Diving into Droplets

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As a wide range of applications are developed for droplet-based microfluidics, investigators grapple with the challenges of manipulating tiny volumes.

Flask. Test tube. Ninety-six-well plate. These are examples of ways to handle defined volumes of samples and reagents on the macroscale. But what about manipulating discrete aliquots of samples and reagents on the microscale? How can individual micro- to picoliter volumes be generated, transported, mixed, split, and analyzed?

The answer is droplet-based microfluidics, an emerging field that is less than a decade old. Investigators contend that droplet-based systems offer greater flexibility in throughput and scalability over continuous-flow-based devices. With droplets, the possibility of manipulating numerous samples and reagents as individual packets becomes feasible.

Some experts predict that future droplet-based devices will be analogous to electronic microprocessors. Peter Gascoyne of the University of Texas M. D. Anderson Cancer Center says, "One can envision general-purpose, programmable droplet processors that could be embedded within any number of machines, instruments, and controllers, [in the same way] general-purpose electronic microcontrollers are deployed today in a wide range of diverse systems." Because samples and reagents are handled as tiny, individual packages of information, droplet-based microfluidics is occasionally referred to as "digital" microfluidics.

The list of potential applications for droplet-based devices is long and diverse. It includes chemical or biochemical analyses, chemical kinetics studies, high-throughput screening, combinatorial chemistry, and fabrication of customized microparticles. In addition, the number of ways to interface droplets with microfabricated devices is

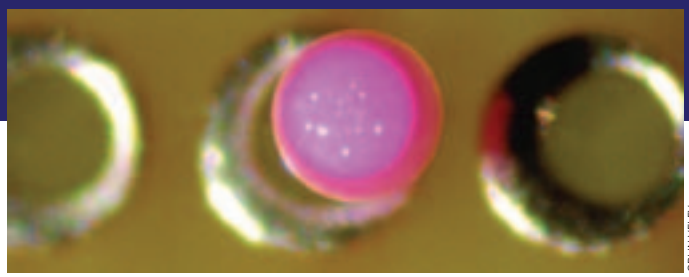

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FIGURE 1. A 500-nL droplet of water containing fluorescent latex particles (pink) is suspended in fluorinated oil. Electrodes (3 silver rings) on the surface generate an electric field to move the droplet by dielectrophoresis. The particles collect at the top of the droplet because of controlled evaporation.

bewildering. Droplets can be inside closed channels, sandwiched between two plates, or positioned on open surfaces; they can be made to scoot over solid surfaces or float on liquids; and they can be maneuvered by electric fields, vibrations, optics, pressure, or thermocapillary forces.

Because the pros and cons of each approach are still being teased out, investigators are careful not to dismiss any particular method. Thomas Jones of the University of Rochester states, "All these schemes are all different wrinkles on the same bedsheet. They all need to be looked at."

Making a move on droplets

The ways to move droplets within the confines of a microfabricated device are numerous, and it's not possible to comprehensively explore all of them in this article. Electrowetting and dielectrophoresis are two popular techniques for moving droplets in a microfluidic device. Other examples include thermocapillary forces, optical trapping (*1*), and vibrations (*2*).

Electrowetting requires a droplet to be in contact with a surface that has an embedded array of electrodes. The droplet has to partially wet the surface and needs to be large enough to bridge the gap between one electrode and the next. When a voltage is applied, the stronger side of the electric field decreases the contact angle the liquid makes with the surface, causing the liquid to wet more. "[If] the droplet is close to overlapping an adjacent electrode, then you can get the droplet to crawl over," says Robin Garrell at the University of California, Los Angeles. "[When] you turn off the voltage, the droplet wants to stay together. It stays together on the place where it was wetting. So the droplet 'pops' over to the second location."

In dielectrophoresis, the droplet doesn't have to contact a surface, but the liquid needs to have a higher dielectric permittivity than the surrounding medium (*3*). An electric-field gradient is applied in the vicinity of the droplet. "If you have a droplet in the vicinity of a high-field region, there will be a tendency to pull the droplet into the highest-field region. This is now a bulk force that works on the whole dielectric body," explains Gascoyne.

In some cases, the distinction between electrowetting and dielectrophoresis can be blurred. "You can sit down and write out perfectly general expressions for all of the electrical forces at work in the presence of a droplet," says Gascoyne. "The general case comes out with a mixture of electrowetting and dielectrophoresis. The physics shows [that] in any [electric-field-driven droplet] device, you probably have a little bit of both forces at work, even though you can design the device to emphasize one or the other."

With thermocapillary forces, a droplet gets propelled by a difference in temperature between its front and back regions. The thermal gradient causes the droplet to move toward the colder end, where the surface tension is largest. "One can generate a thermal gradient by digitally activating resistive microheaters embedded within the substrate or by directly heating the gas–liquid interface with laser irradiation," explains Sandra Troian of Princeton University. "Such digital control over the local temperature allows us to program a variety of thermal maps useful not only for droplet propulsion but also mixing, splitting, coalescence, and thermal cycling."

Some researchers use a combination of actuation methods. David Weitz and colleagues at Harvard University, for instance, use both pressure-driven flow and dielectrophoresis to move droplets through microfluidic channels.

When actuation approaches require the droplet to be in contact with the surface, a phenomenon called contact line pinning must be overcome. "We're all familiar with this day to day, when we look at a glass windshield or a shower enclosure. When you see a droplet hang down but [it] doesn't roll, it's stuck [or] pinned. There are forces that keep the molecules from moving on," says Garrell.

Troian explains how contact line pinning affects the power needed to run a device. "Some of the power input into the system is wasted in trying first to dislodge the liquid droplet because you've got to overcome [the] pinning caused by surface roughness and chemical heterogeneities. Once the droplet is dislodged, the remaining power generates droplet motion," she says.

Experts say that the surface can be tweaked to make it easier to move droplets, because very smooth, clean surfaces help to circumvent contact line pinning. Going to the opposite extreme can also help. Superhydrophobic surfaces, for example, are extremely rough and bumpy, and this lowers the contact angle between the liquid and the surface and, in turn, reduces friction.

How do you decide which actuation method to use? Manoj Chaudhury of Lehigh University thinks the choice is primarily related to two factors—the application and the end user. Devices used for laboratory-scale analyses need not be disposable and cheap because researchers can meticulously clean and reuse them. The actuation method most pertinent to the analysis at hand will be used without consideration of cost or disposability.

However, if the devices are aimed at clinical or pharmaceutical applications, which require single-use devices that can be manufactured cheaply on a mass scale, the choice of actuation method could be critical. "The general users would prefer to use a plate that they will put on a device, do the test, and throw the plate away," suggests Chaudhury. "The mechanisms that can actuate the drop somewhat remotely on a surface, that won't have complicated designs [for actuation], that can be detached from the main device and be disposable, that kind of technology will ultimately win," he says.

However, Chaudhury emphasizes that researchers in the field haven't yet determined which actuation methods will be

most relevant for the various applications. "Right now, this field is still in initial development so there aren't established principles of which [actuation] method would work best for [different] applications yet. I believe this will become established over time and after the field settles down," concurs Orlin Velev of North Carolina State University.

Housing droplets

Devices based on droplets tend to have one of three designs. Droplets can be inside closed channels, sandwiched between two plates, or positioned on a surface that is either exposed to air or covered. Often, the actuation method influences the device geometry. Moving droplets by thermocapillary forces, for instance, requires droplets to be on an open plate because a free, exposed interface, like that of a gas–liquid, is necessary. Pressure-driven flows move droplets through channels. Electrowetting and dielectrophoresis can be used to push droplets in channels or on surfaces.

Besides the actuation method, the type of application is also a significant factor in the device design. For example, because MALDI MS requires a laser to access matrix-mixed samples, droplets of peptides, water, and matrix have been moved on an open plate by electrowetting to carry out proteomics analyses (*4*).

Ease of fabrication of the device is also a consideration. Garrell says that the surface or sandwich geometry with embedded electrodes is simpler to fabricate and allows the device to be easily reconfigured. "You could use a single platform for lots of different applications [and] lots of types of manipulations without having to re-engineer the device," she explains. "You can process all the samples identically or uniquely. Ten droplets can all be manipulated in the same way, or each can be directed through a unique sequence of steps. . . . I guess in principle you could do the same in channels, but it's a lot harder to play the mix-and-match game."

But some investigators feel that channel-based devices aren't really more difficult to fabricate and say that the droplet actuation may be easier to control in channels than on surfaces. Darren Link of Raindance Technologies points out that a large array of electrodes embedded in a surface will require control over individual electrodes to move droplets. He says of the channel-based devices, "Instead of having to control thousands of different electrodes, we control several. That simplifies things tremendously in terms of building a chip." But other experts say that the control of embedded electrodes in surfaces can be readily automated.

Size is another factor. Droplets on surfaces usually tend to have volumes ranging from a few microliters to hundreds of nanoliters; droplets in channels can range in volume from the low picoliters to even femtoliters. "We're trying to use droplets as nanoscale reaction vessels for subcellular particles or single cells. In this case, the size of the droplet matters a lot," says Daniel Chiu of the University of Washington. "[If] you want to control the reactivity of the contents of a subcellular organelle or a few molecules, then you have to use a droplet that is a few microns or less in diameter. . . . If your end goal is to work with smaller samples and get higher sensitivities, droplets in channels will work better at the moment because they tend to be smaller," he explains.

The interface between the droplet and the device is another factor in the design. Droplets can be either exposed to air or encapsulated within an immiscible liquid. Exposure of the droplet to the air makes evaporation a concern, but Jim Sterling of the Keck Graduate Institute says, "You need either to humidify the chamber so evaporation doesn't occur quickly or ensure the assay you're doing is completed before the volumes change substantially."

Evaporation, instead of being a nuisance, can be put to work. Velev says, "By evaporating part of the droplet, we can induce mixing inside the droplet. Mixing is a big problem in microfluidics. . . . We can control evaporation in our case by controlling the humidity of the air above the surface of the chip" (Figure 1).

Several investigators prefer to encapsulate or float droplets in immiscible liquids, such as silicone oil, perfluorinated oil, or bromododecane, to prevent evaporation and keep the droplets isolated from the surroundings. Isolation is a concern because many biochemical applications involve proteins that adsorb to the surfaces of the device (*5*). "The oil keeps the contents within the droplet from biofouling the surfaces. That works really well for proteins," says Richard Fair of Duke University. "It may not work very well for really small molecules, which might just seep into the oil."

But some worry that immiscible liquids will hamper biological assays. "One case is when you have cells in water. They need to breathe!" says Chang-Jin "CJ" Kim of the University of California, Los Angeles. For some applications, such as sample preparation for MALDI MS, the presence of immiscible liquids interferes with the analysis.

If droplets are allowed to make contact with the surface, then investigators have to address the biofouling issue. Garrell says that with the plate geometry, "You can simply not reuse a path. . . . We can make a lot of redundant passages and not reuse them." The second alternative is to not give biomolecules the time to adsorb—this is accomplished by rapidly moving the droplets along a surface. A third alternative is to move droplets containing sur-

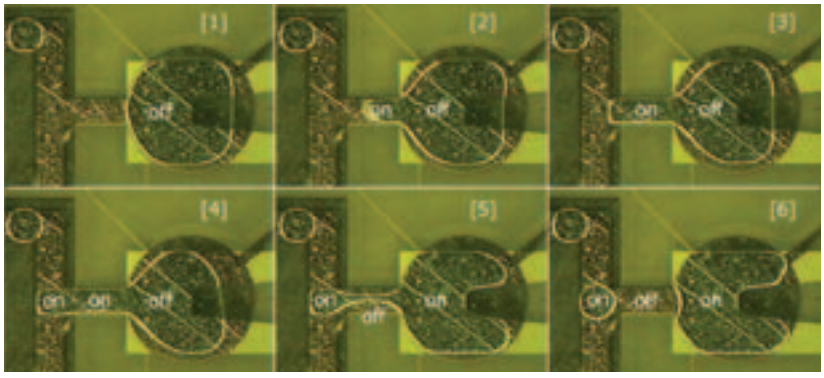

FIGURE 2. Droplets are formed by electrowetting from a reservoir. (Adapted with permission from *Lab Chip* **2004**, *4*, 310–315. Copyright 2004 Royal Society of Chemistry.)

factants behind the droplets containing the biomolecules. The "scrubbing bubbles" remove any type of material left behind on the surface.

But having the droplet on a slick interface, such as an oil, makes droplet movement easier. "When you have a free droplet in a liquid, it's very easy to move that droplet because friction is very low. You only have viscous friction from the liquid, but you don't have friction or contact-angle hysteresis, which you have on the surface," says Velev.

In the end, the device design boils down to different strokes for different folks. Investigators adapt geometries and interfaces that best suit the application or the phenomenon that they are studying.

Creating droplets

"Among the several physical actions you can apply to droplets, creation is the most challenging," says Kim. Because droplets must be introduced into the device with a defined size and volume, investigators have been playing with a variety of approaches. For one, Ali Nadim of the Keck Graduate Institute says he and his colleagues have interfaced their surface-based devices with liquid-handling instruments that can automatically pipette droplets onto a surface.

Another way to create droplets is to design a series of electrodes on which the electrowetting properties of the liquid are manipulated (Figure 2). To dispense droplets from a reservoir, researchers use electrowetting to distort the fluid; this pulls out a projection of liquid into the device and onto the series of electrodes. "By actuating three or four different electrodes, we drop the voltage on the intermediate electrodes. The pressure is greater in the center of the projection, which pushes liquid into the outer portion of the projection and back into the reservoir," describes Fair. "We're playing a game here of creating a differential pressure by electrowetting, which allows us to pinch off droplets."

Thermocapillary forces can also be used to generate droplets of various sizes. "We've recently demonstrated how selective activation of a linear array of microheaters can be used to pull a liquid finger from a large reservoir onto a narrow stripe and to scissor off the exact volume needed. Liquid fingers can also be split into a series of microdroplets of different sizes, which are then moved in different directions," says Troian.

Jones says that capillary instability can also be used to create droplets. Liquid is stretched out from a reservoir as a projection along a long electrode. When the voltage is turned off, the capillary pressure breaks up the stretched column of liquid into droplets. "We can get 20–30-pL droplets, [in] large numbers, if we design the electrodes the right way. . . . The droplets appear at regular sites that can be determined in advance," he explains.

Injectors have been used to draw out droplets into immiscible liquids. In this approach, Gascoyne explains that the liquid in the injector is pressurized to start forming a droplet. If voltage is applied to a nearby electrode, an inhomogeneous electric field is generated. If the fluid has a higher dielectric constant than the immiscible liquid, the droplet gets drawn from the injector into the device. "If you pulse the field, then the droplet will come off. . . . You need to provide it with an acoustic shock to distort the membrane [of the droplet], and that will propagate into a break," says Gascoyne.

In channel-based devices, a T-junction is commonly used to create droplets encapsulated in an immiscible liquid. The viscous drag of the inner, usually aqueous, fluid is balanced within the immiscible liquid so that the surface tension of the fluid breaks and droplets are generated. According to Weitz, another way to generate droplets is to take advantage of the Rayleigh–Plateau instability in a stream of fluid. If a stream has a local depression in radius, then the internal pressure is locally increased. Weitz explains, "The pressure goes up, and it drives the fluid towards the wider region. That tends to make a drop. It turns out that, from an equilibrium point of view, drops are more stable than a stream of fluid just because the total surface tension is reduced."

Where are the droplets going?

Experts say that one of the most significant advantages droplet-based microfluidics offer is throughput. "We imagine moving hundreds and thousands or even tens of thousands of droplets simultaneously," says Aaron Wheeler of the University of Toronto. And Sterling points out that droplet-based microfluidic devices are well suited for automation. If assays can be automated, then the devices "could be incorporated into some process that biopharma would use in drug discovery or in microarray gene expression studies," he says.

However, hurdles exist. One could be the individual control of actuation of potentially thousands of droplets in an automated device. "The algorithms for being able to move droplets around, so they don't accidentally find each other when you don't want them to find each other, may be challenging," says Wheeler. "The other critical element, in addition to droplet movement, is droplet sensing. The most useful integrated devices will need to have feedback." Sensors must be incorporated into the microfluidic devices to ensure that the droplets have been successfully moved and have carried out the appropriate functions (e.g., *6*).

Another challenge that applies not just to the droplet-based microfluidic devices but to any microfluidic device is that "their dimensions are incompatible with the dimensions of standard systems that are used in research labs," states Fair. "Everyone is doing their sample preparation in 96-well plates. But how do you get that material onto the chip?"

Although experts acknowledge that a lot of work lies ahead, the enthusiasm for droplet-based microfluidic devices is infectious. Link says, "This is incredibly powerful [technology] for screening pharmaceuticals, for health care, and for diagnostics."

References

(1) He, M.; et al. *Anal. Chem.* **2005**, *77*, 1539–1544.
(2) Daniel, S.; et al. *Langmuir* **2005**, *21*, 4240–4248.
(3) Gascoyne, P. R. C.; et al. *Lab Chip* **2004**, *4*, 299–309.
(4) Wheeler, A. R.; et al. *Anal. Chem.* **2005**, *77*, 534–540.
(5) Mukhopadhyay, R.; *Anal. Chem.* **2005**, *77*, 429 A–432 A.
(6) Valentino, J. P.; et al. *Appl. Phys. Lett.* **2005**, *86*, 184101:1–3.

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