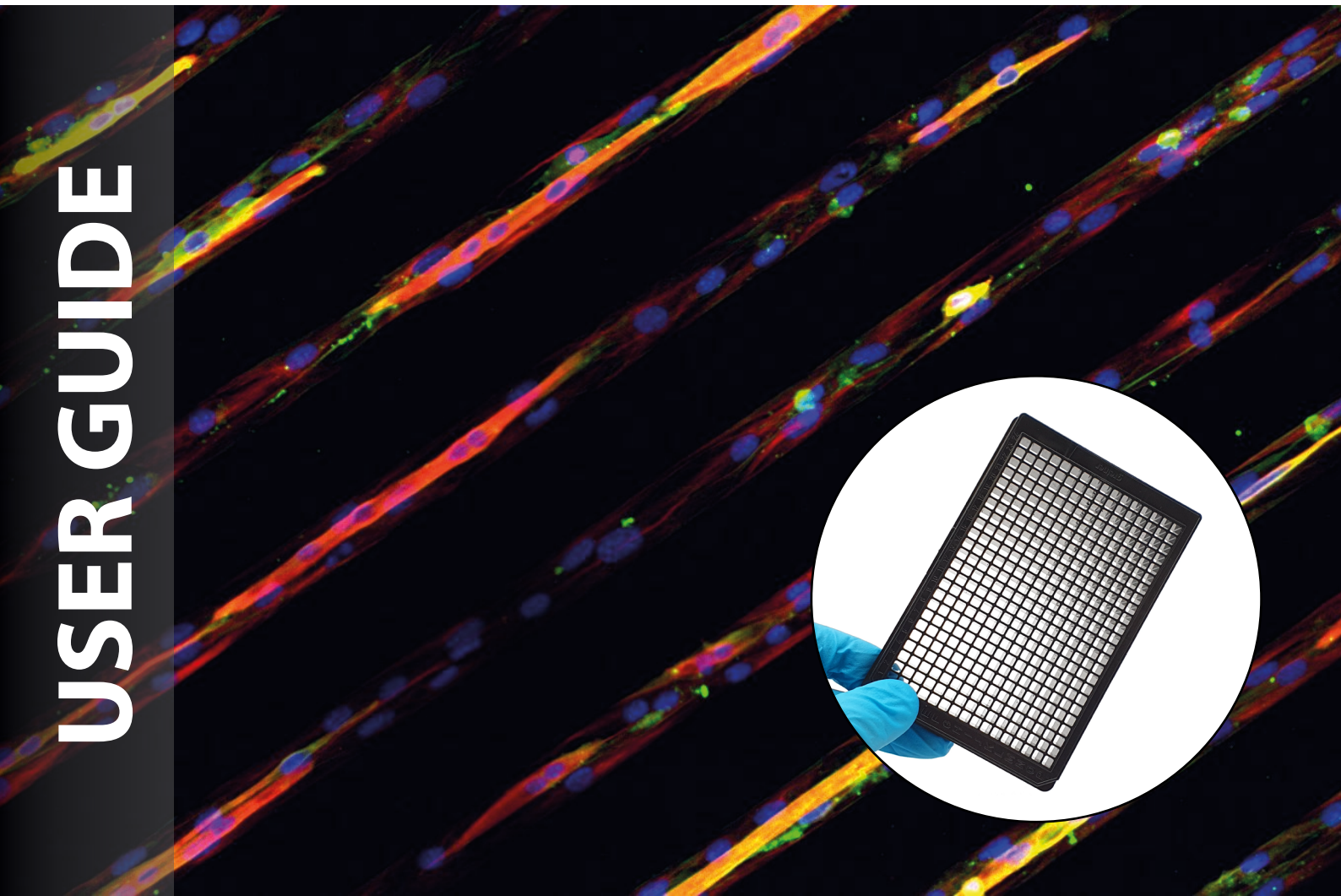
4Dcell
EXPLORE BETTER.

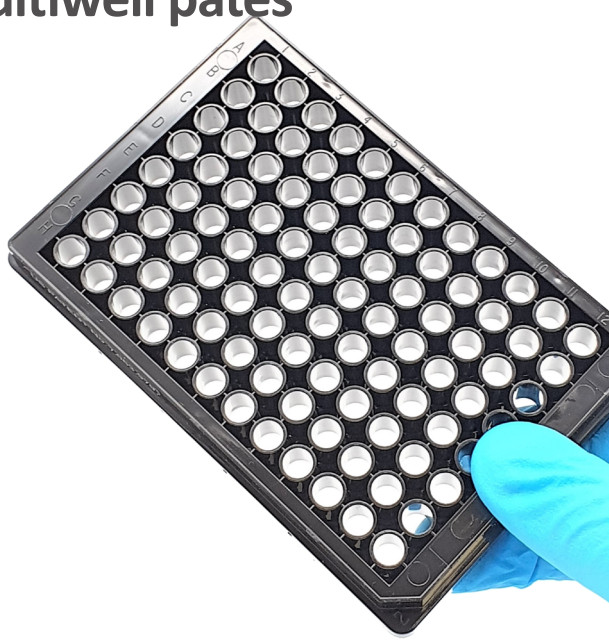
4Dcell micropatterned multiwell plates

User protocol

USER GUIDE



How to use 4Dcell micropatterned multiwell pates



Materials included

Micropatterned 96-well or 384-well plates
Adaptable lid

Materials not included

20-200 µL single or multichannel micropipette
Distilled water
PBS

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.

Shipping & storage

The micropatterned 96-well or 384-well plates are shipped in sterile sealed bags. Each plate is provided with a sterile transparent lid packed individually. The plates can be stored unused at room temperature for up to six months from the date of delivery.

Before starting

To avoid contaminations, only manipulate the plates using aseptic techniques in a laminar flow hood.

The micropatterns consist of a thin layer of polymer bound to the surface of the glass. To avoid scratching this layer, pipette solutions in and out of the wells carefully and avoid touching the surface of the glass.

The timing of protein and/or cell incubation can vary depending on your needs. The protocol suggested by 4Dcell was optimized for HeLa cells. You may need to adapt this protocol for your cell type.

Incubation with an adhesion protein is optional. We suggest only using an adhesive protein if your cell type does not readily adhere to glass substrates. Several adhesive coating proteins can be employed if needed (e.g. fibronectin, laminin, or collagen).

Protocol

a. Open the individual plastic bag containing the plate using aseptic technique in a laminar flow hood.

If coating the plate with an adhesion protein is necessary, proceed to step a.1. Otherwise, proceed directly to step b.

a.1. *Optional: Pipette 100 µL (50 µL for the 384-well plate) of your adhesive protein in each well*[1)(2)].

a.2. *Optional: Incubate for 30 min to 1 hour at room temperature.*

a.3. *Optional: Discard the adhesive protein solution from the wells*

a.4. *Optional: Wash each well 3x with 200 µL (100 µL for the 384-well plate) of distilled water and allow them to air dry before proceeding to the cell seeding step.*

b. Prepare a cell solution. We recommend 30 000 – 40 000 cells/mL. Prepare at least 20 mL (40 mL for the 384-well plate) to ensure you have enough solution for your entire plate[3].

c. Pipet 200 µL *(100 µL for the 384-well plate)* of cell suspension into each well. To prevent cells gathering in the center of the wells, do not unnecessarily move the plate at this stage. Cell adhesion can be encouraged by placing the plate on a warm surface (30°C) before pipetting and not touching the plate during or after pipetting.

d. Incubate the plate containing the cells for 1 hour (or more, depending on cell type) at 37°C, 5% CO_2.

e. Remove medium and unattached cells and wash with pre-warmed culture medium or sterile PBS[4].

f. Discard medium or PBS, add 200 µL of fresh pre-warmed culture medium per well and incubate cells overnight (or for the desired time).

g. Compare the patterns created by the cells with the micropatterned plate map (provided in the brochure).

Notes

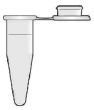
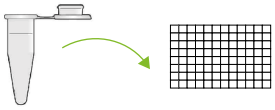
(1) The fibronectin solution provided by 4Dcell has a concentration of 50 µg/mL. It can be adjusted according to the cell type.

(2) The patterns can be visualized using fluorescent fibrinogen or other fluorescent protein. The fibrinogen solution provided by 4Dcell has a concentration of 20 µg/mL.

(3) The seeding density is optimized for HeLa cells. The number of cells added and the adhesion or culture times could vary for other cell types.
Make sure the cell suspension is thoroughly re-suspended before pipetting into the wells. It is better to use cells that are at a confluency around 50% or less.
High confluency of cells before re-suspension and seeding onto the plate will promote cell-cell adhesion and cell cluster formation, which is not suitable for single cell patterning.

(4) Removing the unattached cells will reduce cell clustering and bridging between micropatterns.

Essential steps

	Step	Description
	1. Optional: coating with adhesive protein	Addition of an extracellular matrix protein to each well (e.g fibronectin, collagen laminin
	2. Optional: incubation	Incubation for 30-60 minutes at RT
	3. Optional: 3x wash	Washing of each well x3 with the dedicated volume of distilled water. Air dry the wells.
	4. Preparation of cell solution	Preparation of enough volume of cell solution for the whole plate.
	5. Addition of cell solution to the plate	Addiction of 100 µL of cell solution into each well.
	6. Incubation	Incubation for at least 60 minutes at 37°C, 5% CO_2
	7. Remove unattached cells	Aspirate the medium with unattached cells and wash each well with medium or PBS
	8. Incubation	Incubation of cells at 37°C, 5% CO_2 for the desired period.
	9. Analysis	Compare the patterns created by the cells with the micropat-terned 96-well plate map.