

PhD Thesis title: 'Single-cell Raman spectroscopy of irradiated tumour cells'

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ABSTRACT:

This work describes the development and application of a novel combination of single-cell Raman spectroscopy (RS), automated data processing, and principal component analysis (PCA) for investigating radiation induced biochemical responses in human tumour cells. The developed techniques are first validated for the analysis of large data sets (~200 spectra) obtained from single cells. The effectiveness and robustness of the automated data processing methods is demonstrated, and potential pitfalls that may arise during the implementation of such methods are identified. The techniques are first applied to investigate the inherent sources of spectral variability between single cells of a human prostate tumour cell line (DU145) cultured *in vitro*. PCA is used to identify spectral differences that correlate with cell cycle progression and the changing confluency of a cell culture during the first 3-4 days after sub-culturing. Spectral variability arising from cell cycle progression is (i) expressed as varying intensities of protein and nucleic acid features relative to lipid features, (ii) well correlated with known biochemical changes in cells as they progress through the cell cycle, and (iii) shown to be the most significant source of inherent spectral variability between cells. This characterization provides a foundation for interpreting spectral variability in subsequent studies. The techniques are then applied to study the effects of ionizing radiation on human tumour cells. DU145 cells are cultured *in vitro* and irradiated to doses between 15 and 50 Gy with single fractions of 6 MV photons from a medical linear accelerator. Raman spectra are acquired from irradiated and unirradiated cells, up to 5 days post-irradiation. PCA is used to distinguish radiation induced spectral changes from inherent sources of spectral variability, such as those arising from cell cycle. Radiation induced spectral changes are found to correlate with both the irradiated dose and the incubation time post-irradiation, and to arise from biochemical differences in lipids, nucleic acids, amino acids, and conformational protein structures between irradiated and unirradiated cells. This study is the first use of RS to observe radiation induced biochemical effects in single cells, and is the first use of vibrational spectroscopy to observe such effects independent from cell cycle or cell death related processes. The same methods are then applied to a panel of human tumour cell lines, derived from prostate (DU145, PC3, LNCaP and PacMet), breast (MDA-MB-231 and MCF7) and lung (H460), which vary by p53 gene status and intrinsic radiosensitivity. One radiation induced PCA component is detected for each cell line by statistically significant changes in the PCA score distributions for irradiated samples, as compared to unirradiated samples, in the first 24 to 72 hours post-irradiation. These RS response signatures arise from radiation induced changes in cellular

concentrations of aromatic amino acids, conformational protein structures, and certain nucleic acid and lipid functional groups. Correlation analysis between the radiation induced PCA components separates the cell lines into three unique RS response categories: R1 (H460, MCF7 and PacMet), R2 (MDA-MB-231 and PC3), and R3 (DU145 and LNCaP). These RS categories partially segregate according to radiosensitivity; the R1 and R2 cell lines are radioresistant and the R3 cell lines are radiosensitive (PacMet radiosensitivity (R1) unknown). The R1 and R2 cell lines further segregate according to p53 gene status, corroborated by cell cycle analysis post-irradiation. Preliminary results obtained from a mouse prostate tumour cell line (TRAMP-C2), irradiated both *in vitro* and *in vivo*, indicate that RS signatures of radiation response may also be detectable from tumour cells obtained from an *in vivo* system during radiation therapy treatment. These results indicate the potential for future RS studies designed to investigate, monitor, or predict radiation response.

References to author publications that relate specifically to the dissertation:

1. Matthews, Q., Jirasek, A., Lum, J.J., and Brolo, A.G. "Biochemical signatures of *in vitro* radiation response in human lung, breast and prostate tumour cells observed with Raman spectroscopy". *Physics in Medicine and Biology*, 2011, *56(21)*, 6839-6855.
2. Matthews, Q., Brolo, A.G., Lum, J.J., Duan, X. and Jirasek, A. "Raman spectroscopy of single human tumour cells irradiated *in vitro*". *Physics in Medicine and Biology*, 2011, *56(1)*, 19-38.
3. Matthews, Q., Jirasek, A., Lum, J.J., Duan, X. and Brolo, A.G. "Variability in Raman spectra of single human tumor cells cultured *in vitro*: correlation with cell cycle and culture confluency". *Applied Spectroscopy*, 2010, *64(8)*, 871-887.