# *A novel technique for the extraction of single cell spectra from an IR image*

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In recent years there has been growing interest in the study of single biological cells using infrared micro-spectroscopy. This technique enables the global chemistry of a cell to be analysed and compared from cell to cell. Unfortunately, using a laboratory based infrared microscope and a thermal source is not possible to obtain spectra from an area the size of a cell in a realistic timeframe. In order to get round this limitation researchers have exploited the considerable brightness of a synchrotron source which means a spectrum of a single cell with good signal to noise ratio can be obtained in about 1 min. This, however, is still not a high throughput process since each cell has to be measured sequentially. In addition measurement time at synchrotrons is limited and very expensive.

In this project an alternative method is being developed in which a laboratory based imaging system is used to obtain an infrared image of a large area containing hundreds of cells. The image takes longer to obtain than at a synchrotron but the multiplex advantage means that the total time taken to analyse say 100 cells is faster. Up until now we have not been able to exploit this advantage since there was no way of linking the spectra from the image to a particular cell in the image.

The aim of this work was to perform the extraction of those spectra that are related with a single cell within an IR image. In order to achieve this objective, a MATLAB algorithm was written, it uses as an input the entire IR image and the heat map of the surface, based upon these images the algorithm enables the user to extract the following information:

a) The number of cells identified while performing the algorithm,
b) The precise location of each cell,
c) How many spectra make up each cell, and
d) The average spectrum per cell contained in the field of view.

After doing this (which takes about 1 or 2 seconds in displaying the new data sets and the proper graphs), the user is allowed to discard all those spectra that might come from cell debris by observing simultaneously the visible image of the field of view and the total absorbance map of the IR image.

As a result of the application of this new algorithm we obtain as many averaged spectra as cells contained within the IR image and therefore their location is known as well as reducing the amount of time invested in collecting individual spectra per cell.

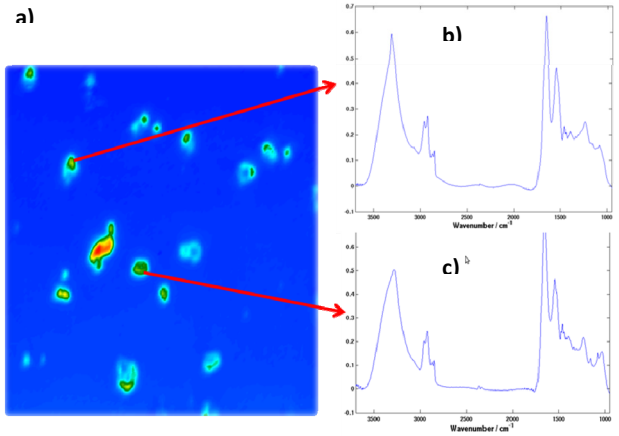


**Figure 1. a)** IR image of several Renal Carcinoma Cells grown and formalin fixed onto a MirrIR slide; **b)** and **c)** show the corresponding averaged spectrum per cell after being corrected by the RMieS algorithm.

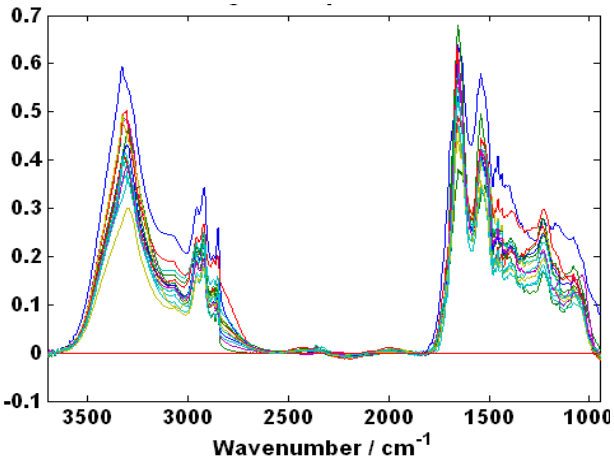


**Figure 2.** Average spectra per cell contained in the IR image after being corrected using the RMieS algorithm.