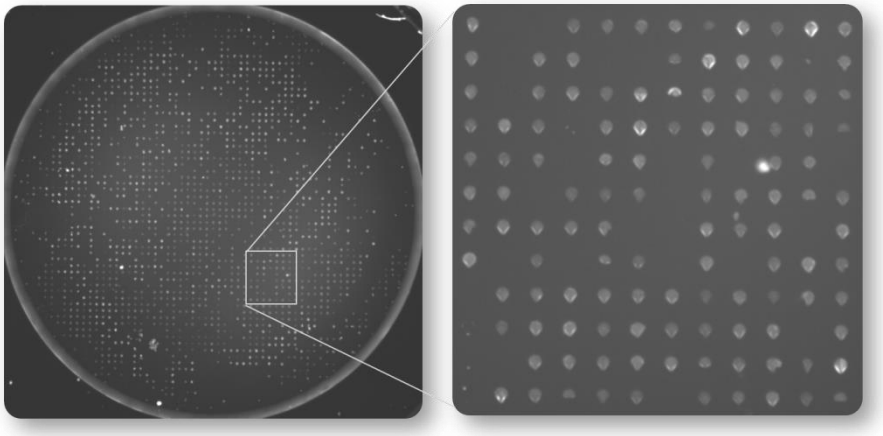
CYTOOplate™

Refrigerate at 4°C on arrival. DO NOT FREEZE. Keep bag sealed until use.

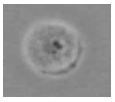
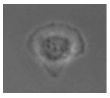
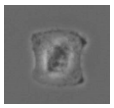
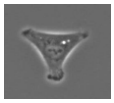
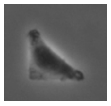
Layout of the Standard CYTOOplate

There are 5 different standard micropattern designs with three possible sizes (small 700µm², medium 1100 µm2; large 1600µm²). The standard CYTOOplate is arrayed with the indicated micropattern throughout (see plate label).
Each well holds approximately 4,000 patterns which are arrayed over the glass surface at a pitch of 80µm (small micropatterns) or 100µm (medium or large micropatterns).



HeLa cells seeded in a crossbow micropatterned CYTOOplate.
Image is courtesy of M. Kwon (Harvard Medical School)

Description of patterns and some applications

	Disc	Crossbow	H	Y	L
Micropatterns					
Cells					
Description	No polarization	Strong polarization	Symmetric organization	Triaxial symmetry organization	Single free edge organization
Noteworthy Applications	Cell arraying Ciliogenesis assays	Cell polarity Organelle positioning Receptor internalization	Cell division Cell-cell junction	Multipolar division	Cytoskeleton rearrangement & Spindle orientation

Upper structure of CYTOOplates

Glass bottom has a thickness of 170µm and is mounted with black polystyrene upper structure of standard SBS format.

Storage

Refrigerate at 4°C on arrival. The bag is sealed with silica gel inside to keep the CYTOOplate dry. Keep the bag sealed and stored at 4°C. We guarantee at least 2 months stability from date of shipment. 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.

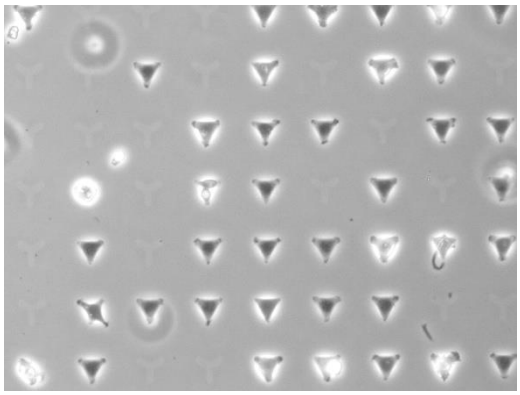
If you have already used CYTOOchips, you have already collected important information on your cell's behavior. This includes adhesion and spreading time, best size and type of micropattern as well as the optimal adhesion protein for your cell lines. In this case the following protocol should be rather straightforward. If you are a first time user of CYTOO products, you will have to follow the simple guidelines added to the protocol to determine these key parameters. Depending on cell behavior, a few plates may be required to fully optimize your protocol.

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.
2. **If you are using a fibronectin coated CYTOOplate, jump to step 3**. 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, polyLysine, Matrigel, 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 (see attached guidelines).
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. As CYTOO's standard micropatterns normalize single cells, cells aggregates will be detrimental to efficient single cell normalization.
4. Count cells and dilute to a concentration of 30,000 cells per ml. Prepare at least 15 mL (450,000 cells) if you will use all 96 wells. A higher volume may be necessary to compensate for dead volumes of dispensing instruments or reservoirs
5. We recommend dispensing 100 µL (3,000 cells) into each well.

This volume and/or cell density may need to be optimized for your specific cell line (see Troubleshooting section for guidelines).

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 non micropatterned 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.

6. Let the cells sediment for 15-45 min under the hood or on the bench at room temperature then move them to the cell incubator. Do not stack the plates.
7. After 3-6 hours, cells should have spread out completely on the micropatterns. Some cell lines may take a little longer. Visualize them regularly under the microscope.



RPE1 cells after full spreading on Y micropatterns. You may notice that the unoccupied micropatterns are slightly visible in phase contrast.

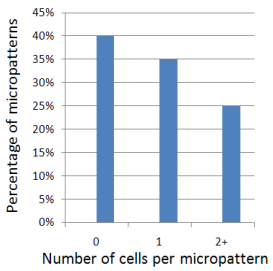
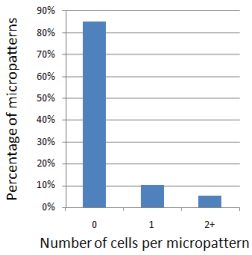
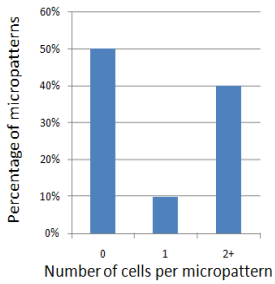
8. Resume your cell assay. Fix within the first 6-8 hours after adhesion to avoid cell division and therefore multiple cells per pattern.
9. After analyzing your cells using all micropattern sizes and geometries, use the layout table to identify the micropattern that works best for your application.

References

See the latest publications using our micropatterns here: www.cytoo.com/publications

Trouble shooting

Observations	Possible solutions
Cells are not homogeneously seeded over the surface of the well.	In Step 4, check that at least 100 µl was added to the well. With less volume, a meniscus effect leads to heterogeneous cell sedimentation. For the same reason, avoid rocking or swirling the dish. In Step 6, be sure to keep cells under the hood for 45 min before moving them to the incubator.
Cells do not spread out on the micropatterns. They keep a round shape and are bright in phase contrast. Cells make blebs.	Check cells on the fully adhesive area in columns 1 and 12. Cells should spread out as in a culture dish. If not, control cell viability or The adhesion protein used might not be the appropriate protein for the adhesion of this cell type. Contact us. or Patterns are too small. Cells suffer from insufficient attachment and spreading. Check larger patterns.
Cells adopt various shapes on a given geometry.	Patterns are too large for the cells moving on it. This induces a large variability in cell phenotypes. Check smaller patterns.
You encounter this distribution of number of cells per micropattern	This is an ideal situation. No need to troubleshoot here!

	
Very few micropatterns are occupied by cells. 	Check cell density after trypsinization. Try higher numbers of cells per well and/or The adhesion protein used might not be the appropriate protein for the adhesion of this cell type. Contact us.
Most patterns are occupied by more than one cell	Check cell density after trypsinization. Make sure the cells are not aggregated after trypsinization. Try lower numbers of cells per well
Micropatterns are either not occupied or occupied by more than one cell. 	This situation is often encountered with cells that tend to clump together (epithelial cells for example). The idea is to try to favor adhesion to the fibronectin (through integrins), versus adhesion between cells (through cadherins). Here are a few ideas: Take great care in dispersing as well as possible the initial cell suspension by pipetting up and down many times Using a medium depleted in calcium (with 300 µM EGTA) while seeding would probably help limit cell aggregation (through cadherins which are calcium-dependent) before adhesion to the surface (through integrins). All media and dishes should be preheated to 37° to help adhesion start immediately (cell clumping is less temperature dependent). Using a diluted cell suspension would also help (less chance of them meeting before they fall to the surface), but of course less micropatterns will be occupied. Treating the cell suspension with Accutase (Invitrogen) before seeding may also be efficient in avoiding cell clumping.

Technical support:

support@cytoo.com

Please use this email address for any questions or requests for information concerning this product.