Electronic Supplementary Material (ESI) for RSC Advances

Supplementary Information

Single microbe trap and release in sub-microfluidics

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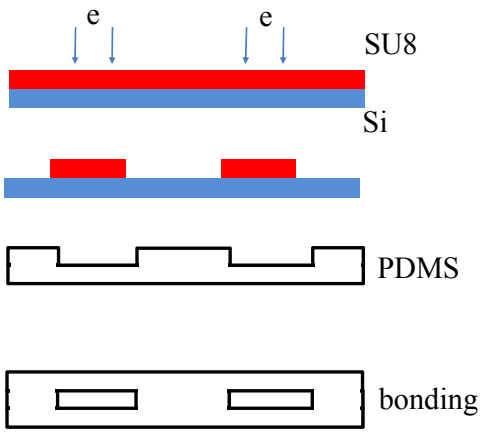
Imaging

For imaging and inspection, an inverted frame microscope (Olympus IX71) was used, equipped with a high-resolution stage (MS-2000, Applied Scientific Instrumentation), a 100×, NA = 1.45 objective (PLAPO100XO/TRIFM-SP, Olympus), as well as an EMCCD camera (iXon DV885 VP, Andor Technology) cooled to −80 °C. The optical resolution was determined at approximately 200 nm by single molecule imaging.

Hybrid Fabrication Method Resolution

To investigate the resolution limits of this hybrid method, the distance of how close two SU8 blocks can be defined without observing any proximity effects was measured at various film thicknesses (39). Resolution levels below 1 µm were possible for µm scale resist thicknesses with this method (Suppl. Fig. 5a, *lower*). The resolution was found to increase when the SU8 pattern is transferred to PDMS. Cross-talk between closely spaced channels was minimal even at sub-micron channel separations where the SU8 molds exhibit proximity effects. (Suppl. Fig. 5a, *upper*). SU8 is also compatible with grey-scale lithography, and thus the micro-channel height can be varied by simply modulating the electron beam dose (Suppl. Fig. 6) (38). Consequently, monolithic microfluidic channels with varying morphologies in all three dimensions are feasible on a single exposure (Suppl. Fig. 5b). This possibility substantially enhances the processing speed and cost by eliminating the need for multiple exposures or complex etching strategies. Three-dimensional control of the channel morphology is critical for microbial immobilization. Microbes can be non-uniform (e.g. peritrichous flagella, Pilli structures) and consequently their dimensions may be orientation dependent. This necessitates the exertion of a three-dimensionally symmetric flow resistance in order to achieve repeatability.

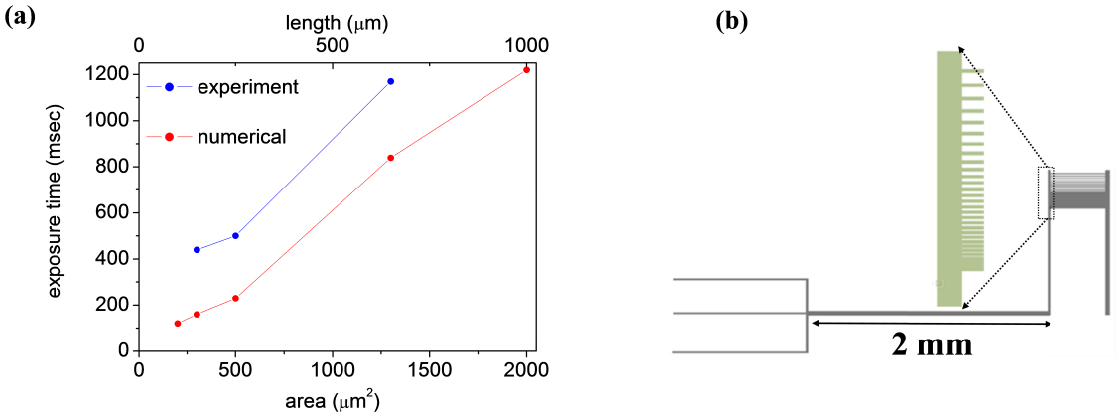
Supplementary Figure 1



Supplementary Figure 1

This schematic illustrates the fabrication strategy employed in this work: electron beam lithography creates the patterns on SU8, which are then transferred to PDMS and bonded to glass coverslips or flat PDMS pieces.

Supplementary Figure 2



Supplementary Figure 2

(a) The relationship between exposure area and required time for the EBPG5000 with a 50 MHz pattern generator at the clearance dose of SU8 used in this work (7 μC/cm^2); the upper axis indicates the corresponding length of the 2 μm wide lines used in this experiment. (b) A relatively complex microfluidic circuit that required 48'' of exposure at 7 μC/cm^2.

Supplementary Figure 3

Supplementary Figure4

Table1: spin-speed conditions

formulation concentration	spin speed	thickness (µm)
40 % (1040)	2000 rpm	2.3
60 % (1060)	4100 rpm	5.6

Table2: pre-exposure bake

temperatures (Celsius)	duration for 1?m - 8 ?m
30 – 130 (ramp)	1500"
130	300"
130 – 30 (ramp)	1500"

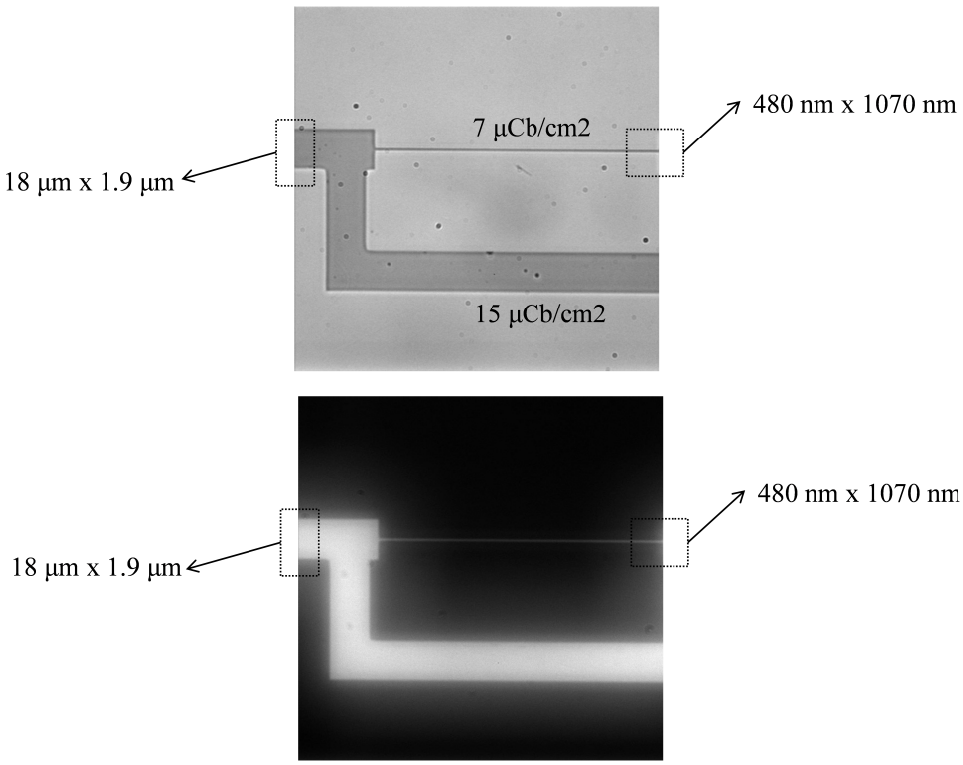
Table3: post-exposure bake

temperatures (Celsius)	duration for 1?m - 8 ?m
30 - 100	1200"
100	900"
100 - 30	5400"

Supplementary Figure 3

Three tables illustrating the processing conditions for SU8 films, namely the spin and pre- as well as post-exposure conditions).

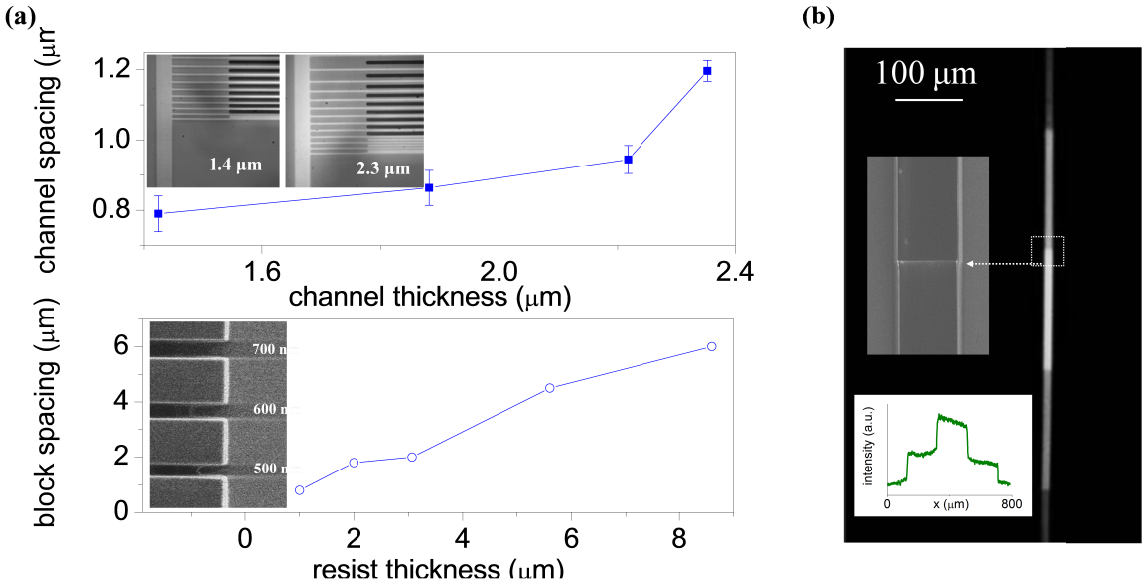
Supplementary Figure 4



Supplementary Figure 4

The integration of millimetre and sub-micron scale features in microfluidics comprising of a 15 µm wide and a 515 nm wide channels is shown in Fig. 1d. Note the strong thickness modulation from 2 µm to 500 nm.

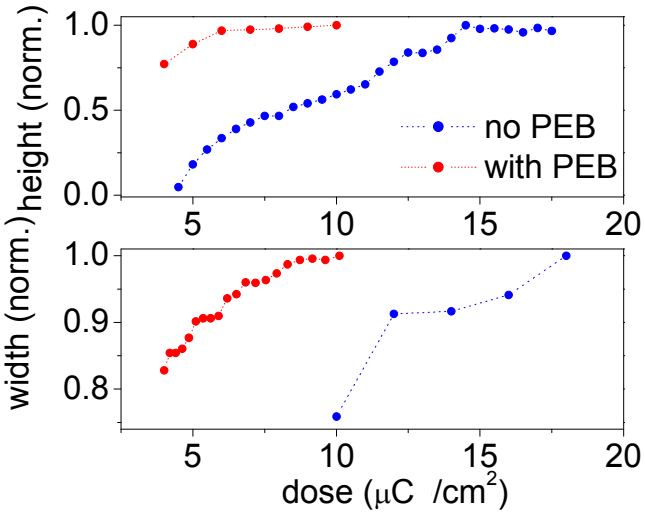
Supplementary Figure 5



Supplementary Figure 5

(a) The resolution variation as a function of thickness for SU8 blocks (*lower*); the corresponding resolution for microfluidic channels, where the resolution is manifested via the absence of cross-talk between filled and empty channels (*upper*). (b) A fluorescent image of a channel containing a passive height modulation illustrating the principle of electron-beam grey-scale lithography applied to microfluidics*;* inset illustrates an SEM image of the surface morphology of the SU8 mould and a fluorescence intensity profile along the channel; each intensity step corresponds to 400 nm thickness variations.

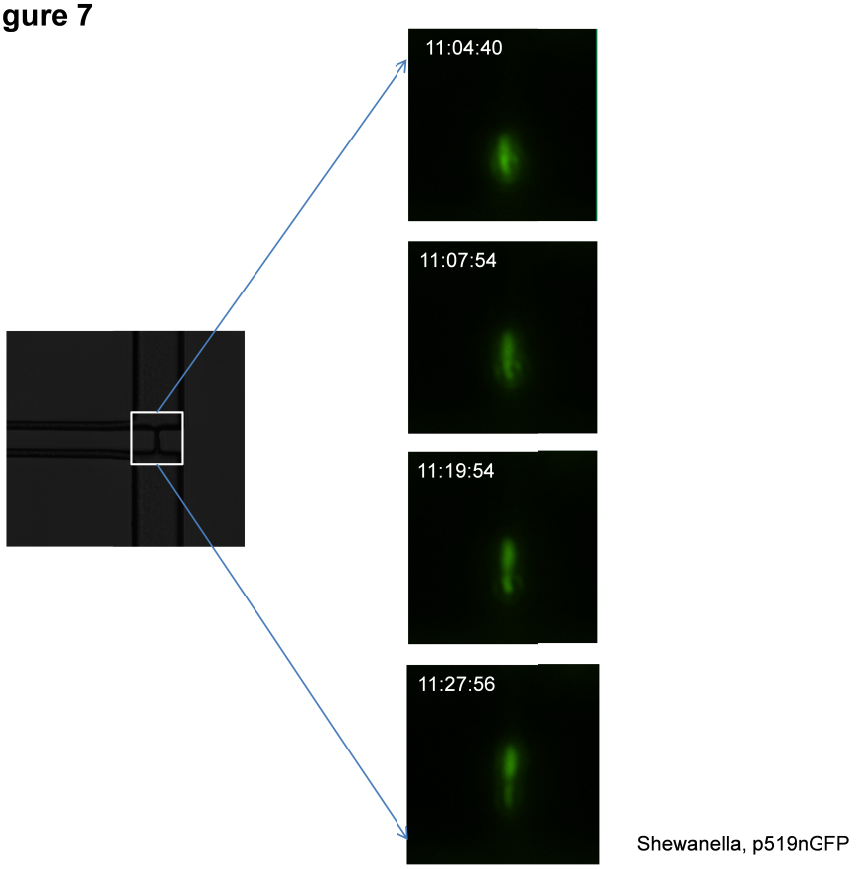
Supplementary Figure 6



Supplementary Figure 6

The width and depth dependence of exposed features in SU8, as a function of exposure dosage. Two conditions are shown, with and without post-exposure bake (PEB).

Supplementary Figure 7



Supplementary Figure 7

Trapped Shewanella bacteria divide under constant flow of 0.7 µL/sec. The cells strain is GL-1 and expresses GFP (p519nGFP) for visualization of the division process.