MICROFLUIDICS

Multiplexing to the max

Researchers demonstrate how microfluidics can be used to both synthesize millions of differentially encoded particles for multiplexed *in vitro* biological detection assays, as well as to decode their identities.

Multiplexed methods allowing researchers to simultaneously screen multiple analytes yield rich information in a single experiment. Such technologies have been enjoying their time in the spotlight and are highly touted for clinical application, though most present tools are prohibitively expensive for routine use.

In array-based technologies, probes are encoded by their position on the array, which allows the user to easily decode which probes detect the target analyte. In suspension-based technologies, in contrast, the probes are attached to a particle that must be uniquely 'bar-coded'. Suspension-based methods have the advantage of solution kinetics and may ultimately offer higher multiplex capacity than arrays. No suspension-based platform described so far, however, has been ideal for implementation in high-multiplex biological assays. Patrick Doyle of the Massachusetts Institute of Technology explains: "Most particle-based technologies really only get you into the hundreds." But that should change with Doyle's recent publication in *Science* of a new procedure for creating a very high-multiplex, simple and cheap platform for biomolecule analysis (Pregibon *et al.*, 2007).

Doyle has been interested in "exploring the use of microfluidics to create particles or materials, which might have morphologies, or geometries, or chemistries, or localization of chemistries that are impossible to make otherwise," he says. Last year, he and his colleagues published a paper in *Nature Materials* describing a method coupling photolithography and microfluidics (Dendukuri *et al.*, 2006). Building upon this work, in their recent *Science* paper, Doyle, his student Daniel Pregibon and Mehmet Toner of Harvard Medical School synthesized unique bar-coded particles by flowing two laminar streams in a microfluidic device, one containing a fluorescent polyethylene glycol (PEG)-diacrylate monomer and the other containing a probe-loaded PEG monomer (**Fig. 1a**). By flashing ultraviolet light through a photomask with a unique graphical pattern on the 'fluorescent' side, the oblong two-sided particles are instantly polymerized in a single step (**Fig. 1b**).

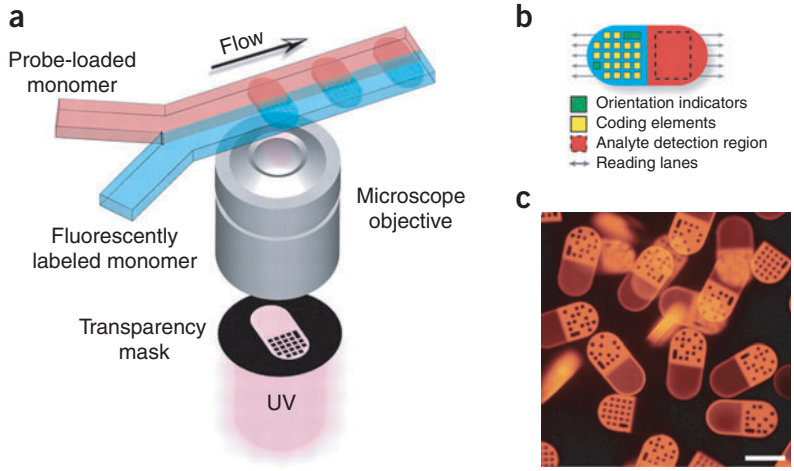

Figure 1 | Synthesis of bar-coded, probe-containing particles. (**a**) Microfluidic particle factory. Laminar flow of two streams containing fluorescent label or probe monomers; ultraviolet light shined through a photomask induces polymerization and formation of the PEG-based particles. (**b**) One side of the particle contains the graphical code and orientation indicators; the other side contains the detection probe. (**c**) Fluorescence images of particles; fluorescence in the probe region indicates target detection. Scale bar, 100 µm. Figure adapted from Pregibon *et al.*; reprinted with permission from AAAS.

As proof of principle, the researchers made oligonucleotide probe–containing particles and incubated them with fluorescently labeled complementary oligonucleotides. They detected hybridization with the complementary sequence when the probe region of the particle became fluorescent (**Fig. 1c**). They then used a second microfluidic platform, "essentially a type of modified flow cytometer," explains Doyle, to decode the particles. Owing to their elongated oval shape, the particles are aligned in the narrow flow-through channel; images are captured of each for decoding.

Doyle envisions that the platform could be used for multiplexed detection of DNA, RNA and proteins. The platform is relatively simple and cheap, and the particles are made out of biologically friendly PEG. They were able to make more than a million different uniquely patterned particles, well more than any other suspension-based platform. However, the researchers have their work cut out for them in speeding up the analysis. "For [this to become] high-throughput you have to be able to analyze many things and do it quickly," explains Doyle. "I think we still have a lot of work to do in terms of our scanning of these particles."

Another intriguing result of this work is the demonstration of the utility of microfluidics in materials synthesis. Says Doyle: "Colleagues have told us that [these two papers] have really made them believe that microfluidics could be a viable solution to create custom microparticles; … this is not just a dream, this is reality."

Allison Doerr

RESEARCH PAPERS

Pregibon, D.C. *et al.* Multifunctional encoded particles for high-throughput biomolecule analysis. *Science* **315**, 1393–1396 (2007).

Dendukuri, D. *et al.* Continuous-flow lithography for high-throughput microparticle synthesis. *Nat. Materials* **5**, 365–369 (2006).