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Author(s):
Läubli, Nino; Nelson, Bradley

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

Nino F. Läubli (nino_laeubli@hotmail.com)
ETH Zurich https://orcid.org/0000-0003-2894-2385
Bradley J. Nelson
ETH Zurich

Method Article

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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 Cornin*g)

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 uL) 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

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