Cell

One Cell at a Time

> Unfortunately, nature seems unaware of our intellectual need for convenience and unity, and very often takes delight in complication and diversity.—Ramón y Cajal (Nobel lecture).

In Ramón y Cajal's lab, science met art. His sketches capture the fine dendritic spines emanating from the pyramidal cells of the frontal cortex; their beauty in a brushstroke. Ramón y Cajal's meticulous drawings of neuronal and glial cells not only appeal to our aesthetic sense—they provide the first evidence of the extraordinary degree of cellular diversity that characterizes our brains.

Fast forward to the present, and the daunting quest of classifying populations of brain cells is hardly over. Complicating matters further, like snowflakes, no two cells are alike, and population-averaged assays may obscure cell-to-cell variability that could be potentially biologically significant. Remarkable progress in next-generation sequencing methodologies now allows researchers to profile thousands of cells in parallel, offering unprecedented insights into the heterogeneity of cells within a population. The latest example of these efforts comes from Sten Linnarsson, Jens Hjerling-Leffler, and colleagues, who used large-scale single-cell RNA sequencing to map brain diversity in the somatosensory cortex and hippocampus at high resolution (Zeisel et al., 2015). They identified nine major classes of neurons, glia, and vascular cells. An even more refined view, based on in-depth clustering analysis, revealed 47 molecularly distinct cellular subclasses and allowed the identification of relevant markers for each of these subclasses, suggestive of subtype specialization dictated by the functional needs of the nervous system. This unbiased survey showcases the power of single-cell transcriptomics in identifying previously uncharacterized cell subtypes existing within established cell classes.

Yet, the genome and transcriptome analysis of a cell only uncovers part of its fingerprint, and the single-cell revolution marches on into other fields, such as proteomics, metabolomics, and epigenomics.

Until recently, single-cell characterization of distinct chromatin states has been difficult. Innovative work from Jay Shendure and co-workers now permits single-cell profiling of chromatin accessibility on a large scale (Cusanovich et al., 2015). In contrast with most contemporary methods for single-cell analysis, their method uses molecular tags to capture chromatin accessibility data on each of many single cells without ever requiring physical isolation of any individual cell. The approach allowed the authors to investigate the epigenomic landscape of more than 15,000 single cells and identify functionally relevant differences in chromatin accessibility between cell types. Contrary to the wide range of mRNA transcripts present in a cell, DNA is at a constant copy number, and as such, single-cell chromatin mapping may need fewer reads than RNA sequencing, illustrating the potential of this method to collect data from an even larger number of cells.

An important application of single-cell sequencing is the ability to reveal rare cell types such as stem cells, short-lived progenitors, circulating tumor cells, or cancer stem cells. For instance, key information of diagnostic and prognostic relevance may be buried in the tremendously complex and heterogeneous tumor microenvironment. Exposing particularly scarce subpopulations of cells, such as cells that have a greater potential to metastasize, develop resistance to drugs, or that can help reconstruct the cancer lineage tree, is, therefore, a research and medical priority. Access to this hidden information requires specific in-depth computational processing tailored to single cells that can account for the noise inherent to single-cell datasets (Buettner et al., 2015).

Morphologically diverse motor axons, labeled by Brainbow 3.0. Image courtesy of Ian Boothby (Jeff Lichtmann lab).

Collectively, these studies yield insights into the molecular landscape of a single cell, and although single-cell sequencing reads accumulate by the day, the approach still faces significant difficulties such as technical variability, bias, and sensitivity. Additionally, the computational challenges associated with the interpretation of such complex and immense volumes of data are still substantial.

An additional challenge in the field of single-cell heterogeneity relates to the ability to combine and integrate different sets of "omics" data from the same cell. To this end, the group of Alexander van Oudenaarden has developed a method that allows simultaneous sequencing of genomic DNA and mRNA in a single cell. They find that genes with high transcriptional variability are generally associated with reduced genomic copy number and vice-versa, suggesting that copy number variations could result in gene expression differences between cells (Dey et al., 2015). Such methodology will help elucidate the correlation between molecular variability and phenotypic diversity—a fundamental question in biology. Ultimately, however, it is the convergence of integrated single-cell "omics" approaches and other tools that retain information on the spatial positioning of cells within

a microenvironment that will help us interpret cellular noise and heterogeneity, comprehend complicated networks, and pave the way to understand how complex multicellular organisms work. In the meantime, many questions remain. It is now universally accepted that cells are intrinsically noisy and, as such, molecularly heterogeneous, and the emerging notion is that cellular heterogeneity may be a functional trait—for instance, for coping with stress or changing environmental conditions. But which heterogeneity components serve a biological function and which ones can be ignored? What defines a cell type or a new functional state? To address such questions, one also needs to fully understand the sources of noise and how they can complicate the interpretability of the data, such as in the case of noise arising from transcriptional bursts. It is also critical to distinguish technical from biological noise and ultimately learn how the latter is regulated. A collaborative effort from the van Oudenaarden laboratory and the groups of Nils Blüthgen and Debora Marks sheds some light on this question by establishing a role for microRNAs in controlling cellular protein expression noise (Schmiedel et al., 2015). The authors combine mathematical modeling with a synthetic reporter gene approach to demonstrate that combinatorial miRNA regulation dampens noise by preferentially targeting genes that are expressed at low levels and for which the intrinsic noise could be too high and possibly detrimental to the system.

Heterogeneity is a constant presence in nature, as illustrated by the varied pigments and patterns of the sea slug *Hypselodoris villafranca*. Photo courtesy of Pedro S. Koch.

The belief that information residing in single cells is key to understanding the organism as a whole has motivated many transformative advances in single-cell technology and continues to generate an even greater number of challenges.

Santiago Ramón y Cajal, the Spanish boy who loved to draw and grew up to be the father of modern neuroscience, discovered that brain cells come in all flavors. But he had absolutely no idea how many. Neither do we. Not yet.

REFERENCES

Buettner, F., Natarajan, K.N., Casale, F.P., Proserpio, V., Scialdone, A., Theis, F.J., Teichmann, S.A., Marioni, J.C., and Stegle, O. (2015). Nat. Biotechnol. *33*, 155–160.

Cusanovich, D.A., Daza, R., Adey, A., Pliner, H.A., Christiansen, L., Gunderson, K.L., Steemers, F.J., Trapnell, C., and Shendure, J. (2015). Science *348*, 910–914.

Dey, S.S., Kester, L., Spanjaard, B., Bienko, M., and van Oudenaarden, A. (2015). Nat. Biotechnol. *33*, 285–289.

Schmiedel, J.M., Klemm, S.L., Zheng, Y., Sahay, A., Blüthgen, N., Marks, D.S., and van Oudenaarden, A. (2015). Science *348*, 128–132.

Zeisel, A., Muñoz-Manchado, A.B., Codeluppi, S., Lönnerberg, P., La Manno, G., Juréus, A., Marques, S., Munguba, H., He, L., Betsholtz, C., et al. (2015). Science *347*, 1138–1142.

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