Low-dose Studies with Focused X-rays in Cell and Tissue Models: Mechanisms of Bystander and Genomic Instability Responses.

Barry D. Michael[1], Melvyn Folkard[1], Kevin M. Prise[1] and Kathyrn D. Held[2]

[1]Gray Laboratory Cancer Research Trust,
Mount Vernon Hospital, Northwood,
Middlesex, HA6 2JR, U.K.

[2]Radiation Oncology,
Massachusetts General Hospital,
Cox 302, Boston, MA 02114.

Summary

The project uses techniques developed to irradiate individual cells in culture or subcellular regions to determine mechanisms by which they are damaged by extremely low doses of radiation and whether there are threshold-type responses related to the pathways by which cells respond to oxidative damage that occurs as a byproduct of their metabolism.

Background

Cells in the human body are continuously exposed to reactive oxygen species (ROS) produced as side products from normal physiologic processes, in certain disease states and due to exposure to low level background ionizing radiation. Although some of the types of ROS produced endogenously are the same as those produced by low dose ionizing radiation, some species may differ, and the relative quantities and the subcellular distributions of the ROS from the two sources may be quite different. Hence, critical, unanswered questions include:

Are the same cellular damages caused by endogenously produced ROS and ionizing radiation?

Do cells respond in a similar fashion to the damage from endogenous ROS and radiation-induced ROS?

Are the same cellular repair processes elicited by endogenous and radiation-produced ROS?

To address these questions, we will couple use of a unique focused soft X-ray facility for low dose irradiation of individual cells or irradiation of specific subcellular regions of individual cells with studies of effects of ROS produced in cells. We aim to understand the responses of cells to low doses of radiation typically encountered in environmental level exposures. Modern cellular and molecular biology approaches will be used to monitor the responses of cells to the X-ray and cellularly produced ROS.

Goals - 1

- **Determine the dose response of individual cells to low doses of focused soft X-rays, down to doses approaching that of a single electron track.**

- **Determine the response of cells to ROS generated to mimic the endogenous cellular generation of ROS.**

- **Study the interactions between cellular oxidative processes and ionizing radiation.**

Goals - 2

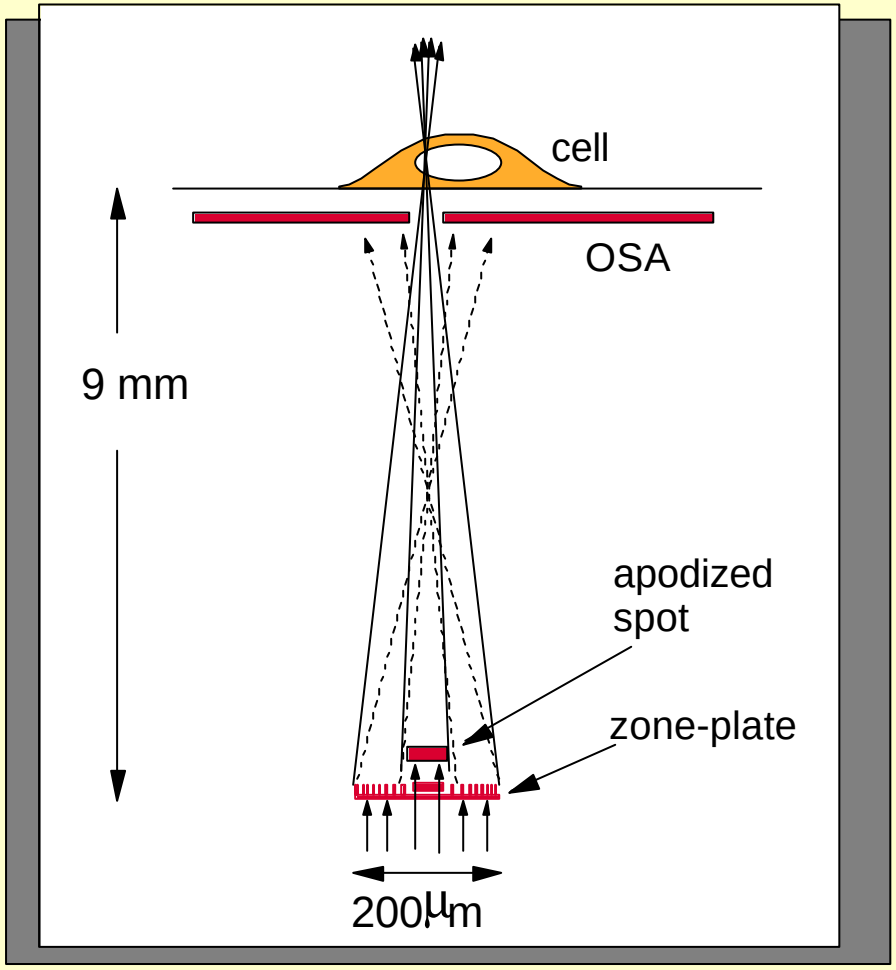
- **Determine the importance of the subcellular distribution of ROS from focused soft X-rays on cellular response by targeting nuclear versus cytoplasmic regions**

- **Determine whether damage deposited in individual cells by focused soft X-rays or chemically generated ROS can elicit a bystander response**

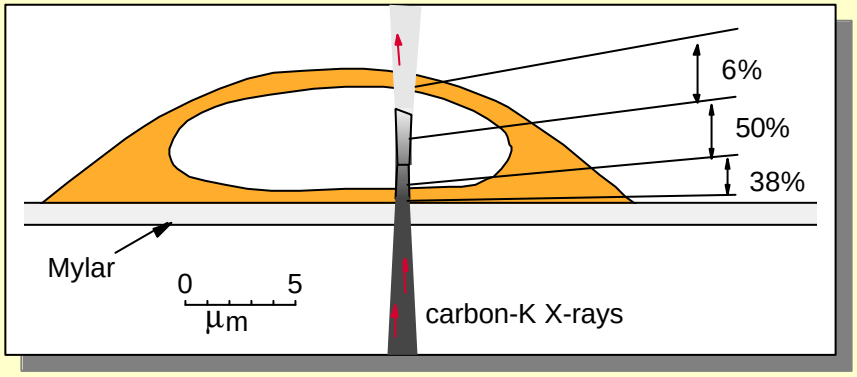
- **Determine if there is a relationship between the bystander response and genomic instability**

- **Develop tissue explant systems for the measurement of low dose effects in multicellular systems**

Spatial Resolution of the Microfocus Source



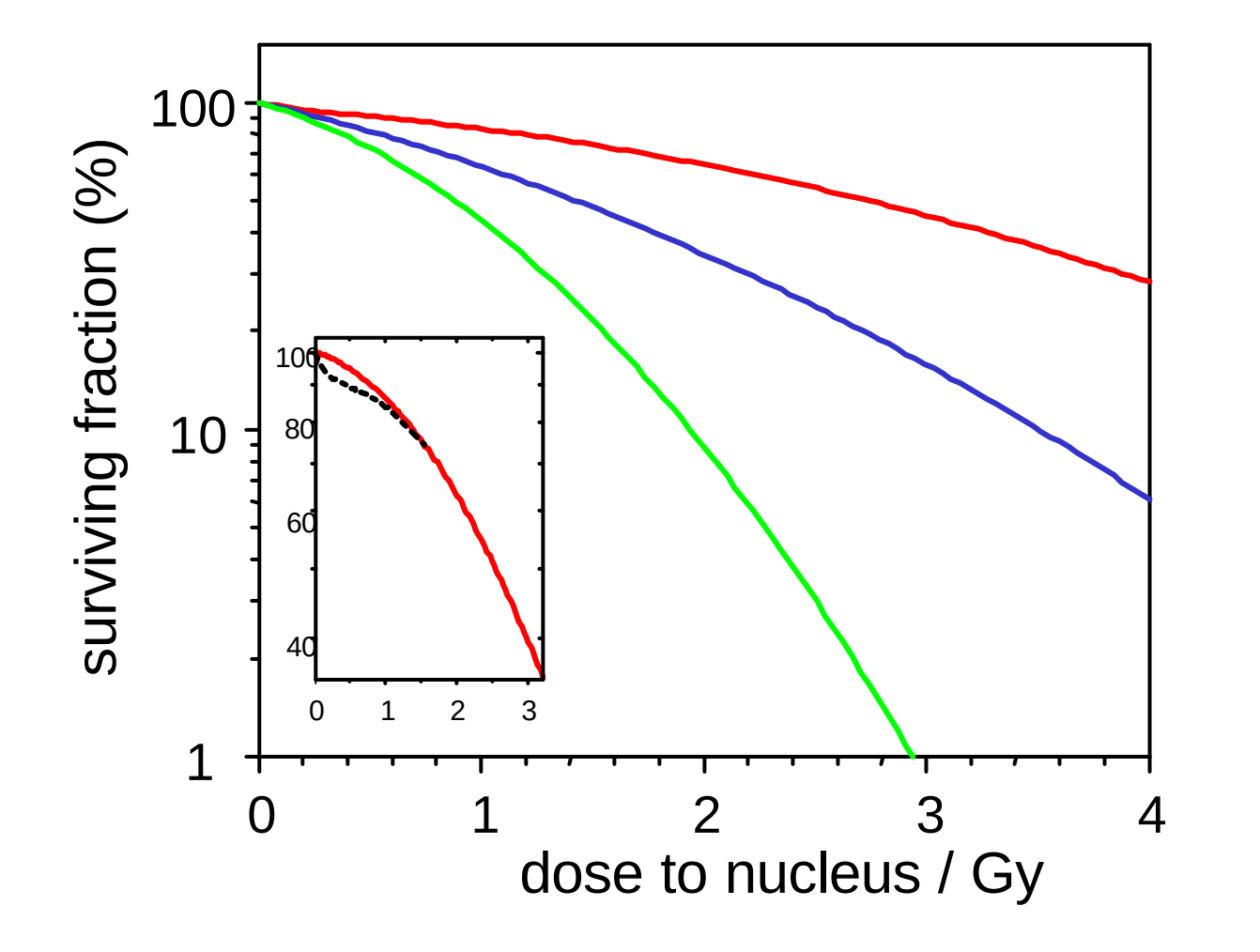
Zone plate alignment and dose distribution across a V79 cell



Preliminary Experiments Measuring Cell Survival

Low dose survival using single cell revisiting assay

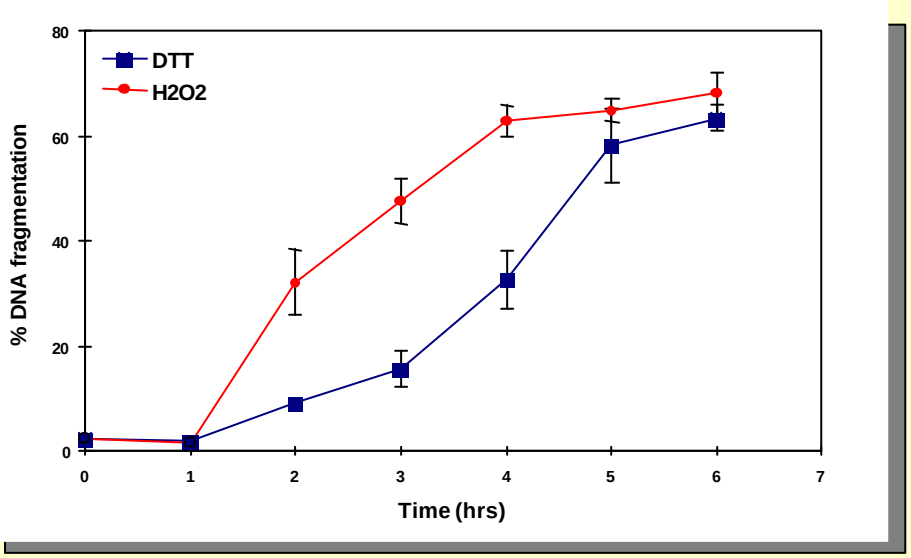
- **240kV X-rays**
- **Al-K X-rays (non-targeted)**
- **C-K X-rays (targeted)**



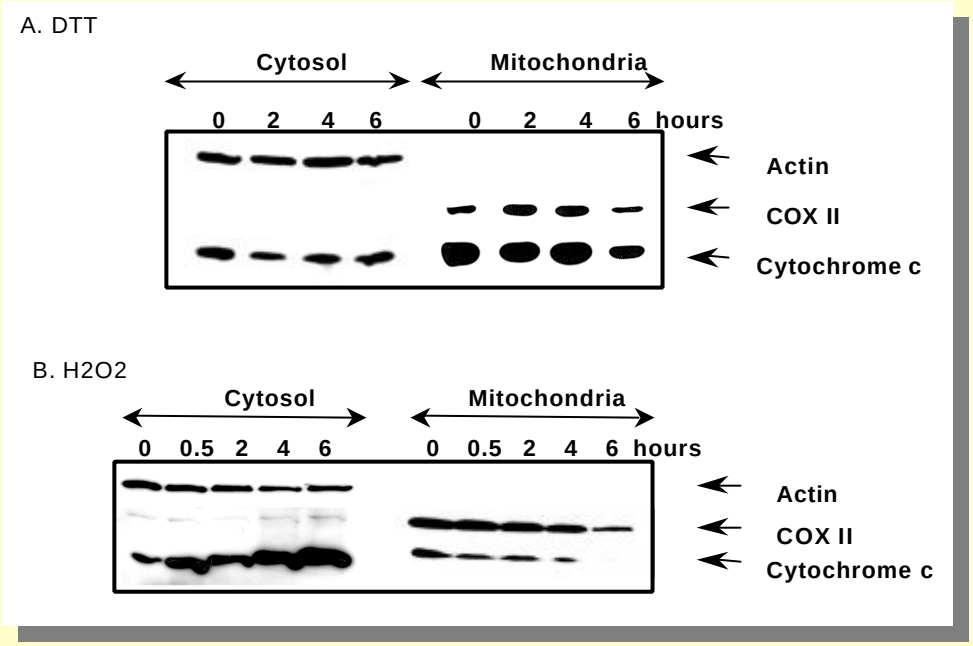
Endogenous ROS versus Ionizing Radiation

	Endogenous ROS	*Ionizing Radiation*
Relative yields of products	$O_2^{-\bullet}$ and H_2O_2 in highest yield; $^{\bullet}OH$ only in presence of metals	$^{\bullet}OH$ in highest yield
Cellular distribution	Primarily produced in mitochondria	Produced randomly along tracks throughout a cell, but thought to be most effective in DNA
Microdistribution	As single molecules (apart from site-specific)	In clusters (MDS)
Timing of production	Slow, chronic release	Instantaneous production during radiation

Reactive Oxygen Species - 1

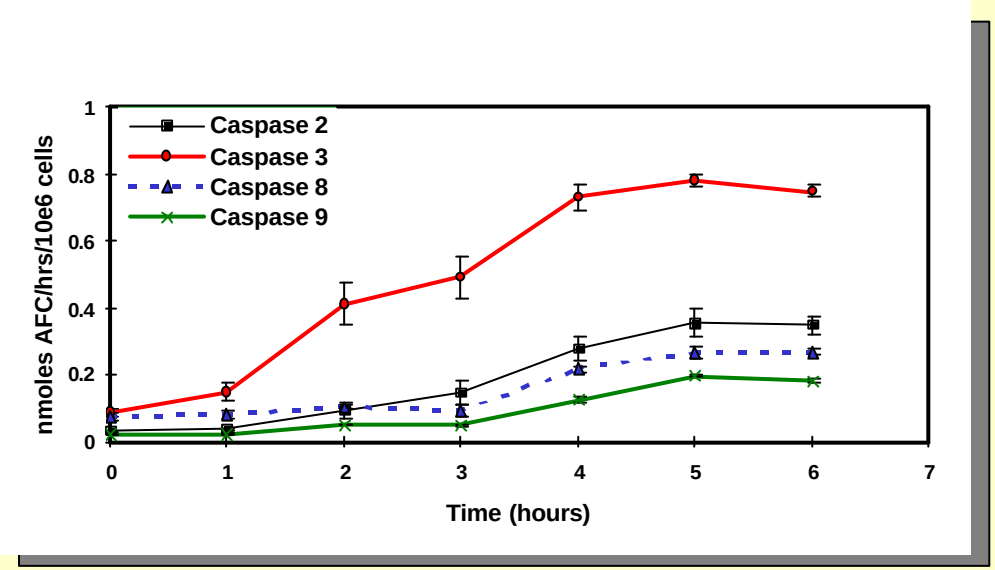


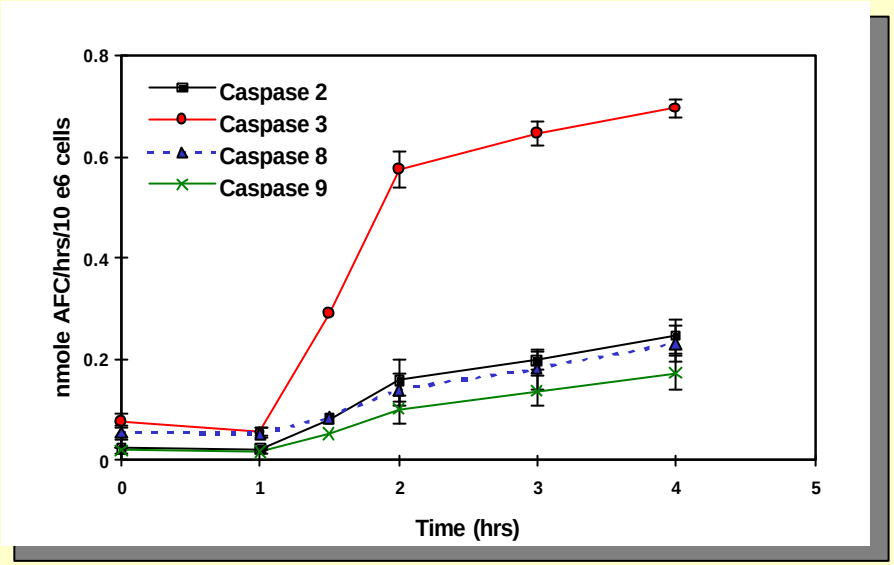
Apoptosis in HL-60 cells treated with 2mM DTT or 100mM H_2O_2



Cytochrome c release from mitochondria in HL-60 cells treated with 2mM DTT or 100mM H_2O_2

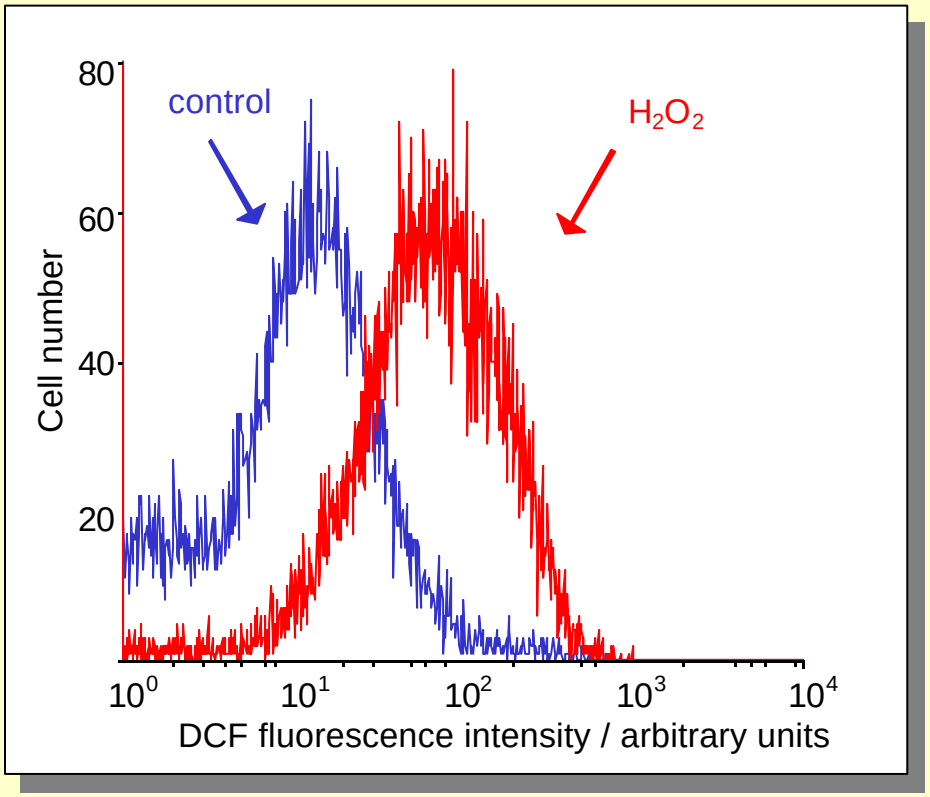
Reactive Oxygen Species - 2



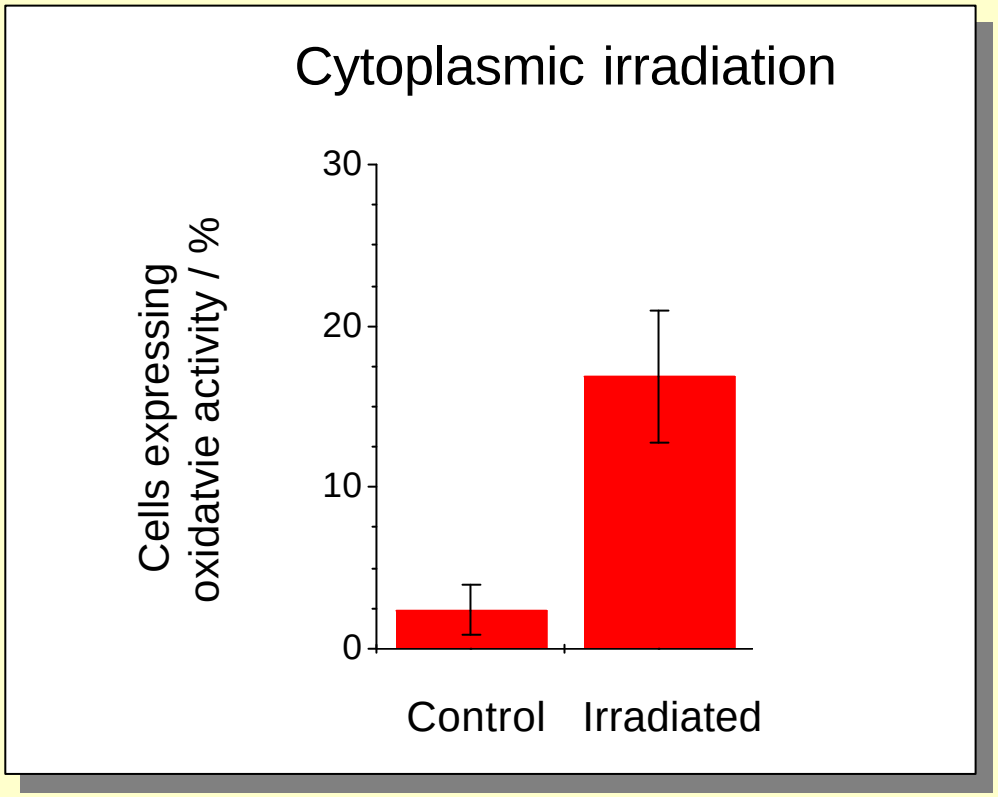


Caspase activity in HL-60 cells treated with 2mM DTT (left) or 100mM H_2O_2 (right)

Oxidative activity induced by cytoplasmic irradiation



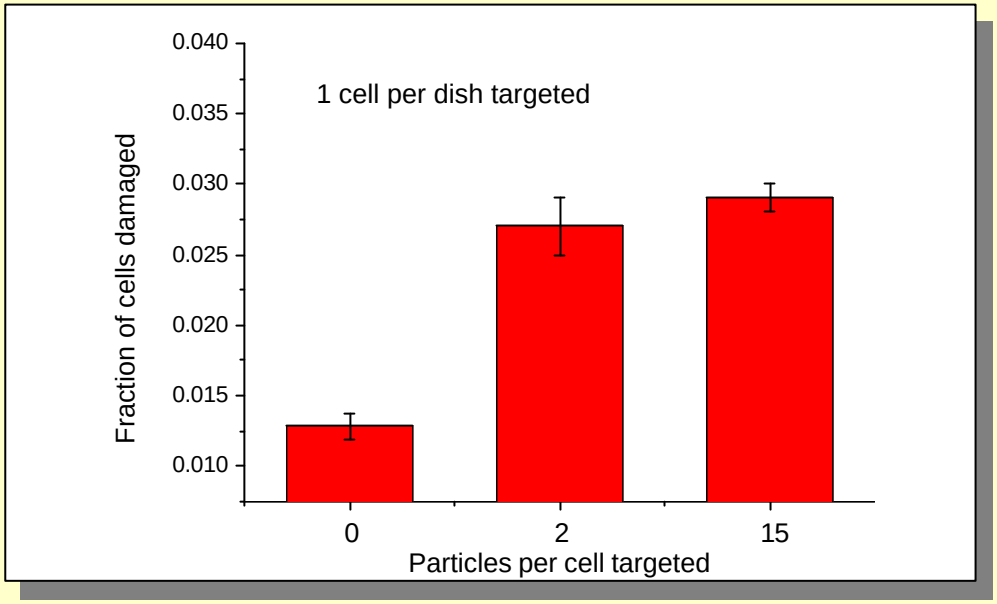
Oxidative activity in human fibroblasts treated with hydrogen peroxide



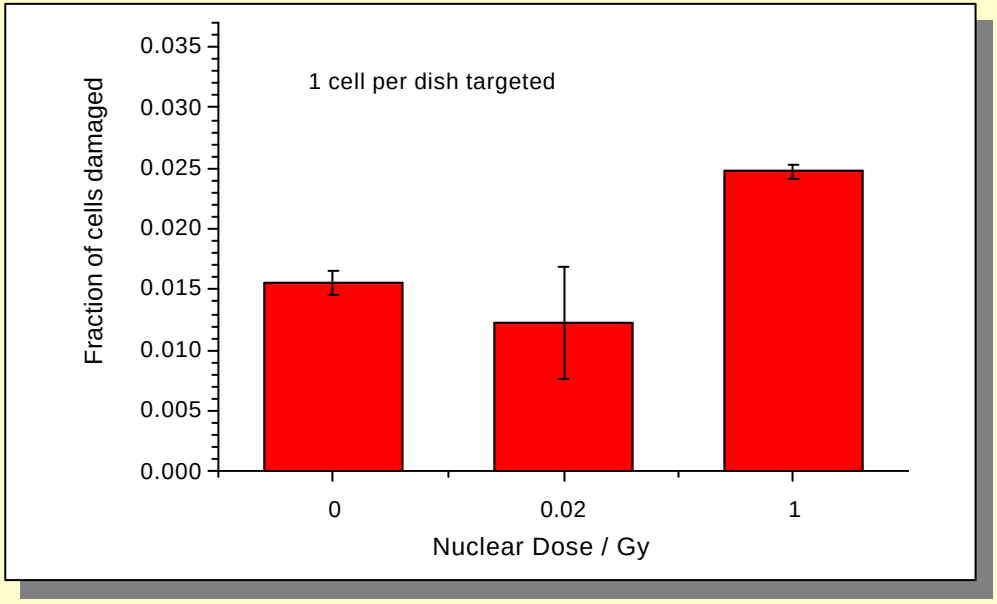
Oxidative activity in human fibroblasts irradiated through the cytoplasm with a-particles

Radiation-induced bystander effects

Targeted α-particles



Focused soft X-rays



Current experiments

- **We are currently targeting X-rays to different regions of the cell to determine biological effectiveness measured as loss of clonogenic survival or chromosomal damage. The aim is to simulate protection level doses approaching that of a single electron track.**

- **The interactions between targeted and non-targeted cells will also be measured to determine the degree of bystander response.**

- **We are also characterising the pathways leading to oxidative stress-induced apoptosis.**