



DROPLETS FOR HIGH THROUGHPUT MICROFLUIDIC BIOASSAYS

Namwon Kim
Ph.D. Candidate

Faculty Advisor: Dr. Michael C. Murphy and Dr. Dimitris E. Nikitopoulos

ABSTRACT

Continuous delivery of samples/reagents using pressure-driven segmented flow in microchannels is a promising technology for high throughput microfluidic bioassays.¹ Separation of target sample/reagent cocktails and encapsulation within an inert carrier fluid provides many advantages over single phase flow. It reduces the sample/reagent volume, opens the door for multiplexing (different cocktails per segment), limits nonspecific adsorption of the cocktail ingredients on channel walls, and provides isolation to avoid cross-contamination. In order to achieve these advantages and control these segmented two-phase flows it is necessary to understand their generation and transport characteristics as influenced by geometrical miniaturization, channel wall properties, the effects of surfactants and operating conditions.

Liquid-liquid segmented flows in microchannels fabricated on polymer test chips were investigated experimentally. Polymer test chips were prepared using hot embossing of polycarbonate (PC) or polymethylmethacrylate (PMMA) sheets with micro-milled brass mold inserts. Three different configurations of microchannels were used in experiments designed to examine the effects of the injection channel geometry on the characteristics and stability of the resulting segmented two-phase flows. Surface modification was also used to render the polymer channel walls hydrophobic in order to examine wettability effects. The carrier fluid was biocompatible perfluorocarbon mixed with a nonionic fluorosoluble surfactant and the dispersed fluid was deionized water representing a typical analytical.

Two-phase flow maps and the operating windows over which different types of segmented flow can be stably sustained were not universal and strongly dependent on the configuration of the micro-injection system. Representative flow regimes are shown in Figure 1. The injection channel cross-section size and the area expansion ratio (ER) with respect to the test channel were significant influencing parameters. This is evident in Figure 2, where flow pattern maps and the transitions between flow regimes in the superficial velocity space are shown. The topological

characteristics of the dispersed segmented flows (droplet and plug size and pitch) as functions of carrier fluid volumetric flow ratio (β_C) were determined by image processing of frames acquired with a CCD camera with bright field illumination on a microscope. Velocities of the dispersed droplet and plug flows were measured using double-pulsed laser illumination and were found to be 1.46 and 1.25 times faster than the mixture superficial velocity for the segmented flow, respectively (Figure 3).

As a practical application of multiphase flow in high throughput bio assay, endonuclease-inhibitor activity within continuously segmented droplets is being monitored by using fluorescence cross-correlation spectroscopy (FCCS), which is a sensitive tool for monitoring coincident signals of two spectrally distinct fluorophores from a small number of molecules to generate information on enzyme activity² (Figure 4). Droplets flowing in the microchannels were monitored with the dual-color emission FCCS instrument shown in Figure 5. The optical system was demonstrated to monitor regularly spaced droplets loaded with single fluorescent beads at delivery rates from 40-60 droplets s^{-1} (Figure 6).

ACKNOWLEDGMENTS

This work was partially funded by grants from the National Science Foundation under grant EPS-0346411, MRI grant NSF-9977576 (CTS), and NSF EPS-0701491/LEQSF (2007-10)-CyberRII-02 as well as the State of Louisiana Board of Regents under grant LEQSF (2005-06)-ENH-TR-20.

REFERENCES

- [1] M. Curcio and J. Roeraade, "Continuous segmented-flow polymerase chain reaction for high-throughput miniaturized DNA amplification," *Analytical Chemistry*, 75, 1 (2003)
- [2] Kettling, U.; Koltermann, A.; Schwille, P.; Eigen, M., Real-time enzyme kinetics monitored by dual-color fluorescence cross-correlation spectroscopy. *Proc. Natl. Acad. Sci.*, 95, 1416 (1998)

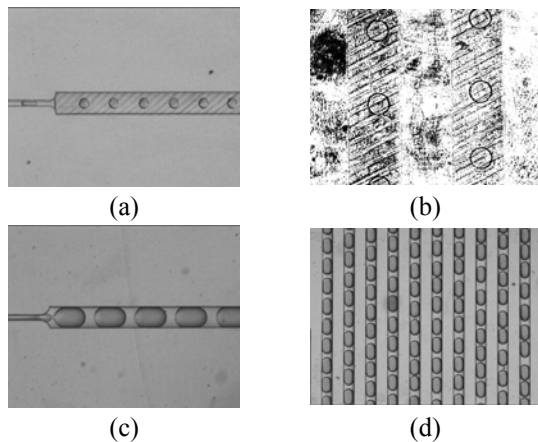


Figure 1. Liquid-liquid segmented flows from the Type I chip (ER=16): (a) Droplet flow in the expansion area ($\beta_C = 0.93$); (b) Droplet flow in the test channel with, $\beta_C = 0.95$, under laser illumination; Liquid-liquid segmented flow regimes from Type II chip (ER=4): (c) Plug flow in the expansion area ($\beta_C = 0.4$) (d) Plug flow in the test channel ($\beta_C = 0.4$)

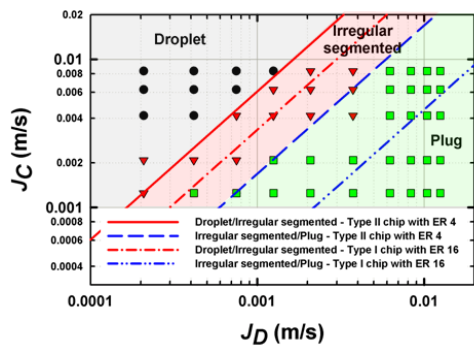


Figure 2. Liquid-liquid segmented flow regime map and regime-transition lines observed from Type I chip, ER=16. (●: Droplet flow, ▼: Irregular segmented flow, and ■: Plug flow)

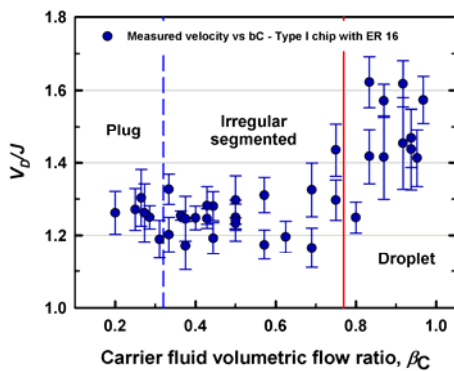


Figure 3. Measured dispersed fluid velocity (V_D) from the Type I chip (ER=16), scaled by the mixture superficial velocity ($J = J_C + J_D$) as a function of β_C .

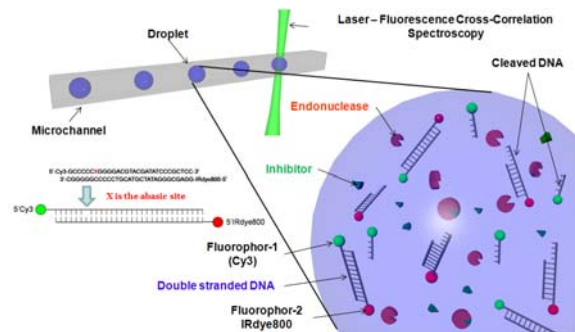
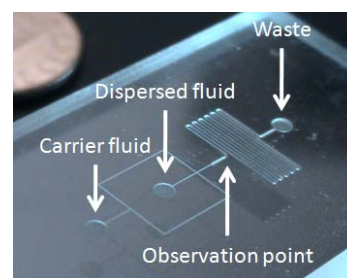
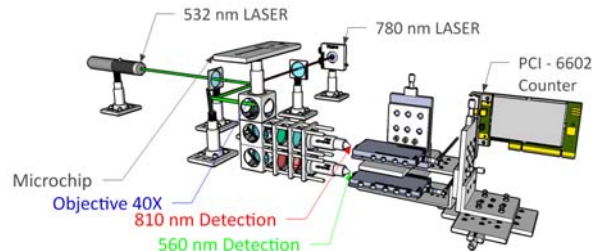


Figure 4. Monitoring of enzyme activity in droplet for high throughput bioassay using fluorescence cross-correlation spectroscopy (FCCS)

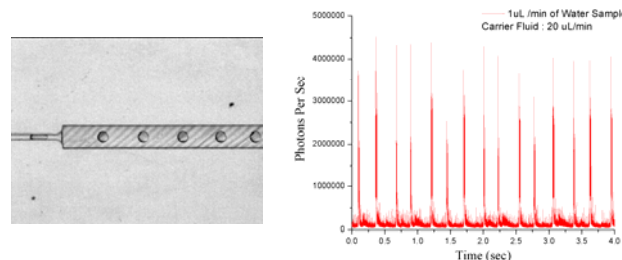


(a)



(b)

Figure 5. (a) Configuration of the polymeric microfluidic chip for generating droplet two-phase flow and (b) instrumentation setup for two-color FCCS



(a)

(b)

Figure 6. (a) Droplets containing fluorescent microspheres and (b) fluorescence signals from droplets delivered at 1 $\mu\text{l}/\text{min}$ with 20 $\mu\text{l}/\text{min}$ carrier fluid volumetric flow rate