Submission ID #: 61877
Scriptwriter Name: Domnic Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18872533

Title: Design and Development of a Three-Dimensionally Printed Microscope Mask Alignment Adapter for the Fabrication of Multilayer Microfluidic Devices

Authors and Affiliations:

Celine R. Garcia[1*], Zhenya Ding[1*], Hilario C. Garza[1], Wei Li[1]

[1]Department of Chemical Engineering, Texas Tech University, Lubbock, TX, USA

Corresponding Authors:
Wei Li (wei.li@ttu.edu)

Email Addresses for All Authors:
celine.r.garcia@ttu.edu
zhenya.ding@ttu.edu
chris.garza40@yahoo.com
wei.li@ttu.edu

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 14
Number of Shots: 29

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. This protocol is significant as it allows for the generation of a cost-effective and easy-to-use platform that aids in the production of precise multi-layer microfluidic master molds.

1.1.1. *2.2.1*
NOTE: Shot 2.3.1 was not filmed. So, authors suggested to use 2.2.1 or 2.1.1 or 2.1.2 for shot 1.1.1.

1.2. The main advantage of the technique is that it only requires the use of the 3D printed platform and standard laboratory equipment commonly found in laboratories that produce microfluidic devices.

1.2.1. *3.12.1.*

OPTIONAL:

1.3. Visual demonstration of this protocol is critical to demonstrate how to customize and use the 3D printed Microscope Mask Alignment Adapter.

1.3.1. *3.7.1.*

Protocol

2. Designing the MMAA

2.1. Obtain the dimensions of the tray of the available UV light emission system to be the upper bound for the dimensions of the wafer holder **[1].** Measure the diameter of the inner circular rim, the inner height of the UV light emission system's tray, the total width, and length of the tray. Using a computer design application, apply these dimensions to customize the wafer holder to fit within the UV light emission system's tray **[2].**

NOTE: VO narration for step 2.1 was changed.

2.1.1. Talent measuring the dimensions (width and length) of the tray.

2.1.2. Talent measuring the inner dimensions (height and diameter of circular rim) of the tray.

2.2. Measure the length between the screws and the width of the screws on the available upright microscope stage that hold the slide holder in place. Using a computer design application, apply these dimensions to customize the magnetic holder to fit the available microscope to allow for easy and precise fixation of the MMAA to the microscope **[1-TXT].**

2.2.1. Talent measuring the length between the screws and the widths of the screws of microscope stage. **TEXT: MMAA- microscope mask alignment adapter**

NOTE: Shot 2.3.1 was not filmed as the software not working.

3. Creation of bilayer master mold using the MMAA (photolithography) and determination of the alignment error

3.1. Use a 4-inch silicon wafer with the appropriate photoresist to create the first layer of the master mold, ensuring that the thickness is greater than the subsequent layers for easy identification of the alignment markers **[1].** Use a light-colored marker pen to color the first layer's alignment markers on all four sides **[2].**

3.1.1. Talent spin-coating photoresist onto silicon wafer to create desired thickness of the first layer.

3.1.2. Talent coloring the alignment markers of first layer with a pen.

3.2. Using the photoresist manufacturer's instructions, initiate the second layer of the master mold by spin-coating the photoresist onto the wafer **[1]** and performing the soft bake **[2]**.

Insert the coated wafer into the wafer holder of the MMAA **[3]** and fix the coated wafer to the MMAA using tape **[4].**

- 3.2.1. Talent performing spin-coating to prepare second layer of master mold.
- 3.2.2. Talent performing soft bake of the prepared wafer.
- 3.2.3. Talent inserting the coated wafer into the wafer holder of MMAA.
- 3.2.4. Talent fixing the wafer using tape.

3.3. Attach the wafer holder to the available upright microscope using the magnetic microscope fastener **[1]**. Move the position of the MMAA using the x- and y-direction knobs of the microscope stage until one of the colored alignment markers on the wafer is in view through the microscope lens **[2].** *Videographer: This step is important!*

- 3.3.1. Talent attaching the wafer holder to the microscope using magnetic fastener.
- 3.3.2. Talent positioning the viewing of colored markers on the wafers using knobs of microscope stage.

3.4. Remove the wafer holder from the microscope stage and insert the second-layer photomask into the wafer holder on top of the coated wafer. Ensure that the first-layer's colored alignment markers can be partially seen through the alignment markers on the photomask and that the straight edge of the photomask is superimposed with the straight edge of the silicon wafer **[1].**

- 3.4.1. Talent inserting the second-layer photomask into the wafer holder and adjusting the position of the photomask.

  NOTE: Additional action was performed in shot 3.4.1 and VO narration was edited.

3.5. Attach the wafer holder back onto the microscope stage and attach the photomask to a scissor lift through one of the side cut-outs with tape **[1]**. Use the scissor lift to adjust the z-direction position of the photomask until it lies right above the coated wafer **[2].** *Videographer: This step is important!*

- 3.5.1. Talent attaching the wafer holder to the microscope using magnetic fastener and attaching the photomask to a scissor lift.

  NOTE: Additional action was performed in shot 3.5.1 and VO narration was edited.

- 3.5.2. Talent adjusting the position of the photomask to lie above the coated wafer.

3.6. While keeping the photomask still, look through the microscope lens and identify the first-layer's colored alignment markers beneath the alignment markers of the photomask using the x- and y-direction knobs of the microscope stage to move the position of the MMAA **[1]**. *Videographer: This step is important!*

3.6.1. Talent observing the first-layer's colored alignment markers through the microscope.

3.7. Adjust the position of the MMAA until the alignment marker on the photomask is superimposed with the colored alignment marker on the first layer **[1].** *Videographer: This step is difficult and important!*

3.7.1. Talent adjusting the position of the alignment marker.

3.8. Carefully apply a slight force to the photomask **[1]** and use tape to secure the photomask in place on top of the coated wafer **[2]**. Detach the photomask from the scissor lift and ensure all four alignment markers on the photomask are in alignment with the four alignment markers on the first layer **[3].** *Videographer: This step is difficult and important!*

3.8.1. Talent applying slight force on the photomask.

3.8.2. Talent securing the photomask on top of coated wafer using tape.

3.8.3. Talent detaching the photomask from the scissor lift and checking alignment of other alignment markers.

3.9. Post-alignment, carefully detach the wafer holder from the microscope stage **[1]**. Insert the glass top plate on top of the wafer and photomask to decrease the gap between the two pieces **[2].** Place the entire wafer holder into the available UV light exposure system and perform exposure of the second layer **[3].**

3.9.1. Talent detaching the wafer holder from the microscope stage.

3.9.2. Talent inserting glass top plate on top of wafer and photomask.

3.9.3. Talent placing entire wafer holder into UV light exposure system.

3.10. Remove the wafer holder from the UV light exposure system **[1]**, then remove the coated wafer from the wafer holder **[2]** and detach the photomask from the wafer **[3]**. Complete the post-bake and developing of the second layer following the photoresist manufacturer's instructions **[4].**

3.10.1. Talent removing the wafer holder from the UV exposure system.

3.10.2. Talent removing the coated wafer from the wafer holder.

3.10.3. Talent detaching the photomask from the wafer.

3.10.4. Talent performing post-bake of the second layer.

3.11. Retrieve the master mold and place it on the stage of the upright microscope to determine the gap distance between the first layer and second layer **[1]**. Measure the distance by which the second layer is shifted and misaligned from the first layer on the microchannel structures **[2].** *Videographer: This step is important!*

3.11.1. Talent placing the master mold on the stage of the microscope for observation.

3.11.2. Talent measuring the distance of shift of the second layer from the first layer.

3.12. Use the upright microscope to determine whether the PDMS chip contains channel walls that are straight with clear device edges. Additionally, check the PDMS chip for any possible defects that may hinder device functionality **[1-TXT].**

3.12.1. Talent observing PDMS chip under microscope for straight channel walls. **TEXT: PDMS- poly(dimethylsiloxane)**

Results

4. Design, customization, and characterization of the master molds fabricated using MMAA

4.1. Through the optimization and use of the MMAA, multilayer master molds with minimal alignment error were fabricated. This system and the described protocol were used for the alignment of the markers on the photomask with the markers on the initial layer of the master mold **[1].**

4.1.1. LAB MEDIA: Figure 6.

4.2. The double-layer SU-8 master mold for a microfluidic device with a herringbone pattern was fabricated and shown to have a gap distance of less than 5 micrometers between the two layers **[1].** The two-layer master mold was then used to fabricate PDMS microchips **[2].**

4.2.1. LAB MEDIA: Figure 5.

4.2.2. LAB MEDIA: Figure 7A and 7D.

4.3. Scanning electron microscope images show that the microfluidic device with the herringbone pattern contains clear edges, straight-channel walls, and well-aligned layers, which are essential for proper device functionality **[1]**.

4.3.1. LAB MEDIA: Figure 7B and 7C.

4.4. In addition, a four-layer master mold with simple circular features was created using the MMAA to show successful alignment of a multilayer master mold **[1].** Profilometer data confirms the four distinct layers of the master mold **[2].**

4.4.1. LAB MEDIA: Figure 8A.

4.4.2. LAB MEDIA: Figure 8B.

Conclusion

5. Conclusion Interview Statements

5.1. It is important to have patience and work slowly when aligning the first- and second-layers' alignment markers and while fixing the photomask to the coated wafer.

5.1.1. *Suggested B-roll: 3.7.1 - 3.8.3.*

5.2. This procedure can be used for the production of many different multi-layer master molds allowing researchers from smaller labs to explore more complex microfluidic device designs.

5.2.1. *3.11.1.*