

4Dcell
EXPLORE BETTER.

micropatterningkit
complete.handy.simple

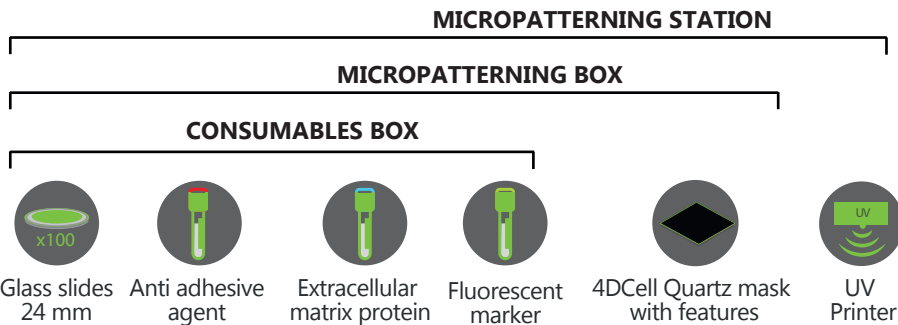
USER GUIDE



Innovative cell culture systems.

Material included

Material not included



Ethanol 70%
 Kimwipes
 Parafilm
 Plastic tweezers
 Distilled water
 Microliter pipette
 Millipore H₂O






Fig. 1: Micropatterning kit: UV Printer, 4Dcell optical mask, micropatterning box, glass slides, micropatterning chemicals.

How to use the UV Printer (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.

Micropatterning protocol

A. PREPARING THE SLIDE

-  1 Make sure there is no dust: wash the slides in ethanol 70% and dry them. Use wipers to absorb any remaining droplets.
-  2 Using the deep UV Printer, activate the slides for 5 min. You can put the slides on a microscope slide or any glass support to avoid contact with the bottom of the lamp box. Directly use the slides after activation.
-  3 Incubate 30 min in PEG⁽¹⁾ at room temperature: add 50 μ L of the PEG solution (yellow tube) on a parafilm. Put the activated side of the slide on the drop !

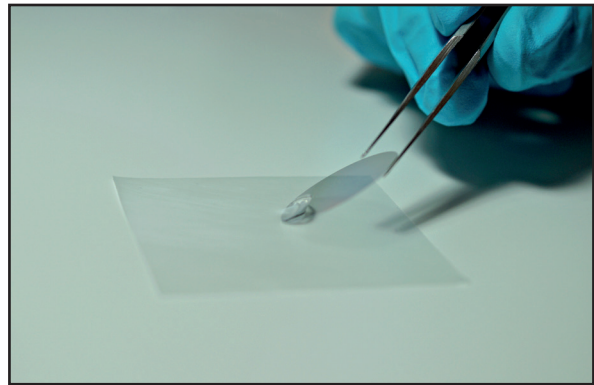
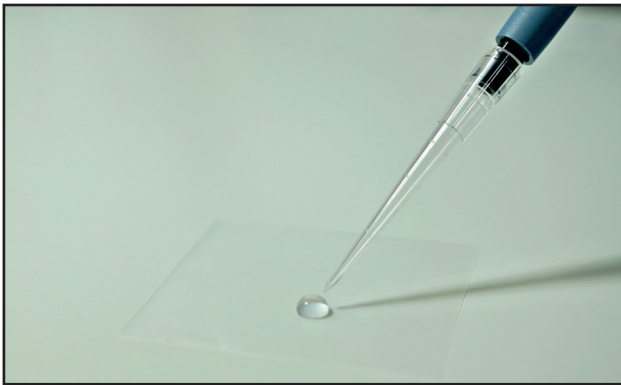



Fig.2: Illustration of point A.3

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

-  4 Remove the slides with tweezers. Rinse the PEG with distilled water in a Fluorodish or a 6-well cell culture plate. Place the activated side facing you and add water on the edge of the well. Wash twice. Take the slides with tweezers. Let the drop drip down the glass. Use a paper to absorb any remaining droplets. Be careful not to touch the activated side with paper.

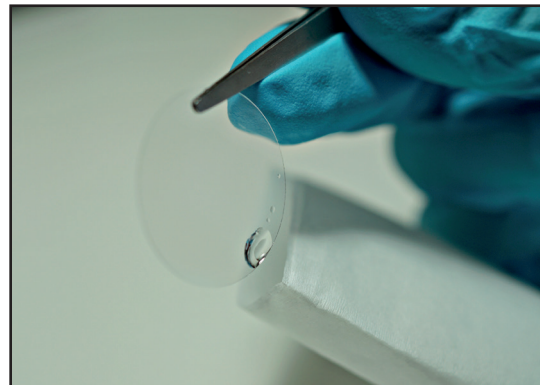
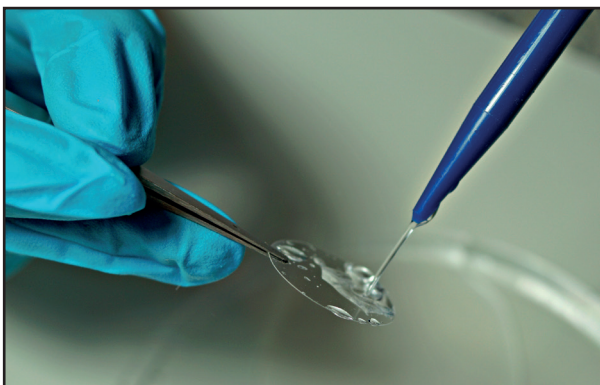


Fig.3: Illustration of point A.4

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

B. UV ILLUMINATION (Wear gloves to manipulate the mask)

- 1 Wash the 4Dcell quartz mask with absolute ethanol⁽²⁾. To dry it, you can either air dry or use wipes.
- 2 Using the deep UV Printer, illuminate the 4Dcell mask 10min with its brown side facing the lamp.⁽³⁾
This makes the brown side of the mask hydrophilic.
- 3 Add 3 μ L of millipore H₂O onto the brown side of the 4Dcell quartz mask. Place each drop of 3 μ L on the edge of a circle defining the patterns.
 Trick: place the drop at the extremity of a round mask to facilitate point 4.

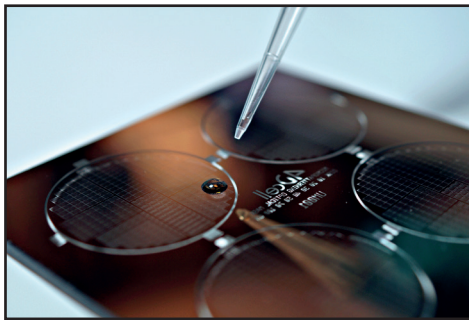


Fig.4 Left: Illustration of point B.3; Right: Illustration of point B.4

- 4 Gently drop off the PEGylated side of the slide on the mask. Start to place the slide near the water drop and slowly drop it off on the circle where the micropattern is. You can use a plastic tip to press the slide against the mask and thereby spread the drop⁽⁴⁾ and avoid any bubbles.
- 5 Using the deep UV Printer, illuminate the 4Dcell mask 10min with the silver side facing the lamp.
- 6 Add 5 mL of distilled water on the mask to help detach the slides from it. Let it incubate for 5 min.
- 7 Take off the slide with tweezers⁽⁵⁾. You can bring the slide to the closest edge of the mask. Be careful not to scratch the mask, and catch it with tweezers.







Fig.5: Illustration of point B.7

- 8 Incubate the activated side 1h at room temperature with a combination of fibronectin and fibrinogen: on a parafilm, add 50 μ L of fibronectin (blue tube) and 10 μ L of fibrinogen (green tube), mix them with your micropipette and let your slide incubate.
- 9 Rinse with water. You can stock the slides in a fridge at +4°C.

(2) Use a soft tissue for cleaning not to scratch the mask. You can use Kimwipes which are delicate task wipers.
 (3) Use spacers (not provided) to avoid contact with the bottom of the lamp box. You can use two microscope slides and put the extremity of each side of the mask on it.
 (4) Every resting air bubble will cause enlargement of underlying patterns! This accounts also for airbubbles that arise due to evaporation during illumination. A solution to avoid evaporation can be to put a very large glass coverslip sealed with water onto the 25 mm coverslips.
 (5) Do not use metal tongs to avoid stripes on the mask

C. PLATING OF THE CELLS

-  Prepare 100.000 to 200.000 cells for each 25 mm slide.
-  Detach cells ⁽⁶⁾.
-  Centrifuge cells to get rid of the medium used to detach cells.
-  Resuspend cells in 1 mL of the culture medium for each slide (mix extensively with a 1mL pipette to separate the cells from each other): put your slide in a petri dish with the patterned side facing you. Add the culture medium onto the slide with the micropipette.

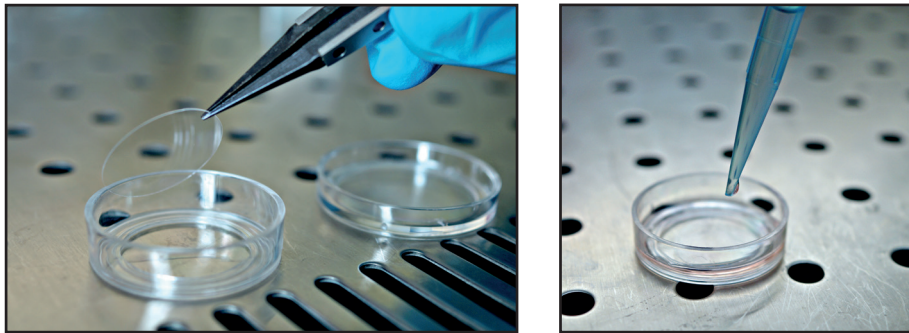


Fig.6: Illustration of point C.4

-  Wash the unattached cells 20-30 min after plating with equilibrated hot medium, without serum in it⁽⁷⁾. Cells should be spread on the patterns 1h after plating.

For this step, the times depend on the type of cells used and in which conditions.

(6) It is better to have cells at a confluency around 50%. High confluency of cells will promote cell-cell adhesion and give you clusters of cells, which is bad for single cell patterning.

(7) An efficient washing is obtained by adding medium from one side and aspirate from the other side at the same time.

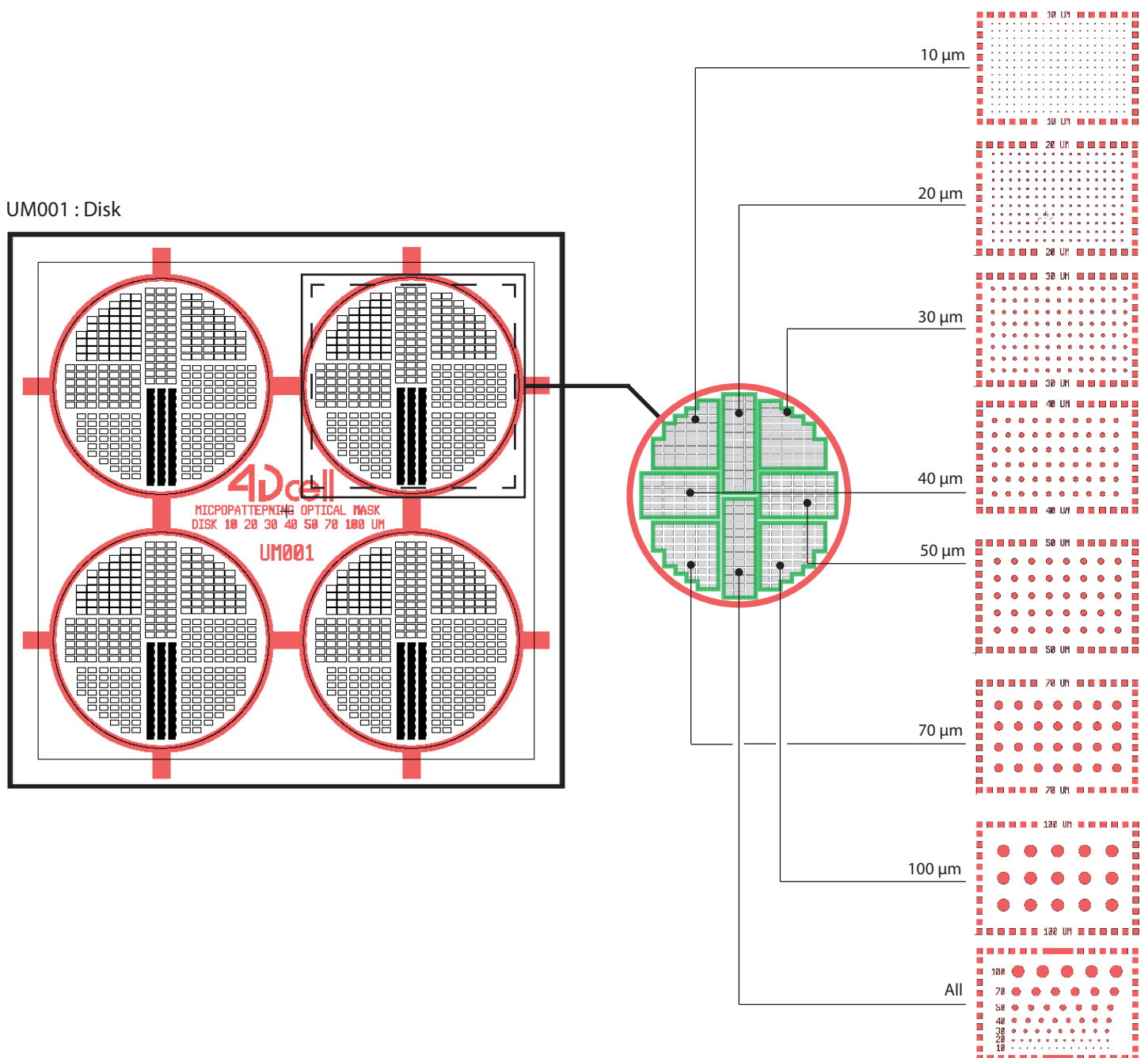
4DCell Quartz mask specifications

Photomask 2.5 inch allowing photoprinting of 4 slides simultaneously

a. Structure of the 'Round' pattern optical mask

Example of applications of 'Round' pattern:

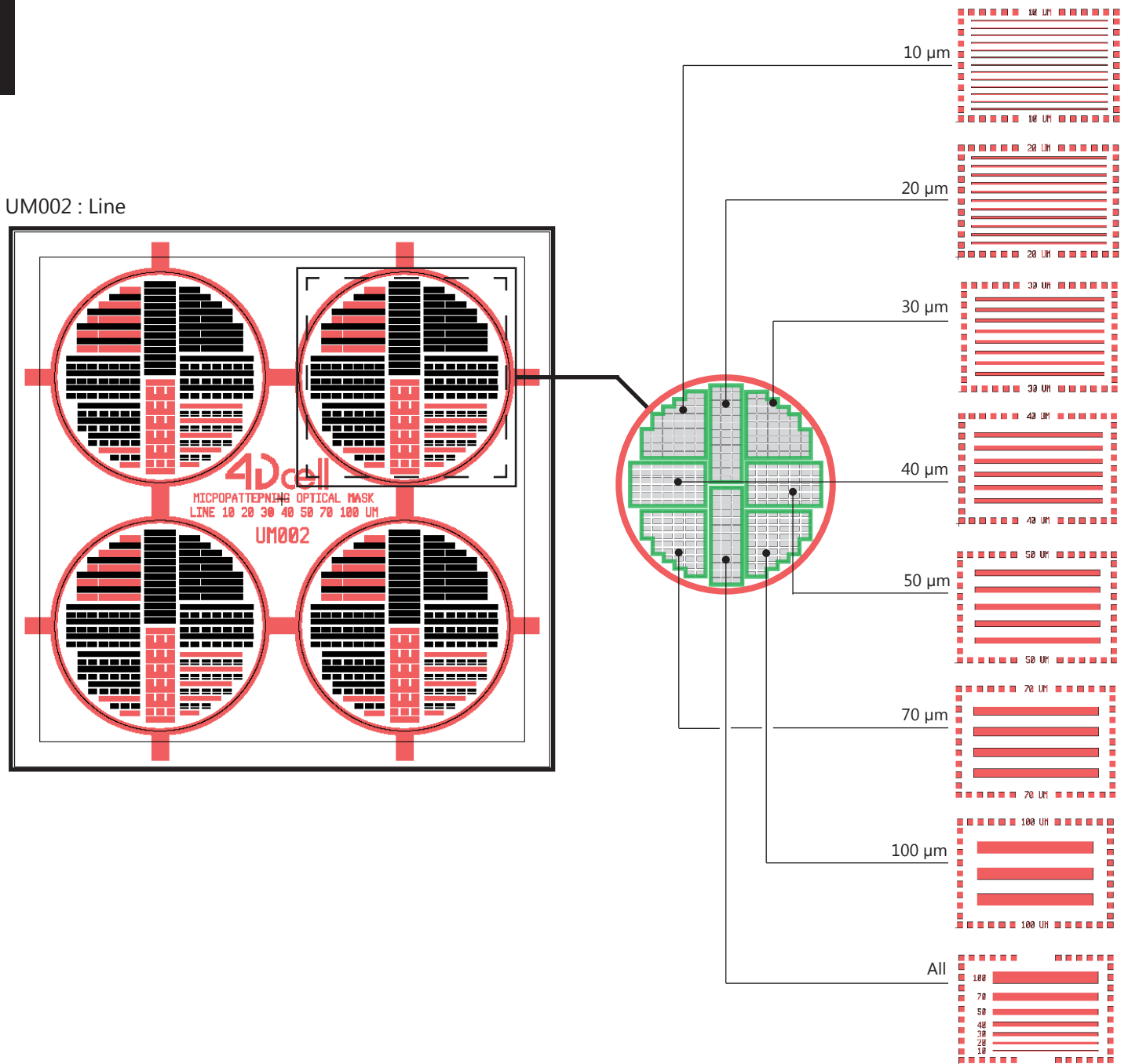
- Standardize cell shape
- Control the position of cells
- Control cell adhesion surface
- Free cell polarisation
- Small patterns can be used to make attached quasi-spherical cells (mimic detached cells)



b. Structure of the 'Line' pattern optical mask

Example of applications of 'Line' pattern:

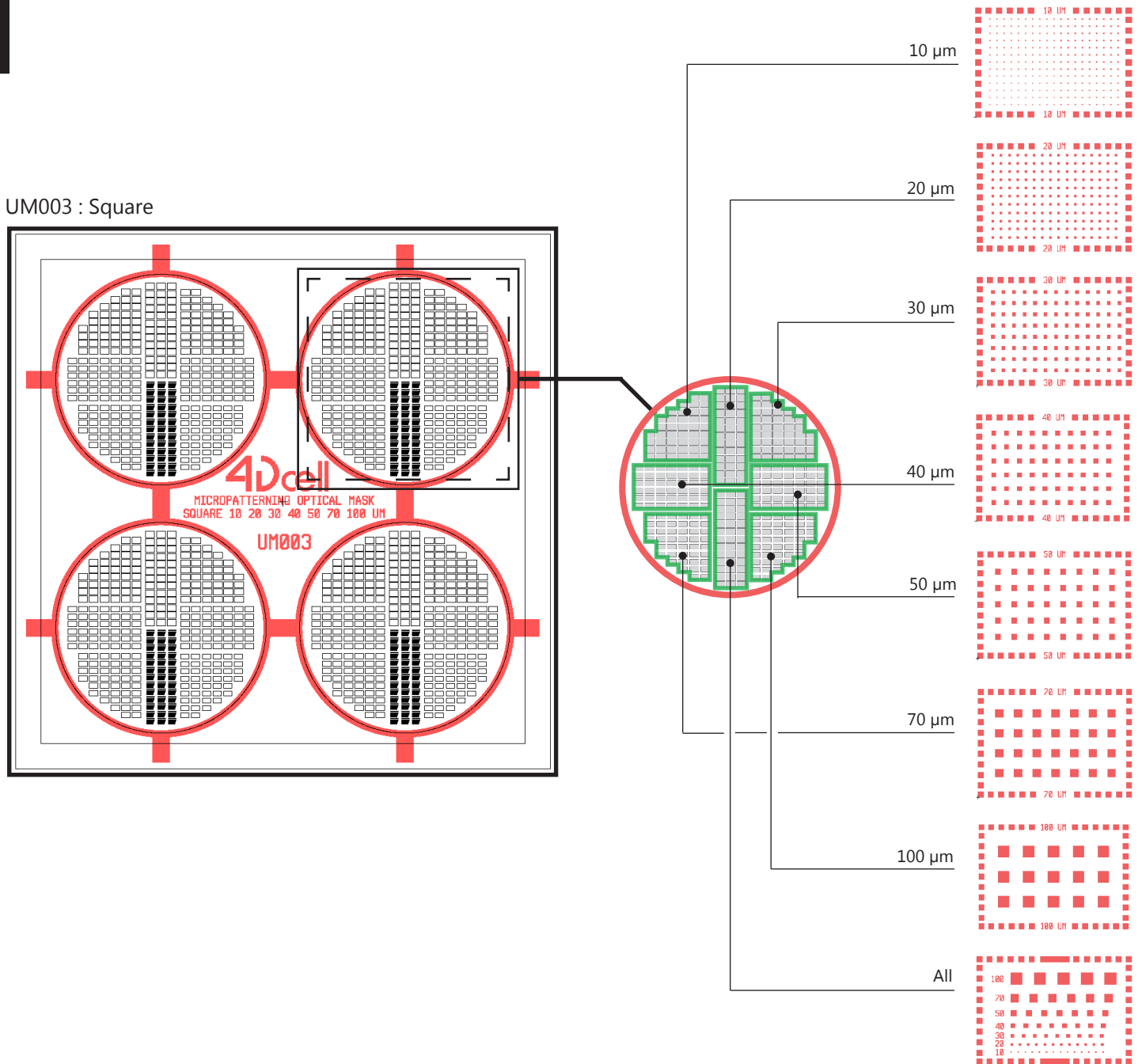
- Cell migration
- Neuron studies
- Cell-cell interaction



c. Structure of the 'Square' pattern optical mask

Example of applications of 'Square' pattern:

- Restrict area of migration of cells without imposing a shape (on large squares)
- Can impose specific axes to the cell division
- Study of pseudopodes at the tips of the squares

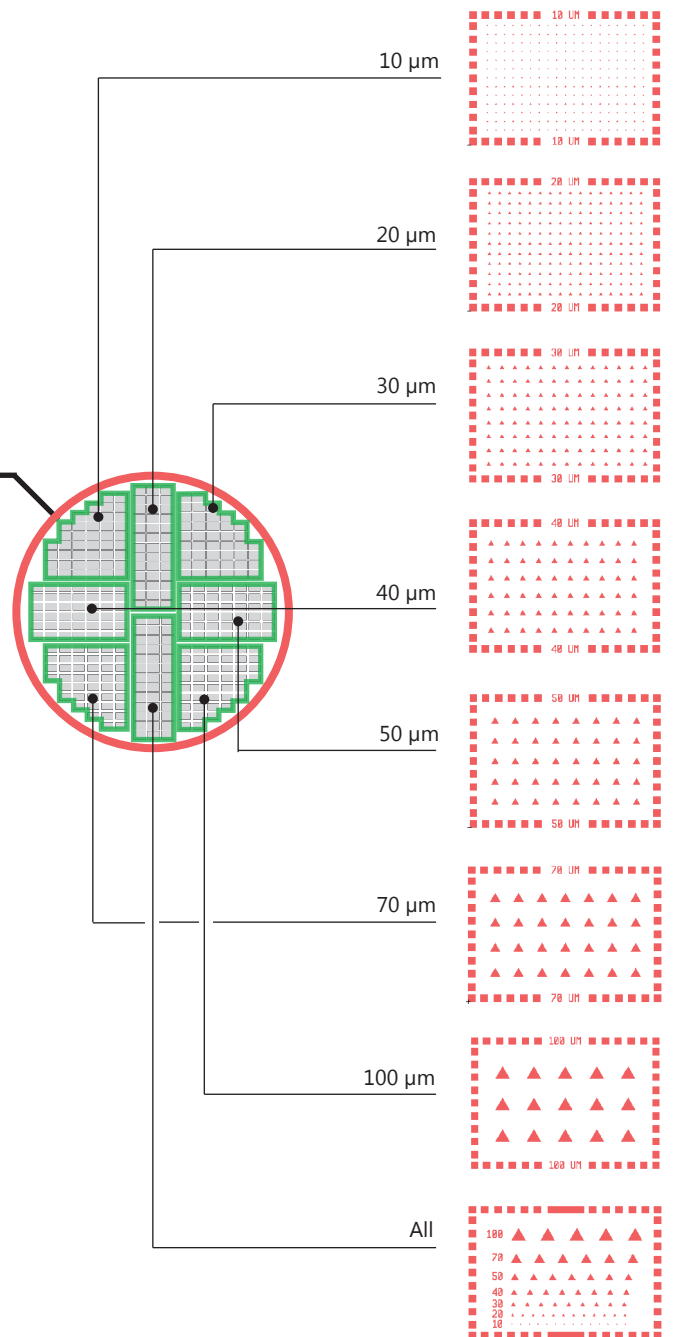
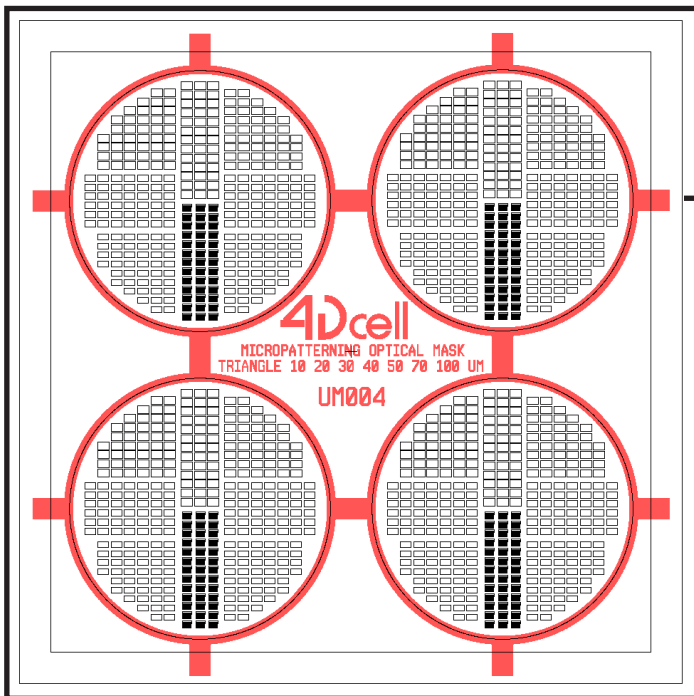


d. Structure of the 'Triangle' pattern optical mask

Example of applications of 'Triangle' pattern:

- Can impose specific axes of polarisation to the cell
- Can impose specific axes to the cell division

UM004 : Triangle



**Product specifications****Included in the consumables box**

Glass slides diameter, thickness	25mm, 0.13-0.16µm
Number of slides	100
Extracellular matrix protein, volume	Fibronectine, 2mL x 5 tubes, stored at +4°C
Fluorescent marker, volume	Fibrinogen, 2mL x 1 tube, stored at -20°C
Anti-adhesive agent, volume	PEG (Poly-Ethylen-Glycol), 2mL 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	2.2mm
Number of slides simultaneously printed	4
Micropattern geometries	Disk, line, square, triangle, rectangles, grids
Micropattern sizes	10, 20, 30, 40, 50, 70, 100µm
UV Printer	Deep UV (wavelength <200 nm), Bioforce - ProCleaner



The link between biophysics and biology

Based on their laboratory experiments, 4DCell teams offer a product whose innovation lies in its flexibility of use. Our goal is to make the researcher's life easier providing affordable biophysical technologies, with a custom-made use. This without ever cutting back on quality, thanks to 4DCell selection of the best chemicals and materials on the market.



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Products & Prices

Please note that some goods may vary in style, color or detail from the image shown. We reserve the right to change prices at any time without notice. Important : read all Instructions, warnings and precautions prior to use.

Limitation of warranty

4DCELL explicitly disclaims all warranties, express or implied, for any period during which the goods are operated or stored not in accordance with the technical specifications. 4DCELL does not assume any liability arising out of any application or use of any product or circuit and specifically disclaims any and all liability, including without limitation consequential or incidental damages. All operating parameters, including without limitation recommended parameters, must be validated for each customer's applications by customer's technical experts. Recommended parameters can and do vary in different applications. 4DCELL reserves the right, without further notice, (i) to change the product specifications and/or the information in this document and (ii) to improve reliability, functions and design of this product. The foregoing warranty shall not apply to defects resulting from improper or inadequate maintenance by the Customer, Customer-supplied software or interfacing, unauthorized modification or misuse, operation outside of the environmental specifications for the product, or improper site preparation and maintenance.

Exclusive remedies

The remedies provided herein are the customer's sole and exclusive remedies. 4DCELL shall not be liable for any direct, indirect, special, incidental, or consequential damages, whether based on contract, tort, or any other legal theory. No other warranty is expressed or implied. 4DCELL specifically disclaims the implied warranties of merchantability and fitness for a particular purpose.

Assistance

4DCELL Sales and Service office for further information on 4DCELL full line of Support Programs. See: www.4dcell.com. The following general safety precautions must be observed during all phases of operation, service, and repair of this instrument. Failure to comply with these precautions or with specific warnings elsewhere in this manual violates safety standards of design, manufacture, and intended use of the instrument. 4DCell assumes no liability for the customer's failure to comply with these requirements.

General

Caution for research use only: wear proper body protection during experience. Do not operate in an explosive environment. Do not operate in wet/damp conditions. Do not use this product as safety or emergency stop devices or in any other application where failure of the product could result in personal injury. The protective features of this product may be impaired if it is used in a manner not specified in the operating instructions.

Before installing, handling, using or servicing this product, please consult the data sheet and user guide. Failure to comply with these instructions could result in death or serious injury. If the buyer shall purchase or use 4DCELL products for any unintended or unauthorized application, the buyer shall defend, indemnify and hold harmless 4DCELL and its officers, employees, subsidiaries, affiliates and distributors against all claims, costs, damages and expenses, and reasonable attorney fees arising out of, directly or indirectly, any claim of personal injury or death associated with such unintended or unauthorized use, even if 4DCELL shall be allegedly negligent with respect to the design or the manufacture of the product.

ESD precautions

The inherent design of this component causes it to be sensitive to electrostatic discharge (ESD). To prevent ESD-induced damage and/or degradation, take customary and statutory ESD precautions when handling this product.

Environmental conditions

This instrument is intended for indoor use. It is designed to operate at a maximum relative humidity of 60% and at altitudes of up to 2000 meters. Operating temperature range is +5°C to 50°C. Do not operate in an explosive atmosphere. Do not operate the instrument in the presence of flammable gases or fumes. Before applying power, verify that the line voltage matches the product's input voltage requirements and the correct fuse is installed. Use only the specified line cord for this product and make sure the line cord is certified for the country of use.

Fuses

Only fuses with the required rated current, voltage, and specified type (normal blow, time delay, etc.) should be used. Do not use repaired fuses or short circuited fuse holders. To do so could cause a shock or fire hazard. Keep away from live circuits Operating personnel must not remove instrument covers.

Component replacement

Component replacement and internal adjustments must be made by qualified service personnel from 4DCELL company only, subject to the loss of the warranty. Do not replace components with power cable connected. Under certain conditions, dangerous voltages may exist even with the power cable removed. To avoid injuries, always disconnect power, discharge circuits and remove external voltage sources before touching components. Do not service or adjust alone. Do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present. Do not substitute parts or modify instrument. Because of the danger of introducing additional hazards, do not install substitute parts or perform any unauthorized modification to the instrument. Return the instrument to an 4DCELL Office for service and repair to ensure that safety features are maintained. Instruments which appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified service personnel.

CE compliance

This product meets the essential requirements of applicable European Directives, as amended for CE marking, as follows: Electromagnetic Compatibility COUNCIL DIRECTIVE 89/336/EEC of 3 May 1989. This directive has been amended by the following Council Directives: (i) 92/59/eec of 29 June 1992 (General Product Safety) ; (ii) 93/68/eec of 22 July 1993 (CE Marking directive); (iii) 99/5/ec: Directive of Radio Equipment & Telecommunications Terminal Equipment (R&TTE).

Delivery

Delivery dates are indicative. 4DCELL shall not be liable for any delay in delivery. Claims arising from delivery delays will not lead to any financial compensation from 4DCELL company. In case of major delays in delivery dates, claims will be treated amicably.