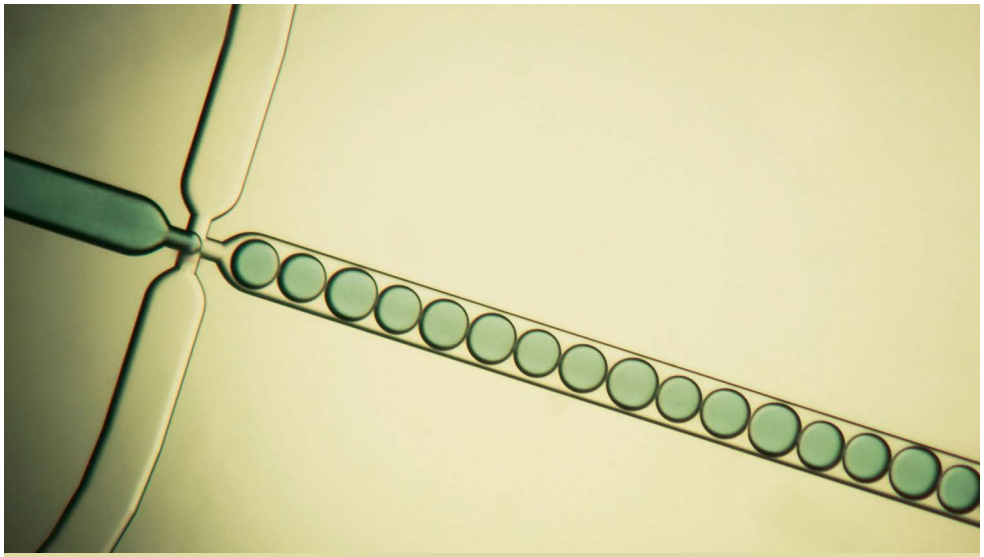

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MILESTONE 11

Sequencing one cell at a time

Bulk analysis of tissue-based gene expression results in averages that mask contributions of individual cell types. Single-cell genomic approaches are needed to understand developmental processes, homeostatic function and disease initiation and progression. The development of single-cell techniques has enabled analysis of cell states, rare cell types, developmental trajectories, lineage relationships, diseased states and tumour heterogeneity.

Laying the groundwork for these studies, in 2009 Tang et al. performed the first whole-transcriptome analysis of a single mouse blastomere, adapting an amplification method to generate longer cDNAs in an efficient, unbiased manner. This method could detect the expression of 75% more genes than traditional microarrays, while finding new splice junctions.

Islam et al. were among the pioneers who piloted scaled-up single-cell RNA sequencing (scRNA-seq). Sequencing 85 cells of two distinct types, they used single-cell expression profiles to generate 2D maps that clustered cells based on similarities in expression. They could visualize distinct cell populations without a priori knowledge of markers previously used to classify these cell types.

Subsequently, Ramsköld et al. developed Smart-seq to enable single-cell analysis of more cells with enhanced read coverage. Testing the method on circulating tumour cells from melanomas, they were able to identify potential biomarkers for melanoma circulating tumour cells based on distinct gene expression patterns. Compared with the approach of Tang et al., the method showed improved identification of single-nucleotide polymorphisms and alternative transcript isoforms, while quantifying sensitivity and accuracy.

To increase scalability by another three orders of magnitude, allowing for single-cell analysis of entire tissues, the technology had to transition to high-throughput functionality. Two groups dedicated to this goal both turned to microfluidics.

In 2015, Macosko et al. introduced Drop-seq for profiling thousands of cells by encapsulating them into nanolitre-sized droplets, each of which is linked to a unique barcode that connects each mRNA transcript to its cell of origin. Sequencing 44,808 mouse retinal cells identified 39 distinct cell clusters, creating a gene expression atlas with known and newly discovered retinal cell types.

The same year, Klein et al. developed inDrop, which also separates cells into individual droplets that contain hydrogels carrying barcoded primers to connect each cell to its transcriptome. They sequenced >10,000 mouse embryonic stem cells and tracked the onset of differentiation following leukaemia inhibitory factor withdrawal, revealing the population dynamics of differentiating embryonic stem cells. With this technical advance enabling the rapid and efficient sequencing of >10,000 cells, huge datasets were generated — this called for the development of computational techniques to keep pace. Previous strategies needed to be modified to integrate data from different experiments, species, time points or treatment groups.

Researchers were keen to apply these high-throughput methods to the investigation of different tissues at the single-cell level. Haber et al. profiled individual murine small intestine epithelial cells directly after harvest or following organoid culture. Cells clustered into 15 distinct subpopulations, revealing unexpected diversity and two previously unknown subtypes of Tuft cell. This group also analysed regional differences between the duodenum, jejunum and ileum and cellular response to bacterial and helminth infections.

> “single-cell techniques [have] enabled analysis of cell states, … diseased states and tumour heterogeneity”

Another example to illustrate potential applications of scRNA-seq is in the context of characterizing single cells from first-trimester placentas to study the early maternal–fetal interface, as conducted by Vento-Tormo and colleagues. By analysing this specialized tissue, they identified subsets of perivascular and stromal cells, as well as natural killer cells with unique immunomodulatory profiles.

Since the first whole-transcriptome analysis of a single cell in 2009, the community has improved technologies from both engineering and computational perspectives to enable unprecedented insight into biological processes in development, health and disease.

Aline Lückgen,
Nature Communications

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