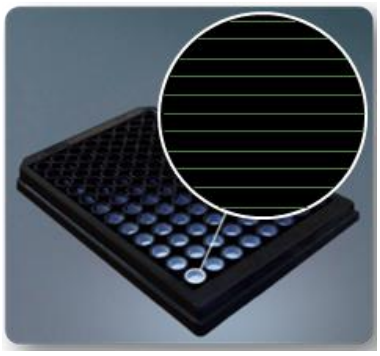
CYTOOplates™ *Motility*

User Manual

For Research Use only

Refrigerate at 4°C on arrival. Do not freeze.
Keep bag sealed. Expiry date is indicated on the bag.

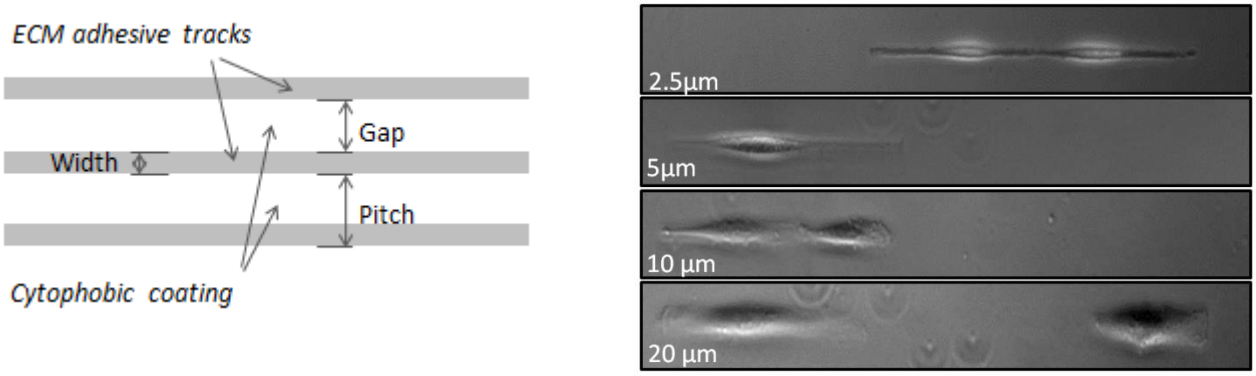
General description



CYTOOplates *Motility* are glass-bottom microplates, bearing arrays of adhesive lines in each well. Each CYTOOplate offers 4 different line widths for 1D cell migration (2.5, 5, 10 and 20 µm), with a line-to-line gap of 45 µm.

CYTOOplates *Motility* are available in standard 96well format. The glass bottom has a thickness of 170µm (1.5) and is mounted with a black polystyrene upper structure. CYTOOplates are supplied with lids. They are compatible with all leading HCS instruments and standard inverted microscopes.

Micropattern design



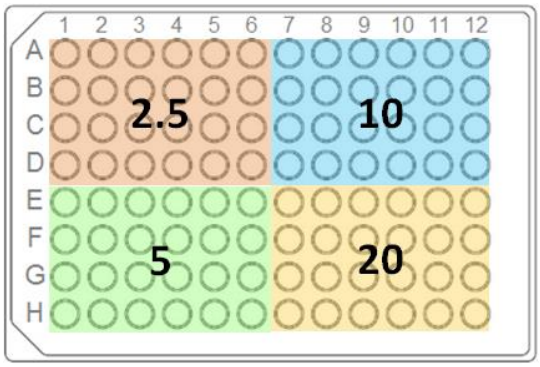
(A) (B)

(A) Micropattern design on the CYTOOplates Motility. (B) HUVEC cells cultured on fibronectin-coated adhesive tracks. Courtesy of M. Chatelais, University Hospital Nantes.

Micropattern	Line 2.5 µm	Line 5 µm	Line 10 µm	Line 20 µm
Width, µm	2.5	5	10	20
Gap, µm	45	45	45	45
Pitch, µm	47.5	50	55	65

Layouts

CYTOOplates *Motility* have been designed to study 1D cell motility on lines of different widths (from 2.5 µm to 20 µm). There are 24 identical wells for each line width organized in quadrants.



24 identical wells per zone
Well diameter: 6.3 mm

Protein coatings

CYTOOplates *Motility* are available with Fibronectin coatings (unlabeled or fluorescent in far red) or as Ready-to-coat (Activated)**.** Activated micropatterns can be coated with a protein of your choice (Collagen, Poly-L-lysin, Laminin, Matrigel®, antibodies etc). Validated coating protocols are available. Please contact us.

Packaging

CYTOOplates are packaged individually sealed in an aluminum bag under protective atmosphere and with silica gel inside to keep the CYTOOplate dry.

Storage

Refrigerate at 4°C on arrival. The shelf life from the date of production is 6 months. The expiration date is printed on the bag. Once opened, use rapidly. **DO NOT FREEZE** at any time or the glass bottom may become detached from the upper frame.

Instructions for use

For Research Use only

THIS PROTOCOL IS OPTIMIZED FOR HELA AND RPE1 CELLS AND WAS USED FOR THE FIRST WORLD CELL RACE[1]. TIMES FOR ATTACHMENT AND SPREADING MAY VARY CONSIDERABLY DEPENDING ON YOUR CELL TYPE AND MUST BE REEVALUATED.

When using CYTOOplates motility for the first time with a given cell line, you will have to follow the guidelines below to optimize the seeding density.

1. CYTOOplates are packaged individually and have to be stored at 4°C. Before you start your experiment, remove the CYTOOplate from its packaging under the cell culture hood and let it warm to room temperature.

   If you are using a fibronectin (FN or FN650) coated CYTOOplate, jump to step 3.

2. If you are using an Activated "ready-to-coat" CYTOOplate, you need to coat the micropatterned wells with the appropriate adhesion protein (such as collagen, laminin, etc). Typical coating protocols use a protein concentration of 20-50µg/ml in 100µl PBS per well, and incubation times run for 1-2 hours at room temperature. After this incubation time, wells need to be rinsed extensively without drying out the wells (please see specific CYTOO protein coating protocol).

3. Collect cells by trypsinization and centrifuge using your usual settings. Resuspend the cells by gentle but thorough pipetting and check that they are properly individualized under a microscope. Cells aggregates will be detrimental to efficient cell migration experiments on adhesive tracks.

4. Count cells and adjust the suspension to a density of 10,000 cells per ml for seeding. This density is indicative and may vary considerably depending on the type of experiment you plan to carry out, line width used, as well as experiment protocol. Namely you may consider lowering cell density if you are use proliferating cells and start your measurement 24 hours after cell seeding.

5. Prepare at least 15ml (150,000 cells) to fill the wells of a 96 well CYTOOplate (see the Table below).

Suspension density, cells per ml	**10,000**
Well diameter	6.3 mm
Total well volume	0.34 ml
Recommended dispensed volume per well	0.1 ml
Number of cells per well	1,000
Volume to prepare per plate	15 ml

A higher volume may be necessary to compensate for dead volumes of dispensing instruments or reservoirs. This volume and/or cell density may also need to be optimized for your specific cell line and application.

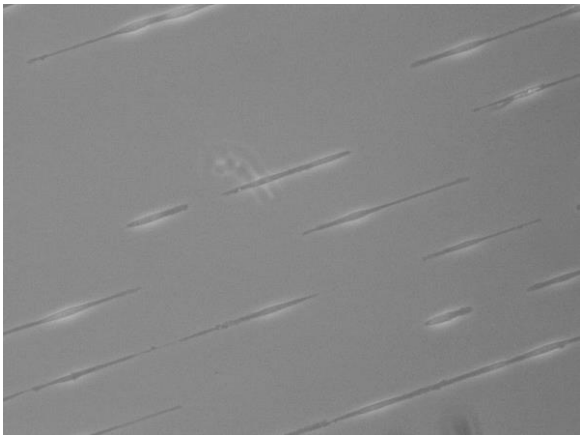
[1] Mauiri et al. The first World Cell Race. Curr Biol. 2012 Sep 11;22(17):R673-5.

6. Place your tips vertically above the micropatterned surface and dispense cells slowly. Avoid touching the micropatterned surface as this will damage the cytophobic surface and create aberrant areas where cells can adhere.

THE PLATE SHOULD BE MOVED AS LITTLE AS POSSIBLE TO AVOID INDUCING ROTATING MOVEMENTS IN THE MEDIUM AS THIS WILL TEND TO RESULT IN A HETEROGENEOUS SEEDING OF THE WELL'S SURFACE.

7. Place the plate in a cell incubator.

8. After 3-6 hours, cells should have spread out on the lines. Some cell lines may take a little longer. Visualize them regularly under the microscope.



RPE1 cells after full spreading on adhesive lines. Cells imaged in phase contrast.

DO NOT STACK THE PLATES.

9. Imaging or drug treatments can be started immediately or the next day. For efficient cell tracking, you may consider labeling the nuclei with Hoechst dye (incubating live cells with 5 ng/mL Hoechst dye diluted in normal growth medium). The cells can be imaged using fluorescence and phase contrast microscopy every 5 to 10 min over 24-48 hours.

Troubleshooting

Observation	Possible solutions
Cells are not homogeneously seeded over the surface of the well. There are more cells in the center of the well and very few at the periphery.	In **Steps 6 and 7**, avoid any movement of the CYTOOplate such as rocking or swirling.
Cells do not spread out on the lines. They keep a round shape and are bright in phase contrast. Cells make blebs.	The adhesion protein used to make the lines might not be the appropriate protein for the adhesion of this cell type. Contact us. We can offer micropatterns without adhesive proteins ("Activated" surface) that can be used as such, or with a different adhesive protein.
There are too many cells per line to observe single cell migration.	Decrease cell seeding density.

Technical support

If you have a problem or question concerning this product please contact us online: www.cytoo.com/support.

References

- Pouthas et al. In migrating cells the Golgi complex and the position of the centrosome depend on geometrical constraints of the substratum, J of Cell Sci 2008.
- Doyle et al. One-dimensional topography underlies three-dimensional fibrillar cell migration, JCB 2009.
- Sharma et al. Reconstitution of in vivo macrophage-tumor cell pairing and streaming motility, IntraVital 2012.
- Devalliere et al. LNK (SH2B3) is a key regulator of integrin signaling in endothelial cells and targets α-parvin to control cell adhesion and migration, FASEB J 2012.
- Maiuri et al. The first World Cell Race, Curr Biol 2012.
- Wissner-Gross et al. Large-scale analysis of neurite growth dynamics on micropatterned substrates., Integr. Biol. 2010.

Reordering information

Cat. No.	Product	Description
CYTOOplates 96 wells		
20-031-10	CYTOOplate 96 RW *Motility* FN	Microplate 96 wells (round); Black PS; Glass bottom 170 µm; Continuous lines; Width 2.5, 5, 10, 20 µm; Gap 45 µm; 24 identical wells per pattern type; Fibronectin; Qty: 1
20-031-13	CYTOOplate 96 RW *Motility* FN650	Microplate 96 wells (round); Black PS; Glass bottom 170 µm; Continuous lines; Width 2.5, 5, 10, 20 µm; Gap 45 µm; 24 identical wells per pattern type; Fibronectin fluorescently labeled (Ex. wavelength 650 nm); Qty: 1
20-031-00	CYTOOplate 96 RW *Motility* A*	Microplate 96 wells (round); Black PS; Glass bottom 170 µm; Continuous lines; Width 2.5, 5, 10, 20 µm; Gap 45 µm; 24 identical wells per pattern type; Ready-to-coat (Activated); Qty: 1

*Pre-activated micropatterned CYTOOplates for adsorption of the protein of your choice (Collagen, Laminin, Matrigel®, specific antibodies etc.). Protein may be fluorescently labeled. Contact us for recommended coating protocols and specific needs.

For sales inquiries please contact us online www.cytoo.com/contact-us.

