Standard Operating Procedure for Protein Photopatterning in Enclosed Channels with the Alveole PRIMO Tool

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2. SOP: PRIMO Patterning in Enclosed Channels

Objective: This SOP is intended to provide additional instructions for photopatterning with PRIMO directly within simple microfluidic channels. New PRIMO users should first read the PRIMO Patterning on PDMS Surfaces SOP. Further explanation of the approach for patterning in channels can be found in our E241 Winter 2019 Final Report, posted on the SNF website.

2.1 Channel Fabrication

2.1.1 Additional Materials & Equipment:
- UV absorbing dye (e.g. yellow food dye)
- Silicone/PDMS sheet of known thickness
- Silhouette Cameo® cutting machine

2.1.2 Stencil Preparation
A quick and simple way to fabricate microchannels for patterning experiments is to sandwich a thin PDMS stencil between two planar substrates (e.g. glass coverslips). To create stencils, we used the Cameo Silhouette tool located next to the PRIMO. If using the same tool, first design a pattern in the free Silhouette Studio software. The dimensions of this pattern will determine the channel length and width, while the thickness of the material sheet to be cut will determine the channel height. Users may find it convenient to set the overall width of the stencil to slightly less than the width of your underlying glass slide or PDMS substrate for alignment purposes. If using a standard 22x22 mm square glass coverslip, design the channel length accordingly such that the outlet ports will not be blocked. We found that designing a large inlet hole and smaller outlet facilitated fluid transfer in later steps. In designing the channel width, keep in mind that the size of the DMD projection is roughly 300 x 500 μm. In general, a wider channel may help to avoid edge effects impacting patterns, such as leakage of reagents into between the stencil and substrate. Once you have finalized a design, you may wish to cut multiple stencils at once for future use.

The following steps summarize the process to use the Cameo Silhouette tool:

1. Open Silhouette Studio.
2. Turn on machine by using the button at the right side.

Figure 1: Silhouette Cameo® cutting machine and "sticky mat" for holding PDMS sheets in place.

3. Define artboard shape in the software.
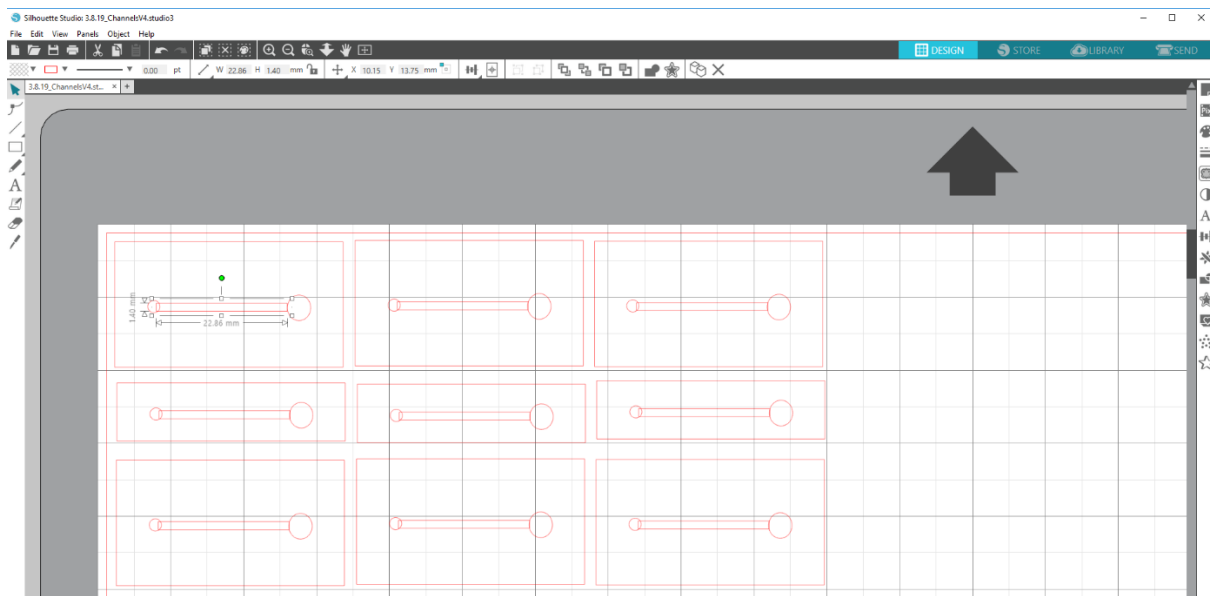4. In "Design" tab draw your design and then save.

Figure 2: Example of a stencil designed in Silhouette Studio for use in microchannels.

5. Go to the "Send" tab.
6. Use the red circle at the top called "tool 1".
7. Settings: PDMS_oneside, cut, ratchet blade, cut. Black dial should be on 5; Speed = 1, Force = 7; Passes = 1.
8. Remove blue sheet from sticky mat. Cut pieces of PDMS, remove one side and place PDMS face up.
9. Load by placing mat up against white cylinders and press "Load" on the Silhouette screen.
10. On bottom of the software screen press "Send".

11. Press "Unload' on the Silhouette screen when complete.
12. Return the plastic backing to the PDMS to keep it clean.

Note: these cutting steps were developed by other SNF users and are also located under the top panel of the device. You may need to make slight modifications to the dial, speed or force settings to achieve desired results.

2.1.3   Channel Preparation

Prepare clean glass coverslips or PDMS substrates on top of coverslips as described in the PRIMO Patterning on PDMS Surfaces SOP.

Plasma treat the substrates which will form the floor and ceiling of the eventual microchannel. This may be optional provided that your substrates are sufficiently clean, but plasma treatment will increase hydrophilicity of the surfaces and make channel wetting easier with the added potential of improving the quality of poly-l-lysine and PEG adsorption and ultimately the passivation effectiveness.

We recommend cleaning stencils with 70% ethanol and drying prior to use. After plasma treatment of the glass or PDMS substrates, use forceps to carefully place the stencil onto the bottom substrate before adding the upper coverslip or PDMS slab in place to sandwich the stencil. It may be necessary to press gently on the upper substrate to ensure a secure bond.

When placing stencils on the coverslip or PDMS surface, it can be difficult to avoid distortion of the channel shape since the thin PDMS is quite flexible. One solution can be to leave the plastic backing on one side of the stencil during application and only remove it once the stencil has securely stuck in place. Wider channels will also provide more margin for error (i.e. avoid total channel blockage due to distortion).

2.2   Substrate Preparation and Reagent Transfer in Microchannels

We have found that a simple and repeatable method for flushing of reagents through these microchannels is by capillary filling and wicking rather than pressure-driven flow.

Place a large droplet of the reagent (e.g. PBS) at the channel inlet and then place the tip of a kimwipe at the outlet to wick liquid through. Try to avoid creating bubbles in the channel as these can be difficult to remove and interfere with proper rinsing and incubation.

Repeat this process for each incubation and rinsing step of the standard PRIMO protocol for patterning on PDMS (i.e. PLL, HEPES rinse, PEG-SVA, PBS rinse, PLPP, PBS rinse, protein solution, rinse). For less expensive reagents and for the rinses especially, we recommended flushing through with several hundred microliters to ensure complete coating of the channel. The wicking method is a little bit trickier for reagents that must be conserved (e.g. PLPP), but it is possible to go slow if you are careful about how saturated the Kimwipe is and how much contact and pressure is applied to the outlet.

As previously mentioned, it is essential to avoid drying of the channels after incubating the PEG-SVA. When using PBS, the crystals formed with evaporation will stretch the PEG chains and interfere with passivation. One strategy for maintaining hydration during short term sample storage is to place the device in a petri dish along with a Kimwipe soaked in PBS/water and wrap the dish with parafilm.

2.3 Calibration

Perform calibration as described in the PRIMO Patterning on PDMS Surfaces SOP. Calibration on a substrate matching the lower surface of the channel should be sufficient for patterning on both surfaces. It is helpful to plan ahead and think about what focus planes you will need to identify to perform the calibration and patterning before making channels, in case this requires physical scoring of the glass or PDMS or addition of sharpie marks at specific locations (such as between the stencil and glass).

2.4 Patterning

Follow the steps described in the PRIMO Patterning on PDMS Surfaces SOP. To restrict pattern formation to the bottom surface of the channel, a soluble UV absorbing dye can be mixed with the PLPP. We have found that a 25% mixture by volume of yellow food dye in PLPP will provide sufficient absorption of the 375 nm PRIMO wavelength to block undesired patterning on the surface of a 200 μm tall channel.

Some considerations for patterning channels:

- Thin coverslips may sag in the middle (on the order of 100 μm) due to the weight of the device layers. You will need to take this into account when setting the focus and particularly for patterning large arrays within channels.
- Be careful about drying of the PLPP/dye mixture during long exposures (e.g. large patterns)
- We advise protecting devices from ambient light exposure while the channel is filled with PLPP

2.5 Protein Incubation and Pattern Visualization

- Incubate with a fluorescent protein solution and visualize patterns as described in the PRIMO Patterning on PDMS Surfaces SOP.
- We have had the best results for signal to noise ratio after rinsing with several hundred microliters of PBS, followed by several hundred microliters of DI water

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