all cell types? It is just too early to answer these questions, particularly considering the transcriptome of unexplored parts of the mammalian brain, where the cortex alone contains more than 600 cell types, but it is likely to be orders of magnitude larger than the number of genes. Defining RNA variants and their function in the relevant cells will keep us occupied for some time.

1. Djebali, S. *et al. Nat. Methods* **5**, 629–635 (2008).
2. Salehi-Ashtiani, K. *et al. Nat. Methods* **5**, 597–600 (2008).
3. Carninci, P. *et al. Genome Res.* **10**, 1617–1630 (2000).
4. Lander, E.S. *et al. Nature* **409**, 860–921 (2001).
5. Waterston, R.H. *et al. Nature* **420**, 520–562 (2002).
6. Carninci, P. *et al. Science* **309**, 1559–1563 (2005).
7. Kapranov, P. *et al. Science* **316**, 1484–1488 (2007).
8. Birney, E. *et al. Nature* **447**, 799–816 (2007).

Microfluidics: streamlining discovery in worm biology

S Elizabeth Hulme, Sergey S Shevkoplyas & Aravinthan Samuel

Advances in the application of microfluidics technology to biological assays using the model organism *Caenorhabditis elegans* help to automate otherwise time-consuming experiments.

The nematode *C. elegans* has several unique advantages for the study of animal biology, but automated methods are only beginning to be applied to this model organism. In the June and July issues of *Nature Methods*, two independent groups apply microfluidic technology to enable precise manipulation and automated sorting of the worm[1,2].

Among other advantages of *C. elegans*, laboratory cultivation of the worms is fast and cheap because an egg grows to a self-fertilizing, millimeter-long adult in ~3 days. Each worm lays hundreds of eggs, and thousands of worms can be grown on a single Petri dish seeded with bacterial food. The worm is anatomically simple, with precisely 959 cells, which arise from the fertilized egg along a fixed lineage. Combined with powerful forward and reverse genetics methods, this organism is an ideal system for characterization or manipulation of gene expression at the cellular level.

The standard first lesson for a worm biologist is how to move and place worms one by one using a thin platinum wire. For a worm microscopist, the first lesson is how to immobilize individual worms using glue or anesthesia for high-resolution imaging. These basic manipulations are not onerous (after a little practice) when applied to individual worms, but when thousands of worms must be handled with great care and precision to carry out genetic screens or to acquire meaningful statistics, they can easily become prohibitively time-consuming and extremely tedious.

One way to address the need for high-throughput and precise worm manipulation is through the use of microfluidics[3]. The worm's tiny size and ability to live in liquid media make it ideal for microfluidic devices. Consequently, several microfluidic tools have recently emerged for studying *C. elegans*. In a microfluidic device, the precisely controlled flow of fluid—instead of the hands of the worm biologist—handles the worms. A microfluidic chip for rapidly changing the chemical environments of individual trapped worms has already proven its value in studies of the worm chemosensory system[4]. Additionally, a network of microfluidic traps has been previously developed to immobilize hundreds of worms rapidly and automatically in stereotyped configurations for imaging and microsurgery[5], and a microfluidic device has been designed for the rapid immobilization of worms for imaging[6].

Two recent papers in *Nature Methods* describe how the combination of microfluidic technology with sophisticated methods in optics and microscopy can produce new capabilities for worm biologists[1,2]. The first is from the group of Adela Ben-Yakar at University of Texas–Austin, who, in an earlier study, pioneered the application of femtosecond laser surgery in *C. elegans*[7]. They used tightly focused ultrafast laser

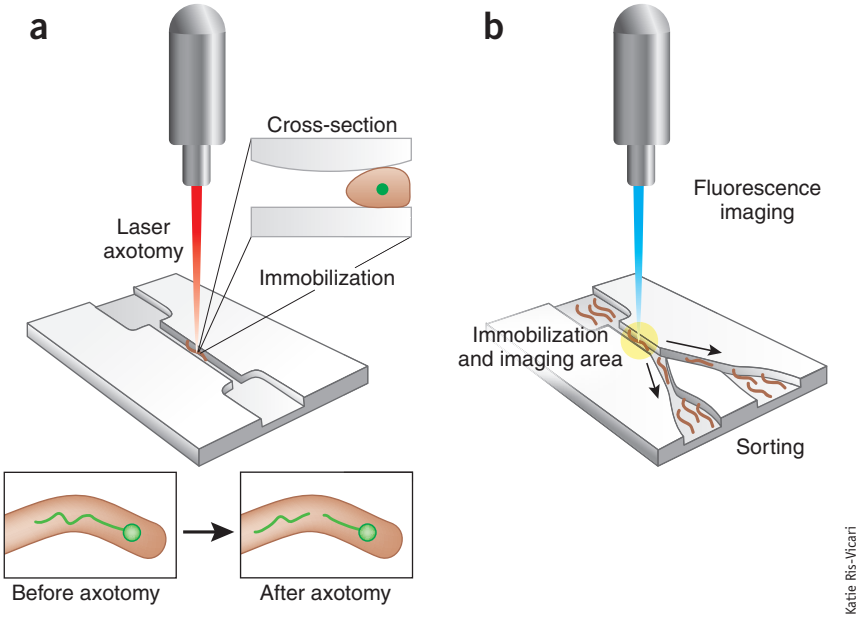

Figure 1 | Two new applications of microfluidics to study *C. elegans*. (**a**) Worms are immobilized and positioned for high-resolution axotomy with an ultrafast laser[1]. (**b**) Worms are sorted at high-throughput based on imaging of desired phenotypes[2].

S. Elizabeth Hulme and Sergey S. Shevkoplyas are in the Department of Chemistry and Chemical Biology, and Aravinthan Samuel is in the Department of Physics and Center for Brain Science at Harvard University, Cambridge, Massachusetts 02138 USA.
e-mail: samuel@physics.harvard.edu

pulses to perform nanoaxotomy, that is, to cut individual axons in the worm nervous system, and showed that the axons can actually regenerate after being cut in this way. In the new study presented in *Nature Methods*, they describe a device that can position individual worms so that axons can be targeted with an ultrafast laser for this purpose[1] (**Fig. 1a**). An attractive feature of this device is that it also allows for post-operative care by shunting the worms into individual chambers after surgery, where they can recover as their neurons grow back. This device could facilitate high-throughput analyses of nervous system regeneration after nanoaxotomy, perhaps enabling the identification of molecules that affect axon regeneration.

In the second paper, the group of Hang Lu at the Georgia Institute of Technology demonstrates how computer vision can be used to automate the visualization and screening of worms as they are rapidly processed through the microfluidic device[2]. Much like a flow cytometer—or, in this case, a flow wormmeter—the device uses multiparametric analysis of several characteristics—such as numbers of fluorescently labeled neurons or number and position of synapses—to identify and sort phenotypes without human intervention (**Fig. 1b**). In test runs, their device automatically sorted over 100 worms per hour based on computer-mediated recognition of morphological features, and the system detected rare mutants spiked into a sample. The device should therefore enable high-throughput screens for worms with subtle but quantifiable changes in the anatomy of the nervous system—for example, mutations that affect axon guidance or synapse formation—or of other cell or tissue types. It may be most gratefully embraced by bleary-eyed graduate students and postdocs who now conduct such screens by hand.

These new devices show how microfluidics can be joined with other technologies to generate powerful tools that could, in principle, drive new investigations in worm biology. For these microfluidic devices to be actually useful, the devices must be usable; that is, these tools must be simple and robust. The ultimate test for the usability of these devices is whether researchers who are not experts in microfluidics—such as most worm biologists—will use them to discover new biology. We encourage you to try!

1. Guo, S.X. *et al. Nat. Methods* **5**, 531–533 (2008).
2. Chung, K., Crane, M.M. & Lu, H. *Nat. Methods* **5**, 637–643 (2008).
3. Whitesides, G.M. *Nature* **442**, 368–373 (2006).
4. Chronis, N., Zimmer, M. & Bargmann, C.I. *Nat. Methods* **4**, 727–731 (2007).
5. Hulme, S.E., Shevkoplyas, S.S., Apfeld, J., Fontana, W. & Whitesides, G.M. *Lab Chip* **7**, 1515–1523 (2007).
6. Rohde, C.B., Zeng, F., Gonzalez-Rubio, R., Angel, M. & Yanik, M.F. *Proc. Natl. Acad. Sci. USA* **104**, 13891–13895 (2007).
7. Yanik, M.F. *et al. Nature* **432**, 822 (2004).