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Microfluidic platform for on-demand generation of spatially indexed combinatorial droplets

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SUPPLEMENTAL DATA

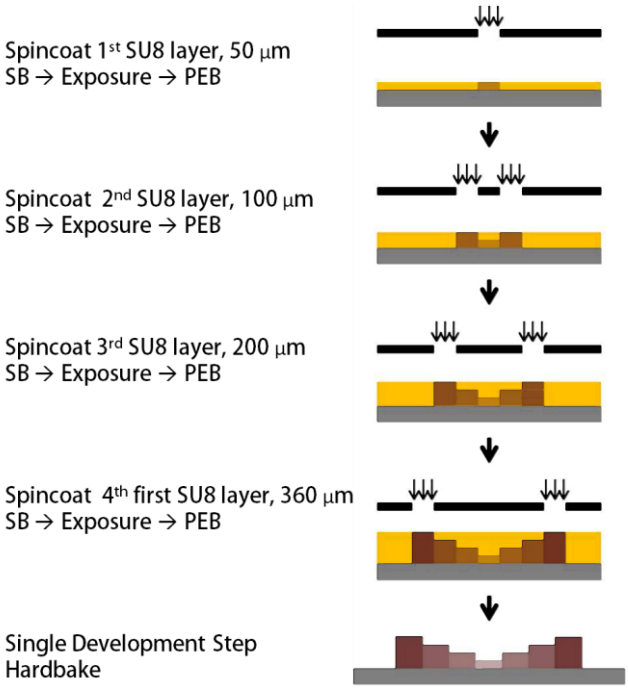
S.1 Mold Fabrication

The work flow used for fabricating the fluidic layer mold is illustrated in Supp. Fig. 1. The mold consists of five different layers of photoresist with heights of 25 µm, 50 µm, 100 µm, 200 µm and 360 µm. The photoresist used for the 25 µm layer was SPR 220-7.0 (Rohm & Haas), while the rest of the layers were fabricated using SU-8 3050 (MicroChem). Initially a 25um tall SPR 220-7.0 layer was spin coated on a silicon wafer. This layer was patterned using photolithography and hard baked to generate a rounded channel cross section, required for effective valve closure. Rest of the layers were fabricated by stacking and patterning multiple layers of SU-8 3050 on the wafer. All the steps required for standard photolithography (Soft Bake, Exposure and Post Exposure Bake) are conducted for each layer of SU-8 3050, except the developing step. This step is conducted in common for all layers after the last SU-8 layer is patterned to remove excess unexposed photoresist from the wafer (Supp. Fig. 1). This technique was found to be very effective in preventing generation of bubbles and non-uniform coating of photoresist on the wafer due to the presence of features from earlier layers on the wafer.

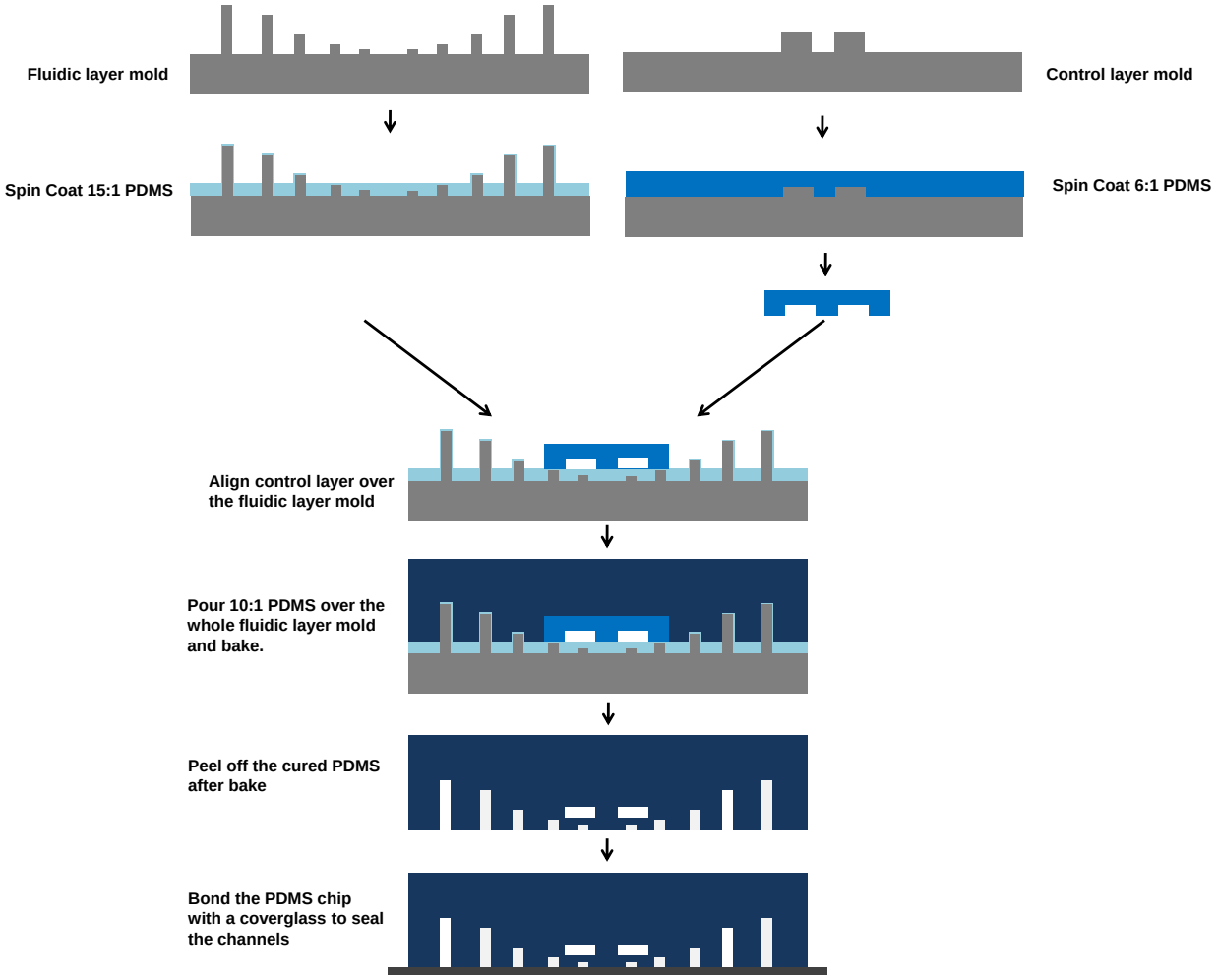
S.2 Device Fabrication and Operation

The microfluidic device for our experiments was fabricated using multilayer soft lithography technique. Standard dual layer microfluidic devices with push-down valves fabricated using polydimethylsiloxane (PDMS) require shallow fluidic channels to make sure the layer of PDMS between the fluidic and control layer is sufficiently thin (~50um) for complete closure of valves at low pressures (<30psi). The requirement of shallow fluidic channels is incompatible with our chip design. So we developed a modified fabrication process for our device. This modified soft

lithography process is outlined in Supp. Fig. 2. For this modified fabrication process, three different batches of PDMS were mixed. These varied in composition, and base to crosslinking agent ratios of 15:1, 10:1 and 6:1 were used, respectively. These batches were thoroughly mixed and degassed prior to use for device fabrication. The control layer mold was spin coated with a thick layer (~1 mm) of 6:1 PDMS and baked at 80^{o}C for 7 mins. A thin layer of 15:1 PDMS was spin coated on the fluidic layer mold. The device was designed such that the valve regions on the device were placed in areas surrounded by shallow fluidic channels, ensuring uniform coverage of these regions with a thin layer of PDMS. The PDMS on the fluidic layer mold was then baked at 80^{o}C for 6 minutes. The PDMS was removed from the control layer mold and the control layer was cut to the exact size of the valve regions on the device, while not covering any channels higher than 50 µm on the device (Supp. Fig. 2). The control layer PDMS pieces were aligned with baked PDMS layer on the fluidic layer mold under a stereoscope. The fluidic layer mold with the aligned control layer was baked at 80^{o}C for 20 mins to promote adhesion between the control layer and the fluidic layer. Following this, 49.5 g of 10:1 PDMS was poured on the fluidic layer mold, covering all features on the fluidic layer mold with a 3-4 mm thick layer of PDMS. The fluidic layer mold was then baked for at least 30 minutes at 80^{o}C. Following this, the PDMS was removed from the fluidic layer mold and individual devices were cut. Fluidic access holes were then punched into the device and the device was bonded to a coverglass through oxygen plasma treatment.



Supp. Fig. 1: Microfabrication Process with Single Developing Step. Four consecutive layers of SU8 photoresist are spin coated and patterned on a single silicon wafer using photolithography. Each layer undergoes all standard photolithography steps like soft bake (SB), exposure and post-exposure bake (PEB). However, the developing step is conducted in common for all layers after patterning the last photoresist layer.



Supp. Fig. 2: Modified microfluidic device fabrication: The process flow used for fabrication of our microfluidic devices to accommodate tall channels in the fluidic layer while maintaining the functionality of push-down valves. (6:1, 10:1 and 15:1 refer to the ratio of the base to curing agent used to mix a batch of PDMS)

S.3 Fusion Zone design

An important aspect of our microfluidic device is the robust synchronization-free fusion mechanism. This mechanism utilizes the cross-sectional area of the central channel on the microfluidic device for the merging operation. Supp. Fig. 3 demonstrates the design criteria of the fusion region on our device. In our current design, the dimensions of the fusion zone, defined as the distance between the first and last reagent inlet (3800 μm), height (50 μm) and width (100 μm) of the central channel determine the volume of the fusion zone (19 nL). This volume of the fusion zone corresponds to the minimum volume of the sample daughter droplet, such that the droplet spans the entire length of all the reagent injection sites on the chip. As a result, the

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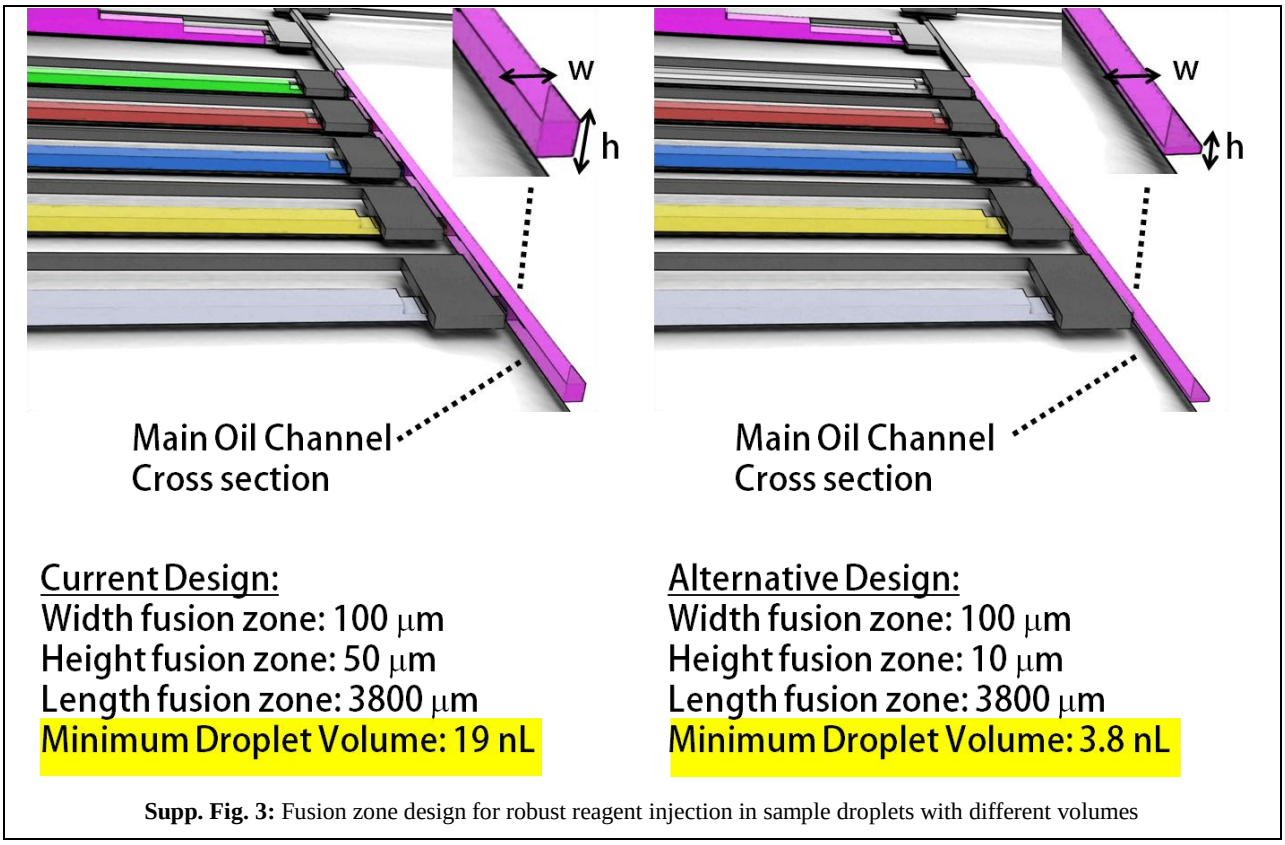
sample daughter droplet position doesn't need to be finely controlled to inject different reagents in it. If there is a need for smaller reaction volumes, the cross-sectional area of the central channel can be modified to reduce the minimum volume of the sample daughter droplet required. For instance, reducing the fusion zone channel height from 50 μm to 10 μm will results in reduction in minimum required droplet volume from 19 nL to 3.8 nL (Supp. Fig. 3). A similar approach can be used to accommodate more than four reagent inlets on the chip.



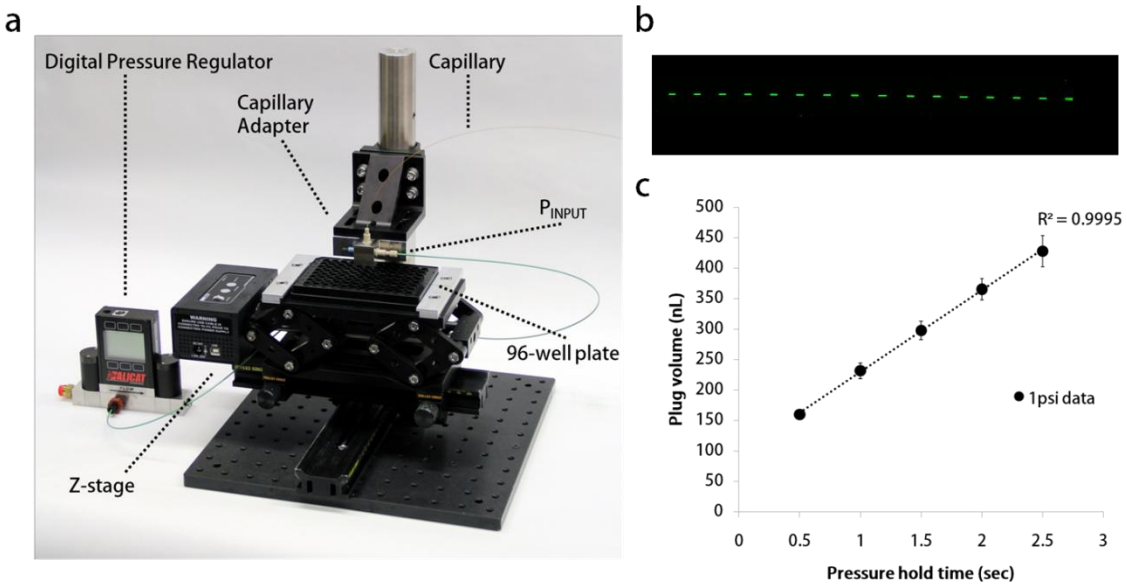
Supp. Fig. 3: Fusion zone design for robust reagent injection in sample droplets with different volumes

S.4 Serial Sample Loading System

The figure below shows a photograph of the actual assembled Serial Sample Loading (SSL) system for sample library generation. The SSL system consists of 1) a custom-made capillary adapter, 2) an automated Z-stage, 3) manual X- and Y- stages 4) a multi-well plate and 5) an Electronic Pressure Controller. The capillary adapter was designed in Solidworks (Solidworks Corp.) and then fabricated by the staff at the Physical Sciences Machine Shop at Johns Hopkins University. A motorized Lab Jack (L490MZ/M, Thorlabs) was used as the automated Z-stage. The manual X and Y stages were purchased from Melles Griot(Albuquerque, NM). In its current

form, the SSL system was designed to be compatible with the Costar 96-well plates (Corning). The Electronic Pressure Controller (PCD-100PSIG-D-PCV03, Alicat Scientific) used in the SSL system is a dual valve pressure controller designed for pressure control in a closed volume. All other structural components of the SSL system were purchased from Thorlabs (Newton, NJ). Custom software developed in LabVIEW was used to control the Z-motion of the automated Z-stage and the injection pressure applied by the Electronic Pressure Controller.

The capillary adapter in the SSL system features three different ports, which are designed for accepting, a microcapillary input, a pressure input and an output for gauging pressure inside a sealed sample well. We attached NanoPorts (Idex Health and Science) at these three ports, for consistent leak free connections with tubing corresponding to each port. The bottom surface of the capillary adapter also holds a silicone sealing ring, fabricated from Silicone Septa (1395-32SS, Corning), used to seal a sample well with the capillary adapter. All the three ports on the capillary adapter are routed to the bottom surface of the capillary adapter, where they open into a sealed sample well. For most of our experiments, a silica microcapillary (360 µm OD and 200 µm ID) was attached to the capillary input of the capillary adapter, unless specified otherwise. The pressure input was connected to the output of the pressure controller. The pressure gauge port was unused and kept plugged for all the experiments. Prior to use, the silica microcapillary is treated with Aquapel^{TM} (PPG Industries).



Supp. Fig. 4: Custom Serial Sample Loading (SSL) System a) Photograph of the custom SSL system. The SSL system features: 1) a custom-made Capillary Adapter, 2) an automated Z-stage, 3) a 96-well plate, 4) Manual X and Y stages and 5) an Electronic Pressure Controller. b) A fluorescence image of sample plugs containing Lamda DNA stained with PicoGreen. These sample plugs were generated in a silica microcapillary using the SSL system, without any visual feedback during plug generation due to transparent nature of the sample. The image indicates high uniformity of the sample plugs despite lack of visual feedback. The lack of fluorescence background in the area between sample plugs (carrier fluid) also indicates minimal sticking of sample to the inner wall of the capillary preventing chances of cross contamination between sample plugs. c) A plot of sample plug volume versus duration of peak pressure application (T_{peak}) at constant peak pressure (P_{peak}: 1psi). The linear relationship between sample plug volume and T_{peak} indicates the capability of our SSL system to vary sample plug volume in a predictable manner. The small error bars also indicate the uniformity of the sample plugs generated for identical loading conditions.