IR microspectroscopy of single live cells

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The objective of the present study was to establish the conditions for biologically relevant infrared spectroscopy of single living cells in cell culture under aqueous media, using the high brilliance synchrotron light source to achieve optimal S/N at high spatial resolution. This is experimentally challenging, but brings a number of advantages: in addition to the obvious issues of avoiding possible artefacts and increasing the perceived relevance for biomedical scientists, the use of living cells raises the possibility of monitoring the spectral changes resulting from perturbation of a single cell in real time. In analogy to the use of difference spectroscopy for isolated biomolecules, this should yield detailed spectral data due the precise background subtraction, and is thus more likely to yield insights at the molecular level.

Various human colorectal cancer cultures were studied (HT29, sw480, WIDR, CaCo2), together with human fibroblasts (primary culture) and human umbilical vein endothelial cells as healthy controls, in both confluent and non-confluent cultures. Cell cultures were grown on CaF_2 windows, which were then assembled into a flow cuvette with a 12 µm pathlength.

For each cell type, we recorded the spectra of individual cells in groups of 50 from a number of independent cultures. This aspect of our study targets the question of whether area measurements covering a number of cells are a suitable approach for diagnostic applications, i.e. whether the average alone is sufficient to describe a cell population and distinguish it from other populations.

We also collected data from the nuclei and cytoplasm of several hundred cells in order to assess the spectral differences between these cellular compartments. In all of these experiments, the spectral differences are a very small proportion of the total absorbance, so that some experimentation with advanced statistical techniques is still needed to confirm significant trends.

Finally, we carried out some initial experiments using treatment of the cells to assess the potential of the technique for recording biologically relevant changes. The first goal of was to establish baseline conditions for stable long-term (up to 8 hr) monitoring of single living cells, and to establish the reproducibility of such baseline measurements across individual cells from the same cell culture and across different cultures.

Vanadate and hydrogen peroxide treatments were used as models of perturbation of protein phosphorylation and sulfhydryl bridge oxidation/reduction, respectively. A clear spectral change was observed on addition of vanadate to the cell culture medium reservoir, which appeared not to be attributable merely to the spectrum of the reagent itself. The spectral region affected was consistent with the expected inhibition of protein phosphorylation. Hydrogen peroxide treatment appeared to produce spectral changes in the region expected for -SH vibrations. However, these conclusions are still quite preliminary and need to be checked for reproducibility.