Protein Photopatterning in 3D with the Alveole PRIMO

ENGR 241: Autumn 2018- Winter 2019

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1. Executive Summary

The purpose of this document is to summarize the results of a two-quarter project to expand the use of the Alveole PRIMO system at the Stanford Nanofabrication Facility (SNF) for protein patterning in three-dimensional microenvironments. While two-dimensional surfaces have typically been used for micropatterning, this project seeks to leverage some unique advantage of the contactless PRIMO approach for direct bio-functionalization of more complex structures such as microfluidic channels. The first half of the project discusses key considerations for patterning through thick PDMS substrates, including depth of field limitations and pattern distortion on slanted surfaces. These results set up the second set of experiments, which focus on challenges encountered when patterning directly within enclosed microfluidic channels. This includes a unique approach to shield surfaces from UV illumination with a soluble UV absorbing dye and thus selectively pattern biomolecules to desired faces inside confined spaces. The report also discusses important practical factors such as calibration and substrate preparation which impact pattern quality. Detailed SOPs are provided in supplementary documents to be made available to the SNF community. These SOPs include step by step instructions for new users to the PRIMO tool, as well as tips for patterning on PDMS substrates and directly within microfluidic devices.

2. Acknowledgment

We would like to express our sincerest gratitude to Gaspard Pardon, Swaroop Kommera, and Tony Ricco for the guidance and support they provided throughout this project. We are really grateful to them and this work would not have been possible without their mentorship.

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4. Background

4.1 Motivation

Organs consist of cells residing within highly organized tissues. Physical and spatial cues such as topography and patterning of extracellular matrix proteins direct cell behavior and function. For example, the retina is composed of multiple layers of neurons which convert visible light images into electrical signals and transmit them to the brain. The retinal photoreceptors are the primary light detecting cells, whereas pigment epithelial cells play a crucial role in supporting photoreceptor cell functions including phagocytosis and regeneration of visual pigments via the visual cycle [1]. The interconnection of these cells plays a crucial role in the functioning of the retina.

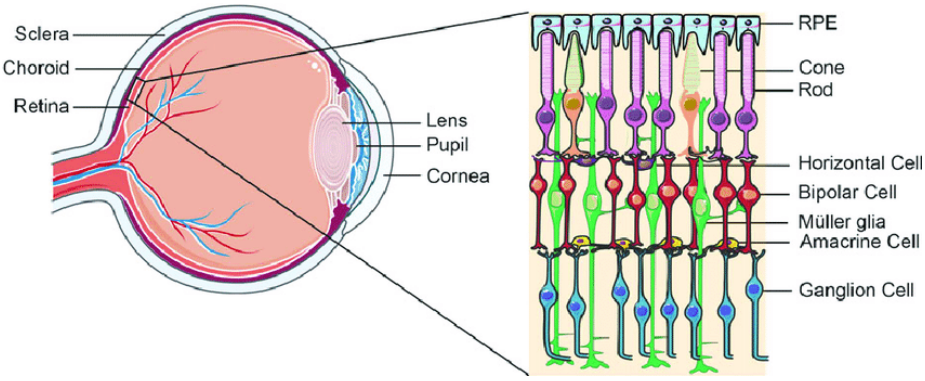


Figure 1: Structure of the eye and retinal cross-section [1].

While these spatial and biophysical cues are essential to healthy and pathological function, standard cell culture techniques fail to recapitulate the native tissue organization and structural complexity. Better control of the 3D cellular microenvironment (e.g. by micropatterning) is crucial for basic biological research and has widespread applications in quantitative mechanobiology, tissue engineering, disease modeling, drug discovery, and biosensor development.

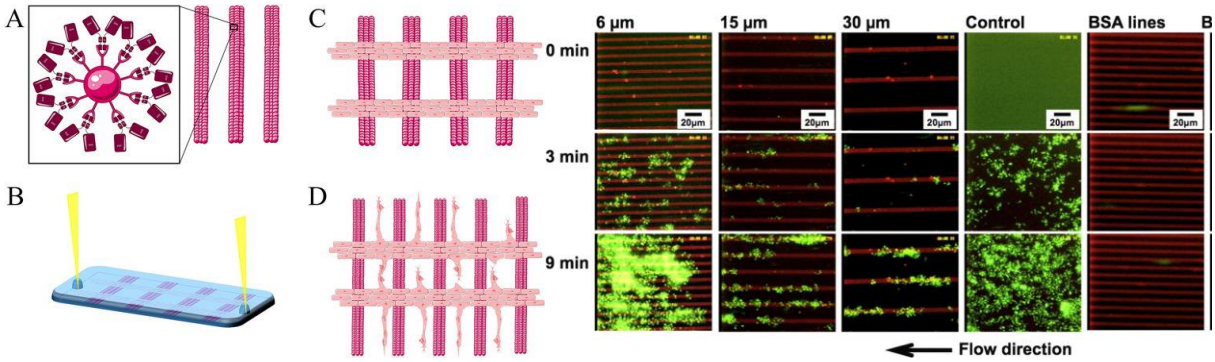


Figure 2: Applications of biomolecule micropatterning include control of vascularization for tissue engineering and biophysical tools. Left: Microfabrication techniques for spatial pattern of Notch ligands are used to control angiogenetic sprouting of human endothelial cells.[2] Right: Fibrinogen patterns surrounded by a protein-resistant BSA background are used to study platelet adhesion dynamics, test pharmaceutical compounds, and refine computational models of blood clotting. [3]

Micropatterning techniques allow for co-cultures with precise localization of different cell types and show promise in the advancement of organ-on-chip systems. Recently, spatial patterning of surface-immobilized Notch ligands with microcontact printing has been used to control angiogenic sprouting in vitro and demonstrates the potential of spatial control of vascularization for applications in tissue engineering and regenerative medicine [2].

Alternatively, high resolution multi-protein microarrays can be used as biophysical tools to study the dynamics of platelet adhesion or endothelial cell alignment and inflammation under physiological flow [3].

4.2 A Brief Introduction to the Alveole PRIMO System

A common method for micropatterning is microcontact printing with elastomeric stamps. However, this technique relies on physical contact between the substrate and thus suffers from poor alignment capability, limited pattern resolution, and limited surface compatibility. In contrast, the Alveole PRIMO system is based on a maskless, contactless photopatterning approach which integrates into a standard inverted microscope and enables rapid and flexible prototyping of high-resolution biomolecule patterns without extensive fabrication experience or equipment.

The optical setup uses a UV laser diode (375 nm) and a digital micro-mirror device (DMD) to project patterns directly onto a surface. Local control of UV exposure by grayscale patterns with the DMD allows for controlled gradients of protein density. Crucially, PRIMO is capable of multi-protein alignment and precise positioning on micro structured substrates (e.g. on the curved surfaces of a microwell) using the microscope motorized stage.

4.2.1 PRIMO Workflow

Briefly, the principle of operation is as follows: The substrate of interest (e.g. glass coverslip) is coated with an anti-fouling polymer such as polyethylene glycol (PEG) to prevent adhesion of biomolecules to the surface. Alveole's proprietary photoinitiator (PLPP) is added, and the PRIMO module projects a user-defined pattern onto the surface through the microscope objective. UV illumination activates the PLPP to locally degrade the PEG brush by photoscission. Proteins incubated onto the substrate selectively bind to the exposed regions, forming a micropattern to which cells will adhere [4].

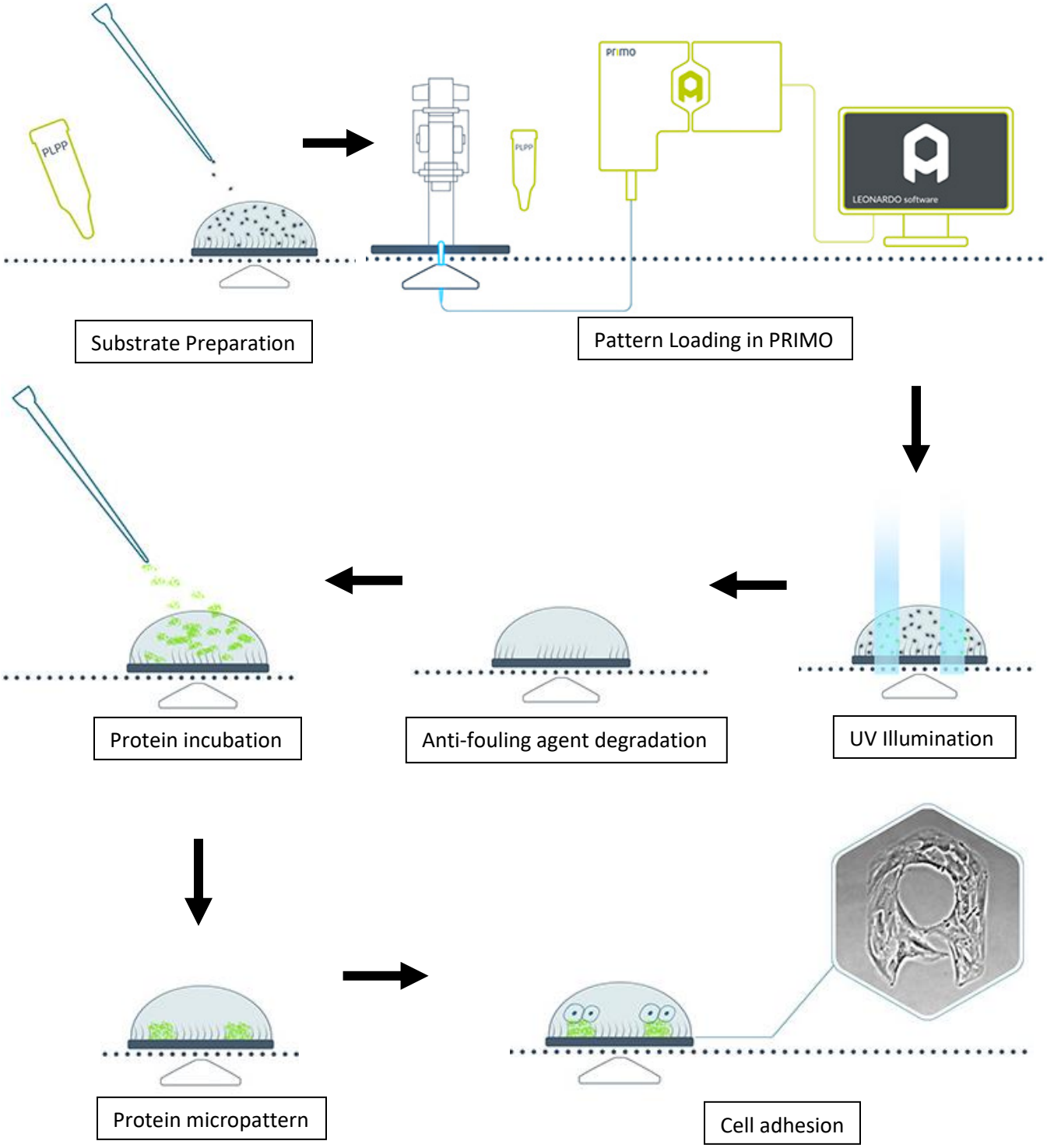


Figure 3: A simplified PRIMO workflow schematic demonstrating the major steps (Adapted from Alveole https://www.alveolelab.com/our-products/plpp-photoactivatable-reagent/])

5. Project Goals

While a relatively new technology, PRIMO has commonly been used thus far at SNF for patterning of simple 2D substrates such as glass coverslips, as previously documented in an excellent E241 report by Erica Castillo and Joy Franco. The overall goal of the project described here was to build off these results and expand the capabilities of the Alveole PRIMO tool at SNF for protein photopatterning on 2.5D and 3D substrates. This will benefit the SNF community by developing a toolbox for researchers to create custom, well-defined 3D cellular microenvironments by rapid and flexible photopatterning.

Part of the motivation for this project originated from an idea for protein patterning within microfluidic channels mimicking the stiffness and branched geometry of a human blood vessel to study the effect of spatial cues on vascular endothelial cell behavior. Creation of such a device requires several technological advancements. One of the first steps was to develop an understanding of the capabilities of PRIMO for generation of protein patterns on substrates beyond flat, glass coverslips.

In the fall quarter we explored the limitations of PRIMO for patterning on polydimethylsiloxane (PDMS), an elastomer commonly used to make microfluidic chips. This included attempting to pattern through thick PDMS substrates and across multiple focal planes, as would be encountered when patterning on the inner surface of microfluidic channels. In the winter quarter, we focused on identifying the challenges encountered when using PRIMO directly within an enclosed space (i.e. microfluidic channel) and developing strategies for selective photopatterning of biomolecules to desired surfaces while maintaining high resolution.

6. Fall Quarter (2.5D PDMS Patterning) Methods

PDMS was chosen as a substrate of interest for PRIMO due to its excellent optical properties, biocompatibility, and tunable stiffness - another key biophysical cue for cell biology. Furthermore, PDMS is commonly used to create microfluidic channels and topographies or microstructures such as microwells.

Given the objective of the project, the goal for the autumn quarter was to develop an understanding of the key considerations for generating protein patterns on 2.5D PDMS substrates. Generation of such patterns requires an understanding of several factors:

1. What depth of field can we achieve for projected patterns while maintaining acceptable resolution (i.e. can we simultaneously create patterns spanning multiple focal planes with a single exposure?
2. How will pattern distortion through PDMS on slanted surfaces affect pattern quality?
3. Through what thickness of PDMS can we pattern while maintaining acceptable resolution?
4. How does the optimal PRIMO dosage change for new substrates?

These questions will influence pattern design and stitching when creating features which span multiple focal planes, set limitations on feature sizes and microfluidic channel geometries which can be patterned, and determine parameters for PRIMO exposure. In order to understand these factors, we used a simple test system for characterizing the performance of PRIMO photopatterning on slanted PDMS surfaces.

6.1 Preparing PDMS Wedges

PDMS wedges were prepared by mixing the prepolymer and curing agent in 10:1 ratio by weight. The PDMS was then poured onto glass slides set at defined angles inside a petri dish and then left overnight

for curing. Extra devices of each thickness were prepared for later use in calibration. (Note: PDMS should be cast on a smooth surface such as glass slide to maintain optical transparency.)

After curing, PDMS samples and glass slides were plasma air etched for 60 seconds and bonded together. (Note: Plasma treatment is optional and was not done for every experiment.)

Score marks were placed at the z height matching the thickness of the control sample which would be used for calibration (see image below). After marking the PDMS sample, a stencil was placed with the score mark passing through the middle of the stencil well.

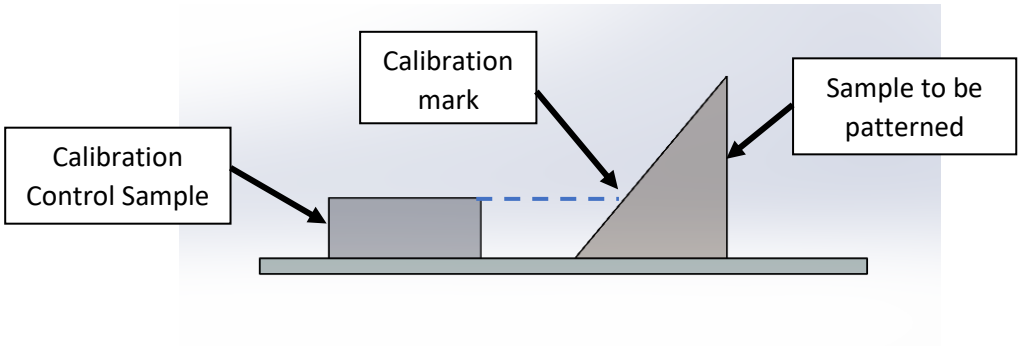


Figure 4: Calibration at the desired z plane with planar PDMS slab

The prepared sample was passivated with PLL + PEG-SVA per the PRIMO PROTOCOL: PHOTOPATTERNING ON PDMS WITH PRIMO Rev 2.

6.2 PRIMO Patterning

Calibration of the PRIMO tool is always important for ensuring quality results, and especially so when placing additional components in the optical path or using a new substrate such as PDMS. Tube lens calibration will correct for stigmatism and ensure that the DMD laser projection plane is conjugated with the objective focal plane.

A droplet of UV highlighter was pipetted onto the PDMS control surface and covered with a coverslip as shown in the figure below. PDMS is hydrophobic and the coverslip was used to prevent the highlighter droplet from shrinking. Placing a glass coverslip on top of the highlighter caused some visible reflections in the calibration image, so generally we removed the coverslip immediately prior to calibration. Calibration of the tool was then performed as described in the SOP accompanying this document.

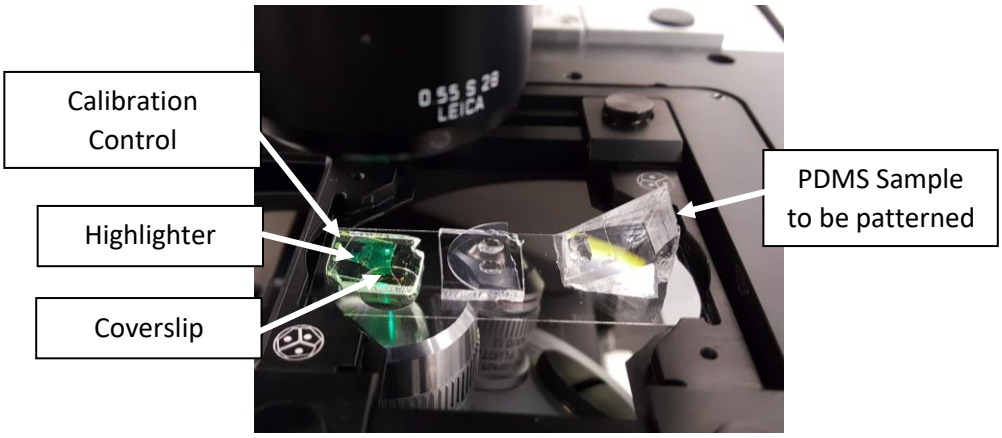


Figure 5: Calibration and patterning setup

After calibration, the Leonardo software was used to pattern PDMS substrates. The test image shown below was the primary pattern used for characterization. This pattern consisted of lines and spaces 1, 2, 4, 8, 16, 32, and 64 pixels wide. This was used to assess pattern resolution for different conditions.

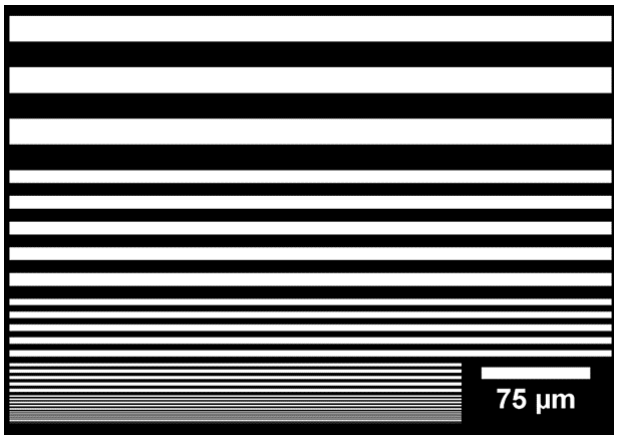


Figure 6: Pattern used for PDMS flat and wedge experiments

For PDMS wedges, this test pattern was oriented with respect to the slanted surface such that the length of the columns extended across multiple focal planes (i.e. the left side of the pattern was at a higher focal plane than the right side). The pattern was centered on the score mark corresponding to the calibration plane, such that the pattern would be in good focus at the centerline and drift out of focus equally on both sides.

A range of UV exposures were tested to determine the optimal dosage (i.e. avoid overexposure while providing sufficient degradation of the passivation layer). After exposure the wells were incubated overnight with fluorescent protein.

6.3 Pattern Analysis

The patterns were imaged using the Leica microscope. To assess pattern quality, line profiles of fluorescent intensity along the patterns were generated in ImageJ.

7. Fall Quarter (2.5D PDMS Patterning): Results

There are several key considerations to be aware of when patterning in 2.5D/3D:

- Depth of field
- Pattern distortion/stretching
- Resolution degradation for different thickness (related to working distance)
- Exposure/dosage optimization for PDMS with this setup

7.1 Evaluating Depth of Field

As expected, the highest resolution is achieved in the middle of the pattern (section 3), where the focal plane of the projected UV pattern intersects the substrate. Section 1 which is furthest away from the center of the pattern shows greatest blurring of features.

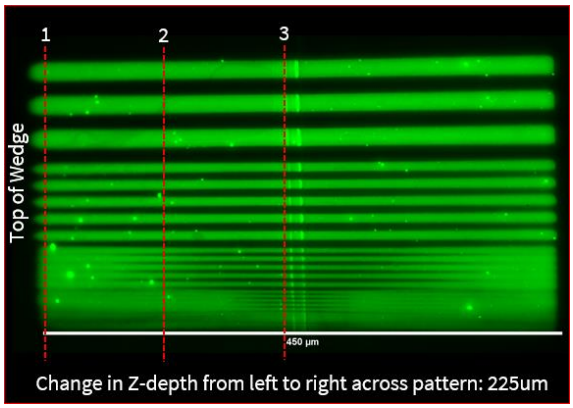


Figure 7: Pattern

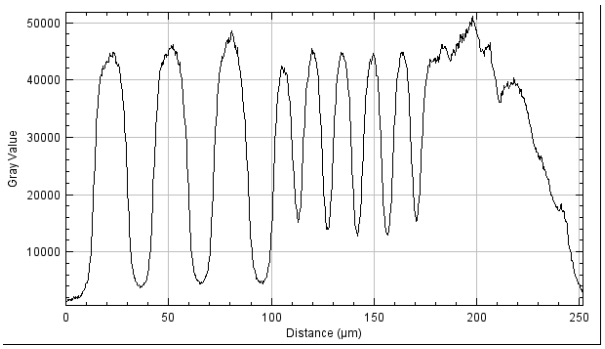


Figure 8: Intensity at Section 1

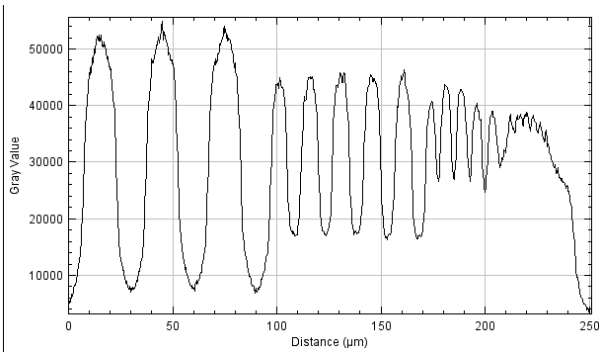


Figure 9: Intensity at Section 2

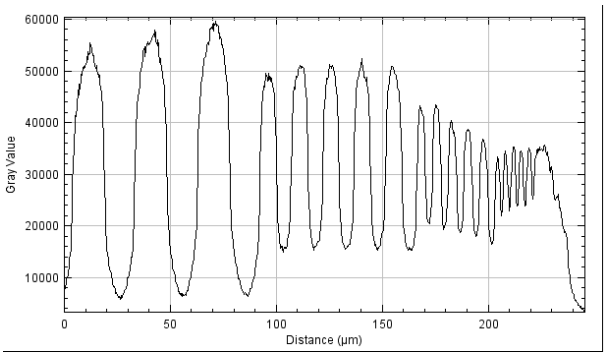
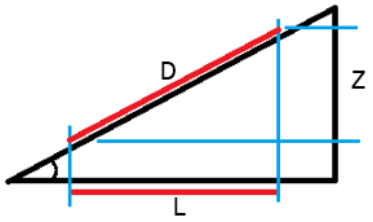


Figure 10: Intensity at Section 3

In order to apply this information towards future guidelines for patterning on 3D surfaces, an acceptable resolution threshold may be specified in order to determine the maximum z-range which can be spanned by a single laser projection. When creating large patterns, the loss in resolution should be considered to determine the size of each pattern to stitch together. This may depend on the feature size (line width and spacing), as the relative distortion of the pattern is greater for thinner lines.

7.2 Pattern Distortion/Stretching

As the PDMS surface is angled with respect to the DMD projection, the pattern gets stretched. This may be compensated for by deliberate design of patterns to be smaller than the desired result.



D = L / cos(theta)
Z = L*tan(theta)
D = Elongation length
L = Original pattern length
Z = Height of pattern

7.3 Pattern Resolution Degrades with Increasing Thickness

As demonstrated in the calibration images below, the maximum achievable pattern resolution degrades as thickness of the PDMS substrate increases. Alveole reports that patterning through PDMS should be possible for layers up to 2 mm thick using the 20x Nikon S plan flour objective (default on SNF setup), with resolution remaining at 1 μm for layers up to 250 μm. Again, the acceptable thickness of PDMS through which you can pattern will depend on the acceptable pattern resolution as dictated by your specific application. However, based on these preliminary results we would suggest limiting PDMS substrate thickness to under 1 mm. This could potentially be expanded with the use of a longer working distance objective.

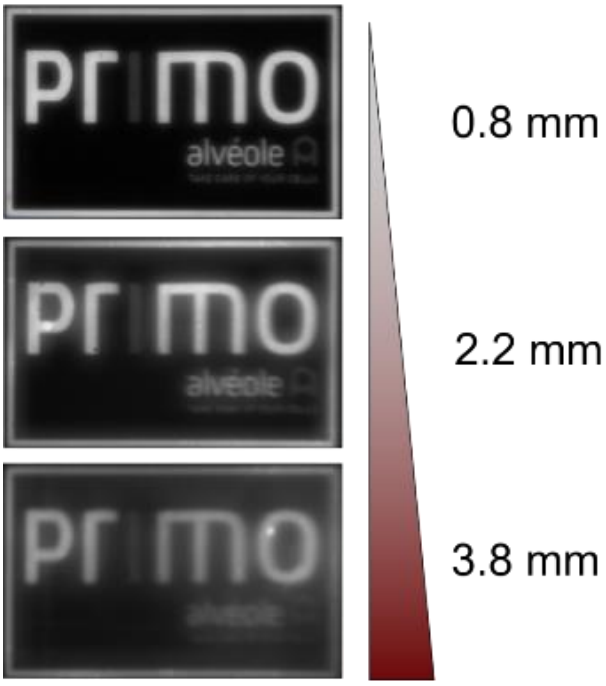


Figure 11: Pattern degradation with increasing thickness

7.4 Exposure Dosage Optimization for PDMS

UV dosage levels affects the resolution of the patterns as shown in figures below. Higher UV doses produce clear overexposure, as indicated by rounding of the corners. The optimal dosage with this setup was determined to be between 750 mJ/mm^2 and 1000 mJ/mm^2. Users will need to determine the correct dosage for a given application depending on parameters such as substrate thickness.

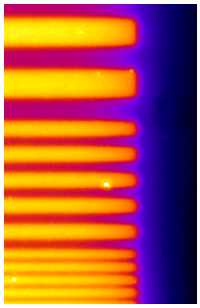


Figure 12: Pattern when exposed to 1000 mJ/mm^2

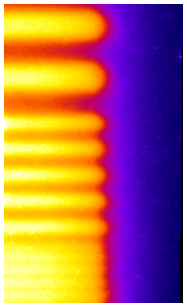


Figure 13: Pattern when exposed to 1500 mJ/mm^2

8. Winter Quarter: Patterning in Enclosed Channels

As briefly discussed in section 5, the second phase of the project was working towards selective patterning of biomolecules in confined spaces. To the best of our knowledge, at the time of this project there have been no reports using PRIMO for protein patterning directly within microfluidic channels. We sought to leverage the advantages of the PRIMO optical system and explore its limitations in this capacity. Specifically, contactless pattern generation within enclosed channels could provide a rapid and flexible approach for fabrication of experimental systems to study the role of biophysical cues in cellular function and advance the in-vitro modeling of human organ systems (e.g. blood vessel on a chip). More generally, the ability to rapidly define spatial patterns of functional biomolecules within arbitrarily complex, user-defined geometries would be a powerful tool for precise engineering of the three-dimensional cellular microenvironment. Our aim was to begin laying the groundwork for such a toolbox for PRIMO users at SNF and beyond.

8.1 Channel Fabrication

For the winter quarter, a simplified rectangular microchannel was chosen to troubleshoot issues we expected to encounter when moving from flat surfaces to enclosed geometries (e.g. reagent handling and rinsing, optical shadowing and reflection effects inside the channel.)

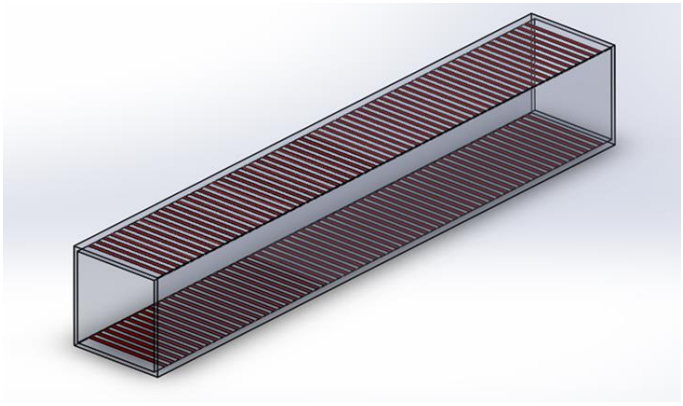


Figure 14: Idealized model of a rectangular microchannel with multiple patterned surfaces

PDMS microchannels are often cast from molds of negative photoresist (e.g. SU-8). To allow for quick, flexible generation of simple channels for patterning and to accommodate reagent transfer steps by pipetting, we chose to instead create channels by sandwiching PDMS stencils between glass coverslips and/or PDMS substrates.

Stencils were cut from a sheet of 200 μm thick PDMS using the Silhouette CAMEO® machine located next to the PRIMO device in SNF. For the final patterning experiments a channel width of 1500 μm was selected, though some experiments were performed in narrower 500 μm channels. Given the PRIMO's field of view of 300 μm by 500 μm, a wider channel allows for stitching of multiple patterns or the avoidance of edge effects. Channels were between 2 to 3 cm in length.

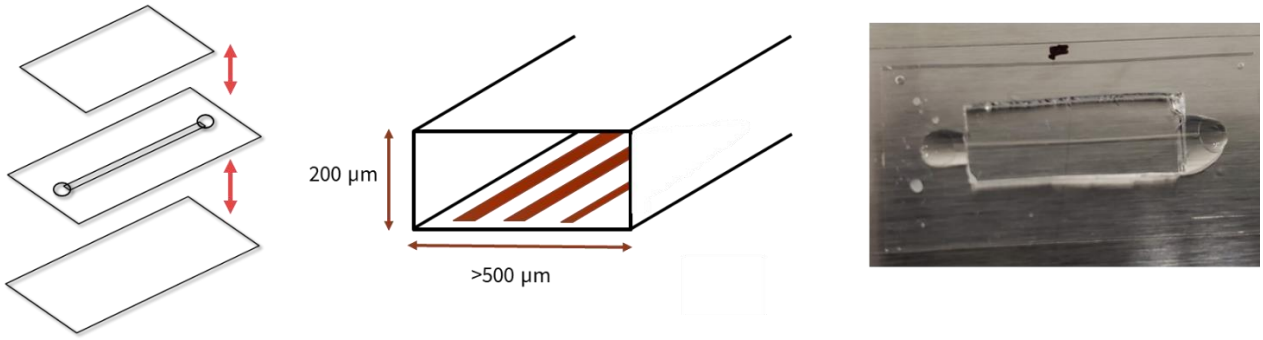


Figure 15: Schematic of channel fabrication by layering of substrates (left), channel cross section (middle), and an image of an actual wetted device with a PDMS upper layer (right)

8.2 Experimental Setup

The contactless PRIMO optical setup and photochemistry theoretically allows for generation of patterns directly within confined but optically transparent spaces. However, if all inner surfaces of a channel are coated in the same manner and exposed to the PLPP photoinitiator, the UV illumination will degrade the anti-fouling layer on any surface in the device that it passes through. We would expect that when attempting to pattern the "floor" of a microchannel by focusing on the bottom surface, a corresponding pattern would be produced on the top surface of the channel. While the projected light is nearly collimated, we have seen from the PDMS wedge experiments that there is a limited depth of field. Therefore, we would expect that for sufficiently tall channels the corresponding out-of-focus pattern on the upper "ceiling" would lose resolution. Likewise, when attempting to pattern the top surface of a microchannel, we would expect to generate a lower quality, blurred pattern on the corresponding bottom face.

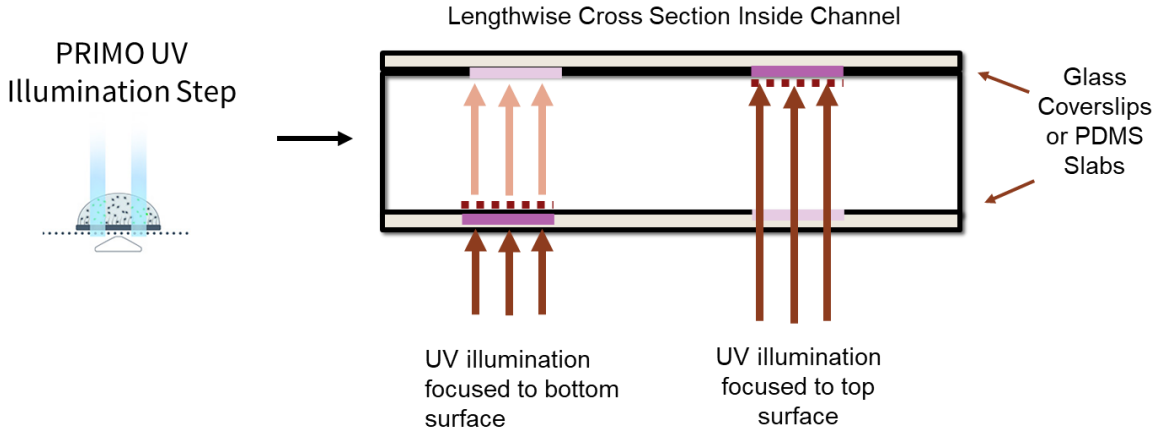


Figure 16: Pattern exposure through microfluidic channels produces multiple intersections with the coated inner surfaces. UV illumination focused at the bottom surface to create an in-focus pattern (purple bar) will also reach the upper surface to produce an out-of-focus pattern (pink bar). The reverse will be true for patterns focused to the top surface.

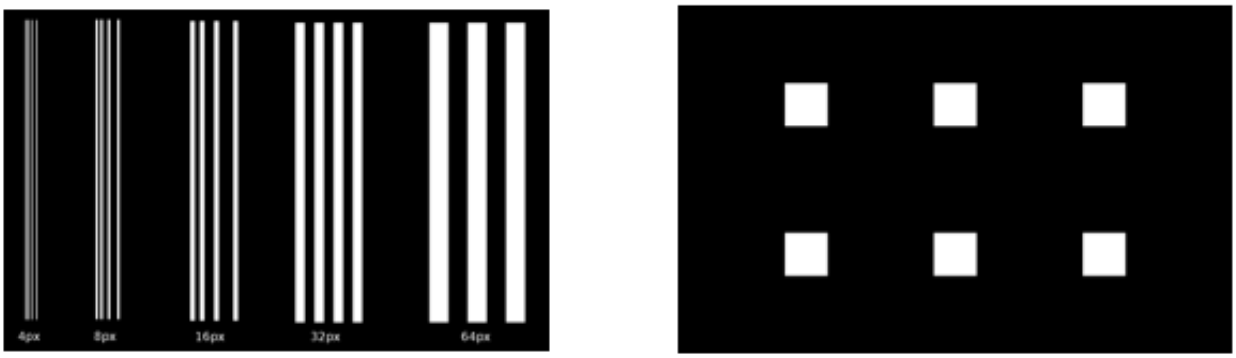


Figure 17: Images used to assess pattern quality in microfluidic channels. Column widths ranged from 4 pixels (~ 1.1um) to 64 pixels (~ 17.6um). Square boxes were 140 pixels across (~ 38.5um).

8.3 Contactless Generation of Protein Patterns Directly Within Microfluidic Channels

Here we demonstrate the first use of PRIMO at SNF to generate protein micropatterns directly within an enclosed microfluidic channel.

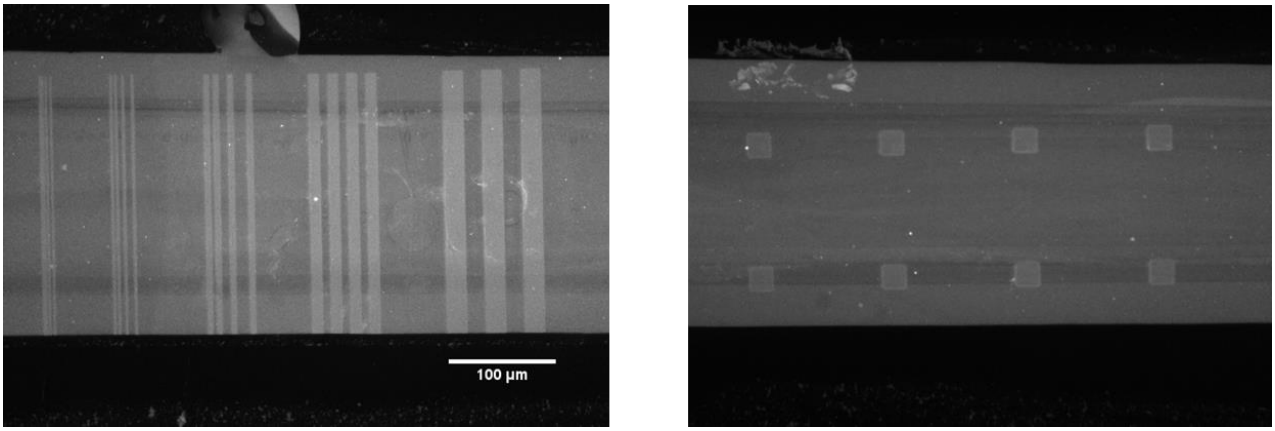


Figure 18: First demonstration of pattern generation directly on the bottom glass surface of microchannels

As discussed in section 6.2, proper calibration plays a critical role in determining pattern quality. Because the opposing faces of the microfluidic channel have a relatively small separation, we used the bottom surface alone for the calibration. The small channel height allows the calibration to remain reasonably accurate for both the surfaces. In order to pattern different surfaces within the channel, the only change was adjustment of the focal plane prior to UV illumination. We then tested at several incremental focal planes to study pattern degradation and confirm our predictions for out-of-focus patterns.

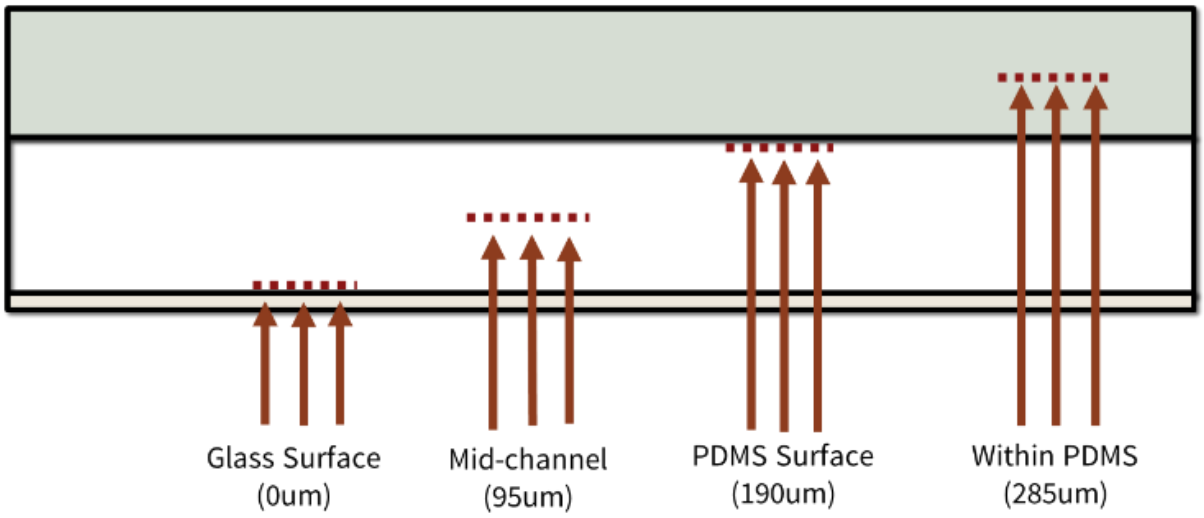


Figure 19: Focal plane for pattern calibration assessment

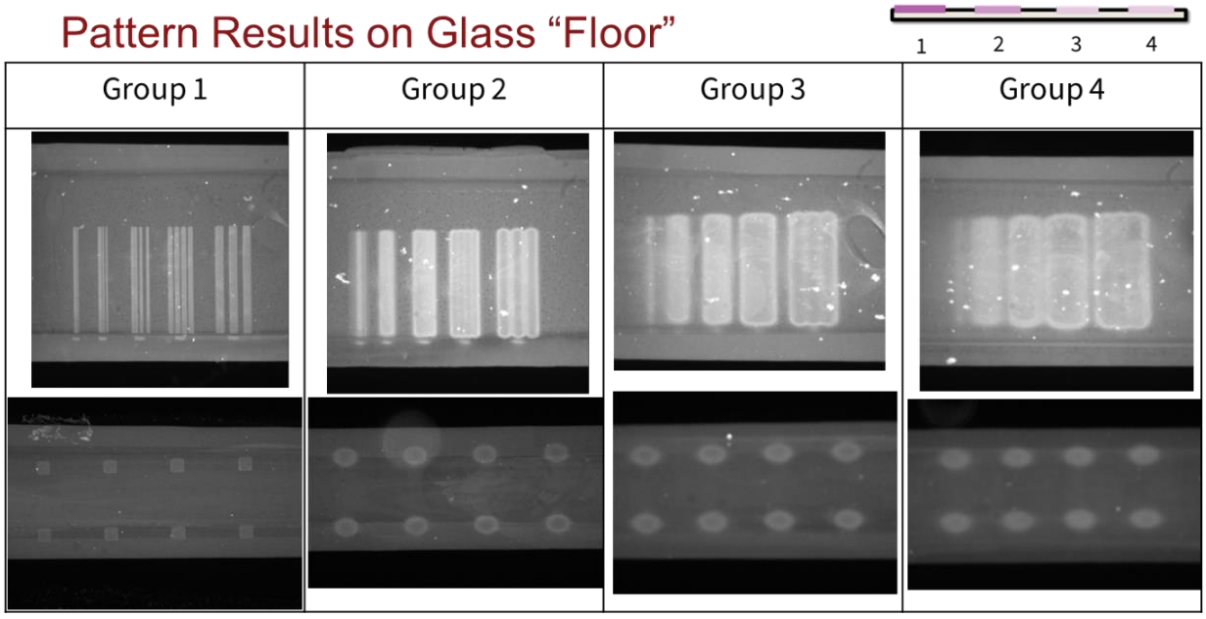


Figure 20: Resulting pattern on bottom glass surface of channel as the focal plane for illumination is incrementally raised. UV Dosage = 1000mJ/mm²

As expected, figure 20 shows that the resulting pattern on the glass floor of the channel has best resolution when the illumination is focused to the corresponding plane (group 1). As the focal plane moves farther away (groups 2 – 4), the pattern becomes increasingly out of focus and blurred.

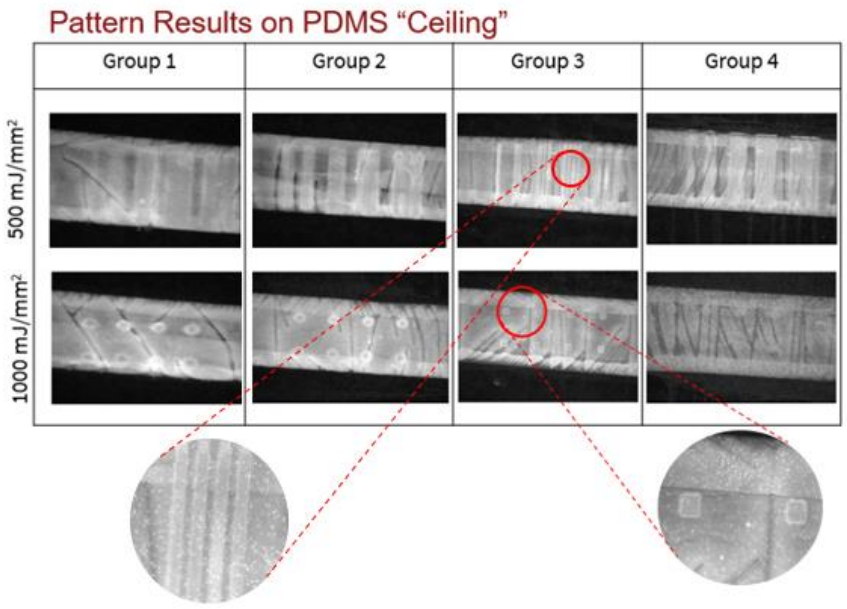


Figure 21: Resulting pattern on top PDMS surface of channel as the focal plane for illumination is incrementally raised

Figure 21 shows patterns generated on the top PDMS surface of the channel. As expected, the highest resolution pattern is obtained when the focal plane is set to the ceiling of the channel (Group 3). NOTE: The high background signal in these patterns was attributed to inadequate passivation, which is discussed in detail in section 8.6 The resolution of the pattern is not affected due to the high background, therefore this experiment was an adequate assessment of the relevant focal planes.

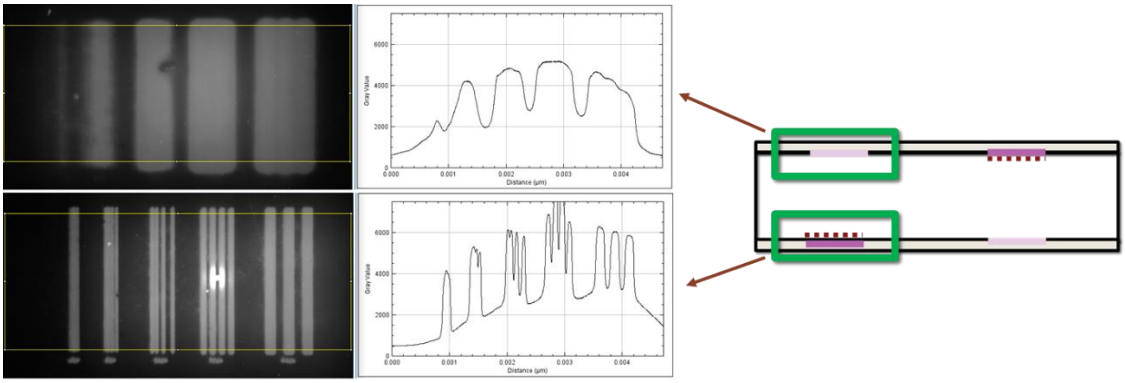


Figure 22: Patterning within 200μm-tall glass channels produces undesired "bleedthrough" pattern on opposing out-of-focus face

As expected, generating an in-focus pattern on the channel floor produces a corresponding out-of-focus pattern on the channel ceiling (Figure 22). The next section discusses a strategy to restrict this undesirable "bleedthrough". For short channels, it may be possible to simultaneously pattern both the faces of the channel by focusing on the midplane of the channel.

Note: Patterning inside channels with PLPP seemed to cause scattering of light as compared with what is observed while patterning within normal wells, though the effect on pattern quality is currently unclear.

8.4 Using UV Absorbing Dye to Restrict Pattern Formation in Enclosed Spaces

To address the issue of undesired out-of-focus pattern formation within microchannels, we explored methods of blocking UV light from passing through to the upper surface. The approach we developed was to fill the channel with a soluble dye which has high absorbance at the 375 nm PRIMO wavelengths.

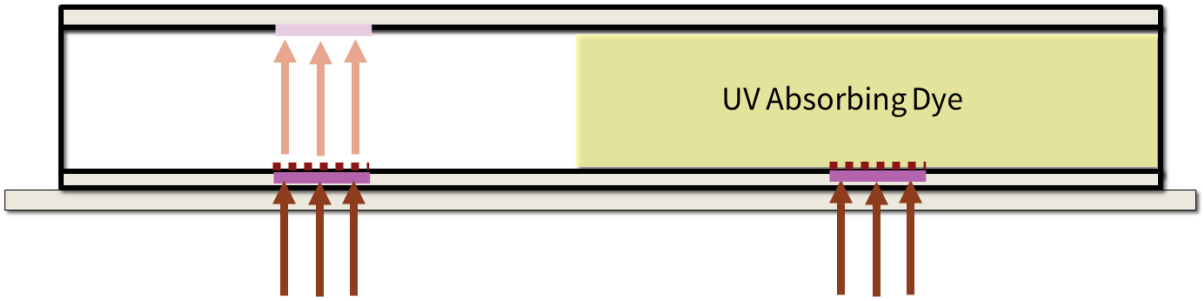


Figure 23: Experimental setup for patterning with dye

For these preliminary experiments, we selected water soluble yellow food dye, which has a peak absorbance around 420 nm (see figure 32). This dye was mixed at different ratios with the PLPP photoiniator to determine the minimum dye concentration that will allow complete blockage of the UV light during patterning. A minimum concentration of 25% dye by volume diluted in PLPP was required to block the PRIMO laser for a 200 µm tall channel.

8.4.1 Yellow Food Dye Blocks Undesired Pattern Formation

The dye experiment was conducted with two experimental setups, one with the microfluidic channel bottom and top surfaces formed by glass coverslips (see figure 24) and another with the bottom and top surfaces formed by PDMS. Channels were filled halfway with full concentration PLPP as used for standard patterning, and halfway with the PLPP-dye mixture. Matching patterns were exposed in each region of the channel to assess the impact of the dye on bleedthrough to the upper surface.

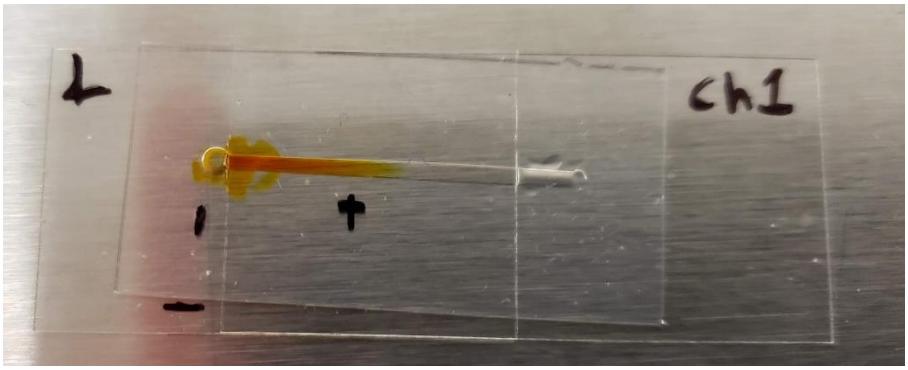


Figure 24: Channel formed with glass coverslip filled halfway with PLPP (right) and a mixture of PLPP and yellow dye (left)

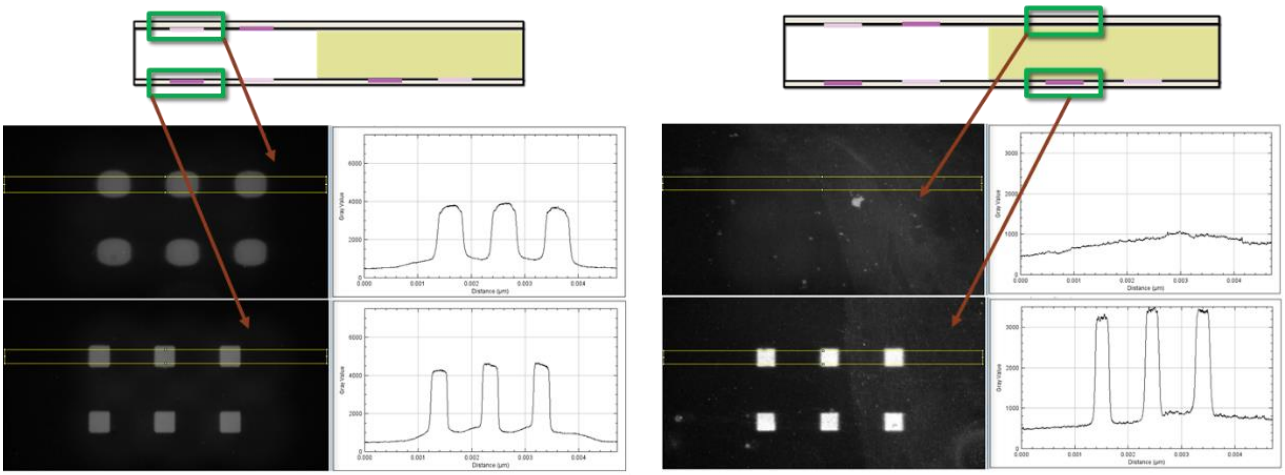


Figure 25: Result for channel formed by PDMS as top and bottom surfaces, 25% dye in PLPP

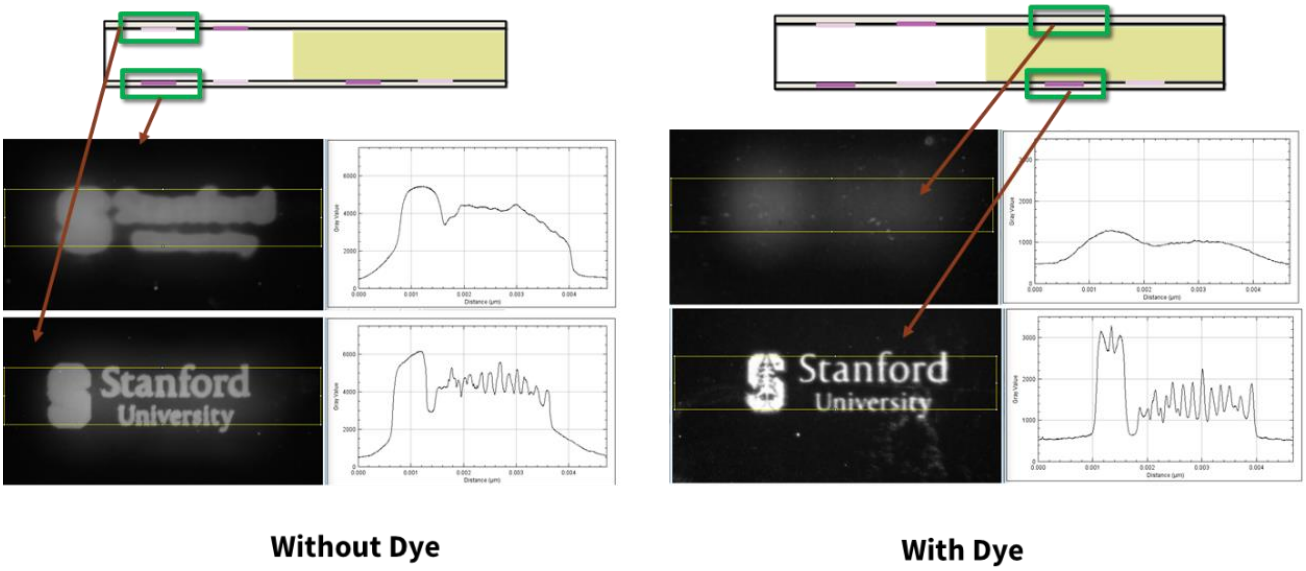


Figure 26: Result for channel formed by PDMS as top and bottom surfaces, 25% dye in PLPP

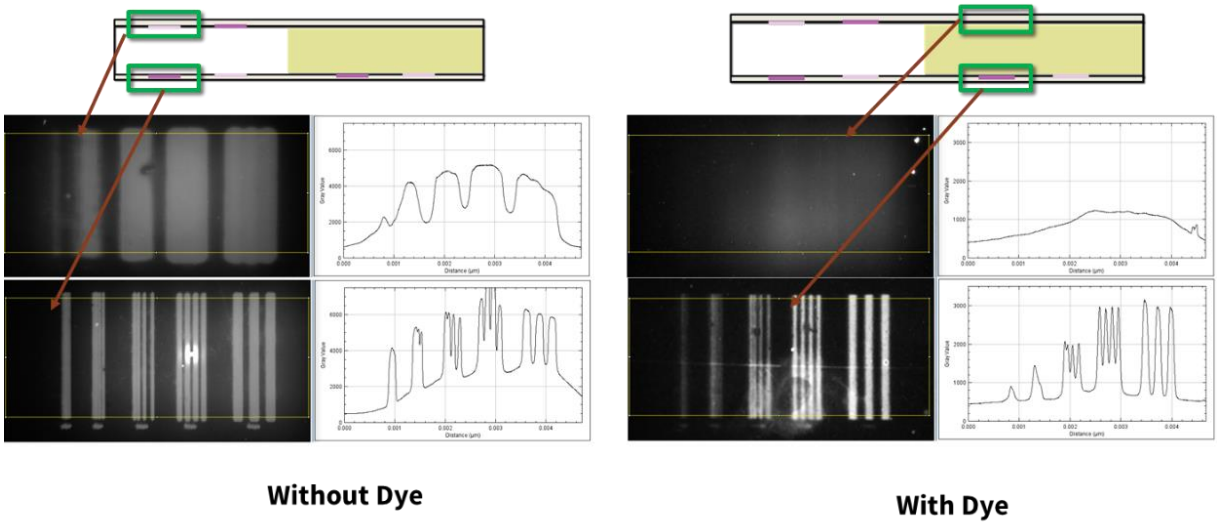


Figure 27: Result for channel formed by PDMS as top and bottom surfaces, 25% dye in PLPP

Figures 25, 26, and 27 demonstrate the ability of dye to block undesired pattern formation. The channel sections without dye clearly show residual pattern formation on the upper channel faces while the sections containing dye are relatively free of bleedthrough. The presence of the dye does not appear to impede the intended pattern generation on the bottom surface of the channel.

As these patterns were imaged through the bottom of intact devices using standard widefield fluorescence microscopy, it seems likely that the background signal on the upper channel surface in the dye regions may actually be lower than what is measured due to out-of-focus contributions. Using confocal microscopy or splitting the channels apart to image the two faces separately could confirm this suspicion. However, we encountered some difficulties with splitting the channels apart to image the two faces separately. Regardless, the prevention of pattern generation appears sufficient for our purposes.

The integration of a UV absorbing component into the PRIMO workflow may also have the added benefit of allow for longer exposure times while conserving PLPP, as well as potentially reducing the impact of stray reflections inside of three dimensional microstructures or channels.

8.4.2 Pilot Study: Patterning Opposing Surfaces of a Microchannel with UV Absorbing Dye

With a strategy established for localizing pattern formation to the bottom surface of a microchannel while preventing UV exposure to the upper surface, our next step was to target pattern generation on the opposing face. This may be used to generate identical protein microarrays on opposing faces while avoiding bleedthrough and maintaining sharp pattern resolution. Alternatively, the ability to independently pattern distinct biomolecules on multiple sides of a microstructure could be useful for the study of tissues where cell polarity is crucial for function (e.g. the apical-basal polarity of epithelial layers) or to study migration driven by spatial patterning of biochemical cues (e.g. axon guidance of growing neurons). More broadly, the ability to localize pattern formation to selected surfaces in three-dimensional space can be a powerful tool for rapid and flexible engineering of cellular microenvironments.

As a proof of principle for this approach, we exposed the bottom surface of a glass microchannel using yellow dye diluted into the PLPP photoinitiator as described in previous sections. We then flipped the channels on the microscope stage, readjusted the focal plane, and exposed the original top surface.

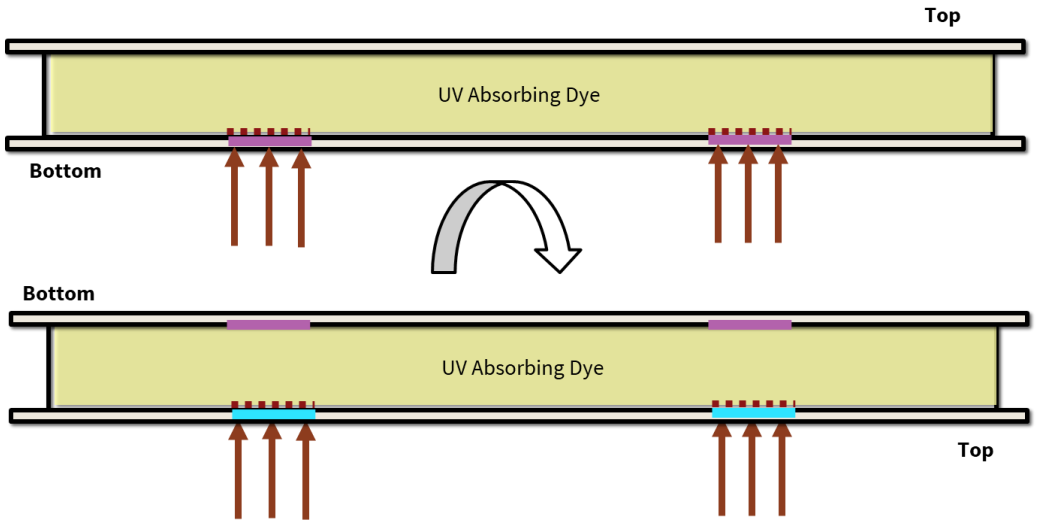


Figure 28: Experimental setup for patterning both faces of the channel

As previously mentioned, one advantage of PRIMO is the ability to use the motorized stage for substrate realignment. This allows for repositioning of patterns between exposure or rinsing steps to specific features of the microstructure or even to previous patterns if a fluorescent biomolecule has already been incubated. In our case, this allowed for repositioning of a patterned array within the microchannel boundaries after flipping the device to pattern on the opposing surface.

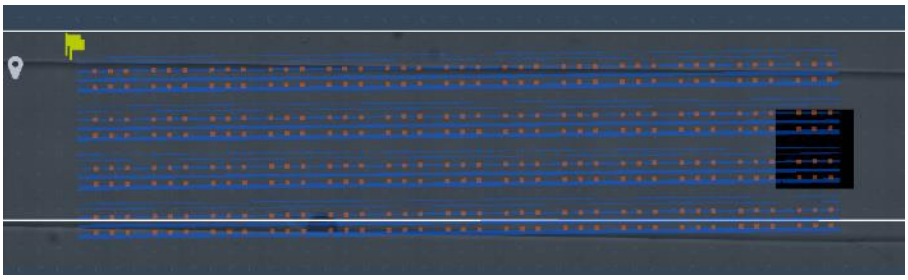


Figure 29: Screenshot of Leonardo software allowing for realignment of overlapping arrays of squares and rectangles

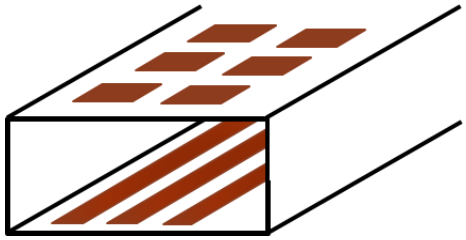


Figure 30: Target result of unique patterns on opposing faces of a microchannel

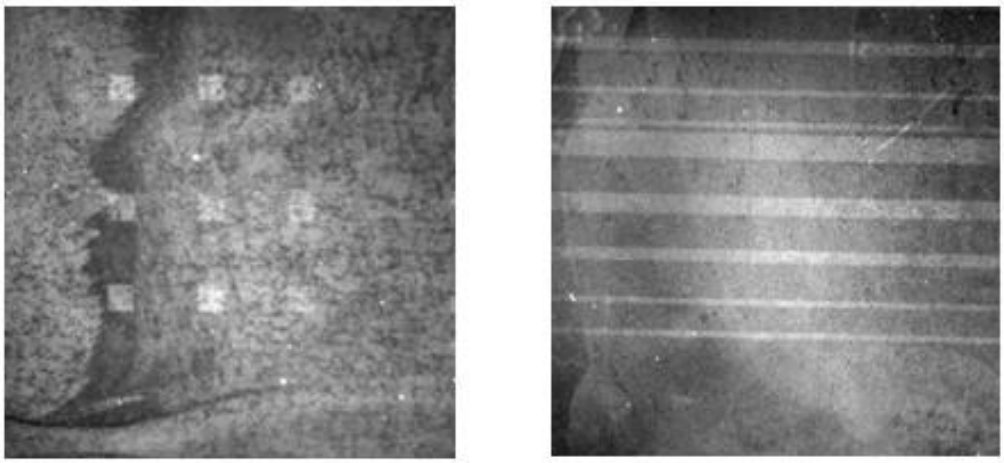


Figure 31: Experimental result

As shown in the figure above, we were able to generate distinct patterns on opposing faces at the same XY stage position within a microchannel. Unfortunately, the high background signal resulting from poor passivation limits analysis of bleedthrough. However, as a proof of principle this result is a promising step towards our end goals.

8.5 Dye Characterization

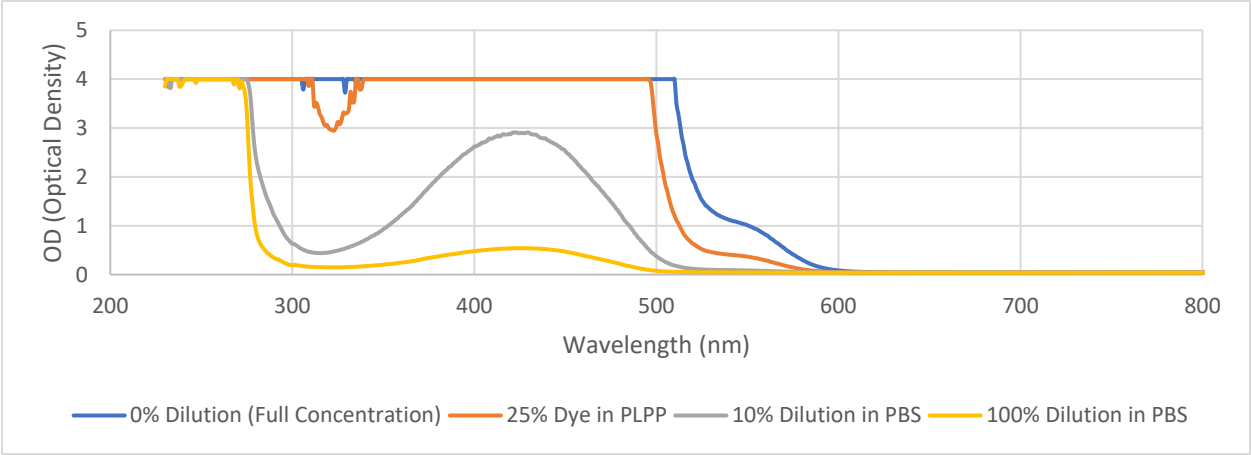


Figure 32: Optical density vs wavelength of the food dye used in the experiment at different dilutions

Optical density (OD) for the yellow dye used in our experiments was measured as a function of wavelength using a Tecan Infinite M1000 Plate Reader. The horizontal line at OD=4 indicates saturation or nearly complete absorption of the corresponding wavelength by the dye. At 25% dilution of dye in PLPP (the concentration used for experiments),the UV laser is completely absorbed by the dye. However, the ability of the dye to block pattern formation will directly depend on the channel height. Dilution of the dye in PBS allows us to observe a peak absorbance at around 420nm.

8.6 Substrate Passivation: Challenges and Recommendations

Many of our experimental results discussed show high background and low signal to noise ratio (SNR) between the desired pattern and the background. Acceptable SNR is between 2 to 10 depending on substrate type, thickness, magnification, NA, camera, UV dose. The main factor contributing to the high background signals observed is likely inadequate passivation of the surface, which causes the protein to also adhere to areas which have not been exposed. The following factors may help to improve the passivation and improve the SNR of the pattern:

- Whenever possible, fresh reagents should be used for each experiment.
- Be sure to properly store all reagents at their specified storage temperatures and prepare aliquots to avoid frequent freeze/rethaw cycles.
- Channels or wells should not be allowed to dry out after PEG-SVA step, particularly if using PBS as a storage buffer, because the crystals formed with evaporation will stretch the PEG chains.
- Plasma treatment/general cleanliness of the surface improves PLL adsorption and ultimately improves PEG-SVA passivation.
- Rinsing several times with PBS or DI water without drying the surface will generally improve SNR.
- The PEG-SVA may have a limited lifetime (i.e. it may lose passivation properties after several days). Therefore we recommend that patterning should be completed within 1-2 days of substrate preparation. The exact lifetime of PEG-SVA was not determined but patterning within 24 hours showed acceptable results.

9. Conclusions

9.1 Summary of Contributions

- Fall:
    - Explored limitations of PRIMO photopatterning on 2.5D PDMS substrates
    - Key considerations: substrate thickness, minimum desired feature size, depth of field, dosage
- Winter
    - Established protocol for selective patterning directly within confined spaces (e.g. microfluidic channels) using a UV absorbing dye

9.2 Future Possibilities

While the soluble yellow dye provided a convenient proof of concept for our experiments and performed well enough to illustrate the concept, there are likely alternative compounds which may be more well-characterized and exhibit higher UV absorption at the 375 nm PRIMO wavelength. Future users might consider Coumarin 1, Sudan I, and suspensions of titanium oxide microparticles or polystyrene beads as starting points. An entirely different approach from using a soluble UV absorber could be to treat distinct surfaces of a device in such a way that the anti-fouling coating in selected regions is not degraded even when exposed to UV.

Another intriguing direction is the integration of a UV absorbing component directly into the PDMS substrate. This could be used to localize pattern formation to opposing faces of a membrane (e.g. for organ on chip applications in which fabrication steps prevent microcontact printing methods).

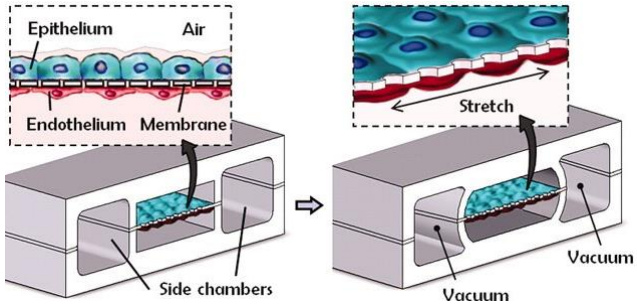


Figure 33: Microfluidic organ-on-chip mimicking the alveolar-capillary boundary in a human lung, with distinct cell layers on opposing faces of a porous membrane [7].

We hope that PRIMO users at SNF and elsewhere can build off the results summarized in this report for photopatterning inside complex custom 3D microfluidic devices.

10. Budget and Team Member Contributions

Item	Amount
Budget Allocated	$10,000
Materials/Supplies Cost	$1,337
Equipment Usage Cost	$811
Training Cost	$480
Total Expenses	$2,628

Team Member Contributions: Chinmay and Frank contributed equally to this project, including the experiments, analysis, presentations, and preparation of reports.

11. Additional Resources

- For updated PRIMO protocols: contact Alveole After-Sales Support: https://www.alveolelab.com/contact-after-sales/
- For a review of the many additional ways to passivate PDMS: Zhang et al., J. Med. Biol. Eng., 2015
- For tutorial videos from Alveole: https://www.alveolelab.com/resources-support-center/tutorial-videos/

12. References

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