4Dcell
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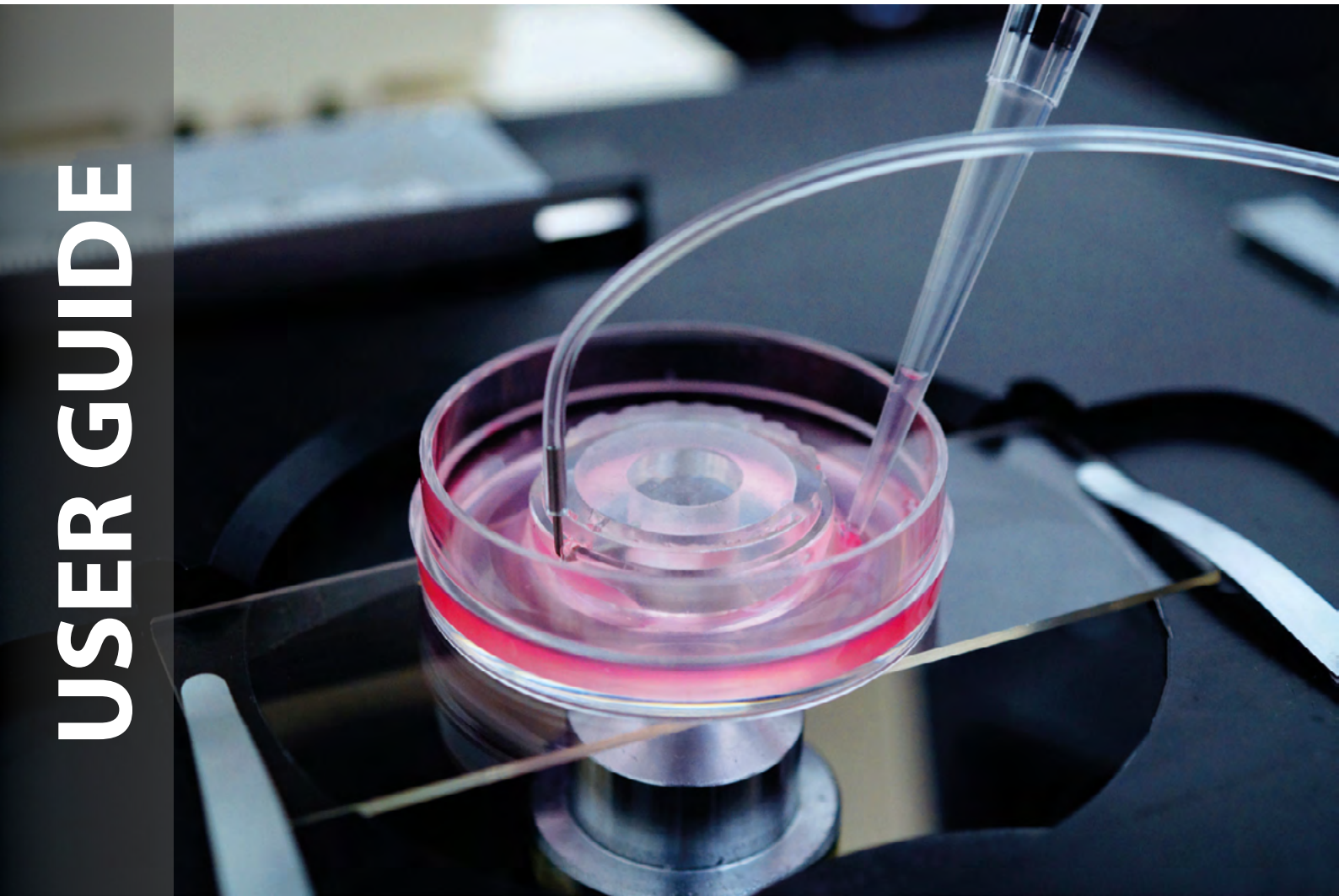
4Dcell dynamic cell confiner

Cobalt - Autonomous vacuum pump

User protocol

USER GUIDE



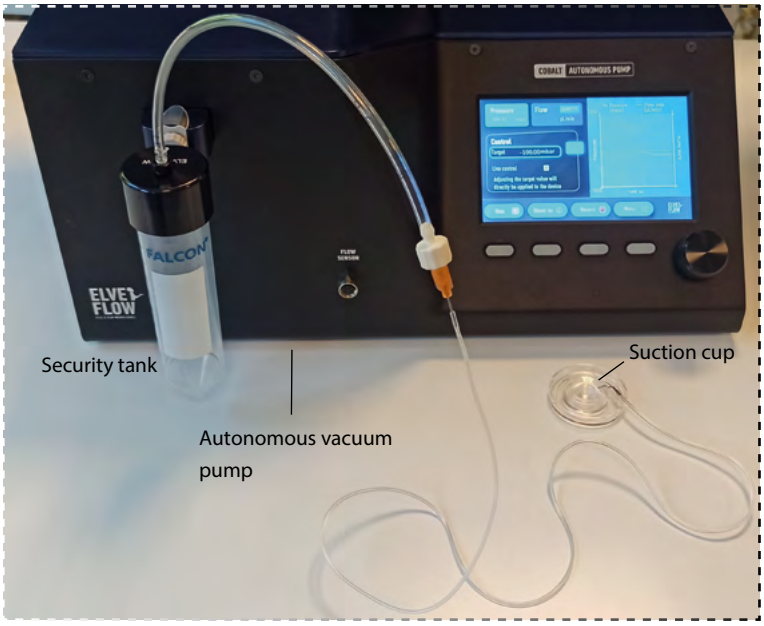
How to use 4Dcell dynamic cell confiner

The 4Dcell dynamic confiner kit enables the confinement of cells or other biological specimens between two parallel surfaces to a defined micrometer height with sub-micrometer precision.

Cells can be cultured in a standard cell culture dish, with either a plastic or glass bottom.
The confinement height (the distance between the substrate on which cells are seeded and the device itself) is determined by micropillars made of polydimethylsiloxane (PDMS).
The micropillars are fabricated on a 10 mm glass coverslip, which is attached to a PDMS piston, the so-called suction cup. The lowering of the confinement coverslip towards the cells is controlled by a vacuum pump.

Material Included in the kit

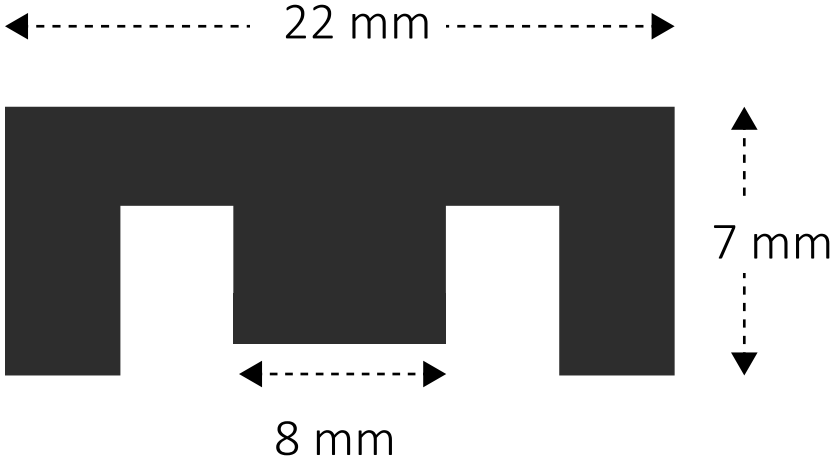
Autonomous vacuum pump
Confinement Coverslips
Suction Cup
Kit of connectors (partially assembled)
Security Tank (Falcon tube with adapted lid)
Humidity filter
Tape
Scalpel
Extra kit of connectors
(to replace parts when necessary)



Other recommended material

70% ethanol
Distilled water
Tweezers

Suction Cup dimensions



1. Assembly of the autonomous vaccuum pump

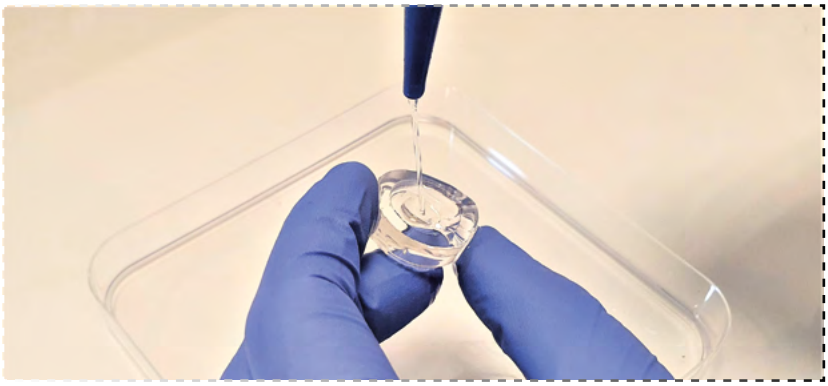
The autonomous vacuum pump can be set up according to Elveflow's manual provided with the kit (via email).

Please note that this device should be assembled before setting up the biological experiments to avoid any unforeseen events.

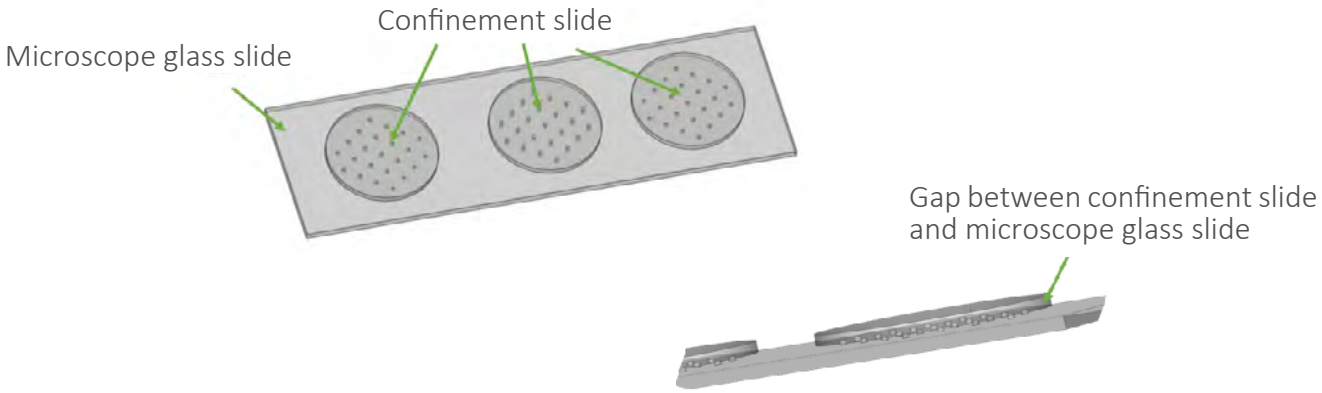
⚠ Please note that when the live control mode is selected, the pressure can be adjusted in real time using the knob.

2. Preparing the suction cup and confinement coverslip

Before performing any experiment, the suction cup must be cleaned. If the device has dust particles attached to it, use the tape provided in the kit to gently remove them.
After removing large dust particles, clean the suction cup with 70% ethanol, and dry it carefully with lint-free absorbing paper (e.g. kimwipe) or an air gun.
Pay attention especially to the bottom of the suction cup, where it will be in touch with the cell substrate and with the confinement coverslip.
Be careful not to leave the device in contact with ethanol for a long time, or it will absorb ethanol and release it into the cell medium during your experiments.



Confinement coverslips are individually shipped according to the micropillars height. The micropillars are placed on a glass slide facing down. You can use a blade to gently remove the confinement slide (be careful not to break it). Place the blade in the small gap between the confinement slide and the microscope glass slide. Afterwards, tilt the blade so that the confinement slide detaches from the microscope glass slide. Be careful not to lose the orientation of the coverslip.

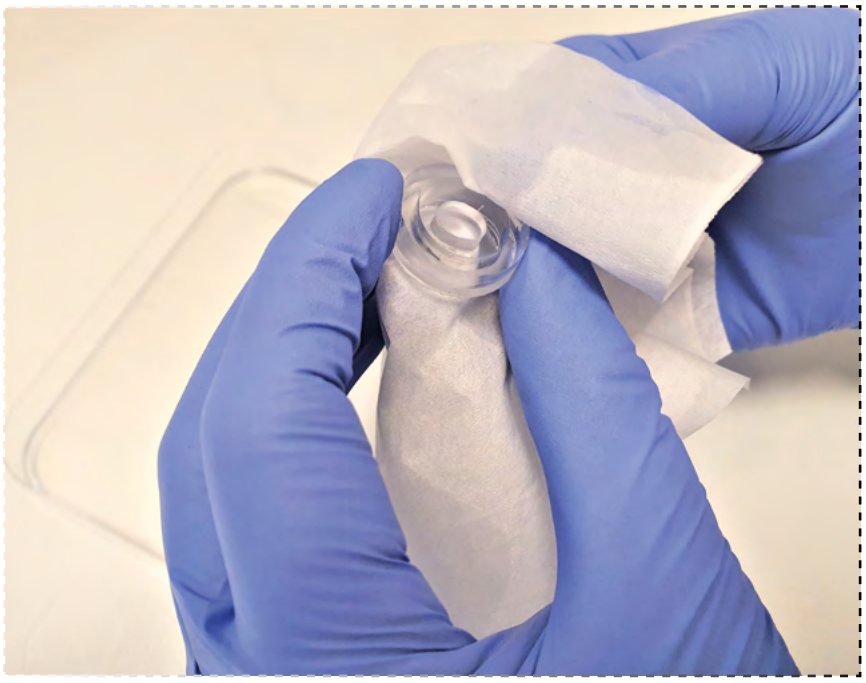


At least 1 h before the experiment, incubate the confinement coverslip and the suction cup in cell culture medium. Use the same medium that will be used for the experiment. The structured PDMS side of the slide should face upwards during incubation. Be careful not to lose the orientation of the coverslip.

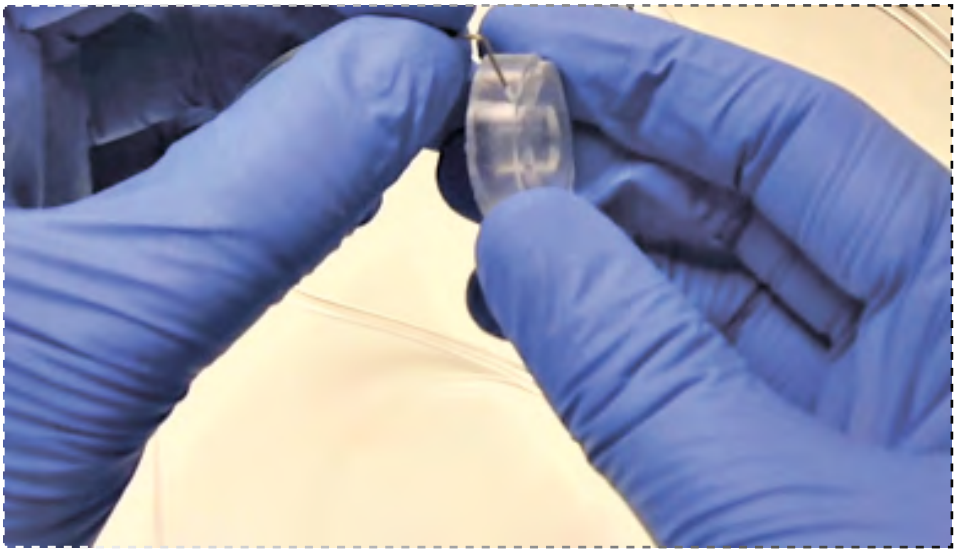
3. Assembling the dynamic confiner

Place the adapted lid on the Facon tube. After, plug the black connector from the pre assembled kit of tubes and connectors to the lid. Finally plug it to the autonomous pressure controller, as shown in the image below:

Remove the suction cup from the medium and dry it with absorbing paper. Pay special attention to the bottom face of the device, as it should be dried carefully to ensure proper adhesion of the confinement slide.

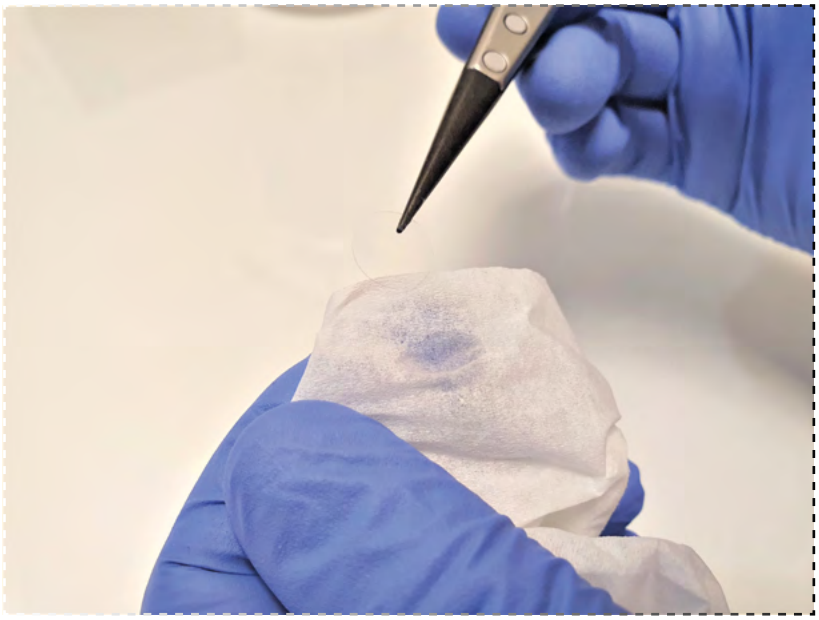


Plug the thin tube to the suction cup using the metallic capillary tube.



⚠ After connecting the suction cup to the autonomous pressure controller, you can verify that there are no leaks in your system by placing the suction cup on a clean surface (for example a container similar to the one used for your cells) and verifying that the piston moves down when you increase the pressure to -100 mbar. This should be visible as a divot in the center of the suction cup.

Prepare an absorbing paper to dry the back side of the confinement slide. Pick up the confinement coverslip with tweezers, and dry its back side on the absorbing paper by placing it onto the paper.



Place the confinement slide onto the piston of the cell confiner. The structured side faces up. The slide should stick naturally onto the piston. Please note that if the back side of the coverslip or the bottom side of the suction cup are not completely dry, they will not stick together and the confinement slide can be lost during your experiment.

4. Cell culture

This is an example for adherent cells cultured in a Petri dish. 4Dcell provides petri dishes with the kit, although you can use any flat-bottomed dish you would like that can accomodate the suction cup.

To ensure cell adherence, the petri dish can be coated with fibronectin or other ECM matrix of your choice. Incubate for 1 hour at room temperature.

Remove the fibronectin solution.

Re-suspend the cells at the desired density in 2mL of cell culture medium and pipette into the petri dish. Let the cells adhere to the substrate (the timing depends on the cell type). This implies that the cells need to be seeded just before the experiment. Please note that the level of medium should never reach the capillary connector (that connects the suction cup to vacuum pump), otherwise the medium will be aspirated.

5. Confining cells

Put the device in contact with the cell culture substrate (e.g. petri dish). At this point, cells can be observed before confinement.

Be careful not to deform the device so that a good seal can be achieved. Also, be careful not to push on the center of the device, or the cells can be crushed. The petri dish will not move during the confinement operation, and several cells can be observed simultaneously.

Tune the controller to - 3 kPa (-30 mbar). At this pressure, if the device is well sealed, the piston should start lowering, but the confinement slide does not yet touch the cells. Please be aware that you can only read the vacuum/pressure value on the AF1 controller.

To confine cells, slowly decrease the pressure down to -10 kPa (-100 mbar). By observing the cells during this time, you should see the confinement slide touching them at approximately - 5 kPa (-50 mbar).

Confinement can be released by increasing the pressure back to -3 kPa.

At this point the confinement can also be repeated in the same position by increasing the pressure back to -10 kPa (-100 mbar).

After finishing your experiments, you can clean the suction cup with water and store it in a dry place. For future use, it can be sterilized with ethanol.