

## How to grow cells inside a $\mu$ -(micro-)channel

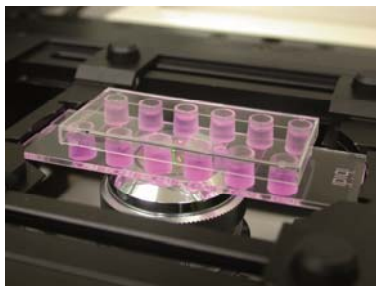
This application note illustrates how to grow adherent cells inside a cell culture micro-channel. Cell seeding, medium exchange, and optical properties will be described. Additionally, the main differences between cell culture channels and standard open well formats are shown.

### 1. Cell Seeding

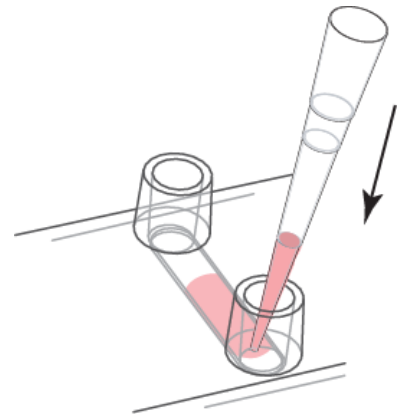
To show the cell seeding and  $\mu$ -Slide handling the  $\mu$ -Slide VI 0.4 is used for demonstration. Prepare the cell suspension (e.g.  $3 \times 10^5$  cells/ml) as usual and apply  $30 \mu\text{l}$  into the channel. Put the pipet tip right on the channel's inlet and point at the channel as shown. Dispense quick and fill the whole channel.



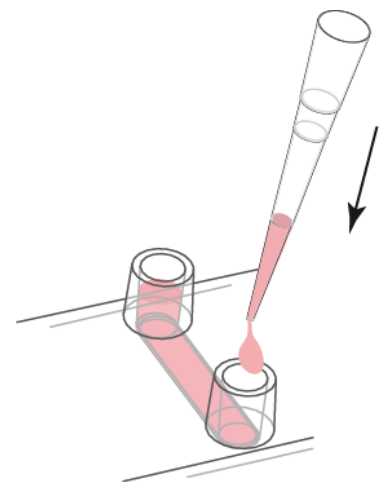
After cell attachment, fill  $60 \mu\text{l}$  cell-free medium into each reservoir as shown. Don't trap air bubbles. If you do the filling step after cell adhesion no cells will be flushed out of the channel. In case you want to fill the reservoirs immediately after cell seeding please pipet carefully.



The  $\mu$ -Slide VI 0.4 filled with cells and culture medium on a microscopic stage.



Filling in a cell suspension into one cell culture channel of the  $\mu$ -Slide VI 0.4.

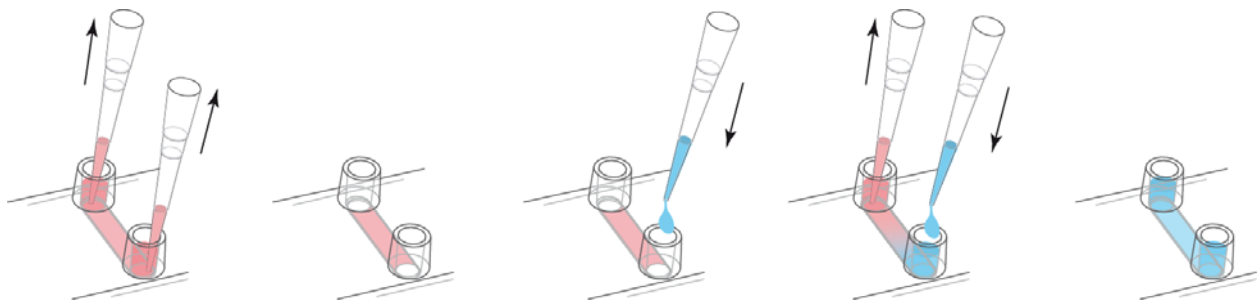


Filling the Luer reservoirs of the  $\mu$ -Slide VI 0.4.

## 2. Medium Exchange

### a. Continuous Medium Exchange – Recommended in Standard Use

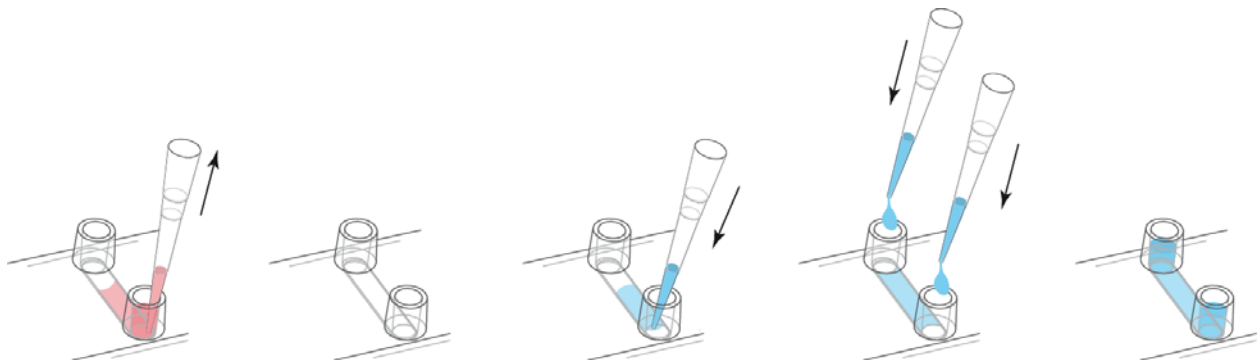
For continuous medium changing remove the old medium from the reservoirs first. Then, add an appropriate amount of fresh medium into one reservoir and aspirate from the other reservoir at the same time. Carefully use a cell culture aspirator. We recommend a volume that is at least 3 times the channel volume. Refill the reservoirs.



Continuous exchange of medium with 3 times the channel volume.

### b. Complete Medium Exchange – Recommended for Expensive Liquids Only

For replacing only the channel volume, empty the reservoirs first. Then, put the pipet tip right on the channel inlet and aspirate the liquid out of the channel carefully. Use a cell culture aspirator to completely remove all liquid. To refill the channel inject fresh medium directly into the channel using the channel volume. Avoid trapping air bubbles. Refill the reservoirs.



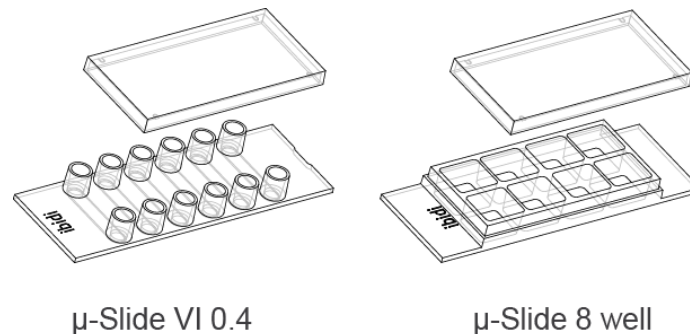
Emptying the channel completely may lead to the formation of air bubbles after re-filling.

Complete exchange of medium with channel and reservoir volume only.

## Application Note 03

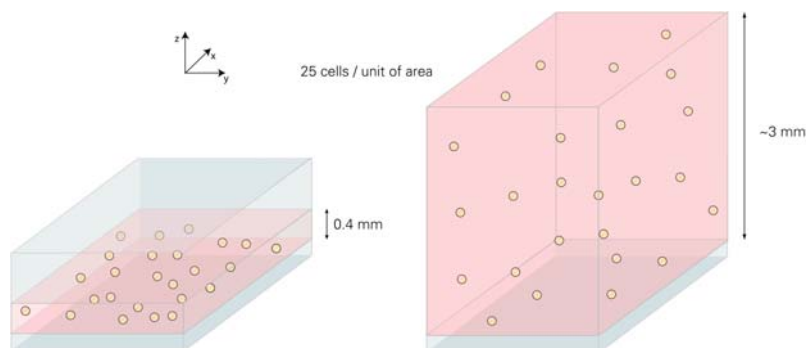
### 3. Channel vs. Open Well

In the following section we compare the properties of the  $\mu$ -Slide VI 0.4 (cell culture channel) with the  $\mu$ -Slide 8 well (open format).



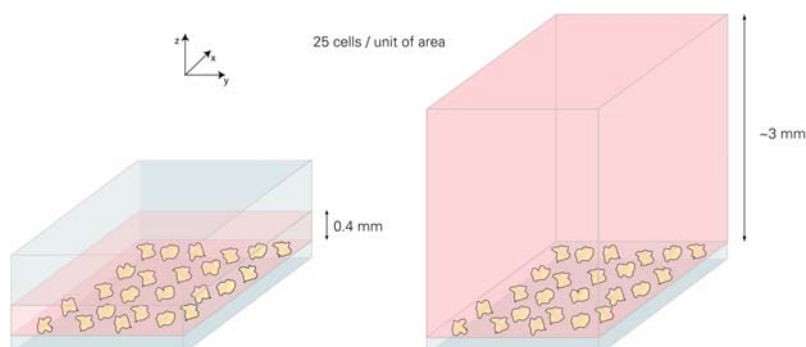
For seeding cells the height of the liquid inside the structure is the main difference. A small area section of both systems is shown below. The distance between bottom and ceiling inside the  $\mu$ -Slide VI 0.4 is 400  $\mu$ m. A filled  $\mu$ -Slide 8 well doesn't have a ceiling. Here, the culture medium stands approx. 3 mm. Effectively, the channel is 7.5 times thinner than the open format.

For cell seeding different cell densities have to be applied to get same amount of cells on the surface. In this example the goal is to seed 25 cells/unit of area. Since the height of liquid inside the  $\mu$ -Slide 8 well is 7.5 times bigger the applied cell concentration has to be lower with the same factor.



**Geometry-based different heights of liquid inside  $\mu$ -Slide VI 0.4 and  $\mu$ -Slide 8 well. In the example on the left, identical growth areas and number of cells but different volumes lead to different cell concentrations necessary.**

To get comparable degrees of confluency we recommend the use of  $3 \dots 7 \times 10^5$  cells/ml ( $\mu$ -Slide VI 0.4) and  $4 \dots 9 \times 10^4$  cells/ml ( $\mu$ -Slide 8 well). That is the same factor of  $\sim 7.5$  between the two geometries. After cell adhesion the number of cells per unit of area is identical.



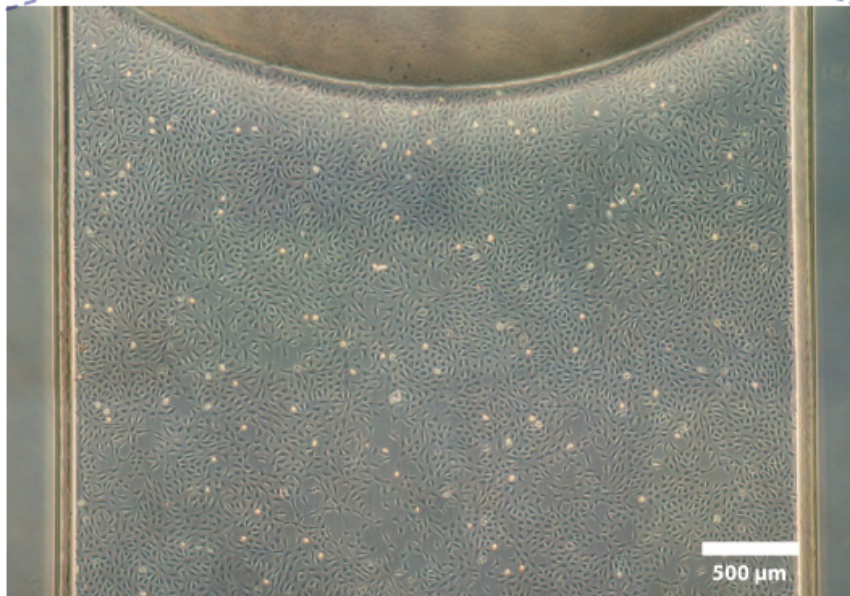
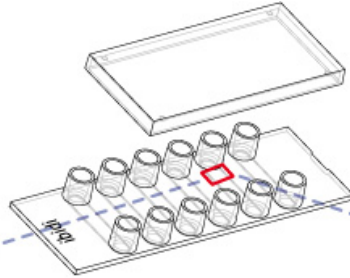
**Different cell concentrations result in the same grade of confluency due to different heights.**

## 4. Advantages of a Channel $\mu$ -Slide

The channel principle exhibits strong advantages compared to standard open well formats.

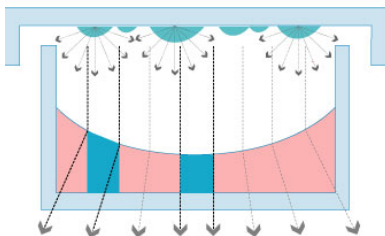
### a. Undisturbed phase contrast microscopy

The channel geometry enables unconfined use of phase contrast microscopy. The whole growth area can be visualized with the phase contrast technique because there is no disturbing meniscus.

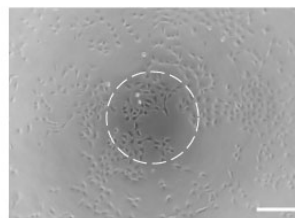


Unlike in open wells the channel does not disturb the beam path of the phase contrast microscope.

(a)

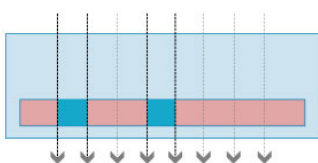


(b)

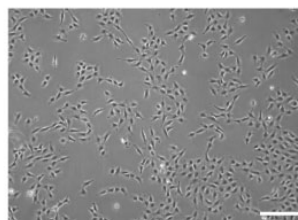


**Open well:**  
The meniscus is disturbing the phase contrast effect. Only the center provides convenient contrast.

(c)



(d)



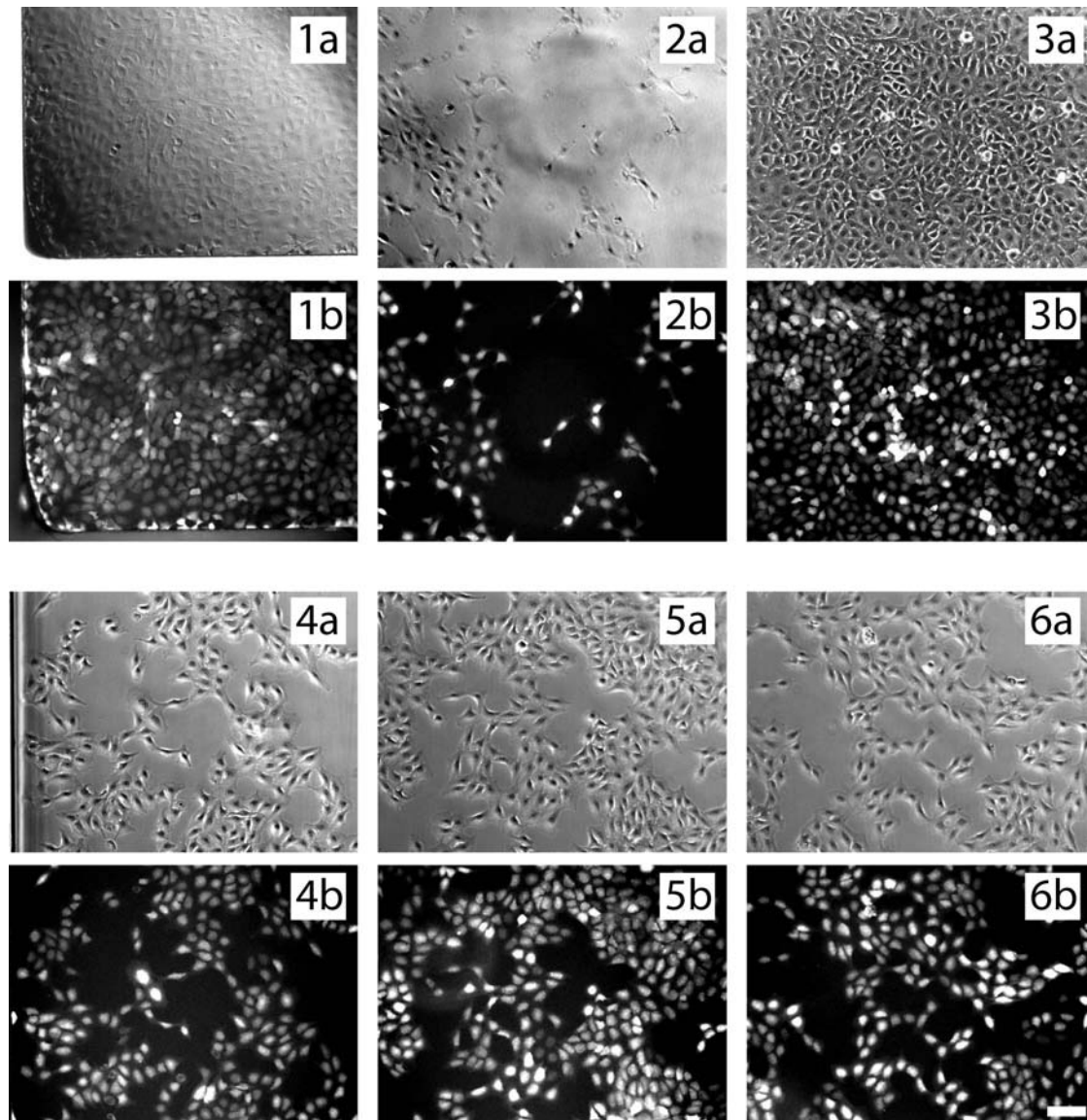
**Channel:**  
In a channel geometry the beam path is always aligned. Phase contrast microscopy is possible independent from the location.

## Application Note 03

### b. Homogenous Cell Distribution

In channels, the homogeneity of adherent cells is much better because edge effects are minimized.

The following microscopic images in phase contrast (a) and fluorescence (b) modes show the inhomogeneity of cell distribution in an open well (1-3) and the homogeneity in a cell culture channel (4-6).



- 1 Open well, edge
- 2 Open well, random location
- 3 Open well, center part
- 4 Channel, edge
- 5 Channel, random location
- 6 Channel, center part