Large-Scale Microfluidic Device Fabrication for Non-Equilibrium RNA Kinetic Experiments

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Primary CNF Tools Used: Plasma-Therm deep silicon etcher, substrate bonder, dicing saw, ABM contract aligner, Heidelberg mask writer

Abstract:

Here we report the design and implementation of a large-scale fabrication protocol to produce ~ 100 microfluidic mixing devices per wafer. These devices enable non-equilibrium single-molecule fluorescence measurements. This upgraded protocol improves on our previous work by increasing the yield, number and robustness of devices while decreasing the fluorescence background. Such improvements allow kinetic measurements on RNA to be made more routinely, easily and with higher data quality.

Summary of Research:

Non-equilibrium measurements are powerful tools that can be applied to study biological interactions [1]. The rapid initiation of biological reactions and examination of their time evolution, exposes information that is hidden in simple equilibrium experiments. This technique provides details on short lived intermediates, folding pathways and transition states.

While the benefits of non-equilibrium measurements are numerous, such techniques are rarely applied when compared to their equilibrium counterparts. The major bottle-neck is the lack of commercially available systems to perform these experiments. Only the stopped-flow technique is widely used, but requires high sample volumes, limiting the number of kinetic measurements that have been made. Additionally, in cases where kinetic measurements are applied, the experiments report bulk averages. Therefore, sparse or short-lived intermediates are challenging to identify, and careful analysis is required to resolve the presence of other intermediate states along reaction pathways [2].

Coupling microfluidic mixing to single-molecule fluorescence circumvents the above challenges. Examination of single molecules allows resolution of individual subpopulations within a sample, nanomolar concentrations are used, and ~ms timescales are accessible using microfluidics, with sparse sample consumption [3]. We previously designed and fabricated microfluidic mixing devices using SU-8 and glass, however this protocol had some disadvantages. The number of devices per wafer was limited by the use of standard sized glass coverslips, and device robustness and fluorescence background were compromised due to the use of an SU-8 sealing layer.

To bypass these limitations, we developed a fabrication protocol where channels are etched into silicon. Full wafers are subsequently anodically bonded to full sized glass wafers to seal devices. The protocol is schematically shown in Figure 1.

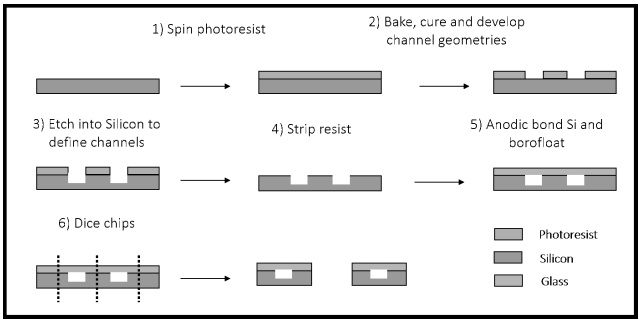

Figure 1: Fabrication process utilized to obtain a large number of robust microfluidic mixing devices.

In brief, a 4 μm thick layer of Shipley resist is spun, baked, and cured on a standard sized silicon wafer. Channel patterns are exposed, and the resist developed. The microfluidic channels are then etched into the silicon using the Plasma-Therm deep silicon etcher — we aim for channel depths of 105 μm. After etching, the resist is removed. The etched silicon piece and a 170 μm thick, full wafer sized borofloat wafer are Piranha cleaned before anodic bonding using the substrate bonder. As a final step, the dicing saw separates individual devices, and liberates channel cross-sections at the edges of each device. Interfacing to the devices is complete by gluing 105 μm outer diameter glass capillary lines into the revealed sockets with UV curable epoxy.

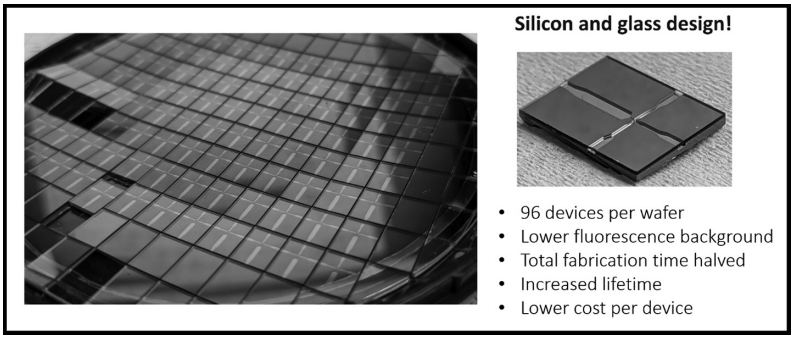

Figure 2: The left shows a wafer of microfluidic devices after the final dicing step. On the right is a stereoscope image of a single device. The thin glass layer can be seen covering the base silicon wafer. Sockets liberated during dicing process are present at the end of the channel and facilitate the connection of fluid lines using UV curable epoxy.

A full wafer of separated devices is shown in Figure 2. Ninety-six devices are made in the same process. On the right, a stereoscope image of a single device from this wafer is shown.

In the stereoscope image, the sockets that interface with external lines are clearly visible. The thin glass layer on top of the silicon base layer is also resolved. Fabrication of devices without an extra adhesive layer in between glass and channel geometries grants higher pressure resilience (as we limit crack propagation at the sealing layer) and reduces the intrinsic fluorescence background from SU-8 and similar epoxies.

Conclusions and Future Steps:

Here we describe an improved fabrication protocol that yields silicon microfluidic mixers as opposed to recent past efforts using glass and SU-8. This new protocol creates many more devices per wafer, at lower cost and reduced fabrication time per piece. We are now positioned to pursue non-equilibrium RNA folding experiments without concern for device robustness, lifetime or inventory.

References:

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