Purpose: To use non-invasive single-cell Raman spectroscopy (RS) and principal component analysis (PCA) to analyze radiation-induced biochemical changes in human tumour cell lines, and to correlate results with known radiosensitivity.

Methods: Six human tumour cell lines, derived from prostate (DU145, PC3, and LNCaP), breast (MDA-MB-231 and MCF7) and lung (H460), were irradiated with single fractions (15, 30 or 50 Gy) of 6 MV photons. Remaining live cells were harvested for RS analysis at 0, 24, 48, and 72 hours post-irradiation, along with unirradiated controls. Single-cell Raman spectra were acquired from 20 cells per sample with a Raman microscope utilizing a 785 nm excitation laser. All spectra (200 per cell line) were post-processed, and the total data set for each cell line was analyzed with PCA using standard algorithms.

Results: One unique radiation-induced PCA component was detected for each cell line by identification of 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. Correlation analysis between radiation-induced PCA components from the different cell lines shows that these RS radiation response signatures fall into 3 distinct RS categories: R1 (H460 and MCF7), R2 (MDA-MB-231 and PC3), and R3 (DU145 and LNCaP). These RS categories segregate according to radiosensitivity and p53 gene status. The R1 and R2 cell lines are radioresistant ($SF2 > 0.6$), whereas the R3 cell lines are radiosensitive ($SF2 < 0.5$). The R1 and R2 cell lines further segregate according to p53 status, as verified by cell cycle analysis post-irradiation.

Conclusions: This study demonstrates that RS is a powerful tool for non-invasive radiobiological investigations, and is a promising technique for advancing the field of personalized radiation therapy.