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Mechanistic Studies for Radiation Exposure to Lens of the Eye

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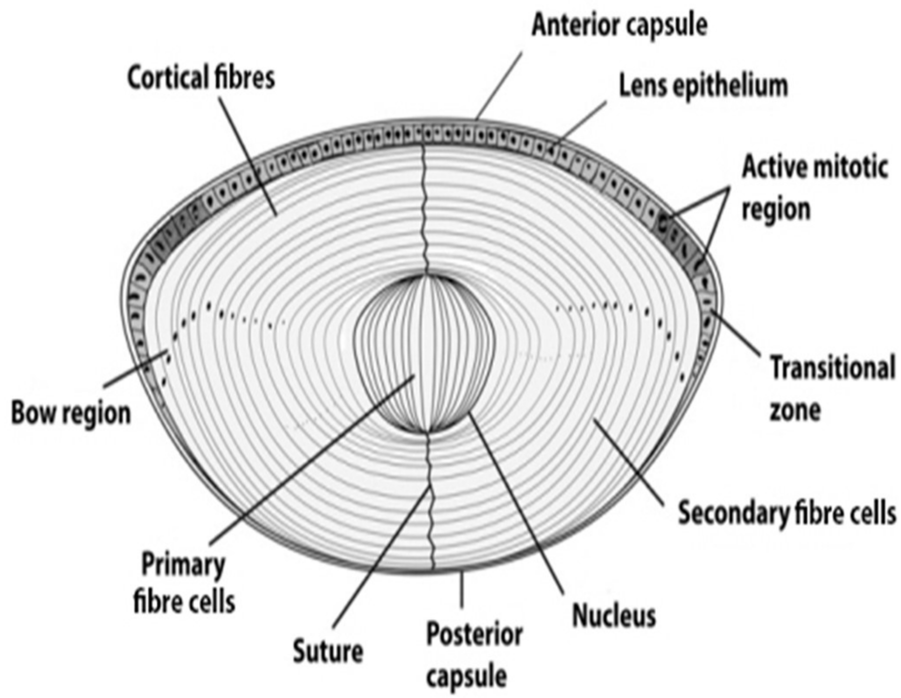
YOUR HEALTH AND SAFETY... OUR PRIORITY.

Schematic of Lens Cells

Generate biological evidence to support an understanding of the underlying mechanisms of radiation induced-damage in lens cells.

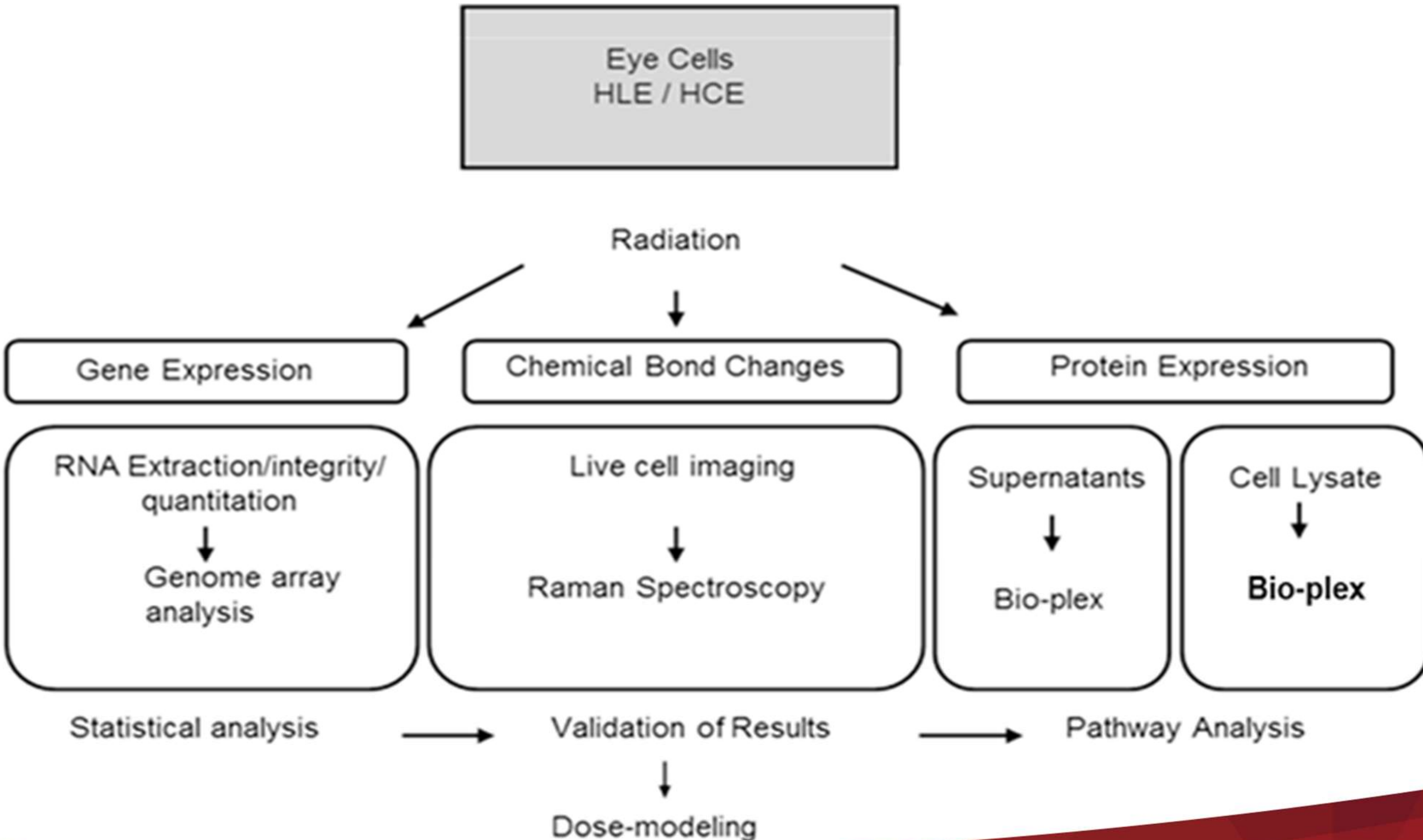
- Radiation causes posterior subcapsular cataracts
- Aberrant lens epithelial cell division results in abnormal differentiation of fiber cells
- This can lead to abnormal accumulation of lens proteins



Specific Objectives

- Identify specific biomolecules that are important contributors to radiation exposure
- Determine molecular pathways involved in early changes to lens epithelial cells following radiation exposure
- Determine if we can identify threshold doses at which these early events occur

Experimental Approach: HLE cells were exposed to doses of 0, 0.01, 0.05, 0.25, 0.5, 2, and 5 Gy of X-ray radiation at two dose rates (1.62 cGy/min and 38.2 cGy/min). Cell culture lysates were collected 20 h post-exposure

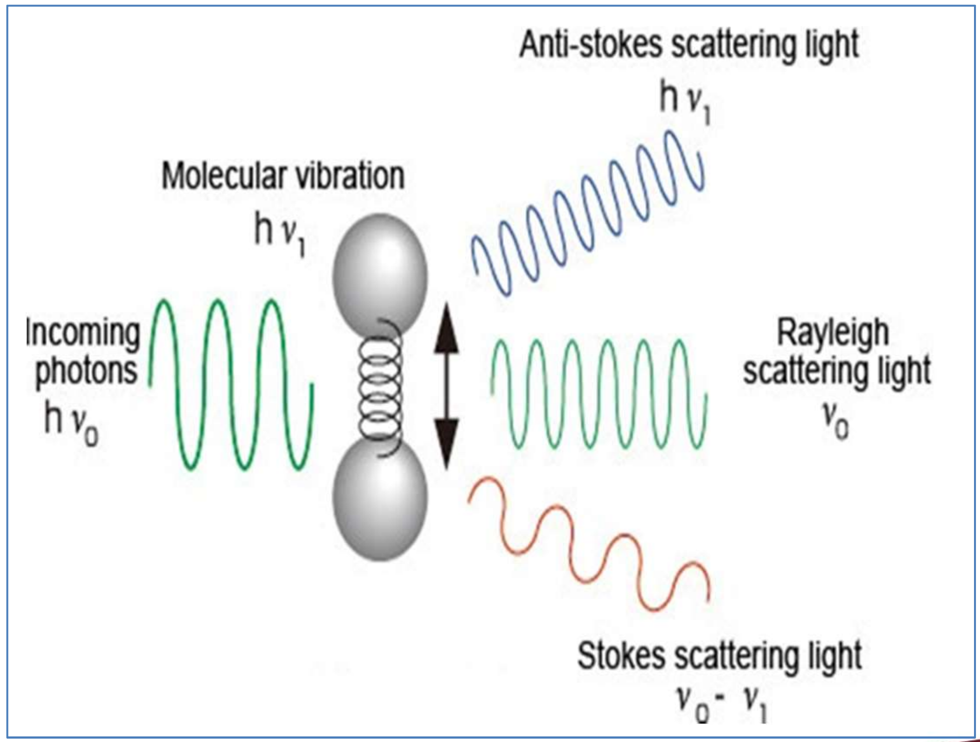


RAMAN SPECTROSCOPY

Collaborators Carleton University
Select slides provided by : Harry Allen and Nyiri Balazs

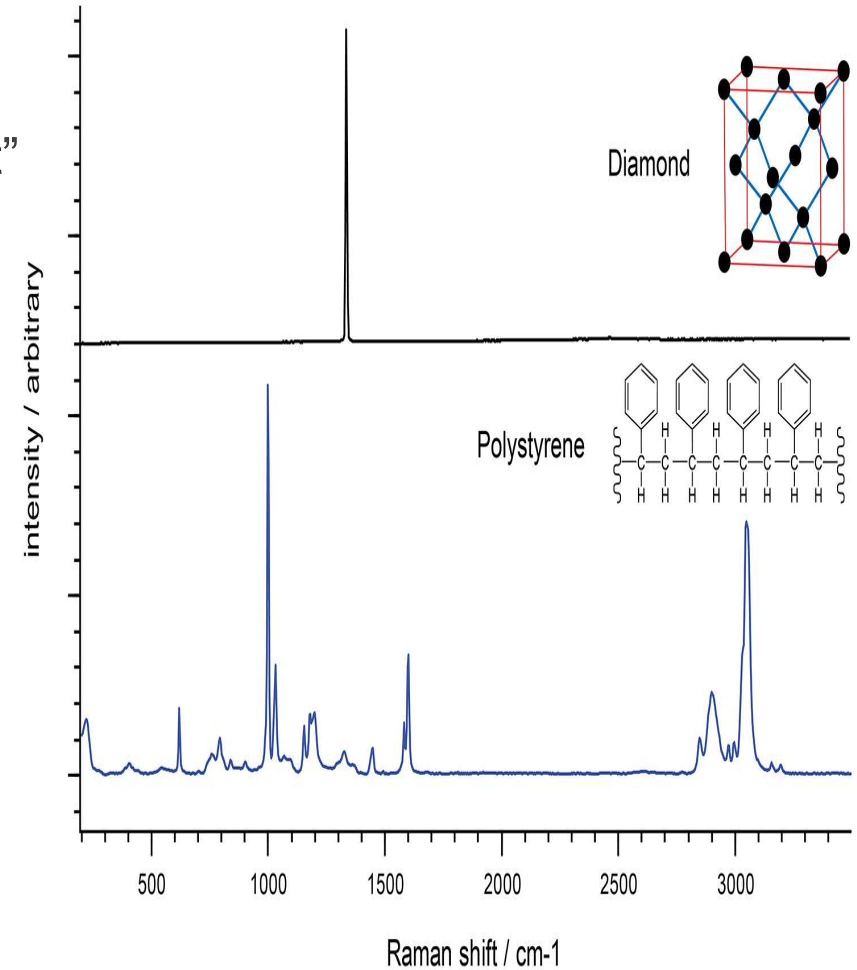
Advantages

- Can provide concurrent molecular composition of live cells/tissues including lipids proteins, DNA
- Minimal to no sample manipulation
- Non-invasive
- Low sample volume
- In-clinic capability



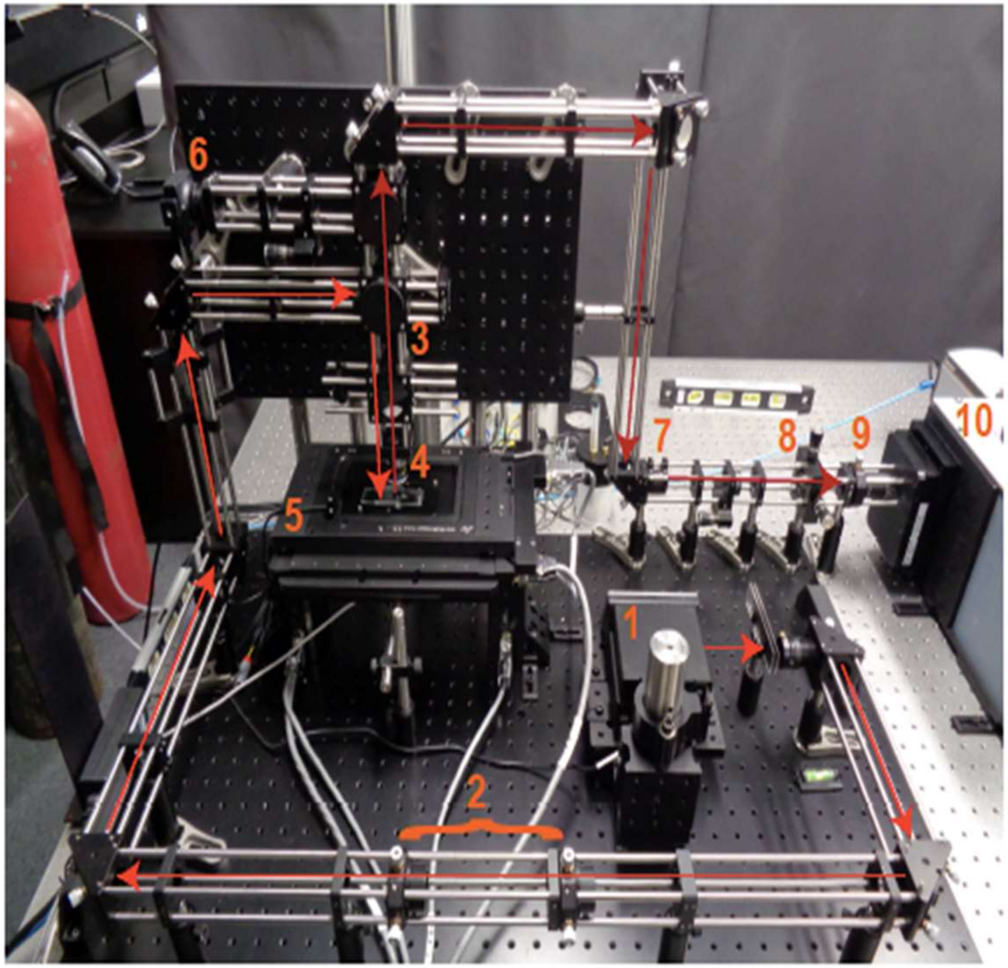
Fingerprint spectrum

- Laser light interacts with molecules in a sample
- Raman scattering generates a “fingerprint” spectrum which can be analysed
- Complexity of spectrum varies with the analyte

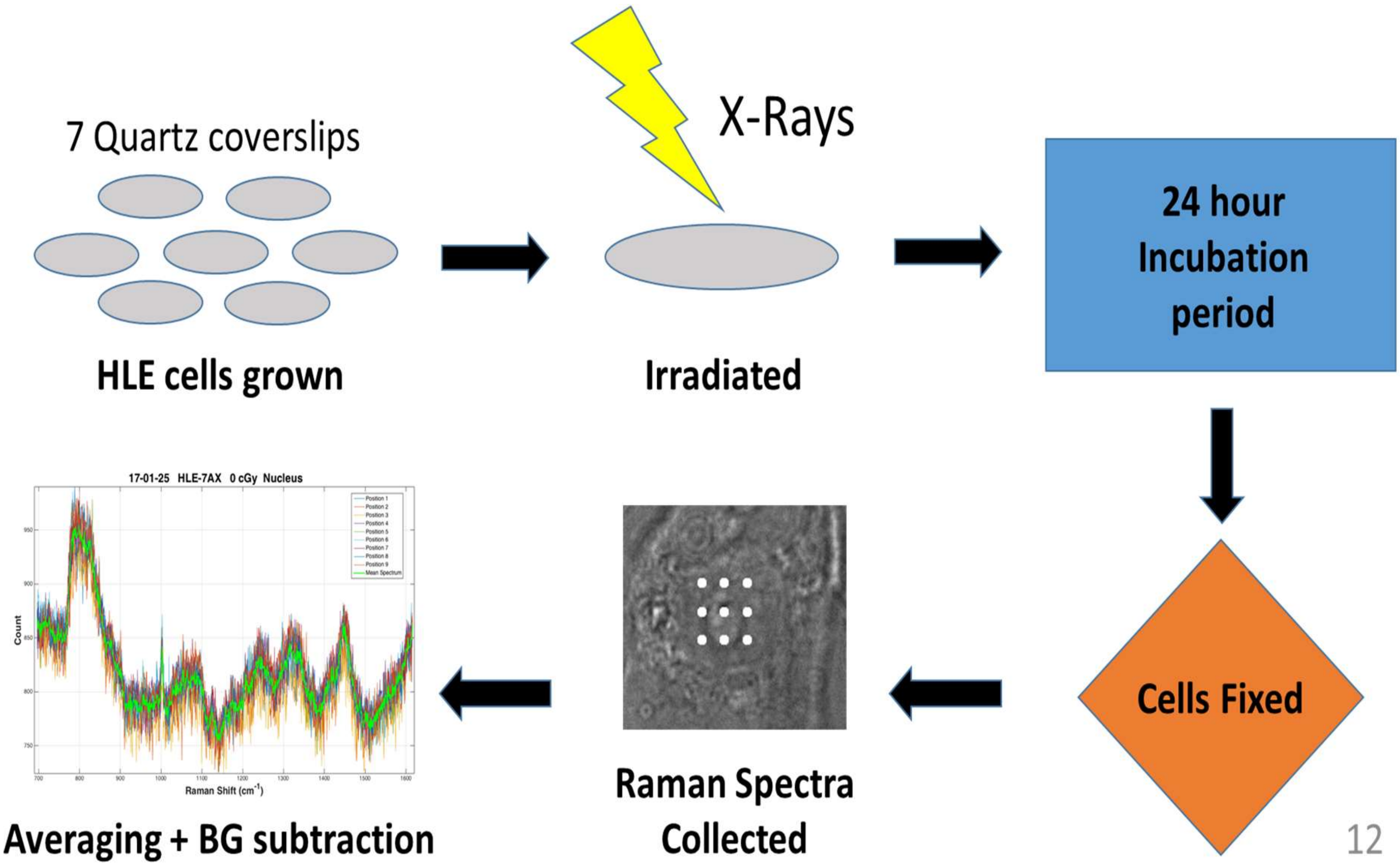


In House Built Confocal Raman Instrumentation

1. 785nm laser
2. Laser beam collimation
3. Dichroic mirror
4. Microscope Objective (60X)
5. Automated x-y-z stage
6. CCD camera
7. Laser rejection filter
8. Pinhole
9. Focusing optics
10. Spectrometer/CCD detector

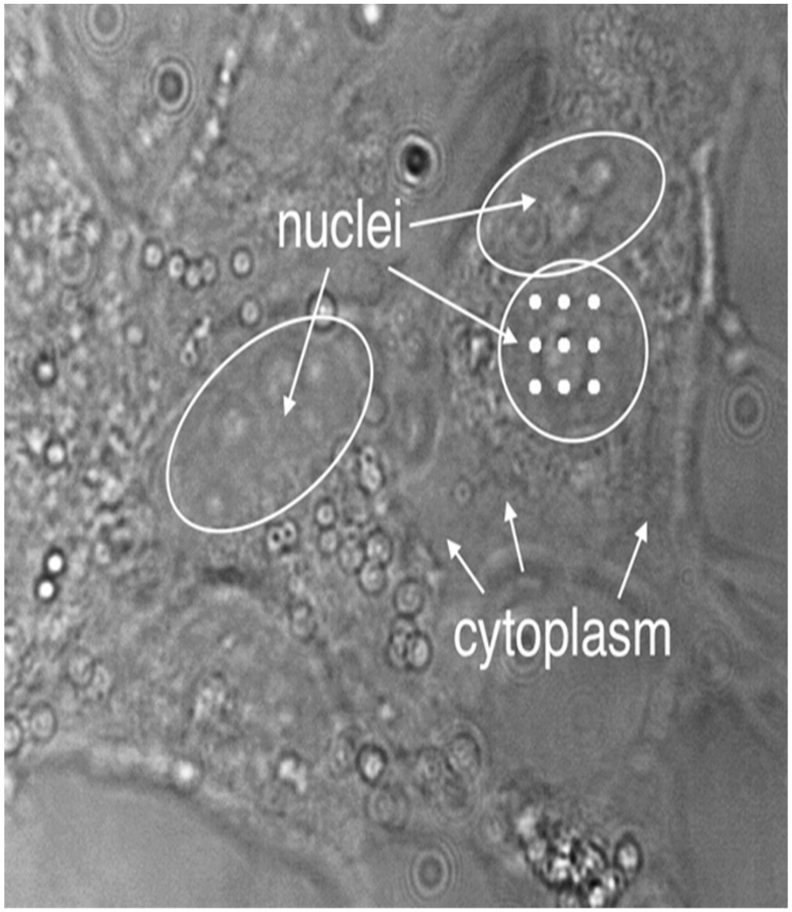


Experiment Overview



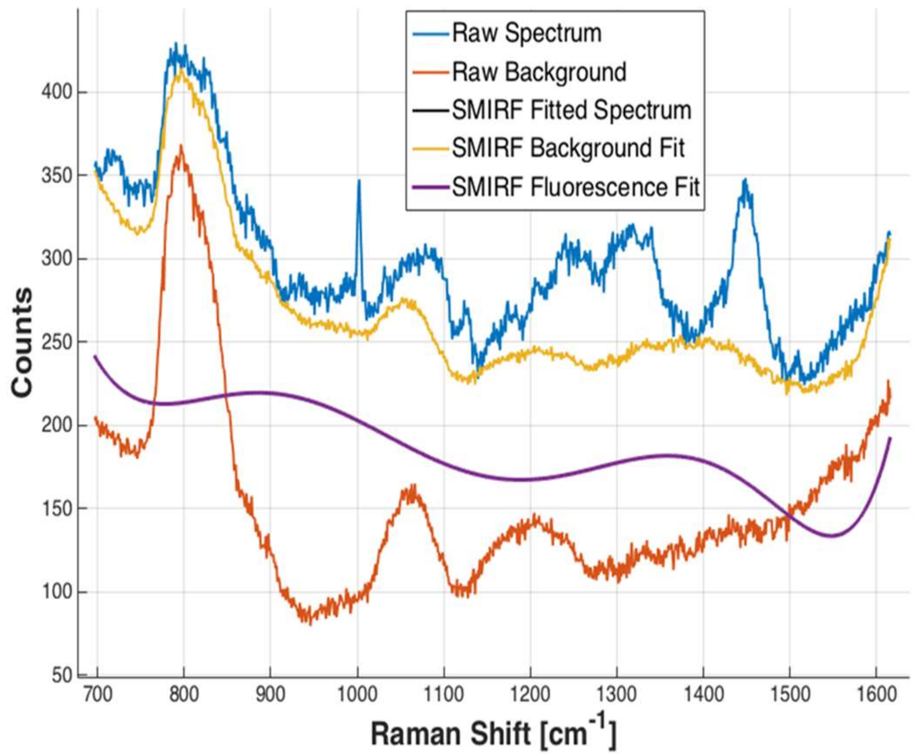
Spectral Acquisitions

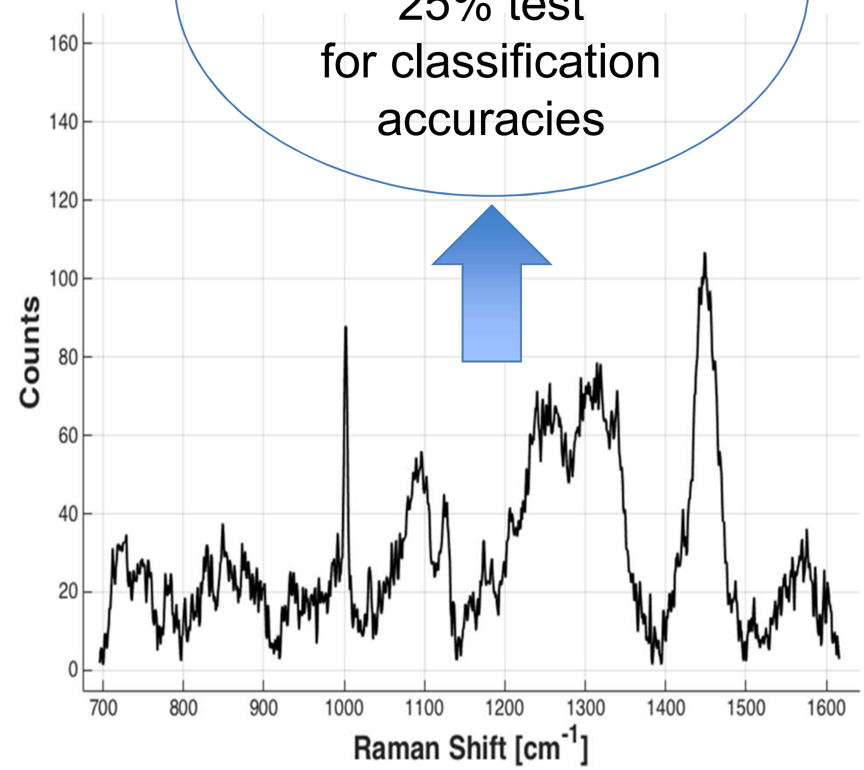
- 20 nucleus and 20 cytoplasm measurements were collected
- Each measurement is the average of 9 one minute spot measurements over a 3x3 grid
- Spectrum collected from 1 micron diameter, 3 micron deep volume



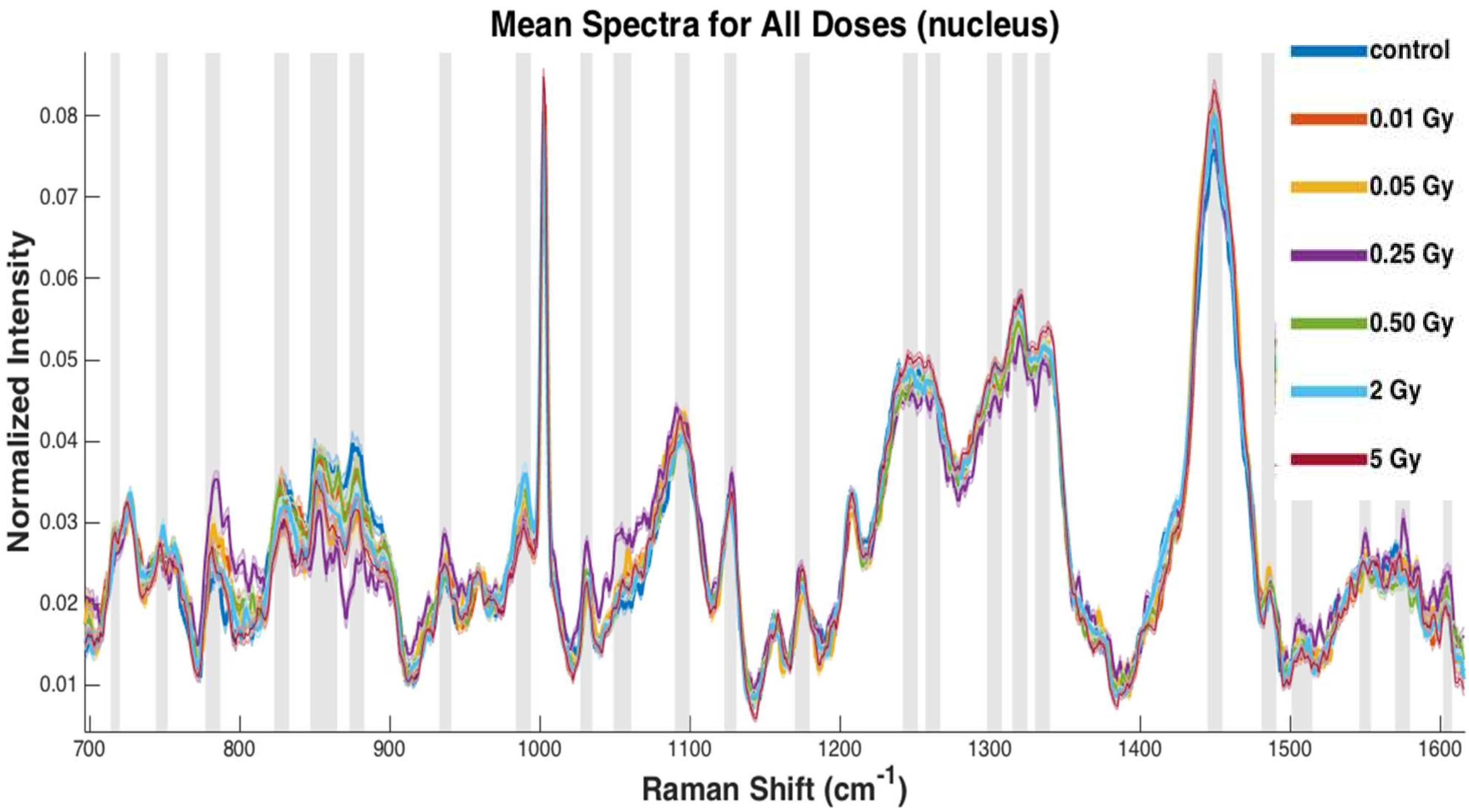
Processing Spectra

PCA-LDA model built using 75% of data and 25% test for classification accuracies



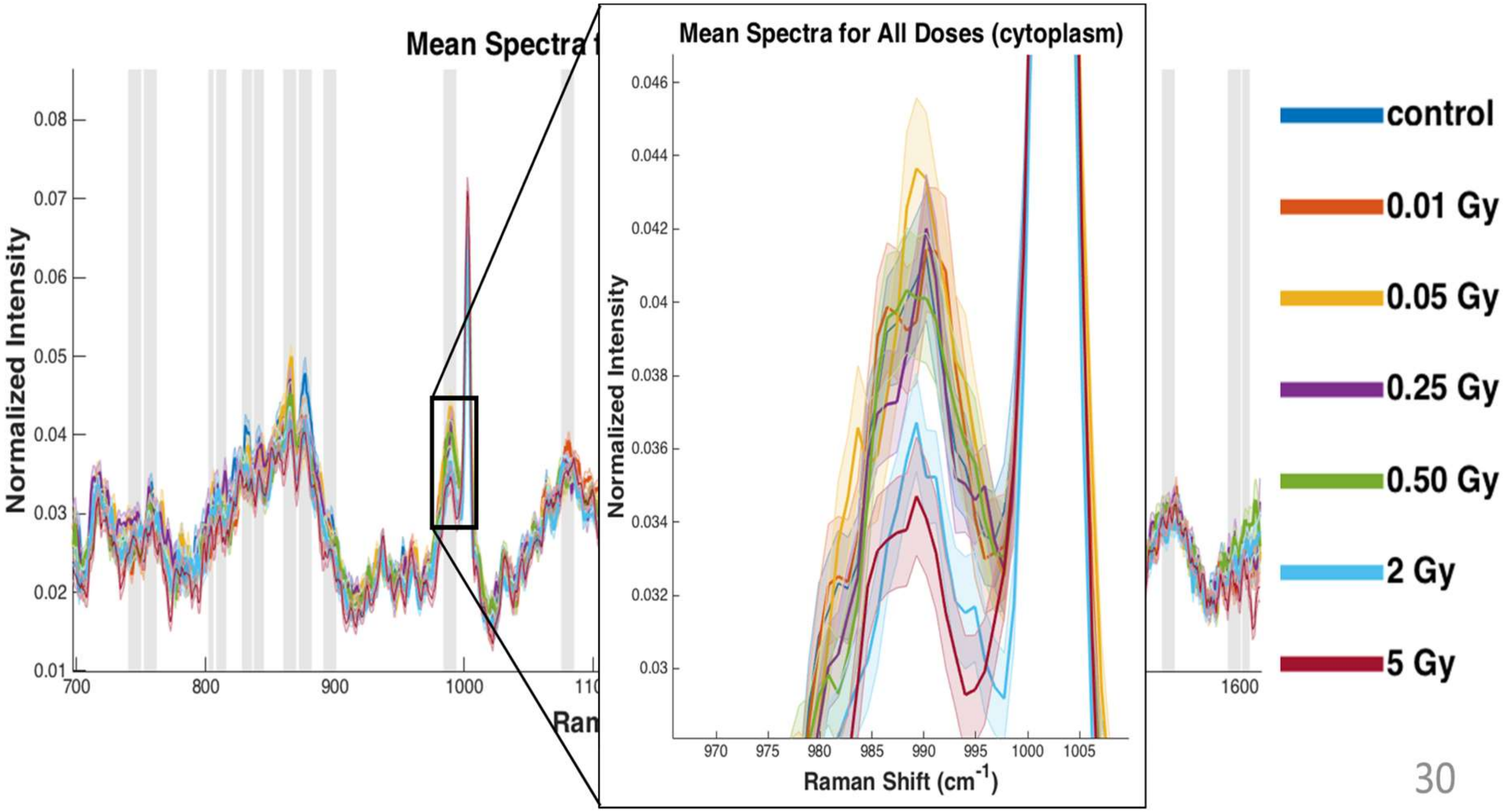


Nucleus



Mean Spectra for All Doses (nucleus)
Normalized Intensity
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
700
800
900
1000
1100
1200
1300
1400
1500
1600
Raman Shift (cm^{-1})
control
0.01 Gy
0.05 Gy
0.25 Gy
0.50 Gy
2 Gy
5 Gy

Cytoplasm

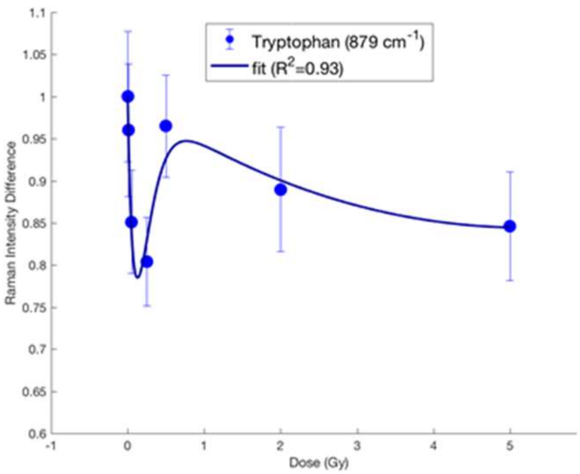


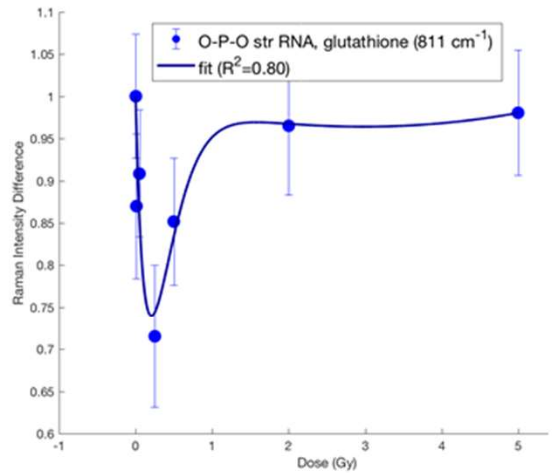
Allen et al., 2018

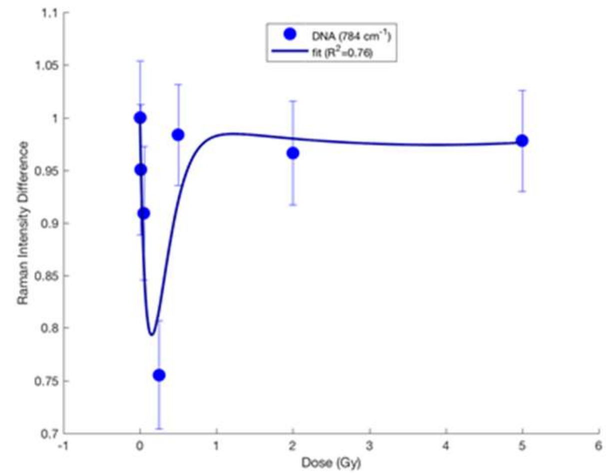
Biological Assignments

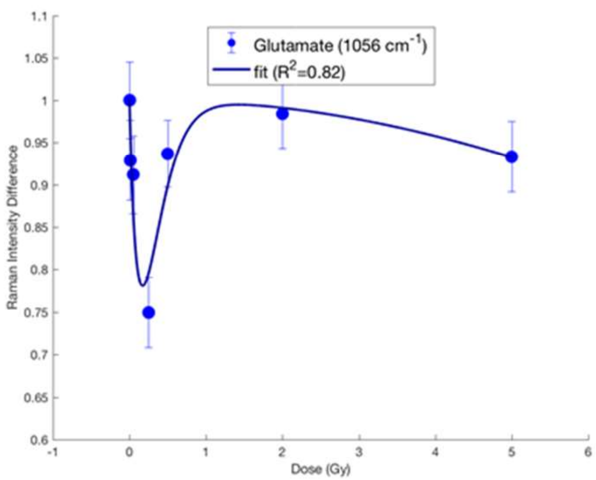
Raman Shift (cm^{-1})	Molecular Assignment	Dose (Gy)			
		0.25	0.5	2	5
	DNA				
782	U, C, T ring br; O-P-O str bk			↓	↓
1551	G	↑			
1577	A, G		↓		
	Proteins				
747	L-Phenylalanine			↑	↑
808	Glutamate	↑	↑	↑	↑
1156	C-C, C-N str			↑	
1204	Amide III; CH2-glycine & proline	↑			
1588	Glycine; Phenylalanine	↓			

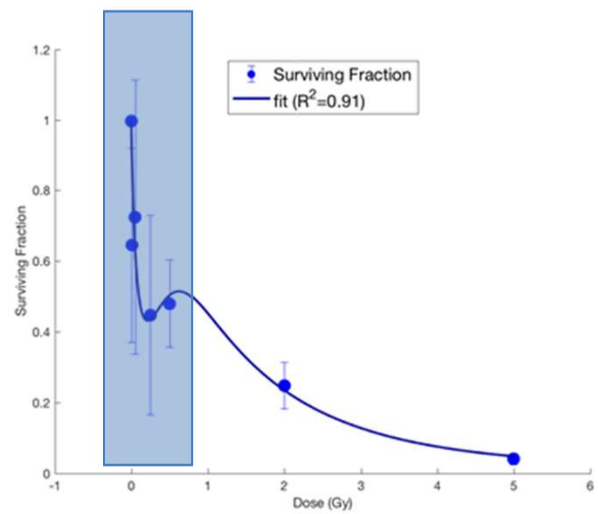
Nucleus – Raman Intensity Difference vs Dose



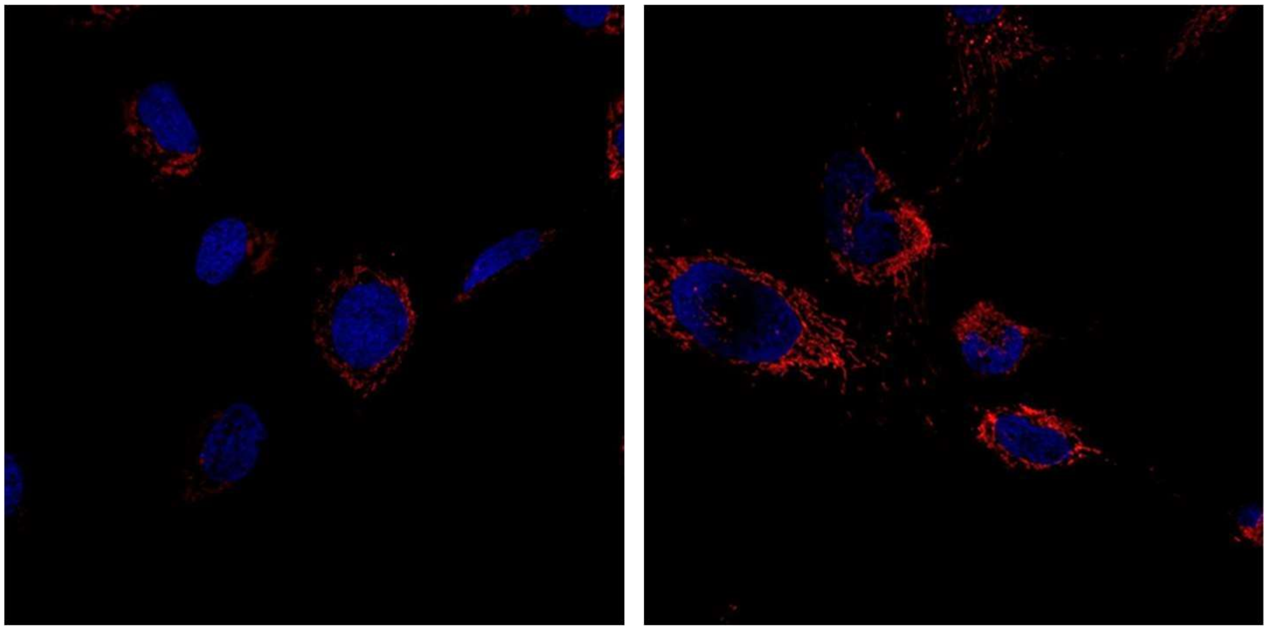






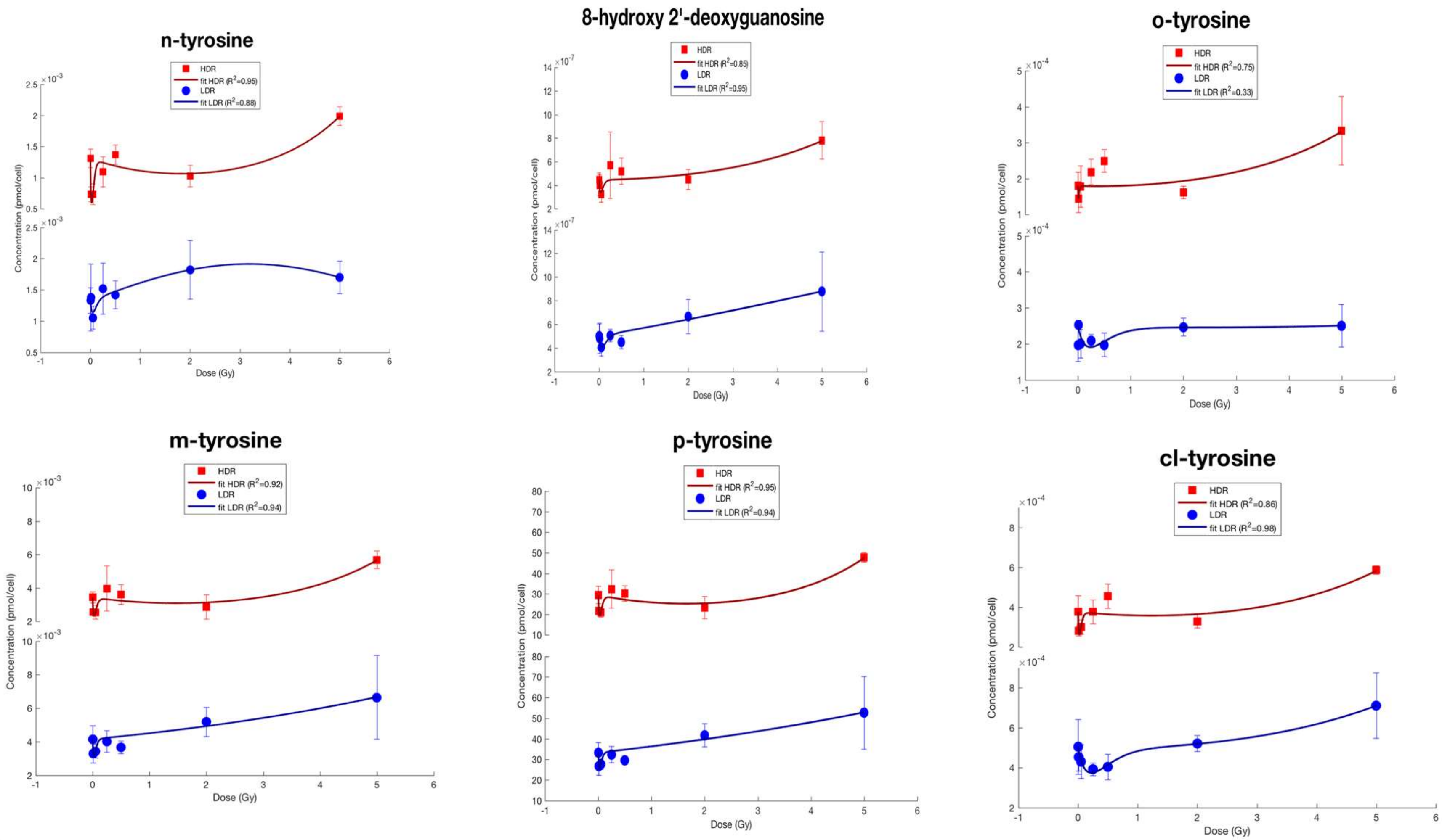


Fluorescent images of control and irradiated HLEs with Hoechst33342 nuclear and MitoProbe mitochondrial membrane potential gradient stain



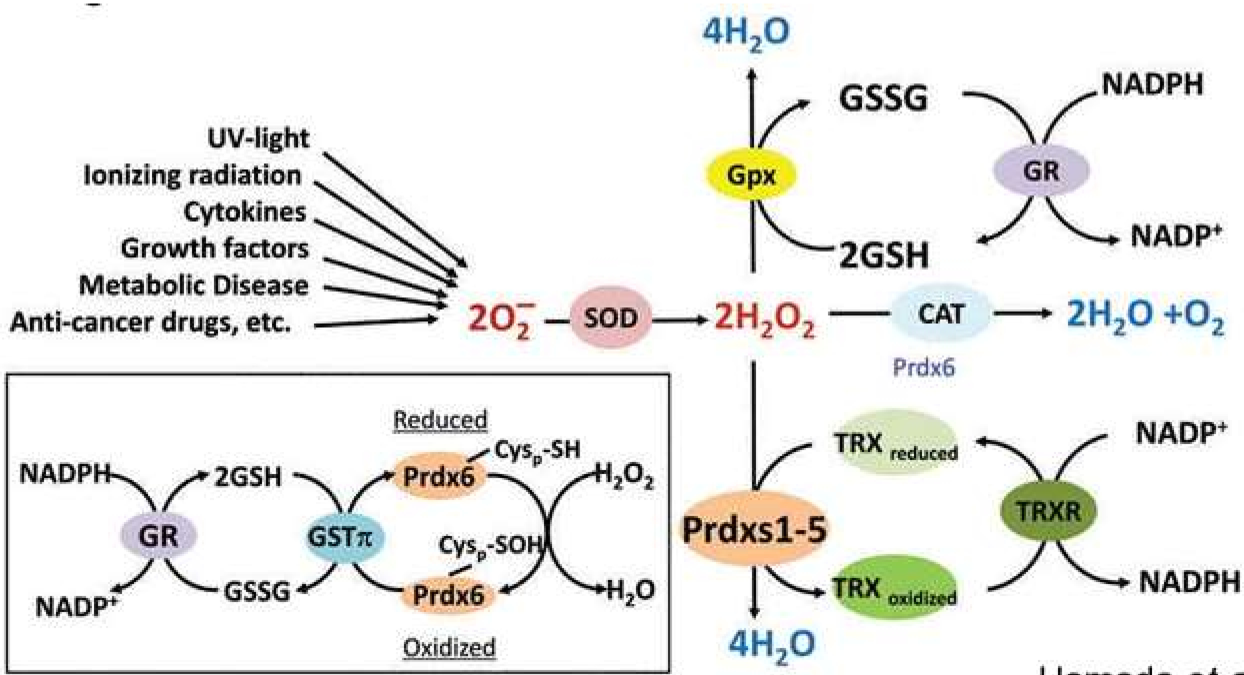
0 Gy   5 Gy

Reactive oxygen/nitrite species



Collaboration: Premkumari Kumarathasan
Bahia et al., 2018

Oxidative Stress



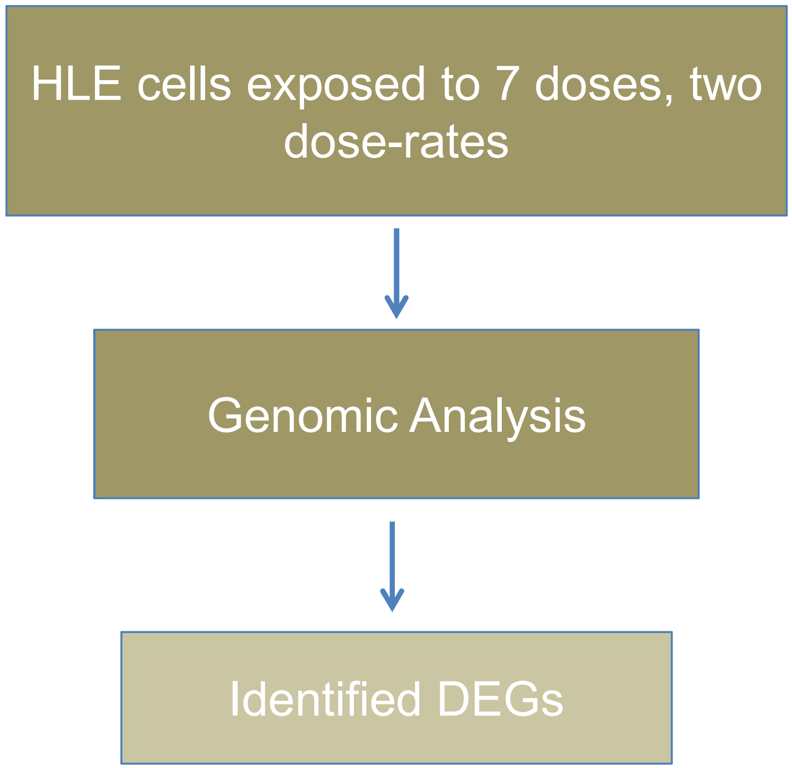
Hamada *et al.*, 2014

ROS leads to degradation, crosslinking and aggregation of lens proteins

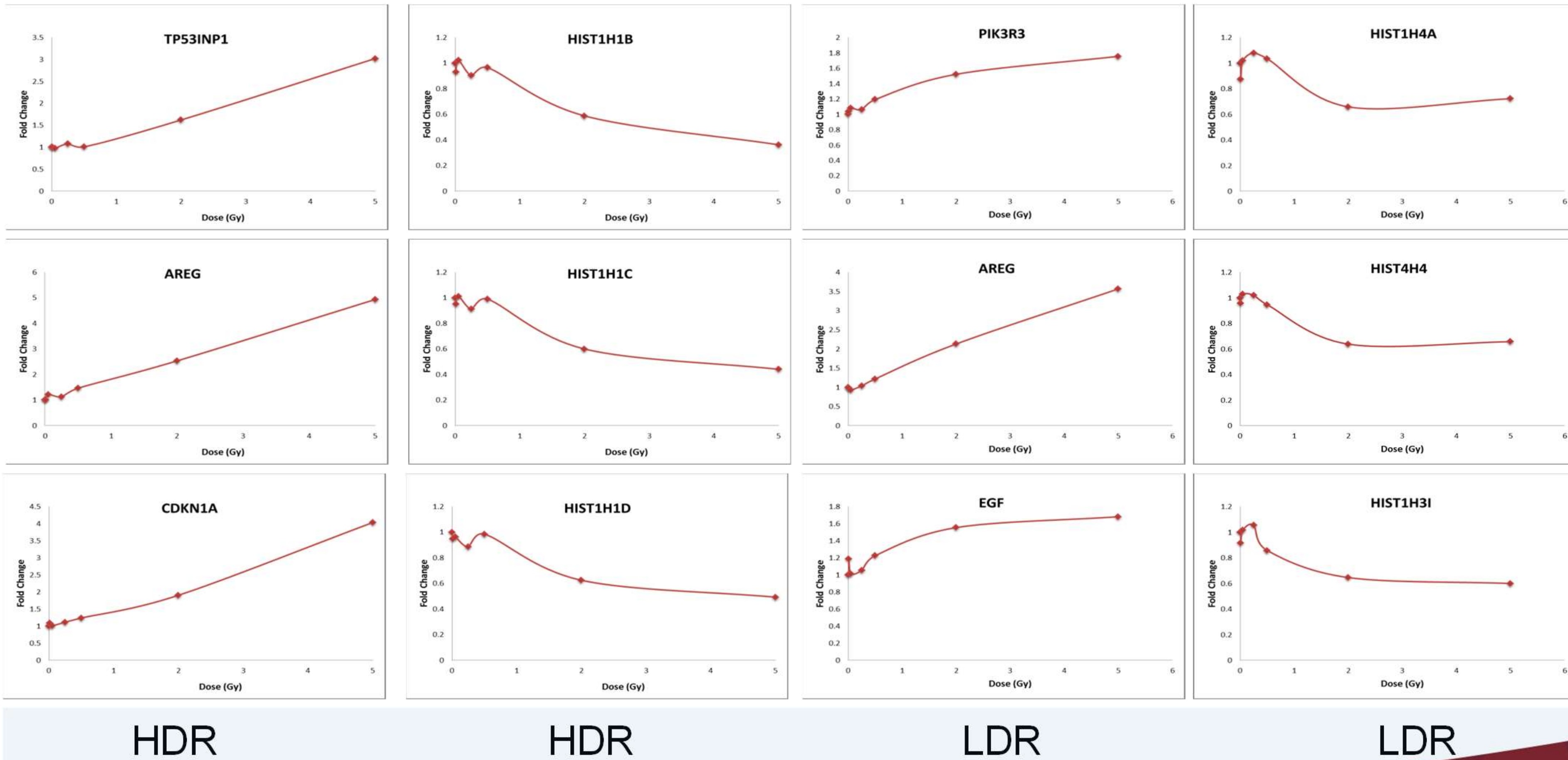
Genomics

Collaborators: Dr Carole Yauk, Health Canada

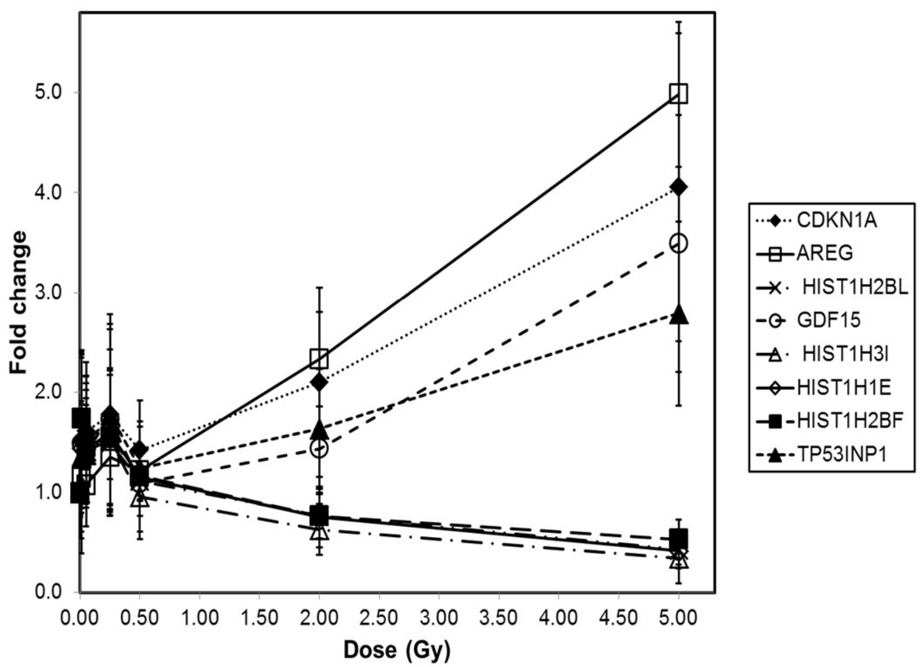
Simplified Workflow



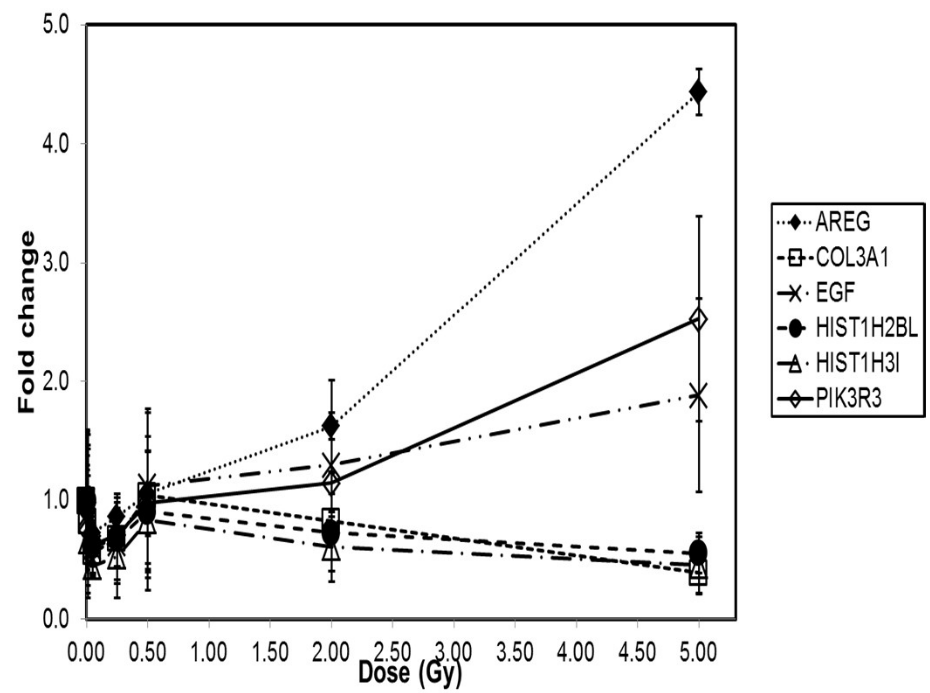
Representative plots of dose and fold change responses for LDR and HDR exposures for a select panel of genes that exhibited statistically significant responses in at least two doses ~1000 DEGs



PCR- pathways associated with low dose responses - amino acid degradation, enzyme inhibition, cell membrane signaling and oxidative stress burden



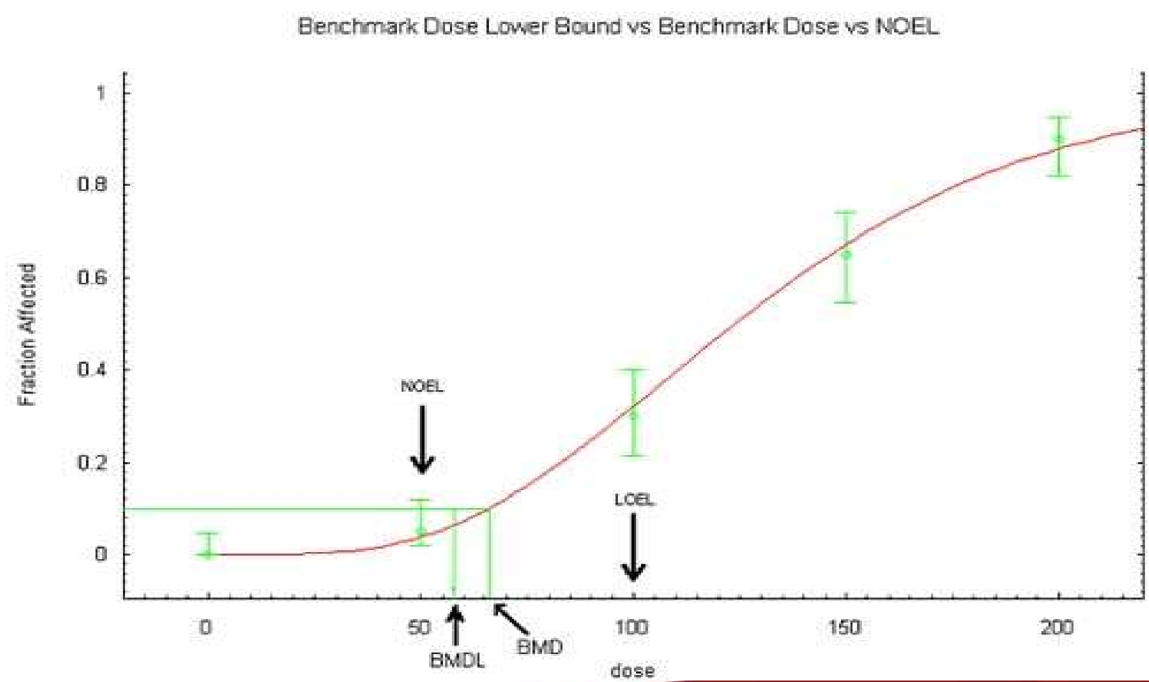
HDR



LDR

BMD modeling

- Method for analyzing dose-response data
- Method identifies the best-fit curve for the dose-response of each gene
- The dose that causes a defined response above the control is marked as the BMD
- Is now being applied to transcriptional data



A table summarizing the BMD responses across pathways and genes. It highlights differences between LDR and HDR exposures.

Exposure Type	Total # of Genes Modeled (#)	BMD Gene Median (Gy)	Minimum Gene BMD (Gy)	Total # of Pathways (#)	BMD Pathway Median (Gy)	Minimum Pathway BMD (Gy)
HDR	985	2.3	0.03	115	**1.43**	0.6
LDR	673	1.86	0.03	17	**2.9**	2.5

HDR pathway and BMD values. HDR exposures induced pathways involved in mitosis, DNA repair, cell cycle arrest, and chromatin reorganization and were enriched in the histone variant genes

GO/Pathway/Gene Set Name	Input Genes	P-value	Percentage	BMD Median
Apoptosis induced DNA fragmentation	5	0.00	38	0.62
Activation of DNA fragmentation factor	5	0.00	38	0.62
Formation of Senescence-Associated Heterochromatin Foci (SAHF)	5	0.00	31	0.62
Protein ubiquitination	12	0.00	16	0.90
E3 ubiquitin ligases ubiquitinate target proteins	12	0.00	21	0.90
Adherens junctions interactions	7	0.00	21	0.92
Ligand-dependent caspase activation	3	0.04	18	0.95
TP53 Regulates Transcription of Death Receptors and Ligands	3	0.02	25	0.95
Constitutive Signaling by AKT1 E17K in Cancer	5	0.01	20	1.04
Ub-specific processing proteases	31	0.00	14	1.04
UCH proteinases	11	0.01	11	1.05
Deubiquitination	33	0.00	11	1.05
SUMOylation	13	0.01	10	1.11
SUMO E3 ligases SUMOylate target proteins	13	0.01	10	1.11
Cellular Senescence	47	0.00	24	1.11
Recruitment and ATM-mediated phosphorylation	23	0.00	30	1.12
Nonhomologous End-Joining (NHEJ)	23	0.00	33	1.12
DNA Double Strand Break Response	23	0.00	30	1.12

LDR pathways and BMD values. LDR induced pathways associated with extracellular matrix responses, cell motility, and collagen assembly and biosynthesis

GO/Pathway/Gene Set Name	Input Genes	P-value	Percentage	BMD Median
Antagonism of Activin by Follistatin	3	0.01	50	2.52
Collagen chain trimerization	14	0.00	18	2.78
Collagen degradation	17	0.01	13	2.78
Collagen biosynthesis and modifying enzymes	18	0.00	15	2.78
MET activates PTK2 signaling	9	0.00	23	2.82
MET promotes cell motility	9	0.00	17	2.82
ECM proteoglycans	18	0.00	16	2.91
Assembly of collagen fibrils and other multimeric structures	18	0.00	16	2.93
Collagen formation	20	0.00	13	2.93
Non-integrin membrane-ECM interactions	17	0.00	19	2.99
Extracellular matrix organization	53	0.00	9	3.03
Integrin cell surface interactions	20	0.00	14	3.05
Syndecan interactions	13	0.00	33	3.06
O-glycosylation of TSR domain-containing proteins	11	0.00	18	3.13
Defective B3GALTL causes Peters-plus syndrome (PpS)	11	0.00	19	3.13
Diseases associated with O-glycosylation of proteins	13	0.03	11	3.13
Crosslinking of collagen fibrils	7	0.04	17	3.78

Conclusions

- Radiation induces complex non-linear biphasic response
- Bio-molecules related to oxidative stress are an important component to radiation-induced damage
- HDR exposures induce pathways involved in cell apoptosis, DNA damage response, cell signaling, and chromatin reorganization related to histone genes
- The median BMD values were 1.4 Gy, but specific pathways were being activated at 0.6 Gy
- The LDR exposures exhibited pathway responses with much higher BMD values (~ 3 Gy) and were centered on extracellular matrix reorganization and collagen biosynthesis/degradation
- Genomic data suggests that the minimal threshold dose for pathway activation is 0.6 Gy for high dose rate exposures and 2.5 Gy for low dose rate exposures

Acknowledgments

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Michelle Vandaloo

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Hamid Moradi

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ICRP

- New threshold of 0.5 Gy was independent of the rate of dose delivery and severity of opacification

- Assuming that exposure to 0.5 Gy of low linear energy transfer (LET) radiation induces 0.5 Gy for single, acute exposure (mainly predicated on A-bomb data)

- A threshold not higher than 0.5 Gy for fractionated/ protracted exposures predicated on Chernobyl data (maximum likelihood central estimates for a threshold ranging from 0.34–0.50 Gy)

- A threshold uncertain for chronic exposures

- Due to lack of evidence, ICRP did not draw any firm conclusions on dose rate effects