# Three-dimensional Manipulation of Hydrogel Microparticles by Positional Change of Light Focus in Stop Flow Lithography

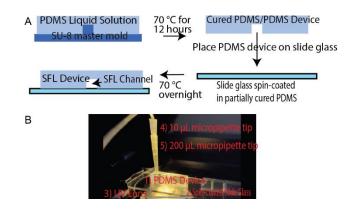
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## **Supporting Information**

## Fabrication of Stop Flow Lithography (SFL) Device

The following procedure is well established in the field of hydrogel microparticle synthesis [1]. The first step of fabricating the SFL devices was creating the Polydimethylsiloxane (PDMS) device. PDMS was used because of its optical transparency, moldability, and gas permeability. Specifically, sticking of synthesized hydrogels to the channel and the disruption of the channel flow are prevented because of the inhibitory terminating oxides produced by the free diffusion of oxygen through the gas permeable PDMS device. 30 g of PDMS elastomer with 1 g of a curing agent were mixed together for 60 seconds until a thick white consistency was produced. Subsequently, the 30 g mixture was poured into a SU-8 master mold, designed on AutoCAD, with a positive imprint 100  $\mu$ m tall and 31.3  $\mu$ m wide and baked in an oven at 70 °C for 12 hours until the mixture fully solidified. Subsequently, the PDMS was separated from the mold, and the inlets and outlet reservoirs were punched into the PDMS with 1.0 mm and 10.0 mm punches, respectively. Each PDMS device had a negative imprint 100  $\mu$ m tall and 31.3  $\mu$ m wide which acted as the SFL device channel. Taller channels lead to taller hydrogels, which was favorable as taller hydrogels would topple, enabling for an analysis of the lateral plane of these particles.

With the remaining PDMS liquid solution, we applied 3mL of solution onto a slide glass and spin-coated the slide glass at 2000 RPM for 30 seconds, evenly coating the glass slide in a thin layer of uncured PDMS. The slide glass was then partially cured in an oven at 70 °C for 30 minutes. The PDMS device was placed on the partially cured slide glass, with negative imprints facing towards the partially cured surface, and the slide glass was placed into the oven overnight at 70 °C to be fully cured (Fig. S1A). The product was a completed SFL device, with a channel connecting the inlet and outlet (Fig. S1B).



**Figure S1:** Outline of synthesis process of SFL device and setup for printing a) Depiction of how SFL device is assembled b) Labelled setup for SFL.

### Detailed Procedure of Particle Recovery, Rinsing, and Photography

After each batch of particles was made, they were first collected from the outlet by dispensing 20  $\mu$ L of PEG 200 into the outlet and pipette flushing to extract the particles. The collected liquid was dispensed in a microtube. This was done one more time to ensure that all particles were removed. After 40  $\mu$ L of hydrogel solution was collected in the microtube, 410  $\mu$ L of phosphate-buffered saline with Tween 20 (PBST), a surfactant, was added to disperse particles and facilitate the next step: rinsing. The solution was vortexed and then centrifuged with a control microtube of 450  $\mu$ L for 20 seconds at 600 RPM. After centrifugation, the top 400  $\mu$ L solution in the microtube was removed via micropipette aspiration. Subsequently, 400  $\mu$ L of PBST was dispensed into the microtube and the solution was vortexed. This process was repeated 2 more times, removing unreacted precursors from the solution and producing a clean solution practically consisting only of hydrogel microparticles and PBST.

Upon rinsing of hydrogels, the solution was vortexed and 10  $\mu$ L of the rinsed solution was pipetted onto a glass slide to be photographed by a light microscope. Visible hydrogels were photographed for analysis. The batches of hydrogels produced by the triangle filter were used for quantitative analysis of uniformity as many could be easily photographed due to the higher number of synthesized particles. Hydrogel images were uploaded to be digitally analyzed on ImageJ.

#### References

1. D. Dendukuri, et al, Stop-flow lithography in a microfluidic device. Lab Chip 7, 818-828 (2007).