Understanding chemometric separation of cell lines: Biochemical signatures versus physical effects

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Previous work has demonstrated it is possible to discriminate prostate cancer cell lines using the vibrational techniques of FTIR spectroscopy and FTIR-photoacoustic spectroscopy[1, 2]. Herein we further demonstrate the combined use of these techniques not only support such previous findings, by application to a different urological cancer, to principal component analysis (PCA), but also to make a firm claim that separation is based on biochemical difference rather than physical differences in individual scattering profiles due to cell morphology.

Cell lines 2245R, 2247R established from primary kidney resections and 2246R from a metastatic pleural nodule were derived from different surgical specimens from different patients with confirmed clear-cell renal carcinoma at the N.C.I., U.S.A. Relative to the other cell lines, 2247R displayed a very aggressive phenotype. On visual inspection they appear mesenchymal-like in structure being smaller and rounded with stellate growth characteristics, expanding radially as compared with the classical epithelial morphology of 2245R and 2246R cells. The 2247R cells proliferate at a greater rate without contact inhibition leading to piling up of the cells in culture. The different morphological appearance and associated differences in growth characteristics of these cell lines enabled us to determine whether chemometric discrimination was based purely on biological differences or scattering properties.
Single point FTIR transflection spectra were taken of global populations using cell mono-layers grown onto MirrIR slides. The data set was pre-processed using an advanced scattering correction programme, which includes recent further understanding of the origins of the 'dispersion artefact' attributed to resonant Mie scattering[3, 4], before PCA. FTIR-Photoacoustic spectroscopy was used as a supportive comparison as the technique is relatively free from scattering effects. Both techniques resulted in the separation of the 2247R cell line from the other two cell lines, suggesting separation based on a biochemical difference as opposed to the cell lines various scattering profiles.
The impact of this result is that not only was the scattering correction program robust, but that the combined spectroscopic and chemometric technique is a valid tool in cell line characterisation, supporting established biological methods.

References:

[1] T. J. Harvey, A. Henderson, E. Gazi, N. W. Clarke, M. Brown, E. C. Faria, R. D. Snook and P. Gardner, *Analyst* 132, 292-295 (2007).
[2] T. J. Harvey, E. Gazi, A. Henderson, R. D. Snook, N. W. Clarke, M. Brown and P. Gardner, *Analyst* 134, 1083-1091 (2009).
[3] P. Bassan, H. J. Byrne, F. Bonnier, J. Lee, P. Dumas and P. Gardner, *The Analyst* 134, 1586-1593 (2009).
[4] P. Bassan, H. J. Byrne, J. Lee, F. Bonnier, C. Clarke, P. Dumas, E. Gazi, M. D. Brown, N. W. Clarke and P. Gardner, *Analyst* 134, 1171-1175 (2009).