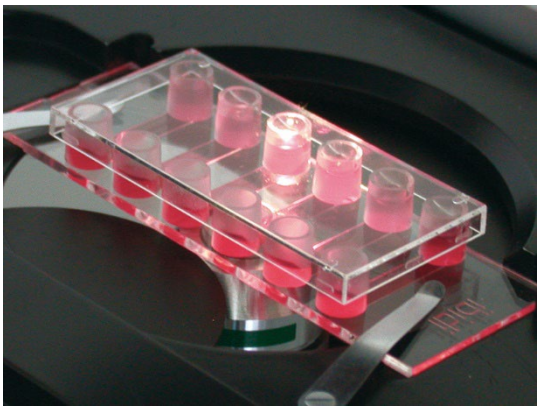
Cell Culture in ibidi Channel Slides: Using the µ-Slide VI 0.4 as an Example



This Application Note illustrates how to grow adherent cells inside the ibidi Channel Slides, using the µ-Slide VI 0.4 as an example. Here, we will describe cell seeding and medium exchange along with the properties and advantages of channel slides.

The µ-Slide VI 0.4 with 6 channels is ideal for static cultivation, treatment, and staining of living and fixed cells. In addition, its ibidi Polymer Coverslip Bottom provides ideal optical conditions for phase contrast and fluorescence microscopy.

Alternatively, the µ-Slide VI 0.4 can be connected to a pump, enabling observation of cells under flow conditions.

Besides the ibiTreat version (tissue culture-treated), the µ-Slide VI 0.4 is available with different coatings (uncoated, Poly-L-Lysine, Collagen I, and Collagen IV). With the µ-Slide VI 0.5 Glass Bottom, ibidi also provides a version with a glass coverslip bottom for special microscopy applications.

Find more detailed information about cell culture and preparation for imaging here.

Related Documents

Instructions µ-Slide VI 0.4 (PDF)

AN 09: Fluorescence Staining Using the µ-Slide VI 0.4 (PDF)

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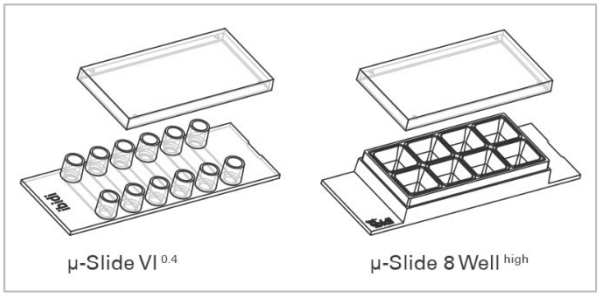
1. Properties of ibidi Channel Slides: A Comparison to Open Wells

The following section compares the µ-Slide VI 0.4 (channel slide) properties with the µ-Slide 8 Well high (open well format). The channel principle exhibits advantages for several applications compared to standard open well formats.

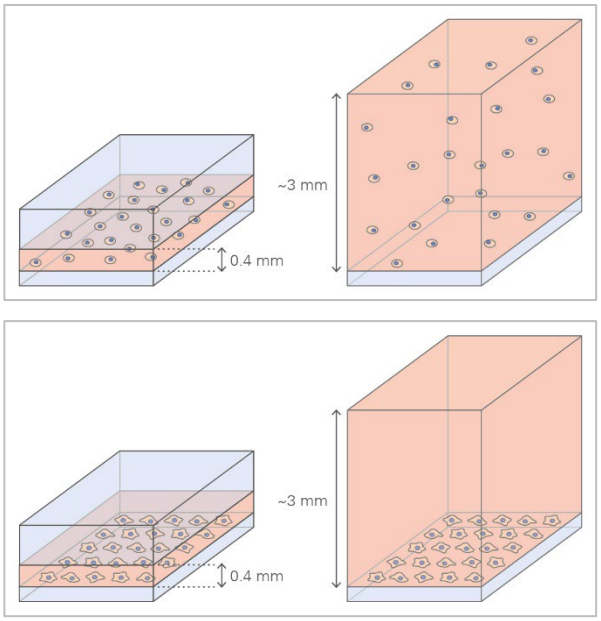


1.1. Cell Seeding Concentrations for Different Slide Formats

A concept to consider when seeding cells in different formats is the height of the liquid inside the labware (filling levels). A channel slide (µ-Slide VI 0.4) and an open well slide (µ-Slide 8 Well high) have different filling levels. Therefore, different concentrations must be used for seeding adherent cells to obtain the same number of cells per surface area.

The channel height and thus the filling level of the µ-Slide VI 0.4 are 0.4 mm. In the µ-Slide 8 Well high, the recommended volume is 300 µl culture medium per well, making the filling level approx. 3 mm. These two values differ by a factor of 7.5. Therefore, for getting the same number of cells that adhere per surface area, the required cell concentration for seeding must be 7.5 x higher in the µ-Slide VI 0.4 than in the µ-Slide 8 Well high.

To get comparable degrees of confluency after cell adhesion, we recommend using 3–7 x 10^5 cells/ml for the µ-Slide VI 0.4 and 4–9 x 10^4 cells/ml for the µ-Slide 8 Well high.

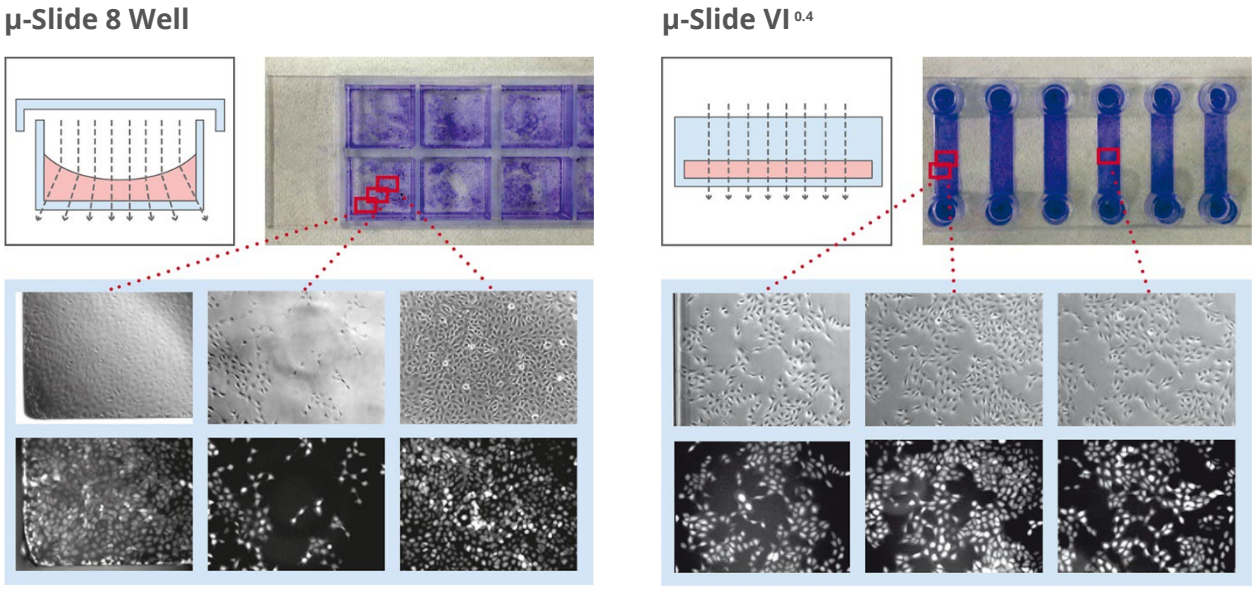


When using the µ-Slide VI 0.4 (left) and the µ-Slide 8 Well high (right), different cell concentrations must be seeded to get the same number of adherent cells per unit area. The example shown here is with 25 cells.

1.2. Homogeneous Cell Distribution

In addition to being optimized for cell culture under flow, the ibidi channel slides format provides the ideal geometry for homogeneous cell distribution.

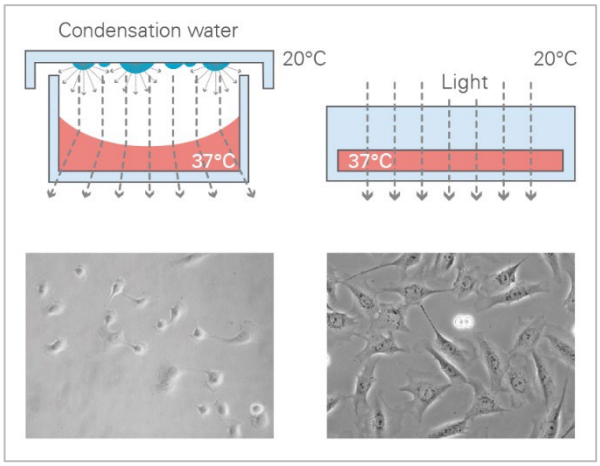
In contrast, cell densities in open wells are very dependent on the handling during cell seeding. Due to the channel's closed geometry, cell densities do not vary depending on their positions inside the slide, Further, they are not affected by handling and treatment during and after cell seeding. To demonstrate the influence of the slide geometry on cell distribution, cultivated cells within the open µ-Slide 8 Well and the channel µ-Slide VI $^{0.4}$ were visualized macroscopically and microscopically, respectively. Macroscopically, the cells cultured in the µ-Slide 8 Well formed characteristic patterns, which is typical for all open well formats. In general, the cell density in the middle of the well reached its maximum, while fewer cells attached to the outer well regions. Some cells congregated to the edges of the well. This inhomogeneous cell distribution is influenced by the meniscus (a natural phenomenon in all open well formats) and handling of the slide (e.g., shaking or movement). In contrast, the cell distribution in the µ-Slide VI $^{0.4}$ stayed homogeneous. Phase contrast and fluorescence microscopy confirmed these differences.



Geometric scheme and cell distribution in the µ-Slide 8 Well (left) and the µ-Slide VI $^{0.4}$ (right). Please note the typical non-homogeneous cell distribution in an open well caused by the natural meniscus effect and handling of the slide. Cells were visualized macroscopically using crystal violet staining (top), and microscopically using phase contrast (middle) and fluorescence microscopy (bottom).

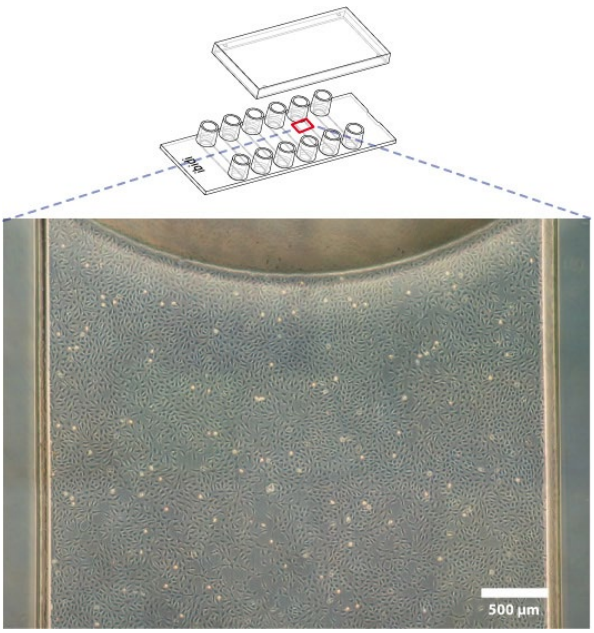
1.3. No Lid—No Condensation

Condensation, which occurs on the lid of the culture vessel after being removed from the incubator, is another effect that disrupts phase contrast microscopy. When using the ibidi channel slides, condensation inside the optical pathway is intrinsically impossible.

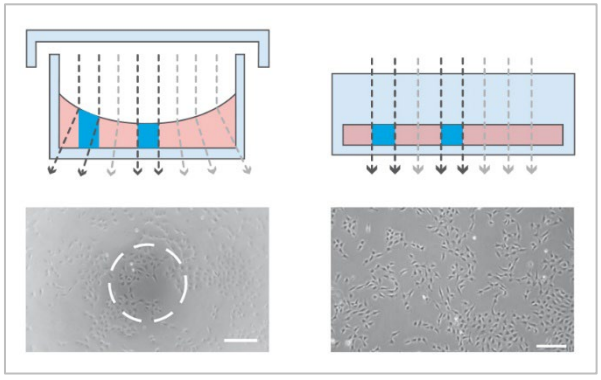


1.4. Optimized Phase Contrast Microscopy

The meniscus formation in open well chambers at the air-water-interphase is a natural and unavoidable effect that can disturb phase contrast microscopy. In contrast, meniscus formation within channel slides (e.g., the ibidi µ-Slide VI 0.4) is geometrically impossible. Therefore, phase contrast microscopy in channel slides is superior to small open wells.



Unlike in open wells, the channel does not disturb the beam path of the phase contrast microscope.



96 well plate or small open well:
Significantly impacted by the meniscus, causing low contrast near the edges.

Channel or parallel plates:
No meniscus, good phase contrast over the entire area.

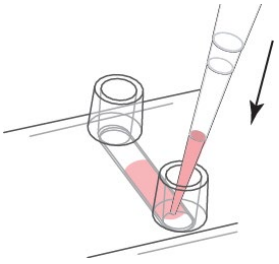
2. Cultivating Cells in the µ-Slide VI $^{0.4}$ Channels

Please read the Instructions before working with the µ-Slide VI $^{0.4}$ ibiTreat. Perform all steps under sterile conditions. Before starting the experiment, prepare adherent cells in a standard cell culture flask (e.g., T75). The cells should be healthy and optimally subconfluent on the day of the experiment.

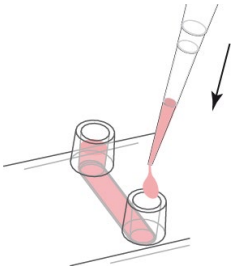
The volumes used in this protocol are valid for the µ-Slide VI $^{0.4}$. Please find the respective volumes for the µ-Slide VI $^{0.5}$ Glass Bottom in the Instructions µ-Slide VI $^{0.5}$ Glass Bottom (PDF).

2.1. Cell Seeding

1. The day before seeding the cells, we recommend placing the cell medium and the µ-Slide into the incubator for equilibration. This will prevent the medium inside the slide, and the slide itself, from allowing air bubbles to form during the incubation time.
2. On the day of the experiment, trypsinize and count cells as usual. Dilute the cell suspension to the desired concentration. Depending on your cell type, application of 3–7x10^5 cells/ml should result in a confluent layer within 2–3 days.
3. Apply 30 µl of the cell suspension into one channel of the µ-Slide. Place the pipet tip directly on the channel inlet and point it towards the channel as shown. Quick dispensing helps to avoid trapped air bubbles. Remove trapped air bubbles from the channel by inclining the µ-Slide and tapping on one edge. After filling all needed channels, cover the reservoirs with the supplied lid. Incubate at 37°C and 5% CO_2 as usual.
4. After cell attachment (time depends on the cell type, usually approx. 1 hour), fill 60 µl cell-free medium into each Luer reservoir as shown. Don't trap air bubbles. Please note: If you do the filling step after cell adhesion, no cells will be flushed out of the channel. In case you need to fill the reservoirs immediately after cell seeding, please pipet carefully.



Filling in a cell suspension into one channel of the µ-Slide VI $^{0.4}$.

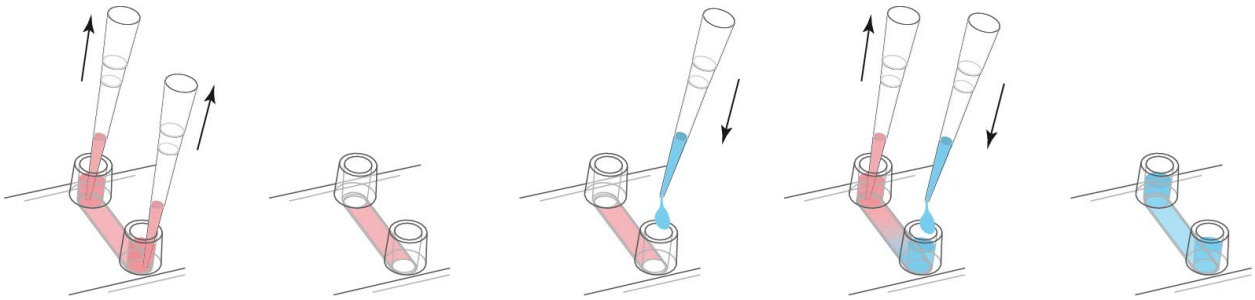


Filling the Luer reservoirs with cell-free culture medium.

2.2. Medium Exchange

Recommended: Continuous Medium Exchange

1. Remove the medium from the reservoirs with a pipette. Do not remove any liquid from the channel itself.
2. Slowly fill 120 µl of fresh medium into one of the reservoirs, which will replace the channel volume by gravity flow. Aspirate from the other reservoir by carefully using a pipette. Attention: Be careful when using a cell culture aspiration device, as this may flush away partially attached cells or clusters.
3. Refill the reservoirs using 60 µl per reservoir.



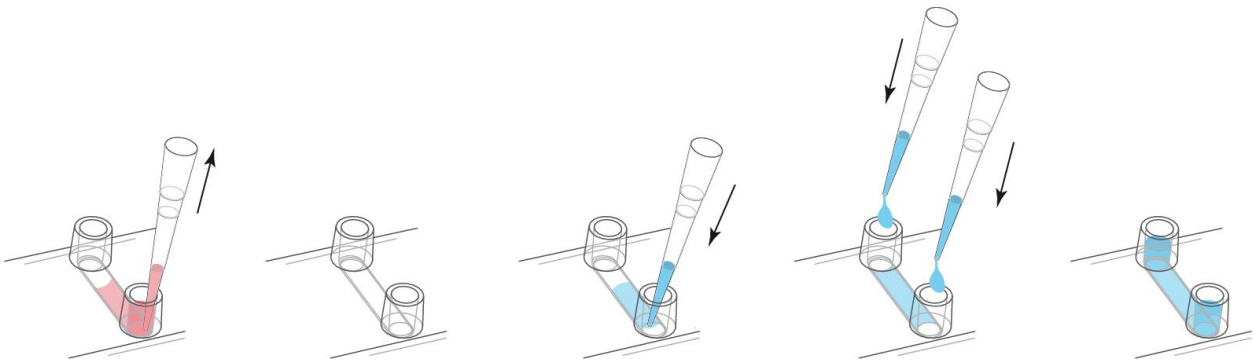
Continuous exchange of medium with minimum 3 times the channel volume.

Recommended for Expensive Liquids Only: Complete Medium Exchange

These steps are recommended only when using expensive supplements, since the cells are more stressed when falling dry. Further, emptying the channel completely may lead to the formation of air bubbles after refilling.

1. Remove the medium from the reservoirs with a pipette.
2. Put the pipet tip directly on the channel inlet and completely aspirate the liquid out of the channel carefully.

   Attention: Be careful when using a cell culture aspiration device, as this may flush away partially attached cells or clusters.
3. To refill the channel, inject 40 µl fresh medium directly into the channel. Avoid trapping air bubbles. Tilt the slide so air will escape upwards while filling the channel with liquid.
4. Refill the reservoirs using 60 µl per reservoir.



Complete exchange of medium with channel and reservoir volume only.