4Dcell
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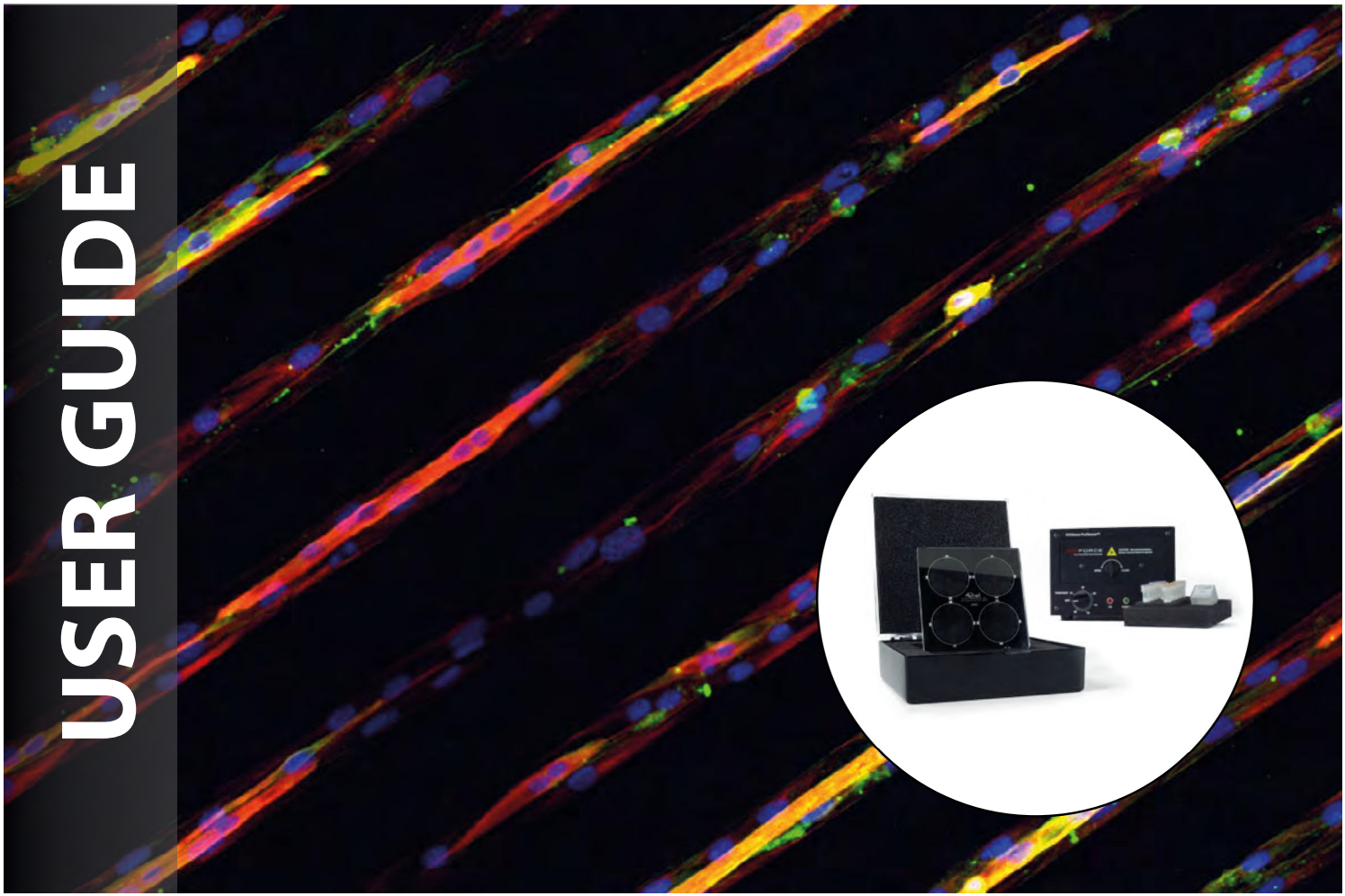
4Dcell micropatterns fabrication kit

User protocol

USER GUIDE



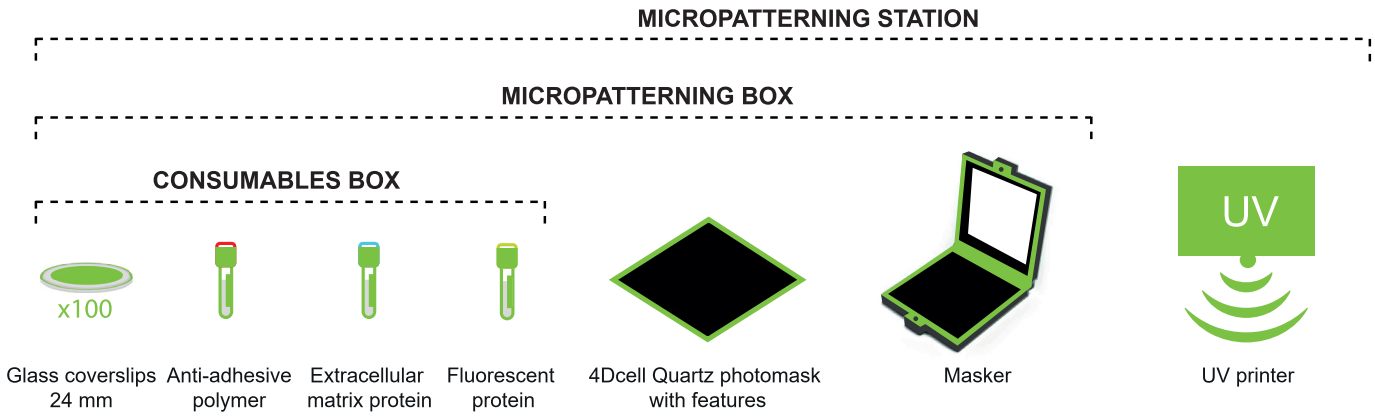
How to use the 4Dcell micropatterns fabrication kit

Materials included

Consumables box
- Glass coverslips 24 mm
- Lyophilized anti-adhesive polymer (PLL-g-PEG) *Store at -20 °C upon reception*
- Buffer solution for anti-adhesive polymer (10 mM HEPES, pH 7.4) *Store at 4 °C upon reception*
- Extracellular matrix protein (fibronectin *50 µg/mL) Store at -20 °C upon reception*
- Fluorescently-labelled protein (fibrinogen-Alexa Fluor 488 *20 µg/mL) Store at -20 °C upon reception*

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
Micropipette

How to use the UV lamp

Manipulate under a hood to ensure aseptic conditions
Press the button ON. Open the drawer, place your sample inside, and close the drawer. Use the button "TURN PAST" to adjust the activation time of your sample (for example, you can turn the button to 5-10 minutes to activate your slide). Make sure that both the "UV" and "POWER" lights are turned on. Once the allocated time is finished, flip the power switch to the "OFF" position and open the drawer to take your sample out.

A. Preparing the PEG solution

Wear gloves to manipulate the coverslips and work in aseptic conditions under a laminar flow hood

The PLL-g-PEG polymer is shipped as a lyophilized powder to ensure longer shelf-life (1 mg/tube). Before preparing the solution, take the tube out of the freezer to allow it to warm up to room temperature (10 minutes). To dissolve the polymer solution, gently tap the tube to ensure so that the powder falls into the bottom of the tube. Add 1 ml of buffer solution to the tube. Close the tube securely and invert to ensure that all inner surfaces of the tube are wetted by the buffer. Transfer the solution back to the falcon tube containing the rest of the buffer solution, for a total of 10 mL of solution (i.e. 0.1 mg/mL).

This solution can now be aliquoted to the sizes most appropriate for your experiments (for example 1 ml for 5 slides) and stored at -20 °C.

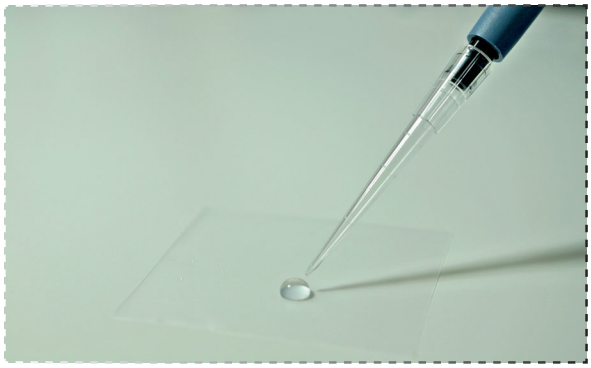
B. Preparing the coverslips

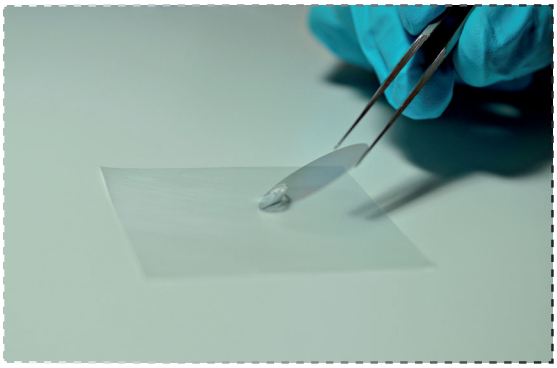
Wear gloves to manipulate the coverslips and work in aseptic conditions under a laminar flow hood

Clean the slides with 70 % ethanol and dry them carefully using lint-free wipes. The coverslips should be free of microfibers or dust after cleaning.

Activate the slides. If using the deep UV lamp, expose the slides for 10 minutes. You can put the slides on a glass support to avoid contact with bottom of the lamp box. If using a plasma cleaner, activate the slides for 3 minutes at maximum intensity. Use the slides immediately slides after activation.

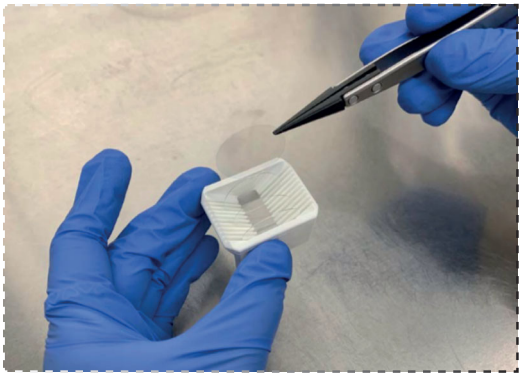
Add a drop of 200 µL of PEG solution to a flat piece of parafilm. Invert the activated face of the coverslip onto the drop. Incubate your coverslips for 30 minutes at room temperature.





Time-saving tip: you can start step C 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 solution. Dry the slide with an air gun, eliminating the remaining droplets or let them air dry on a coverslip rack. Be careful only to touch the treated side of the slide with rubber-tipped tweezers during the rest of the process to avoid scratching the polymer-coated surface.



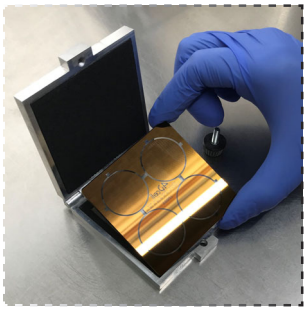
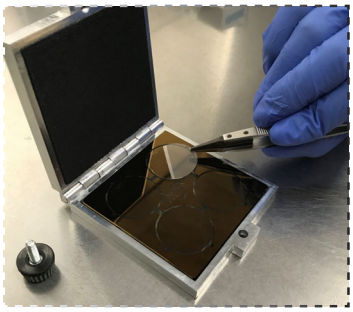
C. Imprinting the micropatterns

Wear gloves to manipulate the mask

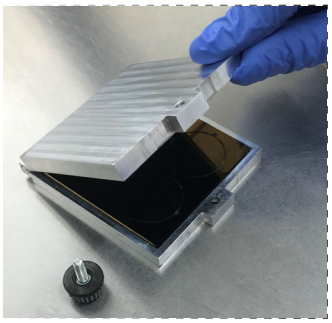
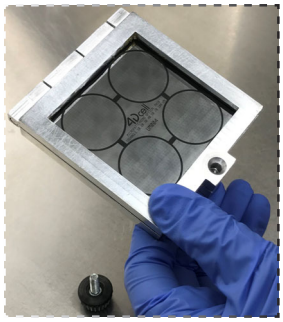
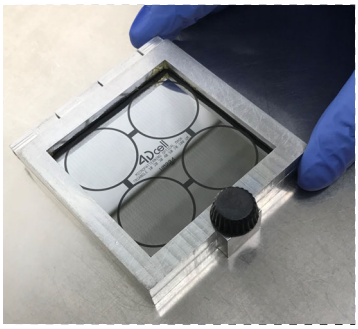
Clean the 4Dcell photomask with 70% ethanol or 99 % isopropanol and lint-free wipes[1]. Ensure no dust or wiping marks are visible on the photomask

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

Gently place your coverslips at their designated spot on the photomask using rubber-tipped tweezers, with the coated side facing the chromium side.

Close the masker. Hold it tightly so that the coverslips don't fall out, turn it upside down and lock it with the screw.

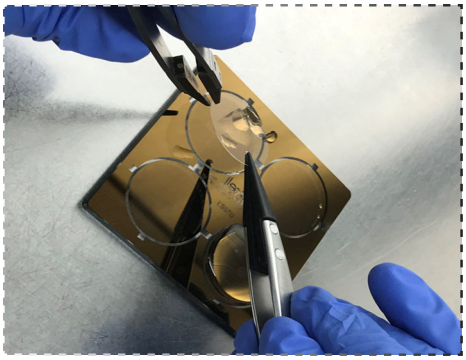
Place it inside the UV printer so that the window of the masker is facing upwards.

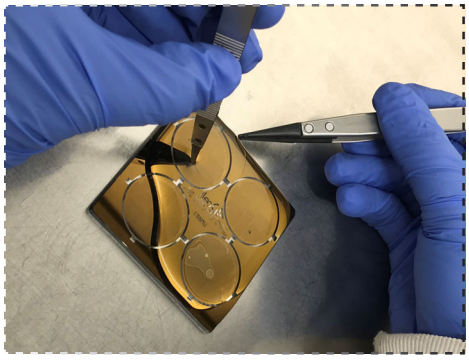
Using the deep UV lamp (below 200 nm), expose the 4Dcell mask for 10 minutes. This step will destroy the anti-adhesive coating in the areas exposed through the mask, creating your micropatterns.


After 10 minutes, turn off the UV lamp and remove the masker from the UV printer. Unscrew the masker, holding it tightly to prevent the coverslips from falling out. Gently turn it over and open the masker and remove the slides using rubber-tipped tweezers.

(1) Use a soft tissue to avoid scratching the mask. You can use Kimwipes or clean-room wipes.

If the coverslips are attached strongly to the mask, add distilled water onto the coverslips and the mask and wait for the coverslips 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 rubber-tipped tweezers[2].





Carefully dry the micropatterned coverslips with an air gun or let them dry on a coverslip rack.

D. (Optional) Extracellular Matrix coating

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 for 30 minutes at room temperature.

Rinse with distilled water and dry with air gun or allow to dry on rack.

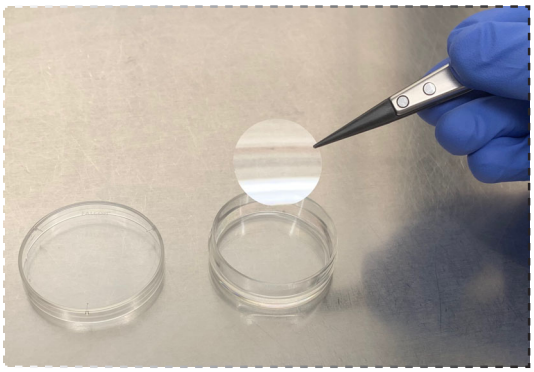
E. Plating cells on the coverslips

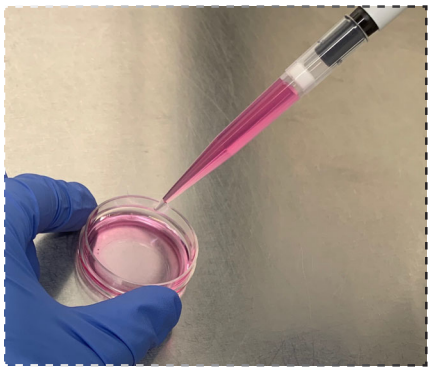
Place each slide in a small petri dish or cell culture plate, the patterned side of the slide facing up.

For our standard designs we recommend 30 000 - 60 000 cells resuspended in up to 400 µL of cell culture medium per slide[2,3].

Pipet 400 µL of cell solution on top of each patterned coverslip[3], and incubate during 1 hour (or more, depending on cell type) under typical culture conditions.

Wash unattached cells with pre-warmed PBS and replace with culture medium.





Incubate cells until the desired analysis time.

(2) These plating instructions are optimized for HeLa cells on 24 mm diameter coverslips therefore the number of cells added, the adhesion or culture time could vary. The cells should not be confluent before plating on the coverslips to reduce cell-cell adhesions and ensure proper resuspension of the cells.
(3) To reduce the likelihood of cells adhering under the coverslip, add medium only on top of the coverslip and take care not to allow the medium to spill off of the coverslip when transferring to the incubator.

Product specifications

Product specifications	Included in the consumables box
Glass coverslip diameter, thickness	24 mm, 0.16-0.19 µm (no. 1.5)
Number of slides	100
Extracellular matrix protein, volume	Fibronectin, 2 mL x 5 tubes, stored at +4°C
Fluorescently-labelled protein, volume	Fibrinogen Alexa Fluor 488, 2 mL x 1 tube, stored at -20°C
Anti-adhesive polymer	PLL-g-PEG (poly-L-lysine-graft-polyethylene glycol) 2 mg, stored at -20°C (1 mg/ml after dissolution in buffer)
Buffer solution	HEPES 10 mM, pH 7.4
	Included in the kit
Optical mask material	Quartz and chromium
Optical mask size	2.5 x 2.5 inches
Optical mask thickness	2.2 mm
Number of coverslips simultaneously printed	4
Micropattern geometries	Disk, line, square, triangle, rectangles, grids or customized
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
UV Lamp	Deep UV (wavelength <200 nm), Bioforce - ProCleaner

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Based on the experience of the R&D team, 4DCell offers products whose innovation lies in their 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 of the R&D team, 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.