MICROTHERMAL TECHNIQUES FOR MIXING, CONCENTRATION, AND HARVESTING OF DNA AND OTHER MICRODROPLET SUSPENSIONS

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ABSTRACT
The use of microprobes to manipulate, concentrate, and sample aqueous droplets within an oil phase on a blank substrate is explored. In particular, this paper introduces a number of thermal-based techniques which make use of a point heat source to perform high speed mixing of droplets at speeds up to 300 rpm, collection and merging of droplets, concentration of suspended particles through controlled evaporation of the droplet, and precipitation of low concentration suspensions such as DNA onto a microprobe tip. Quantities of DNA as low as 10 ng have been sampled on a 15 μm diameter tip.

Keywords: microdroplet, mixer, DNA precipitation, preconcentration

1. INTRODUCTION

Microscale investigations in cellular and biochemical analysis often call for flexibility beyond what can be achieved with micromachined substrates. In such situations, microprobe-based tools can be used for localized sensing and actuation instead of fluidic chips. Among the best known examples are microdroplet-based chemical analysis systems, where micron-scale droplets of aqueous suspensions submerged within a continuous oil phase serve as low-volume, high-speed chemical reactors for quantifying single cell enzyme kinetics [1], concentrating nanoparticles and dissolved solutes [2], detecting low concentrations of molecules [3], and amplifying single molecules of DNA [4]. In contrast with the traditional approach of processing liquids in a microfluidic chip, samples and reagents are manipulated in droplet form using a variety of localized techniques, including micropipettes, optical pressure [5], and electrostatic tips [6]. These systems attain many of the benefits of conventional microfluidics including fast reaction times and low reagent consumption; however, they also offer additional benefits such as reduced adhesion of cells and hydrophobic molecules to channel walls, as well as simplicity and flexibility since no prefabricated chips are required.

This paper introduces a number of micro-thermal techniques for common procedures in microdroplet systems, all of which make use of a heated microprobe tip; namely, we demonstrate high speed mixing of droplets, collection and merging of droplets, concentration of suspended particles, and the aggregation of DNA onto a microprobe tip.

2. CONVECTIVE MIXING OF DROPLETS

A heated metal tip (ϕ=5-620 μm, T=35-45°C) placed in contact with the surface of a thin layer of mineral oil (200-1500 μm) establishes a micro-scale temperature gradient extending radially from the region directly beneath the heat source. The resulting convection currents flow radially outward on the top surface of the liquid pool, and inward below the surface, forming self-circulating toroidal streamlines which have been used to trap small solid particles and droplets [7]. In the previous work, the particles are small compared to the cross-sectional height of the convective flow region, and, as a result, they follow the toroidal streamlines. In the present work, we show that when the height of the

flow region is approximately the same as the droplet diameter (Fig. 1a), the convective currents can rotate and mix the droplet in various patterns depending on the size of the heated tip. In the case where the tip diameter is approximately the same size as the droplet, the droplet rotates about a single axis at speeds up to 300 rpm (Fig. 1b). As the droplet diameter is progressively increased, the rotational speeds fall, and the flow pattern begins to change (Fig. 1c). Eventually, when the tip diameter is small compared to the droplet, a flow pattern composed of two vortices and turbulent eddies is observed instead of rotation (Fig. 1d). Both patterns can be useful for micro mixing within a single droplet.

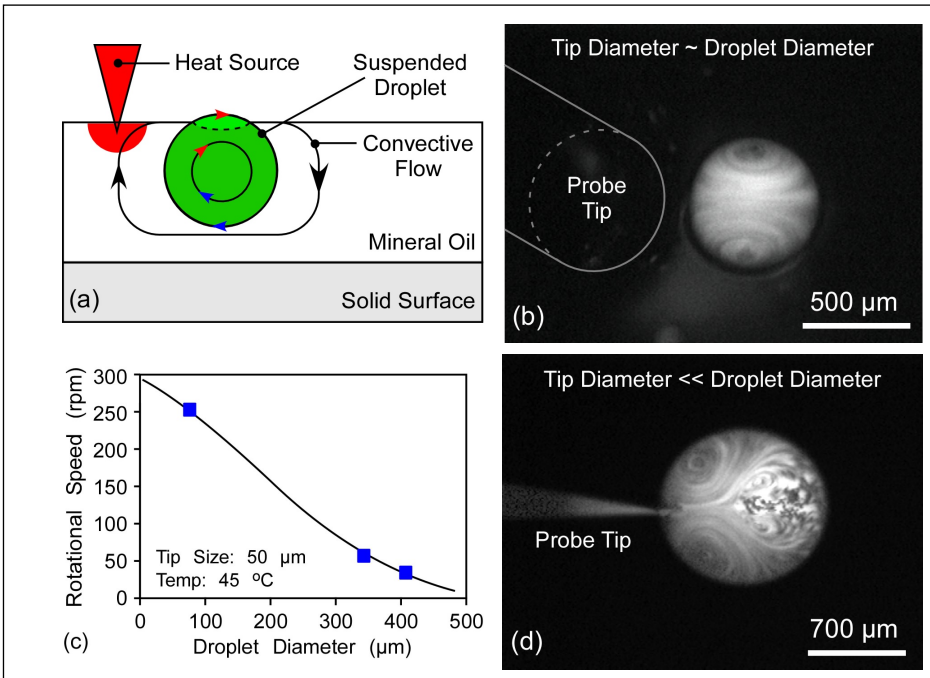

Fig 1: (a) A microprobe tip in contact with a thin oil layer generates a micro-thermal gradient which drives convective flows around and within the droplet. (b) Single axis rotation occurs when a 650 µm tip is placed near a droplet of approximately the same dimensions. (c) The effective rotational speeds using a 50 µm tip fall as droplet diameter is increased due to the change in flow geometry. (d) Vortices and turbulent eddies can be seen instead of rotation when a small (15 µm) tip is used in conjunction with a larger (ϕ=1000 µm) droplet. In both experiments, the tip temperature was ~35-45°C. Flow patterns are visualized using immersed fluorescein particles and a 0.5 second CCD exposure with 490 nm/500 nm excitation/emission filters.

3. DROPLET COLLECTION AND MERGING

The ability to merge discrete droplets is important capability in microdroplet systems, as it allows reagents to be mixed at time scales fast enough to study chemical kinetics [1,6]. To collect and merge droplets, the heated tip is suspended just above the oil layer, and heat transferred to the oil surface drives convection rolls in the same manner as described above. Rotating droplets trapped in the flow collide and merge together without the aid of a surfactant (Fig. 2). The rotation and reduced surface tension due to heating may both assist in droplet merging. By scanning the heat source laterally, several droplets over a large area can be collected and merged.

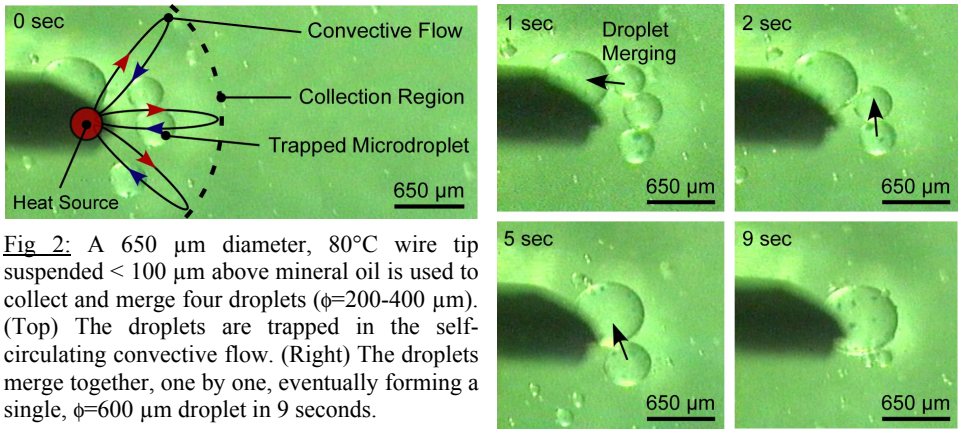

Fig 2: A 650 µm diameter, 80°C wire tip suspended < 100 µm above mineral oil is used to collect and merge four droplets (ϕ=200-400 µm). (Top) The droplets are trapped in the self-circulating convective flow. (Right) The droplets merge together, one by one, eventually forming a single, ϕ=600 µm droplet in 9 seconds.

4. CONCENTRATION OF SUSPENDED PARTICLES

Unlike droplet evaporation on a solid surface, where suspended particles eventually deposit themselves in a circle (the commonly observed 'coffee ring' [8]), particles in an oil-immersed microdroplet aggregate towards the center instead, eventually forming a concentrated solid precipitate after the liquid has completely evaporated. Microdroplet evaporation is, therefore, an effective means to concentrate particles dissolved solutes, but evaporation times for even small (ϕ=10 µm) droplets can be greater than 1 hour [2]. A heated tip placed next to a suspended microdroplet enhances the evaporation rate, allowing controlled evaporation of an 1800 µm droplet in less than 3 minutes (Fig. 3). The linear reduction of the droplet surface area over time is consistent with previous reports [2]. After the droplet has evaporated, the concentrated solids remain in the oil, trapped in the convective flow described above.

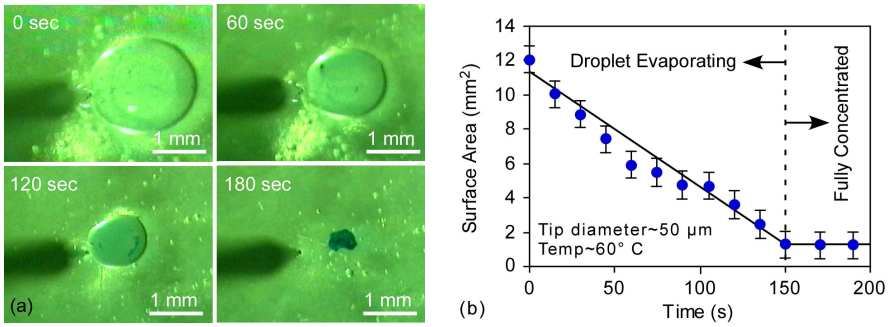

Fig 3: (a) A 60°C, 50 µm tip is placed in contact with the oil near an 1800 µm droplet containing suspended 3 µm polystyrene beads. As the liquid evaporates, the dissolved particles concentrate in the center of the droplet, forming a solid micro-particle suspended in the oil. (b) The surface area of the droplet decreases linearly with time until all the liquid is evaporated at 150 s.

5. AGGREGATION DNA ON A MICROPROBE TIP

If a droplet is evaporated with the heated tip immersed within it, any suspended or dissolved compounds aggregate on the tip as the droplet evaporates. This provides a means to concentrate and 'nanosample' small amounts of solutes onto a probe tip for subsequent analysis using methods such as micro-IR spectroscopy [9]. Fig. 4 shows the aggregate

obtained from 1 µL samples of high concentration calf thymus DNA (10 µg/µL, Invitrogen) and low concentration human male DNA (10 ng/µL, Applied Biosystems) on a ϕ=15 µm metal tip. When the tips are placed in a DNA staining solution, much of the aggregate is washed away; however, fluorescent images show that adsorbed DNA residues remain.

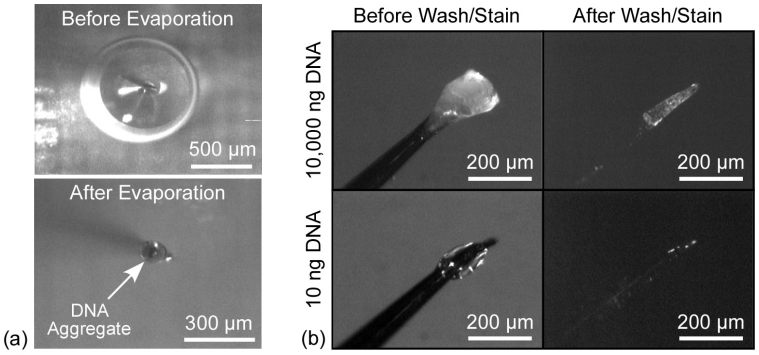

Fig 4: (a) A 15 µm, 40°C tip is lowered into a floating droplet containing DNA suspension. As the liquid evaporates, DNA aggregates onto the tip, and the tip is then removed from the oil. (b) DNA precipitate collected from 1 uL samples of 10 µg/uL Calf Thymus DNA (top) and 10 ng/µL Human Male DNA (bottom), shown in a bright field image before staining (left) and in 490 nm/500 nm fluorescence after immersing in SYBR Green I DNA stain (right).

6. CONCLUSIONS

The experiments described in this effort demonstrate that a heated microprobe tip is a versatile tool that accomplishes several functions in chipless microdroplet analysis systems, including droplet mixing, merging, concentration, and precipitation.

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