Detection of radiation induced changes in human lens epithelial cells using Raman spectroscopy

Harry Allen

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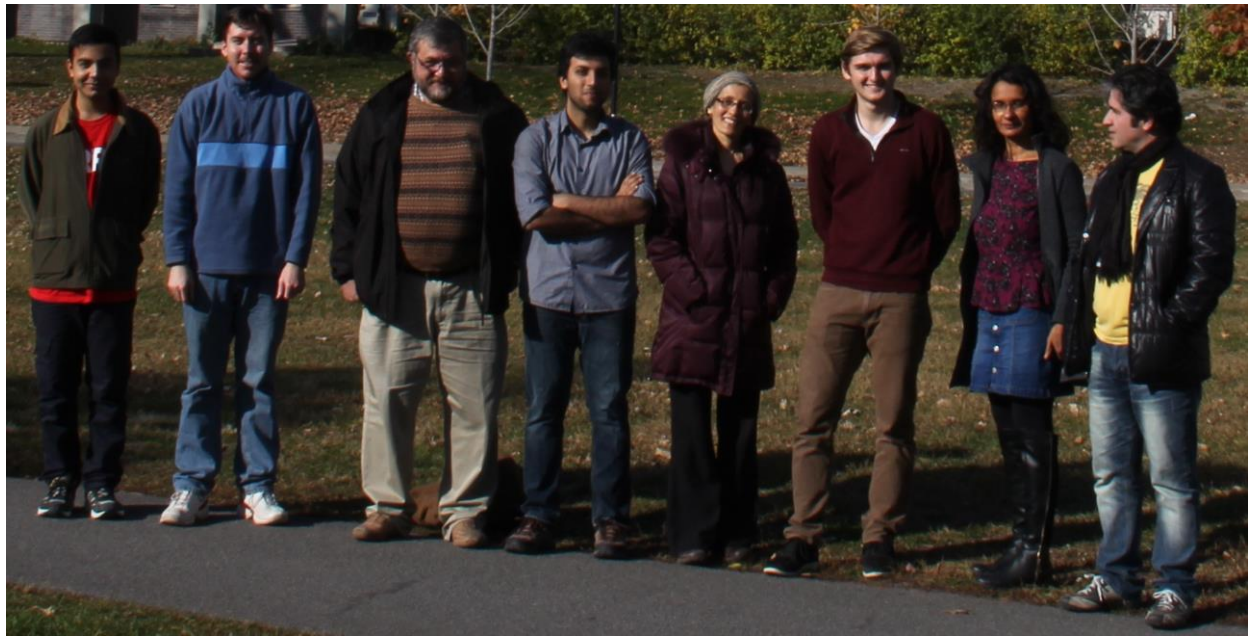


Carleton Biophotonics Research Group

Health Canada Santé Canada

Thank you!

The Ottawa Hospital | L'Hôpital d'Ottawa

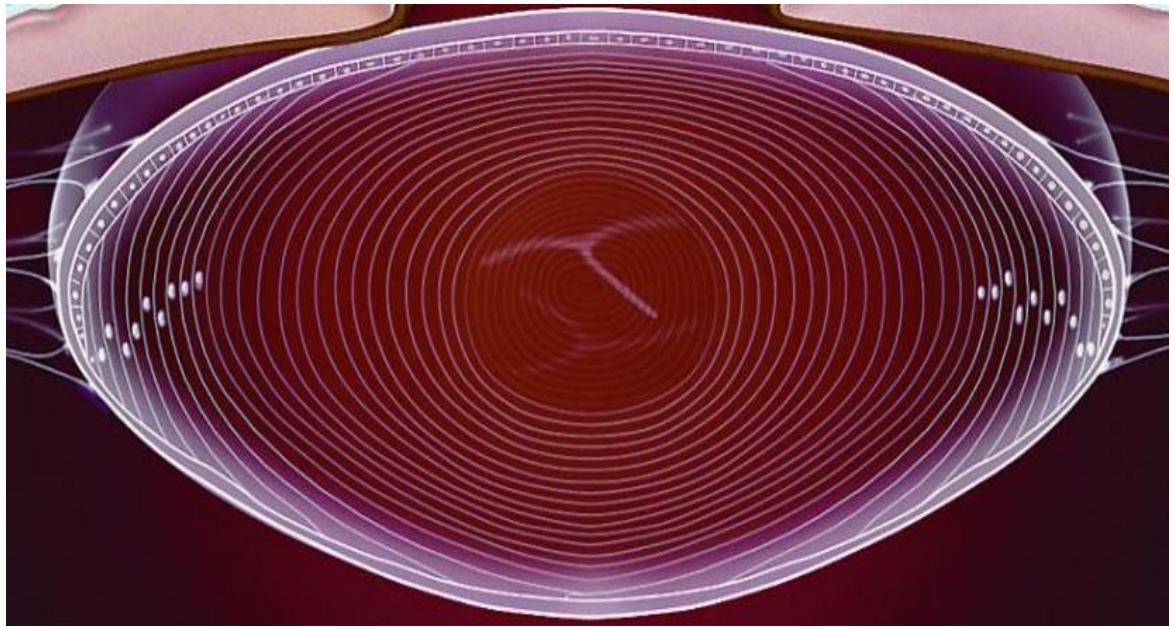
Outline

- Rationale
- Objectives
- Raman Scattering
- Raman Spectroscopy
- Procedure
- Analysis Techniques
- Results
- Conclusions

Rationale

- Lens is radiosensitive
- IR induces cataracts
- Threshold dose was thought to be 2 Gy for detectable opacities
- Recent epidemiological studies suggest 1 Gy threshold for low LET IR.
- ICRP currently suggests a nominal threshold of 0.5 Gy
- Increase in reported injuries due to new diagnostic procedures such as fluoroscopy

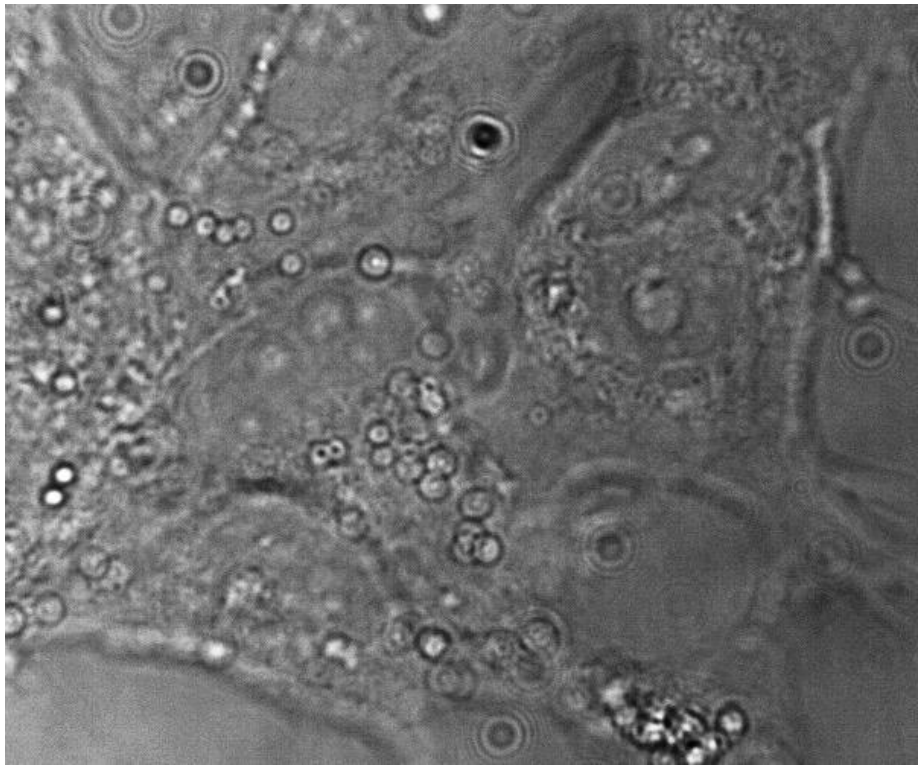
- Need for sensitive assays capable of detecting minute biological changes due to low dose IR exposure



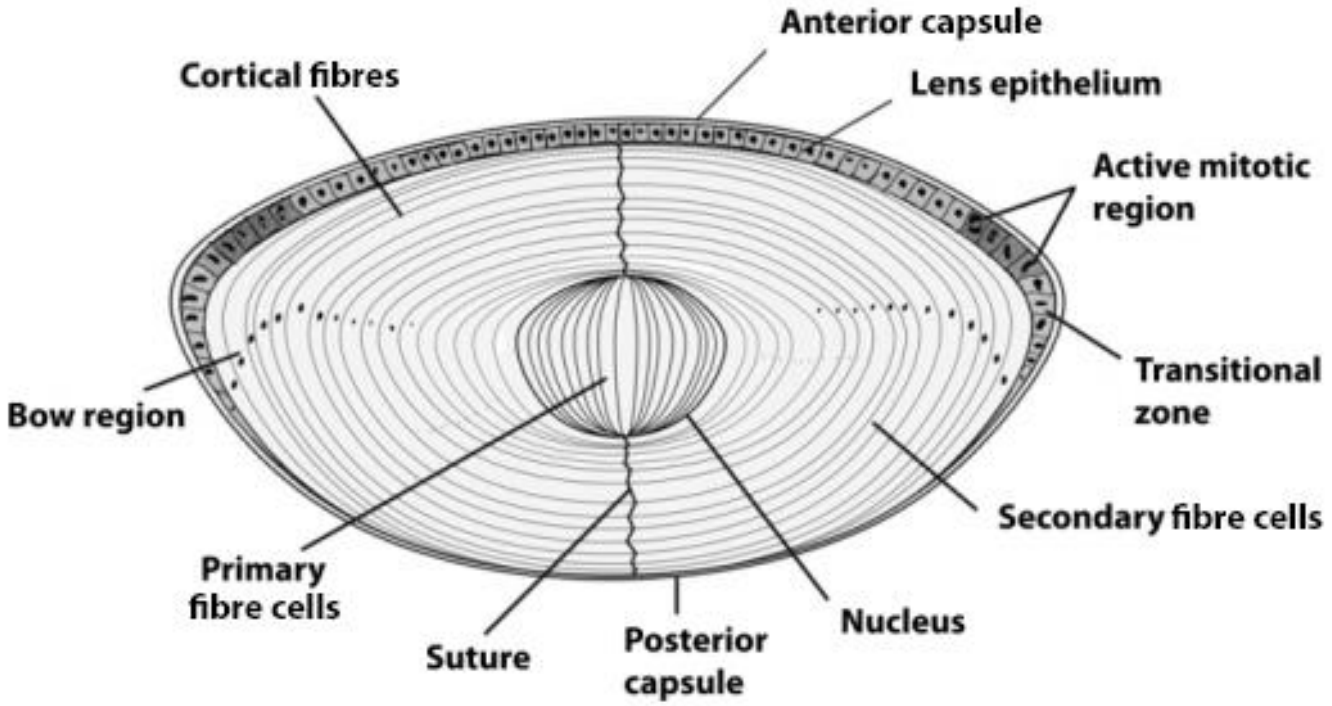
http://www.aao.org

Human Lens Epithelial Cells

- HLE Cells maintain homeostasis of the lens



- Differentiate into lens fiber cells



http://www.abc.net.au/science/drkarl/img/4253961/lens.jpg

Objectives

- Determine whether IR induced changes in Raman spectra of HLE cells are detectable over a range of doses
- Analyse those differences, if present, to determine which biomolecules are changing in concentration
- Determine whether we can discriminate between Raman spectra by dose using multivariate statistical techniques

Doses investigated:

- **0.01 Gy**
- **0.05 Gy**
- **0.25 Gy**

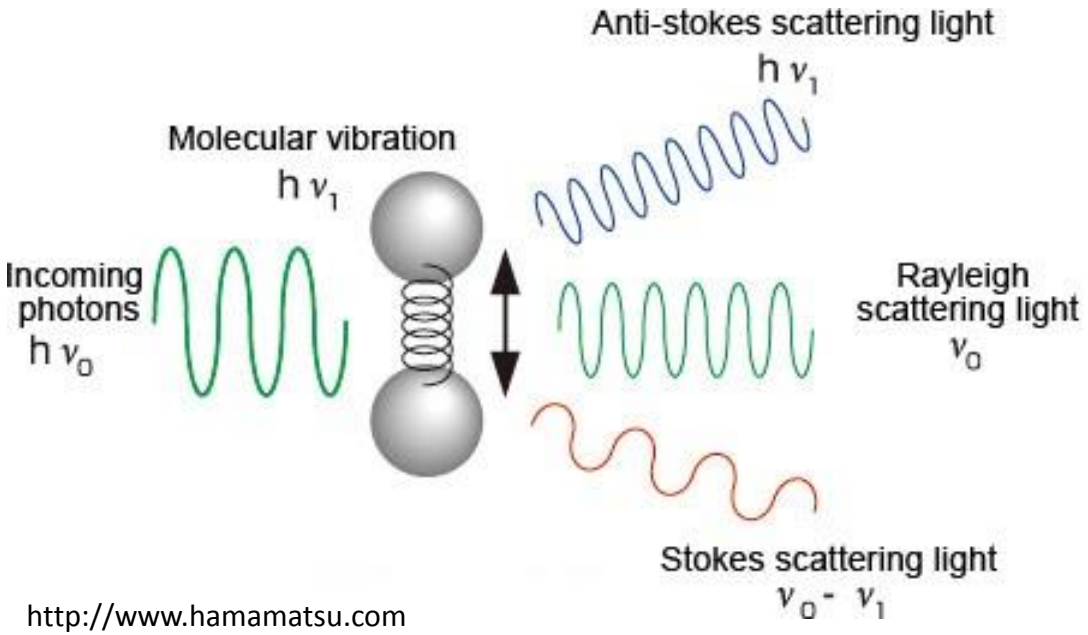
} Low dose range

- **0.5 Gy**
- **2 Gy**
- **5 Gy**

Raman Scattering

- Inelastic scattering of light
- Photon either loses (Stokes) or gains (anti-Stokes) energy
- Molecule reciprocally gains or loses the same amount in vibrational energy
- The difference in energy gives a "Raman Shift" wavenumber

$$\Delta\tilde{\nu} = \frac{1}{\lambda_i} - \frac{1}{\lambda_s} = \frac{E_i - E_s}{hc}$$

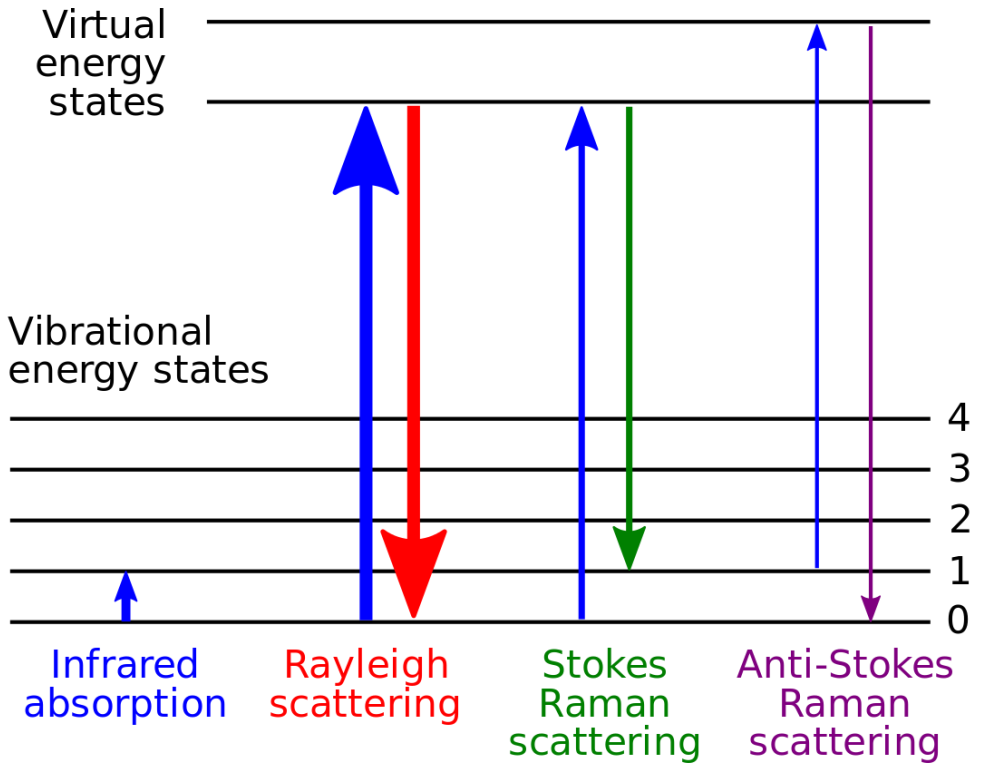


http://www.hamamatsu.com

The probability of a spontaneous Stokes Raman scatter is ~10^{-7}

Raman Scattering

- Quantum nature of a molecule limits its allowed vibrational frequencies to a discrete set characteristic of that molecule
- Raman shift wavenumbers are thus characteristic of the scattering molecule
- Raman scattering thus generates a “fingerprint” spectrum which can be analysed



https://en.wikipedia.org/wiki/Raman_spectroscopy

Raman Scattering

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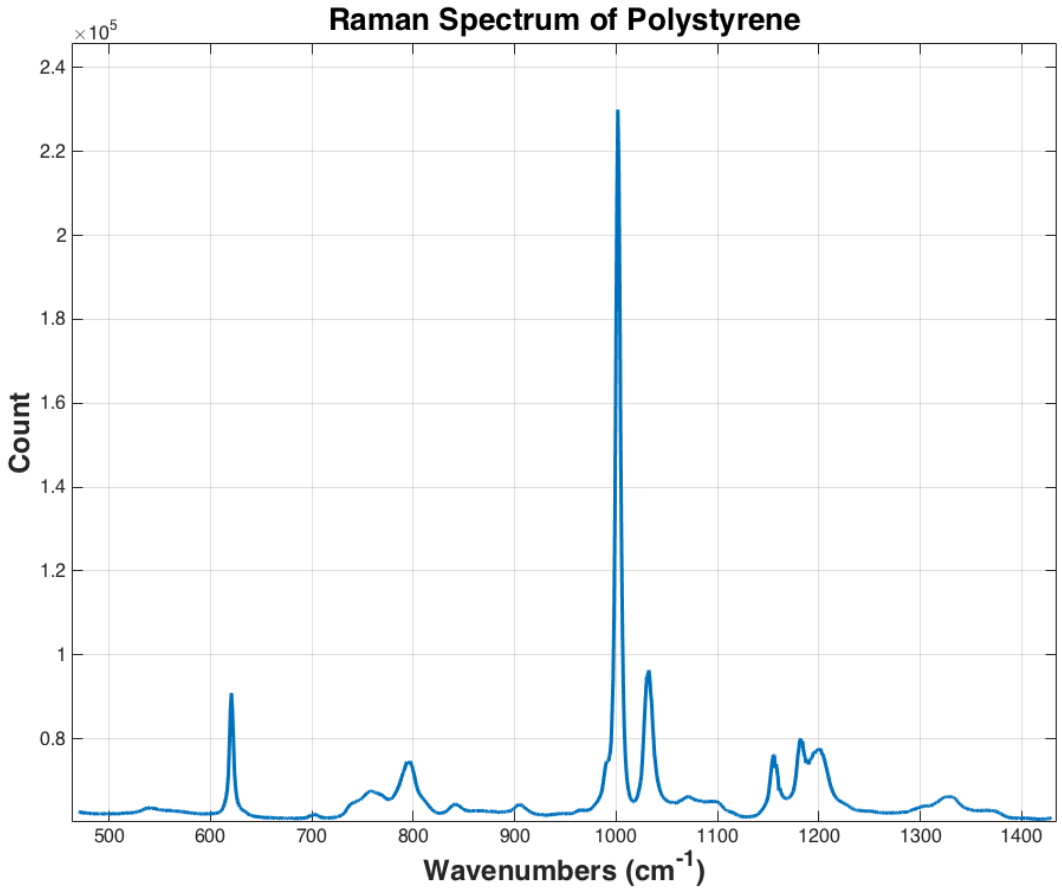
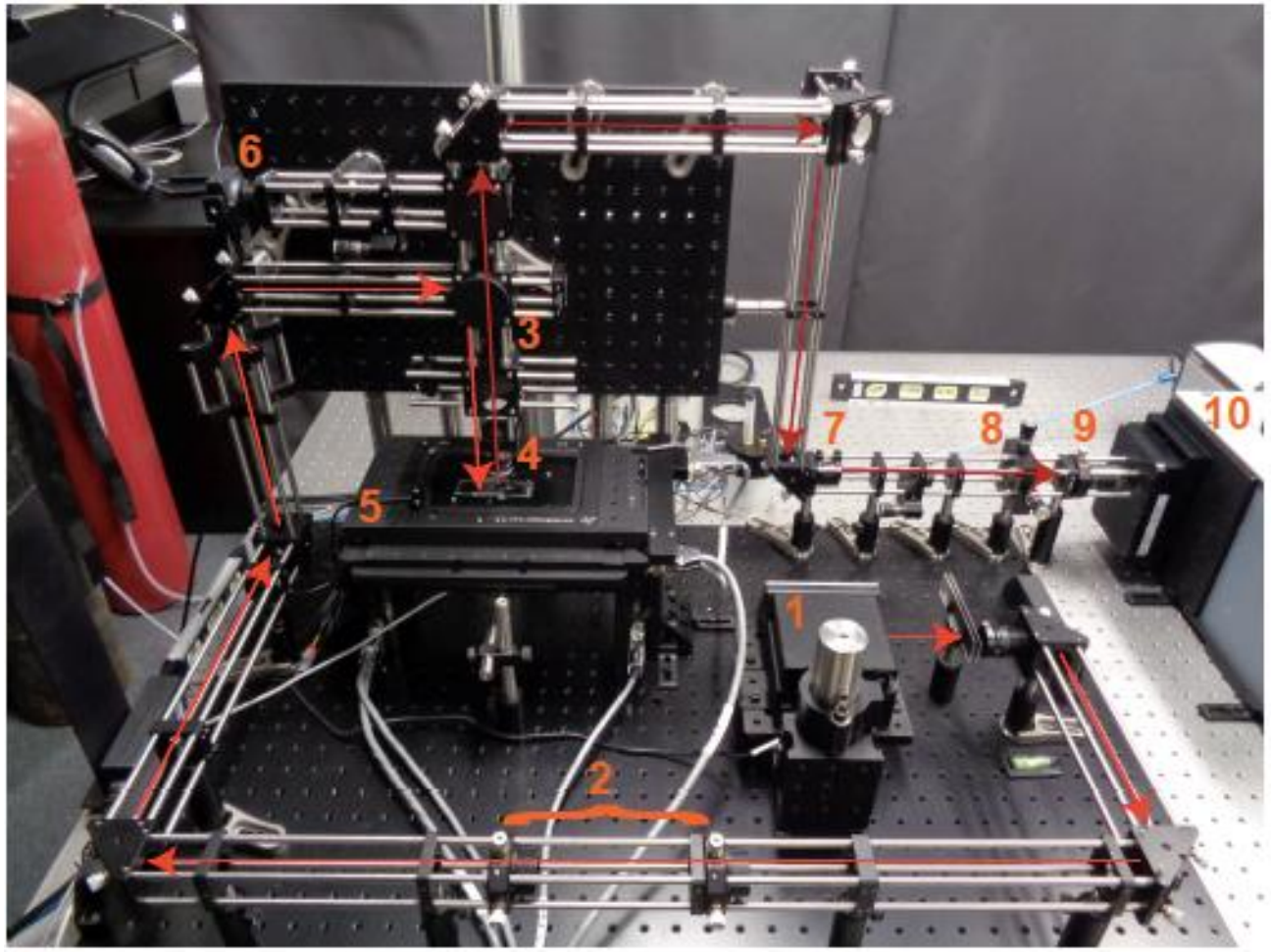
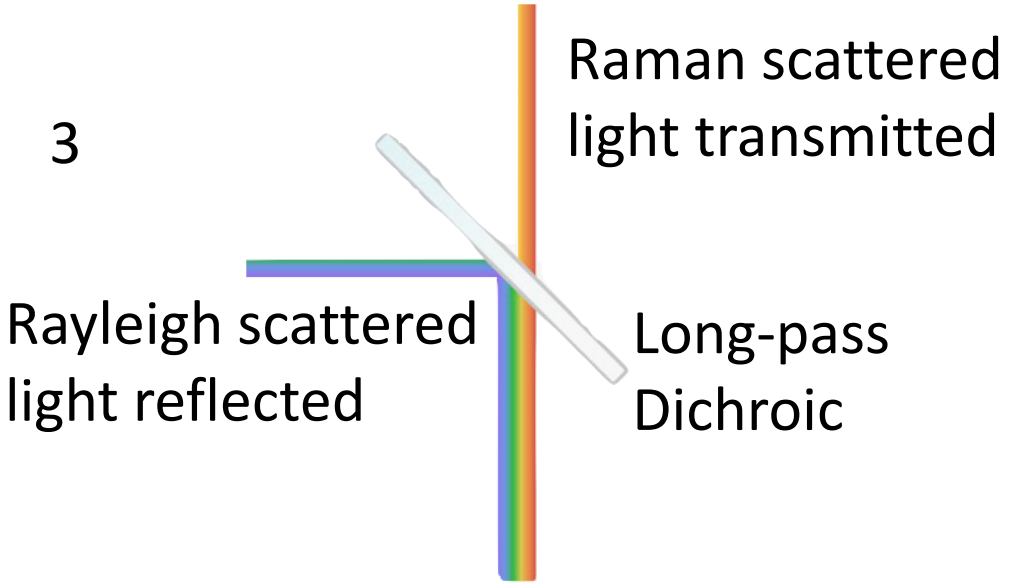
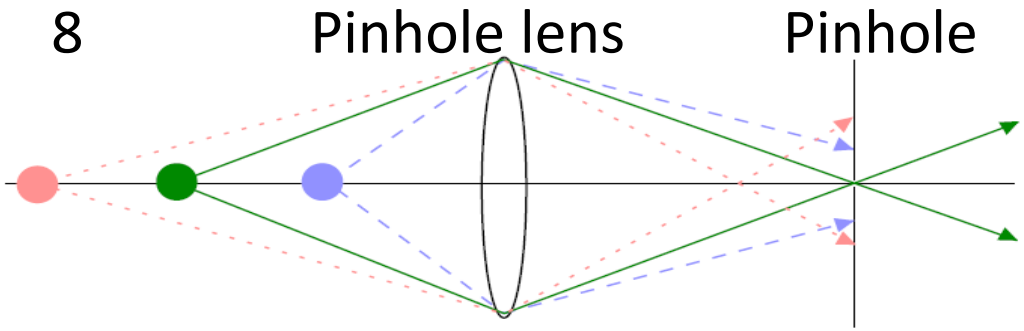


Confocal Raman Microspectroscopy





http://microscopy.duke.edu/learn/introtomicroscopy/confocals.html

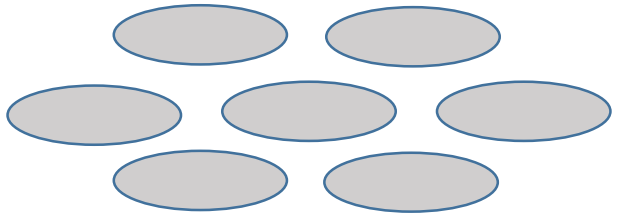


Procedure

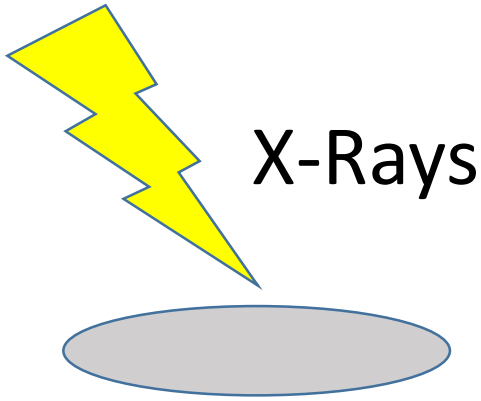
How the data was acquired

Procedure

7 Quartz coverslips



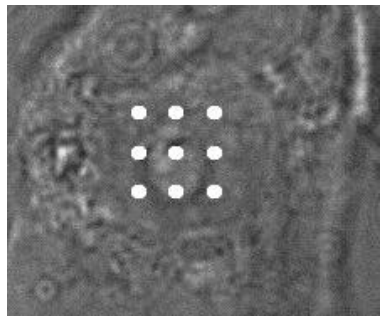
HLE cells grown



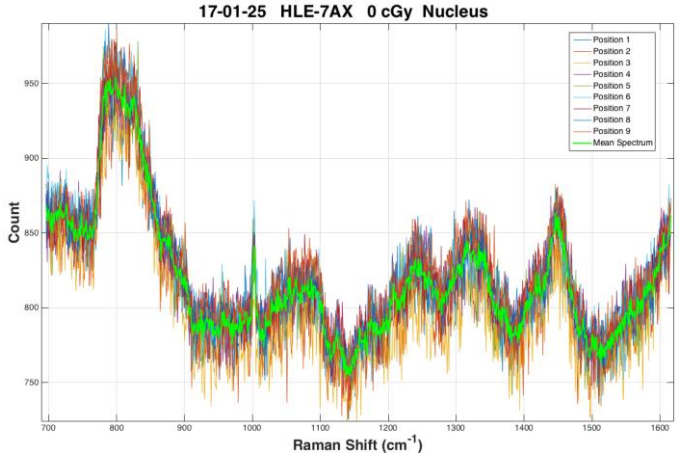
Irradiated

24 hour Incubation period

Cells Fixed



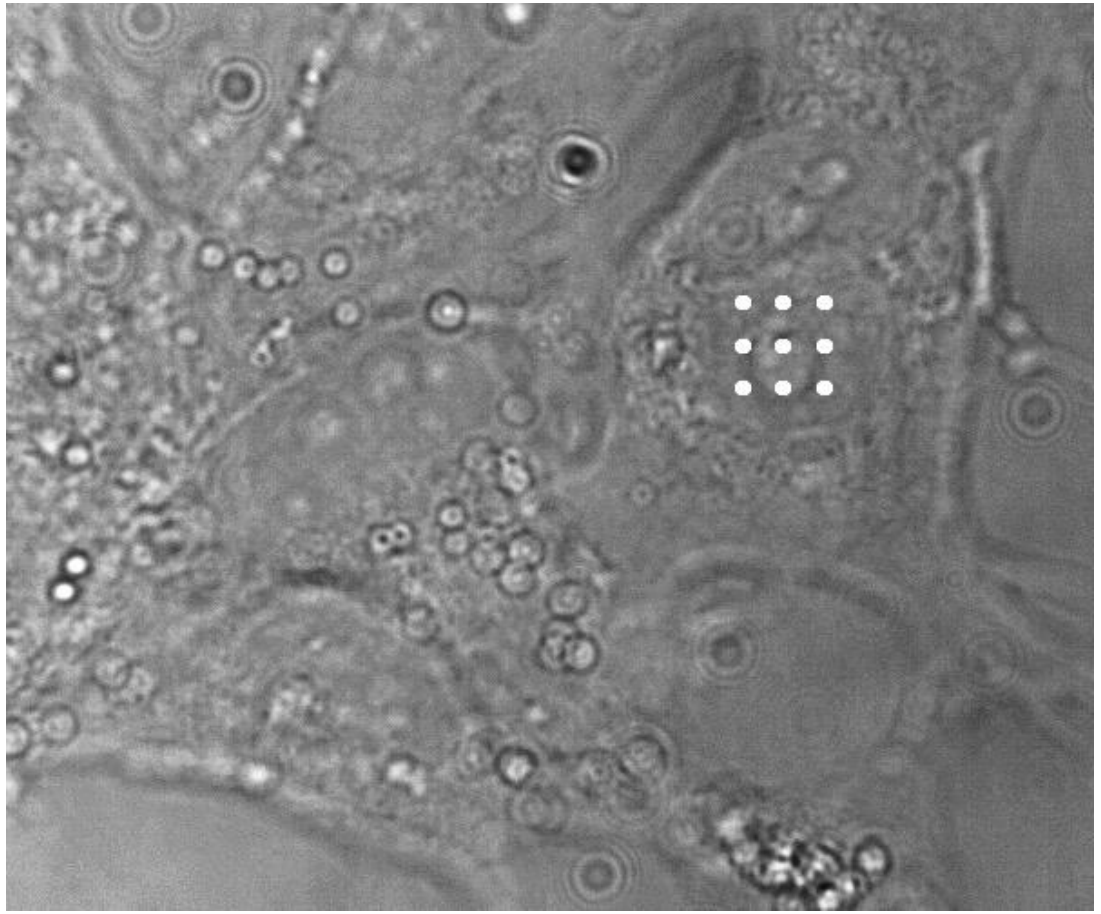
Raman Spectra Collected



Averaging + BG subtraction

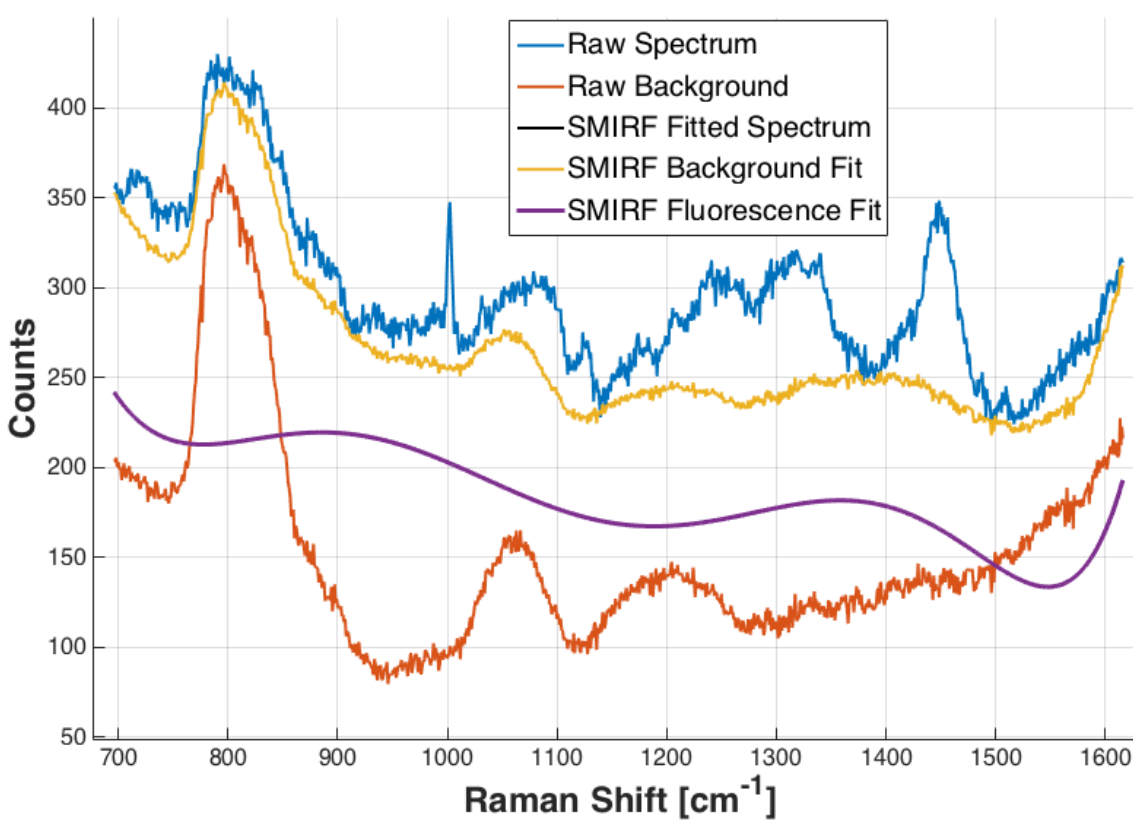
Procedure

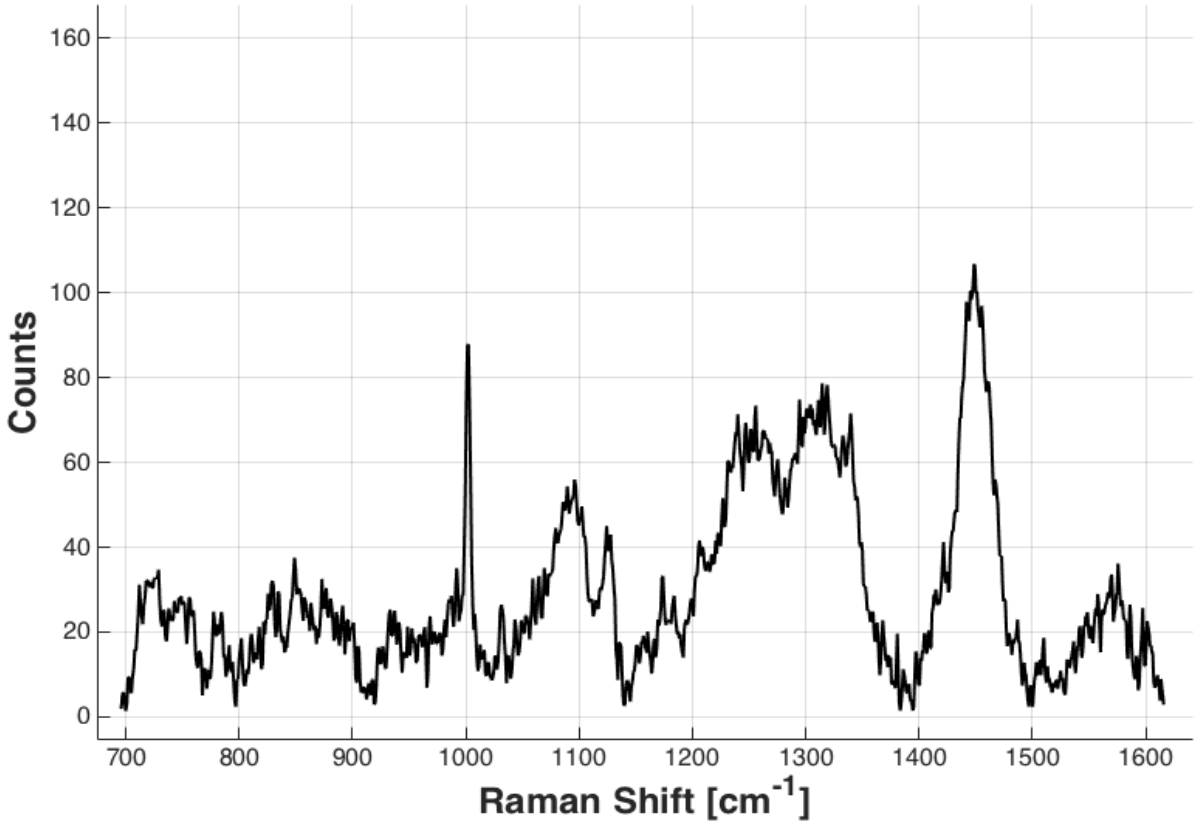
- Raman spectra recorded using the CRM, with a 785 nm laser and a 60x immersion objective
- Sample coverslips with adhered cells immersed in Phosphate-buffered saline (PBS) solution
- 20 nucleus and 20 cytoplasm measurements taken per slip
- Each measurement is the average of 9 one minute spot measurements over a 3x3 grid



Processing Spectra

After spectrum-based method for iterative removal of fluorescence (SMIRF)



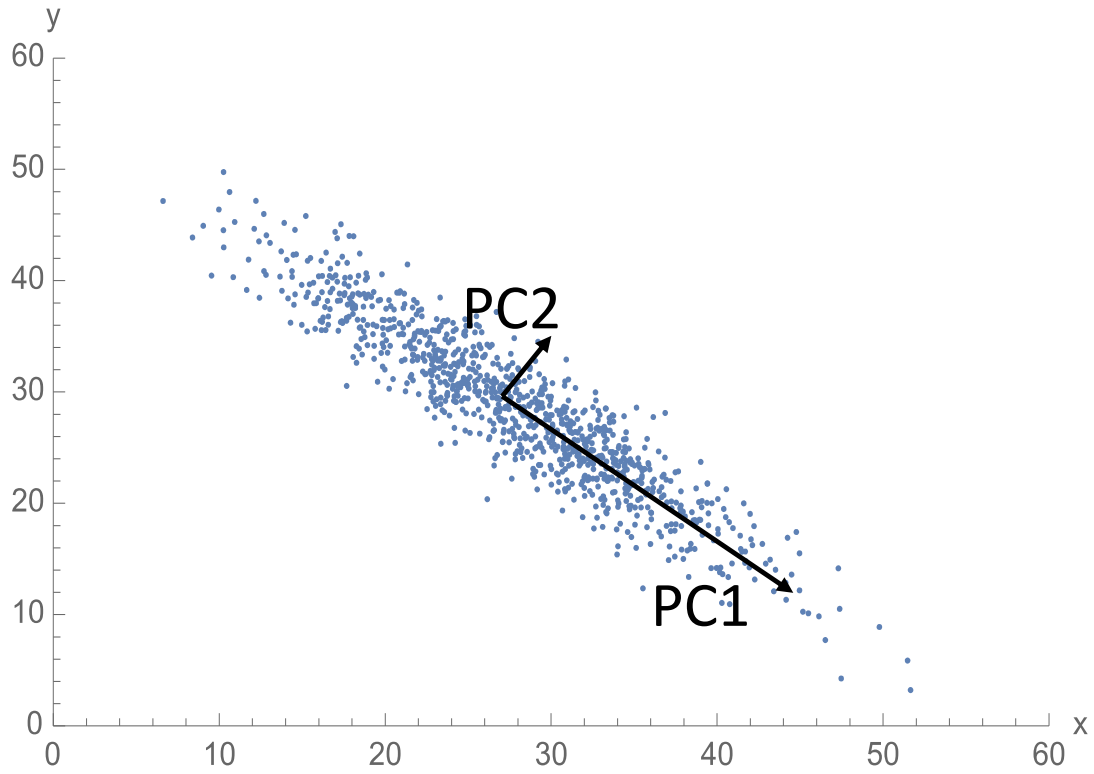


Data Analysis Techniques

- Once we have our data, we need to analyze it
- Spectral differences can be seen visually by simply plotting the mean spectra of different doses together
- We also take the ratio of the area under a peak for a dose to that of the control to see how peak sizes change with respect to dose
- For our classification objectives, we need to test how well we can separate measurements from the 6 doses plus control (0 Gy)
- We are currently using Principal Component Analysis and Linear Discriminant Analysis to do this

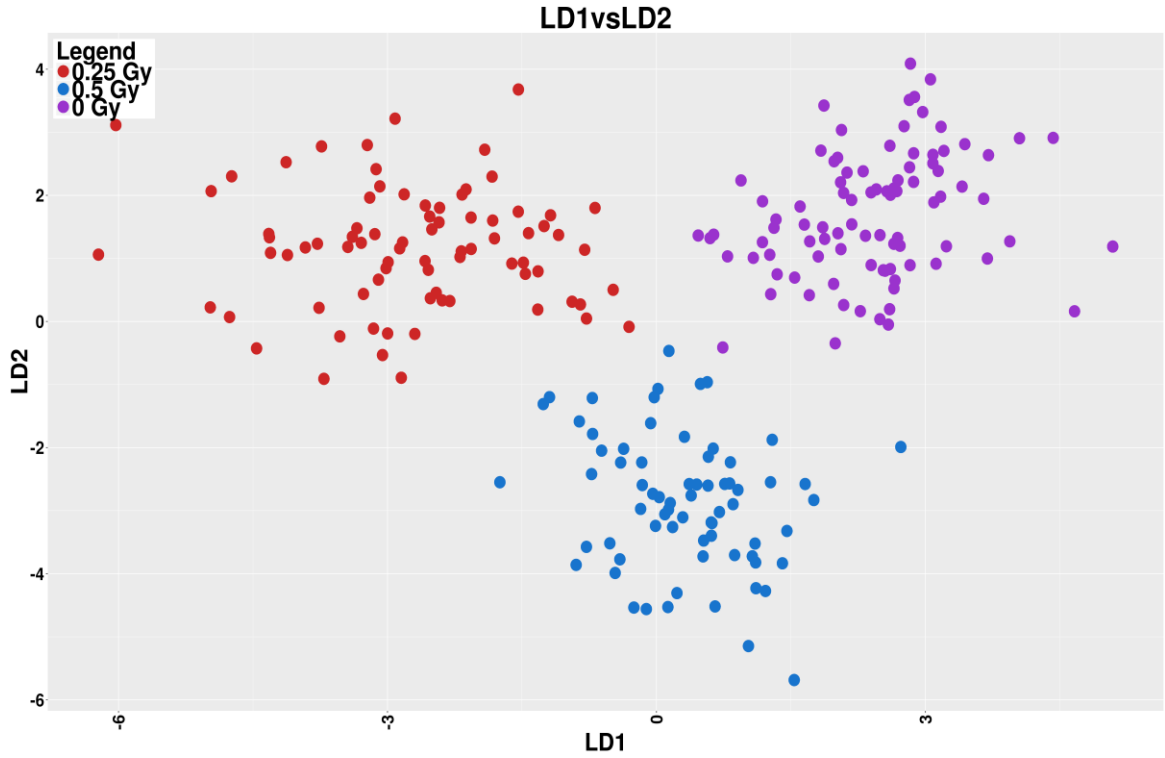
Principal Component Analysis

- Spectra have 1024 wavenumbers with a lot of correlation
- PCA gives uncorrelated variables by finding the eigenvectors of the covariance matrix
- Choose first N PCs, accounting for most (>95%) of the variance
- Data dimensionality reduced from 1024 to less than number of measurements per dose



Linear Discriminant Analysis

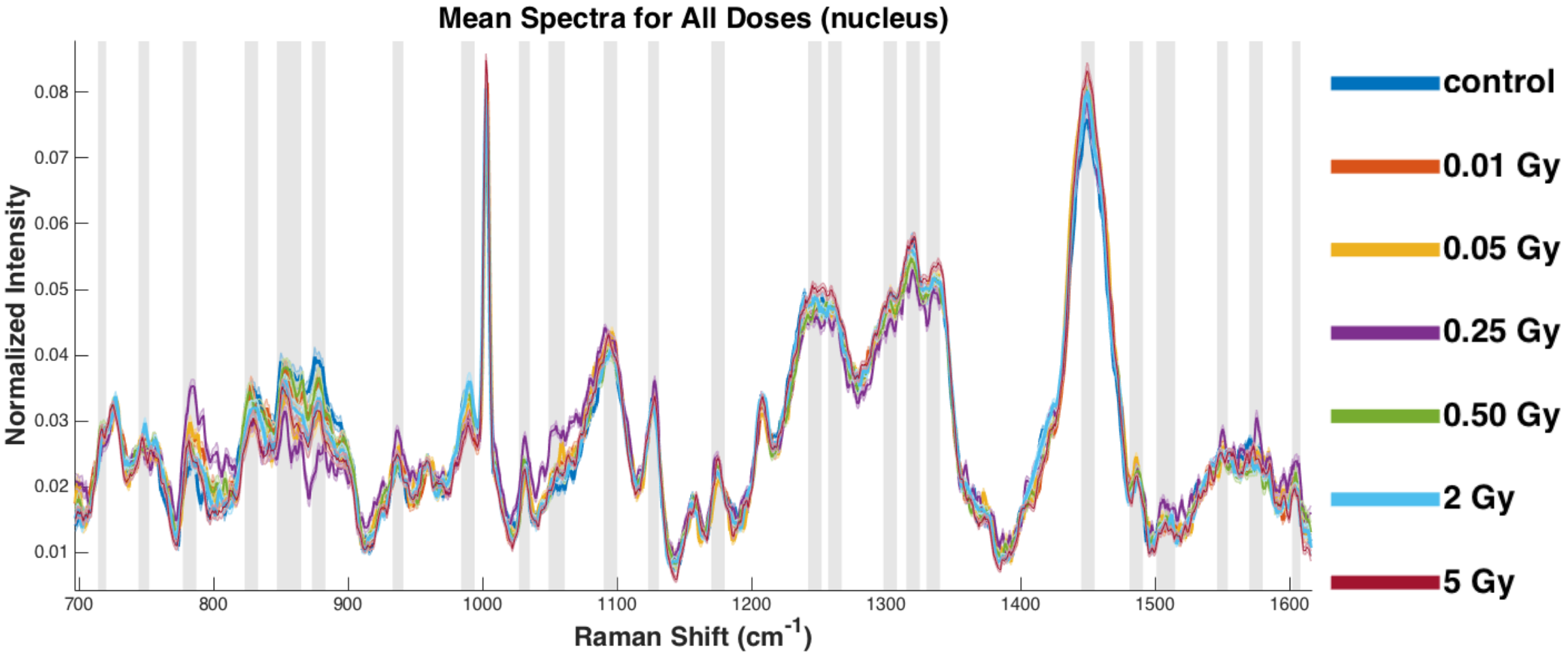
- Wish to discriminate between N classes (doses) in data
- Multiclass LDA projects data onto an N-1 hyperplane, maximizing between class variance and minimizing within class variance
- A distance metric is then used to determine which class mean a data point being tested is closest to in the transformed space



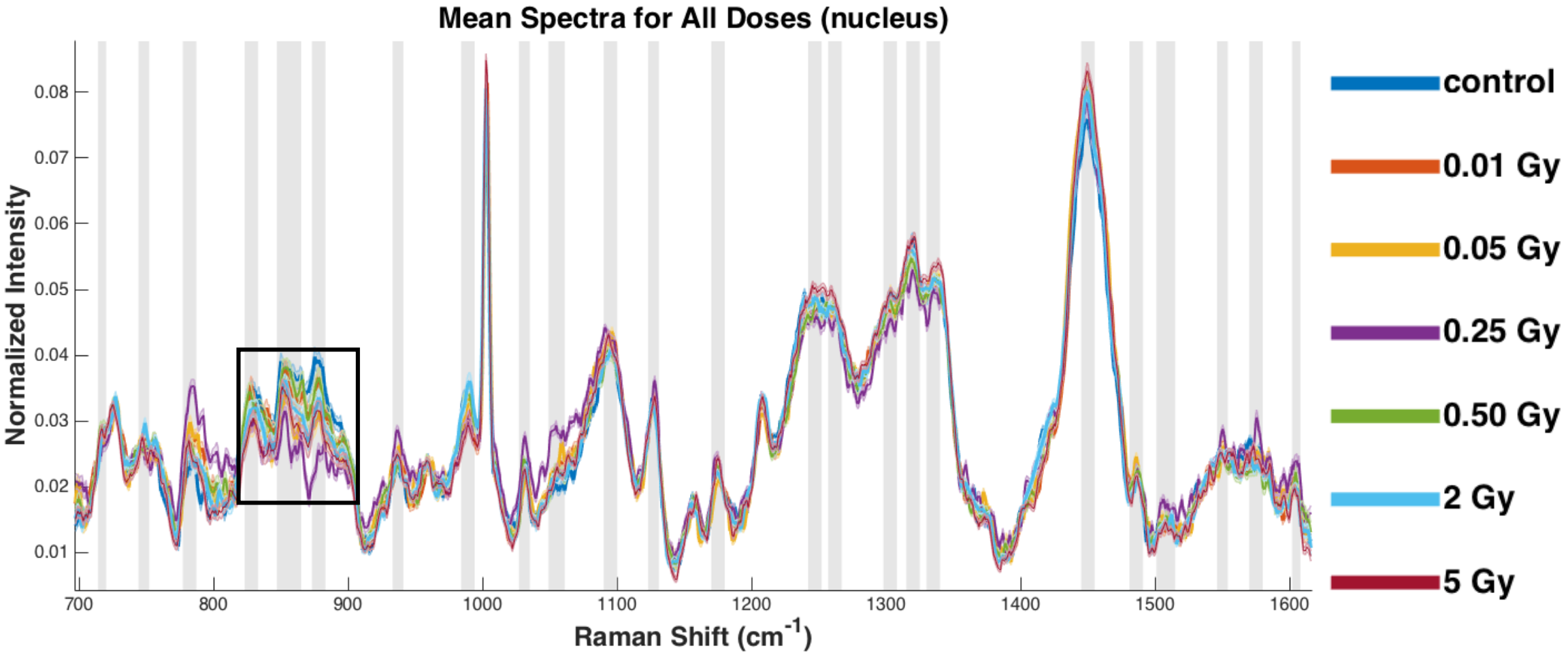
Results

Comparing mean spectra for different doses

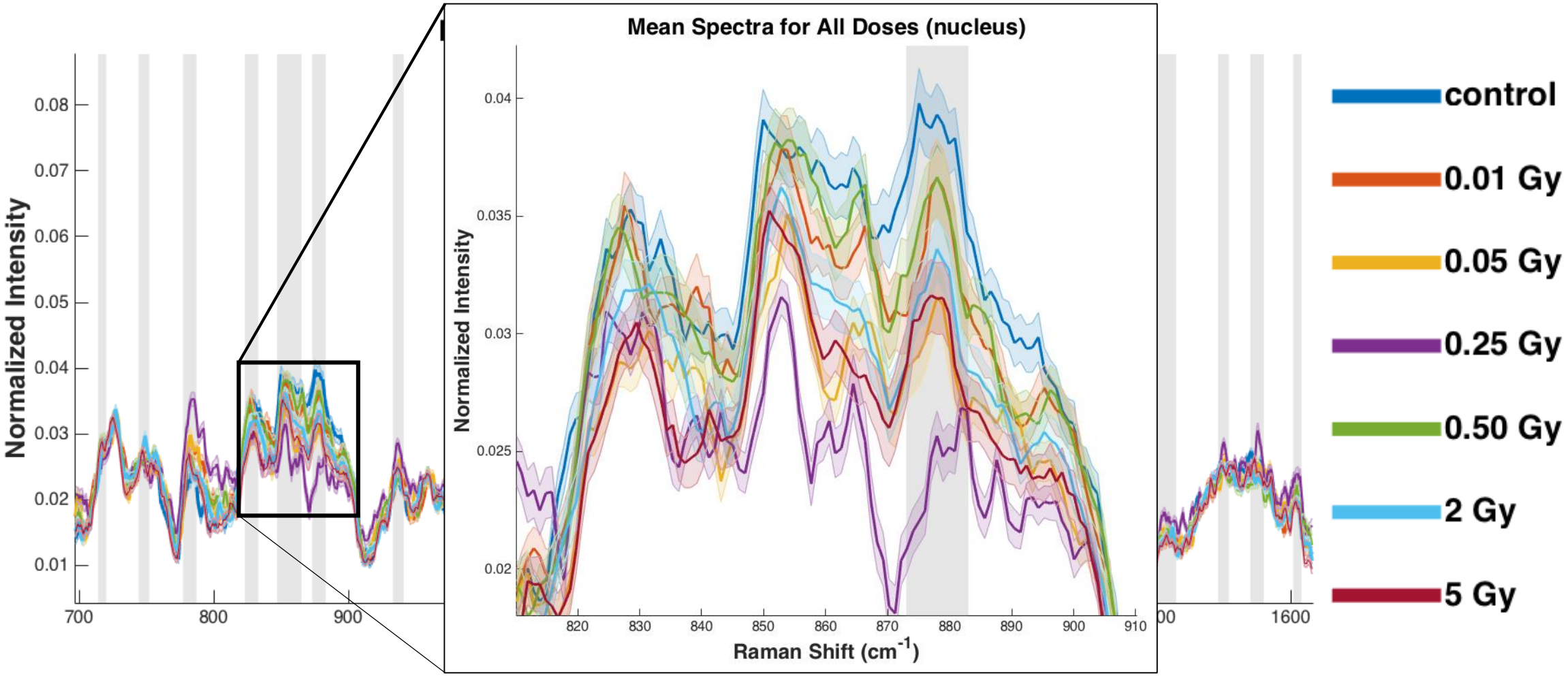
Results: Nucleus Spectra



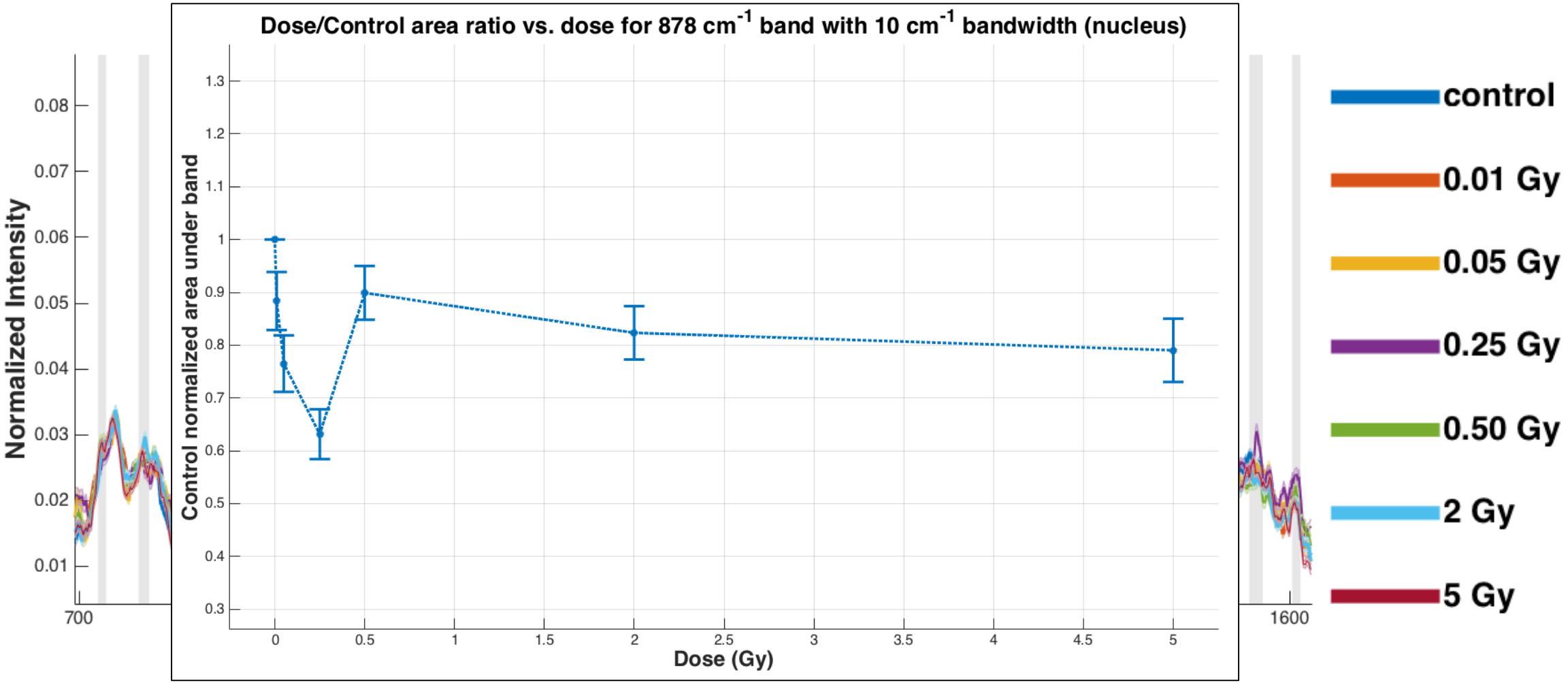
Results: Nucleus Spectra



Results: Nucleus Spectra



Results: Nucleus Spectra



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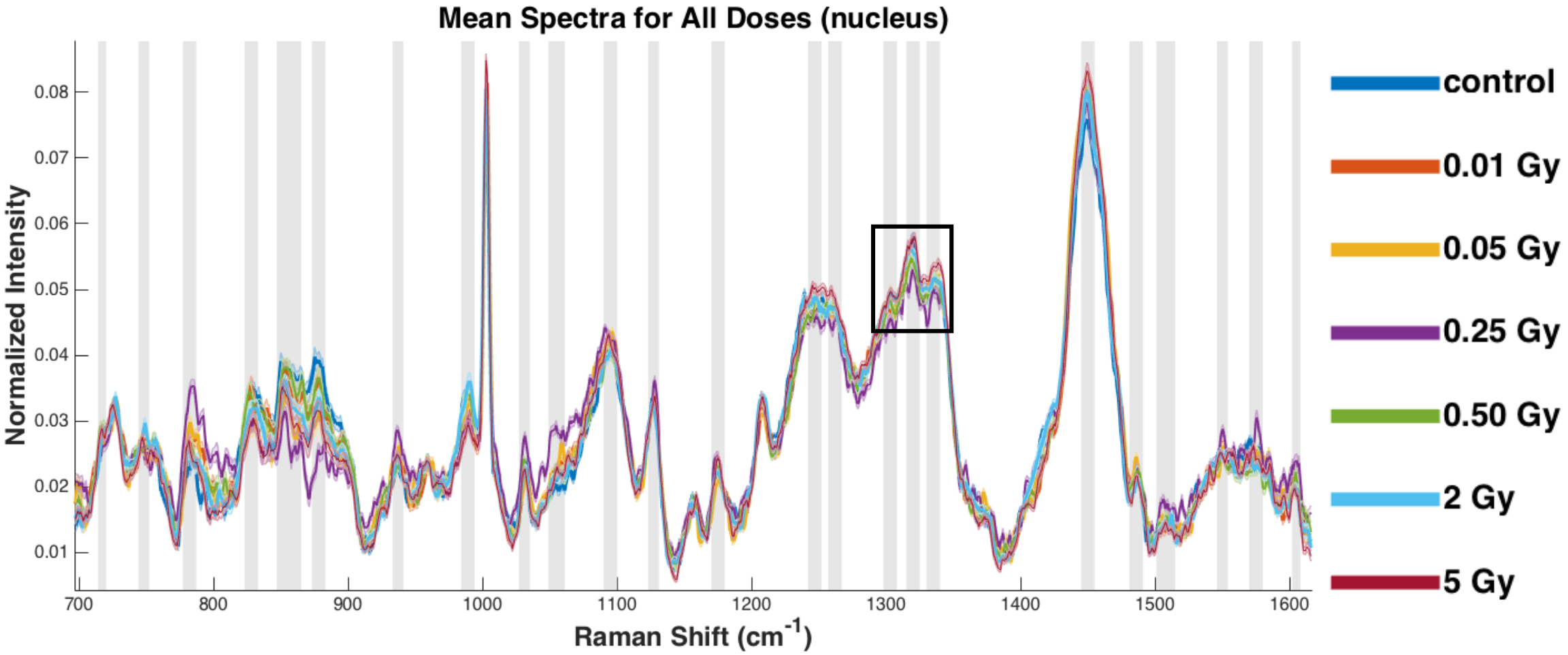

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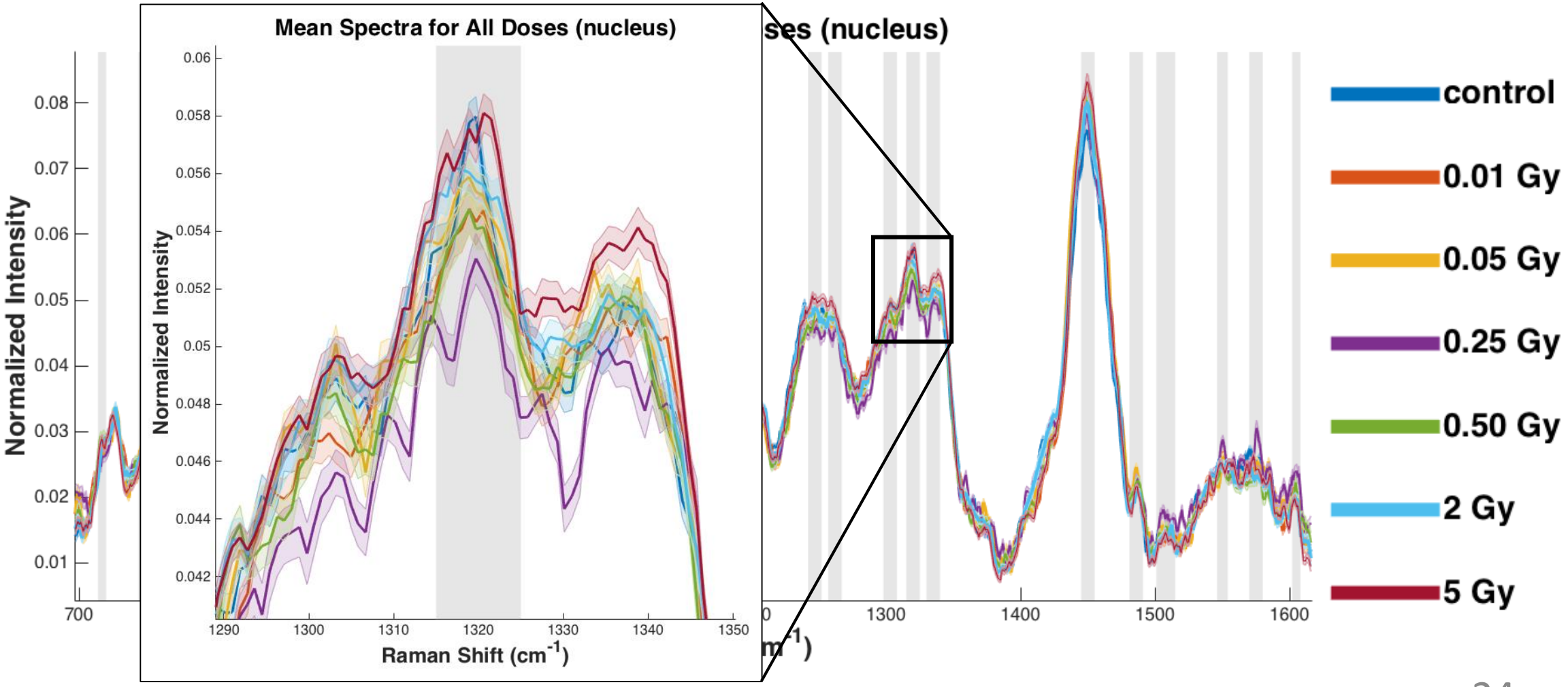

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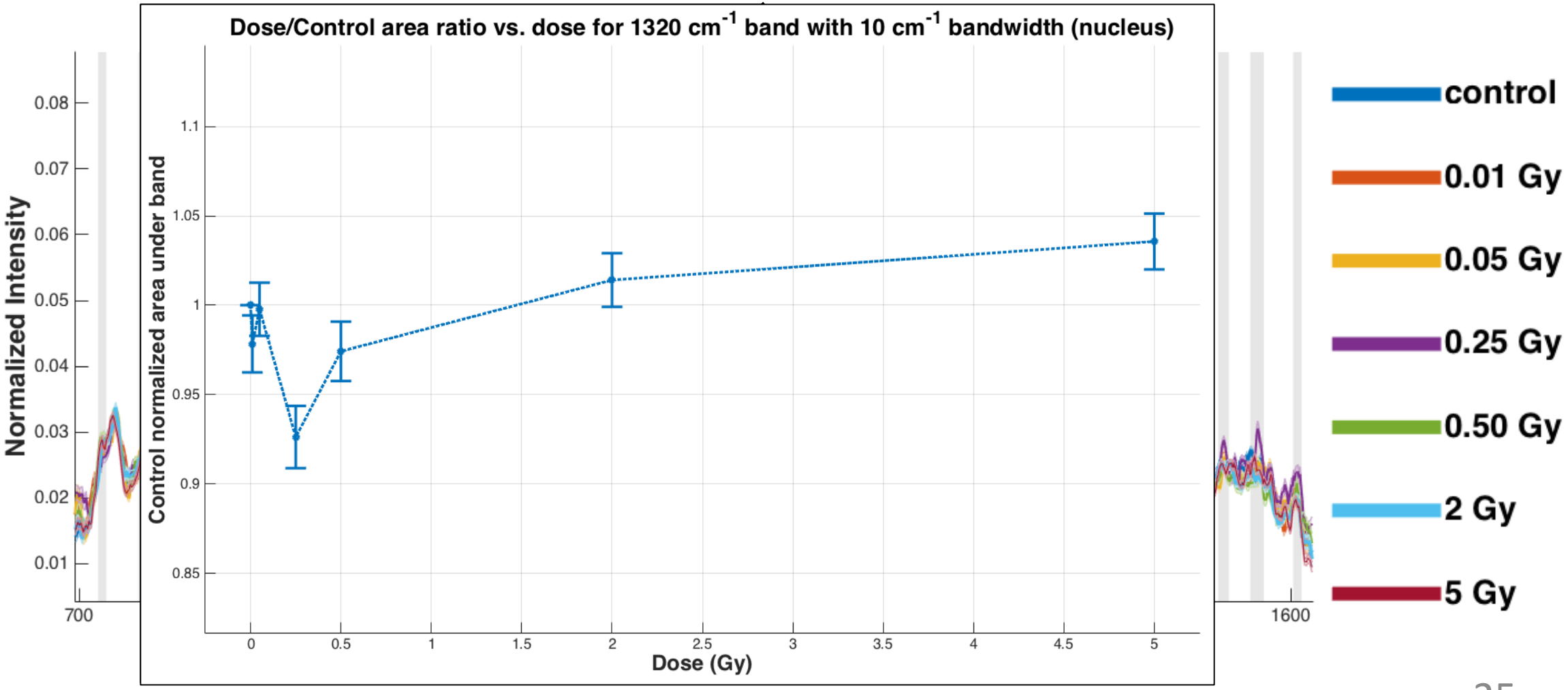
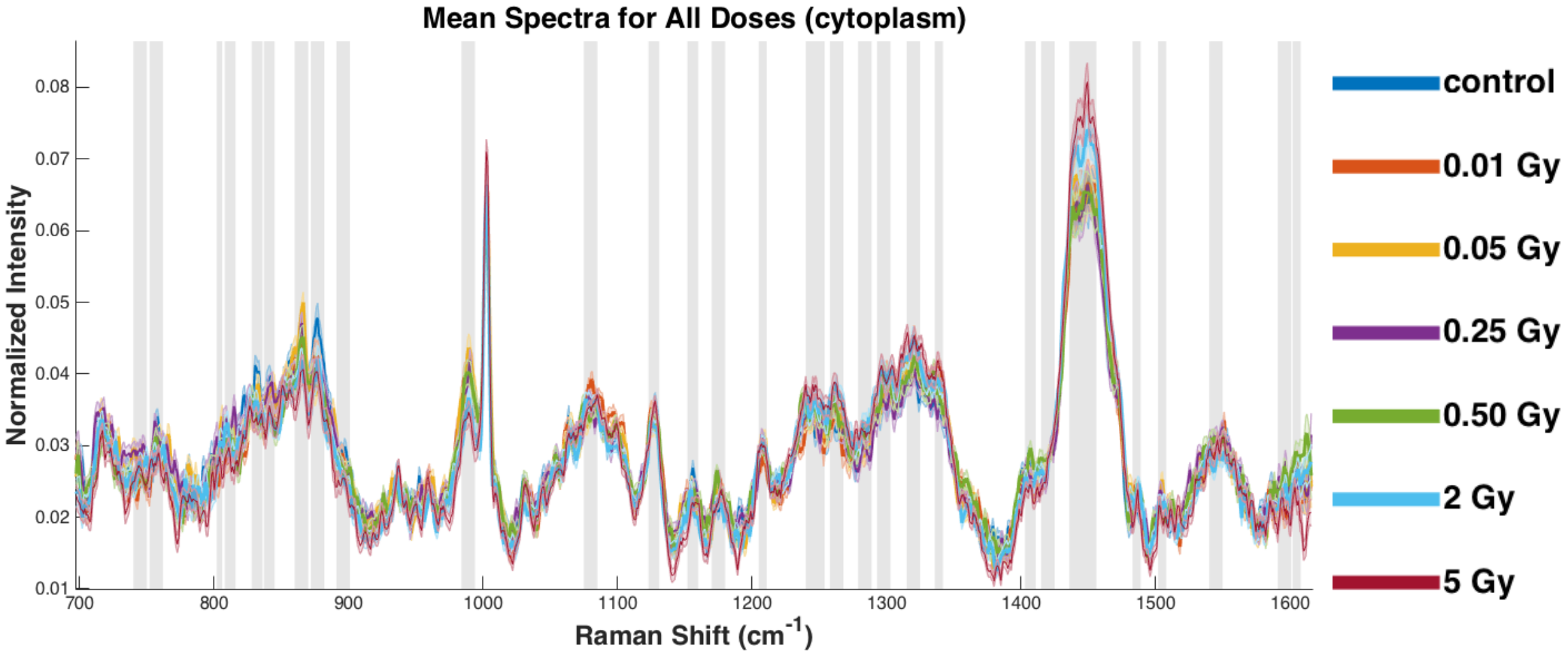


Results: Cytoplasm Spectra



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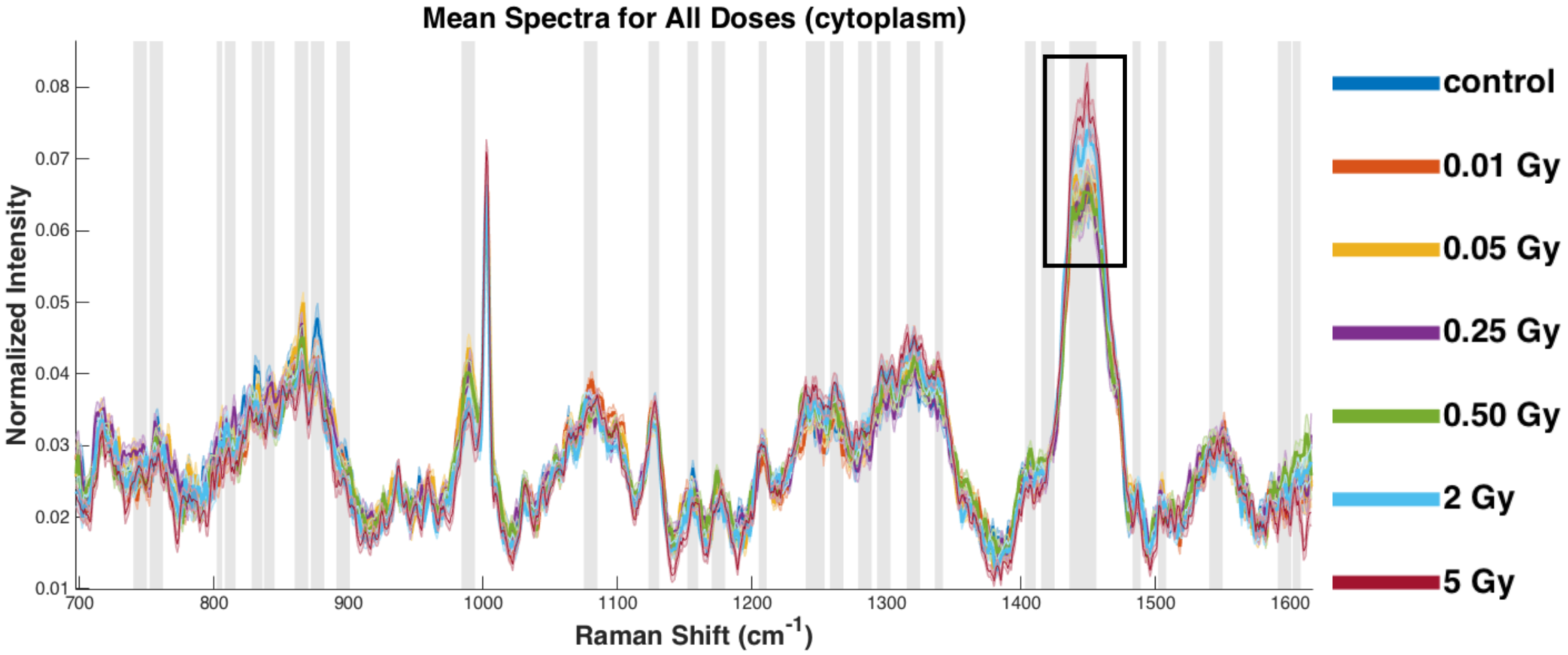

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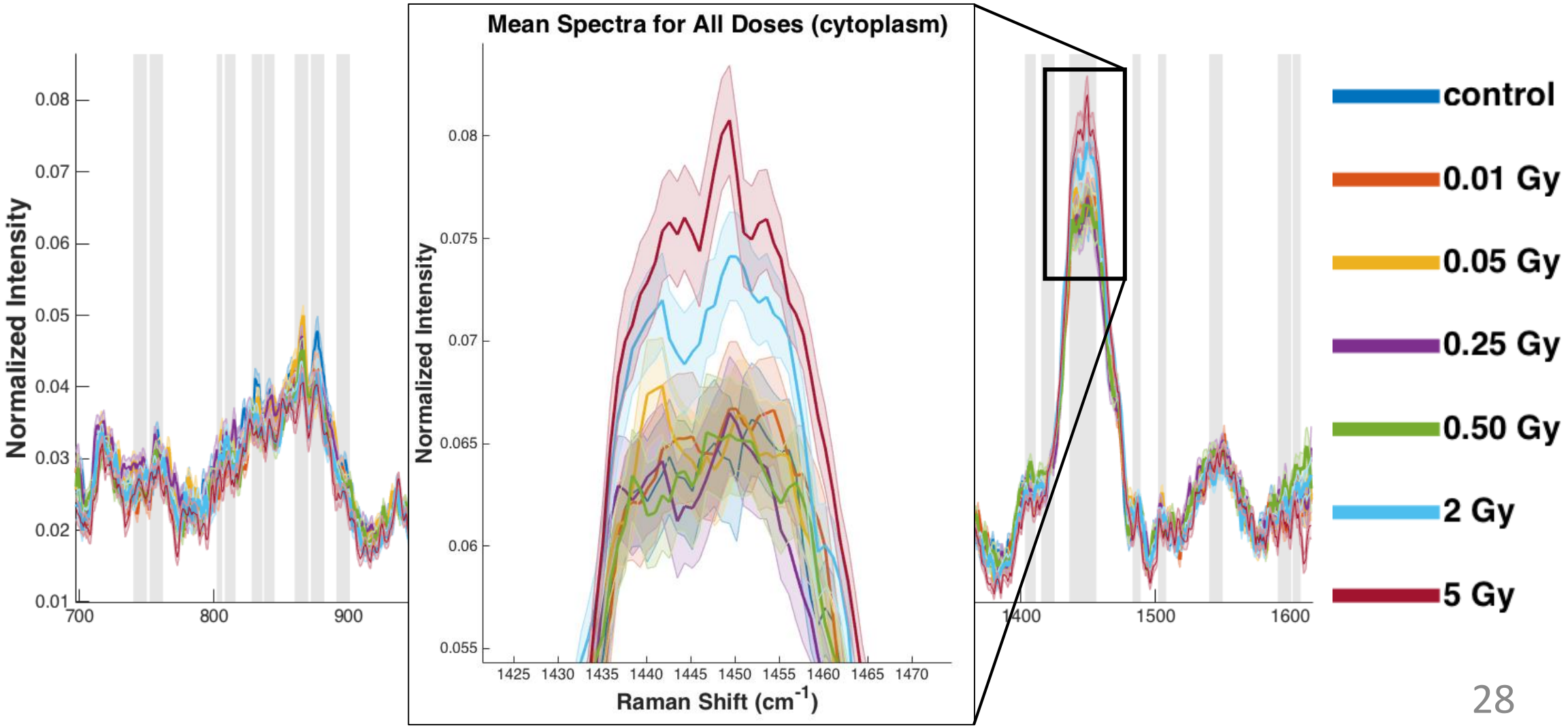
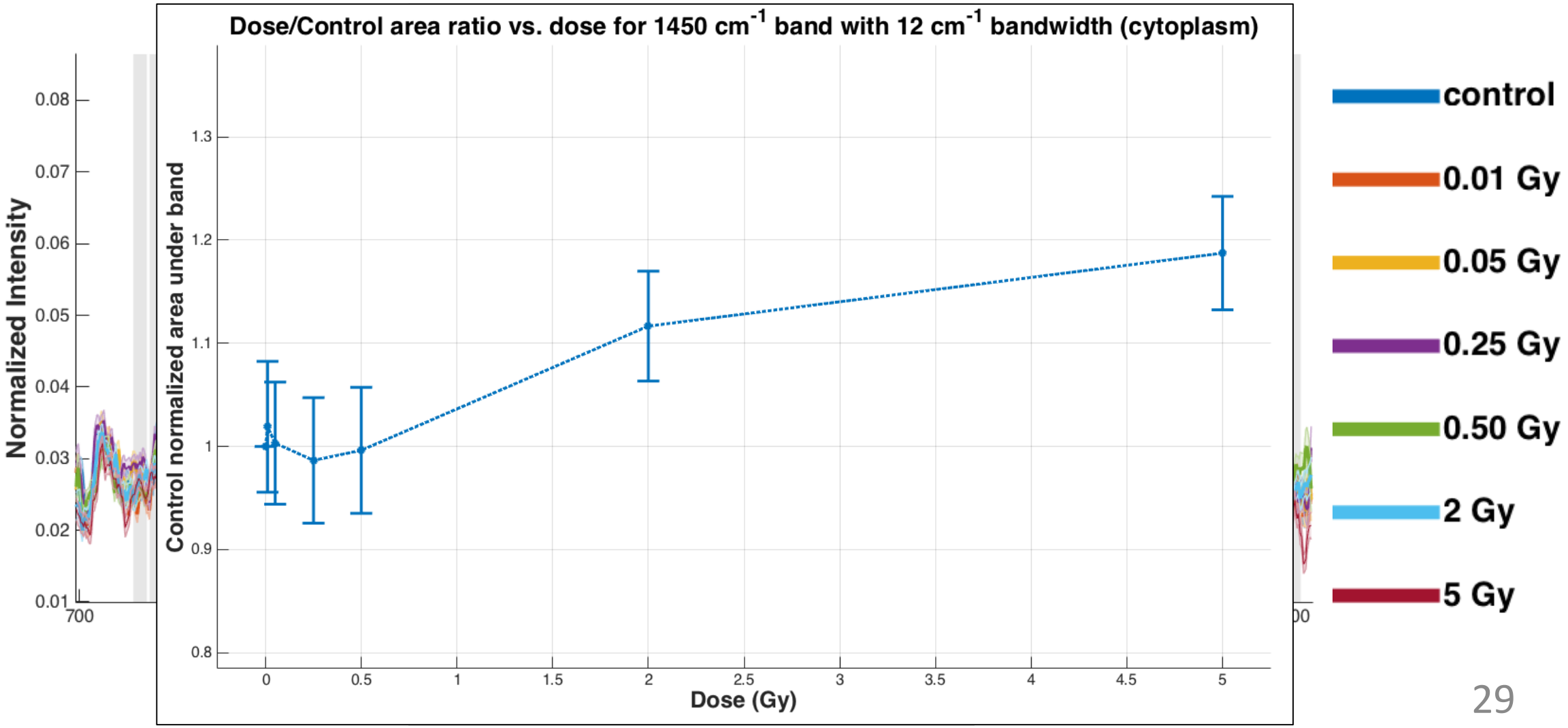
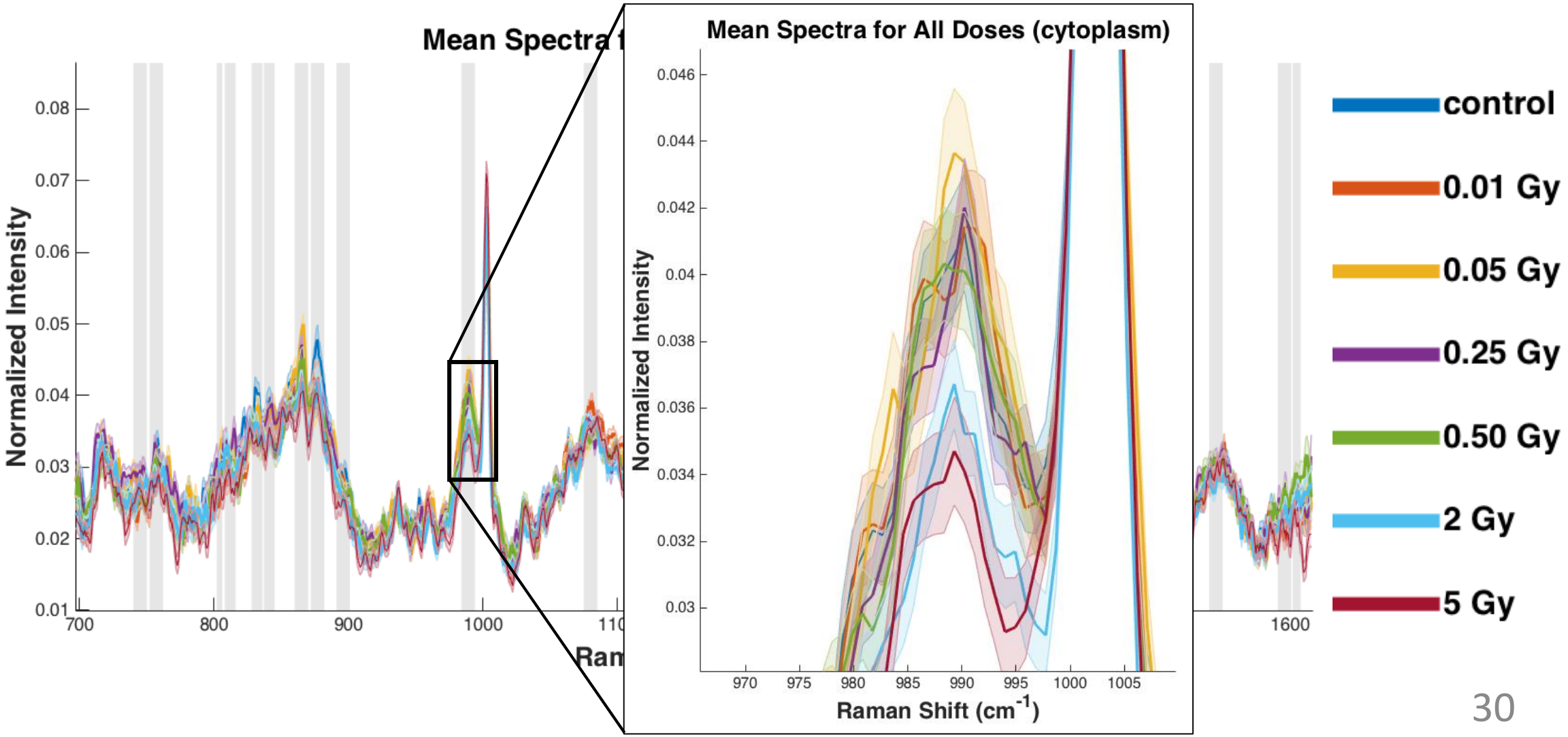


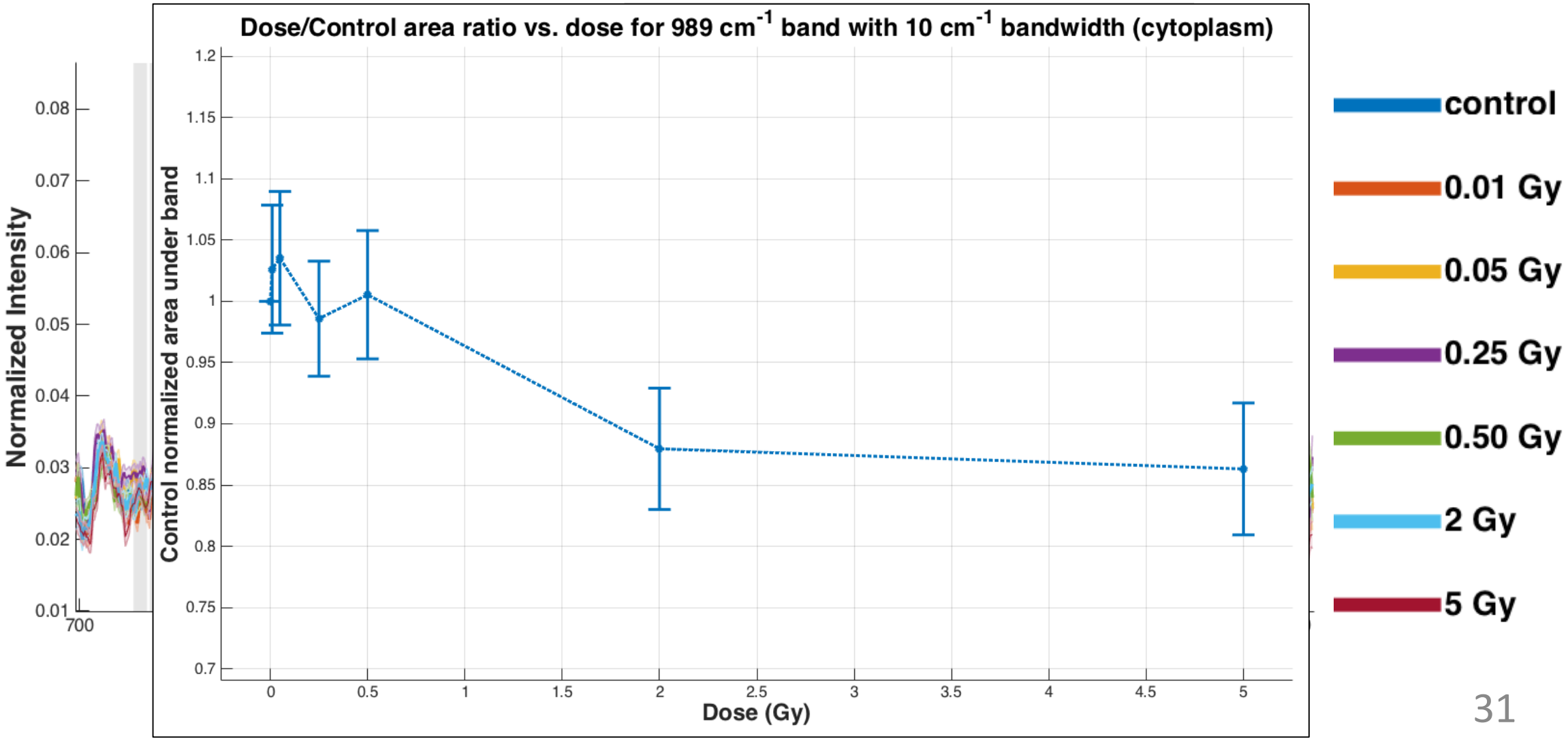
Results: Cytoplasm Spectra



Results: Cytoplasm Spectra



Results: Cytoplasm Spectra



Results

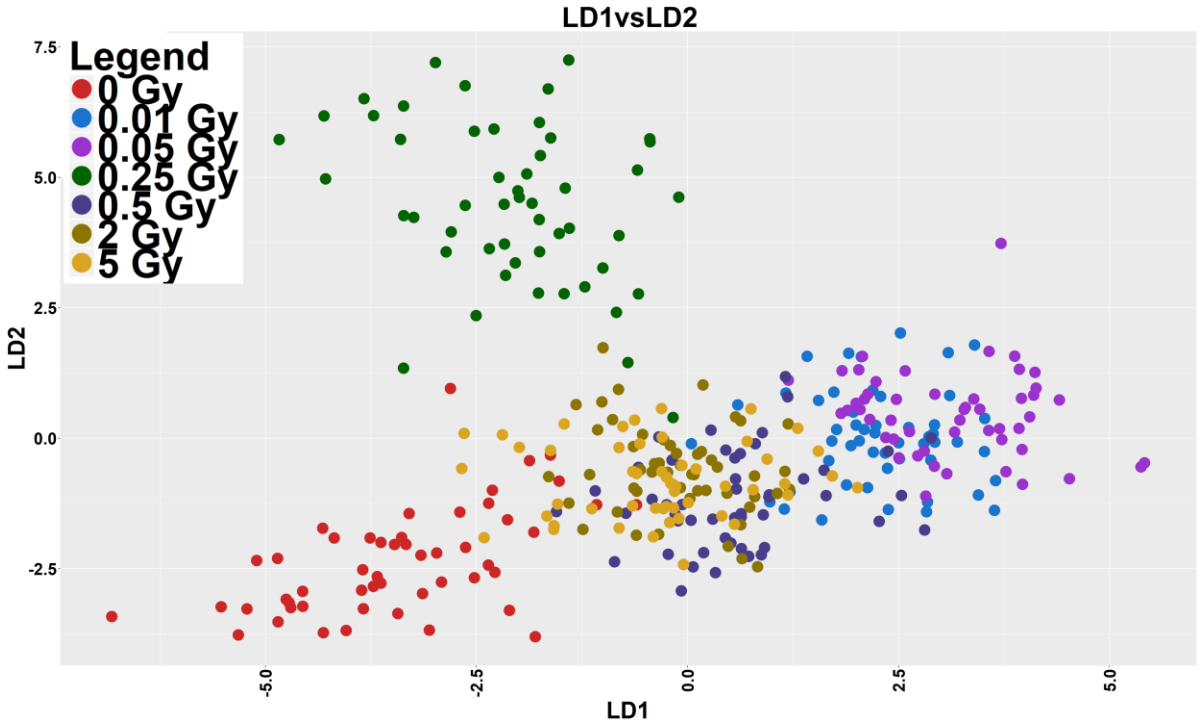
Dose discrimination using PCA-LDA

Results: PCA-LDA

- Data for nucleus, cytoplasm analyzed separately as well as combined
- For each analysis, data is randomly partitioned into ~25% test set and ~75% training set
- Training set is used to find linear discriminants for classification
- 100 different partitions tested and an average accuracy with error is found

Results: PCA-LDA of Nucleus Spectra

- 469 nuclei measurements
- 50 PCs



Average test set accuracy: (92 ± 3)%

Actual Dose

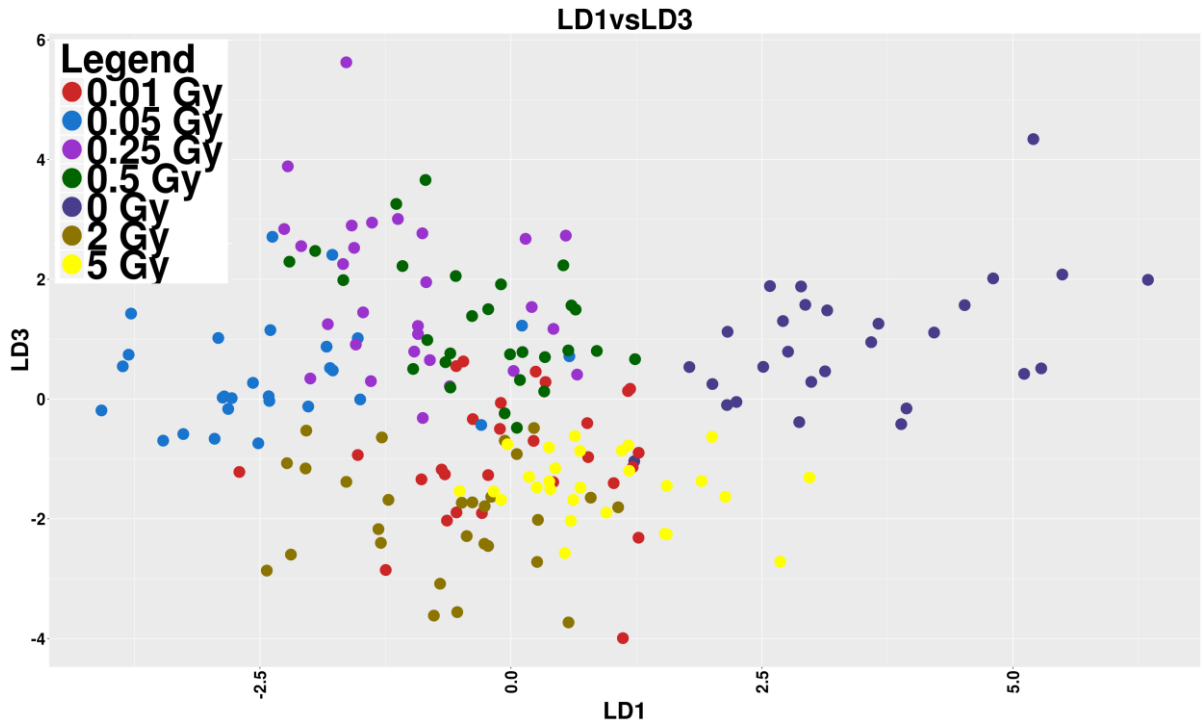
Predicted Dose

	0 Gy	.01 Gy	.05 Gy	.25 Gy	.5 Gy	2 Gy	5 Gy
0 Gy	16	0	0	0	0	0	0
.01 Gy	0	16	0	0	0	0	0
.05 Gy	1	0	14	0	0	1	0
.25 Gy	0	0	0	14	0	1	1
.50 Gy	0	0	0	0	12	1	3
2 Gy	0	0	0	1	0	15	0
5 Gy	0	0	0	0	0	0	16

Table 1: Nucleus Test Data Confusion Matrix

Results: PCA-LDA of Cytoplasm Spectra

- 259 cytoplasm measurements
- 30 PCs



Average test set accuracy: (90 ± 2)%

Actual Dose

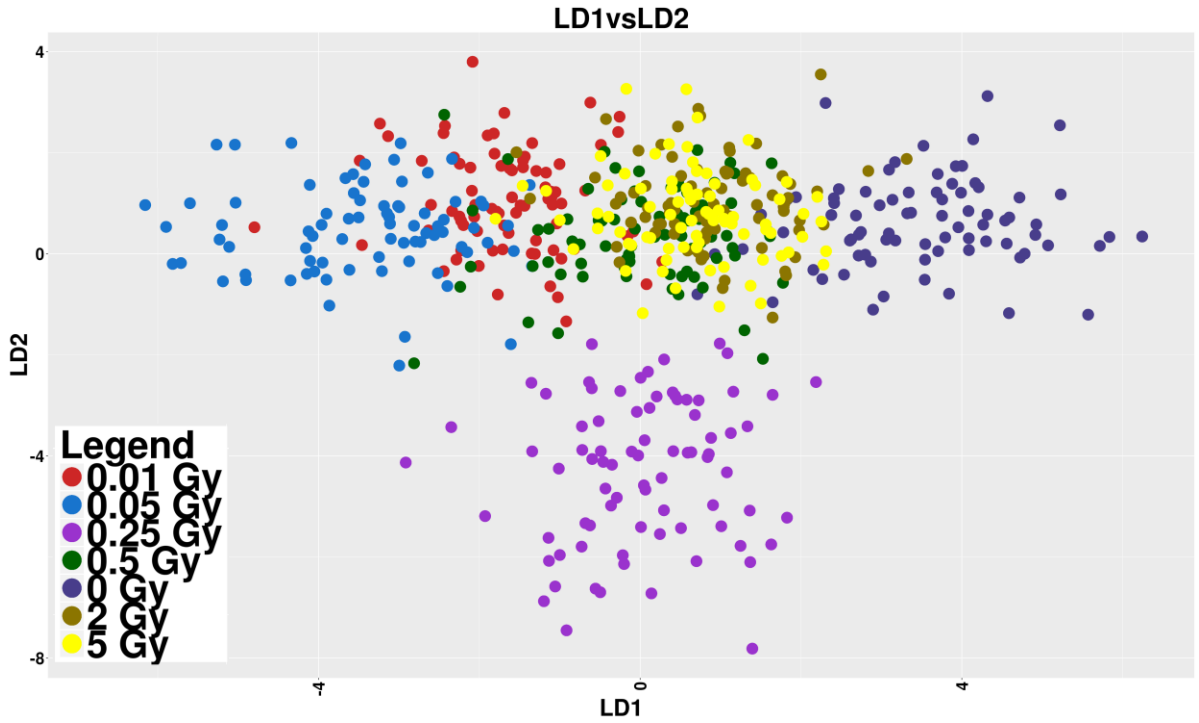
Predicted Dose

	0 Gy	.01 Gy	.05 Gy	.25 Gy	.5 Gy	2 Gy	5 Gy
0 Gy	8	0	0	1	0	0	0
.01 Gy	0	8	1	0	0	0	0
.05 Gy	0	0	7	0	0	1	1
.25 Gy	0	0	0	9	0	0	0
.50 Gy	0	0	0	0	8	0	1
2 Gy	0	0	0	0	0	9	0
5 Gy	0	0	0	0	0	1	8

Table 2: Cytoplasm Test Data Confusion Matrix

Results: PCA-LDA of all Spectra

- 742 combined measurements
- 70 PCs



Average test set accuracy: (94 ± 2)%

Actual Dose ↓

Predicted Dose

	0 Gy	.01 Gy	.05 Gy	.25 Gy	.5 Gy	2 Gy	5 Gy
0 Gy	26	0	0	0	0	0	0
.01 Gy	0	25	0	0	0	0	1
.05 Gy	0	0	25	0	0	0	1
.25 Gy	0	0	0	22	0	3	1
.50 Gy	0	0	0	0	22	2	2
2 Gy	0	0	0	0	0	26	0
5 Gy	1	0	0	0	0	0	25

Table 3: Combined Test Data Confusion Matrix

Summary

- Spectral Differences in Raman spectra of HLE cells are visible
- We can identify bands that show the largest changes, such as proteins, lipids, DNA and glutathione
- Greater than 90% accuracy can be obtained using a 25% test, 75% train model of our data
- Combining nucleus and cytoplasm data increases classification accuracy

Thank you for your time!