MONITORING MIXING DYNAMICS CONFINED WITHIN AQUEOUS MICRODROPLETS WITH 5 μs RESOLUTION

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ABSTRACT
In this paper, we describe the use of spatially-resolved fluorescence lifetime imaging (FLIM) to reconstruct mixing patterns within microdroplets with a 5 μs resolution.

Keywords: droplets, mixing, fluorescence lifetime, FLIM

1. INTRODUCTION

Water-in-oil microdroplets within fluidic channels have the potential to serve as isolated reaction compartments for monitoring real-time dynamics with high efficiency and repeatability. Droplets can be generated at frequencies in excess of 1 kHz. Although mixing within such microdroplets is normally enhanced by chaotic advection the mixing pattern from droplet to droplet is almost identical and reproducible in form. In this paper, we demonstrate that fluorescence lifetime imaging can be used to reconstruct mixing patterns within a droplet with a time resolution of 5 μs.

2. EXPERIMENTAL

Detection setup: A custom built confocal fluorescence detection system for droplet detection is schematically shown in **Figure 1a**.

Lifetime measurements: A PDMS fluidic chip (containing 50 μm wide and 50 μm deep channels) [1], consisting of 2 aqueous inlets, one oil inlet and a winding section was used for all experiments To create a microdroplet system, an oil phase, a 10:1 (v/v) mixture of perfluorodecalin and 1*H*,1*H*,2*H*,2*H*-perfluorooctanol, and aqueous solutions were continuously pumped at a constant flow rate of 2.0 μl min^{-1} (for each phase), resulting in the total flow rate of 4.0 μl min^{-1} (26.67 mm s^{-1}). The first aqueous inlet consisted of 20.0 μM fluorescein 5-isothiocyanate (FITC) (in pH 9.0 buffer) delivered at a flow rate of 0.75 μl min^{-1}. A 2.0 M potassium iodide (KI) solution (in pH 9.0 buffer) was pumped into the second aqueous inlet at a flow rate of 1.25 μl min^{-1}. A schematic of the microfluidic device topology is illustrated in **Figure 1b.** The KI acts as a fluorescence quencher [2] which decreases the fluorescence lifetime of the dye molecule when fully mixed. We use this attribute to map out mixing within single aqueous microdroplets. FLIM data were recorded at the first loop of the winding channel, which is 253.7 μm downstream of the droplet forming region. The detection probe volume was set at a channel depth of 25 μm and was scanned every 1 μm across the 50 μm wide channel in order to reconstruct the mixing pattern for the whole droplet.

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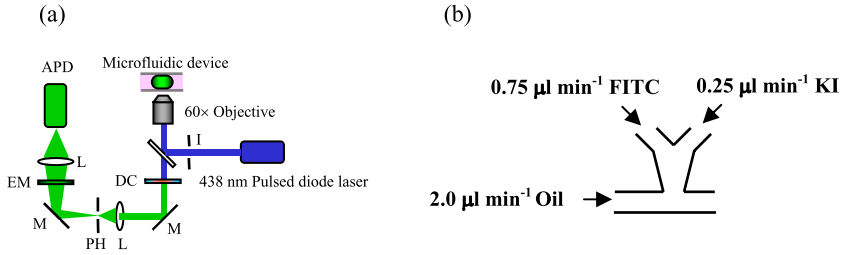

Fig. 1. (a) A home built confocal microscope used for fluorescence lifetime measurements: APD-avalanche photodiode detector, DC-dichroic mirror, EM–emission filter, I-iris, L-lens, M-mirror and PH-pin hole. (b) Schematic of a microfluidic device setup for lifetime measurements.

3. RESULTS AND DISCUSSION

An example of the variation in signal intensity as a function of time is shown in **Figure 2a**. These trajectories were obtained at a position 25 µm deep and 10 µm from the edge of the channel wall. Each peak represents an aqueous droplet transiting the 350 nm wide diffraction limited detection probe volume. In **Figure 2b** the fluorescence lifetime determined using a customized maximum likelihood estimator algorithm is shown for each burst. The algorithm itself is typically used in single molecule lifetime spectroscopy as demonstrated by Edel et al [3]. Each point in this plot represents a fluorescence lifetime measured with a maximum of 20 photons. Using our acquisition electronics, this corresponds to an acquisition time of 5 µs per point. In turn, this corresponds to approximately 600 points per droplet in 1 dimension. A high resolution interpretation of the lifetime trajectory is shown in **Figure 3**. This was obtained by averaging the lifetime of 300 droplets at each and every point. The short lifetimes of ~1.6 ns within the droplets confirm a quenched location and hence complete mixing has taken place at that particular point. A lifetime of 3.7 ns correlates to a perfectly unmixed state. Using this information we show that a droplet can be reconstructed based on fluorescence lifetimes with 5 µs resolution, as shown in **Figure 4**.

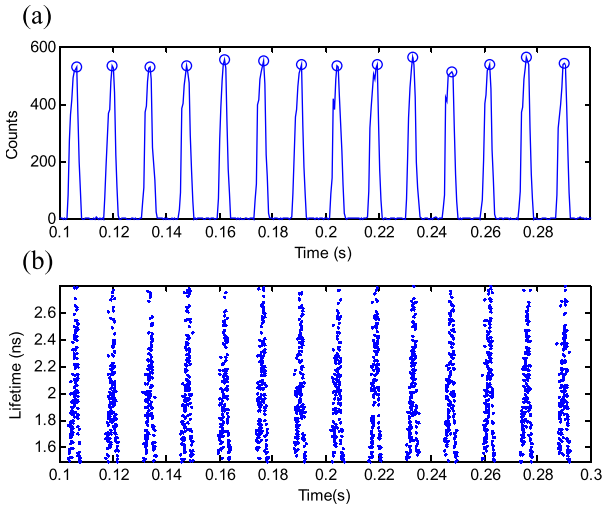

Fig. 2. (a) Intensity trajectory of individual aqueous droplets transiting the detection probe volume. (b) Fluorescence lifetime trajectory of the same droplets.

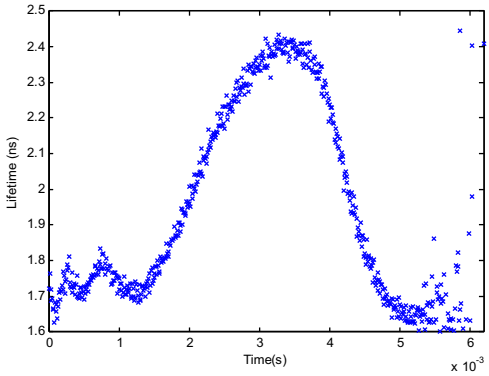

Fig. 3. High resolution lifetime trajectory obtained with 20 photons per point (5 µs). Left most point is the beginning of the droplet transiting the detection probe volume. Right most point is the tail of the droplet.

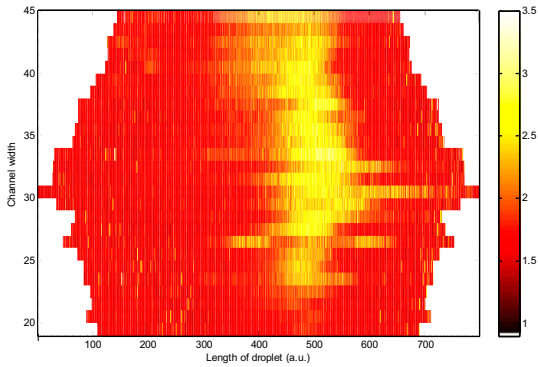

Fig. 4. 2D map of reconstructed droplet at the first loop. The droplets at this point are nearly fully mixed as confirmed by the fluorescence lifetime.

4. CONCLUSIONS AND OUTLOOK

FLIM was successfully used to reconstruct mixing patterns inside droplets with a resolution of 5 µs. Being able to monitor mixing dynamic with such high time resolution will prove to be valuable for monitoring high-speed reaction dynamics and kinetics.

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