ALL-OPTICAL MULTIMODAL LABEL-FREE ANALYSIS FOR HIGH-THROUGHPUT SCREENING OF CELL TYPE AND BEHAVIOUR

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Our laboratory has been interested in developing label-free methods to probe the innate information in immune cells, as well as see how they respond to stimulation. Such information can be highly useful either to classify whether disease is present, or to understand the nature and features of a disease. A complicating factor in trying to understand the features of cells, as well as features of populations of cells is the issue that each cell generally has slightly different morphology as well as chemical makeup.

We developed the first imaging system to combine digital holographic microscopy (quantitative phase) with Raman analysis [1]. The two modes give complementary information on the morphological and chemical features of a cell. Phase measurements are also more dominated by proteins in the cell, whereas Raman measurements show stronger contributions from lipids in the cell. From these results, we were also able to determine that for use in high-throughput analysis, we could simplify the measurement approach. The use of quantitative phase to rapidly image the cells, while Raman is used not in an imaging mode, but in a point-wise manner to collect the “spectral signature” of each cell, allows quantitative morphological features to be extracted, with high signal to noise Raman information. In fact, the smart scanning of the cell, where the Raman excitation spot is scanned rapidly through the cell and then descanned before the detector, can be ideal. This approach allows all the Raman shifted signal to be optically binned at the detector, minimizing readout noise and maximizing the spectral information gained. The variance in measured data was spread throughtout a higher number of principal components when using this optical binning approach that we termed “hybrid scanning”. These results have applications for other scientists working towards high thoughput methods that are challenging due to signal-to-noise considerations.

We will present these results in this presentation, and give an overview of how our methods might be applied in a microfluidic environment, along with some of the challenges that result. Ideally, these methods could be used for fully label-free analysis of the degree of activation of macrophages, as well as the identification and statistics of different lymphocyte types in biofluids. To this end, we recently showed the degree to which different lymphocyte cell lines can be classified, using only single point Raman analysis, with relatively high throughput.

REFERENCES:

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