PRIMO PROTOCOL: Patterning on 96 well plates

Last update 10.12.2018

This protocol is a guideline and you might need to optimize it for each different application. It has been tested for Cellvis glass bottom (#1.5) well plates.

1. Reagents and equipment

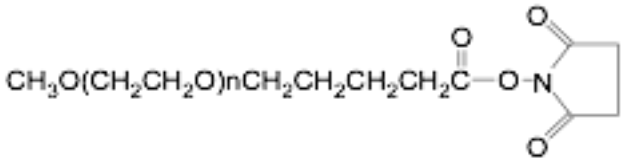
Equipment

- Plasma cleaner
- PRIMO® setup
- 96 well plates

Reagents

- mPEG-SVA (mPEG-Succinimidyl Valerate, MW 5 000, *Laysan bio*)
- Poly-L-Lysine (PLL, *Merck/ Sigma-Aldrich*, ref: P8920)
- HEPES 8,3 < pH < 8,5 at 0,1 M PLPP Gel
- Surfactant Mix
- EtOH
- H_2O mQ
- Protein solution of interest

2. Passivation protocol



mPEG-SVA is a PEG (5kDa) coupled with a Succinimidyl Valerate (SVA) ester. This ester is used for PEGylation of primary amine.

The strategy for glass passivation with PEG-SVA is the following:
1) Coating of the substrate with polylysine (PLL)
2) Coupling of the amines of the polylysine with the SVA ester

- Plasma clean the plate for 1 min.
- Add 60 µl of PLL solution (100 $\mu g.ml^{-1}$ in MQ water) in each well.
- Rinse 3 times with DI water. Remove as much as possible of liquid.
- Make a solution of PEG-SVA in 0,1M HEPES with 8,3 < pH < 8,5 at 70 $mg.ml^{-1}$. If the passivation is not strong enough, you can increase the concentration up to 100 $mg.ml^{-1}$.

As the half-life of the SVA ester is 10 minutes at pH 8,5, the solution must be prepared just before use.

- Wash profusely with MQ water.
- Remove as much as possible of water. You can dry the plate with air flow and keep it for several weeks at 4°C.

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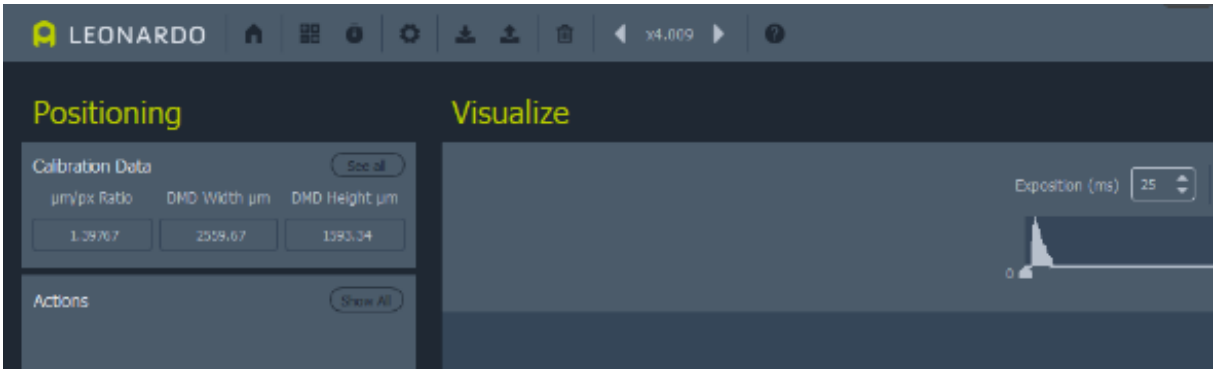
3. Photopatterning with PRIMO: PLPP gel

- ⇨ Mix 2.155 mL of DI water, 44 µl of PLPP gel and 12 µl of Surfactant Mix in a reagent reservoir.
- ⇨ Deposit 20 µl of the mix in each well. Note that the total solution quantity is calculated for 110 wells to accommodate for loss and pipetting difficulties. If you only plan to use few wells, change the quantity accordingly.
- ⇨ Let the solution dry. To speed up the process we can put the plate on a hot plate (90°C max). A transparent gel of photoinitiator will form.

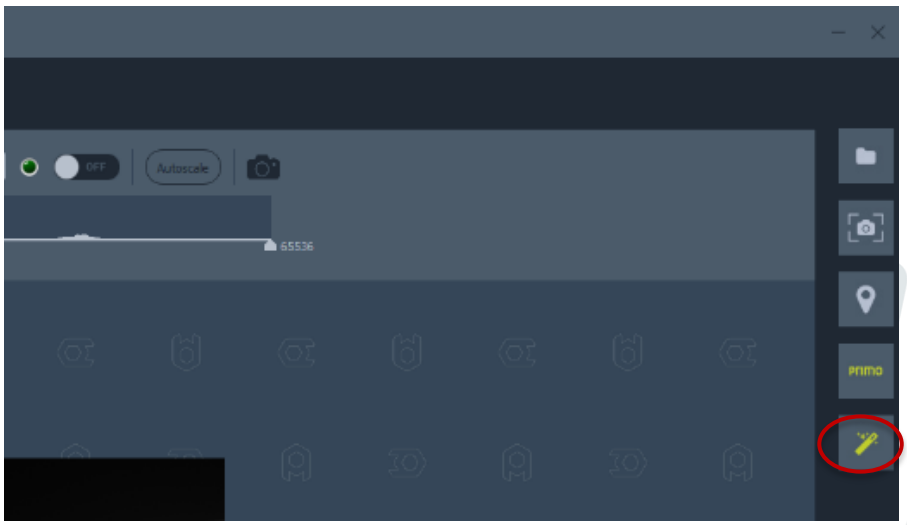
4. Photopatterning with PRIMO: HCS wizard

The HCS wizard on Leonardo allows you to detect the position of your wells and to position automatically the patterns at the center of each well. It's better to detect the well with a low magnification objective (4x) and to make the patterning with a higher magnification objective (20x) for homogeneity reason.

- Place the plate on the microscope holder.
- Select the 4x objective on Leonardo software. Turn the objective turret so that 4x objective is on position.



- Use the HCS wizard to automatically detect and fill the wells.



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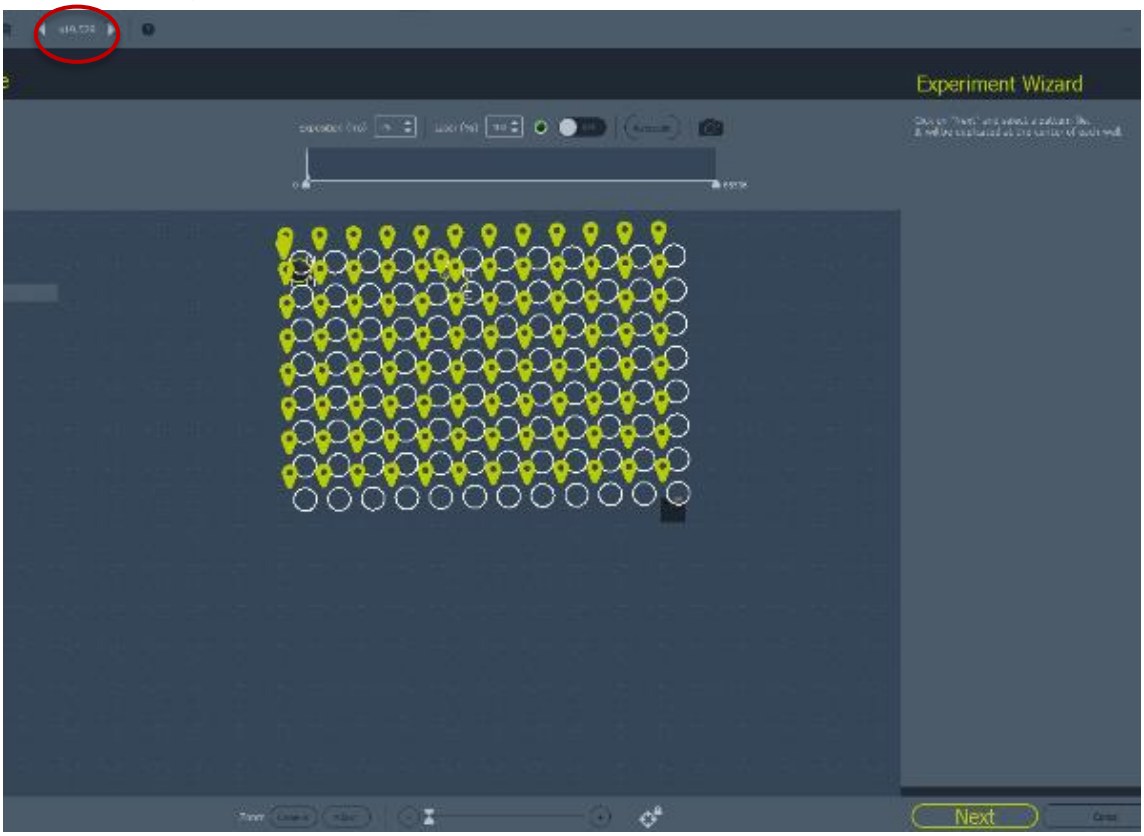
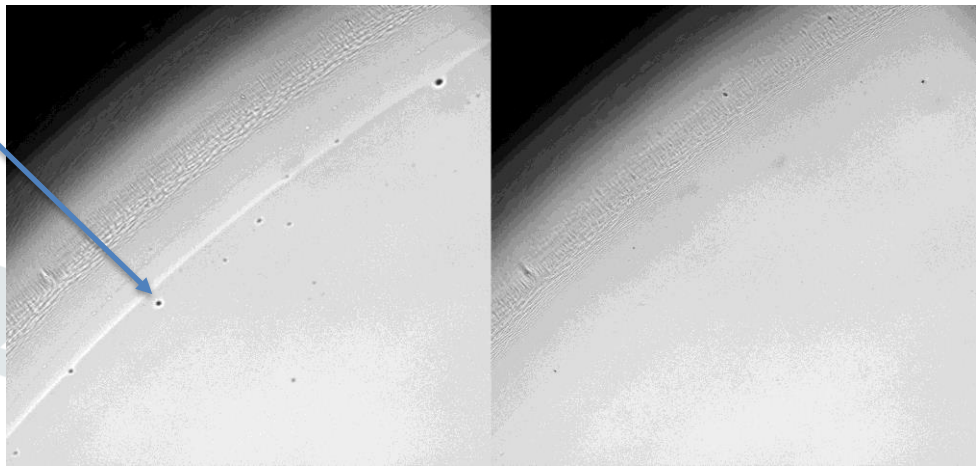
- Follow the instructions of the wizard to detect the well.
- Before to choose your patterns, change the magnification of the objective to 20x. Turn the objective turret so that 20x objective is on position.
- The typical UV dose is 30 mJ.mm^{-2} for PRIMO (laser 375 nm) and 15 mJ.mm^{-2} or PRIMO 2 (LED 365 nm).



- Focus on the surface of the well. You can focus on the PLPP gel:

First option: focus at the border of the well. You can focus on the gel which is inhomogeneous on the borders

Edge of the gel



Unfocused

Focused

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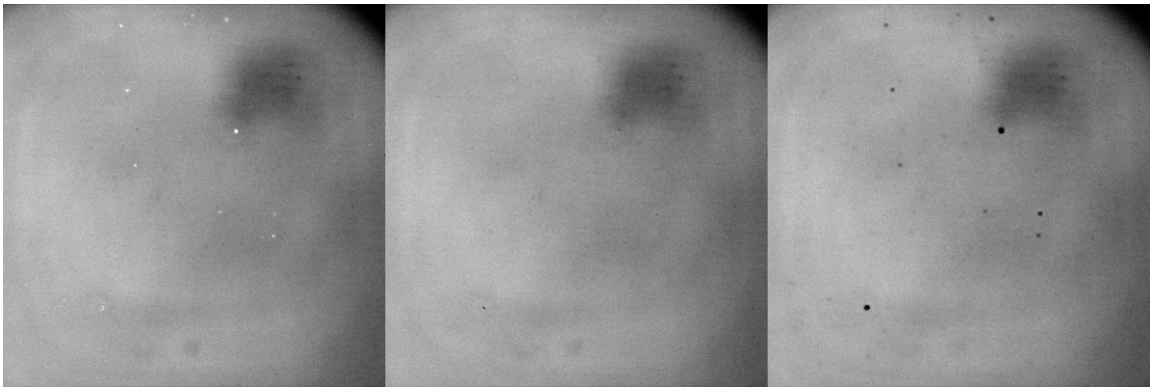
Second option: use dusts inside the gel. Inside the gel dusts are white when the focal plan is below the surface of the gel and black when it is above the surface of the gel. You need to focus at the middle point.



Below Focus | Focused | Above Focus

3. Protein incubation

- Rinse the wells 4 times with PBS
- Add 40 µl of your protein in its dedicated buffer (concentration between 10 and 100 $\mu g.ml^{-1}$) in each well. Incubate for 15 minutes
- Rinse 4 times with PBS. Be careful not to dry or dewet the center of the well
- The patterned plate can be kept for few weeks: rinse each well with DI water and dry it. Keep it at 4°C

5. Cell seeding

This step is really cell type dependent. You will have to adapt it, but here is how we do.

- Rinse your sample with media without dewetting the substrate.
- Add 1.10^4 to 1.10^5 cells per cm^2.
- Carefully put your sample in the incubator and let them adhere for 15 min to 1 hour (or more) depending on your cell type.
- Rinse gently with cell culture media to remove the non-adherent cells.
- If cells are attaching outside the patterns, wait one more hour and pipet over the cells until the one outside of the pattern detach.

6. Related publications

Application note:

- *High-throughput micropatterning on 96 well plates using PRIMO contactless photopatterning system*

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