R^2=0.9908$

γH2AX assay was used to measure DNA double strand break repair.
Biovalidation of Irradiator

γH2AX assay was used to measure DNA double strand break repair.

SpectraMax© i3 high-throughput plate reader used for imaging and analysis.

Biovalidation of Irradiator

γH2AX assay was used to measure DNA double strand break repair.

Summary

• We developed an automated high-throughput irradiator that delivers precise NIST traceable absorbed doses for in vitro radiation biology investigations.

• Irradiator performance characteristics were investigated including radiation output stability, field flatness and symmetry, and absorbed dose verification.

• The system underwent a rigorous biological validation using three known linear effects caused by ionizing radiation.
A "Crystal Ball" Slide

Physical Treatment Planning (DVH)
Model-Based Treatment Planning (TCP, NTCP)
Personalized Treatment Planning

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Questions?