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Other Publication**Author(s):**

Läubli, Nino ; Nelson, Bradley 

Publication date:

2021-05-26

Permanent link:

<https://doi.org/10.3929/ethz-b-000526967>

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Originally published in:

Protocol Exchange, <https://doi.org/10.21203/rs.3.pex-1406/v2>

Fabrication of PDMS-based Acoustofluidic Devices for 3D Access to Single Cells and Small Organisms

Nino F. Läubli (✉ nino_jaeubli@hotmail.com)

ETH Zurich <https://orcid.org/0000-0003-2894-2385>

Bradley J. Nelson

ETH Zurich

Method Article

Keywords: Acoustofluidics, Soft lithography, PDMS

Posted Date: May 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.pex-1406/v2>

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Abstract

Acoustofluidic manipulation has demonstrated significant potential for the investigation and handling of small organisms. In this protocol, we describe the steps required for the fabrication of a PDMS-based device with 150 um high structures to be used for the rotational manipulation of single cells and small organisms.

Introduction

Reagents

- Acetone
- Isopropanol (IPA)
- De-ionized water
- Photoresist: SU-8 2150 (*Kayaku Advanced Materials*)
- Developer: mr-Dev 600 (*Micro Resist Technology GmbH*)
- Silane: (Tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane (CAS 78560-45-9, *ABCR*)
- PDMS: Sylgard 184 (*Dow Corning*)

Equipment

Cleanroom environment including:

- Ultrasonic (US) bath
- Quick Dump Rinse (QDR) bath
- Spin Rinser Dryer
- Two hotplates
- Spin coater
- Mask aligner
- Plasma asher (oxygen)

Lab suitable for polymer casting including:

- Vacuum pump
- Oven

Additional tools:

- Razor blade
- Glass slides
- Piezoelectric transducer, e.g., KPEG-126 (*Kingstate*)
- Glue, e.g., UHU Plus Schnellfest 2-K Epoxy (*UHU GmbH*)

Procedure

Photolithography

Wafer preparation:

- Clean the wafer with acetone in an US bath for 5 minutes.
- Clean the wafer with IPA in an US bath for 5 minutes
- Remove the solvents with de-ionized water using a QDR until a resistivity of 12 MOhm cm is reached.
- Dry the wafer in a spin-rinse-dryer.
- Bake the wafer at 200 °C on a preheated hotplate for 5 minutes.
- Remove the wafer from hotplate and let it cool down to room temperature.

Spin coating:

- Place the cleaned wafer in a spin coater and dispense 4 mL of resist at the center of the substrate. Coat the wafer using the following steps:
 - Time: 15 seconds; Speed: 500 r.p.m.; Acceleration: 200 r.p.m./s
 - Time: 30 seconds; Speed: 3500 r.p.m.; Acceleration: 300 r.p.m./s

Softbake:

- Bake the coated wafer at 65 °C on a preheated hotplate for 5 minutes.
- Increase the temperature of the hotplate to 95 °C and bake the wafer for additional 30 minutes.
- After a total of 35 minutes, turn off the hotplate and let the wafer cool down to 75 °C while still on the hotplate.
- Remove the wafer from the hotplate and let it cool down to room temperature.

Exposure:

- Measure the energy density (mW/cm²) of the UV lamp at a wavelength of 365 nm.
- Load the mask and the wafer into the mask aligner and align them.
- Expose the photoresist until an exposure dose of 260 mJ/cm² is reached.

Post-exposure bake:

- Bake the exposed wafer at 65 °C on a preheated hotplate for 5 minutes.
- Increase the temperature of the hotplate to 95 °C and back the wafer for additional 12 minutes.
- After a total of 17 minutes, remove the wafer from the hotplate and let it cool down to room temperature.

Development:

- Develop the wafer in a Petri dish for 15 minutes. Move manually to improve the circulation of the developer.
- Move the wafer to a second Petri dish with fresh developer for additional 30 seconds.

Rinse, dry and hardbake:

- Rinse the wafer and the developed SU-8 structures with IPA.
- Rinse the wafer with de-ionized water.

- Dry the wafer with a nitrogen gun.
- Hardbake the wafer at 150 °C on a preheated hotplate for 5 minutes.
- Remove the wafer from the hotplate and let it cool down to room temperature.

Silane coating

- Plate the wafer with the SU-8 structures next to a glass slide in a vacuum chamber.
- Add three drops (each about 50 μ L) of silane on the glass slide.
- Close the chamber and start the vacuum pump.
- Once sufficiently low pressure (less than 100 mbar) is reached, turn off the vacuum pump and let silane distribute inside the chamber for 30 minutes.
- Remove the wafer from the vacuum chamber and bake it on a hotplate at 120 °C for 10 minutes.
- Remove the wafer from the hotplate and let it cool down to room temperature.

PDMS casting

- Mix curing agent and pre-polymer with a weight ratio of 1:10 in a disposable cup for 5 minutes.
- Place the wafer in a Petri dish inside a vacuum chamber.
- Cover the wafer with the PDMS and apply a vacuum for 30 minutes to degas the polymer and ensure proper molding of the SU-8 structures. Bleed the vacuum occasionally to accelerate the process and to prevent PDMS from overflowing the Petri dish.
- Cure the PDMS by placing the Petri dish containing the mold and the degassed polymer in an oven and bake it for 1 hour at 80°C.
- Remove the Petri dish with the cured PDMS from the oven and let it cool down to room temperature.

Final steps

- Cut the PDMS and peel it off the silicon wafer. If necessary, punch the connectors using a biopsy punch. For 3D access to the manipulated specimen (see associated publication), cut the fabricated PDMS channels perpendicularly.

- Clean the PDMS, especially the side containing the microchannels, with transparent adhesive tape.
- Place the PDMS (microchannels facing upwards) next to a glass slide in a plasma asher.
- Expose the glass slide and the PDMS to oxygen plasma for 30 seconds.
- Bring the exposed surfaces in contact to chemically seal the structures.
- Fix a piezoelectric transducer used for subsequent acoustic excitation on the glass slide near the PDMS structure using a suitable glue, e.g., two-component epoxy.

Troubleshooting

White clouds during IPA rinse after development:

Clean the wafer with water and dry it. Repeat the second development step.

Time Taken

Photolithography: approx. 2.5 hours

Silane coating and PDMS casting: 2 hours

Manual preparation and bonding: 30 minutes

Anticipated Results

References

Acknowledgements

This work is supported by the ETH Zurich and an interdisciplinary grant from the Swiss National Science Foundation (Grant Number CR22I2_166110) to B.J.N..