UTILISING PAIRED RATIOMETRIC PROBES AND CONFOCAL MICROSCOPY FOR THE MEASUREMENT OF CALCIUM FLUX IN T CELL SIGNALLING

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Calcium flux plays a pivotal role in T cell signalling, controlling both early and late stage effector responses. Fluorescent calcium probes together with flow cytometry have usually been used to detect and measure cumulative calcium ion concentration changes in populations of immune effector cells. Whilst this technique has proven useful for correlating differences in functional outcomes in immune cell populations containing mutated signalling pathways, it cannot be used to discriminate among the heterogeneous activation responses of individual cells, which belong to diverse populations of immune cells.

Here, we utilise paired ratiometric probing and laser scanning confocal microscopy for the measurement of calcium flux within single cells activated on solid state platforms functionalised with stimulatory T cell ligands. The ability to interrogate individual cells in real-time was compared with flow cytometric techniques, and demonstrated heterogeneous calcium flux patterns in T cells responding to presumably ‘equivalent’ ligands. Interestingly, when these individual responding cells were pooled and averaged, they reveal a similar calcium flux pattern observed in the whole population as measured by flow cytometry. These data highlight the importance of analysing single T cell responses to enable functionally significant cells to be distinguished from functionally irrelevant cells.

References:
