Summary of the supplement on single-cell analysis

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Single-cell analysis

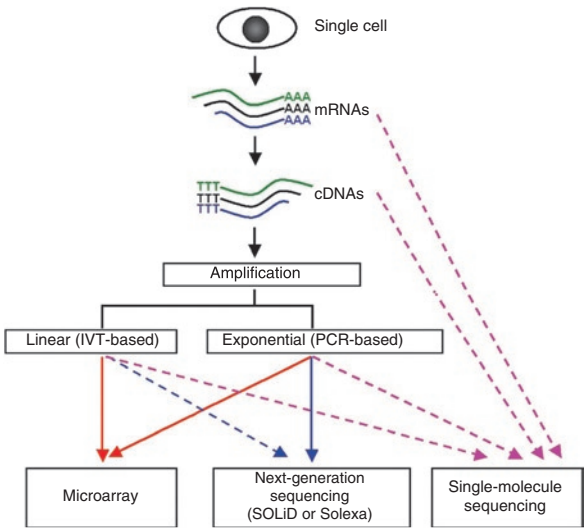
Methods to study single cells

Since the beginning of research on cell biology, say Stephen Quake and Tomer Kalisky in a Commentary, technological advances have driven biological understanding of the single cell. Early microscopes that permitted biologists to observe single cells have led, via molecular marking techniques and flow cytometry, to the ability to rapidly monitor dozens of markers on thousands of individual cells. But the scale of single-cell analysis has not stopped there. The authors discuss methodologies, such as microfluidics, that are enabling highly parallel genome-scale analysis at single-cell resolution. They consider new applications—including haplotyping of human cells and the analysis of complex bacterial populations—for whole-genome sequencing of single cells. (*Nat. Methods* **8**, 311–314, 2011)

Transcriptomes

Methods for single-cell transcriptome profiling

Cells, even when derived from a common tissue source or progenitor, vary in their gene expression, and this in turn influences their behavior and fate. It is thus important to analyze transcriptomes at single-cell resolution. In a Review, Azim Surani and colleagues take the reader through the steps of single-cell transcriptome analysis, from the isolation of single cells to the release and reverse transcription of mRNA and the amplification of the resulting cDNA, followed by DNA microarray analysis or high-throughput sequencing. The authors present available software tools for bioinformatic analysis of sequence data and discuss current limitations of single-cell transcriptome analyses such as the lack of discrimination between sense and antisense strands and the exclusion of non-polyadenylated transcripts. Finally, they describe up-and-coming areas such as single-molecule sequencing for full-length RNAs and the ability to sequence RNA that is actively being translated. (*Nat. Methods* **8**, S6–S11, 2011)
http://www.nature.com/nmeth/journal/v8/n4s/full/nmeth.1557.html

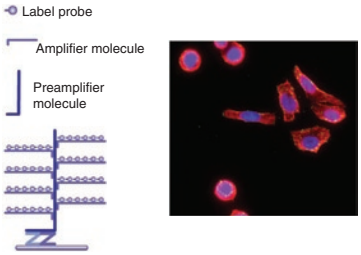


Strategies for single-cell transcriptome analysis.

Transcript imaging

Validating transcripts in single cells

High-throughput sequencing of transcripts in a single cell yields bulk information on what is being transcribed; to follow up on single transcripts in more detail, one needs to visualize the transcripts. In a Review, Alexander van Oudenaarden and Shalev Itzkovitz discuss methods for single-molecule transcript imaging in living and fixed cells. For transcript imaging in fixed cells, they describe fluorescence *in situ* hybridization (FISH) and derivative approaches based on labeled probes. For live cells, the authors compare methods based on gene fusion to the MS2 bacteriophage coat protein and molecular beacons. They discuss imaging technology and data analysis needed to extract information from single-molecule FISH experiments. In an outlook section they provide a glimpse into what is still required to make these methods more sensitive and to combine them with quantitative measurements of DNA and protein for a more complete picture of the expression networks that underlie tissue function. (*Nat. Methods* **8**, S12–S19, 2011)
http://www.nature.com/nmeth/journal/v8/n4s/full/nmeth.1573.html

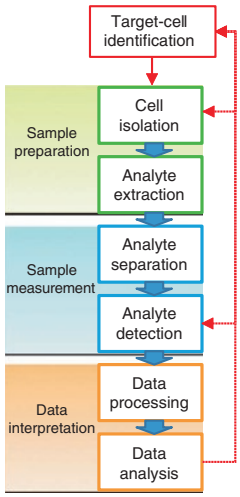


Schematic of a branched probe for transcript imaging.

Metabolomes and peptidomes

Methods for profiling small molecules and peptides in single cells

Metabolites may be small molecules, but they have extremely important regulatory functions in the cell. Their enormous chemical diversity, their wide range of concentrations and the highly dynamic nature of the metabolome, however, make them especially difficult to comprehensively profile. But given this dynamic nature of the metabolome, characterizing small molecules in single cells is even more interesting and important, especially in cells such as neurons and stem cells where heterogeneity is particularly pronounced. In a Review, Jonathan Sweedler and colleagues walk the reader step by step through the process of single-cell metabolome and peptidome analysis, from target-cell identification and isolation to analyte extraction, separation, detection and characterization. They discuss available methods, including microfluidics, chromatography and electrophoresis, nuclear magnetic resonance spectroscopy and a variety of mass spectrometry–based approaches. To date, only a handful of small molecules and peptides at the highest abundance have been identified from single cells, and the existing methods are not sensitive enough to detect proteins. Sweedler and colleagues therefore also discuss the need for sensitivity improvements to make broader-scale single-cell metabolome and proteome profiling a reality. (*Nat. Methods* **8**, S20–S29, 2011)
http://www.nature.com/nmeth/journal/v8/n4s/full/nmeth.1549.html



The main experimental steps for single-cell metabolomic or peptidomic analysis.

Stem cell heterogeneity

Single-cell methods applied to stem cells

Critical for the development, maintenance and repair of biological tissues, stem cells are both dynamic and heterogenous populations of cells. Whether this observed heterogeneity is a consequence of the lack of perfect markers (or combinations of markers) to isolate or define true stem cells, or is due to stochastic processes in these cells, remains incompletely understood. But stem cell heterogeneity can clearly have functional consequences, and analysis of single stem cells is thus important for a complete understanding of these cells and the role that they play in development, maintenance and disease. In a pair of Perspectives, authors discuss methodological approaches to study stem cells in the face of their heterogeneity.

Timm Schroeder discusses the value of continuous long-term live imaging of stem cells *in vitro*, an approach he has in part pioneered, and which takes into account the dynamic and protean nature of these cells. He details the technical and organizational challenges in setting up long-term stem-cell imaging experiments and makes broad recommendations for researchers venturing into this area. Mick Bhatia and Kristin Hope, in turn, describe methods for functional analysis of single stem cells. They describe how, using either physical isolation or *in situ* genetic labeling, the functional output of individual cells can be studied *in vitro* or *in vivo*. They highlight the difficulty of simultaneous molecular and functional definition of single stem cells and methods that are beginning to address this challenge. (*Nat. Methods* **8**, S30–S35, 2011 and *Nat. Methods* **8**, S36–S40, 2011)
http://www.nature.com/nmeth/journal/v8/n4s/full/nmeth.1577.html
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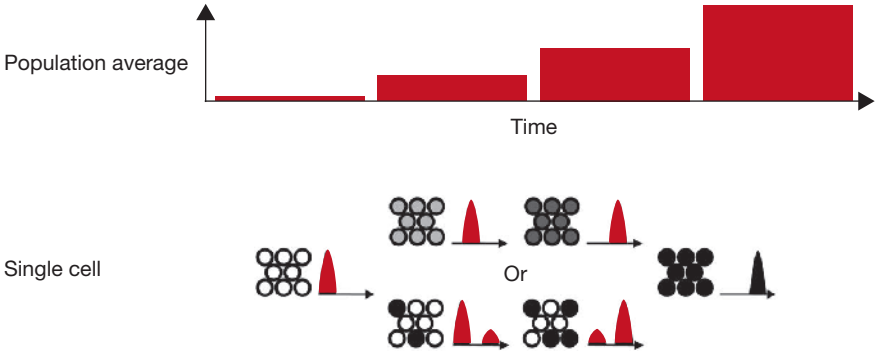

Population measurements can obscure the heterogeneity of single cells.

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