Three-Dimensional molecular Mapping in a Microfluidic Mixing Device Using Fluorescence Lifetime Imaging

Three-dimensional molecular mapping in a microfluidic mixing device using fluorescence lifetime imaging

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Fluorescence lifetime imaging (FLIM) is used to quantitatively map the concentration of a small molecule in three dimensions in a microfluidic mixing device. The resulting experimental data are compared with computational fluid-dynamics (CFD) simulations. A line-scanning semiconfocal FLIM microscope allows the full mixing profile to be imaged in a single scan with submicrometer resolution over an arbitrary channel length from the point of confluence. Following experimental and CFD optimization, mixing times down to 1.3±0.4 ms were achieved with the single-layer microfluidic device. © 2008 Optical Society of America

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Microfluidic systems are attractive for studying reaction kinetics in fluids because of the potential to reduce reagent volumes, time and cost of fabrication, and mixing speeds [1]. To allow accurate measurement of rate constants using a microfluidic mixer, it is important to obtain a comprehensive understanding of the fluid dynamics within the system. It is standard practice to simulate a mixing device design using computational fluid dynamics (CFD) to optimize the microfluidic device and also assist in the interpretation of kinetic data [2]. However, it is normally extremely challenging to validate the CFD simulations. Herein we present a method for directly visualizing fluid dynamics in a microfluidic mixer using optically sectioned fluorescence-lifetime imaging (FLIM) and compare these experimentally obtained results with three-dimensional (3-D) CFD simulations. Previously, steady-state fluorescence has been employed to monitor the mixing of fluids within hydrodynamic focusing devices. These reports have included the use of Förster resonance energy transfer [3], two-photon absorption [4,5], and confocal fluorescence imaging [6]. Although such methods have proven to be successful, intensity imaging is prone to artefacts associated with fluorophore concentration, nonuniform illumination, detection efficiencies, the inner-filter effect, and optical scattering. FLIM has already been shown to provide a robust means of imaging mixing in microfluidic environments [4,7]. Here we demonstrate a novel scheme to provide 3-D chemical concentration mapping over an extended (~cm) field of view. This involves a single automated data-acquisition step that images the (calibrated) change in lifetime due to the presence of a molecular quenching agent [8]. We compare the experimental data to the CFD simulations to validate our technique.

A schematic of the microfluidic mixer device studied here is shown in Fig. 1(a) and was made using soft lithographic techniques [9] that produced channels of 50 μm depth and 67 μm width (±3 μm). Subsequently the surfaces of the glass and polydimethylsiloxane (PDMS) components were treated in an oxygen plasma for 20 s and left for 24 h to bond. Fluids were delivered from two separate syringe pumps

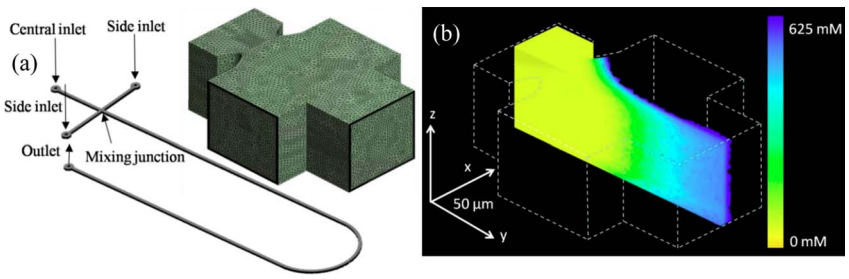

Fig. 1. (Color online) (a) Schematic of channel layout and (inset) CFD mesh of mixing junction. (b) 3-D CFD simulation result of the mixing junction, where the scale bar represents the concentration of NaI within the fluorescein stream. The channel edges are outlined with dashed white lines, the central and side channel flow rates are 1 and 6 μL/min respectively, and the image has been sliced in the y–z plane down the center of the channel to facilitate inspection of the mixing.

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with 1 mL syringes to provide independent flow rates in the central and side channels. Typical flow rates in the inlet channels were between 0.1 and 6.0 μL/min.

3-D CFD simulations of the mixer were performed using the finite-volume-based commercial code, CFX 11 (ANSYS, Canonsburg, Pa.). The flow domain, depicted on the right in Fig. 1(a), with dimensions corresponding to that used in the experiment was discretized using an unstructured mesh formed of tetrahedral elements and prismatic elements near the walls. No slip boundary conditions, $\mathbf{u}=0$ (where $\mathbf{u}$ is the 3-D velocity vector), were used at the domain walls, and Dirichlet boundary conditions for fixed normal velocity along with experimental concentrations of sodium iodide (NaI) and fluorescein were set at the inlets. At the central inlet, fluorescein had a concentration of 500 μM (with no NaI), and at the side inlets the concentration of NaI was 625 mM (with no fluorescein). A mixed Dirichlet–Neumann boundary condition (zero static pressure and zero normal gradient of velocity and concentration) was assigned at the outlet. The diffusivities of NaI in water ($D_{\text{NaI-Wat}}=2\times10^{-9}$ m^2 s^{-1}) and fluorescein in water ($D_{\text{Fl-Wat}}=6\times10^{-10}$ m^2 s^{-1}) obtained from the classical Stokes–Einstein equation for dilute liquid solutions [10] are used in the convection–diffusion equations of the individual components to solve for the simultaneous diffusion processes taking place in the mixer. A study of mesh resolution determined that a maximum cell length of 2.5 μm with an inflated boundary, comprising 10 prismatic layers of 5 μm thickness, produced consistent results, with higher-resolution meshes providing no change in the outcome but requiring more computation. This corresponds to a total of 758,553 volume elements for which the typical computation time was 30 min on a dual-core Intel processor.

The microfluidic mixing process was also studied experimentally using a home-built line-scanning, semiconfocal, hyperspectral FLIM microscope previously reported in [11]. Briefly, the microfluidic channel is illuminated across its width by a line originating from a frequency-doubled Ti:sapphire femtosecond laser (Mai Tai, Spectra-Physics, Mountain View, Calif.) tuned to 460 nm and directed to the sample through an inverted epifluorescence microscope (IX71, Olympus, Tokyo, Japan) with a 60X, 0.8 NA air objective. The resulting line of fluorescence is relayed through a spectrograph (Specim Imspector V8E, Oulo, Finland), a gated optical intensifier (HRI, Kentech Instruments, Wallingford, UK), and finally an EMCCD camera (iXon DV887, Andor, Belfast, UK). Using a stage scanner and z stepper (SCAN IM, Märzhäuser, Wetzlar-Steindorf, Germany), the line is then scanned down the length of the channel at various depths. The result is a five-dimensional data stack (x, y, z, λ, τ) from the microfluidic channel. For this study we did not require the spectral resolution and so integrated over the spectral emission profile to obtain fluorescence lifetime images. Any scattered excitation light was eliminated by a 515 nm long-pass filter. A typical acquisition used 90 steps in the y direction and 10 steps in the z direction, sampling the fluorescence decay profiles with seven time gates of 1 ns width, each with a 0.1 s integration time. The total acquisition time was ~10 min, which is suitable for imaging laminar flow mixers but is too slow to image turbulent mixing. Fluorescence lifetimes were analyzed using a single exponential decay model using custom-written software (LabVIEW, National Instruments, Austin, Tex.).

To characterize the mixing time of the device, 500 μM fluorescein and 625 mM NaI solutions (both in a 500 mM Tris buffer at pH 8.3) were introduced in the central and side channels, respectively, so that the quenching of the fluorescein by iodide ions could be used to report on the mixing of the two fluids. The fluorescence lifetime can report quenching ion concentration [8] and facilitate imaging of the mixing process. The fluorescence lifetime is related to the concentration of quencher Q by the Stern–Volmer equation, $\tau_0/\tau=1+k_q\tau_0\,[Q]$, where τ_0 and τ are the fluorescence lifetimes in the absence and presence of the quencher and k_q is the bimolecular quenching rate coefficient [12]. By measuring τ at various $[Q]$ values, a linear plot of τ_0/τ against $[Q]$ was extracted and a value of 2.7×10^9 M^{-1} s^{-1} was obtained for k_q. This value was then used to calculate the concentration of quencher from the lifetime value.

Figure 1(a) shows a schematic of the channel layout and the corresponding CFD mesh. The inlet channel has a nozzle at the mixing junction to aid the focusing [6]. Figure 1(b) shows the results of the CFD simulation in which the concentration of NaI in the fluorescein stream at a given finite volume is displayed using a color scale. Properties of the fluids were chosen to match those of iodide ions diffusing across a focused fluorescein stream. Such simulations can be invaluable for analyzing and optimizing the performance of such a microfluidic mixing device but are not straightforward to verify the accuracy of such CFD simulations. Figure 2(a) illustrates how a 3-D map of mixing in the microfluidic device can be acquired experimentally using FLIM. This image was rendered using Volocity (Improvision, Coventry, UK) from a stack of optically sectioned concentration maps calculated from the FLIM maps recorded at each z position (using the Stern–Volmer equation). It should be noted that half the image of the focused fluorescein stream is removed in postprocessing to reveal the mixing within. This image is in reasonable agreement with the CFD simulation in Fig. 1(b). We note that the length of the channels in the simulation was truncated to reduce computational load. For a more quantitative analysis, Fig. 2(b) shows the concentration of the second quenching fluid at the center of the channel from the experimental and simulation data, both plotted as a function of distance along the y axis. The differences between the curves are likely to arise from such experimental factors as pressure losses and fabrication defects that the simulation did not take into account.

As noted, fluorescence intensity imaging of fluorophore quenching has been used to assess mixing in a microfluidic mixer [5]; however, such an approach requires two separate measurements (with and with-

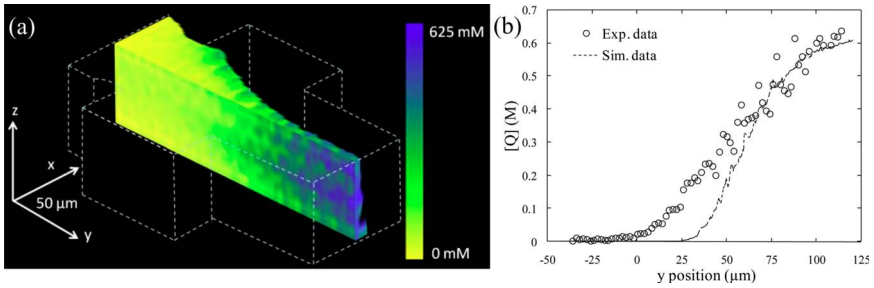

Fig. 2. (Color online) (a) 3-D concentration map of the mixing junction. The central and side channel flow rates are 1 and 6 μL/min, respectively. (b) Profiles of $[Q]$ calculated from the experimental fluorescence lifetime values and the CFD simulation as a function of position in the y direction. The experimental data are shown as circles and the simulation data as a dashed curve.

out the quenching agent), which could introduce errors resulting from changes in the experimental conditions between measurements or from the processing required to normalize intensities, etc. In contrast, the FLIM approach used herein requires only one measurement after calibration. Moreover, the fluorescence lifetime is independent of the excitation/detection efficiency or the fluorescein concentration and is only effected by the concentration of the quencher. A particular advantage of the line-scanning microscope is that the complete mixing process can be imaged down an arbitrary channel length in one acquisition and with high spatial resolution. This is important for continuous-flow experiments, in which each y position along the channel corresponds to a time point during the course of a reaction or mixing process.

Figure 3 compares results from FLIM experiments and CFD simulations to study how the mixing time of the device varies as a function of flow rate. The mixing time is defined as the time the concentration passes from 10% to 90% of the equilibrium concentration and can be calculated from the mixing profile in the y direction along the center of the channel together with the flow rate and channel dimensions. Figure 3(a) shows how the mixing time depends on the ratio of the side to central channel flow rates (α). Changing α changes the width of the focused stream and hence the volume through which the quenching ions have to diffuse to complete mixing [6]. Figure 3(a) indicates that α should be maintained between $\sim$8 and 12 to ensure rapid mixing. The increase of the mixing time at high α values is due to the low flow rates of the central channel. Values of α above 17 are not achievable in the current system, because at this point the side stream begins to flow up the central channel and focusing is lost. Figure 3(b) shows how the mixing time varies as the total flow rate is changed while keeping α constant. It can be seen that increasing the total flow rate beyond $\sim$7 μL/min does not significantly decrease the mixing time. These figures indicate how well the CFD models the actual mixing process and reproduces the variation of mixing time with flow rates. We note that it is often important to optimize flow rates while achieving rapid mixing in order to minimize the use of potentially valuable reagents. For the device studied here, the optimum mixing time was found to be 1.3 ± 0.4 ms when α was 8.25, and the total flow rate was 7 μl/min with the two data sets in reasonable agreement.

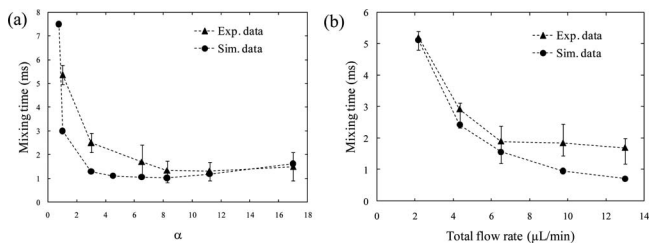

Fig. 3. (a) Mixing times from experimental and simulated data as a function of (a) α (side flow rate/central flow rate) for a fixed total flow rate of 7 μL/min and (b) total flow rate for a fixed α of 6.

We have shown that the use of a line-scanning optically sectioning FLIM microscope is a powerful tool to image 3-D fluid dynamics in microfluidic devices. It possesses the ability to preserve high spatial resolution along arbitrary channel lengths during a single image acquisition. This approach is useful to validate and provide a confidence level in CFD simulations to analyze the performance and optimization of such devices.

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