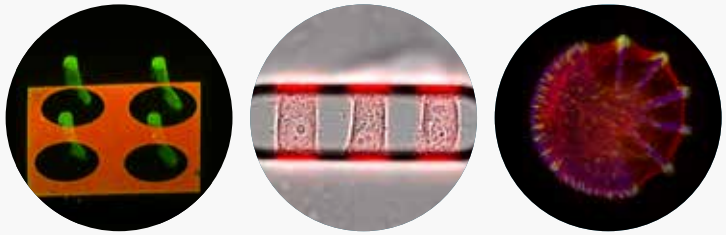
primo

Bioengineering Custom Microenvironments

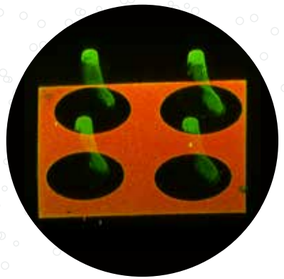


alvéole

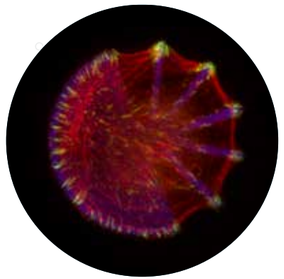
Bioengineering
Custom Cell Microenvironments *in vitro*

One of the challenges confronting cell biologists *in vitro* is to work with **controlled and reproducible microenvironments** to more efficiently study living cells and model diseases.

PRIMO contactless and maskless photopatterning platform allows to engineer custom *in vitro* microenvironments and **fine-tune their mechanical and biochemical properties**, at the micrometer scale with high flexibility and reproducibility.



Top and bottom of PDMS micro-pillars (100 µm high, spaced by 100 µm) patterned with two different biomolecules.



Embryonic fibroblasts from vimentin knockout mice on fibronectin + fibrinogen-A647 (blue) micropattern, actin (red) and focal adhesions (green). Courtesy of A.J. Jimenez and B. Vianay, Physics of cytoskeleton & Morphogenesis lab

Benefits

Time saving & independence

Rapidly optimize your experimental conditions yourself.

High Flexibility

Download any images you want to structure and/or functionalize your substrate.

Versatility

Use your regular cell culture substrates: flat or microstructured, stiff or soft.

We are currently particularly interested in determining the role of the biophysical environment in the establishment of apico-basal polarity in mammary gland cells and in liver cells. The use of PRIMO in this context proved absolutely essential since it allowed us to create artificial microniches in 3D where we could control up to 150 combinations of environmental cues.

Virgile Viasnoff
Associate Professor at MechanoBiology Institute - National University of Singapore, and Director of Research at CNRS

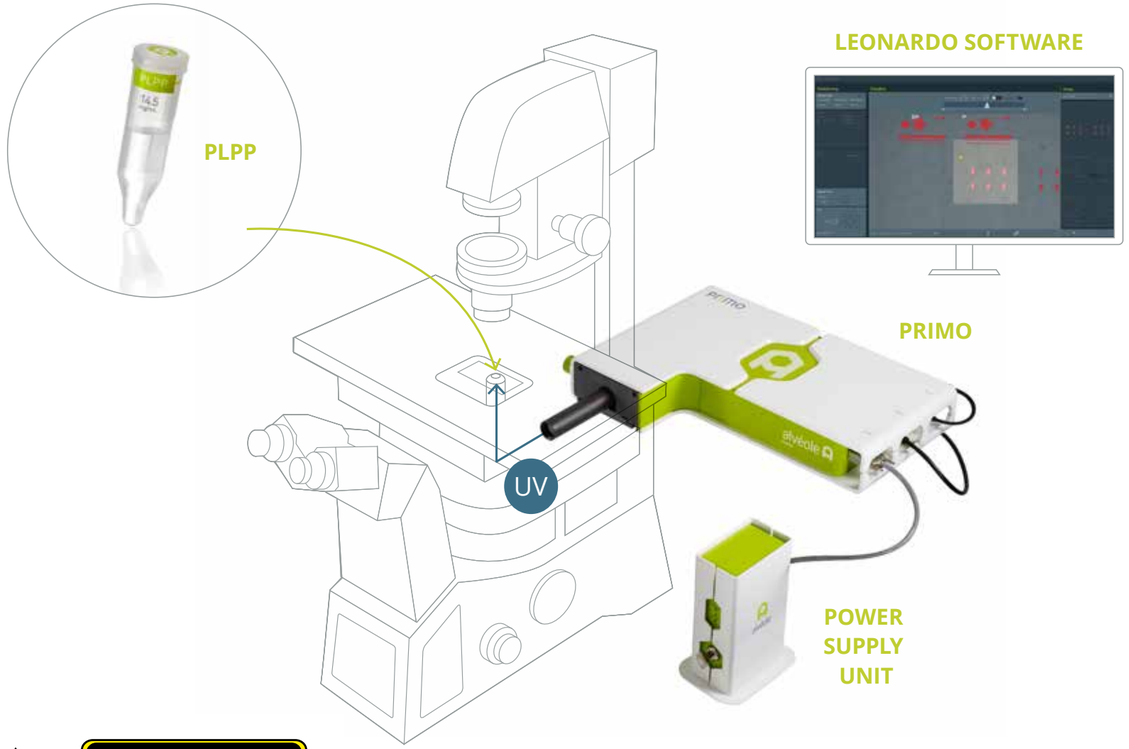
Bioengineering made easy

01 PATTERN DESIGN
Images are drawn in order to create microstructures and/or biomolecule micropatterns on the substrate (compatible with all standard cell culture substrate).

02 SUBSTRATE STRUCTURATION
Photopolymerization
(UV light, λ=375nm):
• Microfabrication with photoresist
• Hydrogel microstructuration

03 SUBSTRATE FUNCTIONALIZATION
Protein micropatterning in 2D or 3D:
1- Anti-fouling coating
2- Addition of PLPP photoinitiator and UV illumination (λ=375nm)
3- Protein adsorption on illuminated areas only.

04 CELL EXPERIMENT
Cells are seeded onto the custom *in vitro* microenvironment. They adhere to the protein micropatterns and adapt to the custom biochemistry, topography and stiffness of the substrate.



Unrivalled performance

GRADIENTS

256

gray levels

MULTI-PROTEIN

3

depending on experimental conditions

Range of 10+ proteins used daily by our users

HIGH RESOLUTION

1.2µm

over the entire illuminated field*

**Approximately 500x300µm, 20x objective.*

ALIGNMENT

on microstructures* or micropatterns

**Automatic detection and patterns positioning*

COMPATIBLE

standard substrates*

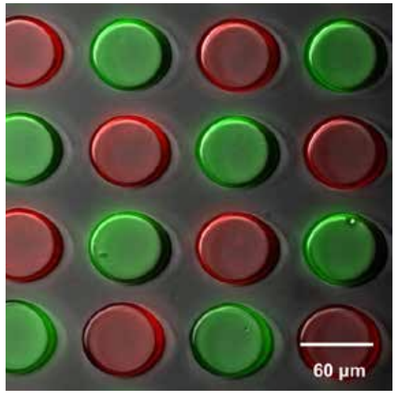
**stiff or soft, flat or microstructured.*

FAST

30sec

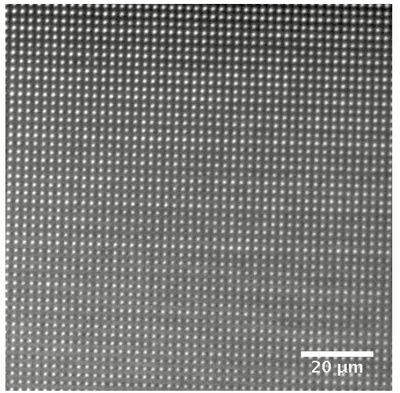
for a full field pattern*

**Approximately 500x300µm, 20x objective.*



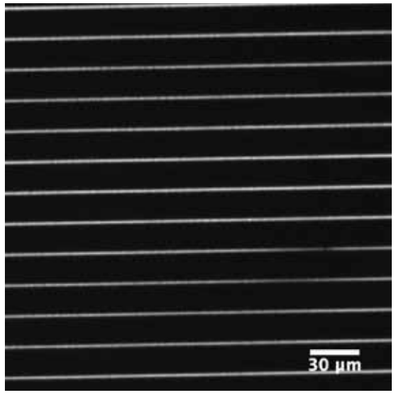
ALIGNMENT & MULTI-PROTEIN:

Sequential photopatterning of Fibrinogen -A488 in green and Protein A-A647 in red onto PDMS micropillars microfabricated with PRIMO.



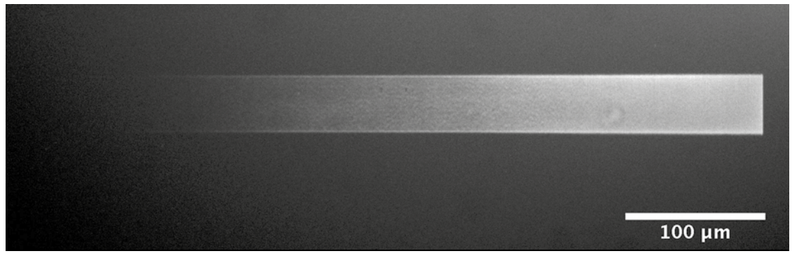
HIGH RESOLUTION:

Epifluorescence microscopy image of 1,5µm dots (spaced by 1,5µm) of ProteinA-488 on PDMS.



HIGH RESOLUTION:

Epifluorescence microscopy image of 2 µm horizontal lines of ProteinA-488 on glass.

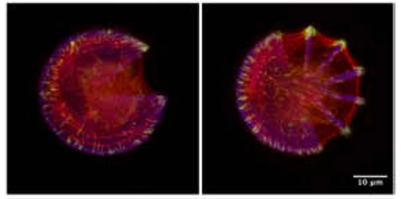


GRADIENTS:

Epifluorescence microscopy image of a gradient of Fibrinogen-A488 on a glass coverslip.

Applications

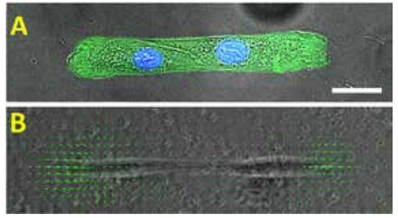
Cell Adhesion Control



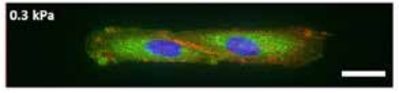
Embryonic fibroblasts from vimentin knockout mice on a fibronectin + fibrinogen-A647 (blue) pattern, actin labelled with phalloidin-A555 (red) and focal adhesions revealed via Anti-Paxillin Antibodies + secondary Antibodies coupled to A488 (green). Courtesy of A.J. Jimenez and B. Vianay, Physics of cytoskeleton & Morphogenesis lab.

Mouse teratocarcinoma cells plated on fibronectin micropatterns.

Force Measurement

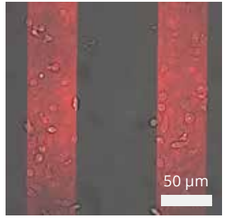


Top: two airway smooth muscle (ASM) cell ensemble on a rectangular gelatin micropattern (green) done with PRIMO on Nusil gel, scale bar = 25µm. Bottom: displacement field (green arrows) calculated from the movement of 1µm beads spin coated on the gel surface.
S. R. Polio, BioRxiv, 2018 - doi.org/10.1101/402842

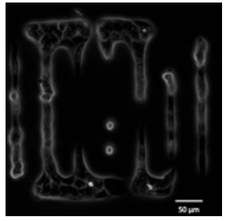


ASM-cells form stable cell-cell junctions on soft substrate and the boundary is marked by β-catenin stain.
S. R. Polio, BioRxiv, 2018 - doi.org/10.1101/402842

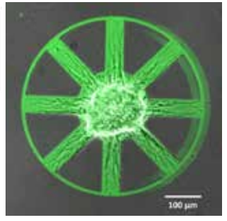
Cell Migration



Primary human T lymphocytes on specific adhesion strips. Courtesy of O. Theodoly.

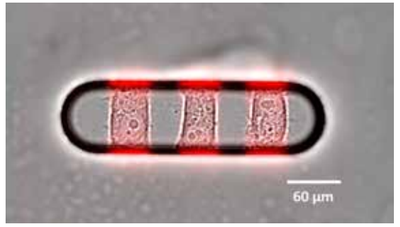


Phase contrast imaging of MDCK cells on a complex micropattern of fibronectin.



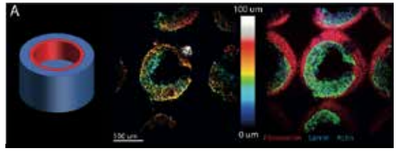
Chicken brain explant at the center of a wheel pattern of laminin-alexa488 (green). Courtesy of H. Ducuing, R. Moore, Y. Lecomte, P.-O. Strale and V. Studer.

3D Cell Confinement



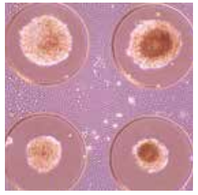
3 hepatocytes HepG2 adhering on patterns of fibronectin on the sides and the bottom of a micro-well. Courtesy of C. Stoecklin and V. Viasnoff.

3D Cell Culture

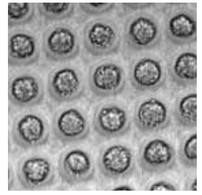


Left panel: scheme of topographically (blue) and chemically (red) photopatterned hydrogels. Middle panel: COS-7 cells seeded on the gel (Z-scale). Right panel: patterned fibronectin (red), actin cytoskeleton (green) and nuclear envelope (blue).
A. Pasturel et al., BioRxiv, 2018.
doi.org/10.1101/370882

Spheroid Formation

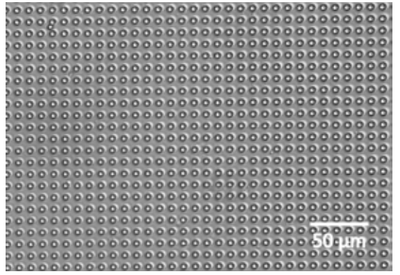


Huh-7 cells forming spheroids on micropatterns of fibrinogen-A488 (wells Ø=500 µm, micropatterns Ø=300 µm).



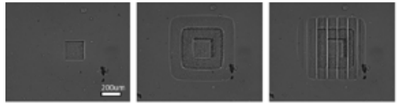
Spheroids of HEK cells in hydrogel microwells (Ø=100 µm, H=175 µm) photopolymerized with PRIMO. Courtesy of A. Pasturel and V. Studer.

Microfabrication



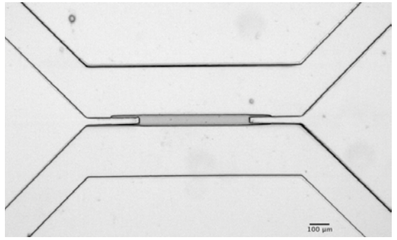
Micro-pillars of 3 µm diameter spaced by 3 µm made by the replica molding of a primary SU8 photoresist master obtained by photolithography with PRIMO.

Hydrogel Structuration



Fabrication with PRIMO of a complex 3D hydrogel structure by sequential potopolymerization of 3 different heights (left and middle image), then sliced by photoscission (right image).
A. Pasturel et al., BioRxiv, 2018.
doi.org/10.1101/370882

Microfluidics



Photopatterning with PRIMO system of pressure-resistant hydrogel-based permeable membrane within PEGDA microfluidic chips. Courtesy of J.-B. Salmon.

A complete bioengineering platform

We have developed complementary products to give you optimized and personalized control over your experimental conditions.

Nomos
Automation
of Experiments

Leonardo
Photopatterning
Software

PRIMO

Stencil
Multi-Well
Solution

PLPP
Photoactivatable
Reagent

alvéole

TAKE CARE OF YOUR CELLS

Alvéole – 30 rue de Campo Formio – 75013 Paris – France

ALVEOLELAB.COM