Microfluidic Mixer for Time-Resolved Single-Molecule Fluorescence Experiments using Flip-Chip Bonded SU-8 Structures

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Primary CNF Tools Used: ABM contact aligner, class 2 resist room, VersaLaser engraver/cutter

Abstract:

We report the fabrication procedure for a microfluidic mixer capable of detecting single, fluorescently labeled biological molecules as they progress through a reaction. Precise flow control and device longevity is achieved using hard materials (SU-8) for fabrication, in place of traditional replica molding.

Summary of Research:

Single-molecule fluorescence experiments are powerful tools for elucidating structural characteristics of biological molecules [1]. Techniques such as fluorescence correlation spectroscopy (FCS) report global molecular size, while Förster resonance energy transfer (FRET) experiments yield distances between labelled residues with Angstrom precision. Critically, these techniques can probe single-molecules, granting detailed information about the underlying population that is smeared out in a bulk measurement. Long measurement times are required to gather statistics from a small (1 μm^3) focal volume, often requiring tens of minutes of data acquisition per condition.

While single-molecule experiments are routinely performed at equilibrium, the dynamic nature of biology demands an approach that embraces the time evolution of these systems. A typical strategy to provide time-resolution in fluorescence experiments exploits microfluidic mixing (e.g., ref 2). Molecules are rapidly introduced to initiate a reaction, then spatially separated to provide time-resolution. These technologies are limited to bulk measurements due to the flow speed and exposure times associated with these devices. Furthermore, the length of time required to obtain a single-molecule data set (typically several hours), paired with the pressures required to precisely control the flow (~ 1000-5000 mbar) presents challenges for conventional soft material approaches, though some have been developed and applied [3]. To meet these criteria, we designed and fabricated microfluidic devices from SU-8 to rapidly mix reagents, then subsequently slow the flow speed to a range compatible with single-molecule detection (~ μm/ms).

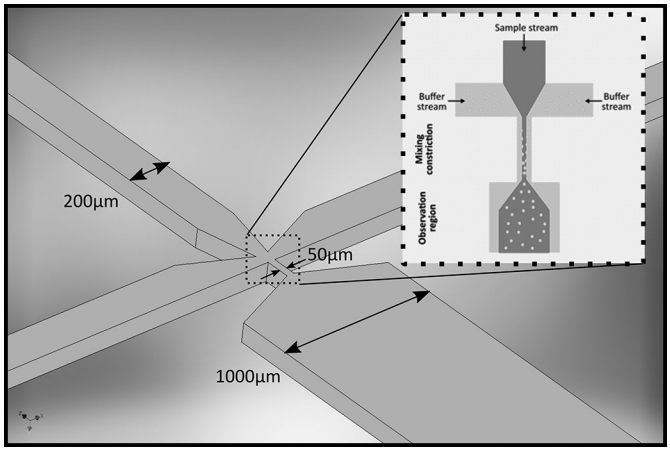

Figure 1: The device design (main panel) is based on the principle of hydrodynamic focusing (inset).

The device design is based on the hydrodynamic focusing principle (Figure 1 inset), where a sample stream is squeezed by flanking buffer streams and forced through a narrow constriction [4]. This yields a micron-sized sample stream in the constriction. Diffusion occurs rapidly across this stream, introducing a reactant from the buffer that initiates a given reaction. This narrow stream is then expanded out into a wide probing channel, which

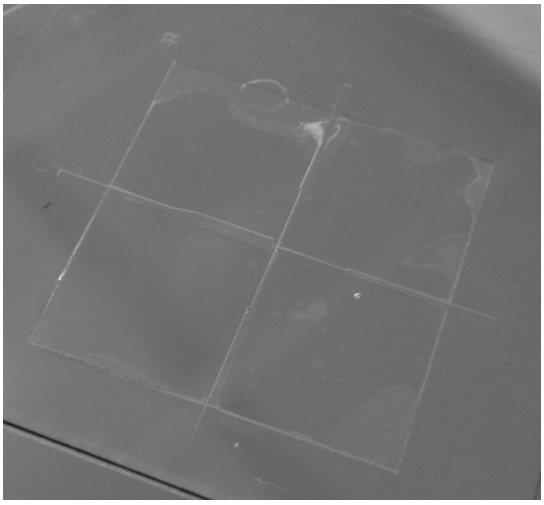

Figure 2: Stereoscopic image of a completed device.

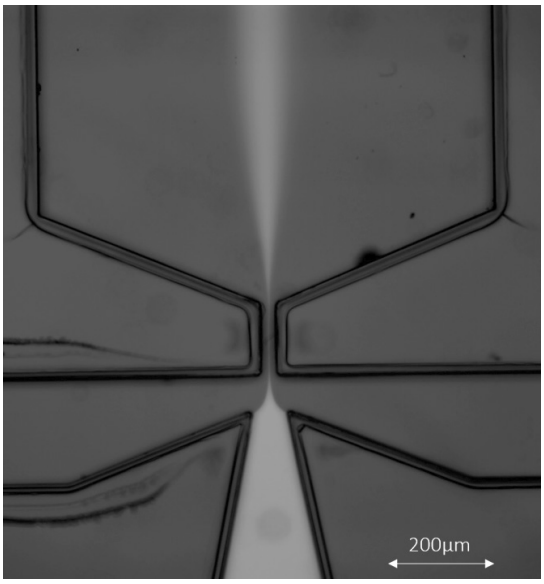

Figure 3: False colored fluorescent image showing a test sample (fluorescent dye Rhodamine 6G, bright) flowing in the device.

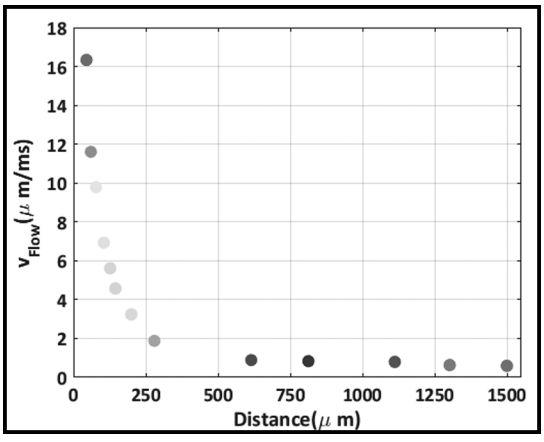

Figure 4: Flow speeds (in microns per millisecond) as a function of distance from the end of the constriction as measured by FCS.

acts to slow the flow speed down to detectable levels. Probing at different spatial locations along the observation channel provides different time delays after the reaction is initiated.

The design in Figure 1 was first replicated in a chrome mask using the Heidelberg 6600 mask writer. The device is then fabricated from four layers: two glass layers that act as windows on the top and bottom, one layer of SU-8 that defines the channel geometry, and one sealing SU-8 layer. In the first process, a 100 μm thick layer of SU-82050 is spun on borofloat, and subsequently exposed, baked and developed to yield the channel geometry. A thin sealing layer of SU-82005 is next deposited over the existing geometry, and a glass cover slide installed on top of this layer. The stack is then baked and exposed through the glass cover slide with the same mask used to pattern the channel geometry. This step acts to polymerize the sealing SU-8 2005 layer in all regions except the underlying channels. Excess SU-8 from the sealing layer that may have entered the channels is then removed by development, yielding fluidic channels which are sealed, clear and flanked solely by glass. A stereoscopic image of a completed device is shown in Figure 2.

To demonstrate the device and flow stream, we used a fluorescent dye (Rhodamine 6G), to act as a control sample, and water on the flanking buffer channels. Figure 3 shows a false colored image of this experiment, where fluorescence intensity is denoted by brightness. The sample stream clearly shrinks in the mixing constriction, before slowing and expanding in the observation region. Measurements of the sample jet widths in the constriction place it between 5-9 μm depending on the flow rates. This results in tunable mixing times between 4-10 ms for added salts and small molecules.

Finally, the flow speed in the observation region can be probed by FCS (Figure 4). For the test system of Rhodamine 6G, the flow can be reduced to less than 1 μm/ms, easily compatible with the dwell time required for confocal microscopy. For this system, the flow in the observation region slowed to single-molecule detectable levels at a time point corresponding to 30 ms after mixing is complete. Thus, in this case the device can access time points ranging from 30-3000 ms in the single-molecule regime.

The mixer lends itself well to our future applications in studying the non-equilibrium collapse of regulatory RNA elements. These motifs sense metabolites and ion levels in the cell, and drastically alter their conformation depending on the ligand concentration [5]. Molecular re-arrangements occur on time-scales ranging from 10 ms to many seconds, but detailed characterization of the sub second structural response is lacking due to the absence of appropriate experimental technology.

References:

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