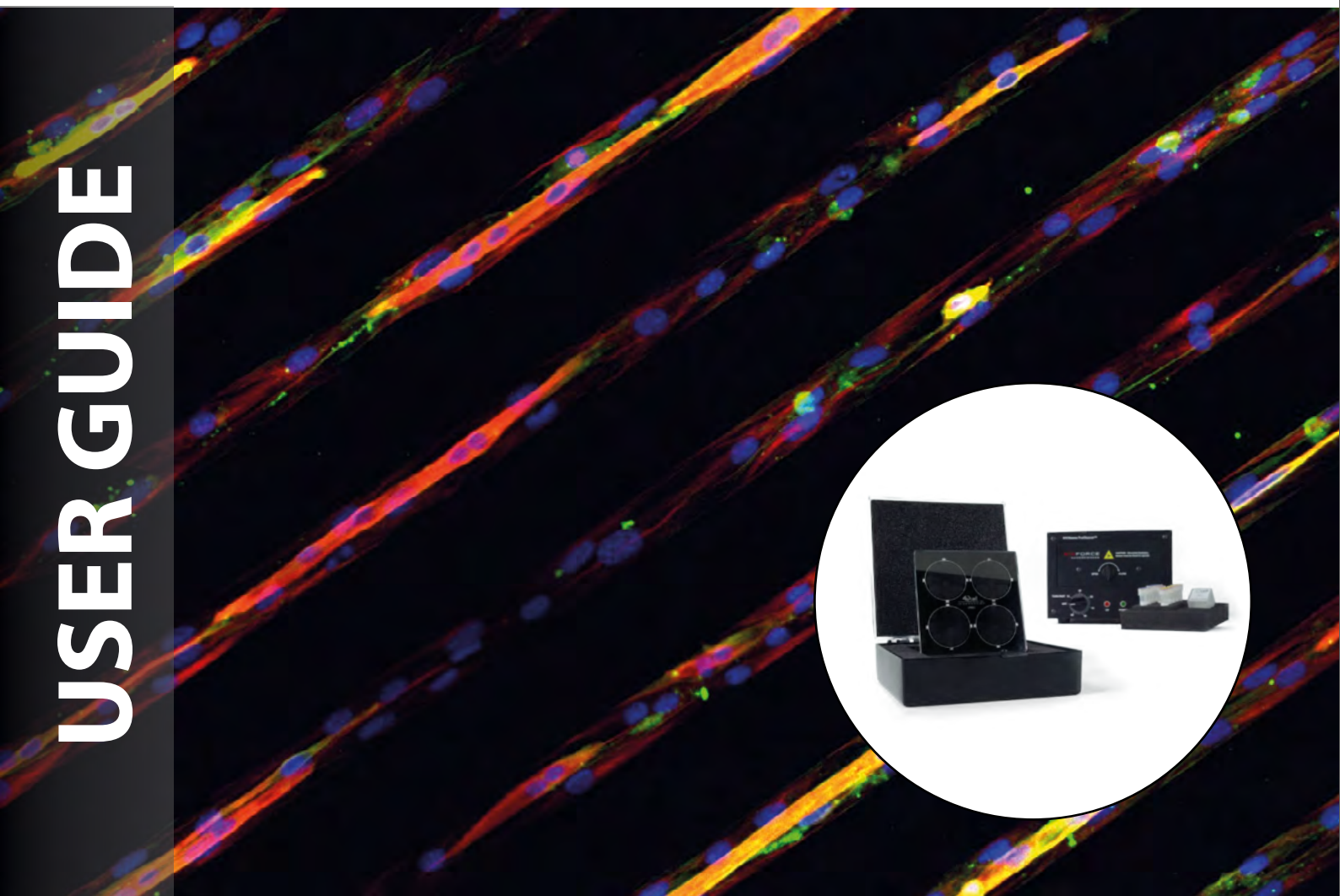
4Dcell
EXPLORE BETTER.

4Dcell micropatterns fabrication kit

User protocol

USER GUIDE



How to use 4Dcell micropatterns fabrication kit

Materials included

Consummable box
- Glass coverslips 24 mm
- Anti adhesive agent
- Extracellular matrix protein
- fluorescent marker

Micropatterning box
- 4Dcell quartz photomask with features
- masker and screw

Micropatterning station
- Deep UV lamp

Materials not included

Ethanol 70%
Kimwipes
Parafilm
Plastic tweezers
Distilled water
Microliter pipette
Millipore H_2O

Optional

> Coating solution *(e.g. fibronectin, collagen, laminin).*
The fibronectin solution provided by 4Dcell has a concentration of 50 µg/mL. It can be adjusted according to the cell type.

> Fluorescent-labelled protein *(e.g. fibrinogen, BSA, collagen, laminin).*
The patterns on the 96-well or 384-well plate can be visualized using fluorescent fibrinogen or other fluorescent protein. The fibrinogen solution provided by 4Dcell has a concentration of 20 µg/mL.

How to use the UV lamp

Manipulate under a hood
Press the button ON. Open the drawer, place your sample and close the drawer. Use the button “TURN PAST” to adjust the activation time of your sample (e.g. for the first manipulation, turn the button less than 10 minutes to activate your slide). Once the time is off, turn the button on “OFF” and open the drawer to take your sample.

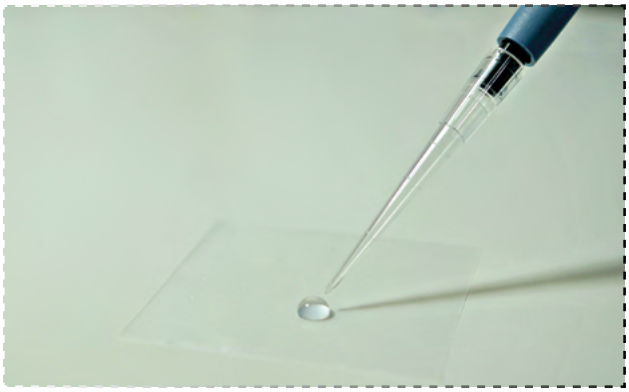
A. Preparing the coverslip

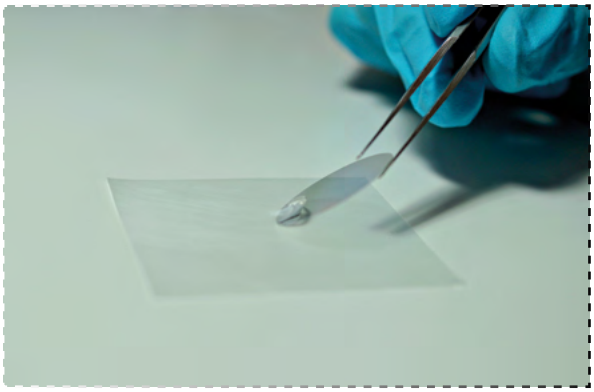
Wear gloves to manipulate the coverslips and work in sterile conditions under the hood

Clean the slides with ethanol 70% and dry them with care using Kimwipes. Slides should be free of microfibers or dust after cleaning.

Using the deep UV lamp, activate the slides for 10 min. You can put the slides on a glass support to avoid contact with bottom of the lamp box. If using plasma cleaner, activate them for 3 minutes at 0.5 Torr. Directly use th slides after activation.

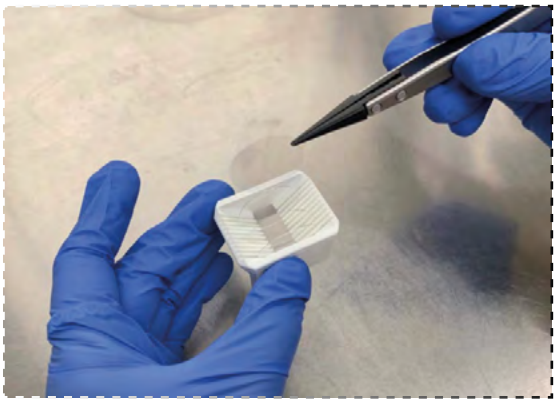
Incubate your slides for 30 min in PEG solution(1) at room temperature: on a flat parafilm add 100µL of PEG solution (yellow tube) and place the activated side of your slide on the drop.





Trick: you can start point B about 15-20 min after this step to synchronize.

After incubation, remove the slide slowly from the parafilm and rinse it with distilled water (using a wash bottle) to remove excess of PEG. Dry the slide with an air gun, eliminating the remaining droplets or let them air dry on a coverslip rack.
Be careful not to touch the treated side of the slide during the rest of the process.



(1) The PEG is very stable and can be kept for month, it can even be resused if you need to use a large volume, just sterile filter it after use.

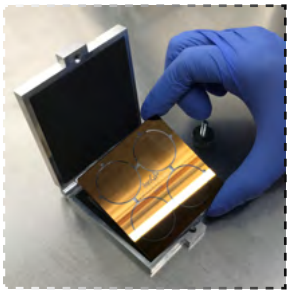
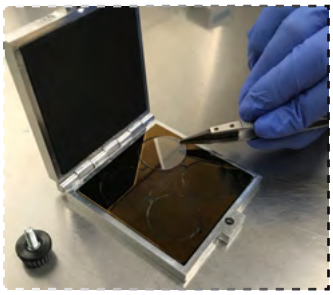
B. UV exposure

Wear gloves to manipulate the mask

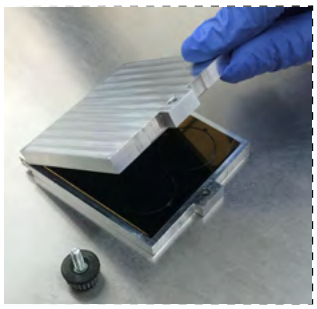
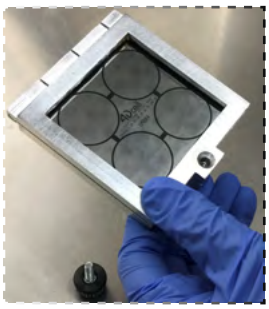
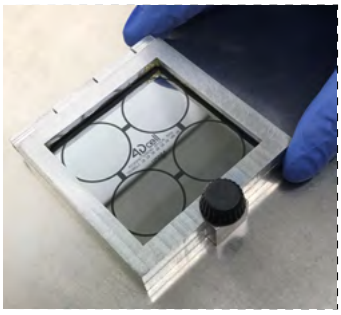
Clean the 4Dcell quartz mask with 70% ethanol or isopropanol and Kimwipes[2].

Once your photomask is cleaned, place it on the Masker with the chromium side (brown side) facing up.

Gently place your slides at their right spot, with coated side on the chromium side.

Close the masker. Holding it tight, turn it upside down once again and lock it with the screw.

Place it inside the UV printer

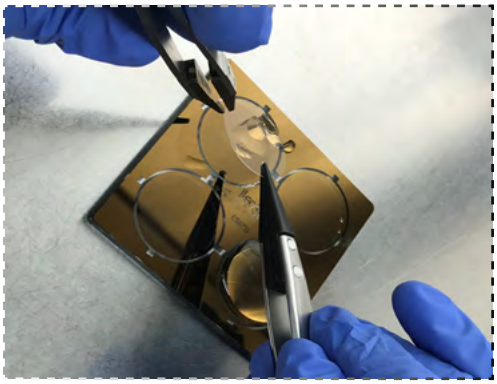
Using the deep UV lamp (below 200 nm), expose the 4Dcell mask during 10 min with the silver side (gray side) facing the lamp. This step will allow the printing of the micropatterns on the coated slides

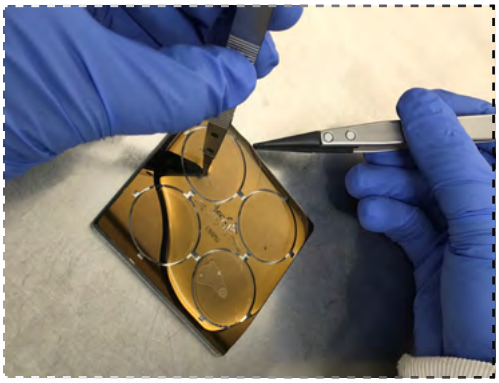

After 10 minutes printing, stop the UV lamp and remove the masker from the UV printer.

Then, gently open the Masker and remove the slides using only plastic tweezers[3]. Be careful while opening the masker because some slides could be already detached from the mask.

(2) Use a soft tissue for cleaning not to scratch the mask. You can use Kimwipes which are delicate task wipers.
(3) To avoid that coverslips and the mask touch the bottom of the UV, you can use two microscope slides as spacers (not provided). Place the microscope slides at the extremity of each side of the mask

If the slides are attached strongly to the mask, add distilled water onto the slides and the mask and wait some minutes for the to detach. The slides will start floating on their own. If not, slip them close to the edge of the mask and pick them up with plastic tweezers[4].





Dry the micropatterned slides with air gun or let them dry on a coverslip rack.

Pipet 100 µL of Fibronectin (blue tube) per slide on a parafilm piece

Place the printed side of the slide on top of the fibronectin drop and incubate during 30 min at room temperature.

Rinse with distilled water and dry with air gun or let it dry on rack.

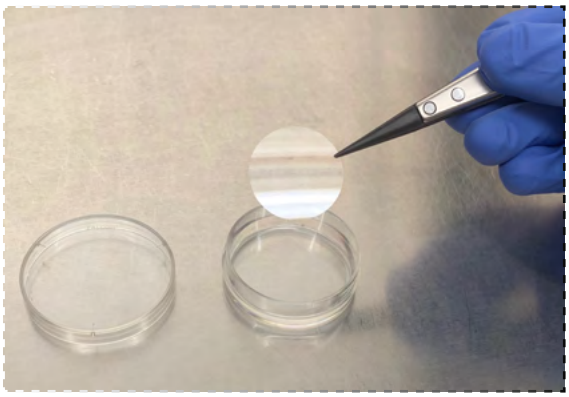
C. Plating of the cells

Place each slide in the small petri dish or cell culture plate as needed. The patterned side of the slide facing up.

Prepare 50.000 to 60.000(6) cells resuspended in 200 µL of cell culure medium per slide[5].

Pipet the 200 µL of cells on top of each patterned slide and incubate the during 1 hout (or more, depending on cell type) at 37°C, 5% CO2.

Wash unattached cells with pre-warmed culture medium or sterile PBS.



Add 2 mL of pre-warmed culture medium per slide and incubate cells overnight for future pattern's analysis[6].

(4) Do not use metal tongs to avoid stripes on the mask
(5) It is suitable to have cells at a confluency around 50%. High confluency of cells will promote cell-cell adhesion and create cluster of cells, which is not suitable for single cell patterning. The volumes are optimized for 2 mm diameter coverslips (standard)
(6) The plating of cell is optimized for HeLa cell line therefore the number of cells added, the adhesion or culture time could vary.

Product specifications

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	Included in the consumables box
Glass slides diameter, thickness	25 mm, 0.13-0.16 µm
Number of slides	100
Extracellular matrix protein, volume	Fibronectine, 2 mL x 5 tubes, stored at +4°C
Fluorescent marker, volume	Fibrinogen, 2 mL x 1 tube, stored at -20°C
Anti-adhesive agent, volume	PEG (Poly-Ethylen-Glycol), 2 mL x 5 tubes, stored at -20°C

	Order separately
Optical mask material	Quartz
Optical mask size	2.5 x 2.5 inches
Optical mask thickness	2.2 mm
Number of slides simultaneously printed	4
Micropattern geometries	Disk, line, square, triangle, rectangles, grids
Micropattern sizes	10, 20, 30, 40, 50, 70, 100 µm
Masker	Aluminium frame and screw Internal dimensions: 64 mm x 64 mm External dimensions: 70 mm x 85 mm x 14 mm
UVO Lamp	Deep UV (wavelength <200 nm), Bioforce - ProCleaner

The link between biophysics and biology

Based on the experience of the R&D team, 4DCell offers other a product whose innovation lies in its flexibility of use.

Our goal is to provide affordable biophysical tools adapted to customized applications, thus meeting your research needs. This is ensured without compromising quality, thanks to the extensive know-how the R&D team has and which we apply to select the best materials and methods to deliver perfect products fabricated to order, in-house in our labs in Montreuil, Paris, France.