Single-droplet dispenser enables optical manipulation of molecules

Carlos López-Mariscal and Kristian Helmerson

A novel microfluidic chip produces single-molecule containers with high precision, facilitating studies of chemical reactions at the lowest possible reagent levels.

Organic molecules participate, and most often play the central roles, in nearly every process relevant to life. Enzymes promote the chemical reactions necessary for metabolism, nucleic acids transfer genetic information in cell nuclei, and certain proteins restrict movement across cell membranes, to name a few examples. These functions are determined by subtle variations in the electronic conformation or the ‘shape’ of the molecule. These changes are difficult (if not impossible) to monitor in bulk, but easy to detect in single molecules.

It is problematic, however, to handle, transport, and analyze molecules individually. Long molecules can be tethered to a microscope slide or attached to larger objects (like functionalized microscopic spheres) that can be easily manipulated using optical tweezers, and studied by concurrent optical spectroscopy. This can provide information on the same timescale as complex processes, although the medium can affect the results. In addition, bringing together different molecular species can prove complicated. A more versatile method is to encase molecules in liquid droplets small enough that, on average, each droplet contains only a single one.

Microfluidic devices—minuscule networks of channels fabricated using optical lithography—have largely been used to produce highly monodisperse emulsions. Droplets are easily generated by slicing the continuous flow of the dispersed phase at channel junctions. They can be engineered to mimic the appropriate environmental characteristics under which particular molecules perform their specific functions. For example, droplets with specific inhibitors or activators can be easily produced using microfluidic chips. However, the typical production rates of a few hundred to tens of thousands of droplets per second makes them difficult to manipulate selectively with optical tweezers. In addition, the smallest droplets produced in this manner enclosure a volume on the order of several femtoliters, too large to ensure that each droplet contains only one molecule.

We have combined flow focusing—an active phenomenon governing the coplanar flow of immiscible liquid phases in an interface—with a novel membrane-actuated microfluidic valve to produce monodisperse emulsions at much lower, adjustable rates and with tunable volume. Flow focusing through a small aperture of two phases results in an abrupt pressure drop in a rapidly narrowing channel. This pressure variation, in turn, produces a Plateau-Rayleigh flow instability, which we have exploited as a mechanism to induce the controlled breakup of the dispersed phase into emulsions of the desired volume (see Figure 1.)

Figure 1. Droplet generation by means of flow focusing. The local curvature of the breakup point determines the sizes of the emulsions (indicated by red arrows). The curvature can be adjusted by regulating the relative viscosity of the continuous and the dispersed phases. Scale bar: 15 μm.

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Figure 2. Dual droplet generators producing a continuous stream of variable-size droplets (top) and a single drop (bottom) simultaneously.

Figure 1). The relative viscosity and flow rate of the continuous and dispersed phases determine the volume of emulsion with a precision of better than one part in one thousand. Incorporating the molecular species of interest at the appropriate concentration in the flow before the break ensures that, on average, only one molecule resides in a single droplet.

The size of emulsions can be further adjusted by mediating the surface interaction of the dispersed and continuous phases at the breakup point. Producing smaller droplets requires more energetic focusing, which can be achieved by increasing the viscosity of the focusing buffer. In general, however, an increase in hydrodynamic energy results in emulsions with kinetic energies significantly higher than typical optical-trap potential energies. We have addressed this problem by including a small amount of surfactant agents that reduce the surface energy at the breakup tip, thereby decreasing the energy required for the formation of droplets of constant volume. A surfactant layer also serves as an individual shielding case for the emulsions.

Ultimately, the minimum volume of the droplets that can be produced in practice using flow-focusing in microfluidics is determined by the relative viscosity of the fluids and aperture size. In addition, other constraints for the fluids used include the refractive-index contrast required for optical manipulation and compatibility with microfluidic-chip materials. A key improvement of our device with respect to current microfluidic technology is the ability to vary the transverse extent of the channel at the point where droplets originate. By injecting pressurized air into adjacent channels, a small deformation in the membranes can be induced, thereby making it possible to fine-tune the size of emulsions independently of the viscosity of the liquid phases, in turn effectively extending the range of emulsions that can be reliably produced towards smaller volumes (see Figure 2). Using our devices, we have been able to produce single droplets with volumes under 1 femtoliter, suitable for performing single-molecule studies and concurrent optical trapping.3

Further progress can be made by integrating our flow-focusing microfluidic with on-chip pumps, which will lead to a significant reduction in device footprint and increased ease of integration with analysis tools such as time-resolved fluorescence detection. Simplicity of design, reliable operation, and the susceptibility of integration with existing optical tools open up the possibility for our device to shed light on single-molecule processes such as protein-enzyme and antigen-antibody interactions using the lowest amounts of reagents possible.

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Author Information

Carlos López-Mariscal and Kristian Helmerson
National Institute of Standards and Technology (NIST)
Gaithersburg, MD

Carlos López-Mariscal is a guest researcher with the NIST. His work primarily focuses on developing integrated technologies for optical manipulation of submicroscopic specimens using optical tweezers. He has promoted the formation of SPIE student chapters in Colombia, Mexico, Scotland, and South Africa. He received the Laser Technology, Engineering, and Applications Scholarship in 2006.

References