Microfluidic Flow Cells for Time-Resolved Fluorescence-Based Studies of Biomolecules

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Primary CNF Tools Used: Heidelberg DWL2000, Oxford PECVD, ABM mask aligner, Oxford 80 RIE, Unaxis 770 Deep Silicon Etch, Suss SB8e Substrate Bonder, DISCO dicing Saw

Abstract:

We report the fabrication of microfluidic flow cells for time-resolved fluorescence measurements of biological molecules. These silicon and glass devices improve upon our previous design by allowing the CNF-fabricated flow cell to be combined with multiple types of microfluidic mixers. The simplified design will enable time-resolved studies of interactions between biological molecules such as nucleic acids and proteins.

Summary of Research:

Single-molecule fluorescence techniques are useful tools for studying dynamic, flexible biological molecules including single-stranded nucleic acids. One such method is fluorescence correlation spectroscopy (FCS) which can provide information about the size and interactions of molecules in solution by measuring the fluctuations in emitted photons from fluorescently labeled molecules in solution as they diffuse in and out of a laser confocal volume [1]. Another useful technique is Förster resonance energy transfer (FRET), which takes advantage of the distance dependence of energy transfer between pairs of fluorescent dyes to measure inter- and intra-molecular distances. At the single molecule level, these measurements provide insight into the dynamics and distribution of conformations in heterogeneous samples such as disordered single-stranded RNA [2]. Microfluidic devices enable time-resolved versions of these experiments, where a biological interaction is initiated and probed as a function of time as the molecules flow along a channel.

Here we describe the fabrication of durable, reusable microfluidic channels that can interface with our lab-built confocal fluorescence microscope as well as different types of microfluidic mixers, providing flexibility in terms of the techniques we can use and the biological systems we can investigate. The design is based on our previously established protocol for fabricating microfluidic mixing devices for time-resolved fluorescence measurements [3]. We now improve upon the earlier design by fabricating a simplified flow cell which can be interfaced with different microfluidic mixers depending on the properties of the system being studied. The flow cell consists of a channel etched into silicon, sealed by borofloat glass to make it compatible with a water immersion objective lens. The fabrication process, outlined in Figure 1, consists of several steps making use of the photolithography, etching, and bonding tools at the CNF. First, a layer of silicon dioxide is deposited onto a silicon wafer using the Oxford PECVD tool. This oxide layer serves as a hard mask for deep etching of the silicon. Next, a layer of photoresist is spun and baked onto the wafer before being exposed through a mask using the ABM contact aligner. After developing to remove the exposed photoresist, we etch

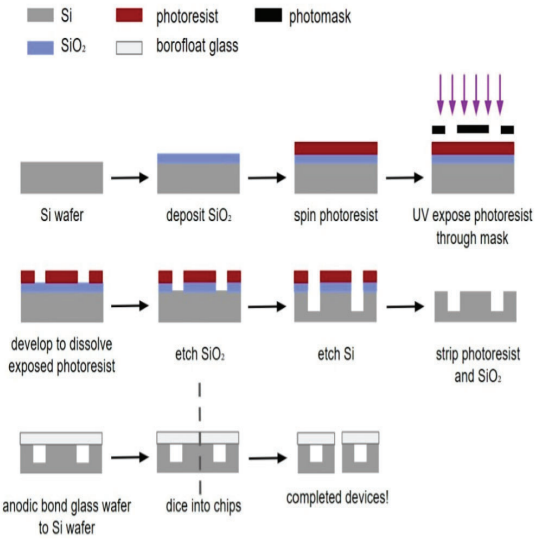

Figure 1: Fabrication process for making many silicon and glass flow cells out of a single wafer.

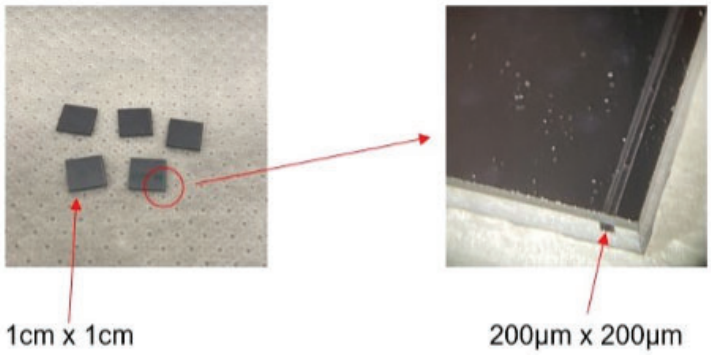

Figure 2: The image on the left shows several flow cells at the end of the fabrication process, after the bonded silicon and glass wafers have been diced. The image on the right is a stereoscope view of one flow cell, with a square opening at the front and the channel visible through the layer of glass.

first through the oxide using the Oxford 81/82, then into the silicon using the Unaxis deep silicon etcher, creating channels that are 200 µm wide and 200 µm deep. To seal the top of the channels, a 200um thick borofloat glass wafer is anodically bonded to the silicon using the Suss SB8E substrate bonder. Finally, the bonded wafers are diced into 1 cm x 1 cm chips. One wafer processed in this way results in about 40 individual chips, each of which contains a 200 µm x 200 µm square channel, sealed on four sides and open on the ends to allow for flow through the channel. A diced wafer and an individual chip are shown in Figure 2.

The completed chips, or flow cells, can then be coupled with a microfluidic mixer to perform time-resolved experiments. The simplicity of the design means that the mixer upstream of the flow cell can be customized for each experiment. Depending on the sizes of the biological molecules being mixed and the timescales of interest, a suitable mixer can be built to efficiently mix the reactant molecules and flow them through the observation channel where fluorescence measurements are recorded. For example, a system in which a small molecule is reacting with a large molecule might employ a coaxial diffusive mixer, while fast mixing of two large molecules requires a different method of mixing such as chaotic advection in a 3D printed mixer [4].

Conclusions and Future Steps:

We have fabricated versatile flow cells which, when paired with microfluidic mixers, will allow us to use time-resolved fluorescence techniques to study a variety of dynamic biological molecules. Future experiments will use these devices to investigate systems such as nucleic acid-protein and nucleic acid-nucleic acid interactions. These experiments will complement the structural information that can be obtained from other techniques, particularly x-ray scattering.

References:

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