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The next frontier in single cell analysis: multimodal studies and clinical translation

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Biological processes are inherently complex. Stochasticity, redundancy, plasticity, and noise are built into fundamental cellular activities from gene transcription to protein expression. A major challenge in biomedical research is to untangle this complexity.

Over the past 30 years, conceptual and technical advances have led the scientific community to appreciate the value of measuring multiple cellular markers at once. The concept was broached as early as 1983, when antibodies coated onto a solid surface were used to isolate cells expressing different surface proteins,[1] but achieved widespread use 20 years later through microarray-based analysis of genomic transcripts. Microarray technology influenced biological research because it demonstrated clearly the wide selection of cellular molecules available for measurement and provided an efficient means to query them. Microarray studies revealed, for example, the identities of molecules associated with immune responses and cell signatures associated with cancer subtypes and patient outcomes. The latter work was influential in developing the concept of personalized or precision medicine, and tied microarrays and lab-on-a-chip platforms into this new paradigm of clinical research.

Microarrays, however, require a large amount of material and assay large numbers of cells together in bulk. Bulk measurements are limited because they average information across heterogeneous cell types and fail to identify the precise cell types involved in a process. A similar problem arises from multiplexed ELISAs for measurement of cytokines in blood or cell culture: the cells secreting the cytokines into the sample matrix cannot be identified.

Single cell analysis overcomes the problems of bulk measurements, but for many years the only available technology—flow cytometry—was incapable of highly multiplexed measurements. The past 5–10 years have brought dramatic changes to the field as multiple flow cytometry platforms can now measure more than 25 cell surface markers simultaneously, and a host of other single cell analysis technologies have emerged. For example, single cell RNA-seq[2] and droplet microfluidics platforms that physically isolate single cells such as Drop-seq[3] have reduced the time, cost, and amount of sample required to measure the transcriptome of individual cells. In parallel, bioinformaticians are tackling the large amount of data that can be generated in single cell analysis and have developed elegant tools for corralling the unusually deep and complex data produced by high parameter single cell analysis platforms.

The current movement in single cell analysis is multimodal characterization. These approaches, which are rapidly replacing one-dimensional single cell analysis in biomedical research, simultaneously combine measurements of transcription with post-transcriptional regulation, epigenetic modifications, and surface protein expression. It is possible that lipid and metabolite composition, and/or cellular morphology may also be analyzed with the transcriptome or proteome. The NIH's National Cancer Institute, for example, is currently soliciting applications for "combinations of tools for multiplex analysis and/or manipulation of single cells to maximize data content over many parameters". Moreover, there is particular interest in application of "tools that enable and transform single cell analysis in clinical tissue biopsies". A recent example of multimodal analysis combines detection of cell surface proteins and cellular mRNA, using a technique called CITE-seq,[4] which uses single cell sequencing to detect antibody–oligonucleotide conjugates that bind proteins, alongside cellular gene transcripts. This tool was recently expanded to allow simultaneous measurement of five or more modalities including TCR clonotypes and CRISPR-mediated perturbations (ECCITE-seq[5]). Bioinformatics tools have been developed to integrate multiomic data,[6] and we are in the early phases of

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evaluating the utility of these algorithms for studying disease.

And so the scientific community is faced with new challenges. We now have a dizzying array of tools that provide us with the potential to comprehensively and accurately characterize the cells involved in a biological process. We are a step away from using these tools widely and efficiently to impact clinical care, but there are large obstacles we must break down first. With a better understanding of the complexity ingrained in cellular systems, how do we smartly choose subsets of markers and cell types to survey, remembering that samples from patients are often limited as are research budgets? Once we know what to measure, there is the critical question of how to measure it, since there are a myriad of technical platforms and data analysis tools from which to choose. As we make measurements, how do we ensure that they are robust—are there general validation and quality control principles we can establish, or are such measures wholly platform-specific? Finally, are highly multiplexed, single cell technologies valuable only as a screening tool to identify simple biomarkers, or can these highly complex technologies (and their associated data analysis algorithms) be used directly for clinical diagnostics? This is a key question that will impact how research institutions plan their investments in these technologies, and will ultimately shape the application and adoption of precision medicine.

We invite authors to submit manuscripts to suggest answers to these questions and related issues for inclusion in a thematic collection focused on multimodal single cell analysis. Here, in this thematic collection, *Lab on a Chip* highlights new advances in the rapidly growing field of single cell analysis.

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