

## SUPPLEMENTARY MATERIAL

### **Design and optimizations of a high-throughput valve-based microfluidic device for single cell compartmentalization and analysis**

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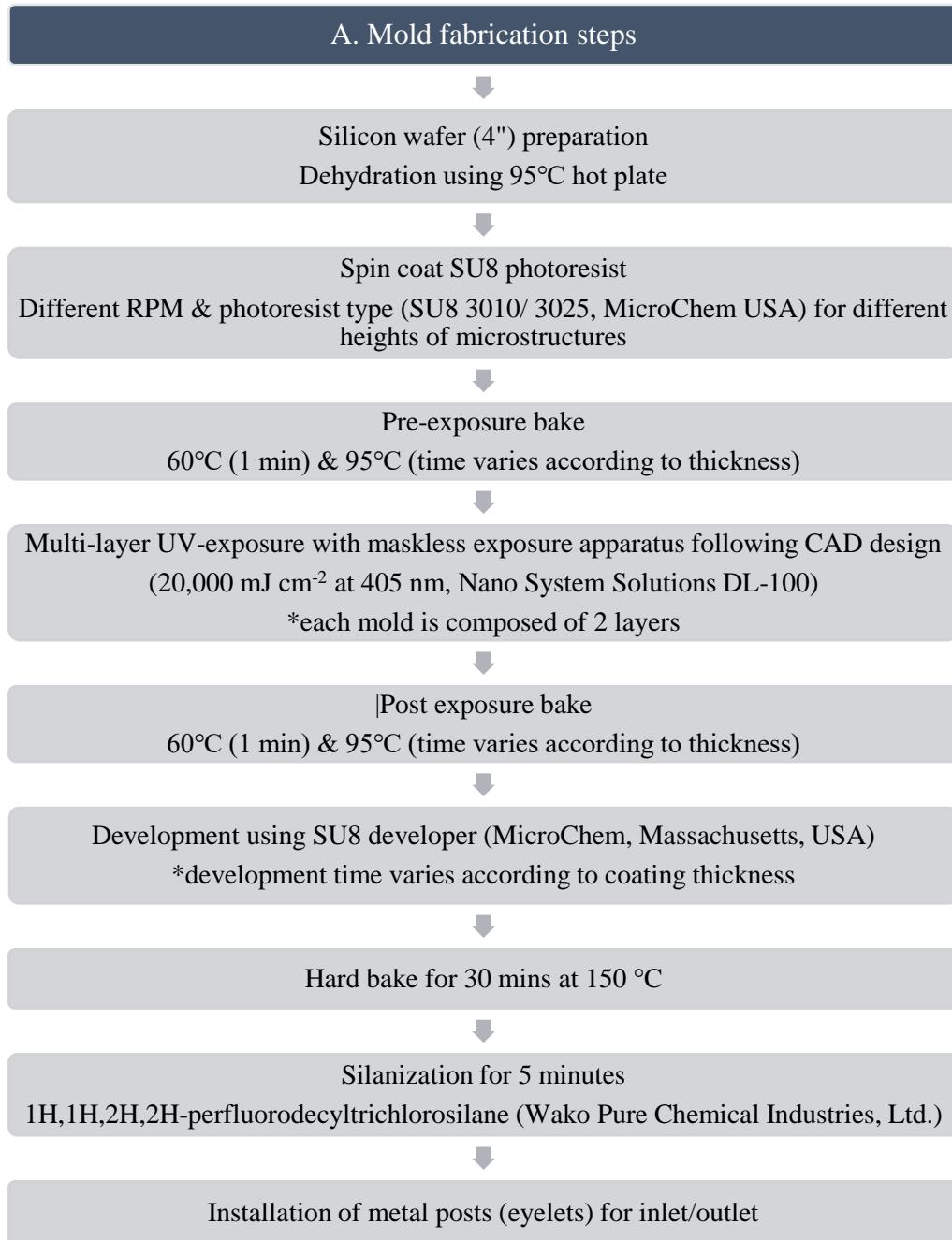
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## I. Fabrication of master molds for PDMS casting and device assembly



**Figure S1.** Outline of the mold fabrication steps

## B. PDMS device assembly

Spin coat about 30  $\mu\text{m}$  PDMS mixture [10:1 base/ curing agent (Sylgard 184 kit, Dow Corning, USA)] on flow layer mold and soft bake for 7 mins on hotplate at 80°C

Pour approx. 1 mm PDMS on control layer mold [5:1 base/curing agent (Sylgard 184 kit, Dow Corning)]. Press COP film on chamber area to create thin film section where thin glass slip will be placed. Soft bake to 15 mins on hotplate at 80°C

Cut the partially cured PDMS control layer, remove COP film and align on top of the flow layer mold with spincoated PDMS

Exposed to O2 plasma dry cleaner for 30s, 75W, place thin glass cover slip. Spin coat about 30  $\mu\text{m}$  5:1 PDMS mix and partially cure at 80°C for 3 mins.

Pour remaining 5:1 PDMS mix evenly around aligned layers

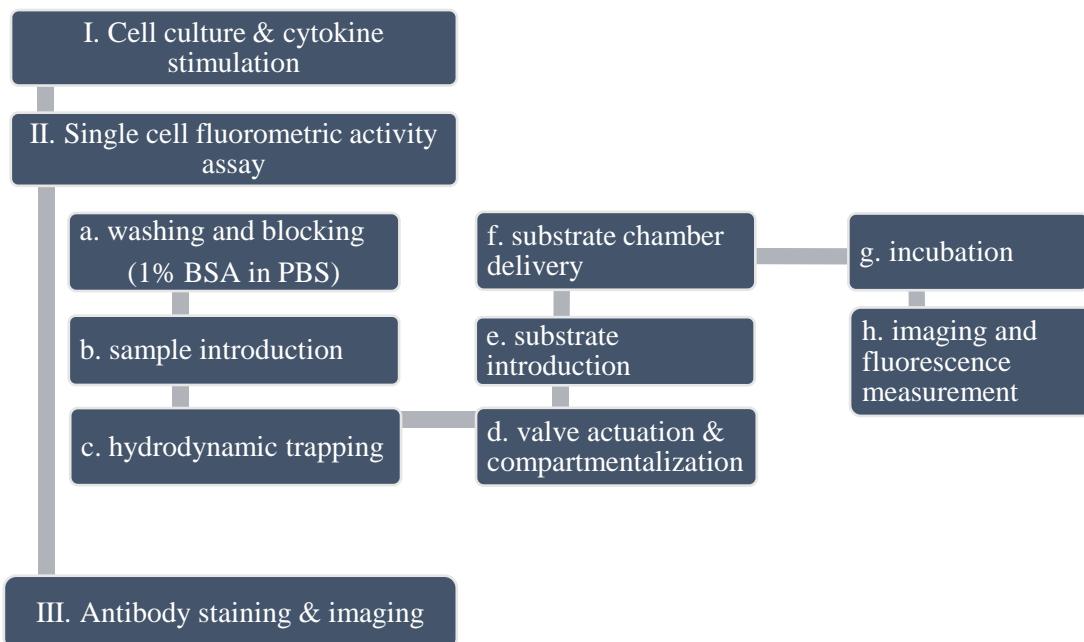
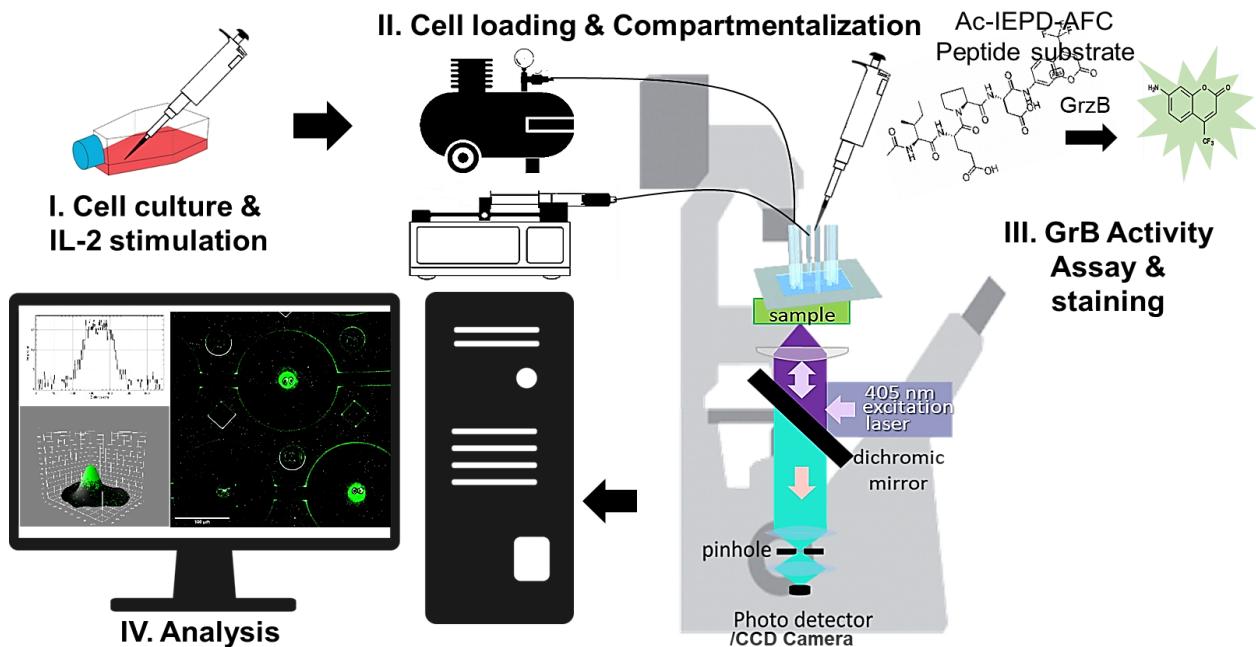
Hard bake at 80°C, overnight

Cut the cured PDMS chip to glass substrate size and carefully peel off

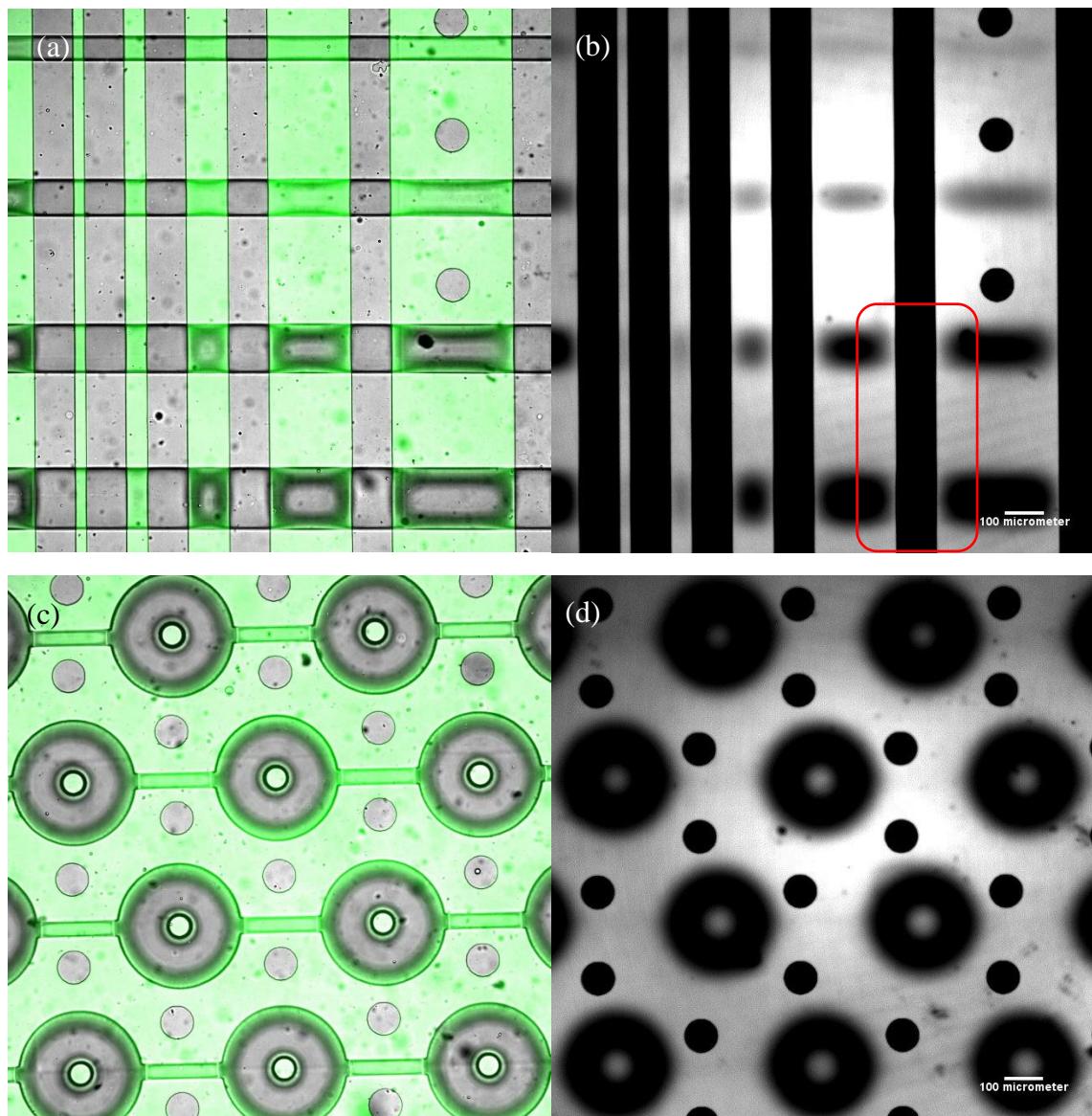
Hydroxylation of the PDMS chip and glass substrate surfaces by O2 plasma treatment (Yamato PDC210) for 50 s, 75 W.

Conformal contact of PDMS chip and glass substrate

**Figure S2.** Outline of the microfluidic device assembly



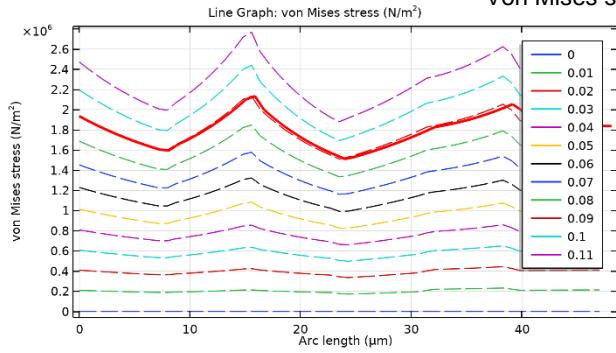
**Figure S4.** Illustration depicting the steps of microfluidic device operation for single cell fluorometric activity assay.



**Figure S3.** Microscope images of fluorescent solution filled microfluidic channels with air actuated valves at 0.14 MPa (a) multicolor (merged) image of the channels and actuated rectangular valves (b) monochrome (488 filter) showing the gaps present at incompletely closed corners (c) multicolor (merged) image of the channels and actuated circular valves (d) monochrome (488 filter) image of sealed microchambers.

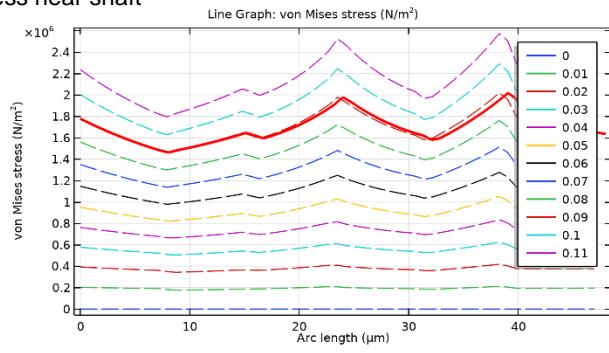
structure A

Von Mises stress near shaft

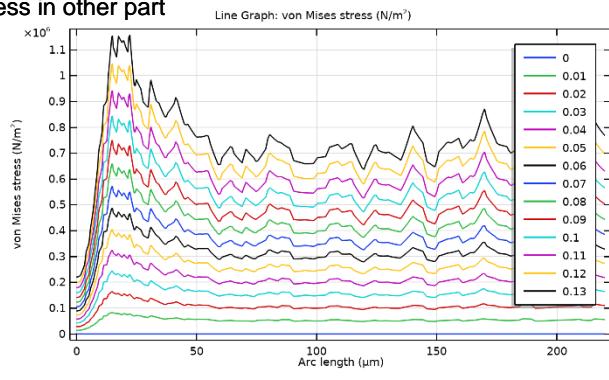
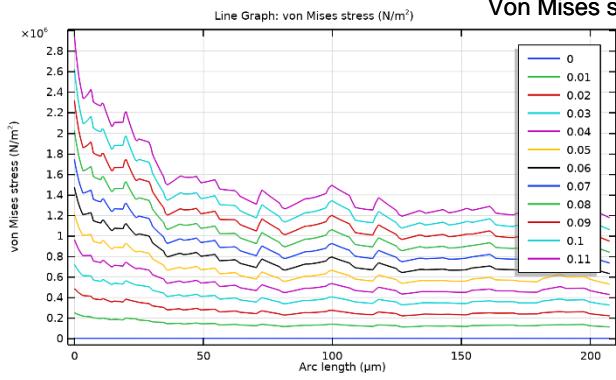


structure B

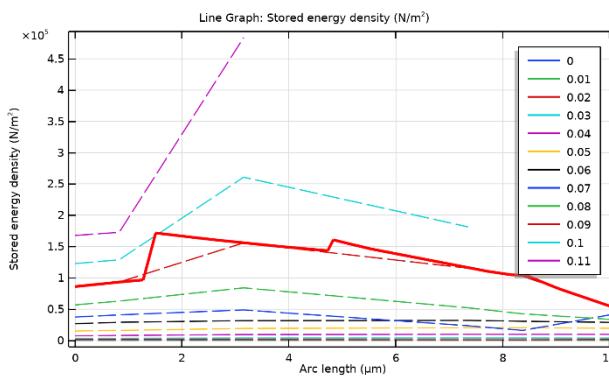
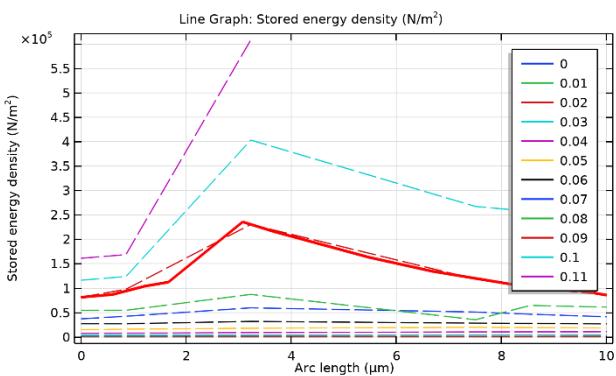
Von Mises stress near shaft



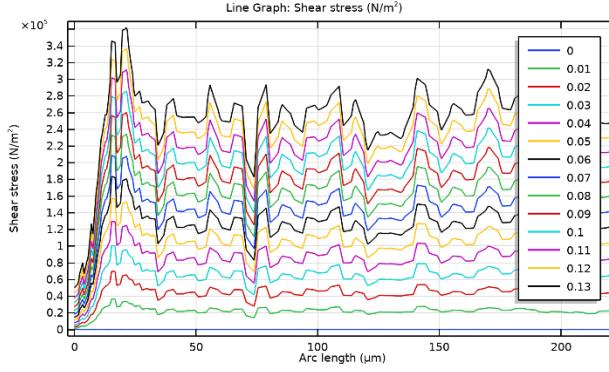
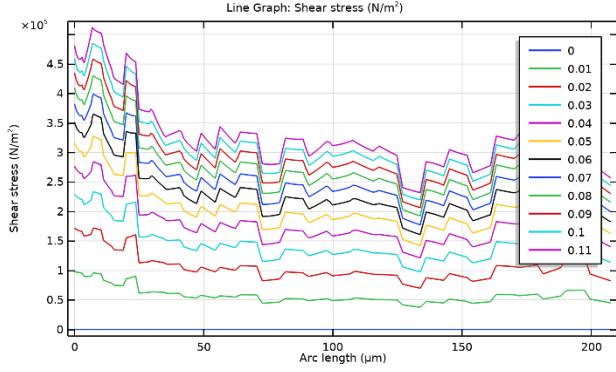
Von Mises stress in other part



stored energy density



shear stress



**Figure S5.** Comparison of the von Mises stress, total stored or strain energy density, and shear stress in structures A and B