RNA in the cell, whereas nonmapping reads are indicative of background noise, autofluorescence or debris.

Another issue is the enormous range in intracellular RNA abundance, which pits the need for a large number of reads against the fundamental optical limitations on the number of spot-like signals that can be reliably detected within a cell. To solve this problem, the authors develop 'partition sequencing', a clever method in which they extend the sequencing primers by a fixed number of bases so that only some of the RNA molecules present in the cell are sequenced. This allows them to tune the number of sequence readouts to avoid saturating the imaging field with too many targets, facilitating robust yet broad sampling of the RNA in a cell.

In a step toward studying multicellular systems, the authors use FISSEQ to detect RNA transcription in a whole-mount *Drosophila* embryo as well as in a mouse embryo and in brain sections. They have not yet sequenced these samples but have shown that, in principle, FISSEQ can be extended to study more complicated biological systems.

FISSEQ has the potential to transform several areas of research. In development, for example, the spatial organization of transcription in a multicellular organism is of critical importance. Many disease processes may also involve spatially heterogeneous gene expression, such as that seen in different areas of tumors. FISSEQ analysis of a tumor biopsy may be useful for guiding treatment programs, in which multiple drugs might be chosen for different components of the tumor.

In its current form, FISSEQ does not provide a complete accounting of the RNA within single cells. Rather, it produces a spatially localized subsampling of the transcriptome, and comparisons with RNA-seq and microarray data suggest that FISSEQ may miss lower-abundance transcripts. Indeed, Lee *et al.*[4] detect a large number of transcripts by only a single read per cell. Whether this level of detection is sufficient depends on the scientific question being asked. As the authors note, it may be enough to distinguish different cell types owing to the sheer number of genes detected, in contrast to lower-throughput methods such as RNA FISH. However, accurately quantifying cell-to-cell variability in the expression of a particular gene would be difficult. In light of the rapid recent advances in sequencing technologies and in the imaging and fluid-handling capabilities of current commercial instruments, it will not be surprising if this limitation is addressed in the near future.

At the same time, FISSEQ should have some competition from new variants of RNA-seq and RNA FISH. Several groups have sequenced RNA from single cells, now up to thousands per study[5], and many groups have been steadily improving single-molecule RNA FISH by increasing multiplex capability[6,7], measuring transcriptional structure in the nucleus[8], achieving single-base resolution[9,10] and shortening assay time[11]. Nevertheless, FISSEQ represents an enormous step forward in the ability to measure gene expression in its spatial context, and we look forward to seeing how it will be applied to yield new biological discoveries.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Wang, Z., Gerstein, M. & Snyder, M. *Nat. Rev. Genet.* **10**, 57–63 (2009).
2. Femino, A.M., Fay, F.S., Fogarty, K. & Singer, R.H. *Science* **280**, 585–590 (1998).
3. Raj, A., van den Bogaard, P., Rifkin, S.A., van Oudenaarden, A. & Tyagi, S. *Nat. Methods* **5**, 877–879 (2008).
4. Lee, J.H. *et al. Science* **343**, 1360–1363 (2014).
5. Jaitin, D.A. *et al. Science* **343**, 776–779 (2014).
6. Lubeck, E. & Cai, L. *Nat. Methods* **9**, 743–748 (2012).
7. Lubeck, E., Coskun, A.F., Zhiyentayev, T., Ahmad, M. & Cai, L. *Nat. Methods* **11**, 360–361 (2014).
8. Levesque, M.J. & Raj, A. *Nat. Methods* **10**, 246–248 (2013).
9. Levesque, M.J., Ginart, P., Wei, Y. & Raj, A. *Nat. Methods* **10**, 865–867 (2013).
10. Hansen, C.H. & van Oudenaarden, A. *Nat. Methods* **10**, 869–871 (2013).
11. Shaffer, S.M., Wu, M.-T., Levesque, M.J. & Raj, A. *PLoS ONE* **8**, e75120 (2013).

Research Highlights

Papers from the literature selected by the Nature Biotechnology editors.
(Follow us on Twitter, @NatureBiotech #nbtHighlight)

Single-cell trajectory detection uncovers progression and regulatory coordination in human B cell development
Bendall, S.C. *et al. Cell* **157**, 714–725 (2014)

Universal computing by DNA origami robots in a living animal
Amir, Y. *et al. Nat. Nanotechnol.* **9**, 353–357 (2014)

Single-cell genomics for dissection of complex malaria infections
Nair, S. *et al. Genome Res.* doi:10.1101/gr.168286.113 (8 May 2014)

Multifunctional wearable devices for diagnosis and therapy of movement disorders
Son, D. *et al. Nat. Nanotechnol.* **9**, 397–404 (2014)

Expression and glycoengineering of functionally active heteromultimeric IgM in plants
Loos, A. *et al. PNAS* **111**, 6263–6268 (2014)