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Raman Vibrational Imaging of Colorectal Cancer Progression

Label-free coherent Raman allows the label-free imaging of cancer phenotypes in live cells, providing simultaneous biochemical and morphological information. Here, we use stimulated Raman imaging (SRS) to investigate colorectal cancer progression. Our prior work used spontaneous Raman to analyse a panel of 4 colorectal cancer cell lines [1]. We followed this work by extending the panel to 5 colorectal cancer cell lines and a healthy colon epithelial cell line model, using hyperspectral SRS imaging.

Raman imaging videos of live cell processes confirmed minimal phototoxicity. We collected a large dataset of adhered live cell images including hyperspectral SRS of the CH-stretching region and selected fingerprint channels (1660 cm^{-1} and 1455 cm^{-1}), revealing stage-dependent biochemical and morphological variation. To develop a pipeline for the analysis of heterogeneous patient samples, cellpose was used to segment the images into single cells (Fig 1c). Fig 1a shows example images and average single-cell SRS spectra for each cell line. Within cell, SRS hyperspectral imaging showed composition heterogeneity of lipid droplet (Fig 1b). Machine-learning analysis of nearly 5,000 single-cell images demonstrated accurate classification of cancer stage with a mean test accuracy of 77.3%, and classification matrix shown in Fig 1d. The machine learning model returned ~100% accuracy when splitting at the image level, highlighting the importance of splitting the dataset at the session level for robust model generalisation.

Together, these studies demonstrate the potential of hyperspectral Raman imaging as a scalable platform for label-free cancer phenotyping. By combining biochemical fingerprints, morphology and computational analysis, coherent Raman approaches show promise for future applications in patient stratification.

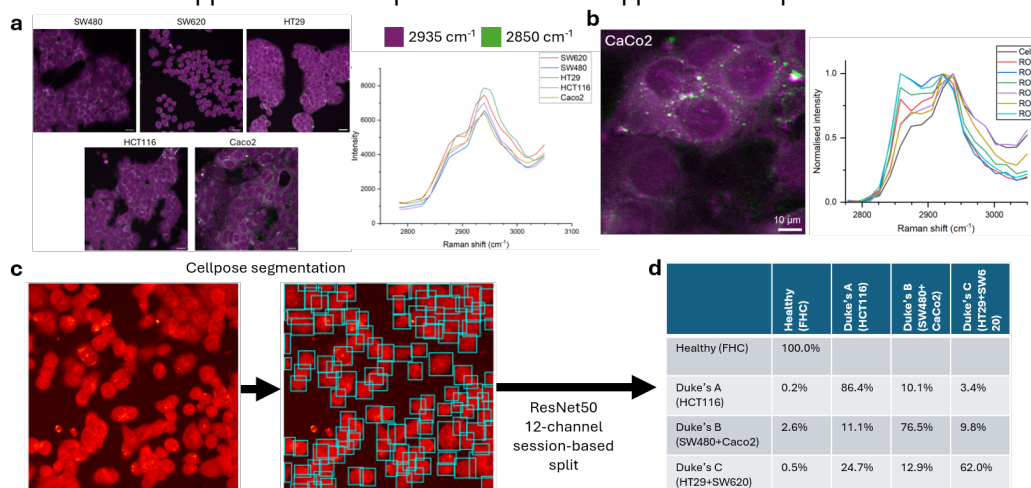


Figure 1: a) SRS images of 5 different live colon cancer cell lines, showing two overlaid channels representing proteins (2950 cm^{-1}) and lipids (2850 cm^{-1}). The average single-cell signal is shown. b) Live CaCo2 cells image, where lipid droplets that did not move during acquisition were identified and their hyperspectral SRS signal heterogeneity is shown. c) Segmentation process to obtain single cell images for machine learning training. d) Classification matrix, where sessions 1 and 2 were used for training and validation, with session 3 used for testing.

References

- [1] J. Gala de Pablo et al., J. Raman Spectrosc. 49 (2018), 1323–1332.
- [2] C. J. Kirkby et al., Analyst. 146 (2020), 581–589.