approach, in which optical analysis is performed on a rapidly moving stream of fluid, but the BD FACSAria instead uses a sorting flow-cell. "This gives you the much higher sensitivity that you need, but maintains extremely efficient sorting," says marketing director Tony Ward. "And you can sort cells that have lower levels of antigen expression than you might be able to see using a jet-in-air approach."

Several alternative systems are available. Beckman Coulter of Fullerton, California, another early entrant into the field, offers its EPICS ALTRA, an established platform for cell sorting. In 2000, Van den Engh launched a new company, Cytopeia, based in Seattle, Washington, whose inFlux Cell Sorter is based on ideas from his academic work. "It's an open system, so you can have access to all the modules and can configure it freely for whatever experiment you want to do," he says. "We're not competing with the other manufacturers for well-established applications; we work with the 10–20% of researchers who have applications that are not done as well on the other machines." And for researchers working with larger objects, Union Biometrica of Holliston, Massachusetts, offers a FACS-like platform for sorting embryos and multicellular clusters (see 'The gentle touch', page 1179).

Most observers agree that cell sorting has probably reached its speed limit, and some scientists are now looking to expand the breadth of flow cytometric analysis and sorting. Mario Roederer of the US National Institutes of Health (NIH) in Bethesda, Maryland, has been a leader in this regard, combining fluorescent dyes and quantum dots to perform experiments involving simultaneous analysis of up to 17 different intracellular and cell-surface markers. Many cell biologists have yet to explore these outer limits, but current commercial sorters can typically accommodate optics for analysing a dozen or more fluorescent parameters. As an immunologist examining very specific cell subtypes, Roederer finds this flexibility invaluable: "We're routinely doing 12- or 15-colour flow-cytometry to try to look for important subsets or functions. In the end, I'm hoping we'll be able to reduce it to a 4- or 5-colour assay with the correct combination of markers."

BD BIOSCIENCES

The BD FACSAria uses a sorting flow-cell.

With all the power that these cell-sorting systems offer, there are still problems to be resolved. "Software is the issue that requires the most effort," says Roederer. "We need tools that can automate the discovery or the analysis of subsets of cells that are present in complex data sets." Ward agrees: "The faster you count particles, the more data get generated and the resulting high degree of data complexity and intersections mean that current approaches to software can be limiting." Both Roederer and Herzenberg have worked to address this, developing two commercially available software packages, FlowJo and FacsXpert, intended to improve the quality of cell-sorting analysis.

Another big factor for many is cost: power and efficiency don't come cheap, and access to high-end machines may be restricted to limited slots in a shared core facility. "I'd like to see cheaper machines that give you five, six or seven colours but that are much less expensive than the mammoth machines," says Herzenberg.

Nevertheless, these instruments receive strong acclaim from users. "I don't want to say it's a way of life," says Roederer, "but it is a way of biology."

Working in bulk

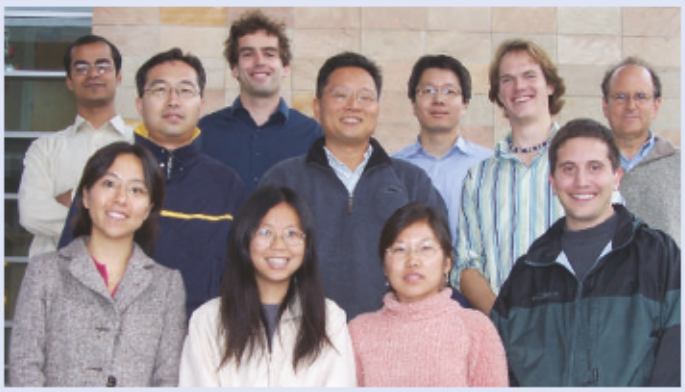
Sometimes, however, all a scientist needs is a way to separate two groups of cells quickly. "FACS can do pretty much everything, but it's expensive," says Steven Woodside, a scientist with StemCell Technologies of Vancouver, British Columbia. "If you want to do more bulk separations, then immunomagnetic separation is a really good option."

The principle is simple. Cells are incubated with paramagnetic beads tagged with antibodies, after which a magnet or array of magnets can be used to either purify cells of interest or remove unwanted cells. Dynal Biotech's Dynabeads — currently available through Invitrogen in Carlsbad, California — were among the first

PLAYING THE FIELD

Researchers have used electrical fields to manipulate nucleic acids and proteins for more than 50 years, but similar systems have only recently begun to emerge for working with whole cells. Dielectrophoresis is nevertheless quickly gaining appeal as a basis for microfluidic cell-sorting.

Hyongsok (Tom) Soh and Patrick Daugherty at the University of California, Santa Barbara, recently demonstrated the feasibility of dielectrophoresis-activated cell sorting, or 'DACS', with a microfluidic chip that uses dielectrophoretic forces to steer bacteria tagged with polystyrene beads into a collection channel (X. Hu *et al. Proc. Natl Acad. Sci. USA* 102, 15757–15761; 2005). Initial experiments showed that one round of DACS could achieve more than 200-fold enrichment of a rare subpopulation of cells at rates of 10,000 bacterial cells per second. They initially tested a single-stage, single-channel device, but Soh believes DACS is ideal for parallel operations. "It is relatively straightforward to design cascaded, sequential sorting stages that operate in parallel," he says. "This allows high purity and cell recovery without sacrificing throughput."



H. SOH

Tom Soh (middle row, centre) and his team have put together prototype microfluidic chips for dielectrophoretic cell-sorting.

Soh is quick to point out that DACS is in no position to usurp fluorescence-activated cell sorting, because of the binary nature of its sorting mechanism; but it shows great promise for high-throughput screening, he says. "We just completed screening the first molecular library and performed epitope mapping with DACS," Soh explains, "and we've shown that it can be faster, cheaper and simpler than commercial assays."

Evotec Technologies in Hamburg, Germany, is also taking advantage of dielectrophoresis for its Cytocon 400 system. The key to this is the CellProcessor microfluidic chip, which contains a three-dimensional array of electrodes that allow users to design and control electrical-field configurations for cell manipulation.

"We developed the CellProcessor platforms for precise and fully automated sorting in a microfluidic environment," says Gabriele Gradl, Evotec's vice-president of cell handling and analysis. "The underlying technology makes cell analysis and isolation reproducible and predictable down to the single-cell level." The resulting platform allows for the delicate manipulation of small numbers of cells, in which the gentle handling provided by combining dielectrophoresis with hydrodynamic flow can be useful.

M.E.