the organoid. There is even evidence of invasion by host microglia, a population that is not normally present in neural organoids *in vitro* owing to their non-neural origin.

The vascularization achieved by transplantation has clear advantages for cerebral organoid development. In particular, transplanted organoids do not undergo the extensive necrosis seen in organoids kept *in vitro*. Neurons in the innermost regions of the tissue appear healthy and produce extensive neural processes decorated with synapses, both within the organoid and in regions of the host cortex where organoid-derived axons project. Finally, the authors show evidence of maturing neuronal activity. Both calcium imaging and multielectrode recordings reveal active neuronal firing, with a progressive maturation from random firing patterns to more synchronized and correlated firing. This pattern of activity is reminiscent of the coordinated bursts seen in the neonatal rodent brain[7].

The improved survival of organoid tissue shown by Mansour *et al.*[2] is exciting, and future studies should assess neuronal maturation in more depth and over a more extended time course. Previous studies with cerebral organoids *in vitro* have also reported synchronized activity[8], and it will be interesting to test whether transplanted organoids show even further maturation to asynchronous networks over longer timescales. One question is whether transplanted organoids can connect with the host brain. To begin to test this, Mansour *et al.*[2] transiently infect the graft with a virus encoding channel rhodopsin and excite the graft while performing recordings in a nearby site of the host brain. The resulting activity is compelling; however, the degree of host–graft connectivity remains to be tested more thoroughly, for example, through retrograde labeling.

While neurons in the grafts are still quite immature, and it is unlikely that human grafts such as these could significantly affect mouse cognition, it is important to consider whether the mice experience changes in their behavior or needs. Mansour *et al.*[2] test grafted mice in a spatial learning experiment and, rather than an improvement, find slight defects compared with untransplanted mice. This is likely because a substantial portion (1–2 mm) of mouse cortex was removed in order to expose the underlying vascular bed of the choroidal fissure.

The surgical procedure raises a potential limitation of the method, namely, that the organoid appears to grow as a large mass in the cavity, up to several millimeters in size. In some ways, the damage to the host brain resembles that caused by growth of a tumor. However, the authors report that transplantation did not affect survival of the host, and it seems the grafts do not spread or metastasize like a malignant tumor would. Nonetheless, limiting damage to the host brain will be important for future studies of host–graft connectivity. For example, prevascularizing the organoid with endothelial cells[9] may bypass the need to transplant at such a deep and destructive location.

The lack of vascularization in brain organoids has been a major hurdle that multiple research groups have been working to solve, so Mansour *et al.*[2] have accomplished quite a feat. While there have been various efforts to vascularize tissue constructs using *in vitro* bioengineering approaches such as microfluidics[10], these approaches can disrupt the self-organizing architecture of an organoid. Transplantation into animals enables vascularization that is more similar to the angiogenesis that occurs during development, and this approach has proved successful for organoids of other tissue types[9,11]. A common site for organoid transplantations is the kidney subcapsule. It would be interesting to test whether highly angiogenic sites other than the brain would also work with brain organoids.

The ability to achieve vascularization and integration of brain organoids with a host is a major leap in studying the developing human brain within the context of a whole organism. There is extensive interaction and feedback between the brain and the body, and this interaction is lacking in existing *in vitro* methods. Integration with a host will allow investigation of the role of host-derived cell types, such as microglia and immune cell types. It will also allow the study of circulating factors like inflammatory signals or even factors coming from the gut microbiome. While much has been learned from the developing mouse brain, the approach of Mansour *et al.*[2] makes it possible to test hypotheses involving interactions of the brain with non-neural tissues, but now with the power of a human model system.

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Boosting the power of single-cell analysis
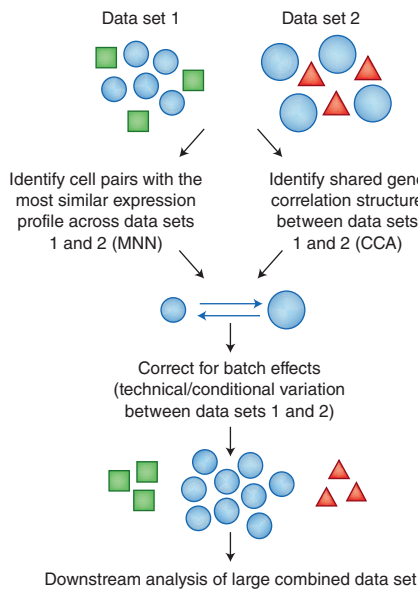
Lu Wen & Fuchou Tang

Data sets from different single-cell RNA-seq experiments are combined with reduced technical error.

Single-cell RNA sequencing (RNA-seq) is transforming our understanding of complex biological systems. Current technologies can measure the transcriptomes of thousands of individual cells in a single experiment, enabling the discovery of rare cell types and cellular heterogeneity that cannot be identified at the population level. However, combining data from different single-cell experiments is technically challenging, and without good tools, there is no way to fully harness the wealth of data that is being generated. In this issue, Haghverdi

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et al.[1] and Butler *et al.*[2] describe two computational approaches that correct for the technical errors, known as batch effects, that arise during meta-analysis of independent single-cell data sets. They show how these methods can uncover new biological variation and new cell types, suggesting the knowledge that can be gained by mining existing data sets.

Recent years have seen rapid improvements in the throughput and cost of single-cell transcriptomics[3,4]. These technological advances have enabled large-scale projects, such as the Human Cell Atlas[5], aimed at generating transcriptome data from millions of single cells to identify new cell populations and functions. Data generation at this scale requires the involvement of many groups over long periods of time, using different reagents,

Data set 1   Data set 2

Identify cell pairs with the
most similar expression
profile across data sets
1 and 2 (MNN)

Identify shared gene
correlation structure
between data sets
1 and 2 (CCA)

Correct for batch effects
(technical/conditional variation
between data sets 1 and 2)

Downstream analysis of large combined data set

Figure 1 Accurate combination of single-cell data sets using mutual nearest neighbors and canonical correlation analysis.

assays, and sequencing platforms. Analyzing these data together would result in systematic variations known as batch effects that, if left uncorrected, could lead to spurious data interpretation. For example, the transcriptomes of two cells may appear more similar if they are measured on the same day using identical experimental conditions than if they are profiled at different times using different techniques. These differences could be interpreted as biological differences when they are actually technical in nature.

Statistical approaches to correct for batch effects, such as limma[6] and ComBat[7], have been developed for bulk RNA-seq analysis. These methods assume that the composition of the cell population in each batch is identical, which is not the case for single-cell data, where extensive technical and biological variation exists between individual cells. Methods to remove unwanted batch effects specifically from single-cell data are therefore needed to accurately integrate data from different experiments. Such data integration would allow analyses of much larger sample sizes without performing additional experiments, and enable comparisons between different cell types in different disease states. Comparisons between different species could also provide evolutionary insights into convergent and divergent transcriptional programs.

Haghverdi et al.[1] and Butler et al.[2] describe important steps toward identifying and correcting batch effects in single-cell transcriptional data. Both groups use a clever strategy that first identifies shared common cell populations across different batches of single-cell RNA-seq data sets and then systematically corrects

for batch effects by quantifying the differences among these shared cell populations (**Fig. 1**).

Haghverdi et al.[1] develop an algorithm that identifies pairs of cells that have similar expression profiles across batches, called mutual nearest neighbors (MNN). The MNN cell pairs are considered to be the same cell type or state, and systematic expression differences between these cells are assumed to be caused by a batch effect. Batch correction vectors that capture these high-dimensional differences are calculated by averaging the differences across many MNN pairs and are subtracted from one batch so that the two data sets can be merged.

Butler et al.[2] use an algorithm called canonical correlation analysis (CCA), which was previously applied for integrating imaging data sets, such as functional MRI. The method identifies a shared gene correlation structure that is conserved between data sets. Then, the data sets are aligned into a conserved low-dimensional space, enabling downstream analyses, such as unbiased clustering of the cells. Rare cell populations that do not overlap between data sets will not be described by this shared structure, and CCA can flag these populations for further analysis.

Both groups use simulated and previously generated single-cell RNA-seq data to validate their approaches and to demonstrate superiority compared with limma and ComBat. Butler et al.[2] successfully align data from interferonbeta-stimulated and non-stimulated human peripheral blood mononuclear cells and identify distinct clusters of cells that were missed by previous cluster analyses. Both groups test their algorithms on mouse hematopoietic stem cell and progenitor cell data generated by two laboratories using two sequencing systems (the fulllength Smart-Seq2 and the 3′ MARS-Seq). They also apply their methods to human pancreatic cells generated by four laboratories using four systems (Cel-Seq, Cel-Seq2, Fluidigm C1, and Smart-Seq2). Both methods correctly merge the cell types that were shared between different batches and both methods clearly outperform limma and ComBat. These results demonstrate that both the MNN and CCA methods can correct batch effects arising in different laboratories using single-cell RNA-Seq techniques as diverse as the full-length and 3′-end assays.

The new approaches[1,2] are compatible with droplet-based, high-throughput technologies that handle tens of thousands of cells. Using the commercial droplet system 10X Genomics Chromium, both groups analyze data on 68,000 peripheral blood mononuclear cells and 4,000 T cells. Haghverdi et al.[1] show that analysis time increases approximately linearly between 7,000 and 70,000 analyzed cells. Butler et al.[2] observe a running time of less than a half hour on a standard laptop for tens of thousands of cells.

Both methods demonstrate excellent performance on a variety of data sets, but given the complex origins and patterns of batch effects, it remains to be seen whether these methods will encounter limitations. In future work, the rare cell populations identified by Butler et al.[2] should be experimentally validated by independent assays. It will also be interesting to see whether these methods could be used with other single-cell molecular profiling methods, such as epigenome sequencing, which have very different data structures[8,9].

A key remaining challenge is to understand in more depth the diverse origins and hidden features of experimental batch effects. This information could lead to further changes in how single-cell data are generated and analyzed. Experimentally, the question could be addressed in part by adding spike-in RNAs for each cell. Currently used ERCC spike-in RNAs have short polyA tails (~20 nt), which have low amplification efficiency. Spike-in RNAs with longer polyA tails (80–150 nt), similar to endogenous mRNAs in a mammalian cell, could overcome this inefficiency and improve quantification of batch effects. Cell controls could also be added to each experimental batch to search for technical variation[3]. Alternatively, controls could consist of reference RNAs that represent an 'averaged' cell (e.g., 10 pg total RNA of a certain cell line from a different species). This approach may be preferable to using cells, which may vary by culture batch. Ideally, for each experimental batch, ~5–10% of the single cells could be split into two equal parts after cell lysis. Half the cells could be processed for RNA-seq analysis and the other half processed with the next batch. This approach, while having no effect on the remaining single-cell samples, would add biologically representative, near-identical technical replicates to two batches of an experiment. In this way, the origins and signatures of the batch effects could be gradually revealed, regardless of the operators, reagents, platforms, or protocols.

These and other new experimental and computational strategies will continue to increase the power of single-cell RNA-seq to decode the enigma of human development and disease.

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