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Objective

The purpose of this study is to **design and lithograph** 3D microstructures that can efficiently **capture cells** per μL of a sample.

These microstructures will be integrated into a digital microfluidic platform for **phenotyping immune cell subpopulations**. Thus, they must also observe a maximum thickness of 100-200 μm .

Background

Microfluidic Devices

Microfluidic devices manipulate and analyze **very small** amounts of fluid through the use of microscale chambers and channels. They are ideal for **rapid, point-of-care diagnosis** [1].

Digital microfluidic systems are droplet-based, not requiring pumps nor valves (Figure 1) [2].

- Electrowetting on dielectric (EWOD) techniques enable manipulation of significantly smaller samples in parallel [1, 2]
- Such platforms allow for **programmed and automated droplet handling**

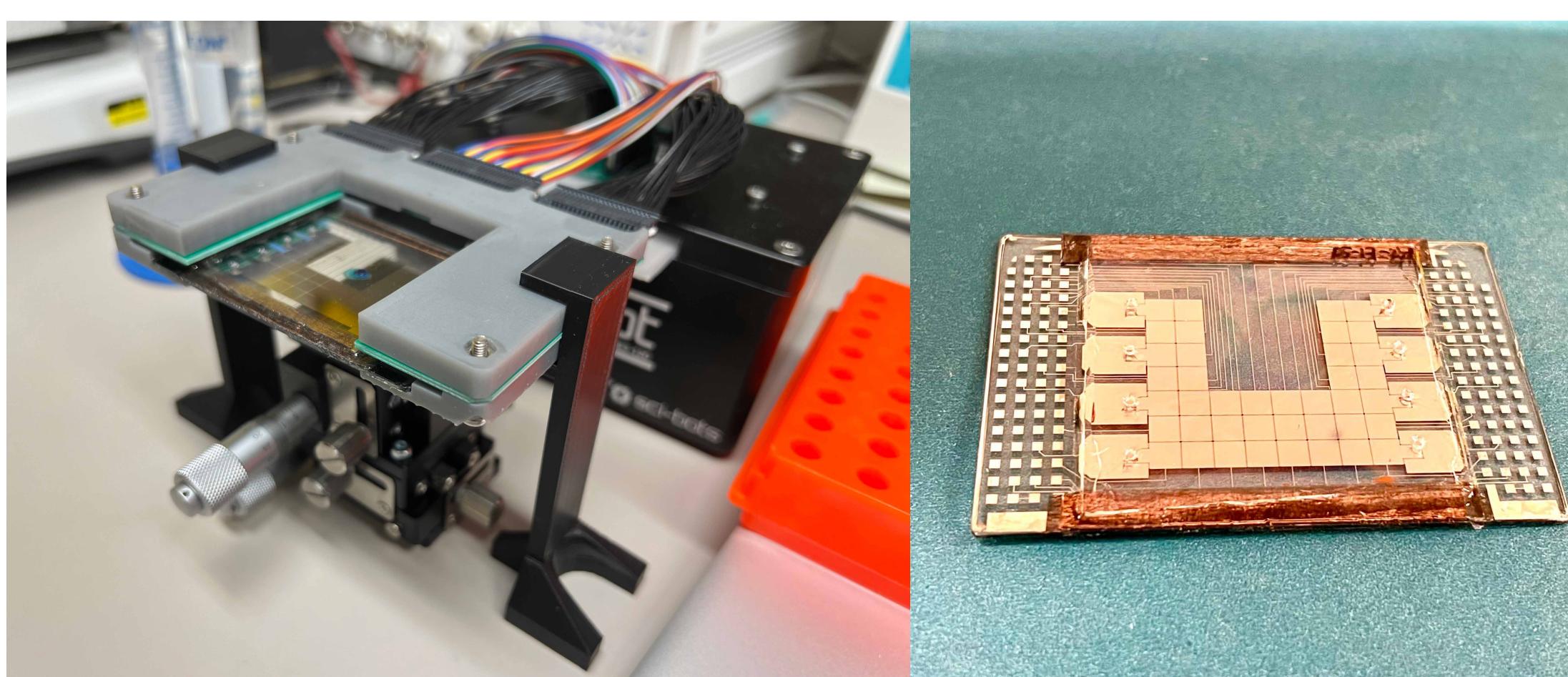


Figure 1. (Left) A DMF device. (Right) Glass substrate for DMF devices. Substrates contain dielectric and hydrophobic layers, as well as electrodes for automatic and reliable droplet control.

Dilase 3D

- Standard photolithography techniques for manufacturing microfluidic device molds is **limited** in geometries
- **3D printing** molds may facilitate fabrication of increasingly complex structures, such as tapered structures and structures of varying depths

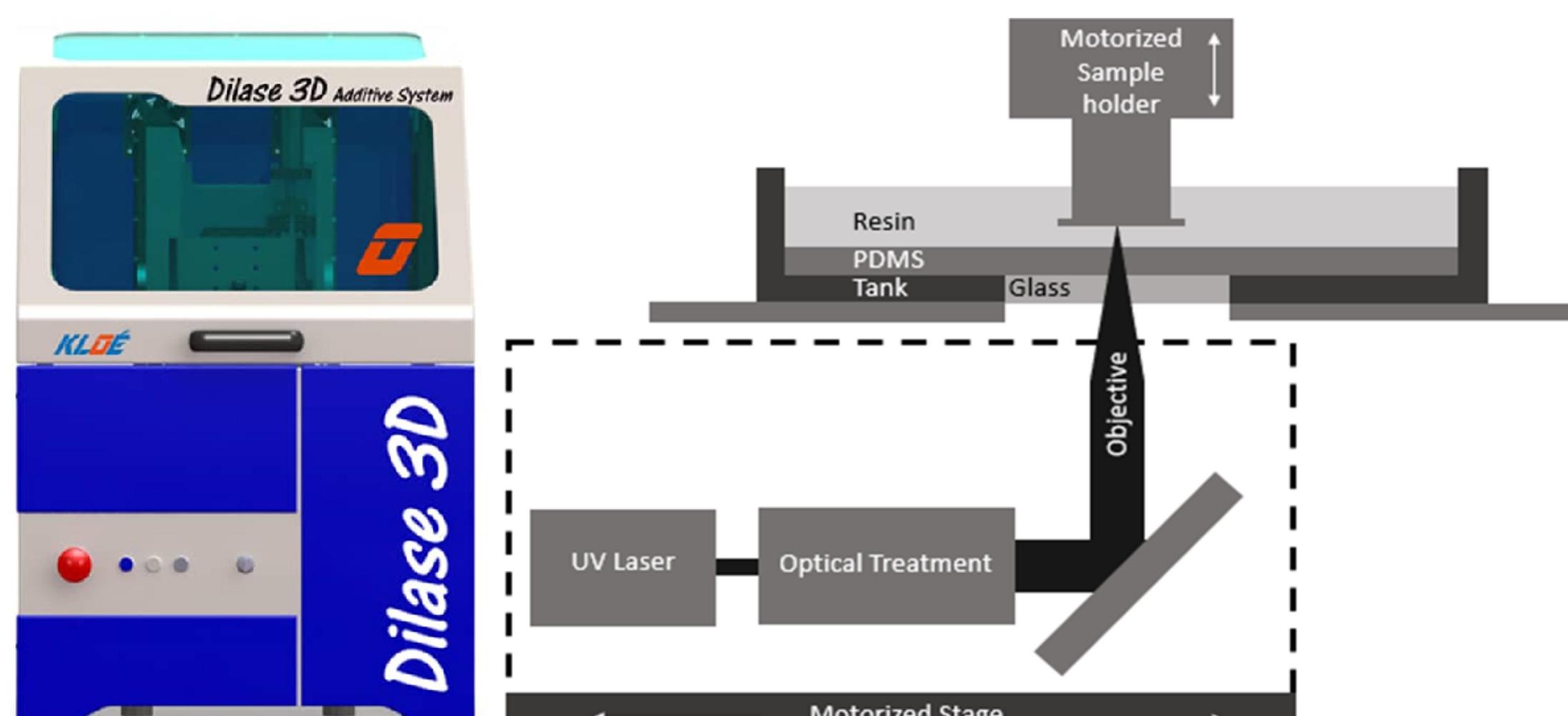


Figure 2. Dilase 3D: a tabletop resin-based micro stereolithography system. Schematic (right) outlines the components of this system. Credit: Renc Saracaydin and Seth Nfonoyim-Hara.

- 375nm UV laser (5 μm and 20 μm objectives) polymerizes **DS3000** Biocompatible resin
- Sample holder travels upward to print layer-by-layer
- 3D models (STLs) are sliced layer-by-layer into individual .LWO files describing laser path and behavior for each layer
- **Modulation** (laser power) and **velocity** (laser speed) control exposure of resin to UV ray
 - Higher modulation or slower velocity \Rightarrow greater exposure
 - Velocity is inversely proportional to print time

Methods

Device Specifications

- Resin mold is the **inverse** of the desired microstructure (Figure 3)
- Up to **6** circular features 2mm in diameter can be printed on a 15 mm x 15 mm x 200 μm square base
- Surface area gain = $2\pi rh$, area loss = $2\pi r^2$
 - When $r = h$ (as in this model), there is **no net change** in surface area
 - Roughness introduced by resin polymerization may increase surface area

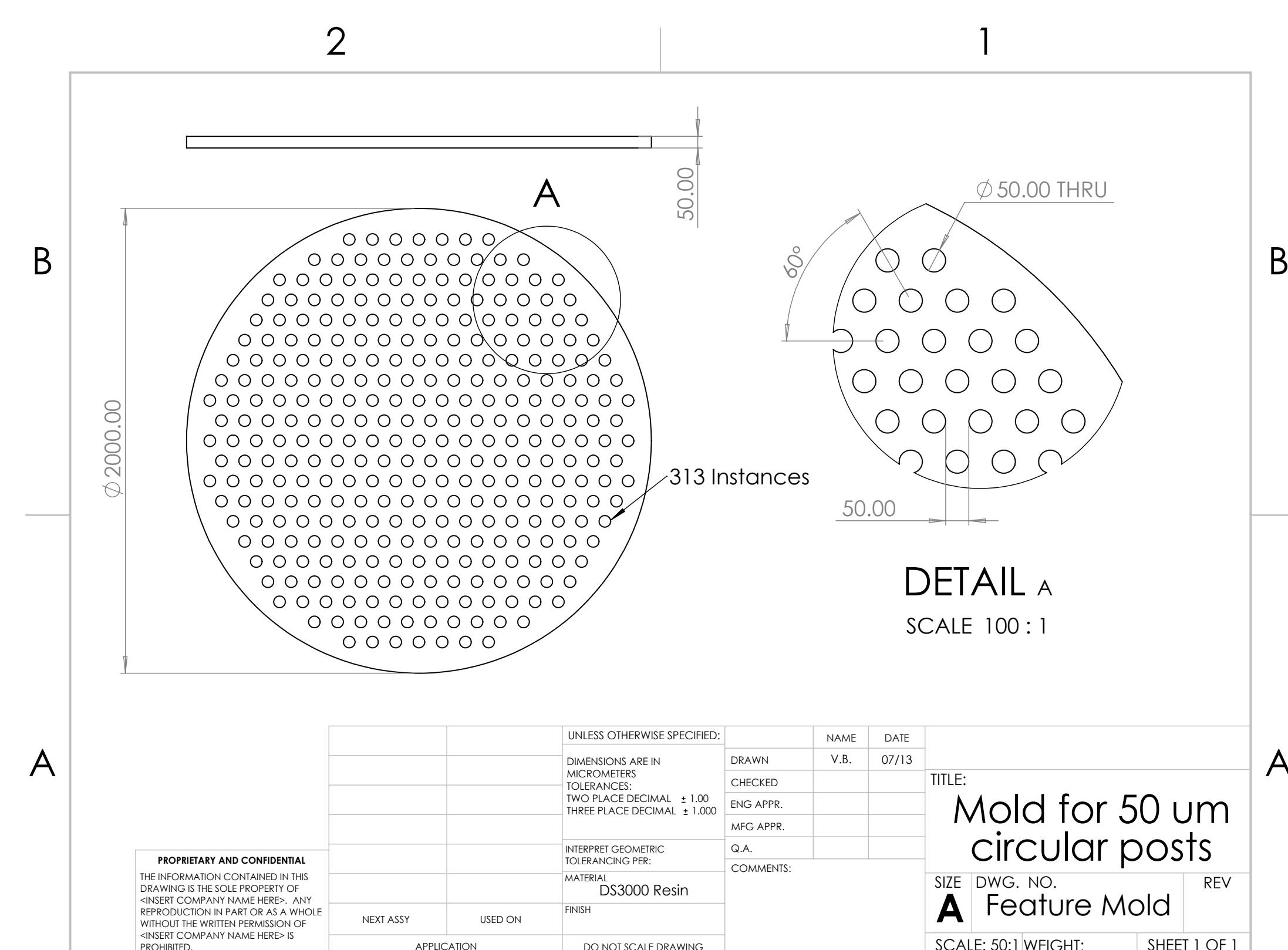


Figure 3. Technical drawing for resin mold containing cylindrical holes 50 μm in diameter, depth, and edge-to-edge distance.

Fabrication

1. Print resin mold using Dilase 3D
2. Treat surface of mold with FluoroPel PFC1101V, a spin-on **hydrophobic** coating
 - Spin-coat for 40 seconds at 2000 RPM
 - Incubate for 10 minutes at 180 °C
3. 10:1 PDMS (Slygard 184) is cast or spin-coated onto the treated mold
 - Spin-coat for 1 minute at 500 RPM
 - Cure at 75 °C overnight

Functionalization

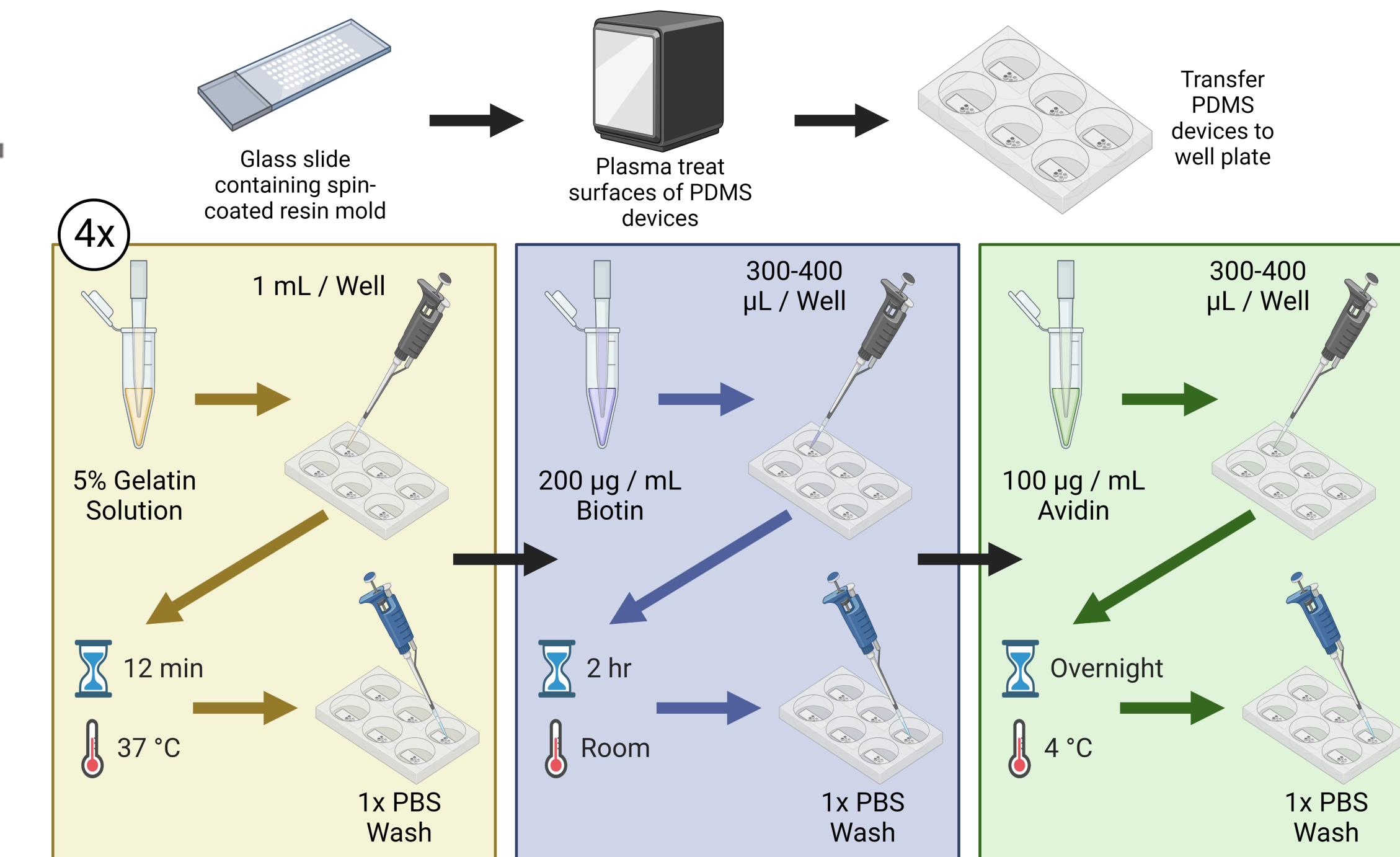


Figure 4. Functionalization of PDMS-based microfluidic devices with gelatin, biotin, and avidin for antibody and cell adhesion.

Results

Table 1. Finalized slicing and exposure parameters for printing a resin-based microfluidic device mold on the Dilase 3D.

Objective (μm)	Slice Height (μm)	Modulation	Velocity (mm/s)
Base	20	50	80%
Features	5	25	15%

- Spin-coating yields a PDMS thickness approximately between **125 - 150 μm**
- Print time shortened to **2 hours** with optimization (Table 1)
- Diameters and depths are $\sim 50 \mu\text{m}$, and the posts are straight

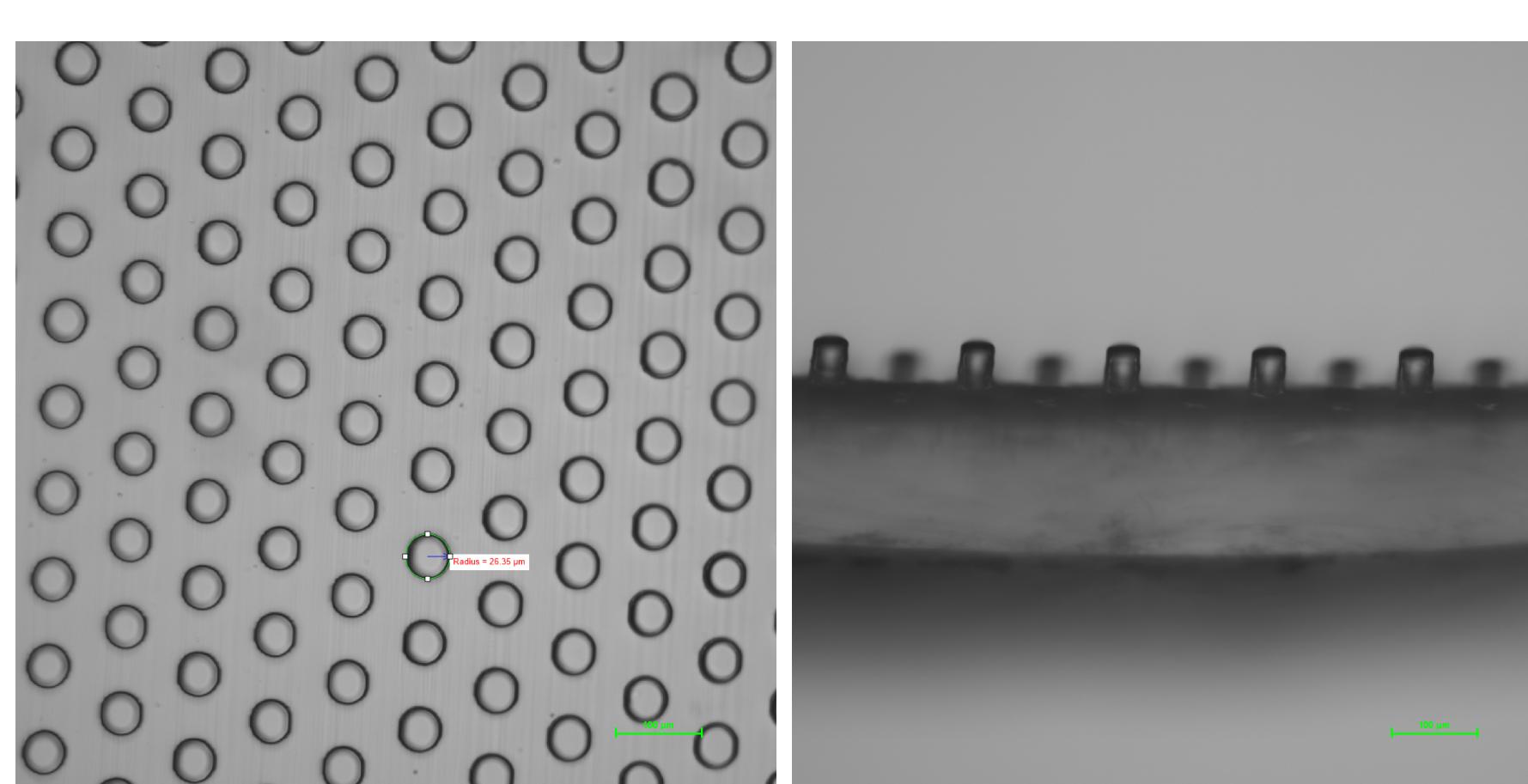


Figure 5. 15% at 50 mm/s, 25 μm feature slice thickness. (Left) PDMS top view. (Right) PDMS side view.

Cell Adhesion Tests

- Devices additionally coated with **CD45+ antibodies** through binding of avidin to biotin
- Entire device **submerged** in solution of peripheral blood mononuclear cells (PBMC) stained with FITC fluorescence dye
 - Excitation results in green light emission
 - Incubated for 1 hour at room temperature
- Figure 6 indicates inclusion of **posts and antibodies** substantially improve cell capture
 - Bright greens near posts, and lack of greens on smooth, post-free surface

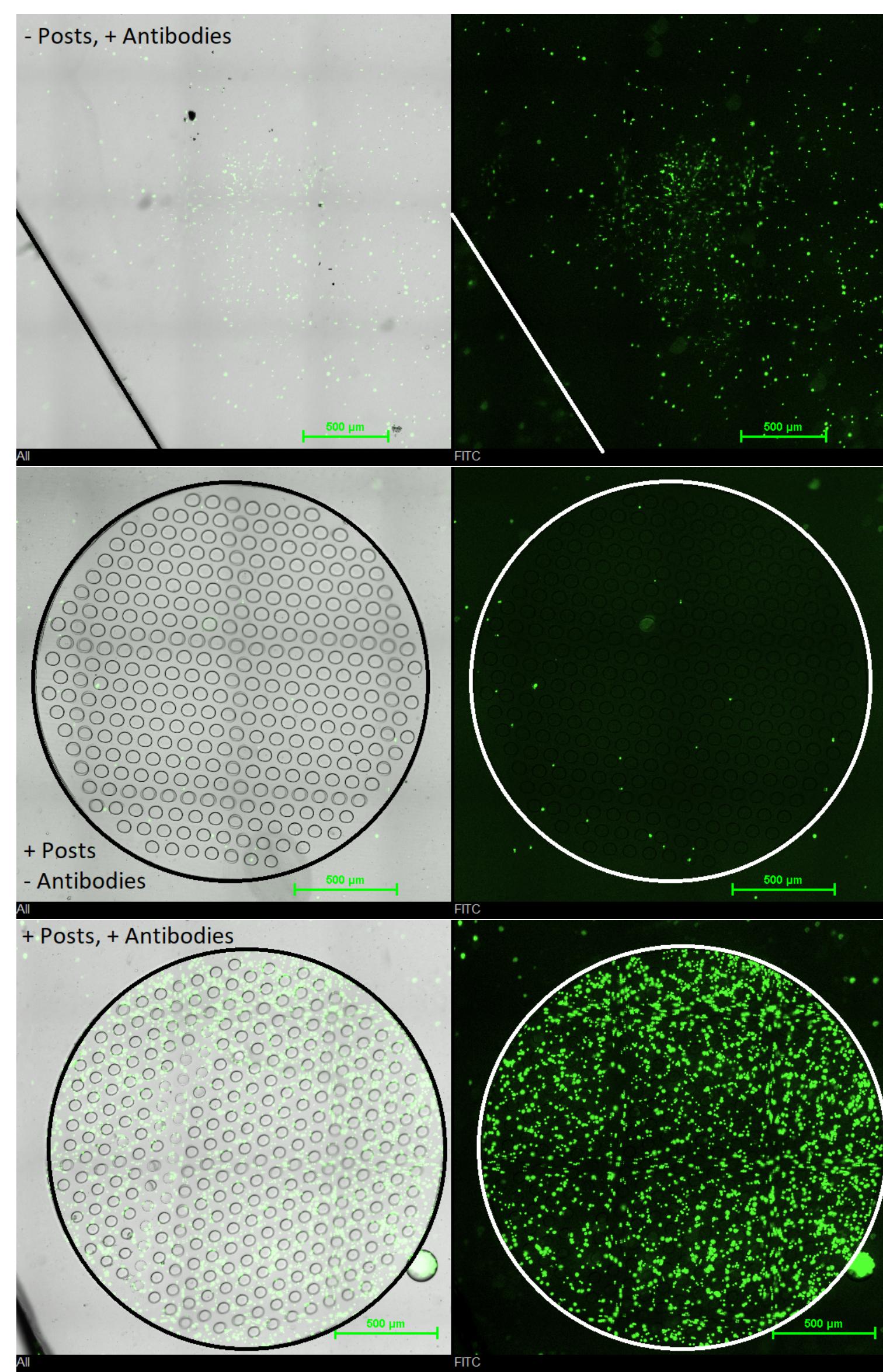


Figure 6. Results of cell adhesion test on PDMS microstructures, viewed under white and fluorescent lights. Brighter greens indicate higher cell counts. (Left) Device without posts, treated with antibodies. (Right) Device with posts, not treated with antibodies. (Bottom) Devices with posts, treated with antibodies.

Challenges

- PDMS degradation occurs rapidly, which negatively affects print quality and accuracy
 - Maximum of **9 hours** of cumulative print time before a tank change
- Optimization is laborious and time-consuming, requiring several sample prints to refine exposure settings
 - Printing parameters will likely vary across different geometries
 - Increasing printing velocities currently result in heavy detail loss
- Low-quality and inconsistent structures are the result of improper parameter selection (Figure 7)

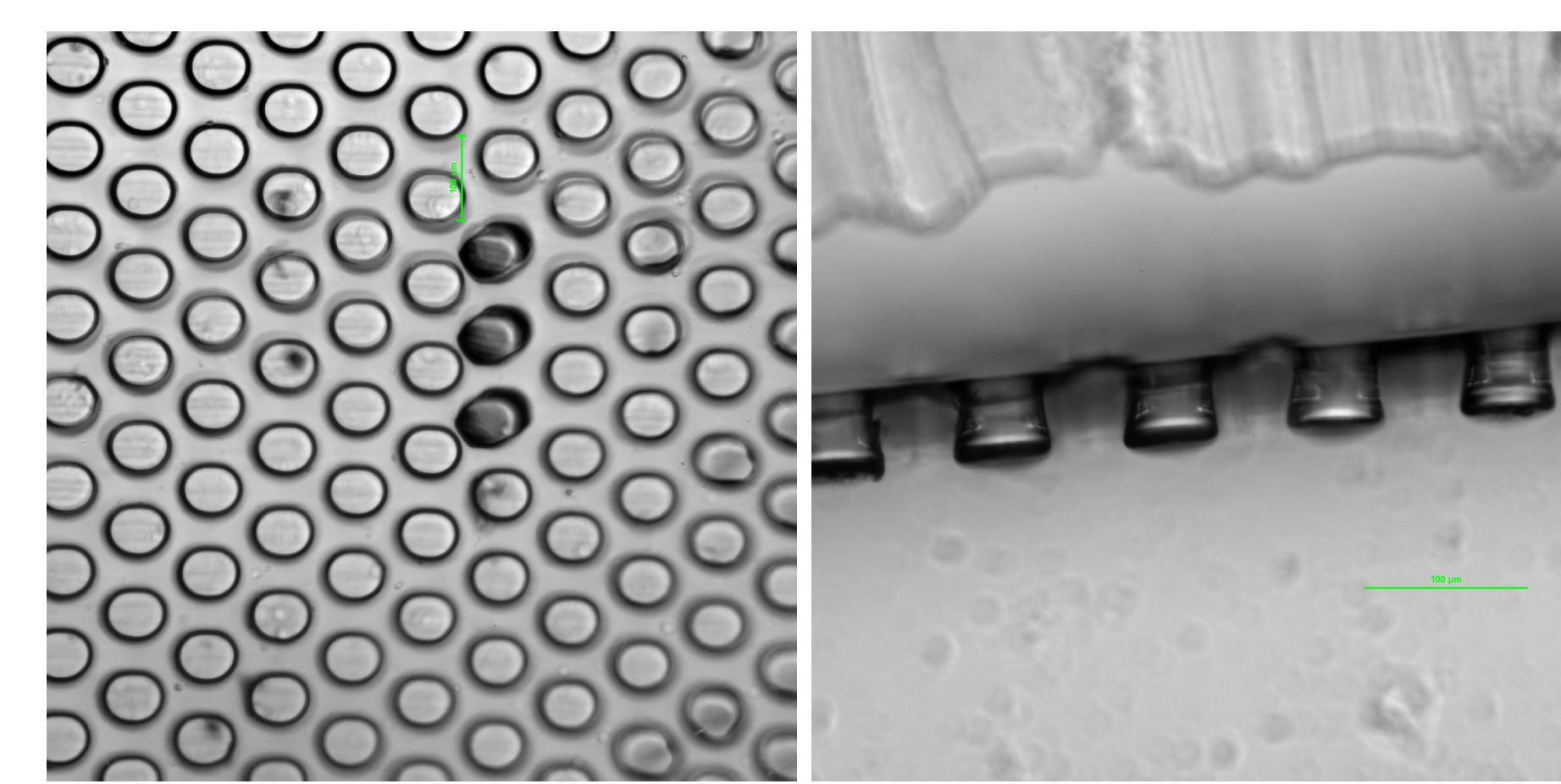


Figure 7. Misshapen and damaged posts form when molds are printed with unoptimized settings.

Conclusions

- 3D printing microfluidic device molds is **viable** under certain circumstances
 - Lacking access to cleanroom facilities
 - Requiring complex structures such as tapered posts (Figure 7)
 - Prototyping for automated production in the future
- Soft, microscale lithography of PDMS using a **resin mold** is **effective**
 - Reliability of a resin mold highly depends on quality of the tank and resin, and inconsistencies may still be present regardless
- **Cylindrical** posts are **practical for capturing cells** in comparison to smooth, flat surfaces
 - Redistribution of surface area provides more opportunities for cells to contact the device

Future Directions

1. Polish the 3D printing process, determining exposure parameters to achieve desired geometries quickly
2. Optimize spin-coating parameters to create devices with thickness < 200 μm for use in a DMF device
3. Continue to refine functionalization procedure to improve cell adhesion to PDMS-based device
4. Evaluate alternative post geometries
 - Shape, size, spacing, count, etc.

References

- [1] Y. Zhang and Y. Liu, "Advances in integrated digital microfluidic platforms for point-of-care diagnosis: a review," *Sensors & Diagnostics*, vol. 1, no. 4, pp. 648–672, 2022.
- [2] E. Samiei, M. Tabrizian, and M. Hoofar, "A review of digital microfluidics as portable platforms for lab-on-a-chip applications," *Lab on a Chip*, vol. 16, no. 13, pp. 2376–2396, 2016.

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