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Can parallel single cell assays support diagnostics in tuberculosis?

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Current biological tools to measure protein and mRNA abundance average the responses of the large number of cells present in a sample, thereby concealing cell-cell heterogeneity and limiting the analysis of rare cell types. While single cell analysis may be more desirable, the tools and methods to do these measurements are not widely available. We have developed a qRT-PCR approach to measure mRNA expression from single cell samples, and are developing a microfluidic ELISA tool to measure multiple proteins simultaneously from single cell samples. These techniques have been used to probe the spectrum and influence of cell-cell heterogeneity on immune responses by defining coordinate response patterns from individual cells and from multiple cells in parallel.

Requirement for success in achieving single cell measurements include: 1) precise nanoliter-volume fluid handling; 2) sufficient sensitivity to detect single cell responses; 3) multiplexing to measure more than one analyte from each cell sample; 4) a means to biochemically process single cell samples; and 5) methods to be able to process multiple samples in parallel. Remarkably, there are technical solutions to these challenges. However, a major limitation is the current lack of understanding of the biological significance of the very heterogeneous properties of individual cells in a population. Biological understanding of cell behavior will require reference datasets to be established so that normal/diseased responses can be classified, and the significance of the absolute abundance of a given protein within a given cell can be understood. Also, current methods focus on candidate genes and proteins, and an additional challenge is identifying which analytes are most appropriately measured to be useful for TB diagnostics and monitoring responses to therapy and vaccination.

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