Communication

Optically Addressed Droplet-Based Protein Assay

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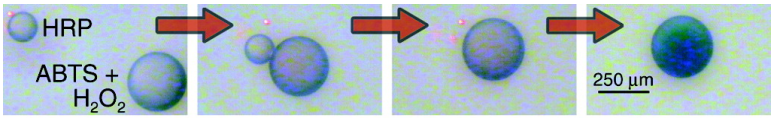

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Optically Addressed Droplet-Based Protein Assay

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In the past decade, there has been a large effort to miniaturize the current analytical laboratory using microfluidic techniques.[1] We have recently demonstrated a microfluidic approach using laser-induced thermal gradients to selectively move small droplets.[2] In this communication, we demonstrate the potential of this technique for analytical applications by performing a protein detection assay in microdrops and discuss some of the challenges when shrinking such a system down to the microscopic scale.

We move small aqueous droplets by inducing a thermal gradient across the width of the droplet as illustrated in Figure 1. This thermal gradient induces a surface energy or surface tension gradient on the droplet surface that is sufficient to move the droplets via the Marangoni effect. We have used these surface tension forces to move droplets over a range of volumes that spans 5 orders of magnitude (~1.7 μL to 14 pL) at speeds of up to 3 mm s^{-1}.[2] In our previous report, we used an argon ion laser beam and added dye to the droplets to produce optical absorption. In this work, we substitute a 1.5-μm (eye-safe) wavelength to heat droplets through the vibrational excitation of the first overtone/combination band of the OH stretch in water rather than through electronic excitation of dye molecules with a visible laser. Infrared heating eliminates potential complications due to unintended excitation of electronic transitions and chromophore photochemistry.

There are many challenges in the field of microfluidic analytical method development that occur due to the physics of fluids on the microscale.[3] For our system, the major effects are due to interfacial phenomena, which become important for small droplets with large surface area-to-volume ratios. For example, to prevent droplet evaporation, we surround our aqueous droplets in an immiscible, nonvolatile liquid (decanol). The use of a binary liquid system also increases droplet mobility on the surface by producing large contact angles.[4] Interfacial effects can also be beneficial; surface tension is the basis of moving the droplets and serves as a driving force for very rapid mixing (<33 ms) after droplet fusion.[2] This latter observation is very important in microfluidics where long diffusion-limited mixing times have been a challenge for channel-based methods.[5,6]

With the introduction of a second liquid phase, other interfacial effects arise due to the partitioning of solute molecules and slow dissolution of the aqueous phase into the surrounding organic fluid.[7] For the assay presented below, the ionic chromogenic substrate remains in the aqueous phase. Aqueous diffusion into the organic phase is slowed sufficiently by prior saturation of the decanol with water. Protein adsorption onto untreated polymer surfaces is another issue for biological sample handling in microfluidic systems. For our system it would lead to sample loss and potential cross-contamination between different droplets. Profile images of our droplets show an exceedingly large contact angle (~180°) and a very small contact perimeter (<10% droplet diameter). Thus, the droplet interface with the polystyrene is less than 0.2% of the total surface area of the droplet, or far less than that for channel-based techniques. If solid surface biofouling becomes an issue, we have demonstrated feasibility of an aqueous assay at the liquid−liquid interface of decanol and a perfluorinated oil. Unfortunately, convection currents and thermal Brownian motion complicate precise droplet control in such a system because there is no contact angle hysteresis to hold the droplets in position. This limitation can be overcome with dedicated optical traps[8,9] or electrostatic trapping[10] at the expense of the simplicity of the optical apparatus used here. Note that, because the index of refraction of water is lower than that of decanol, the water droplets will be repelled by laser beam focus even without optical absorption. However, this radiation pressure force[11] is orders of magnitude smaller than the thermal Marangoni force.[2]

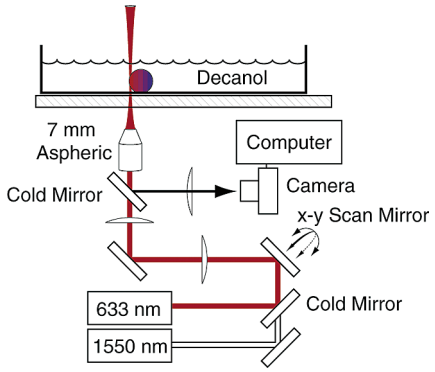

Figure 1. Schematic of apparatus used to control droplets. Aqueous droplets (30−1500 μm in diameter) were deposited in an organic phase of 1-decanol on top of a standard polystyrene Petri dish with a 34-gauge needle (100 μm i.d.) or through direct ejection from a standard inkjet print head. A 1525-nm laser source (New Focus 6262 tunable diode laser pumping a Photonetics Er doped Fiberamp-BT 15, 30 mW total) is focused onto the imaging plane of an inverted microscope stage. A HeNe laser is overlapped with the NIR laser to aid in alignment. The position of the laser beams on the image plane of the microscope stage is controlled by a motorized mirror. Approximately 10% of the NIR light is absorbed by the water O−H stretch. The absorption creates a thermal gradient across the width of the drop sufficient to move the droplets across the surface.

Application of our approach to a chemical assay is presented in Figure 2. One droplet contains an enzyme, horseradish peroxidase (HRP, Sigma), in phosphate buffer (0.1 M pH 6.2), and the other contains an excess of the chromogenic substrates: 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma), and hydrogen peroxide. Upon mixing, the enzyme reacts with the substrates and oxidizes ABTS, resulting in a dark-green droplet (see also, movie S1). The reaction occurs regardless of which drop is moved, demonstrating that we are not heating the droplets above the irreversible denaturing point of HRP.[12,13]

The light transmission through the reacting drop exhibits a smooth single-exponential decay as is expected for this reaction in large excess of substrate (Figure 3). We have observed reactions in droplets as small as 40 μm in diameter. We have also looked at larger droplets with concentrations down to ~3 nM, which corresponds to ~30 *attomoles* of reacting enzyme. The signal-to-noise (S/N) ratio, defined as the ratio of the change in transmission

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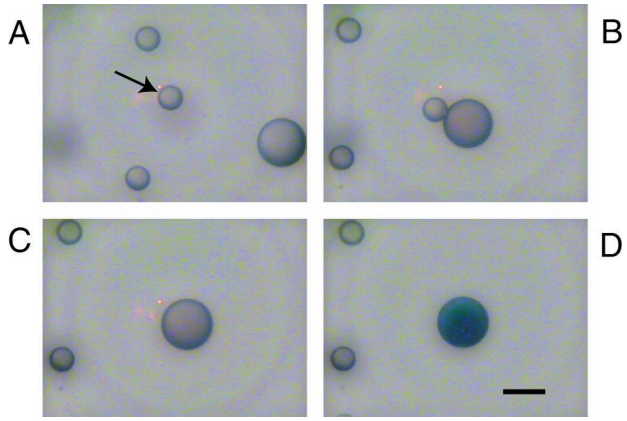

Figure 2. Images extracted from a color video of a chemical assay. The scale bar in (D) represents 250 μm. (A) Two sets of droplets, one containing ABTS and hydrogen peroxide (large drop) and the other containing HRP (small drops). (B) One of the small drops has been moved by the focused laser beam (black arrow) adjacent to a drop containing ABTS. (C) Video frame immediately following (B), the droplets have fused, but not yet reacted. (D) Twenty seconds after the fusion of the droplets, showing the green color of oxidized ABTS.

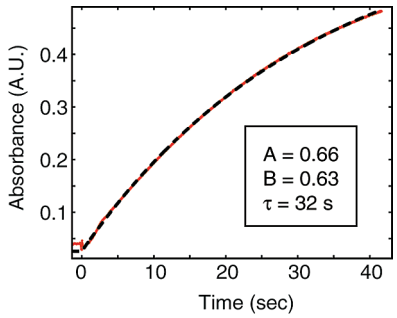

Figure 3. Sample kinetic trace derived from the video sequence of the HRP droplet assay. The experimental data (red dots) were fit to a single exponential described by the equation $A - B\,e^{-t/\tau}$ (black dashed line). The inset shows the fit parameters.

to the fluctuations in transmission versus time, for the lowest enzyme concentration studied was approximately 15. A reasonable lower limit to the S/N of 3 would correspond to a 5-fold reduction in signal. This could be accomplished by reducing droplet diameter from 200 to 40 μm, corresponding to potential detection limits of *zeptomoles* of enzyme.

Because our approach uses heat to move droplets, it is appropriate to consider deleterious thermal effects. Calculations show that the temperature rise is at most ~10 °C across the width of the droplet. Because this temperature rise is present only when the droplet is being moved, the exposure to the temperature rise is short (a few seconds) and is not present during the chemical reaction. The measured rate in Figure 3 agreed with the predicted rate for all the assays, confirming that neither thermal effects nor interfacial effects were influencing assay reactivity. If necessary, the temperature rise could be reduced through more uniform heating, for example, by sweeping the laser beam across the trailing edge of the droplet rather than using a single point focus. Alternatively, using a surrounding liquid with lower viscosity to lower viscous drag would speed droplet movement away from the laser, reducing exposure to thermal gradients.

With a small droplet, one might also consider the influence of the thermal gradient, which can produce convection or mass transport (thermophoresis). Internal convection velocities will be similar to the translation velocity,[14] or roughly 1 mm s^{-1}. In a 100-μm diameter droplet, this velocity produces a shear rate of about 10 s^{-1}, which is far too small to damage molecules.[15] Thermal-gradient-driven mass transport can produce two effects: a transient mass flow and a concentration gradient in steady state.[16] These thermophoretic effects are also small, yielding mass flow rates of about 10 μm s^{-1} and local concentration reductions of about 4 times for 5.6 kbp DNA.[17] We believe that such concentration gradients are unlikely to cause damage or affect reactivity in most cases, particularly as they apply only during droplet motion.

In this report, we have performed a simple assay using a new, highly flexible, optical approach to microfluidics. This approach could be easily scaled through the automation of droplet delivery and motion subsystems. The droplet velocity (in diameters/second) scales with the thermal gradient only, so we expect the technique to work on much smaller droplets. However, the influence of the Kelvin effect on droplet stability and the inability to individually manipulate droplets at spacings of less than the wavelength of light must be considered. This technique should lend itself to a number of applications such as the study of very small fluid volumes, performing parallel assays (high throughput screening), conducting molecular analysis on large numbers of isolated cells, or the advancement of droplet-based combinatorial technologies.

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Supporting Information Available: Video sequence of assay reaction in avi format. This material is available free of charge via the Internet at http://pubs.acs.org.

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