DEPARTMENT OF
Medical Physics
UNIVERSITY OF WISCONSIN SCHOOL OF MEDICINE AND PUBLIC HEALTH

RED

Novel high-throughput irradiators for in vitro radiation sensitivity bioassays

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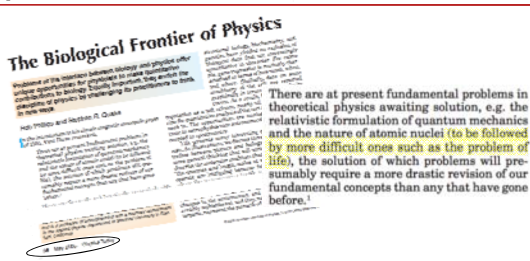
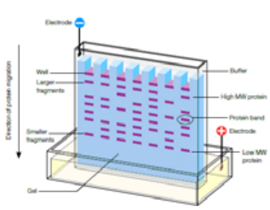
The Biological Frontier

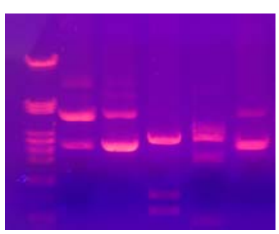

The Biological Frontier of Physics

There are at present fundamental problems in theoretical physics awaiting solution, e.g. the relativistic formulation of quantum mechanics and the nature of atomic nuclei (to be followed by more difficult ones such as the problem of life), the solution of which problems will presumably require a more drastic revision of our fundamental concepts than any that have gone before.[1]

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

The Biological Frontier





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

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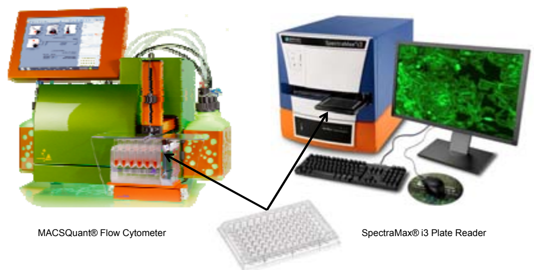

Robotics and automation have paved the way for high-throughput biology.

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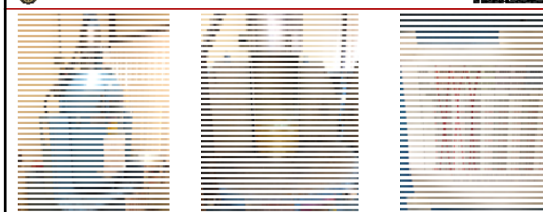
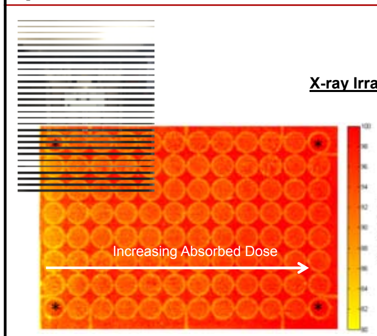


^{137}Cs Irradiators Shortcomings

- Non-tunable dose rate
- Decay Corrections
- Dose Calibration
- Regulatory & Security Issues

The Biological Frontier



X-ray Irradiators Shortcomings:

- "Heel" effect & non-uniformity
- Output drift
- Dose Calibration
- Blanket Dose

Motivation

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CHROMOSOME ABERRATIONS INDUCED BY X-RAYS

KARL SAX

Arnold Arboretum, Harvard University, Jamaica Plain, Massachusetts

Received June 3, 1938

INTRODUCTION

SINCE the discovery by MULLER and STADLER that X-rays induce mutations in animals and plants, a new field has been developed in experimental genetics. This work on radiation genetics has been reviewed

The source of the X-rays was a Coolidge tube with a tungsten target. The line voltage was 120 at 10 ma, and the secondary voltage was 160 kv. No screen was used, and the target distance was about 75 cm. At this distance the tube delivered about 25 r per minute. The dosage used ranged from 75 to 200 r for the analysis of types of chromosome aberrations.

The days of stationary fold-changes in biology are long gone.

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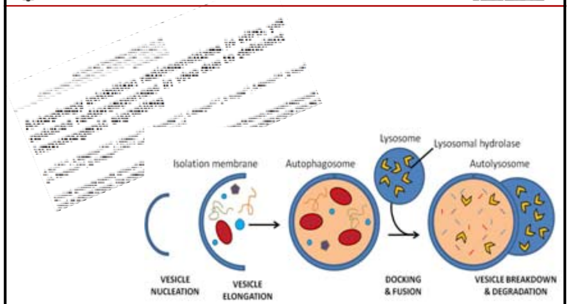

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Motivation

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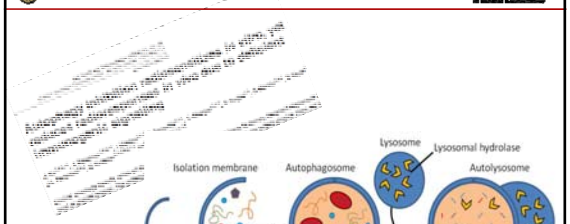

Clonogenic survival and combination studies. Transfected and untransfected cells were plated at different cellular density. The day after, cells were irradiated at doses ranging from 0 to 4 Gy (Cesium = Cs^{137}, 1 Gy/min, gamma irradiator IBL-637 from CIS-BioInternational, IBA, Saclay, France). Then,

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Motivation

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There is a compelling need for an irradiator with <u>precise dosimetry</u> that is compatible with current <u>high-throughout</u> bioassay equipment.

High-throughput Irradiator

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

<u>Design Goals:</u>

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

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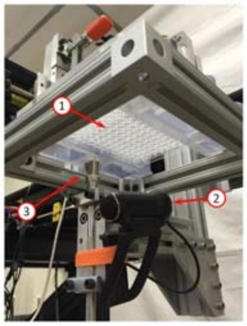
1. Cal-Lab 3-axis precision positioning system
2. Source control rack
3. On-board ionization chamber
4. Irradiator translation sample stage
5. "Beam-eye" video camera
6. Xoft Axxent® eMiniature x-ray source eBx™ Electronic Brachytherapy Source
7. Surface Applicator Collimator

High-throughput Irradiator



1. 96-well cell culture plate
2. “Beam-eye” video camera
3. Surface Applicator Collimator

Images courtesy of Xoft Inc. (a subsidiary of iCad Inc.)

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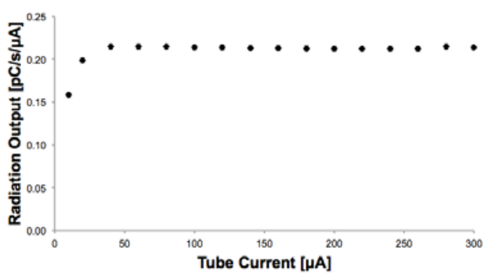
Irradiator Characterization



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

*Error bars are obscured by data points.

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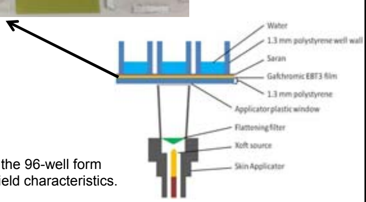
Irradiator Characterization



A special film phantom using the 96-well form factor was machined to test field characteristics.

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Irradiator Characterization

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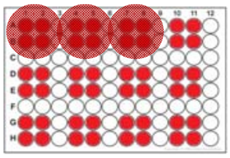

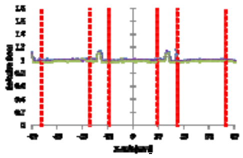

Average Flatness:
In-plane: 2.4 ± 0.2%
Cross-plane: 2.8 ± 1.0%

Average Symmetry:
In plane: 1.1 ± 1.2%
Cross-plane: 0.6 ± 1.0%

Using the 20 mm applicator, groups of 4 wells are irradiated at a time to increase throughput and the number of replicates.

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Irradiator Characterization

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- TLD verification of irradiation dose formalism
  - ^{60}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**

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Irradiator Dose Formulism

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

$$\dot{D}_{water,d} = \underbrace{\dot{M} \cdot N_K \cdot P_{elec} \cdot P_{TP} \cdot P_{cham} \cdot P_{POM}}_{\text{Air Kerma Rate}} \cdot \underbrace{P_{GEO}}_{\text{Dose to Cellular Plane}}$$

Modification to P_{cham} (measured) and P_{GEO} (modeled using MCNP5)

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

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Irradiator Dose Formulism

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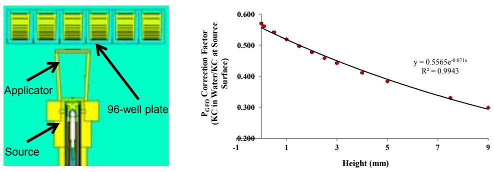
Monte Carlo Correction Factor for Dose to Cellular Plane

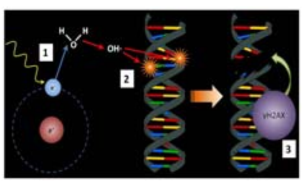


~40% dose lost to 1.3 mm of polystyrene.

Davis SD (2009) Air-kerma strength determination of a miniature x-ray source for brachytherapy application. Madison, WI: University of Wisconsin

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.

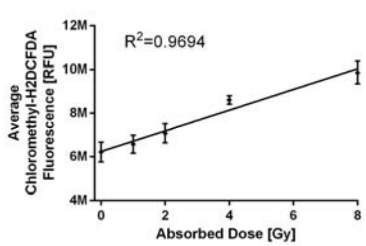


Known linear effects associated with DNA double strand breaks:

1. Intercellular reactive oxygen species production
2. Physical DNA double strand breaks
3. Cellular DNA DSB repair pathway activation via promoter γH2AX

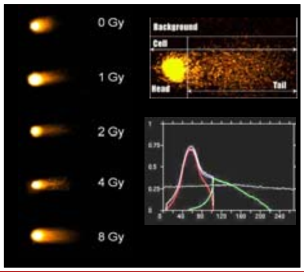
Biovalidation of Irradiator

Intracellular ROS detection with Chloromethyl-H_2DCFDA staining



Biovalidation of Irradiator

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



$$\text{OTM} = \frac{\left(\overline{\text{Tail}} - \overline{\text{Head}}\right)\left(\text{Tail \% DNA}\right)}{100}$$

Absorbed Dose [Gy]	# Comets Counted
0	434
1	372
2	177
4	194
8	273

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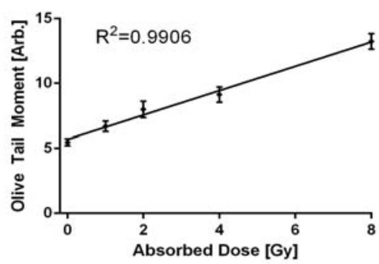

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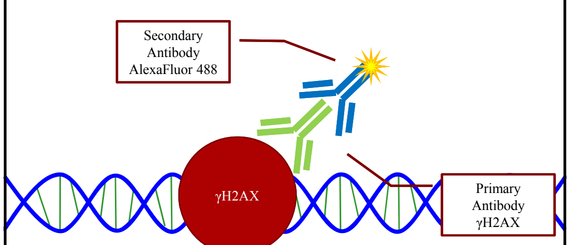
Biovalidation of Irradiator

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



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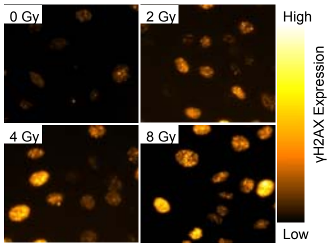

SpectraMax© i3 high-throughput plate reader used for imaging and analysis.

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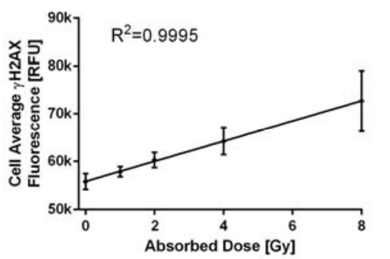

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Summary

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- 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.

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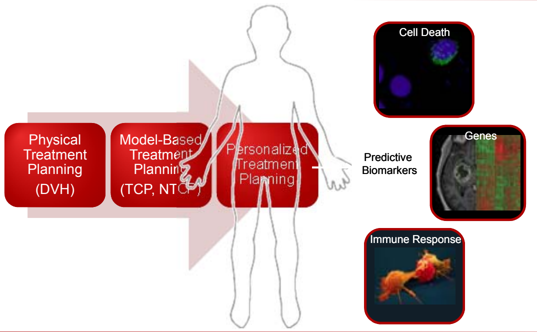
A "Crystal Ball" Slide



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Acknowledgements

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Questions?

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