Detection of Radiation Induced Changes in Human Lens Epithelial Cells Using Raman Spectroscopy

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Background

- Occupational exposure to ionizing radiation
- 0.5 Gy threshold

http://www.magic4walls.com/wallpaper/aircraft-wings-sky-sun-photo-vintage-clouds-on-high-desktop-wallpaper-29288.html

https://www.nasa.gov/multimedia/imagegallery/image_feature_2350.html

https://www.healthcare.siemens.co.in/radiography/digital-x-ray/multix-

Goals

- Can we measure different dosage of radiation non-invasively?
- How low dosage can we distinguish?
- Can we find out the biomolecular changes responsible for the differentiation?

Experiment

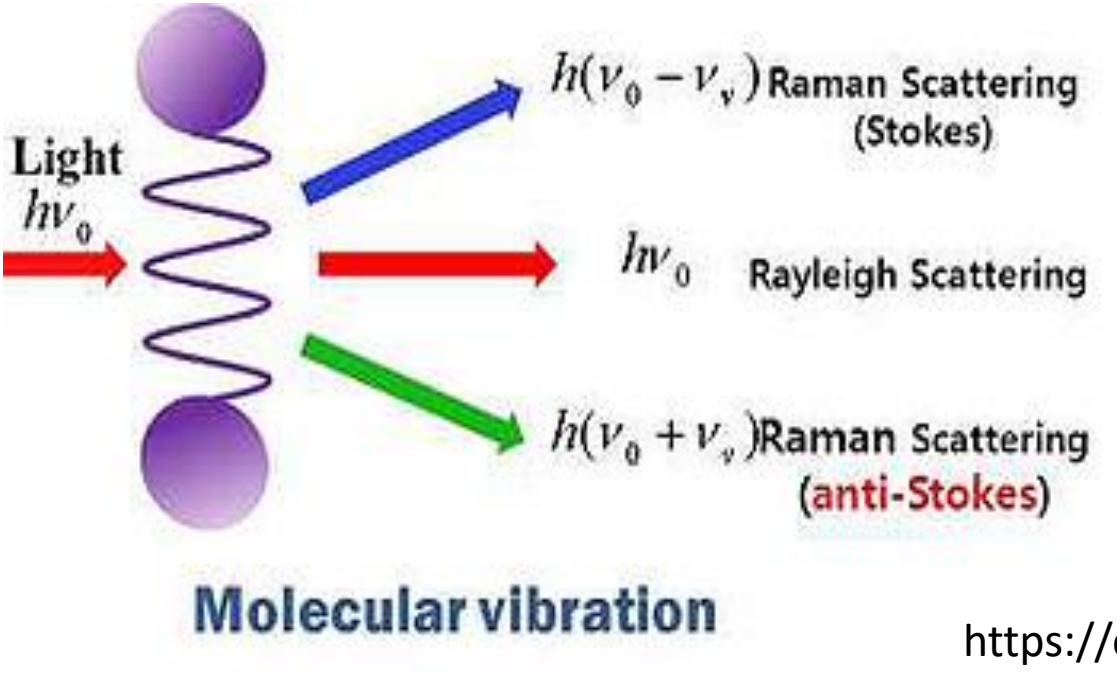
- Collect fixed HLE cells from Health Canada
- Five classes: 0, 0.25, 0.5, 2 and 5 Gy fixed HLE cells
- Target nucleus and cytoplasm (3X3 grid)
- Nucleus: 3x3 grid (3um step size)
- Cytoplasm: 3x3 grid (1um step size)

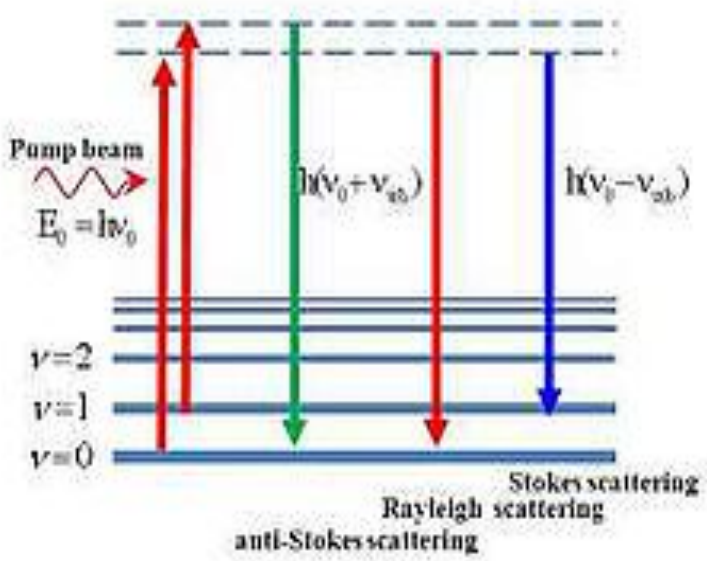
Principles and Techniques

- Raman scattering
- Confocal Microscopy
- Pre-processing
- PCA
- LDA

Raman Scattering

- Inelastic scattering of photons with matter
- Raman spectroscopy: Distribution of intensities at different Raman shifts
- Each material has a unique Raman fingerprint

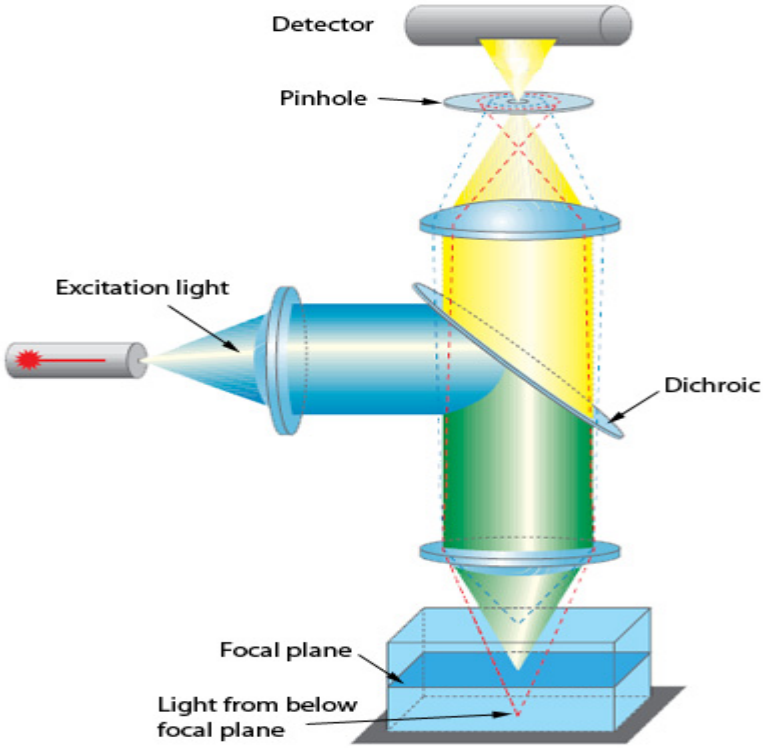


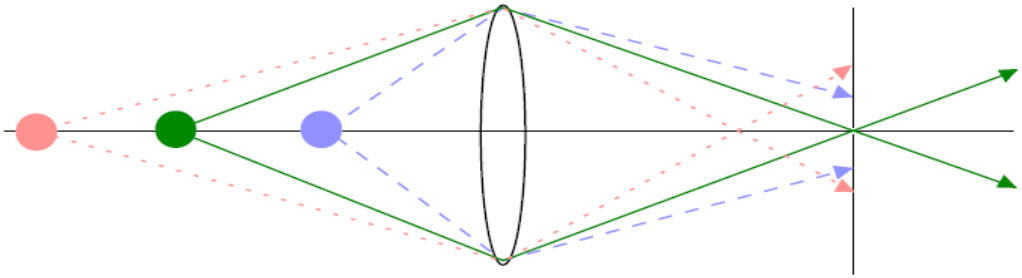


https://en.wikipedia.org/wiki/

Confocal Microscopy

- Provides optical sectioning of sample
- Reduces quartz fluorescence, so better SNR

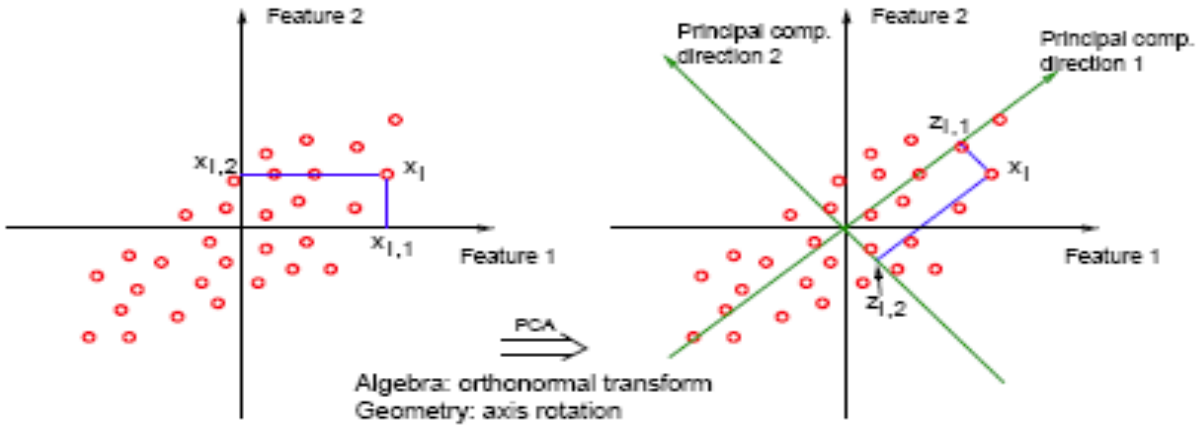




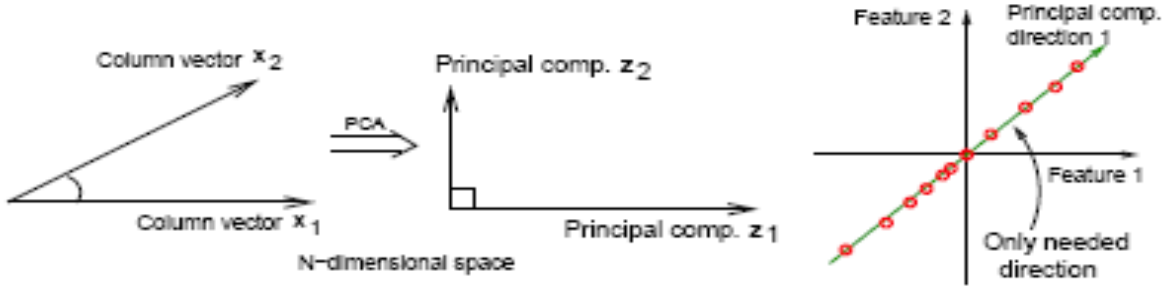
http://microscopy.duke.edu/learn/introtomicroscopy/confocals.html

Principal Component Analysis

- Dimensionality reduction- much more features than data set
- Removes correlated features (PCs are uncorrelated)

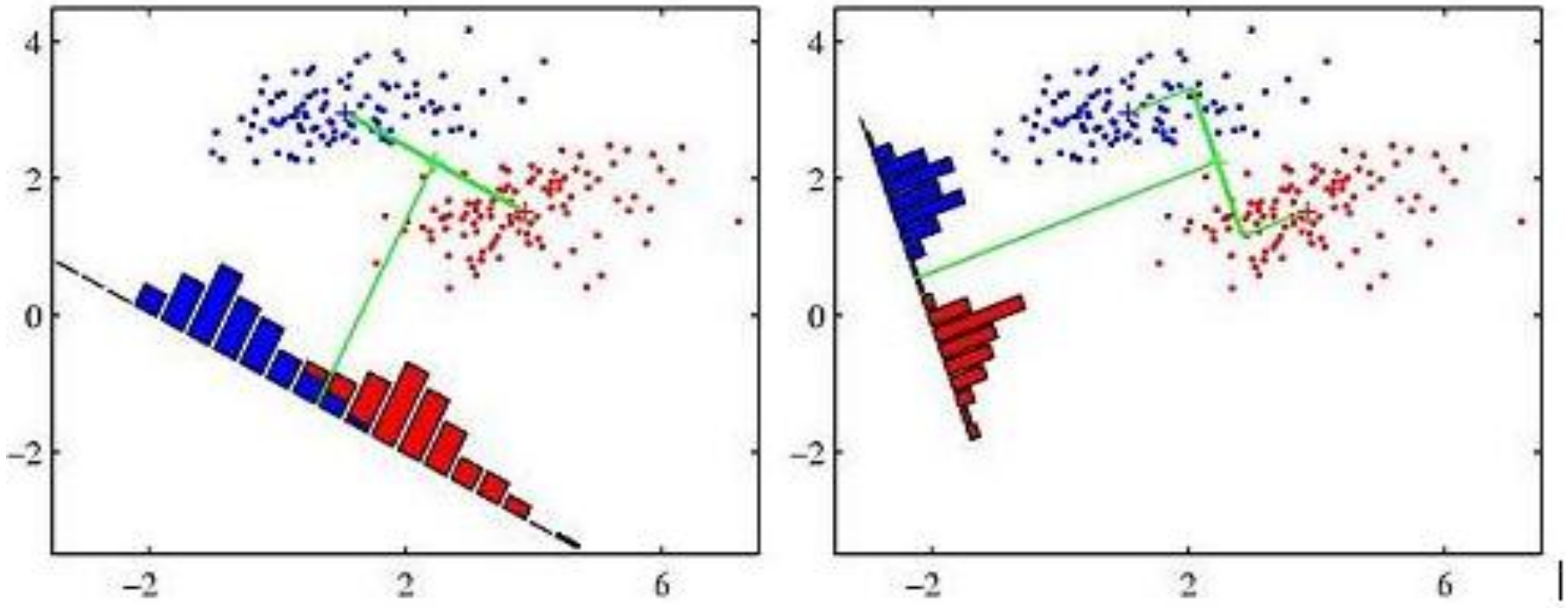


https://onlinecourses.science.psu.edu/stat857/book/export/html/11



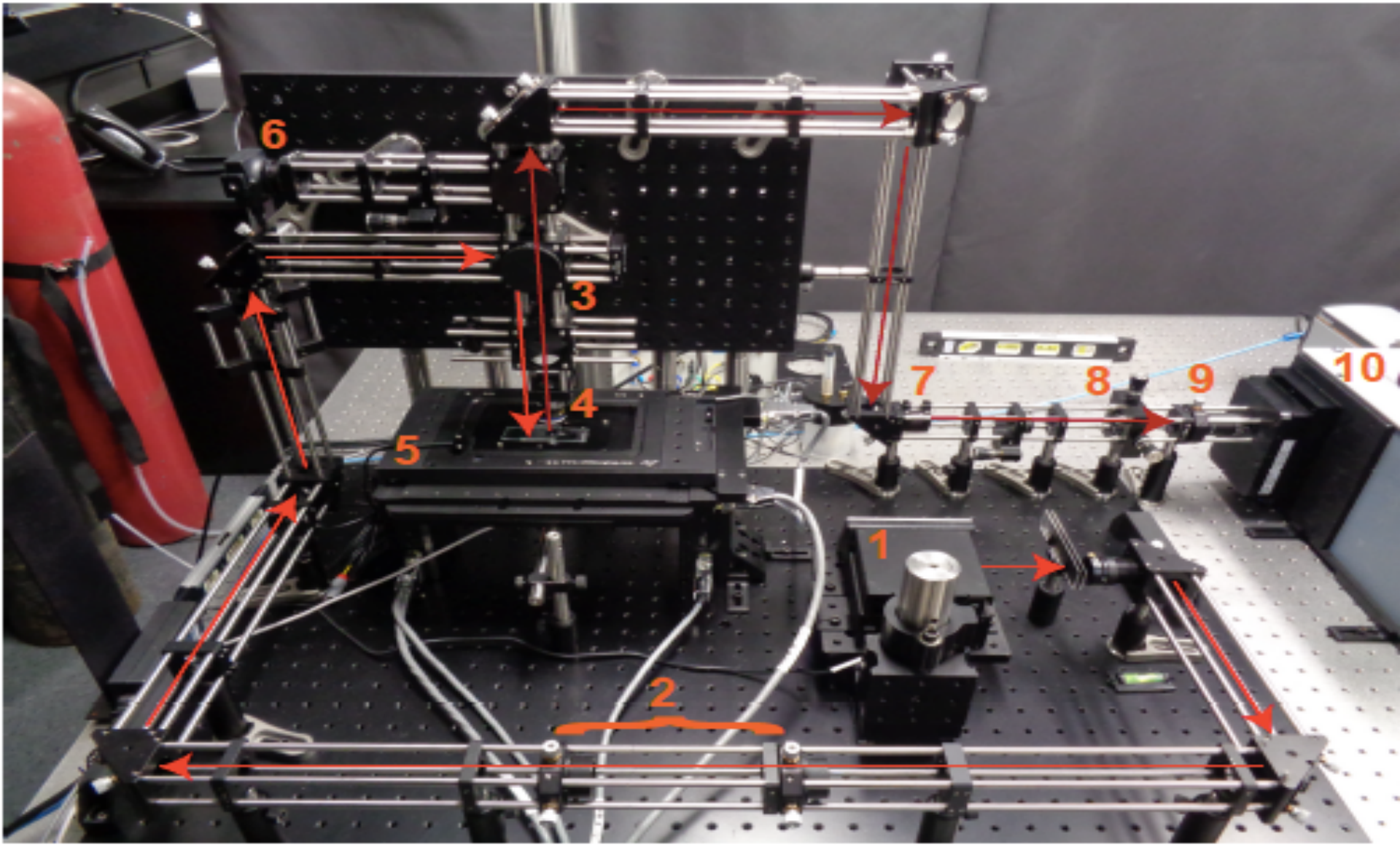
Linear Discriminant Analysis

- Maximize difference in mean divided by sum of scatter to get optimal classification



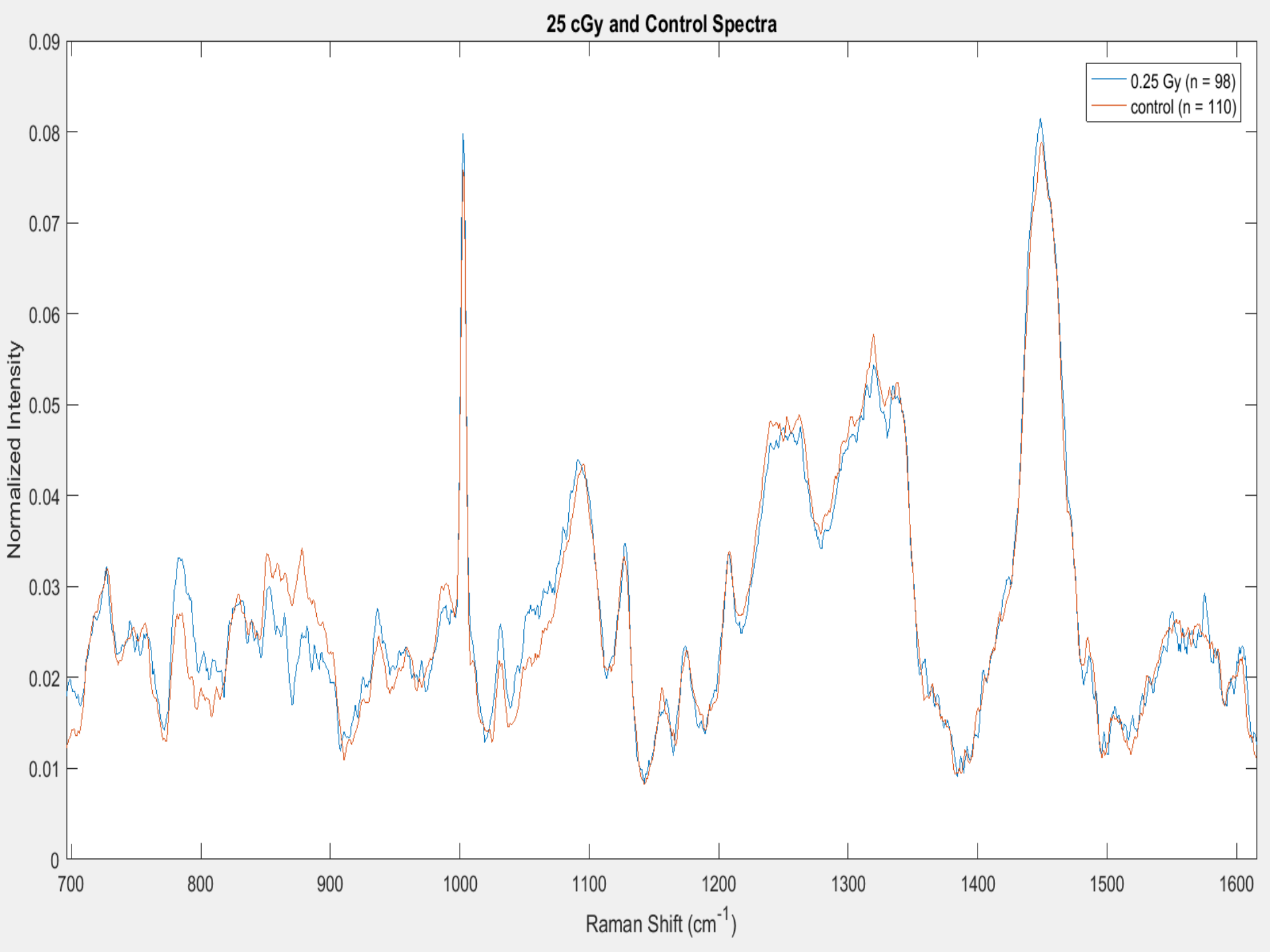
http://stackoverflow.com/questions/33844198/reproduce-fisher-linear-discriminant-figure

Raman Micro-spectrometer



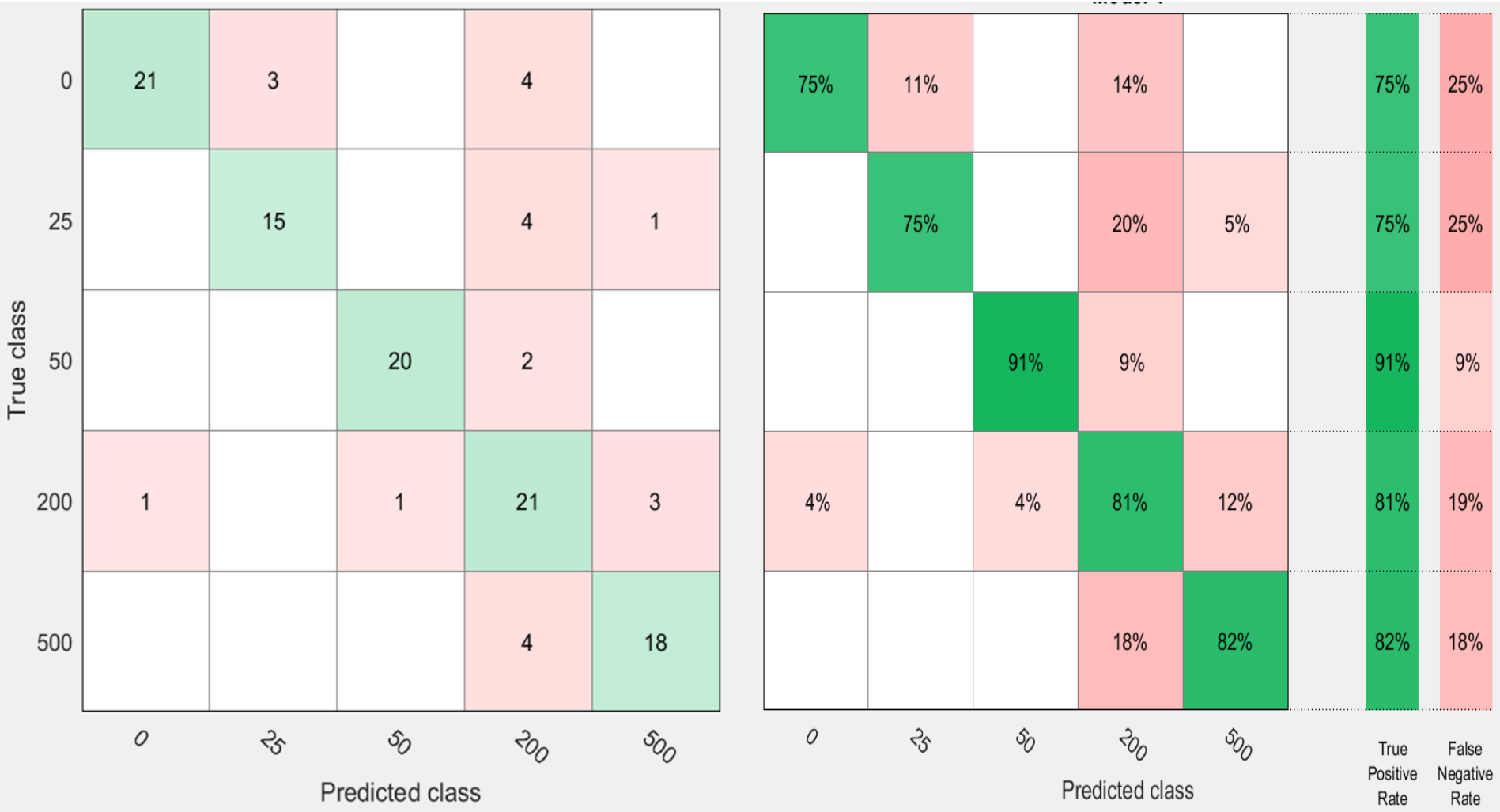
Data Analysis

- 531 nucleus targets
- 346 cytoplasm targets
- PCA-LDA for classification (75% training, 25% training)


25 cGy and Control Spectra
0.25 Gy (n = 98)
control (n = 110)
Normalized Intensity
Raman Shift (cm-1)
0
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
700
800
900
1000
1100
1200
1300
1400
1500
1600

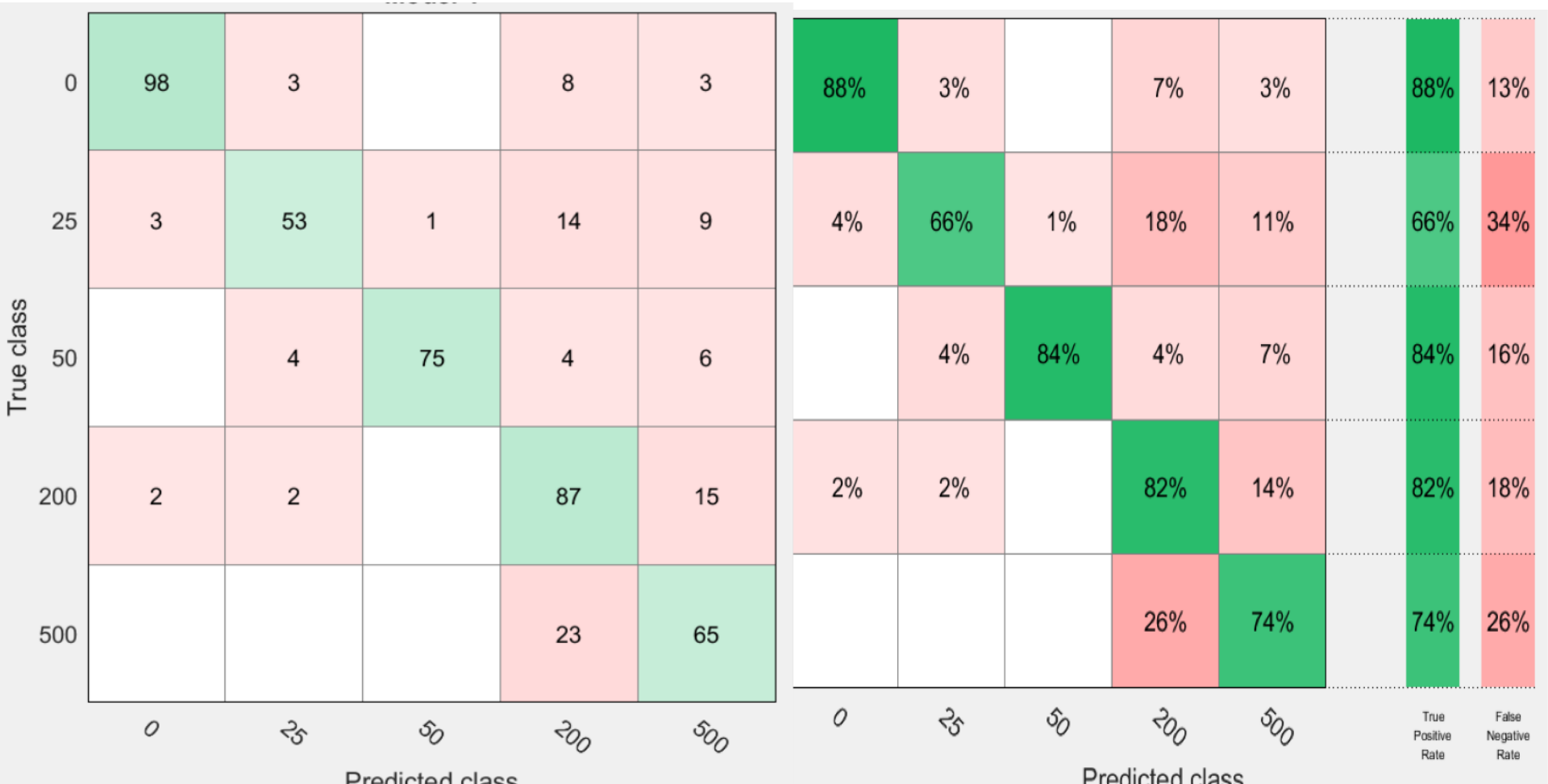
Confusion Matrix for nucleus targets

- 80.5% accuracy (test set)

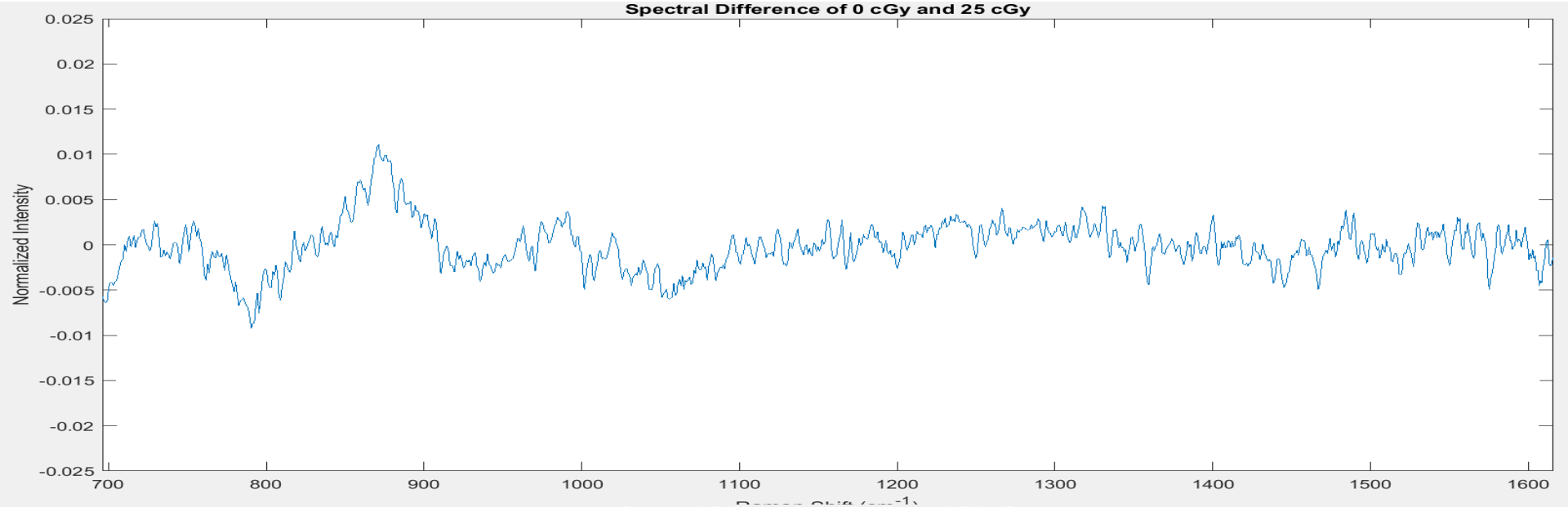


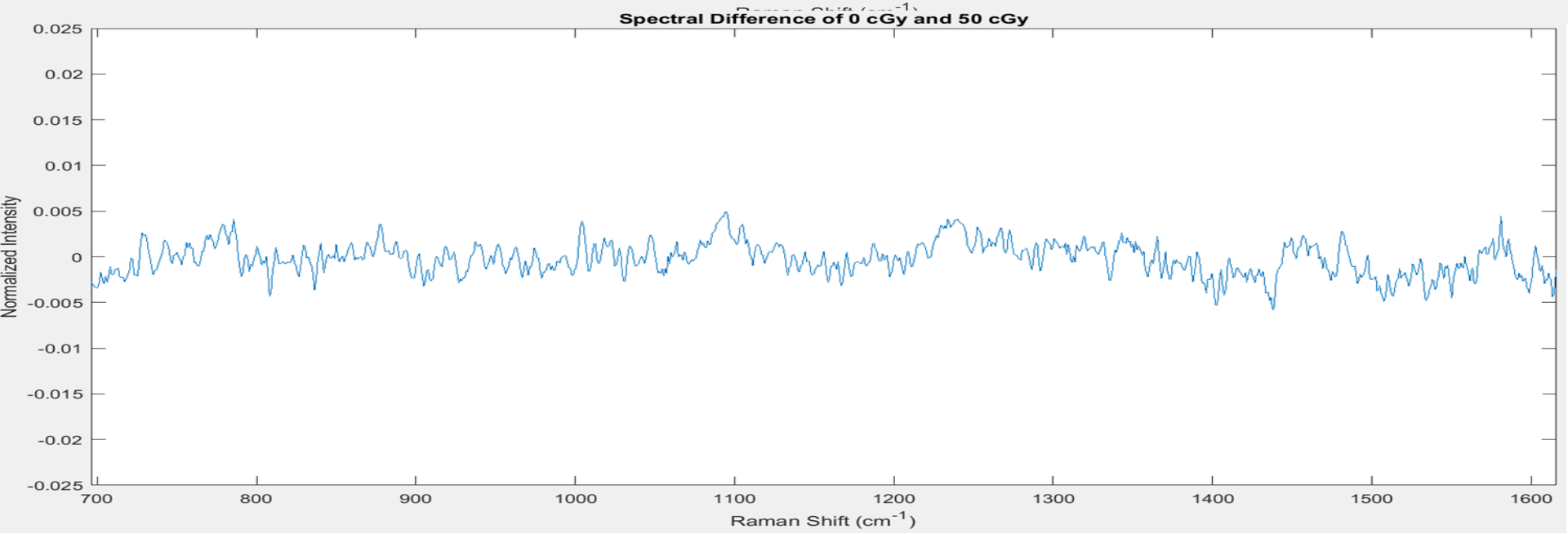
50-fold cross-validation

- 79.5% accuracy

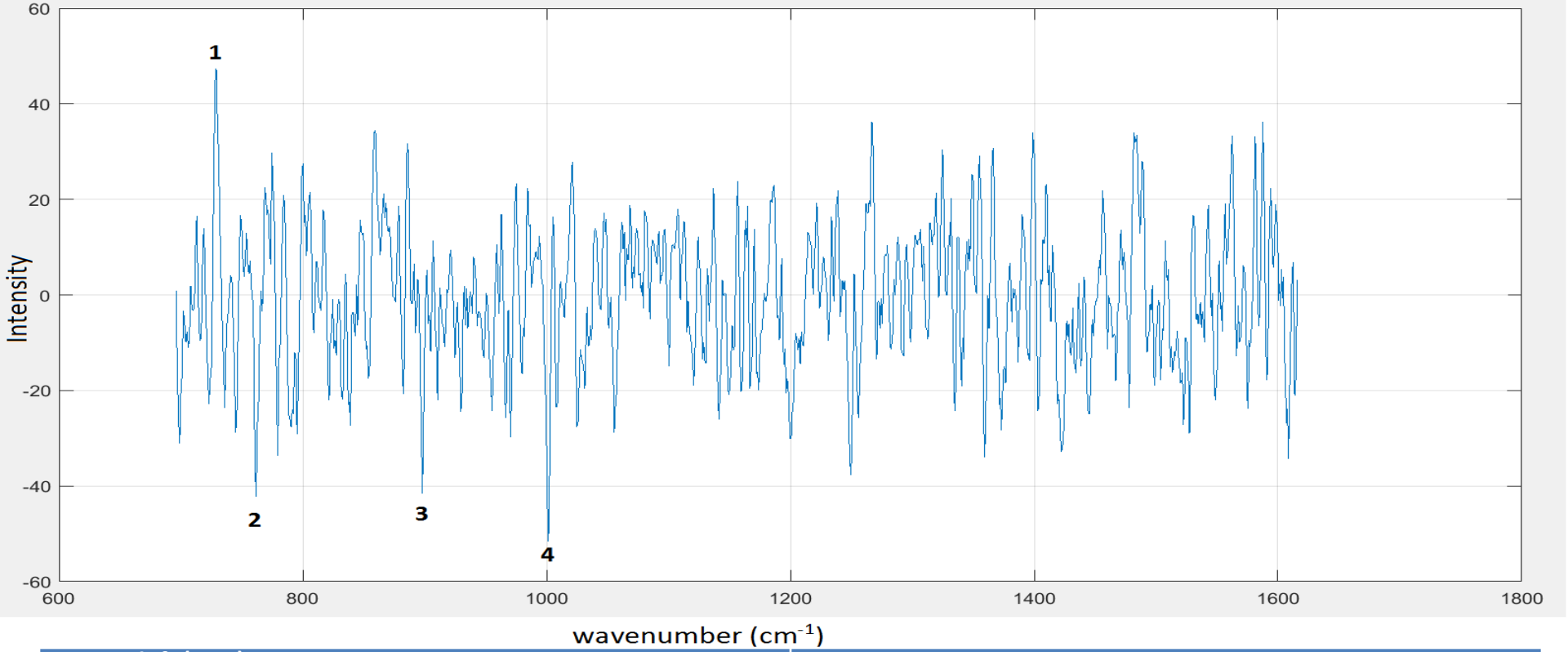


Difference Spectra



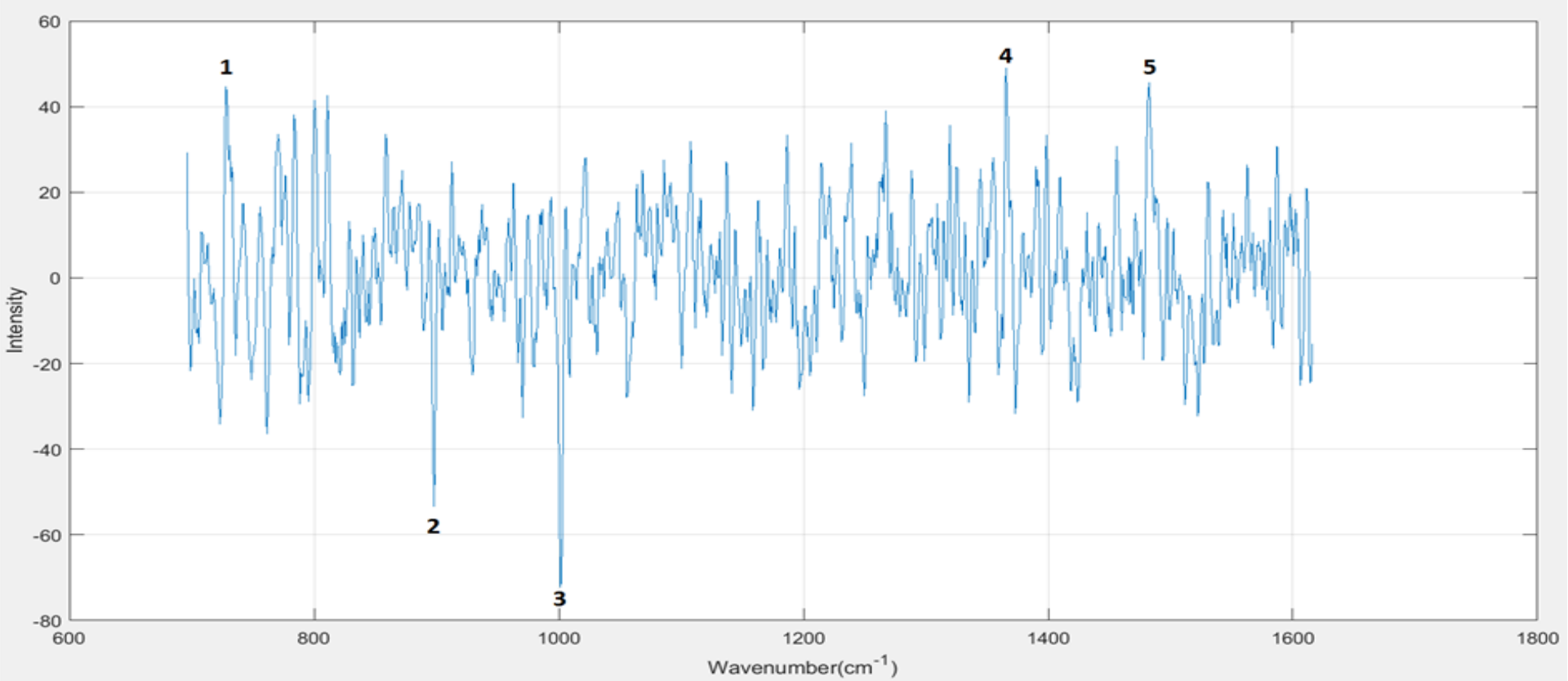


LD Loadings Spectra for 0 and 25cGy



Raman Shift (cm^{-1})	Assignment
729	A (ring breathing mode of DNA/RNA bases)
762	Tryptophan
898	Monosaccharides (β-glucose), (C-O-C) skeletal mode
1002	C-C aromatic ring stretching, Phenylalanine (collagen assignment)

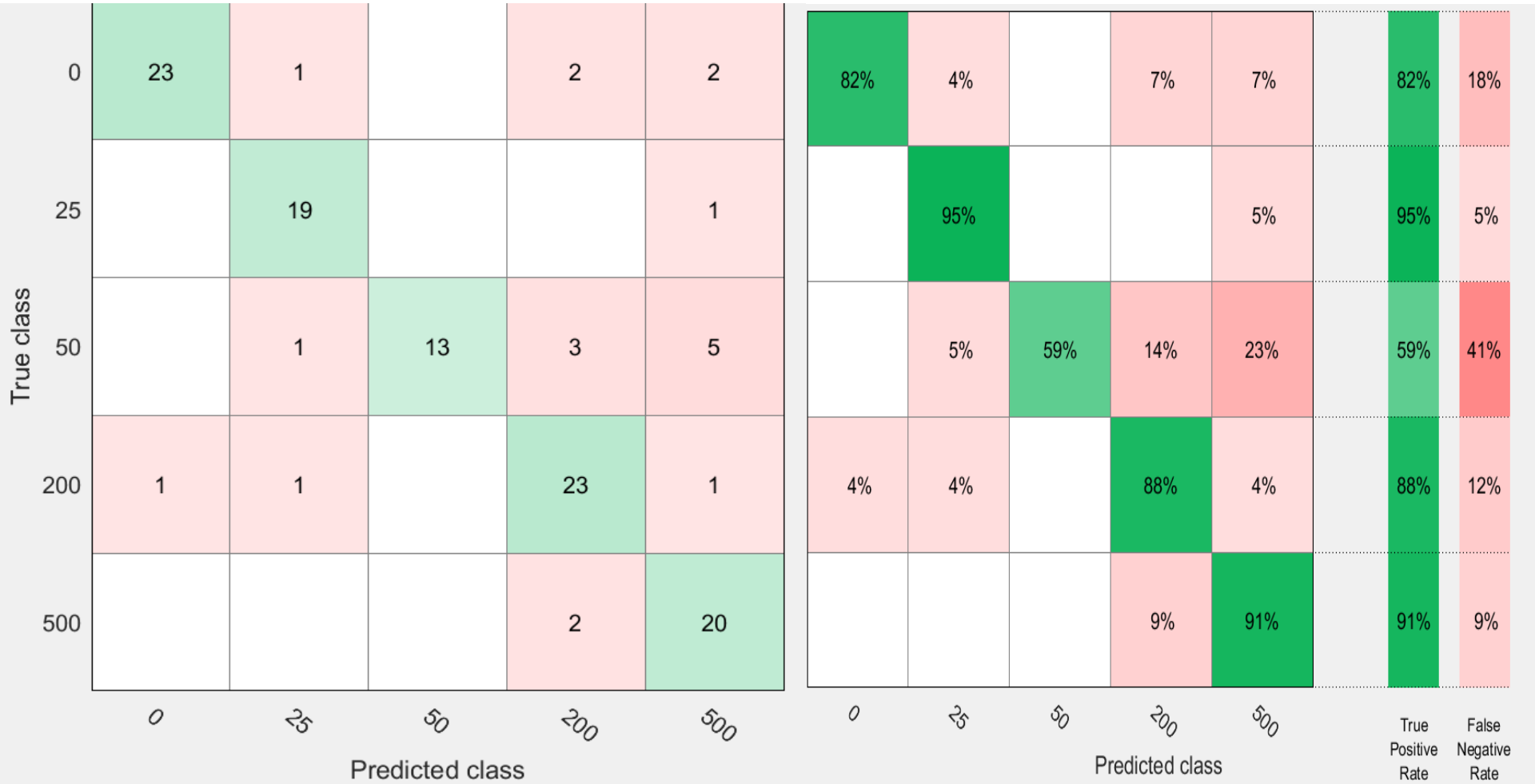
LD Loadings Spectra for 0 and 200cGy



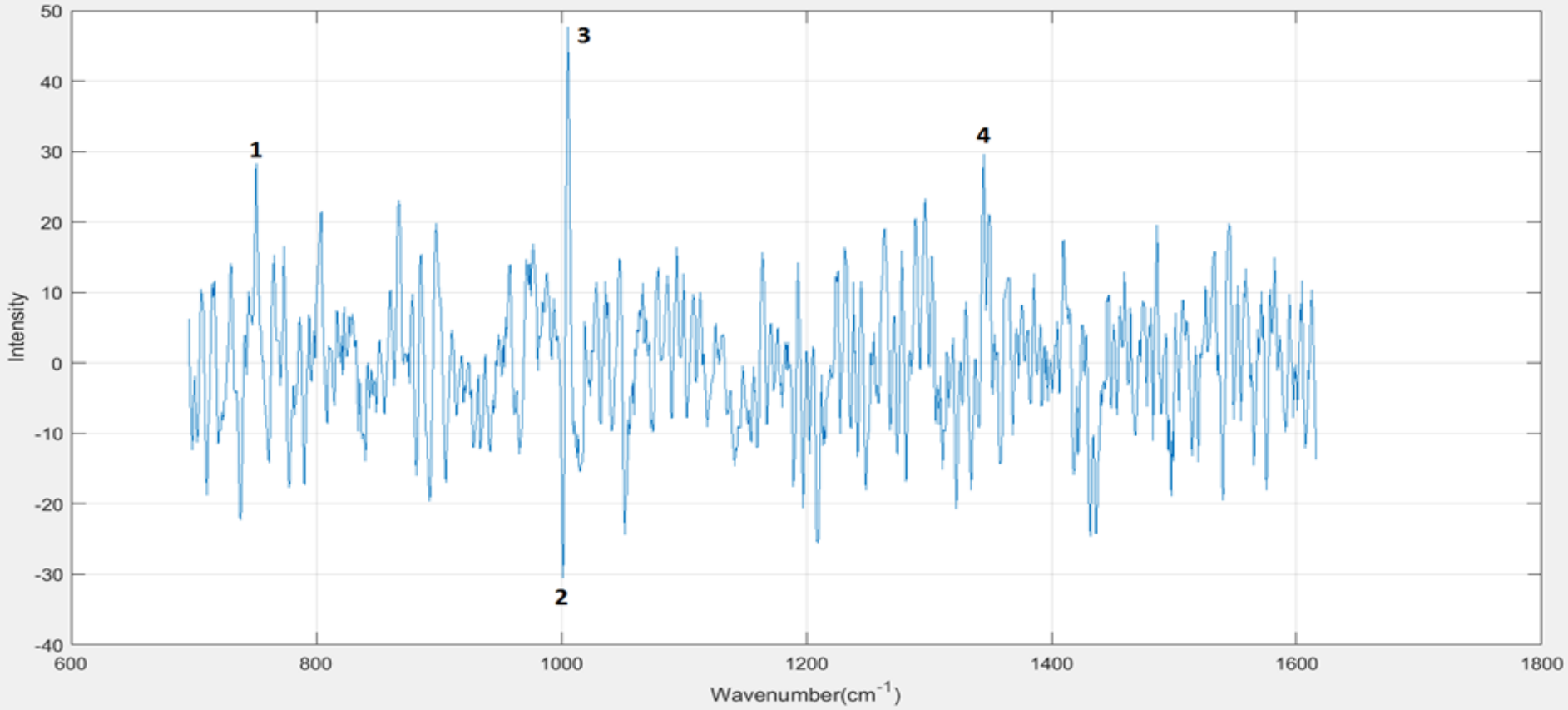
Raman Shift (cm^{-1})	Assignment
728	Ring breathing of tryptophan
898	Monosaccharides (β-glucose), (C-O-C) skeletal mode
1002	C-C aromatic ring stretching, Phenylalanine (collagen assignment)
1365	Tryptophan
1483	Amides

Confusion Matrix for cytoplasm

- 83.1% accuracy (test set)

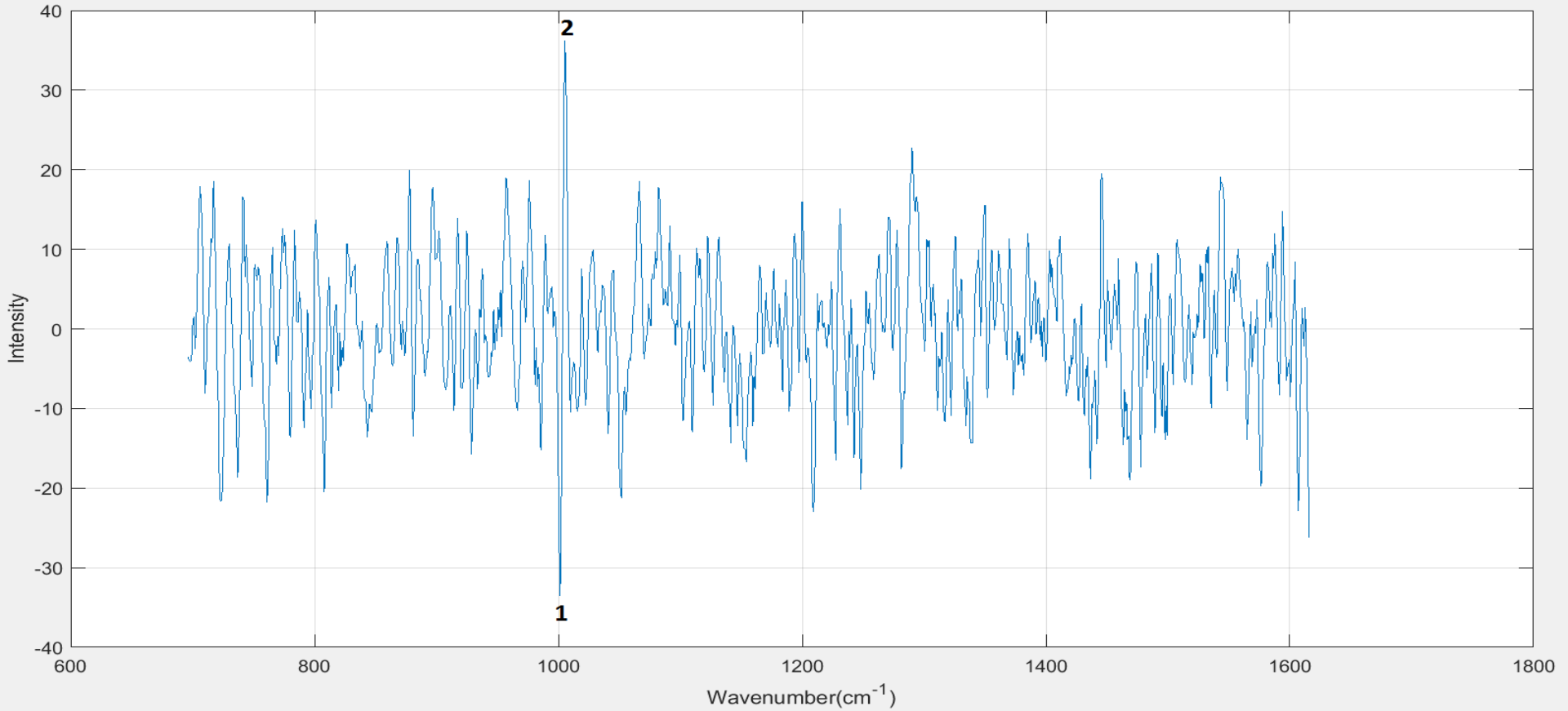


LD Loadings Spectra for 0 and 25cGy



Raman Shift (cm^{-1})	Assignment
751	Mitochondrial DNA
1002	C-C aromatic ring stretching, Phenylalanine (collagen assignment)
1005	Phenylalanine
1345	glucose

LD Loadings Spectra for 0 and 200cGy



Raman Shift (cm^{-1})	Assignment
1002	C-C aromatic ring stretching, Phenylalanine (collagen assignment)
1005	

Conclusion

- An accuracy of ~80% was achieved in classifying different classes
- Molecular concentration changes was determined for irradiated cells.

Challenges and Opportunities

- New non-linear Machine learning algorithms – issue of molecular assignment?
- Automation of data acquisition process- currently 12 hours/cell sample-increasing laser intensity, etc
- Fixed to live cell analysis

Thank You