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A New Technique for Imaging Real-Time Cytokine Secretion

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A NEW TECHNIQUE FOR IMAGING REAL-TIME CYTOKINE SECRETION

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The analysis of single cell cytokine secretion has become an area of great interest in research relating to the immune system and disease. Cytokines, the small proteins that cells secrete for signaling, are used to categorize immune cell's responses to disease, drugs, or other stimulation. The most advanced widespread assay for single cell cytokine secretion is the fluorospot assay, a technique that utilizes multiple fluorescent markers to visualize the secretion of two or more cytokines simultaneously. However, this assay only provides data about the percentage of total cells that are secreting cytokines at a fixed time point, because cells must be removed from the plate before imaging. Therefore, our goal is to develop an assay that retains cells individually, allowing for continuous analysis of single cell secretion over a time course. In this work, we use T cells latently infected with a GFP-tagged HIV virus, which allows us to visualize the activation of the virus and cell secretion in response to this activation. In the assay we developed, a microfluidic device containing pillar traps is used to suspend the cells individually. A channel in the device contains the traps, which are coated in a capture antibody. Flowing through a stimulant activates the latent HIV in the cells. Cytokine secretions are visualized using fluorescently tagged antibodies. We have successfully detected IL-2 secretion by individual J Lat 10.6 cells in a fixed-time version of this assay, and are working to perform this technique over a 6 to 18 hour time course. We will utilize this technique to explore the timing of the activation of latent HIV in relation to cytokine secretion, and will use image processing to quantify the secretion of each cell. The technique we are developing will also be applicable in other contexts, as it will ultimately enable identification and quantification of single cell cytokine secretion over a long period of time.