

# Targeted Micro-irradiation for High Performance Radiobiology Research

David A. Jaffray, PhD

TECHNA Institute, Radiation Medicine Program,  
and Princess Margaret Cancer Centre,  
University Health Network

Departments of Radiation Oncology and Medical Biophysics,  
and the Institute of Biomaterials & Biomedical Engineering,  
University of Toronto

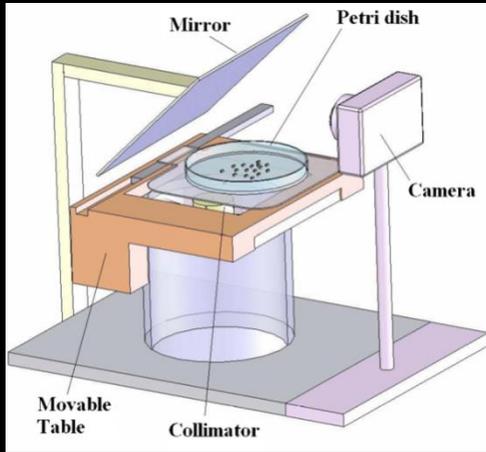
# Conflict of Interest

Presenter is an inventor of some of the technology described herein, portions of which have been licensed for commercial development to Precision X-ray Inc.

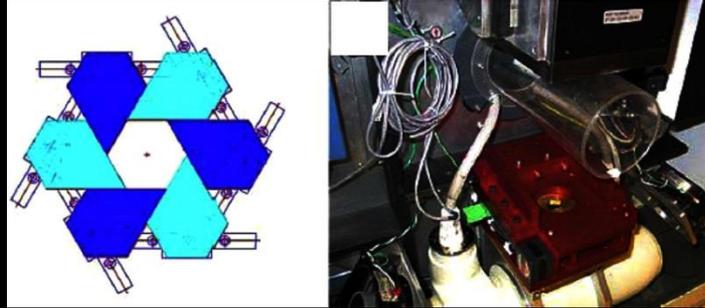
Research developments shown are not necessarily part of the commercial product.

# Advances in Research Irradiators

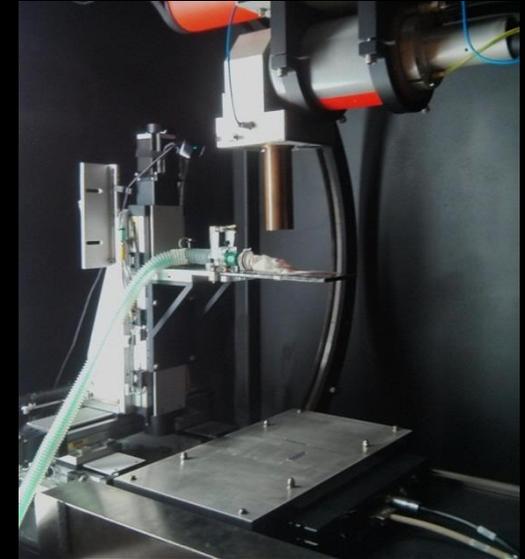
Stanford



Stanford



Princess Margaret



Washington University

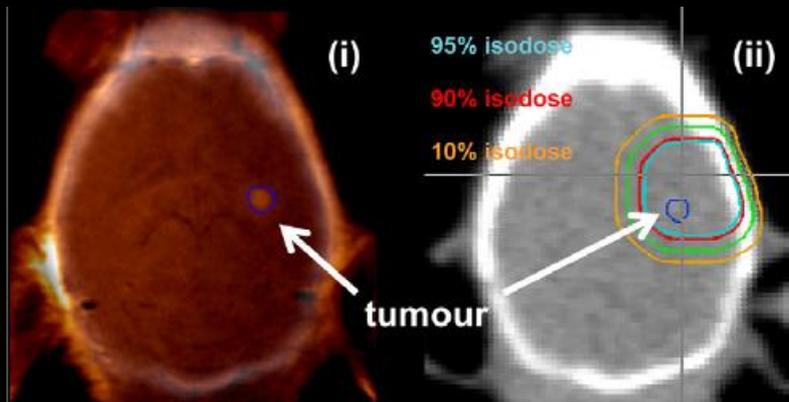


John Hopkins

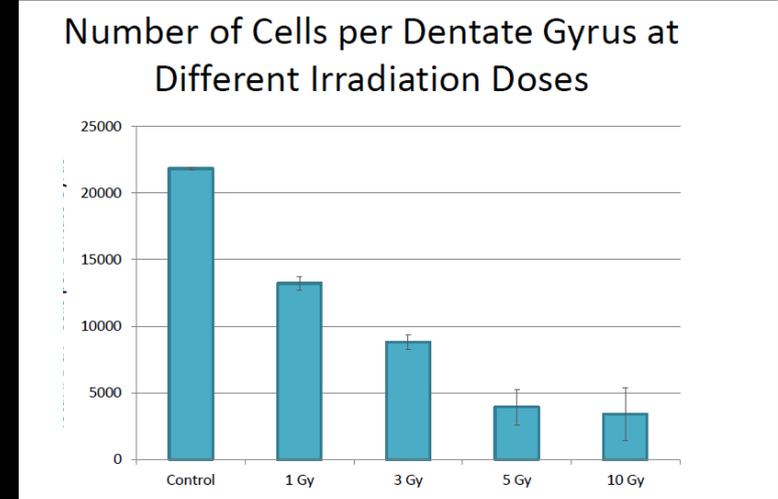
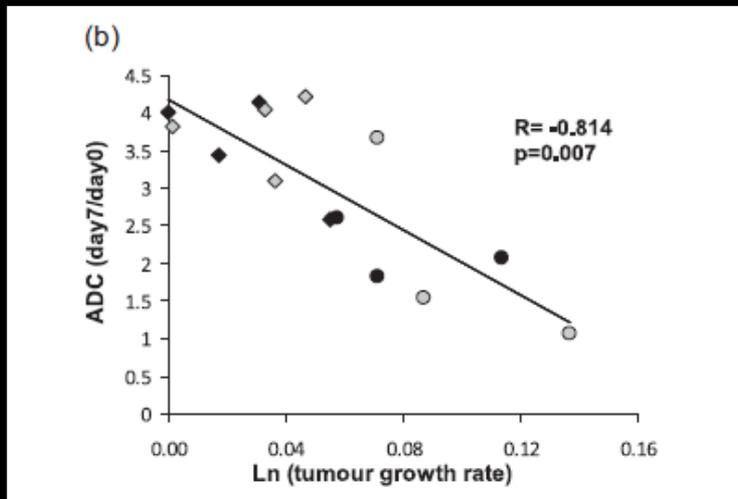
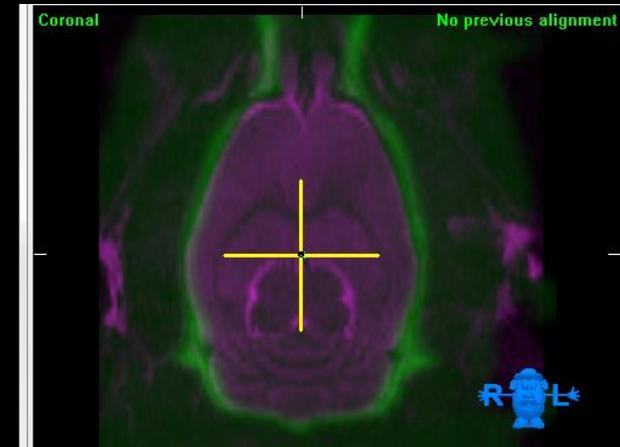
- Rodriguez (2008) *Phys Med Biol*
- Wong (2008) *Int J Radiat Oncol Biol Phys*
- Clarkson (2010) *Med Phys*
- Stojandinovic (2006) *Med Phys*
- Graves (2007) *Med Phys*
- Verhaegen (2011) *Phys Med Biol*

# Pre-clinical Radiotherapy Research

## Imaging Biomarkers for Response Assessment



## Soft Tissue Targeting for Normal Tissue Response Assessment



# XRAD 225Cx Microirradiator

## Treatment:

- 3 kW power
- Dual focal spot (1/5.5 mm at 0.64/3 kW)
- Single beams, arcs, multi- beam/angle

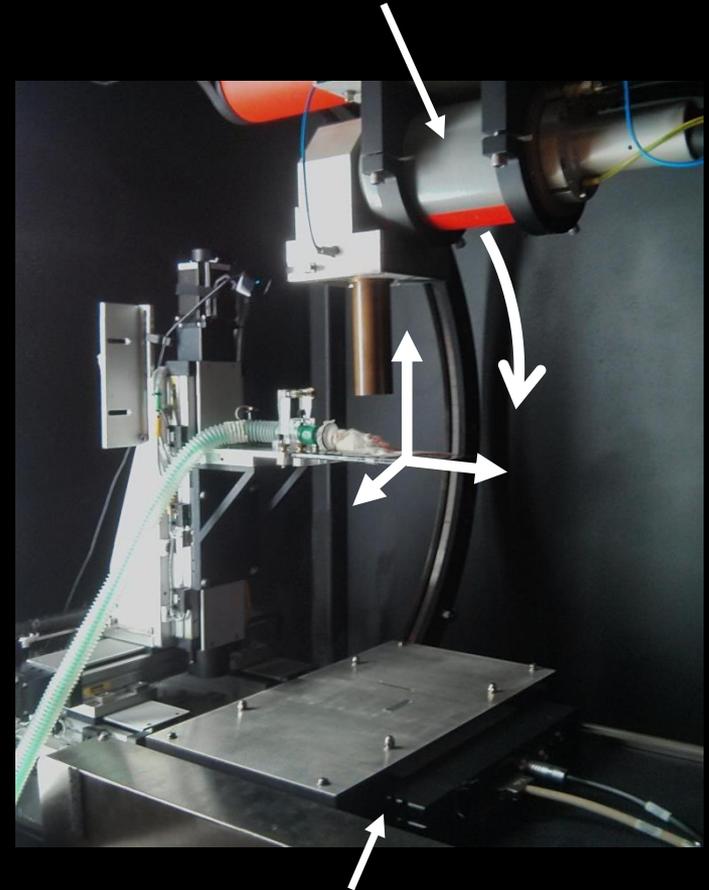
## IGRT Support:

- CBCT or fluoroscopic based setup
- Various (fixed) circular and rectangular collimators

## Calibration:

- Mechanical (flexmaps, Winston-Lutz)
- Imaging (phantom)
- Dosimetric (AAPM TG-61, water phantom, gafchromic film, TLDs)

Comet MXR 225 X-Ray Tube  
GE Isovolt 225 kV Power Supply (GE)



Perkin-Elmer Flat Panel Detector  
(0.2 mm pitch, 30 fps)

# Two-dimensional inverse planning and delivery with a preclinical image-guided micro-irradiator.

*Two-dimensional inverse planning and delivery with a preclinical image guided micro-irradiator.*

*Stewart JM, Lindsay PE, Jaffray DA.*

*Med Phys. 2013 40(10)*

# Planar Dose Optimization

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Empirically Derived  
Dose Kernel

Empirical measurements of dose kernel in phantom meant to simulate small animal geometry



Initial Beam Position &  
Weight Heuristic

A general method to define an initial set of beam positions and weights (beam-on times) to get 'close' to the desired dose



Beam Optimization

Determine optimal set of beam positions and weights for the desired dose

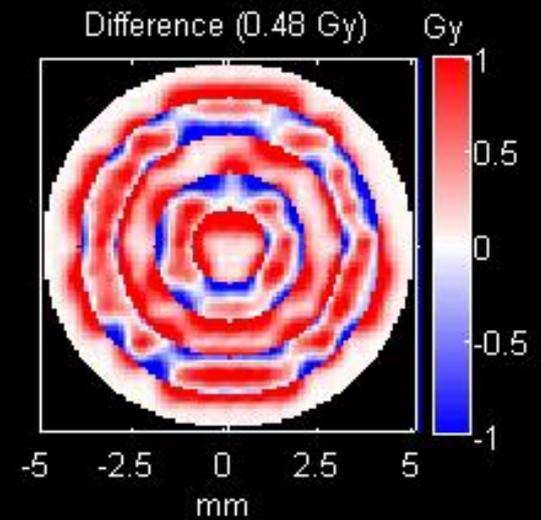
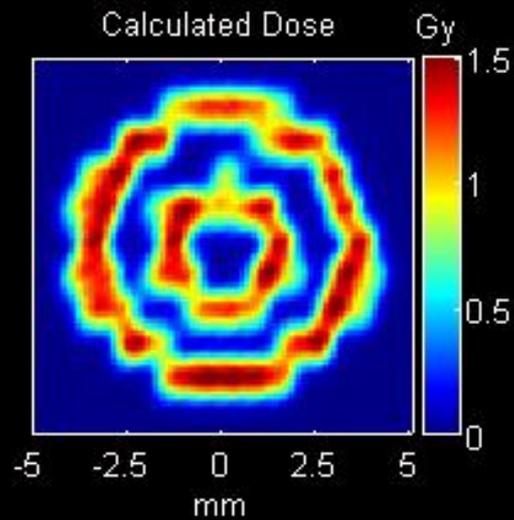
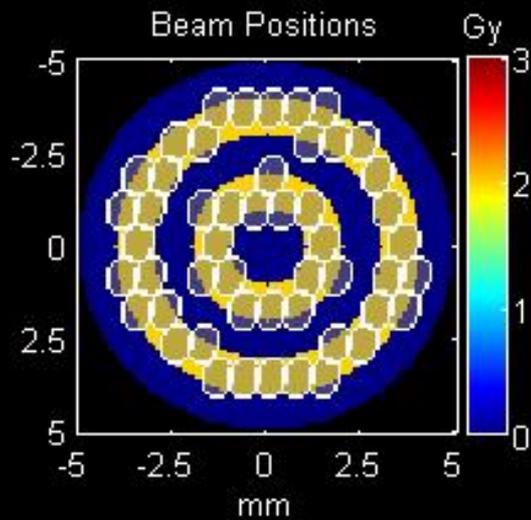


Delivery

Convert beam positions & weights to stage positions and beam-on times and validate delivery

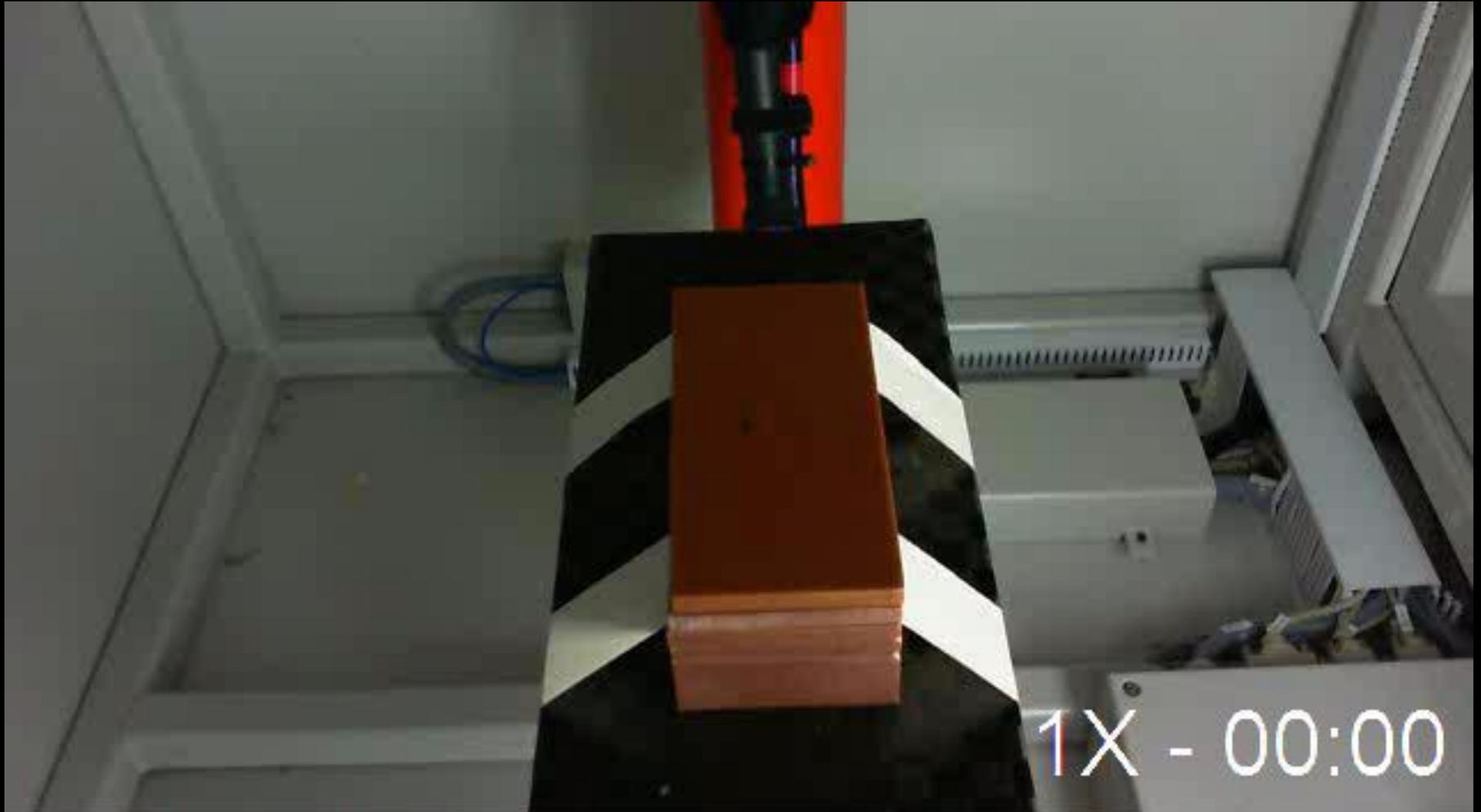
# Planar Dose Optimization

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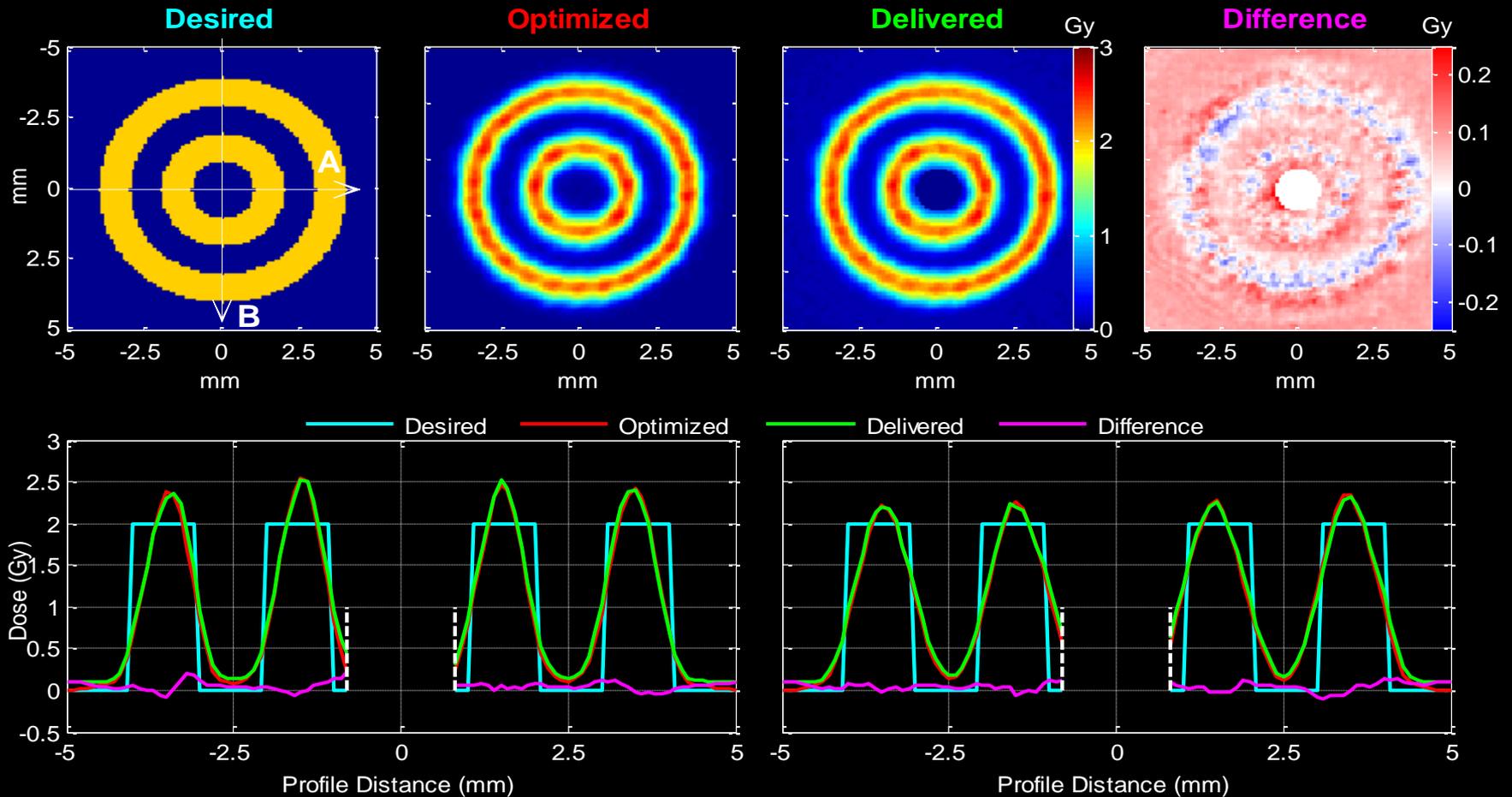


# Planar Dose Optimization

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# Planar Dose Optimization



Absolute difference of optimized and delivered doses along line profiles:

Mean: 6 cGy (horizontal), 6 cGy (vertical)

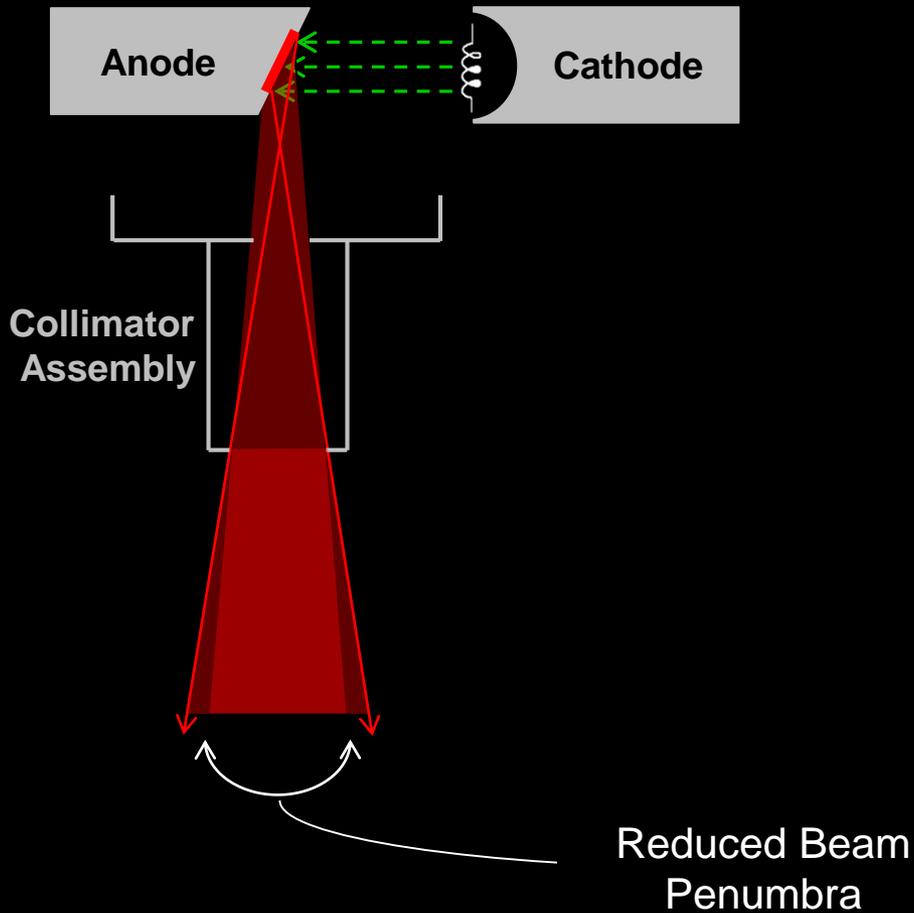
Max: 22 cGy (horizontal), 13 cGy (vertical)

# Dual Focal Spot Optimization for Spatially Varying Dose Painting

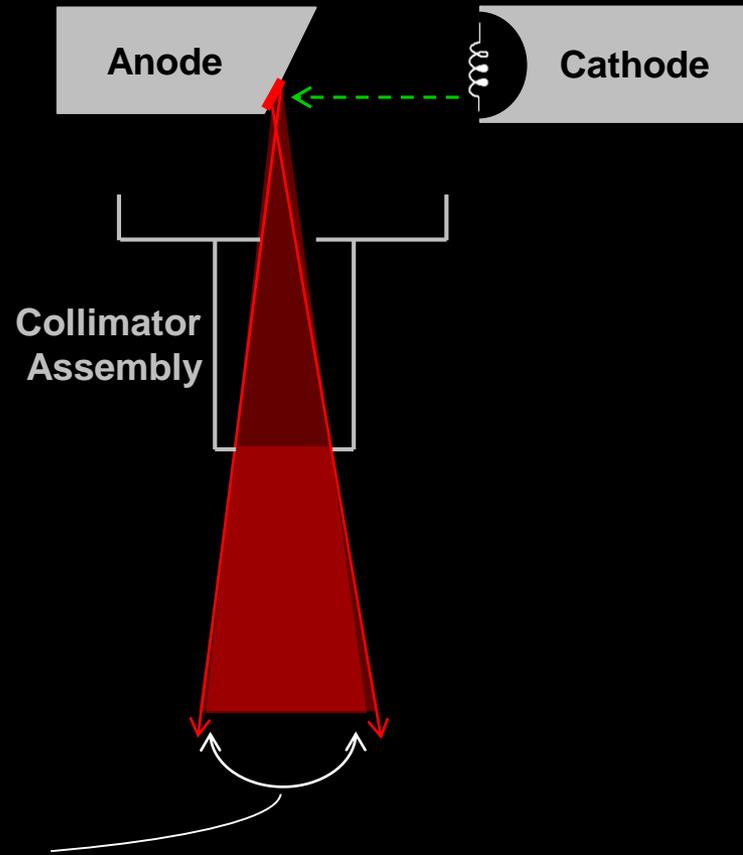
*Dual Focal Spot Optimization for Spatially Varying Dose Painting*  
Stewart JM, Lindsay PE, Jaffray DA.  
*In preparation*

# Dual Focal Spot Optimization

Large Focal Spot (5.5 mm)



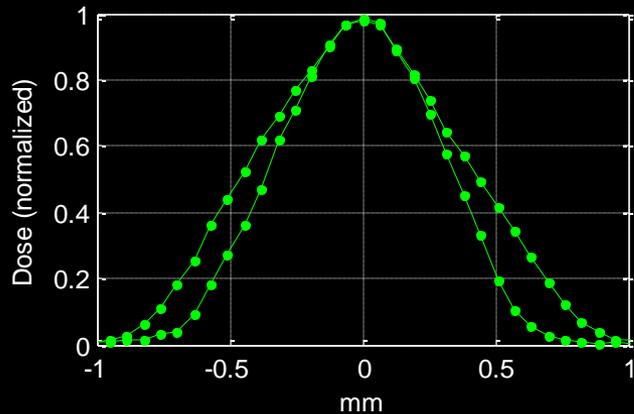
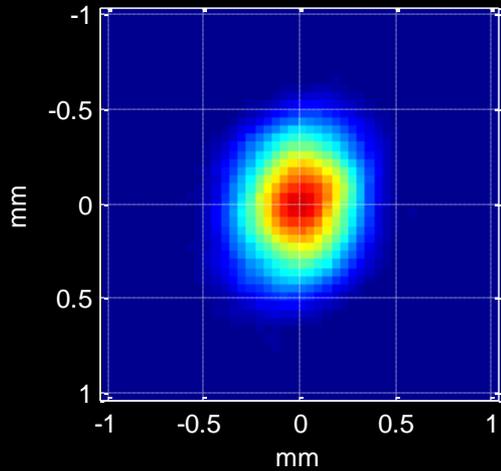
Small Focal Spot (1.0 mm)



# Dual Focal Spot Optimization

## 1 mm Circular Collimator Radiation Fields

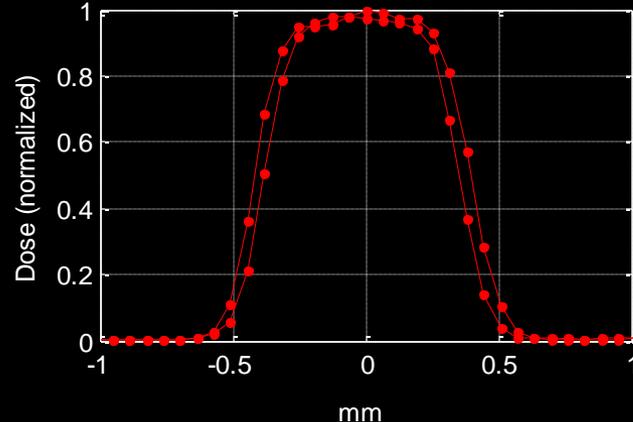
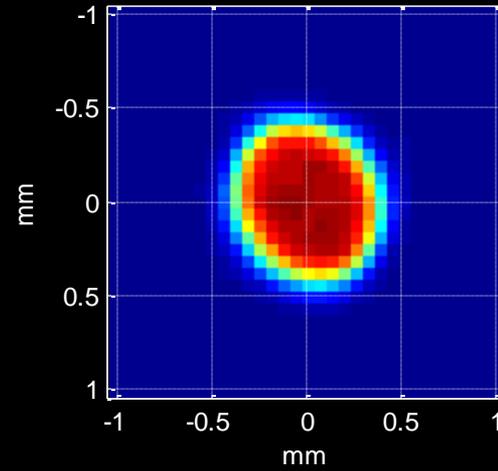
Large Focal Spot



80-20% Dose Falloff: 0.51 mm

Dose Rate: 1.50 Gy/min

Small Focal Spot

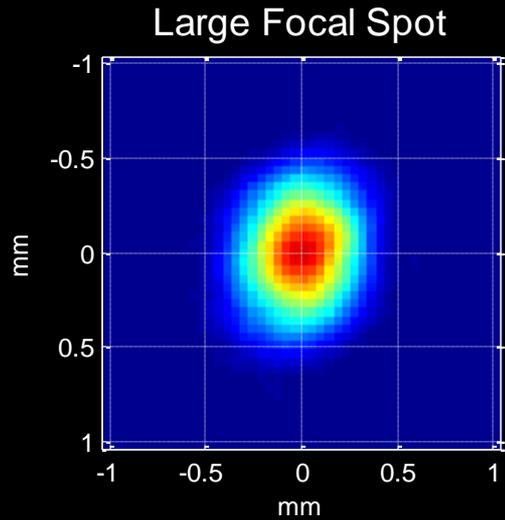


80-20% Dose Falloff: 0.20 mm

Dose Rate: 0.37 Gy/min

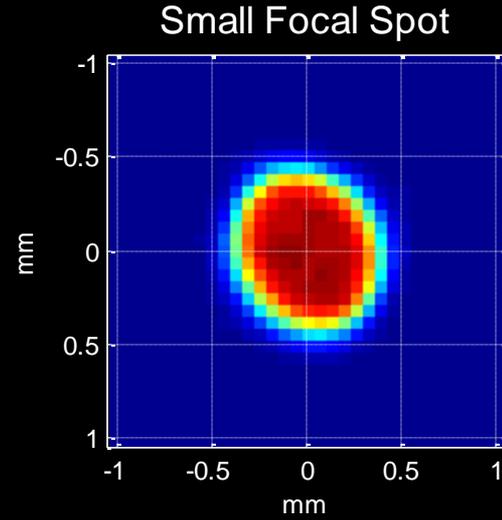
# Dual Focal Spot Optimization

## 1 mm Circular Collimator Radiation Fields



**Large Focal Spot**  
4X Higher Dose Rate

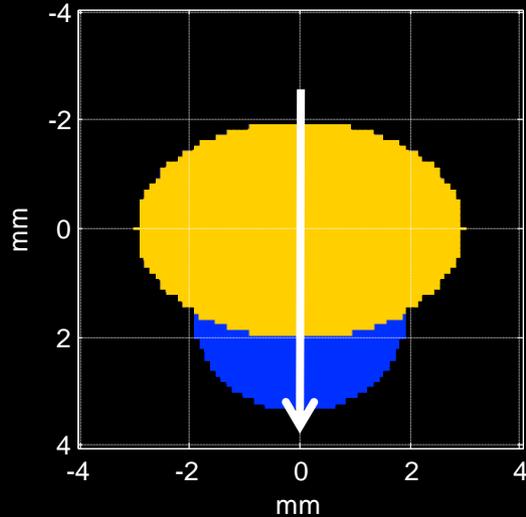
Vs.



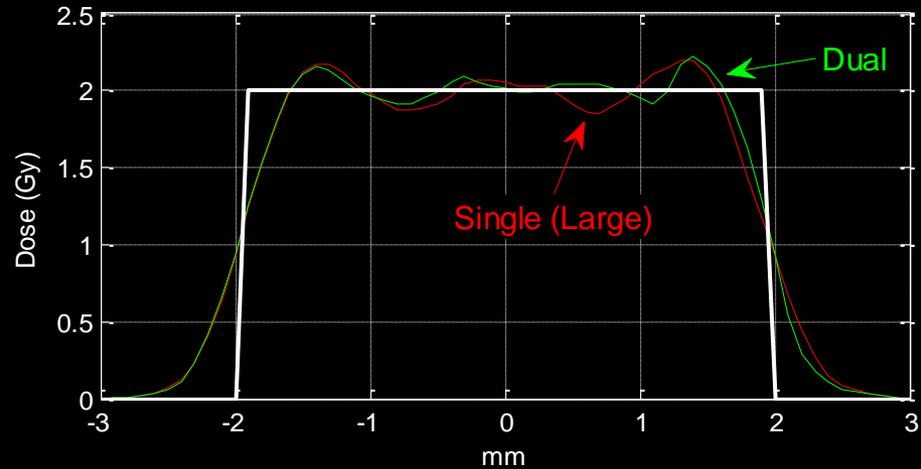
**Small Focal Spot**  
2.5X Sharper Penumbra

# Dual Focal Spot Optimization

Target & Avoidance Region



Vertical Dose Profile



|   | Large Focal Spot | Dual Focal Spot            |
|---|------------------|----------------------------|
| Optimization Time                       | 3.3 min          | 3.5 min                    |
| Delivery Time                           | 37.2 min         | 47.7 min<br>(28% increase) |
| Proportion of Avoidance Region > 0.5 Gy | 17.8%            | 10.6%<br>(40% decrease)    |

\*Limited field size to 1mm circular collimator.

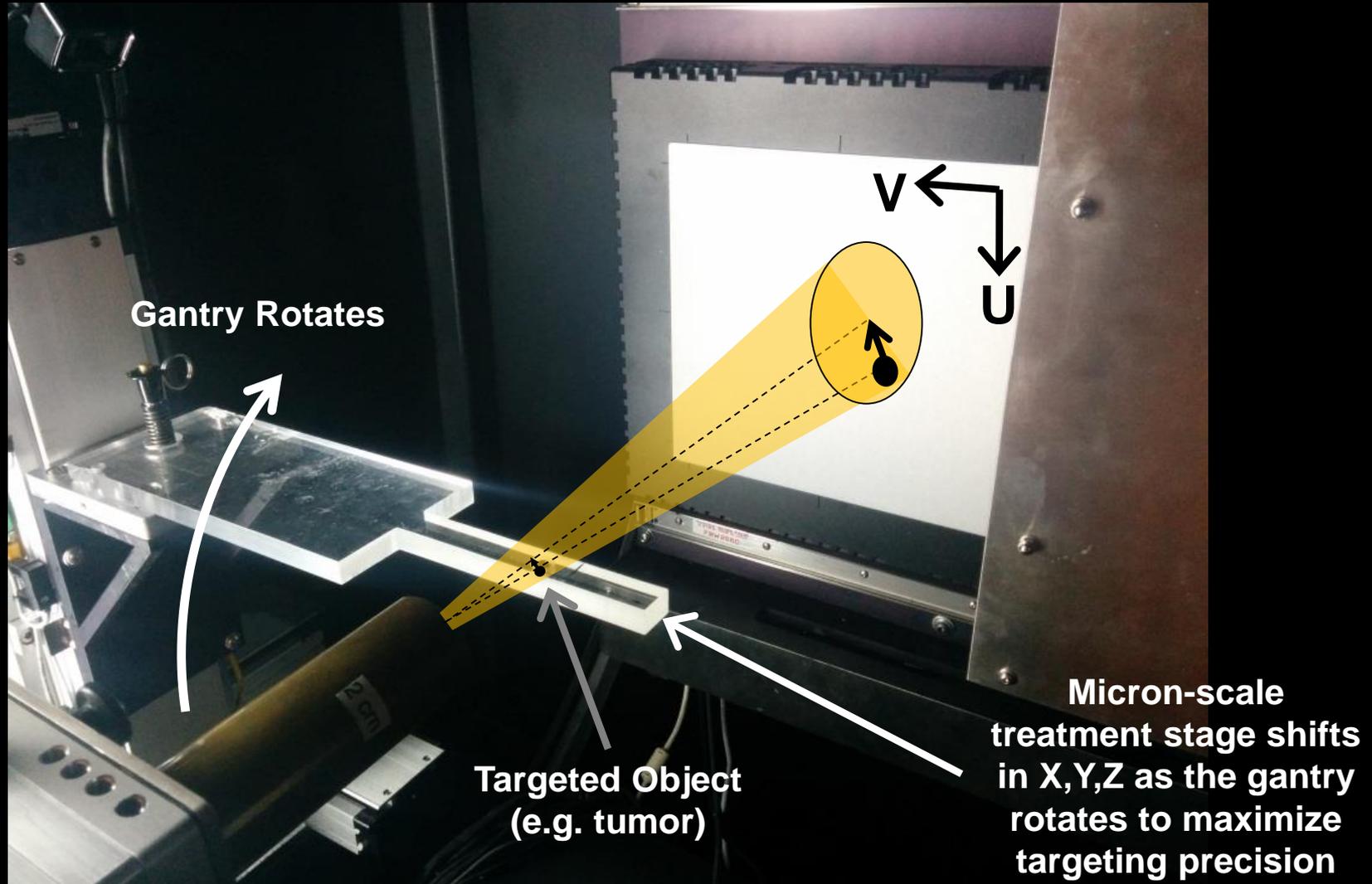
# **Online virtual isocenter based radiation field targeting for high performance small animal micro-irradiation.**

*Online virtual isocenter based radiation field targeting for high performance small animal microirradiation.*

*Stewart JM, Ansell S, Lindsay PE, Jaffray DA.  
Phys Med Biol. 2015 Dec 7;60(23):9031-46*

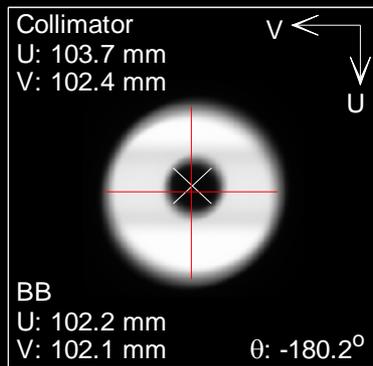
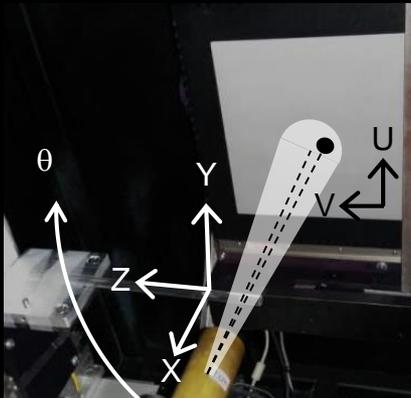
# Radiation Field Targeting – Stage Tracking

After image-guided target is defined, stage tracks to maximize precision of targeting.

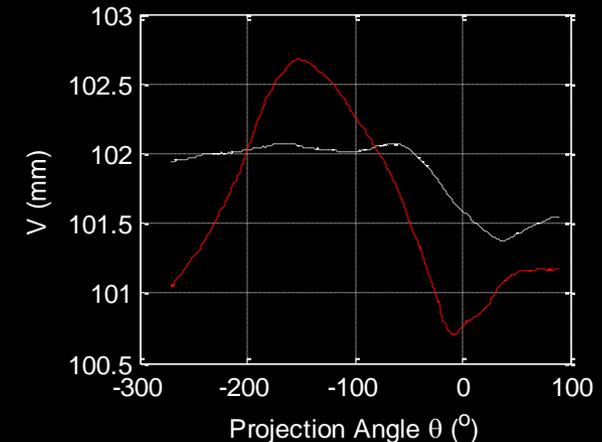
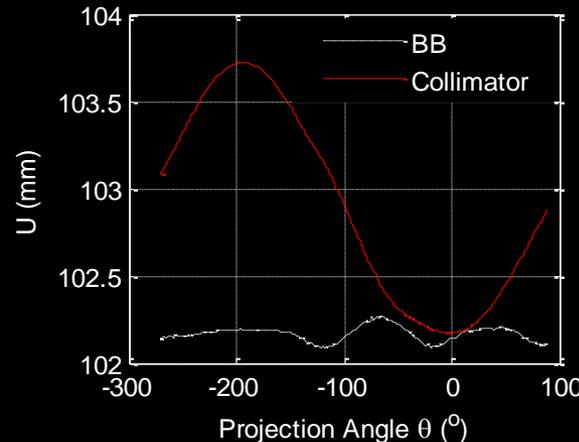


# Radiation Field Targeting – Off-line Scheme

## Targeting Error on Flat Panel at a Single Gantry Angle



## Collect Across All Gantry Angles



Targeting error at isocenter is then

$$x(\theta) = -(1/\bar{M}) (U_{coll}(\theta) - U_{BB}(\theta)) \sin \theta$$

$$y(\theta) = (1/\bar{M}) (U_{coll}(\theta) - U_{BB}(\theta)) \cos \theta$$

$$z(\theta) = (1/\bar{M}) (V_{coll}(\theta) - V_{BB}(\theta))$$

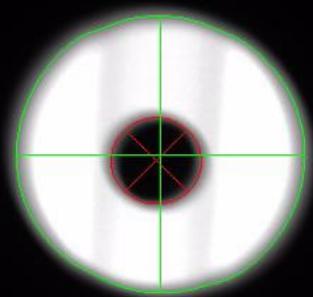
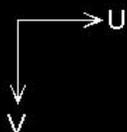
where

$$\bar{M} = SDD / SAD = 2.07$$

is the system magnification factor.

# Radiation Field Targeting – Off-line Scheme

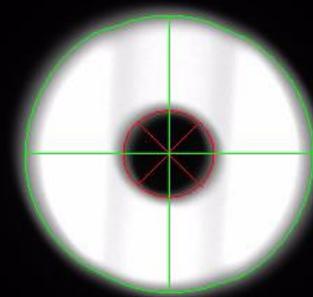
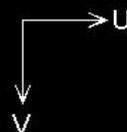
## Without Stage Tracking



$\theta: 89.7^\circ$

| Field       | BB          | Delta       | 2-D Delta Mag |
|-------------|-------------|-------------|---------------|
| U: 0.42 mm  | U: -0.20 mm | U: -0.62 mm | 1.11 mm       |
| V: -1.46 mm | V: -0.54 mm | V: 0.92 mm  |               |

## With Stage Tracking

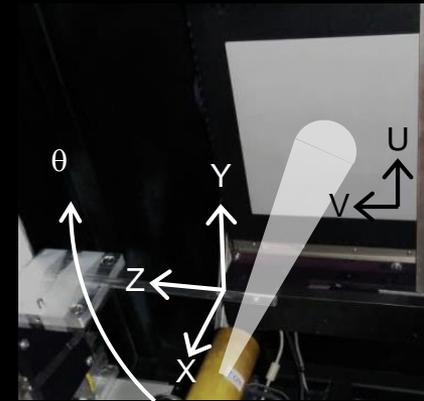


$\theta: 89.8^\circ$

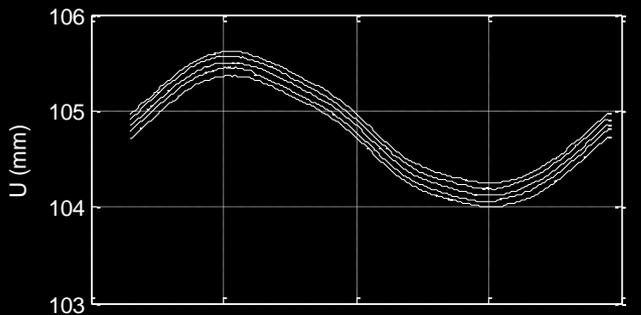
| Field       | BB          | Delta       | 2-D Delta Mag |
|-------------|-------------|-------------|---------------|
| U: 0.38 mm  | U: 0.30 mm  | U: -0.09 mm | 0.09 mm       |
| V: -1.50 mm | V: -1.53 mm | V: -0.03 mm |               |

# Radiation Field Targeting – Off-line Scheme

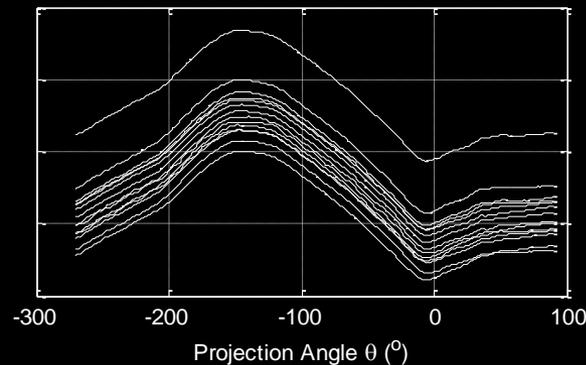
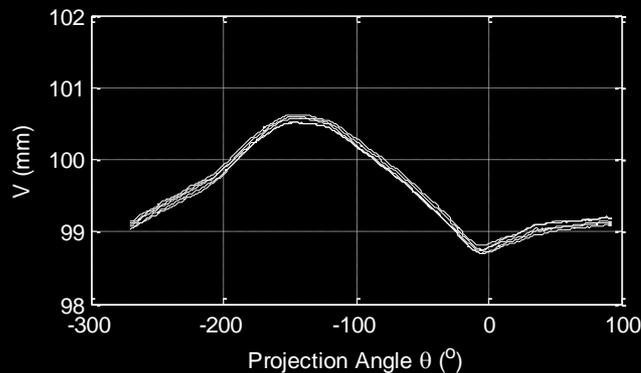
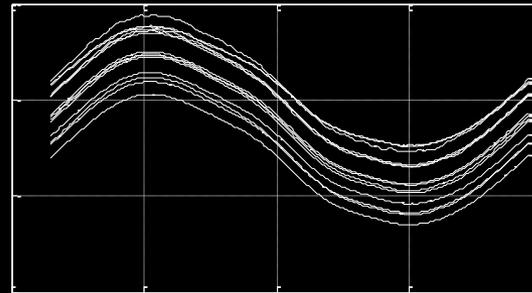
**Radiation field position can vary during an experiment and between different collimators and between focal spots.**



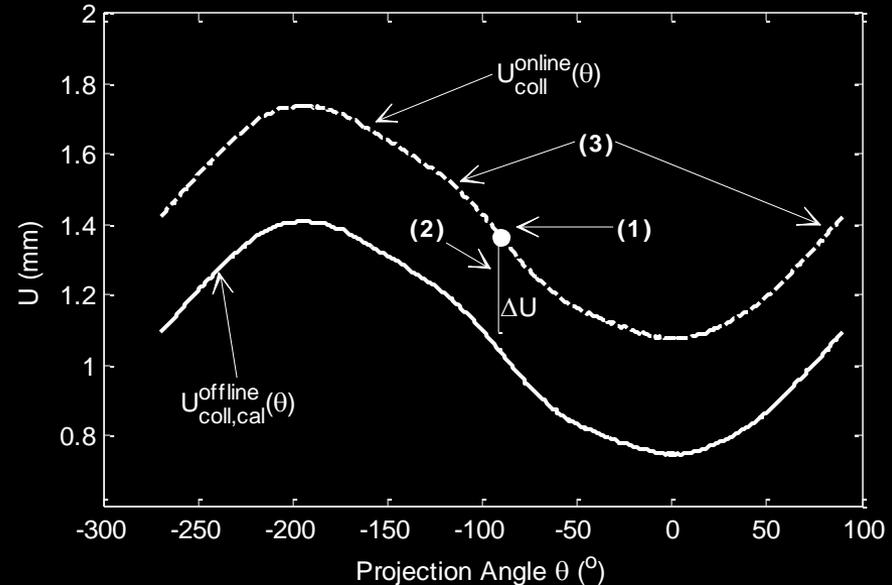
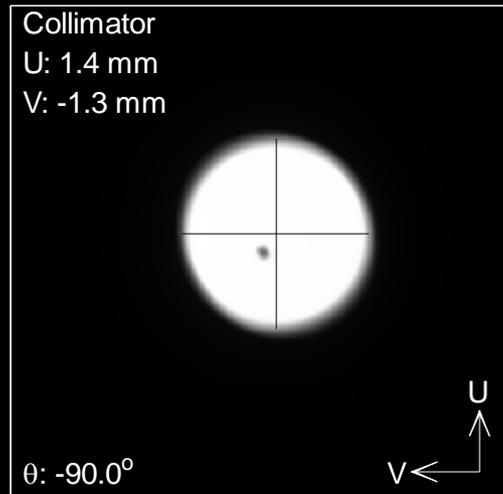
Exemplar Curves for One Collimator (15 mm Circular)



Mean Curve for 13 Collimators



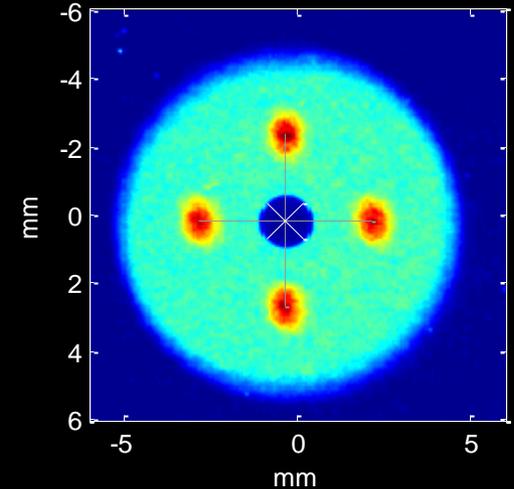
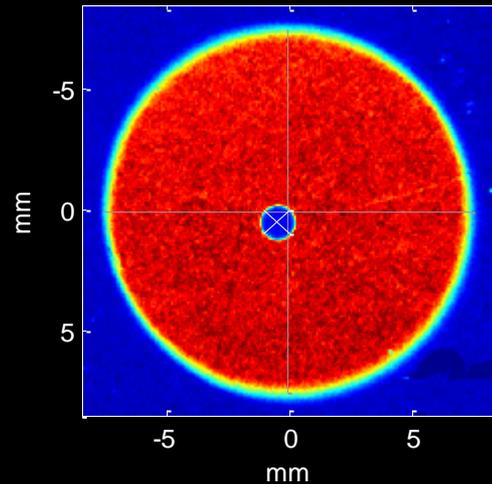
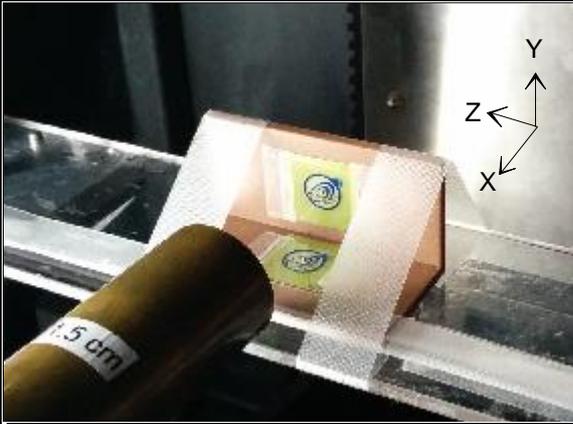
# Radiation Field Targeting – On-line Correction



## Online Virtual Isocenter (OnVI) targeting technique workflow:

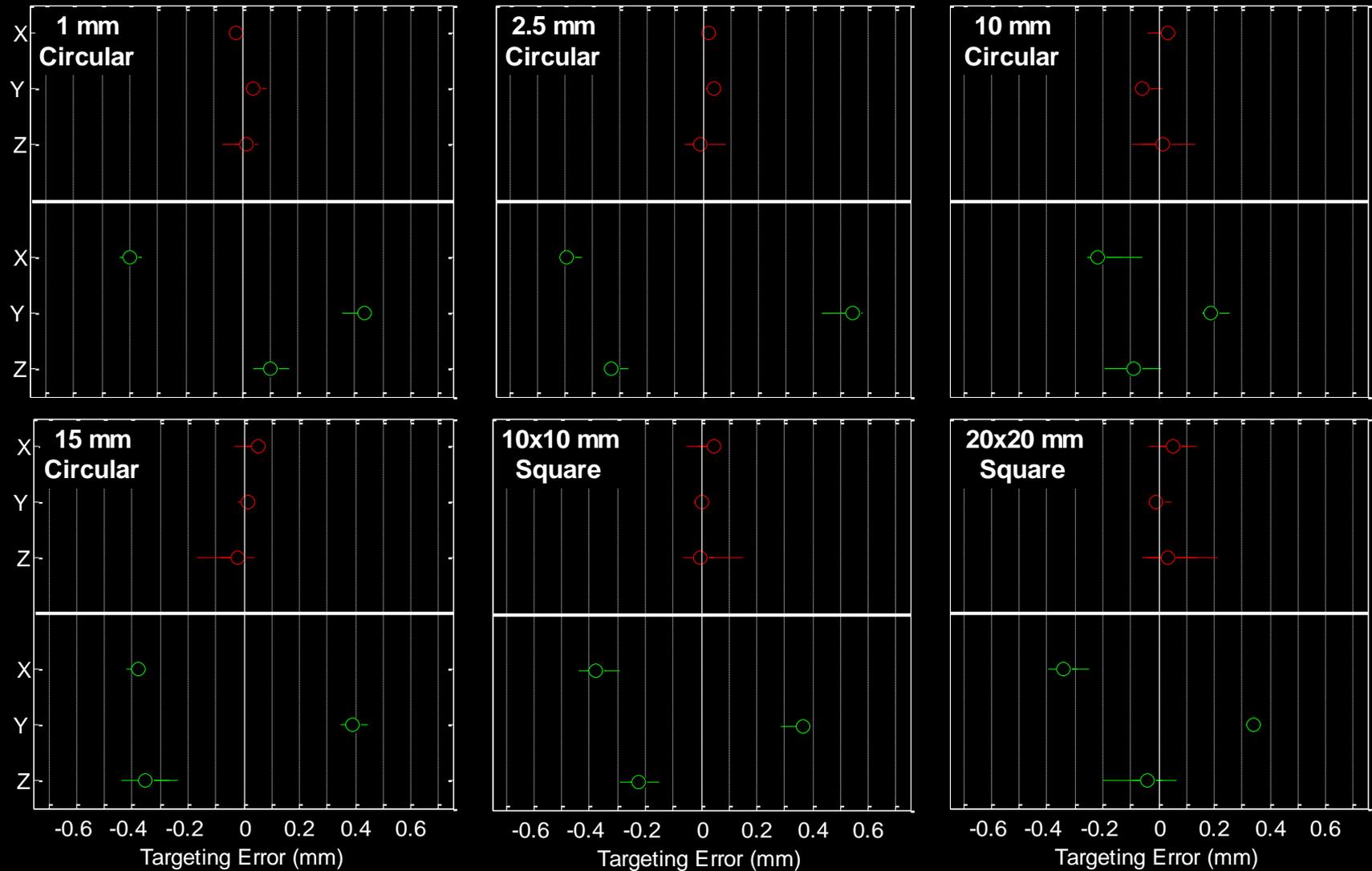
- 1) After insertion of collimator, but prior to irradiation, measure centroid of radiation field (X: 100kVp, 5mA, 1s)
- 2) Determine ( $\Delta U$ ,  $\Delta V$ ) offset from OffVI calibration collimator
- 3) Automatically construct online collimator curve
- 4) Apply online virtual isocenter curve during irradiation

# Validation: End-to-end Targeting Comparison



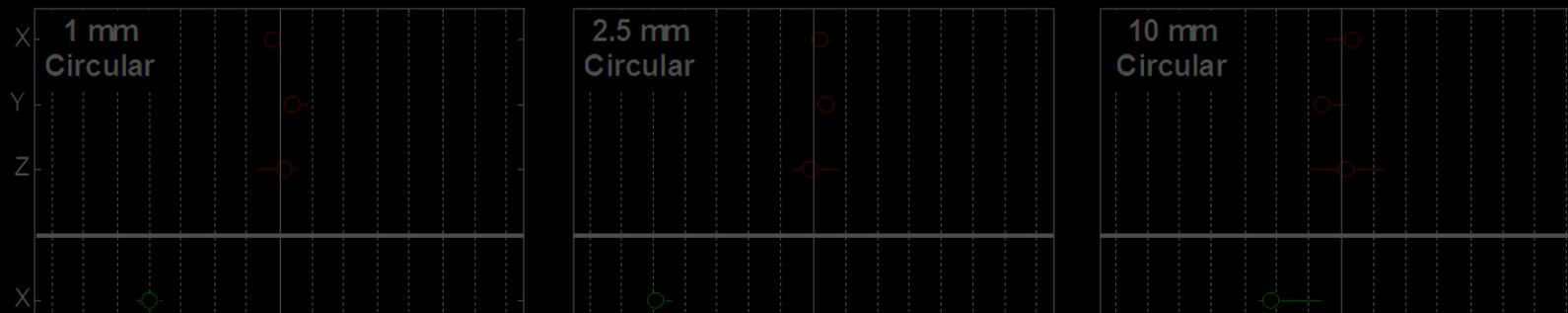
- Phantom setup with CBCT at 80 kVp, 60 mAs and reconstructed with 0.1 mm isotropic voxels
- Ten total irradiations (5 each in the X-Z and Y-Z planes)
- Six different collimators:
  - 1.0, 2.5, 10, and 15 mm circular
  - 10x10 and 20x20 mm square fields

# Results: Sub-100 $\mu$ m Targeting Performance



— Online Virtual Isocenter — Offline Virtual Isocenter

# Results: Sub-100 $\mu$ m Targeting Performance



Number of one-dimensional targeting error measurements within 100 $\mu$ m

OffVI: 16% (19/120)

OnVI: 93% (111/120)



— Online Virtual Isocenter — Offline Virtual Isocenter

# A High-Throughput Robotic Radiobiology Platform

*A High-throughput Robotic Radiobiology Platform.*

*Elliot R, Jaffray A, Vedadi A, Stewart J, Vellanki R, Ryan S, Wouters BG, Jaffray DA*

*Manuscript in preparation; Patent pending*

# Motivation and Objective

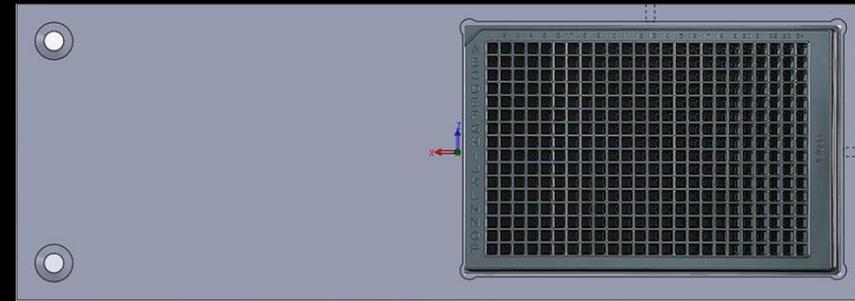
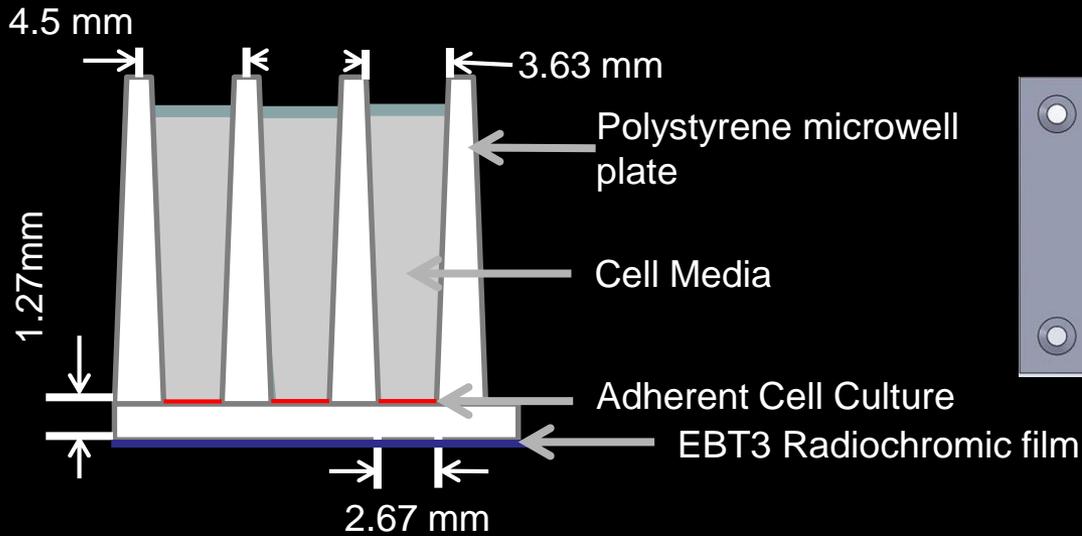
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Overcoming limitations present in manual clonogenic survival assays:

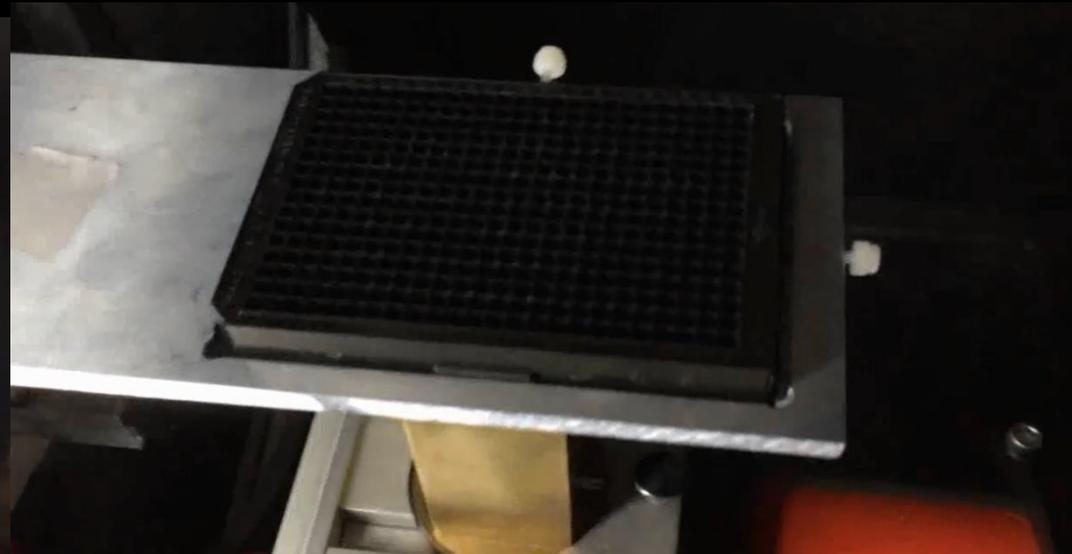
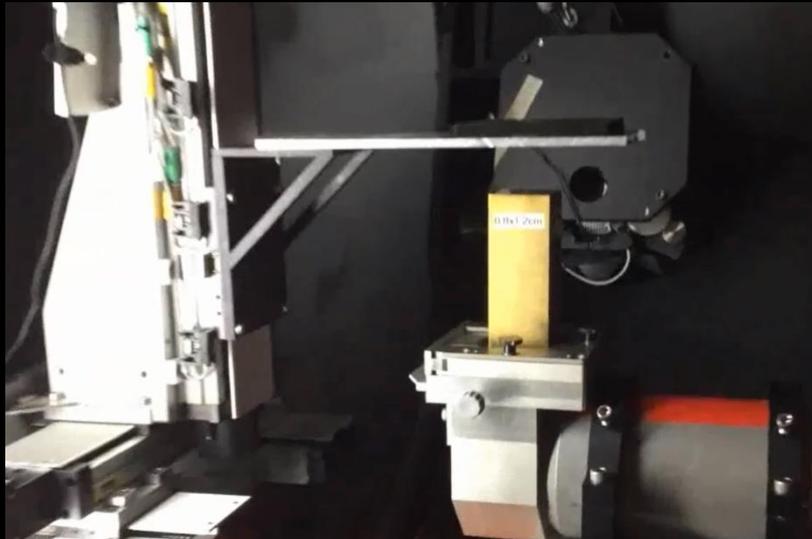
- Labour intensive in preparation and analysis; serial nature limits combined modality studies
- Course control of factors (plating; dose; drug) determining statistical 'dynamic range' of assay
- Long cycle time prevents recursive cell plating approaches; loss in efficiency; irreproducibility
- Unable to study temporal dynamics of colony development

*Approach: Develop a robotic, automated high-throughput clonogenic assay system to accelerate radiobiology research, support combined modality studies, and maximize data extraction.*

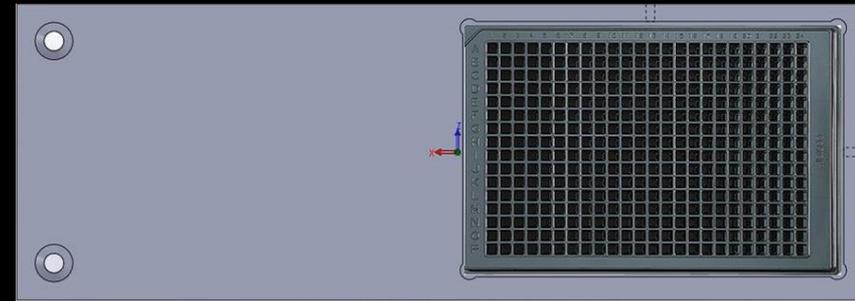
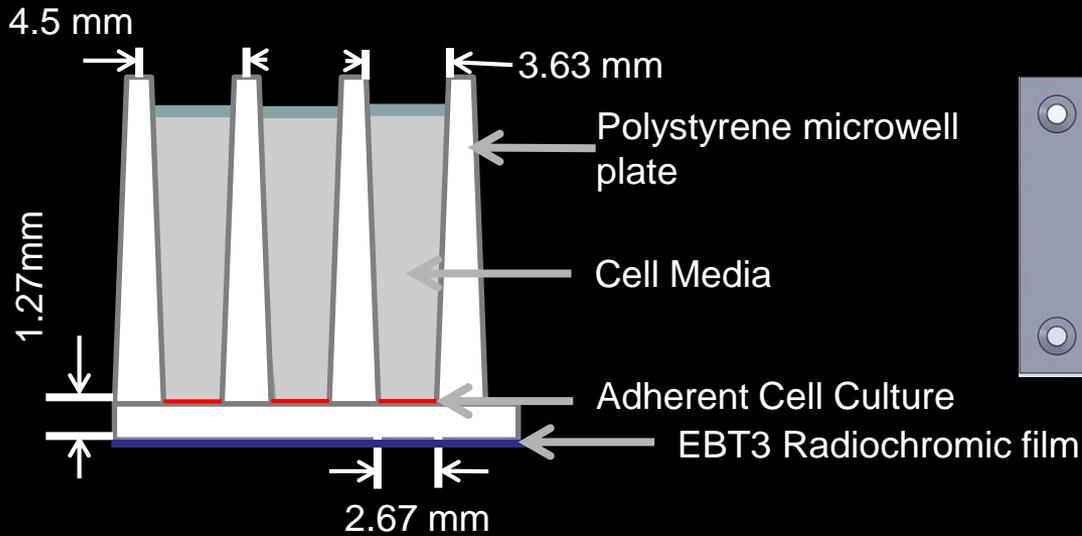
# 384 Microwell Plate: Cell Irradiation



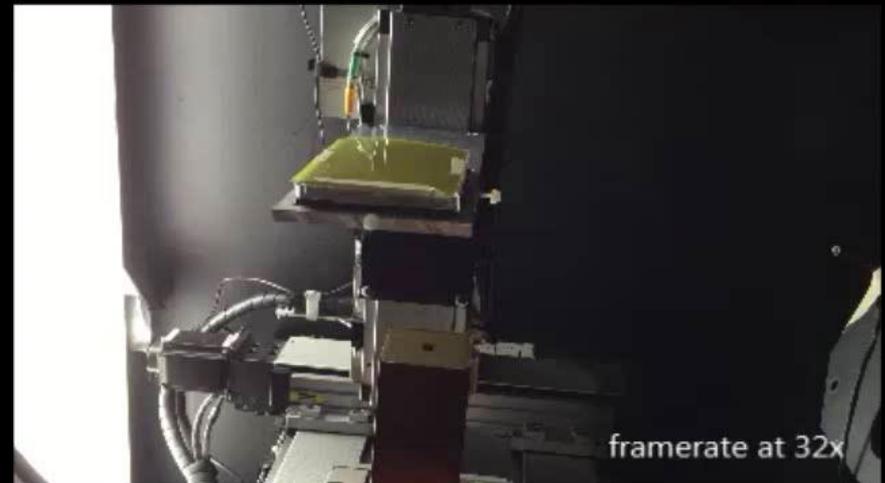
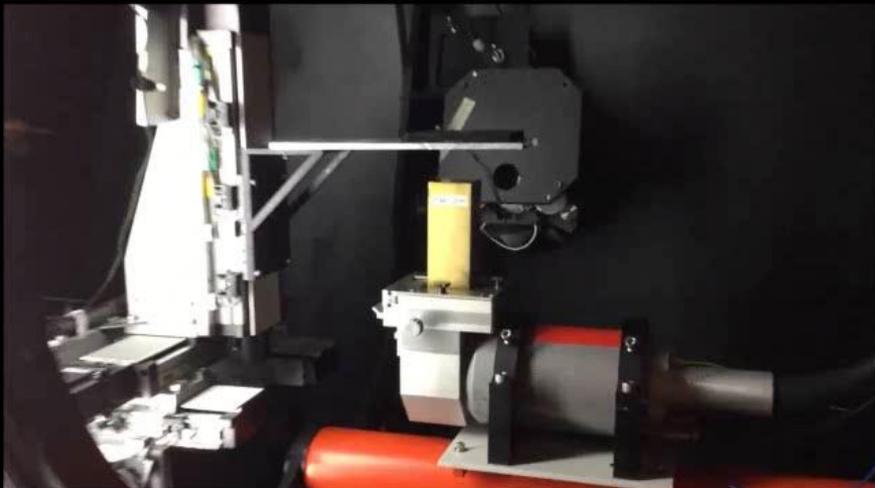
Fitted Microwell Plate Holder  
for Irradiation Unit



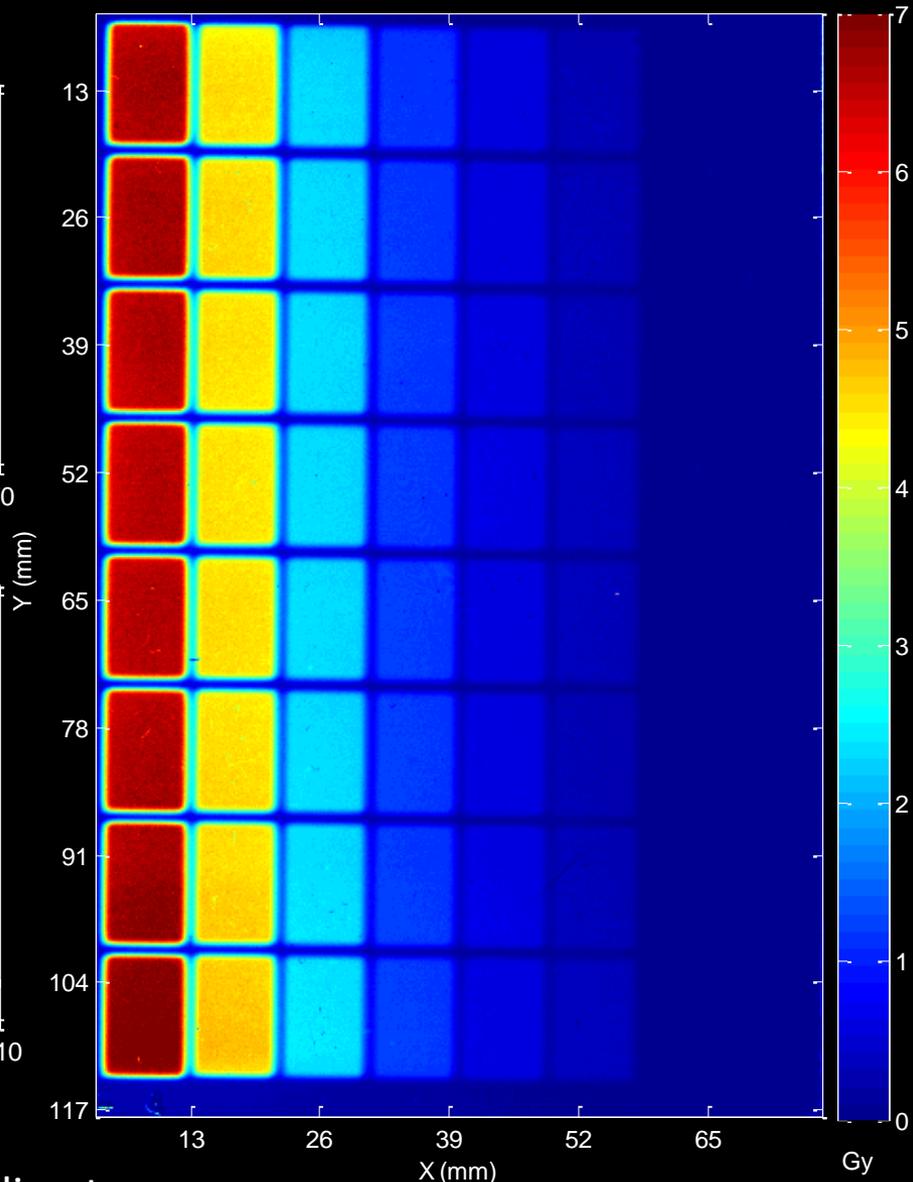
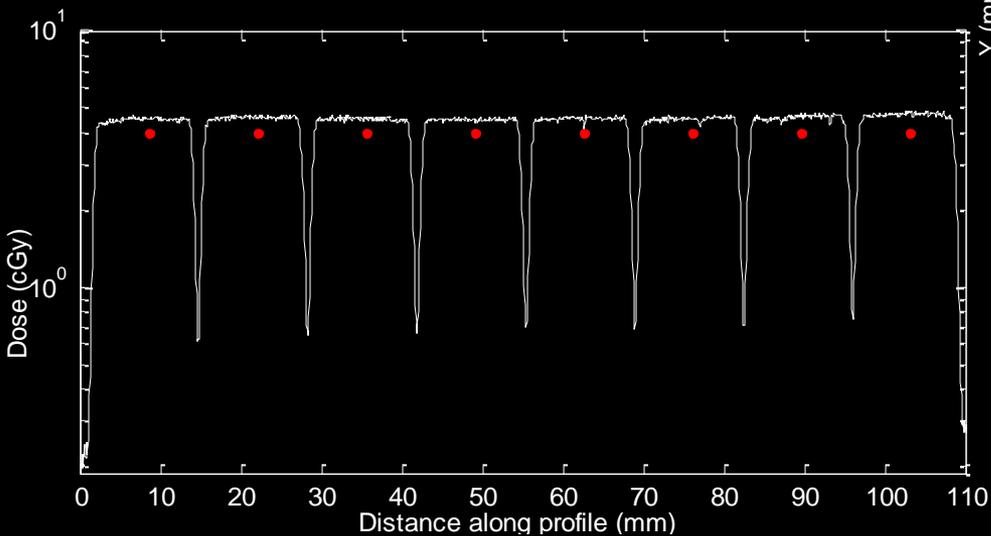
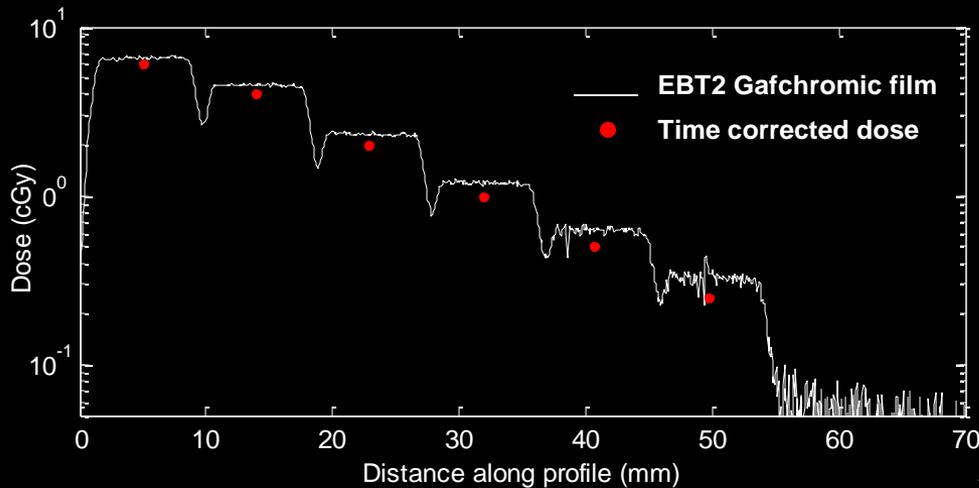
# 384 Microwell Plate: Cell Irradiation



Fitted Microwell Plate Holder  
for Irradiation Unit



# 384 Microwell Plate: Cell Dosimetry



Dose Applied to 3x2 Well Groupings  
225 kVp, 13mA, 2.424 Gy/min, 8mmx12mm Collimator

EBT2 Radiochromic Film

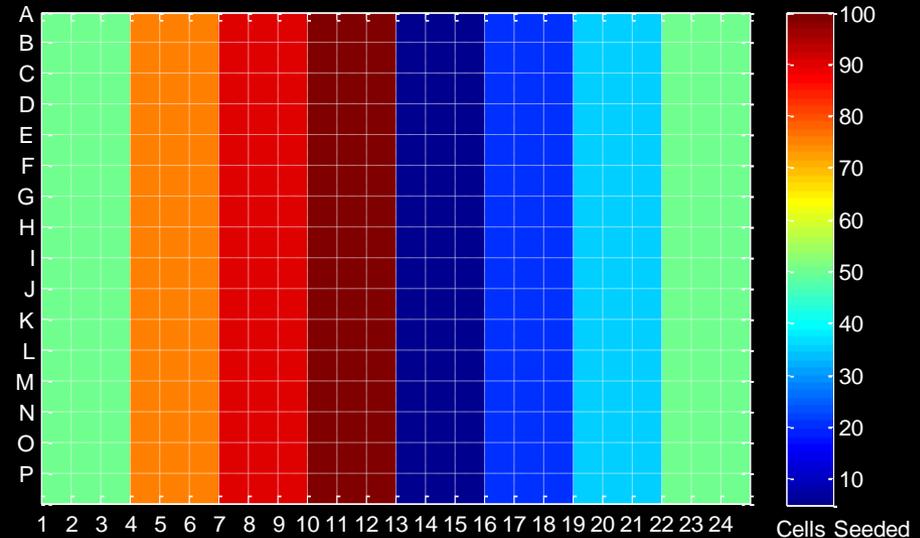
# 384 Microwell Plate: Seeding and Imaging

## Cell Seeding

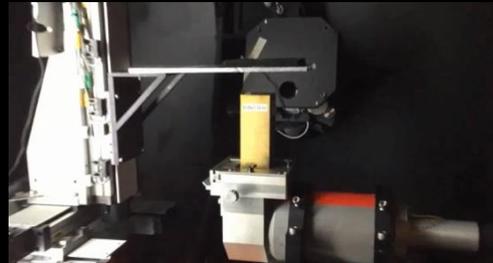
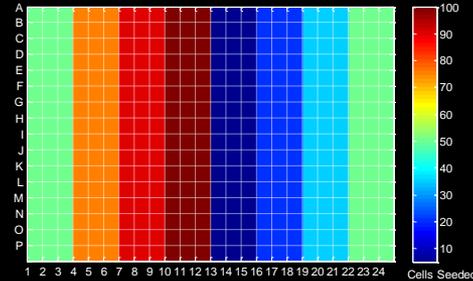
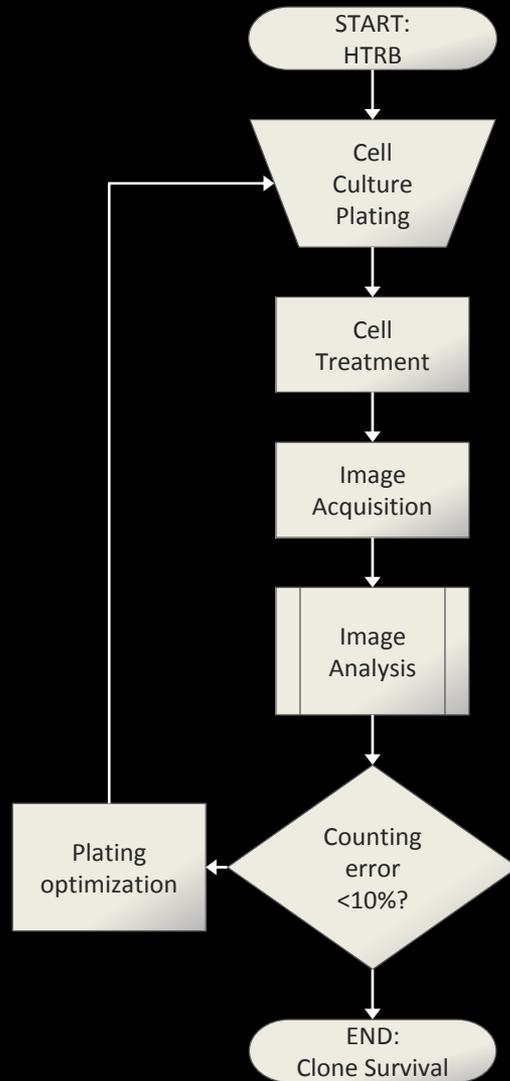
Cell Line: HCT-116 w.t. RFP  
RPMI 1640 with 10% FBS; 5 cells/ $\mu$ L  
Corning 384 plates; 50 $\mu$ L working volume; 5~100 cells/well;  
Incubate for 2hrs at 37°C  
Regions with duplicate cell seeding density; seeding density linked to dose

## Cell Imaging

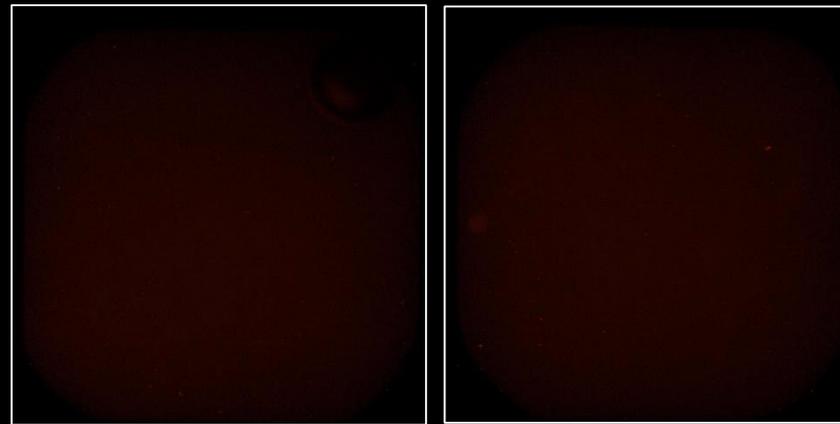
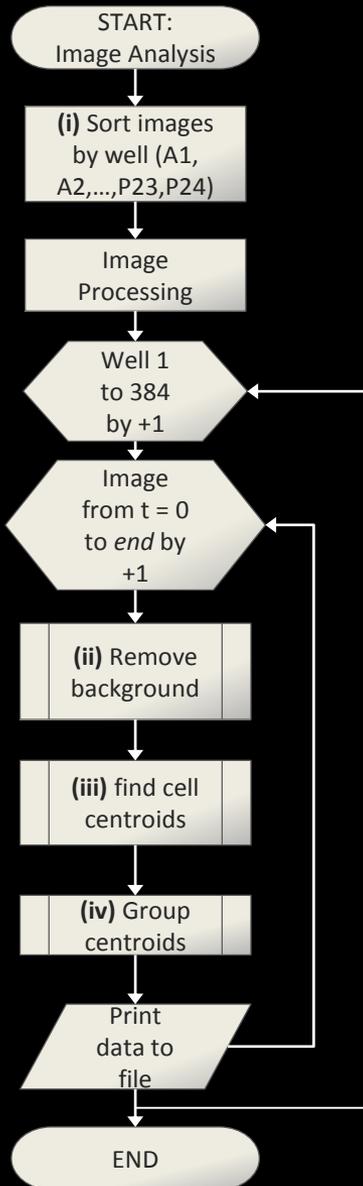
IncuCyte Zoom (Essen Biosciences Inc.)  
Pre- and post-irradiation  
3-6hr Intervals; 4X objective; 2.42 $\mu$ m  
Fluorescent and Phase Images  
Excitation: 488 and 532 nm  
Emission: 588 nm



# High Throughput Radiobiology Workflow

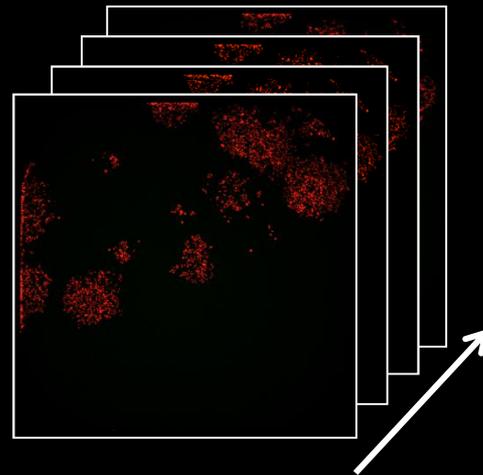


# Imaging Data Pipeline



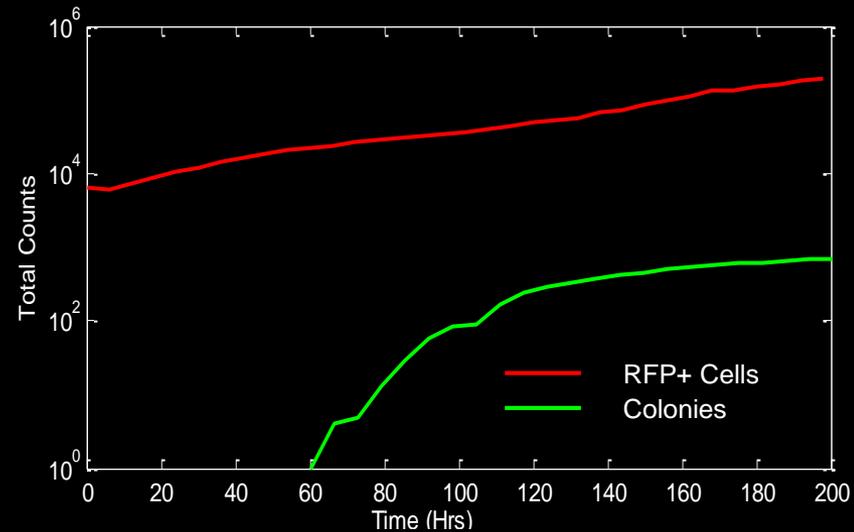
Well O10: 0 Gy

Well H6: 4 Gy



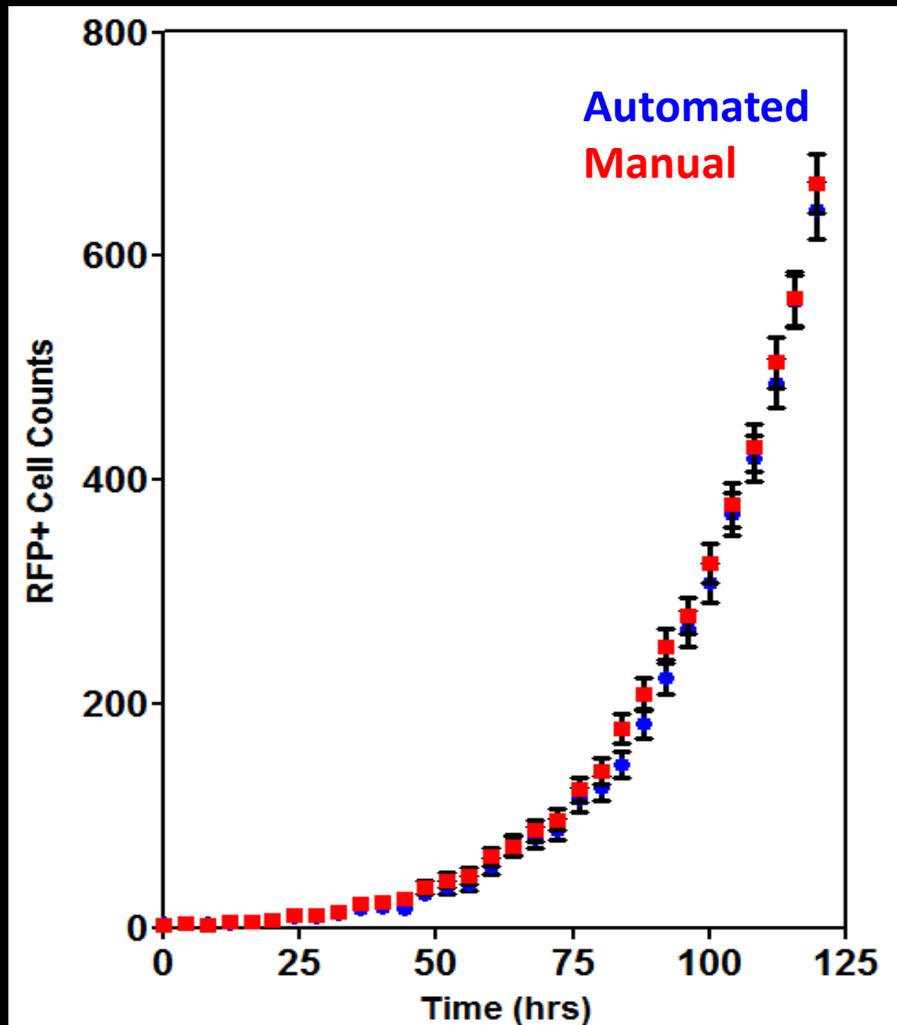
N x 384 images per experiment. Each image file size ~600 kB

39 images per well generates 14976 images  
Upwards of 8.2 GB image data per plate.



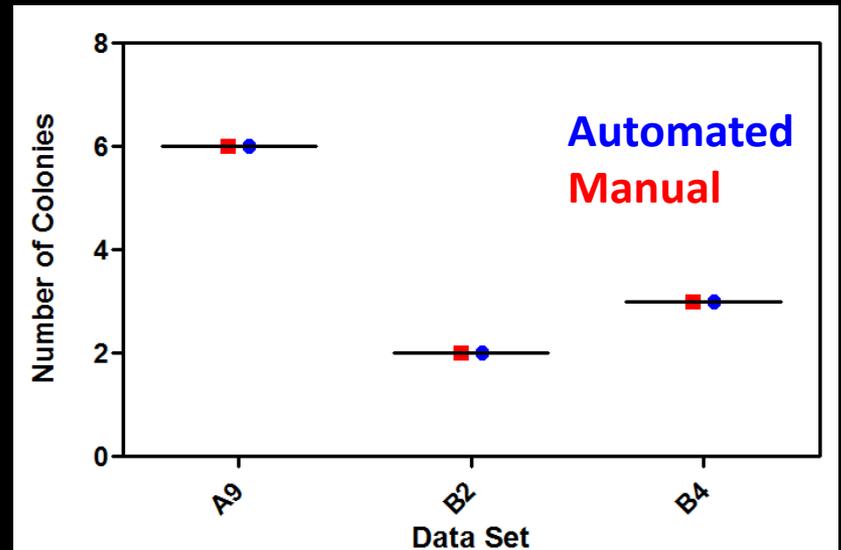
Extracting cell and colony coordinates generates 114 MB of process data; ~2x10<sup>7</sup> measurements

# Algorithm Validation: Cell and Colony Counting

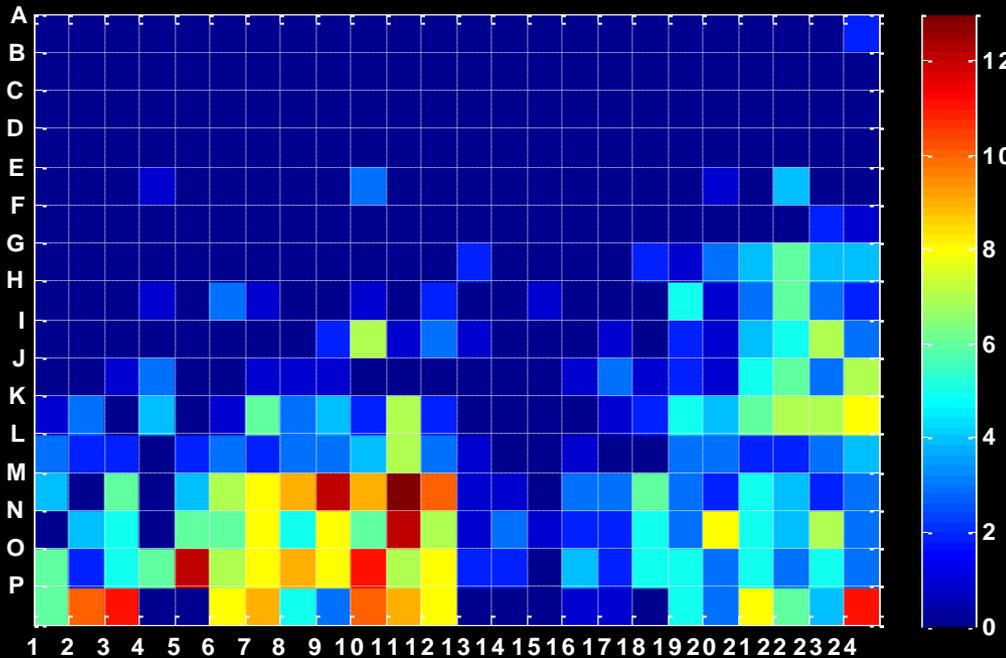


Manual cell and colony counting compared to automated method.

3 Datasets; Human observer.

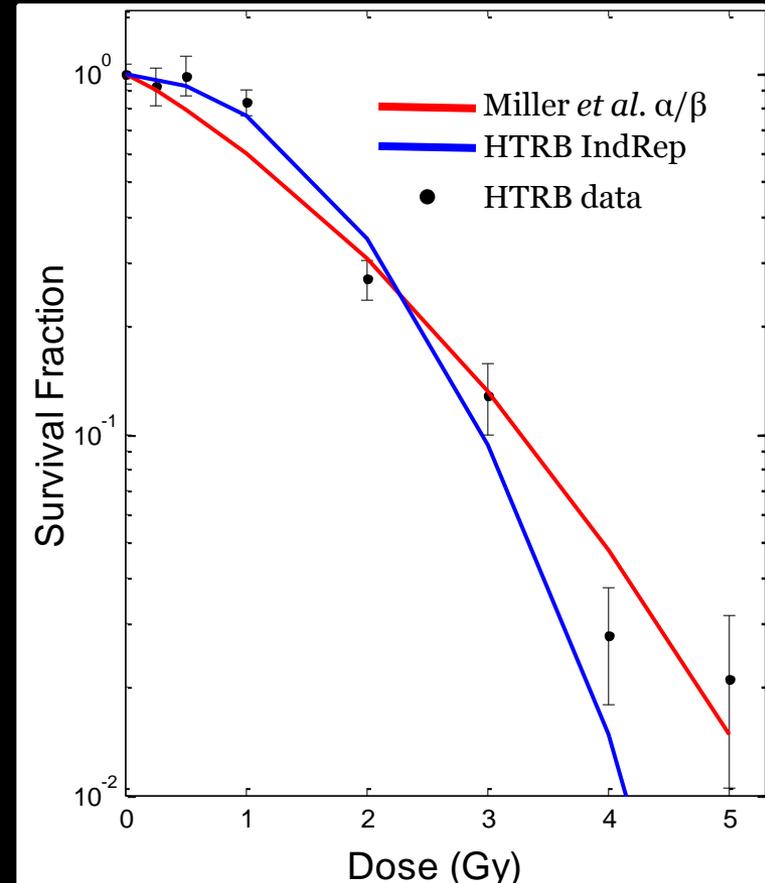


# Result: 384 Microwell Plate Survival Curve

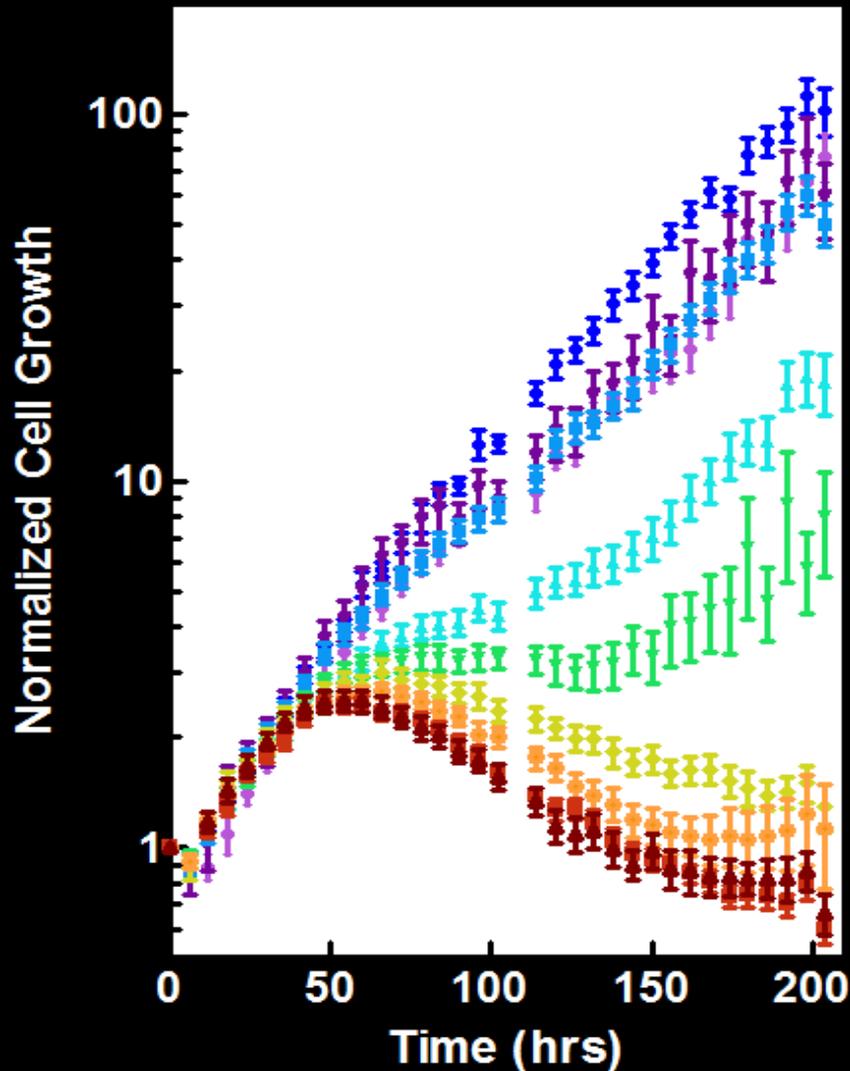


Total Elapsed Time: 15 days  
(includes cell culturing, irradiation, imaging,  
and analysis)  
Labor: 7-9 hrs total

85% decrease in labor;  
80% reduction in experiment turnaround time.



# Result: Includes Cell Proliferation Delay



Additional assays can be performed simultaneous to the colony forming assay.

Can see the knockdown effects of irradiation on cell growth.

Standard methods would require assay to be completed as a completely separate experiment.

- 0 Gy
- 0.25 Gy
- 0.5 Gy
- 1 Gy
- 2 Gy
- 3 Gy
- 4 Gy
- 5 Gy
- 6 Gy
- 8 Gy

# Summary

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On-going advances in micro-irradiation system development.

Enable increased precision and accuracy in radiobiology experiments.

Support experimental validation through replication in other laboratories.

Enables automated techniques to support combinatorial therapies and more agile experimental approaches.

Data handling capacity may allow exciting new observations.



# Acknowledgements

## Preclinical Irradiation Team

|                     |                  |
|---------------------|------------------|
| James Stewart       | Ryan Elliott     |
| Alexander Jaffray   | Ali Vedadi       |
| Ravi Vellanki       | Sarah Ryan       |
| Patricia E. Lindsay | Steve Ansell     |
| Graham Wilson       | Robert Weersink  |
| Justin Grant        | Deborah Scollard |
| Duoaud Shah         | Richard Hill     |
| Brad Wouters        | David Jaffray    |

## Precision X-Ray

Paul DeJean

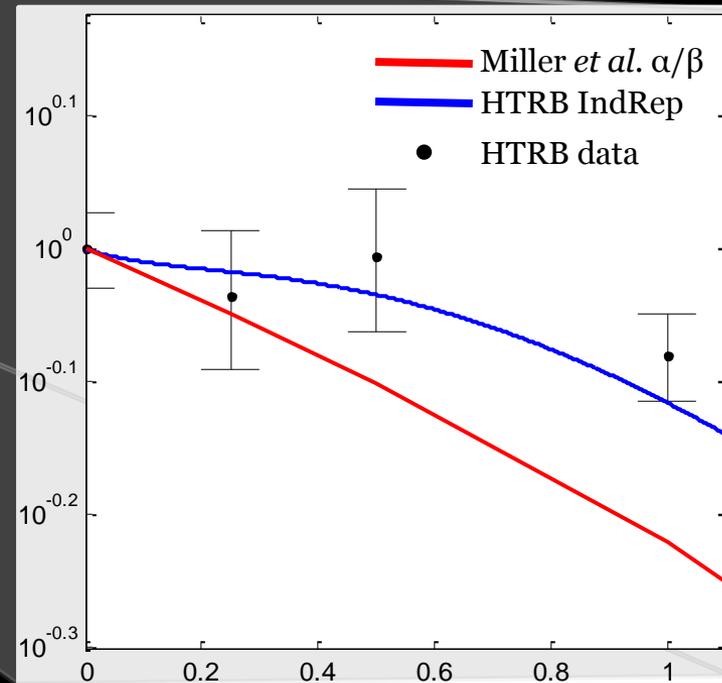
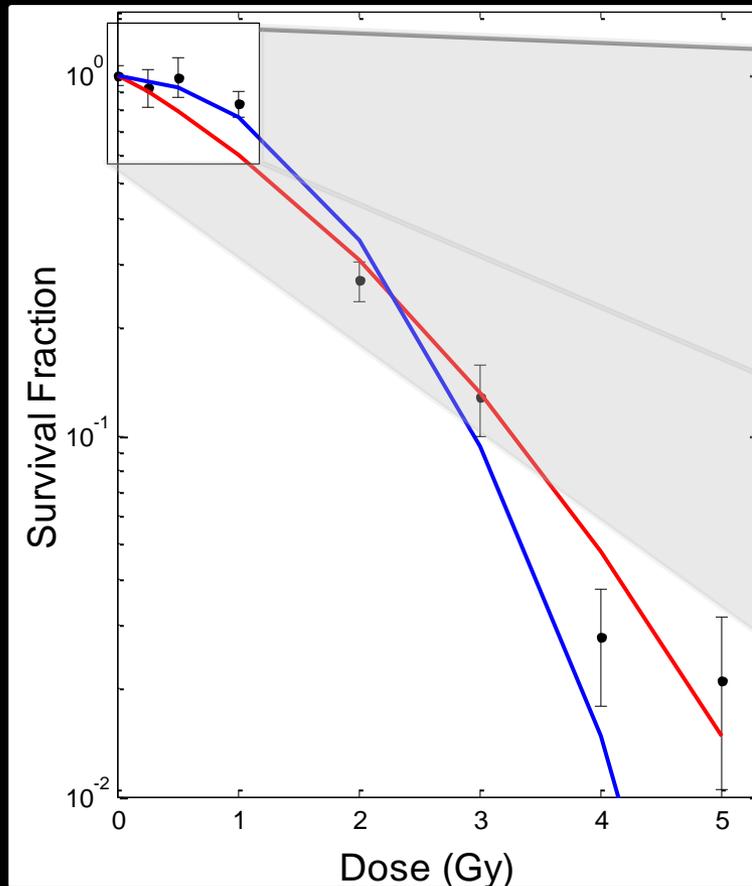
## Orey and Mary Fidani Family Chair in Radiation Physics



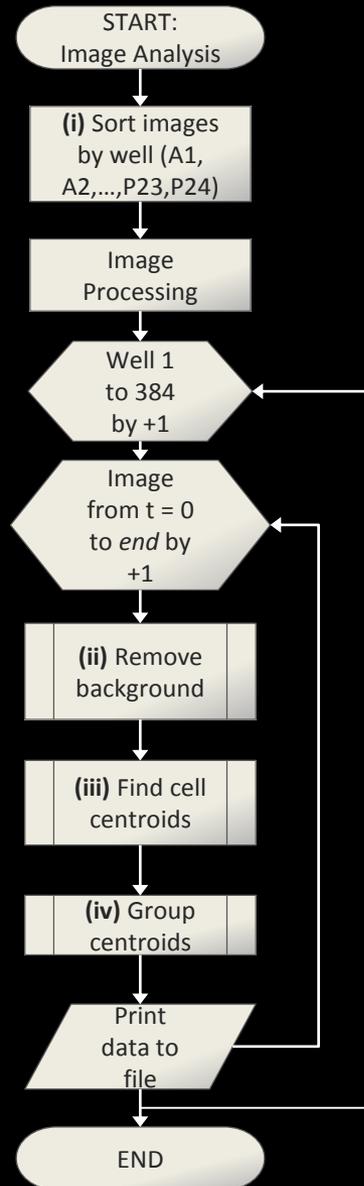
# Result: 384 Microwell Plate Survival Curve

Hyper radiation sensitivity model at low dose, as described by Joiner *et al*

$$p(D) = e^{-\alpha_r D(1 + (\frac{\alpha_s}{\alpha_r} - 1)e^{D/D_c} - \beta D^2)}$$



# High Throughput Workflow



(i)

(iii)

(ii)

(iv)

