Multiscale 3D-printing of microfluidic AFM cantilevers

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• Section 1 - Stereolithography fabrication details
• Section 2 - 2PP-printing strategy, slicing details, and adhesion experimentation
• Section 3 - Hydraulic model
1 Fabrication - Stereolithography

The fabrication process began with the CAD-designs of the fluidic interfacing device, illustrated in figures S1B. An enlarged view of the aperture is shown in figure S1C, around which the base, yurt, and cantilever structures were printed, and the scaffolding that is needed for SL-printing has been designed as illustrated in figures S1A and D. The devices were placed in isopropyl alcohol and ultrasonically cleaned for two minutes, dried with an air gun, and subsequently cured with UV-light for two minutes. The scaffolding was carefully removed from the devices and they were tested for leaks by connecting them to tubing and injecting deionized water. The scaffolding designs and a picture of the device to be mounted in the JPK AFM system can be seen in figures S1D, and S2A and B. The mounting of the device in the AFM holder is pictured in figure S3.

Figure S1: (A) depicts the scaffolding of the SL-printed device, which is detached after printing. The CAD of the device is shown in (B), in which the internal channel is visible in the middle of the device and ending in the aperture at the top (C). The scaffolding of the JPK SL-printed device is depicted in (D). In both scaffolding designs extra ridges were added to support the device during the development and blow drying phases.

Figure S2: CAD (A) and picture (B) of the SL-device to mount in the JPK AFM holder. t denotes the thickness of the device, with $D_t$ and $D_b$ being the diameter of the tubing connection at the top and bottom respectively. The square indentation at the bottom right of the structure was added to identify at which side the aperture was located.
Figure S3: Mounting of the JPK fluidic interfacing device onto an AFM holder. A metal clip clamps the device between it and the white opaque prism. The tubing is connected to the side of the device before mounting.

The specifications of the SL-printer list the minimum in-plane (x and y) resolution as 30 µm, and 25 µm out-of-plane, however, it was found that this does not hold for internal channels. The minimum channel dimensions differed depending on its orientation, and if a channel is printed parallel to the build plate (in-plane), then its dimensions were 150 µm and 90 µm in the x and y direction respectively.

2 Fabrication - 2PP-printing

2.1 printing strategy

A hanging structure printing configuration was used which is called "Dip-in laser lithography" (DiLL), and the printing process is schematically depicted in figure S4. The SL-interfacing device was taped to the bottom of an indium-tin-oxide (ITO) coated glass slide and immersed in the photoresist. The microscope objective is then directly dipped into the same resist droplet.

This printing strategy was devised because in this configuration there exists a chance of scratching the objective lens during initial approach, which can also occur if printing is done close to high-aspect ratio structures that are larger than the working distance of the objective.

Figure S4: 2PP-printing process from initial approach to the determination of the SL-surface height. (a) the focus of the objective is raised and the interface of the glass slide is detected. (b) the objective is lowered an height 'h', which is the combined height of the SL-device and tape. (c) the microscope is positioned under the aperture of the SL-device. (d) the microscope can be raised a distance 'a' after at least one cycle of printing the incremented rectangles. (e), (f), and (g) illustrate the incremental printing of rectangles. A picture of how (g) looks during the actual printing is shown in figure S5B.
Figure S5: A view of how the height test is designed is illustrated in (A). The 10x10x40 µm rectangles are printed at incremental heights of 20 µm until the first rectangle is attached and the printing time of one rectangle is ~11 s. The microscope feed from the 2PP-printer is shown in (B), with the individual printing layers of the SL-substrate visible as horizontal bands. The first three rectangles were not printed on the substrate and remain suspended in the resin, but the fourth was attached. An indicator of this is the movement of the rectangle when the laser repositions, which moves the viscous resin and the unattached rectangles within it. An attached rectangle will not move or change shape.

Figure S6: CAD-design of the base structure that is printed around the aperture of the SL-device, and thereby connecting its channel with the yurt. The slits prevent coalescing of small bubbles that are formed when the femtosecond laser irradiates the SL-device.

2.2 Cantilever slicing

The cantilever is printed in slices, and their thickness and overlap has an influence on the surface roughness, as seen in figure S7. It shows cantilevers with decreasing slice thickness, 5 µm in (A), 3 µm in (B), and approximately 1 µm single voxel thick slices in (C).

These slicing sizes will lead to different structural properties of the cantilever, as this slicing determines how much of the cantilever will be doubly exposed, thereby changing the materials Young’s modulus slightly\[1\]. Overlap between slices should also be sufficient, as that would ensure that slices stay connected during and after illumination, which causes the photopolymer to shrink.

Another effect is the difference in surface roughness. For non-contact measurements such as laser Doppler vibrometry (LDV), a smooth surface is desired for sufficient reflection of the laser. An increase in surface roughness would scatter a higher proportion of the incoming laser light, and this scattering can be reduced by increasing thickness of the slices. The cantilevers with thicker slices have a smoother surface compared to thinner slices, illustrated in figure S7. The internal
Figure S7: 2PP-printed cantilevers with different slice thicknesses. 5 µm slices in (A), 3 µm slices in (B), and approximately 1 µm slices that are a single voxel thick in (C). These single voxel thick slices were printed with a self-written code with a chosen slice overlap of 200 nm, while the cantilevers in (A) and (B) have been printed with an overlap of 1 µm.

Figure S8: (A) 3D composite picture of a 100 x 100 x 100 µm 2PP-block that was printed on a SL-substrate. The blocks with other dimensions were printed in a similar manner. The white arrow indicates the direction of applied force. (B) Results from the adhesion experiment for blocks with dimensions of 200 x 200 x 200 µm. The schematic of how the experiment is performed is inserted in the graph. When the nano indenter tip encounters the block it measures its displacement. The sudden jump in distance is interpreted as the detachment of the block from the SL-substrate.

channel is affected by the slicing as well, as scallops form inside which have sizes comparable to the slice thickness. It was found that slices of 3 µm with a 1 µm overlap would result in a surface roughness that still yielded sufficient signal strength for LDV measurements, while retaining full fluidic functionality.

2.3 Adhesion strength

The strength of adhesion between the 2PP-written base and the SL-interfacing device was investigated. An array of 2PP-cubes was printed onto a SL-printed slate, which in turn was attached onto a substrate using super-glue. The experiment was performed with a nano indenter (Nano Indenter MTS G200, Keysight Technologies) using a Berkovich-tip that applied force on top of the cube. The forces were acting to detach the cube from the slate. A load-range from zero to 500 mN in steps of 1 mN was applied on every cube. The printing of the 2PP blocks resulted in samples as shown in figure S8A, and nano indentation measurements yielded the graph in figure S8B.

Adhesion strength between the SL-device and 2PP-base puts limits on the cantilever design dimensions, as this determines how much pressure can be set on the device before the base detaches. The adhesion strength can be inferred from the nano indentation graph in figure S7B. In this graph the vertical jumps indicate sudden displacements of the nano indenter tip, which has been interpreted as the detachment of the 2PP-blocks. When a cube is loaded it can either detach fully or tear, since the force is applied in the middle of the cube and not at its base. Optical inspection showed that cubes were detached in full, with no tearing observed. The lowest detachment forces were recorded for the 40 000 µm² blocks, and although no standard deviation can be determined, this data can nevertheless be used as a limit for adhesion strength.
It follows that these blocks can withstand a maximum sheer stress of 1.5 MPa or 15 bar, and this is indicative of the maximum pressure the device can handle before detachment of the 2PP-base. During the cell pipetting experiments a pressure of 0.7 MPa or 7 bar was successfully applied with no detachment of the base. The fact that such bonding occurs between the acrylic-based photopolymers of the SL-device and 2PP-structures can possibly be explained by diffusion of some liquid 2PP resist inside the solid SL-substrate. Subsequent laser illumination will then be able to polymerise the internal 2PP resist, thereby creating some interlocking between the two materials.

3 Hydraulic model

A fluidic device can be modelled as an electrical circuit\textsuperscript{[2]} and Figure S9 illustrates the circuit for the fabricated device. If the pressure is taken as an analogy to the voltage and the flow rate $Q$ as current, then the hydraulic resistance for a circular and rectangular channel follows from the Navier-Stokes equations and can be calculated as in equation 1. The values of the volumes, resistances, and capacitances of the individual elements are listed in table S1.

The analogy with the electrical circuit can be taken further to include capacitive elements. The physical reasoning is that the volume of the channel slightly increases due to elastic deformation of the material when a pressure is applied over it, leading to a temporal storage of fluid. This material dilatability is approximately the inverse of the Young’s modulus, and the hydraulic capacitance can be approximated by the equation $C_{hyd} \approx \frac{V}{E}$, where $V$ denotes the undeformed volume (V) in the channel and $E$ the Young’s modulus of the material\textsuperscript{[3]}.

\[ R_{hyd, circ} = \frac{8 \mu L}{\pi r^4} \] (1)

Figure S9: (A) The electrical circuit model of the device, in which a syringe pump is modelled as a current source and where the components are separated based on the channel dimensions. This is illustrated in (B), where (1) denotes the SL-channel, (2) the SL-aperture, (3) the 2PP-yurt channel, and (4) the cantilever channel. All channels are modelled as being cylindrical, with the exception of the rectangular SL-aperture.

<table>
<thead>
<tr>
<th></th>
<th>Volume</th>
<th>Resistance ($\text{Pas m}^3$)</th>
<th>Capacitance ($\mu\text{m}^3$)</th>
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<tbody>
<tr>
<td>Syringe</td>
<td>1000 $\mu$L</td>
<td>$4.88 \times 10^6$</td>
<td>$6.45 \times 10^{-16}$</td>
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<td>Tubing</td>
<td>13.7 $\mu$L</td>
<td>$1.49 \times 10^{13}$</td>
<td>$2.14 \times 10^{-15}$</td>
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<td>SL-channel</td>
<td>32.5 nL</td>
<td>$3.50 \times 10^{11}$</td>
<td>$9.71 \times 10^{-21}$</td>
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<td>SL-aperture</td>
<td>1.82 nL</td>
<td>$2.37 \times 10^{10}$</td>
<td>$5.44 \times 10^{-22}$</td>
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<tr>
<td>2PP-yurt</td>
<td>0.38 nL</td>
<td>$1.97 \times 10^{10}$</td>
<td>$8.25 \times 10^{-23}$</td>
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<tr>
<td>2PP-cantilever</td>
<td>0.214 nL</td>
<td>$4.56 \times 10^{13}$</td>
<td>$4.66 \times 10^{-23}$</td>
</tr>
</tbody>
</table>
\[ R_{\text{hyd,rect}} = \frac{12 \mu L}{wh^3} \left[ 1 - \left( \frac{192h}{\pi^5w} \right) \sum_{i=1,3,5,...}^{\infty} \frac{\tanh(\frac{i\pi w}{2h})}{i^5} \right]^{-1} \] (2)

\[ C_{\text{hyd}} \approx \frac{V}{E} \] (3)