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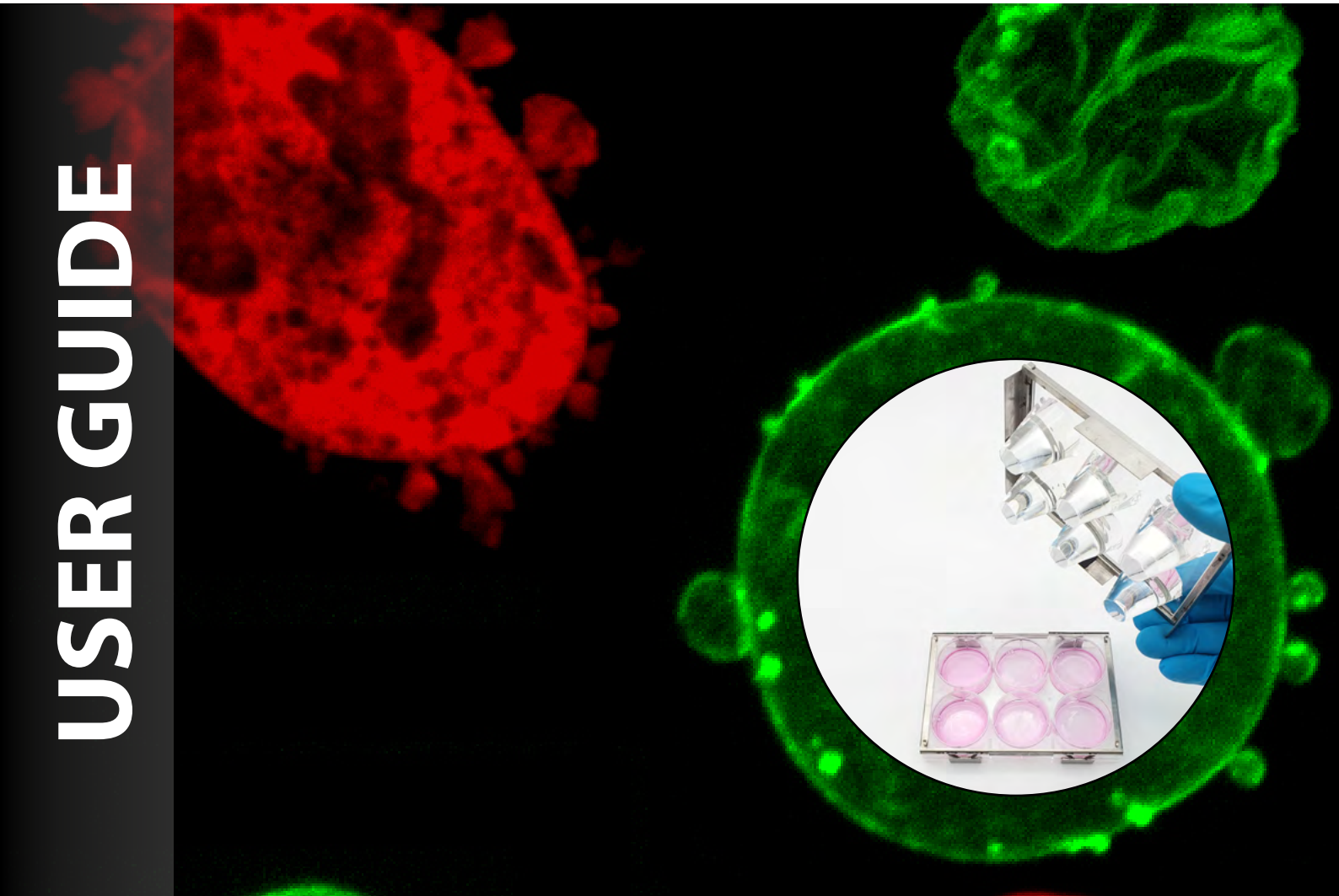
4Dcell static 6-well cell confiner

User protocol- CSOW 620

USER GUIDE



How to use 4Dcell static 6-well confiner ‘CSOW 620’

Support

A video is available online to understand each step to handle perfectly your CSOW 620:
www.4dcell.com/cell-culture-systems/cell-confinement/static-cell-confiner

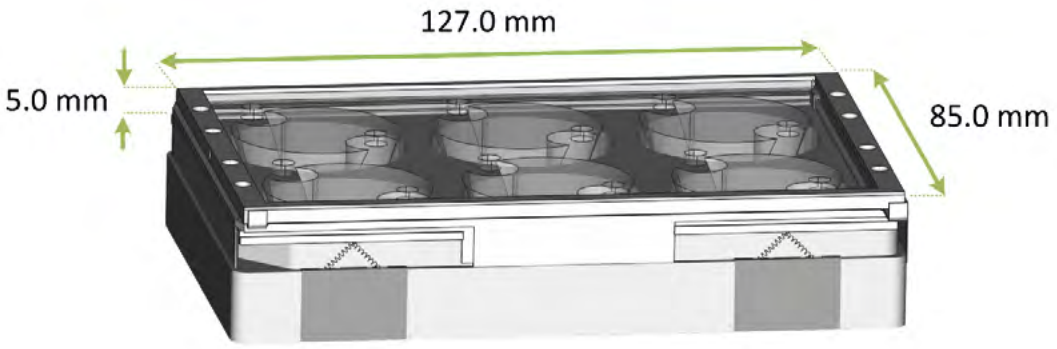
Material Included

4DCell CSOW 620
Confinement slides/coverslips with PDMS micropillars (12 units)
Confinement PDMS pistons (12 units)
Small blade
Tape
Caps for closing the holes on the lid

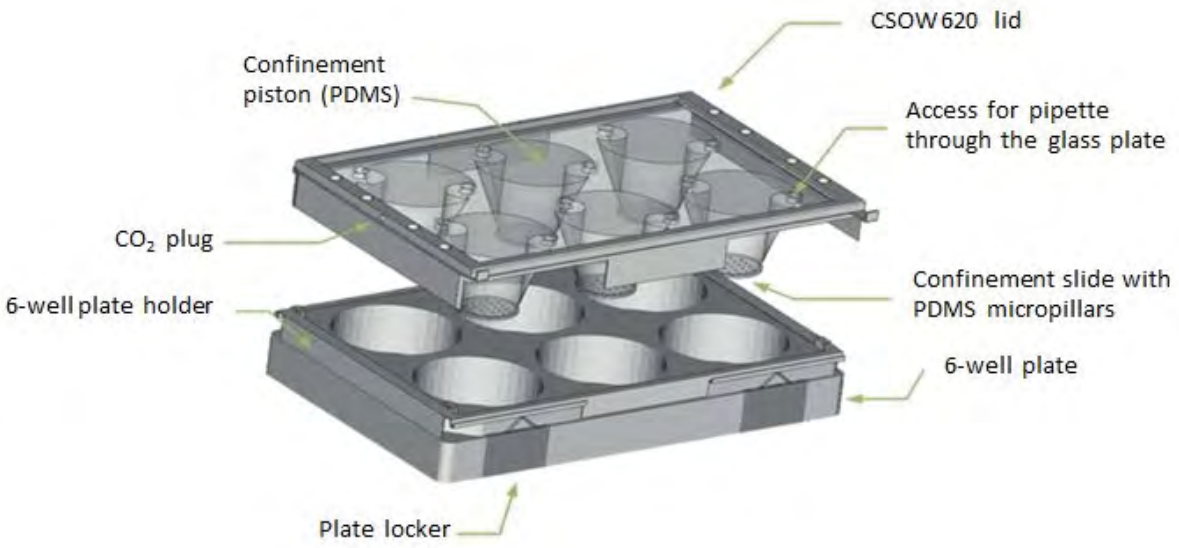
Other recommended material

70% ethanol
Tweezers
6-well plates (Glass Bottom MatTek)

CSOW 620



CSOW 620 parts



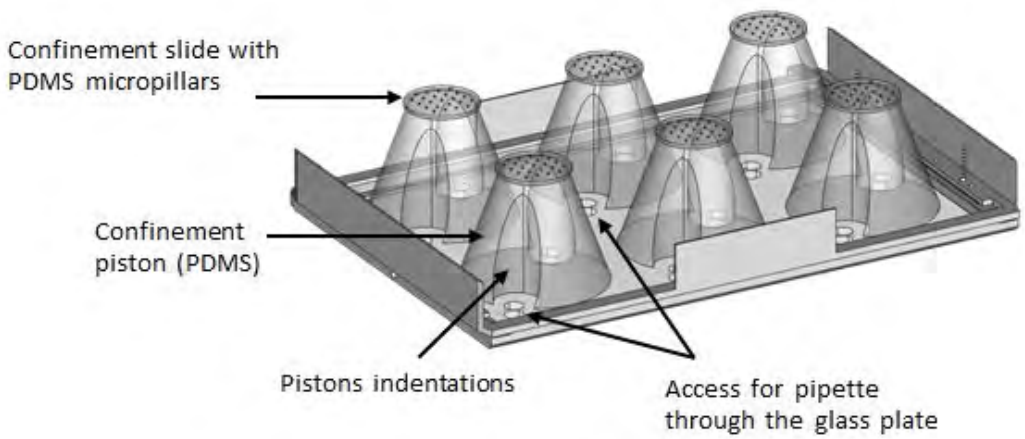
A. Handling and assembling the confiner

Note: The different items of the confiner kit are shipped in sterile bags. To avoid contamination, only manipulate the items using aseptic techniques in a laminar flow hood.

1. Clean the two components of the CSOW 620 confiner, the lid (top) and holder (bottom), using absorbant paper and 70 % ethanol.

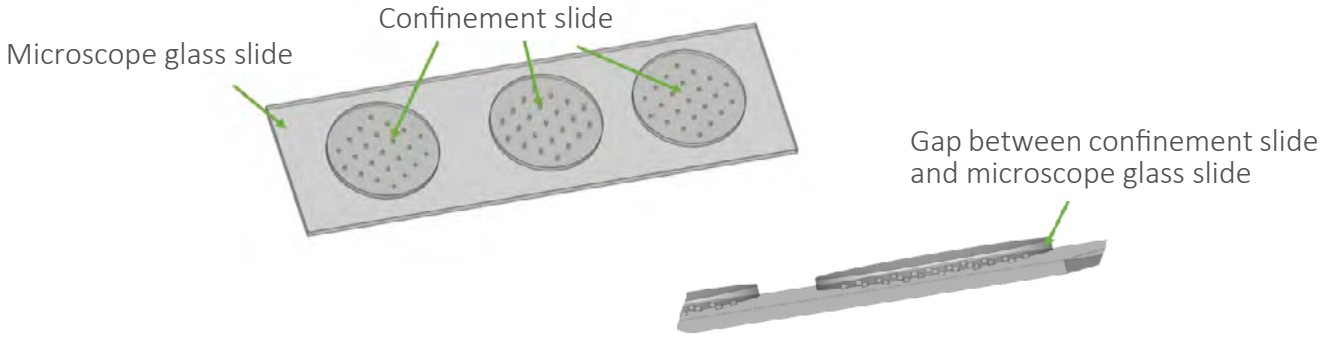
2. Clean the pistons using 70% ethanol. If the PDMS pistons have particles of dust these can hinder the good attachment to the glass and interfere with imaging. In this case, remove the dust particles with the tape provided and rinse once again with 70% ethanol.

3. Attach the pistons to the CSOW 620 lid making sure the indentations of the pistons are aligned with the holes in the glass plate.

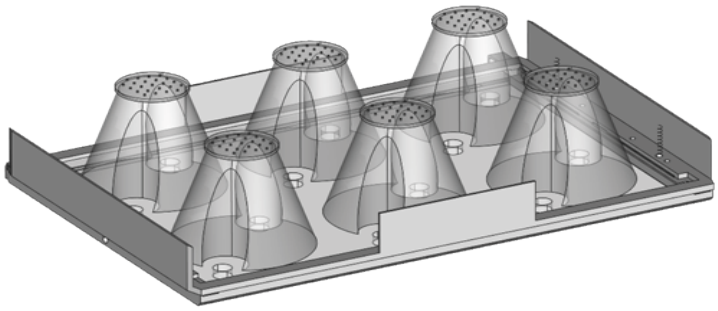


The PDMS is usually sticky, enabling it to be attached to glass very easily. The holes in the lid (access for pipette through the glass plate) can be used to pipette media, aspirate it, etc. The holes can be closed with the white caps provided in the kit. If you find it difficult to remove the caps, please use tweezers to do so.

4. Confinement slides are individually shipped according to confinement height with the pdms pillars 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.

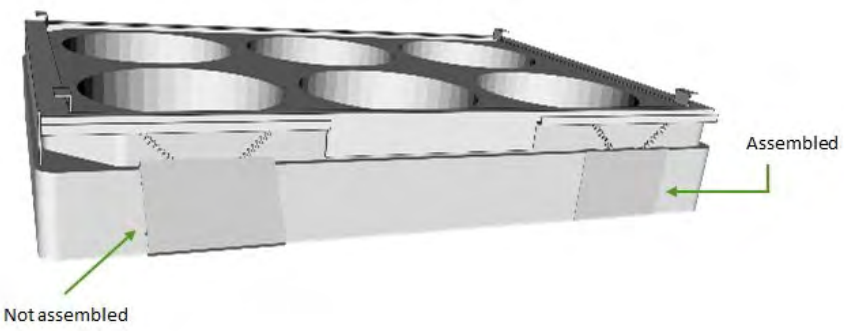


5. Place the confinement slide on the PDMS piston with the pillars facing up as depicted in the figure below. Make sure the slides are well centered on the pistons to avoid breaking the slides once in contact with the inner circle on the bottom of the plate. Avoid creating air bubbles between the PDMS piston and the glass coverslip.

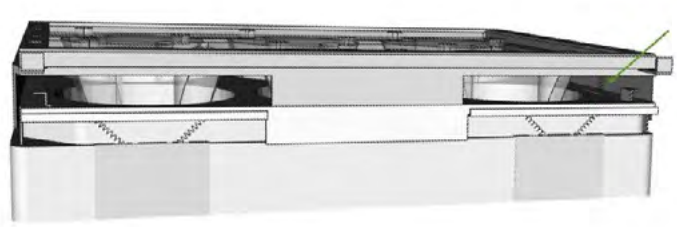


Tip for cell culture (section B of this protocol, page 6): *Incubate the pistons and the confinement slides in culture medium to equilibrate the PDMS for at least one hour. To do this you can put the CSOW 620 lid containing the confinement pistons + the slides on a regular 6-well plate. The springs attached to the inside of the lid will prevent the confinement slides from touching the bottom of the plate. Note that PDMS absorbs small hydrophobic molecules from the medium. Therefore, if drugs are used in the experiment, these drugs should to be included in the medium in this incubation step. You don't need to lock the confiner at this stage (resting position).*

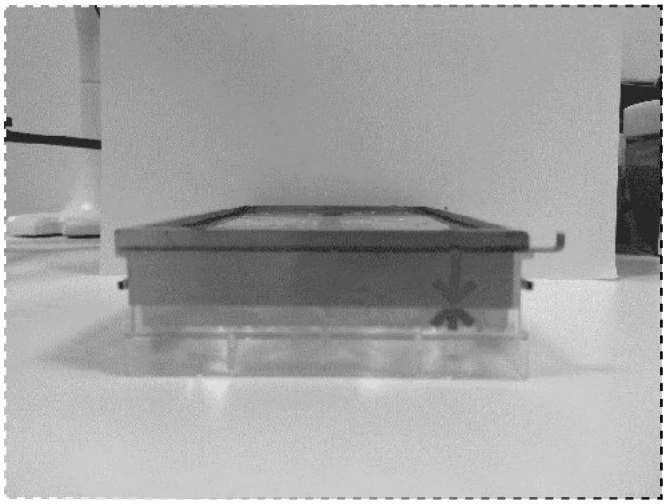
6. To assemble the bottom part of the confiner to the 6-well plate, gently pull the metal pads down so that they grab on to the bottom of the plate. The pads should be pulled one by one, starting with one pad from one side, then the next pad from the diagnonally opposite side, then the last two in the same manner (never pull the two pads from the same side one after the other).



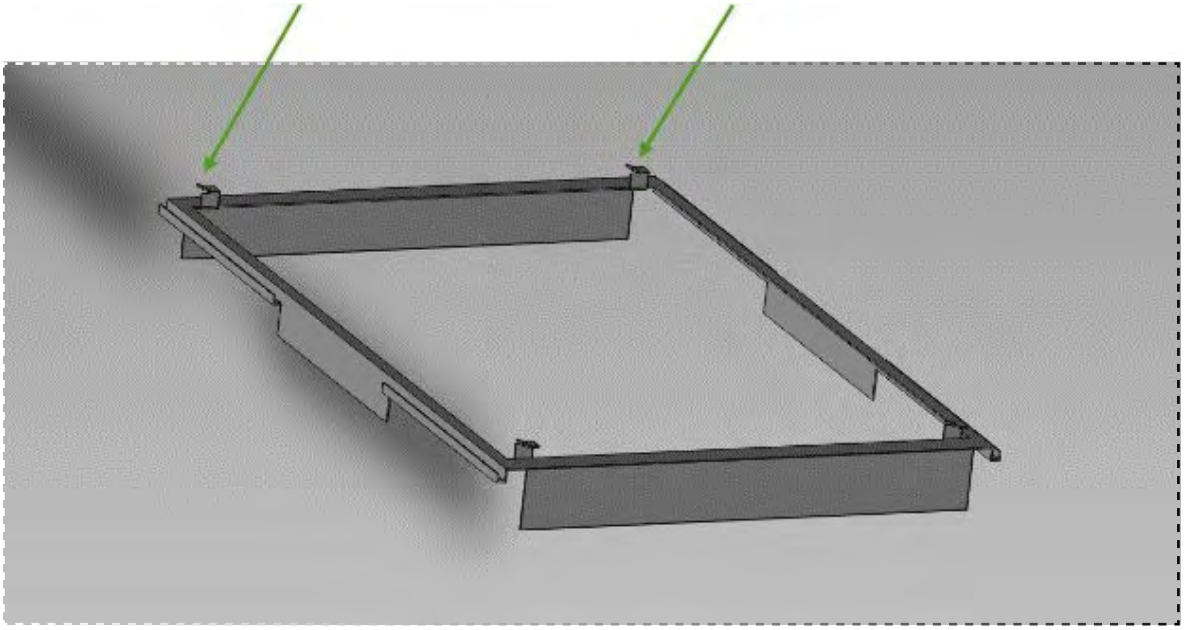
7. Place the lid on the top of the assembled holder. Allow it to be in the resting position, in which the springs hold the lid up.



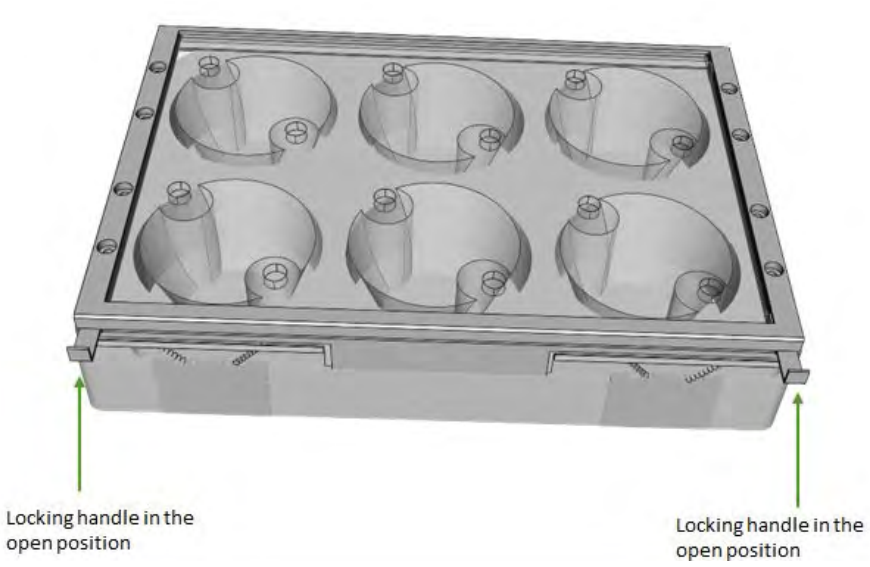
Be sure to align the marks:



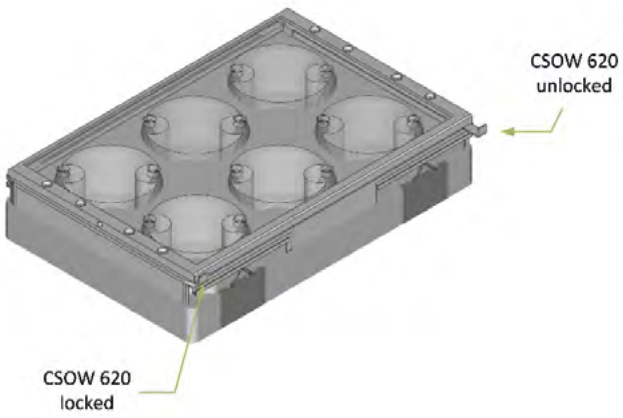
The tilted part of the plate holder should be facing the front when you assemble the lid and the holder.



Please be careful because the locking only works if the plate is assembled in the correct orientation. Make sure that the locking handles are open:



8. To lock the confiner, press the lid down on both left and right sides (as shown below, right image) and simultaneously lock the two handles by pushing them in. If you feel resistance, make sure the plate is assembled in the correct orientation as described in step 7.



9. To unlock the confiner, gently pull the locking handles out and let the lid come up. If it does not come up naturally, gently move it upwards.

10. If you have issues removing the holding pads from the 6-well plate (when needing to change the plate), you can use tweezers to help you remove the metal pads.

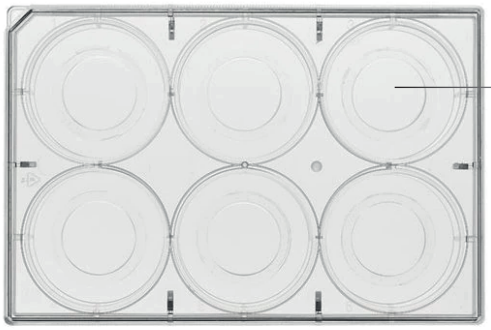
B. Handling and assembling the confiner with cells

11. Make sure the confiner with the pistons are properly cleaned (steps 1 to 2).

12. Proceed to steps 4 to 6.

13. ***Optional: PDMS Stencils.*** If using PDMS stencils to reduce the area available for the cells to adhere, carefully place the PDMS stencils in the wells using clean sterile tweezers. The stencils are shipped clean (dust removed) and sterile. When placing the stencil in the well, care must be taken to place the stencil in the center of the well, without deforming it. Once it is in place, look at the bottom of the plate to ensure that the stencil adheres to the glass on its entire surface*. In areas in which the stencil does not adhere you should see decreased transparency.

14. To ensure cell adherence, the 6-well plate should be coated with fibronectin or any other ECM matrix protein of your choice(1). A volume of 500 µL of fibronectin solution is enough to cover the central circular part of the well. Incubate for 1 hour at room temperature.



15. Remove fibronectin solution.

16. Resuspend the cells at the desired density in 500 µL of cell culture medium (250 µL if using a stencil) and pipette into the wells. Let the cells adhere to the substrate (the timing depends on the cell type).

17. Place the confiner lid on top (after incubation in another 6-well plate for 1 hour in the same temperature and humidity conditions as the cells, as described in step 7)**.

18. To confine the cells, press the lid down on both left and right sides (as shown above in step 8) and simultaneously lock the two handles. If you feel resistance, make sure the plate is assembled in the correct orientation as described in step 7.

19. Add 2 mL of medium through the holes on the lid that give access to the bottom of the plate.

20. To release the confinement, gently pull the locking handles out and let the lid come up. If it does not come up naturally, gently move it upwards (see images on step 8).

**If the surface of the stencil does not adhere to the glass on its whole surface, first try taping it with the tweezers to improve adherence. If the stencil doesn't adhere in some regions, remove it and place it carefully without deforming the shape. Ensure that the stencil does not touch the edges of the plate. If dust particles are visibly interfering with the adhesion of the PDMS, use the tape provided by 4Dcell to clean the surface of the PDMS.*

***Incubate the pistons and the confinement slides in culture medium to equilibrate the PDMS for at least one hour. To do so you can put the CSOW 620 lid containing the confinement pistons + the slides on a regular 6-well plate. The springs inside the lid will prevent the confinement slides from touching the bottom of the plate.*
Note that PDMS absorbs small hydrophobic molecules from the medium. Therefore, if drugs are used in the experiment, these drugs should to be included in the medium in this incubation step. You don't need to lock the confiner at this stage (resting position).

(1) A volume of 500 µL is enough to coat the inner circle of the well glass bottom MatTek 6-well plates.
References: Liu *et al.*, 2015, Cell 160, 659–672