Sound advice

Acoustic technologies facilitate ultralow volumes for high-throughput screening.

BY RICHARD ELLSON AND ROELAND PAPEN

Robotic systems and standardization of fluid containers have facilitated the industrialization of life science analysis. In particular, routine tests in genomics, proteomics, and drug discovery have been modified for high throughput in the range of hundreds of assays per minute and miniaturized from milliliter to microliter volumes (*1*). Conventional transfer techniques rely on the use of solids to transfer liquids, either by adhering them to pins or metering them out through orifices such as a capillaries or piezo nozzles. Numerous instruments have been developed that require a liquid–solid interface to be integral to the transfer process, and over the years, these methods have incrementally improved precision at ever decreasing volumes. Reliable low-volume liquid handling, however, has been elusive.

The reproducible transfer of volumes below 50 nL has proven to be a barrier to miniaturization for many liquid-handling technologies. These systems are often incompatible with many life science applications because of their destructive impact on living cells or inability to work with volatile, corrosive, or saturated solutions.

Liquid handling

Glass capillaries with narrow bore openings have always been interesting reaction vessels for analytical chemistry or as conduits to analytical devices. They are resistant to many solvents and enable miniaturization of liquid volumes while protecting the liquids from evaporation. Liquids can be loaded by taking advantage of capillary action to aspirate liquid from containers by simply dipping the capillaries into the liquid. This mode of filling the capillary vessel is adequate if only one liquid is to be loaded. Capillary loading is commonplace in the life science industry. One example is the Sipper from Caliper (*www.caliper.com*), which acts as a conduit to load nanoliter volumes into lab-on-a-chip devices.

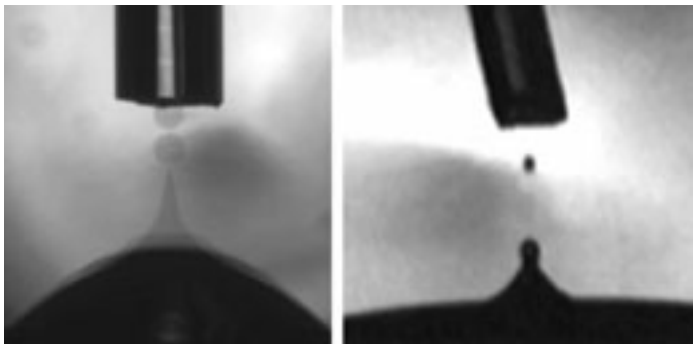

Figure 1. The sound and the flurry. Researchers at Labcyte Inc. have developed a liquid-handling method that harnesses sound energy to project sample droplets at small capillaries. (Adapted with permission from Ref. 6.)

Capillary dipping can trap air bubbles, however, preventing sufficient mixing of the reactants. In addition, when the outside of an inadequately cleaned capillary comes in contact with the source liquid, it can contaminate subsequent experiments. To reduce contamination, Michael Doering from Microdrop (*www.microdrop.de*) used piezoelectric dispensing to load CE capillaries, ejecting droplets smaller than the capillary orifice, thereby eliminating the need to dip the capillaries into the sample liquid (*2*). This method allows loading of multiple chemical entities into the capillary without bubble trapping or cross-contamination. It offers the additional advantage of not requiring a syringe or vacuum device. This approach was later perfected by Deirdre Meldrum of the University of Washington (*www.washington.edu*), who applied it to loading capillaries for submicroliter PCR assembly (*3*).

Despite these improvements, problems with capillaries remain. Dipping them into a source reservoir and sipping fluid require a volume large enough to immerse the capillary, wasting the fluid left behind—the dead volume. Loading with a piezo unit requires filling the piezo capillary, so the dead volume merely shifts from the capillary-loading reservoir to the piezo-loading reservoir. And whereas cleaning the transfer device or only using it once is feasible for glass capillaries, cleaning is more complex for piezo devices, and "single use" is too expensive.

Focused acoustic transfer provides the flexibility needed because it can load directly from the reservoir without contact between the acoustic device, the sample fluid, or the receiver (i.e., the capillary). As shown in Figure 1, a focused acoustic transducer located external to the sample fluid reservoir is positioned to focus acoustic energy just below the surface of the sample liquid. The acoustic vibration ejects a droplet of precise volume toward a target surface or capillary. Many capillaries can be loaded quickly with the same liquid or different liquids using this process, and no cleaning of the device is required. Acoustic transfer is also reliable and precise, in part because of the ability of the device to measure acoustic energy reflected from the fluid interfaces of the well, providing information for both process and quality control.

Acoustic versatility

The isolation of the acoustic-transfer device from the sample liquid provides flexibility

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because the physical surfaces of the transfer device do not play a role in determining the volume of the droplets generated and are not constrained to be compatible with the sample fluid. In particular, the influences of surface roughness and adhesion forces associated with the transfer device are irrelevant to the transfer process. Thus, the acoustic-transfer process is more scalable than conventional methods. The focal spot size is proportional to the acoustic wavelength in the sample fluid, and by adjusting this wavelength, droplets ranging from less than 100 fL to more than 10 µL have been produced. Drops have also been formed from fluids with a wide range of viscosities and surface tensions.

Evaporation or precipitation of the sample fluid will not diminish the robustness of the process as in conventional transfer methods. In fact, sample solutions near saturation can be transferred acoustically even if they contain volatile components (Table 1), because focus can be adjusted to compensate for evaporation, and no precipitate can build up on the acoustic device. In addition, acoustic transfer is gentle enough to move cells, and it is compatible with the existing microplate infrastructure to enable automation and high throughput.

Selection of the material for the container is also flexible and can be made to accommodate the fluid—not the transfer device—as long as it enables sufficient acoustic energy to travel through it. Most solvent-resistant materials are acceptable, including glass, Mylar, and microplate polymers such as polypropylene, cyclic olefins, and polystyrene. Containers with a flat bottom are also preferred because they will not deflect or refocus the acoustic beam.

Acoustics in action

Acoustic transfer provides a more effective method than conventional liquid handling because of the advantages of not having the transfer device in contact with the liquid. Matrix-assisted laser desorption-ionization (MALDI) mass spectrometry potentially offers the ability to identify and quantify proteins on a cell-by-cell basis in tissue slices (*4, 5*). However, this application has not reached its full potential because of the difficulty of depositing the matrix onto the tissue sample in a consistent, reliable manner. Matrix comprises a crystallizing material at near saturation in a volatile solution containing corrosive components such as trifluoroacetic acid and sinapic acid.

Acoustic-transfer devices overcome these challenges because they have no nozzle and do not touch the matrix fluid. Recently, Richard Caprioli of Vanderbilt University (*www.vanderbilt.edu*) and researchers at Labcyte Inc. (*www.labcyte.com*) constructed and demonstrated the operation of a prototype instrument for dispensing matrix onto delicate tissue samples, and early results have shown excellent signal from matrix depositions on the order of 100 pL (Figure 2, *6*).

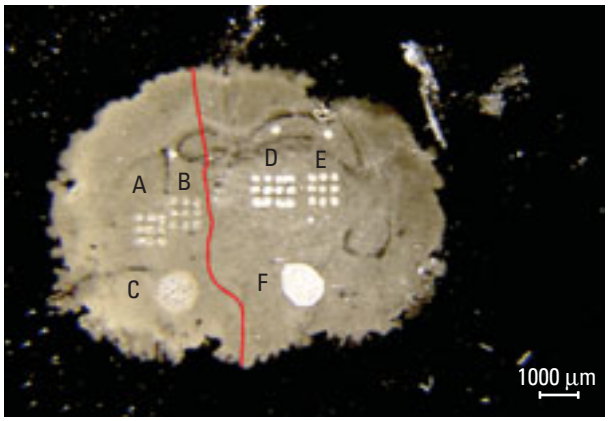

Figure 2. Mass appeal. A cross section of a juvenile mouse brain whose left side was seeded with matrix crystals and then spotted with MALDI matrix by acoustics (A, B, D, E) and pipette (C, F). The acoustic transfers involved 15 (B, E) and 30 (A, D) 120-pL droplets, forming spots ~170 µm in diameter. The pipette transfers were 100-nL formatting spots ~1000 µm in diameter. (Adapted with permission from Ref. 6.)

Getting a handle

The chaotic nature of surface wetting becomes apparent with the reduction in transfer volume, and reliability suffers when either more aggressive solvents or solute precipitation alter the physical characteristics of the surfaces of the transfer device. Decoupling the transfer device from the transfer liquid is one approach for reducing transfer volumes while improving reliability and extending the range of solvents. A generator of focused acoustic energy external to the sample fluid container provides direct transfer of droplets from source reservoirs containing a broad range of solvent systems common to the life sciences.

Table 1

Typical solvents used in analytical chemistry

Solvent	Boiling point, °C	Vapor pressure, mm Hg	Volatility (BuAc = 1)	Example application
Water	100	18	0.3	Buffer solutions, cell assays
Acetonitrile matrix solvent	82	73	5.8	DNA synthesis, MALDI
Ethanol	78	40	1.4	Solvating agent
Methanol	65	97	5.9	Seed crystallization
Acetone	57	184	5.6	Solvating agent
Methylene chloride	40	350	27.5	Synthesis solvent

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