How to grow cells inside a µ-(micro-)channel

This application note illustrates how to grow adherent cells inside a cell culture µ-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.

To show the **cell seeding** and µ-Slide handling the µ-Slide I is used for demonstration. We recommend to use the µ-Slide rack for easy handling after unpacking the slide. Prepare the cell suspension (e.g. 3×10^5 cells/ml) and apply 100 µl into the channel. Put the pipet tip right on the channel inlet and point at the channel as shown below. Dispense quick and fill the whole channel.
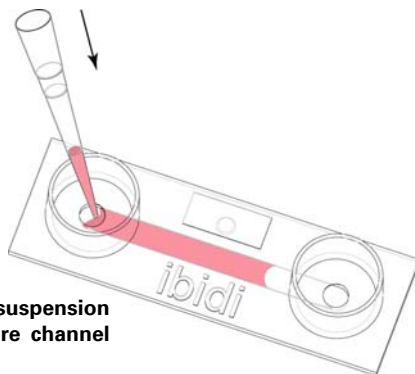

Filling in a cell suspension into the cell culture channel of the µ-Slide I.
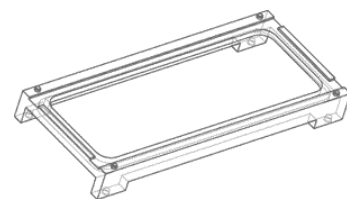
The µ-Slide rack is supportive of parallel handling of 8 µ-Slides.

In case the channel is not completely filled by the cell suspension incline the slide a little bit.

For **filling the reservoirs** (600 µl per reservoir) do not put the pipet tip on the channel inlet. Put 600 µl into each reservoir as shown below. 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.
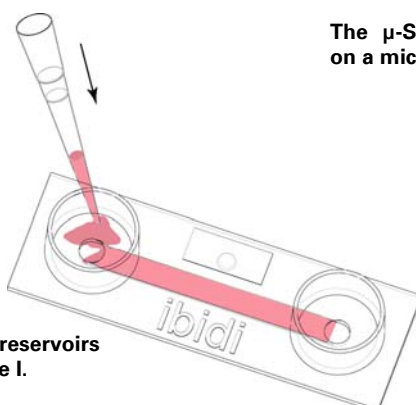
**Filling the reservoirs of the µ-Slide I.**

The µ-Slide I mounted on a microscopic stage.

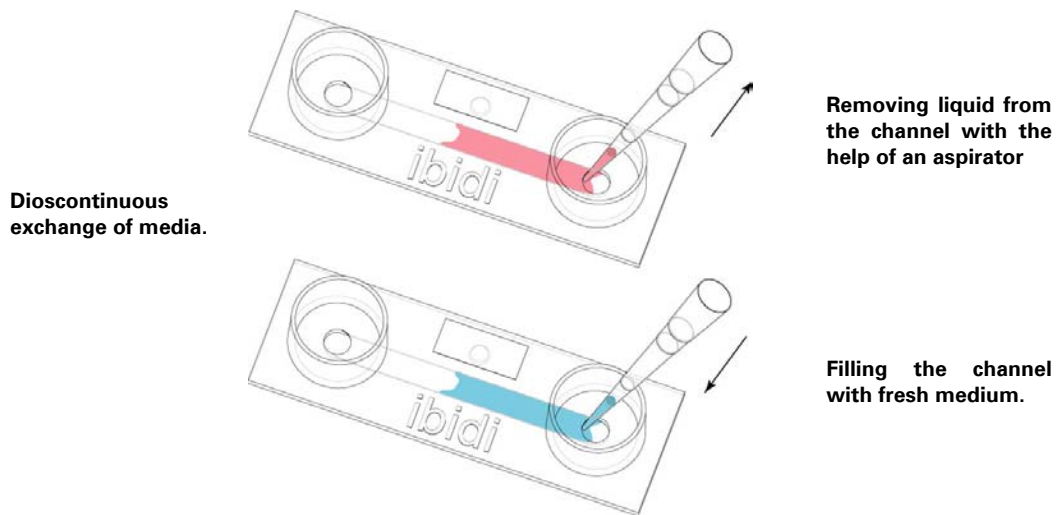After closing the caps the µ-Slide is ready for your experiment.

For **continuous media changing** we recommend to first remove the old medium from the reservoirs. Add fresh medium into one reservoir and aspirate from the other reservoir at the same time. Use an aspirator.


Continuous exchange of media.
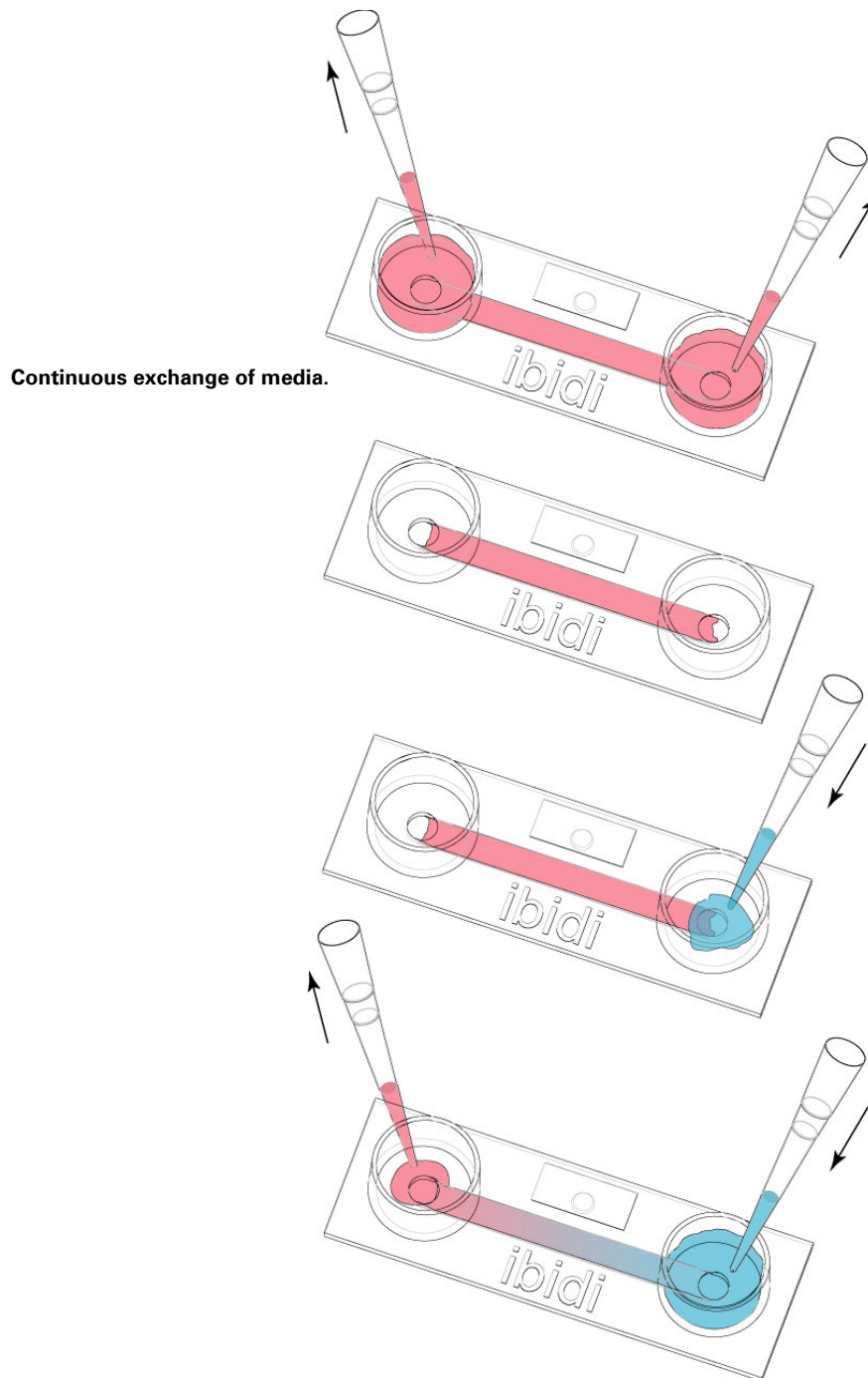
For **replacing only the channel volume** empty the reservoirs first, as described above. Then put the pipet tip right on the channel inlet and aspirate the liquid out of the channel carefully. Aspirate with caution not to flush adherent cells out of the channel.

Application Note 03

Dioscontinuous exchange of media.

Removing liquid from the channel with the help of an aspirator

Filling the channel with fresh medium.

To refill the channel and the reservoirs just add fresh medium directly into the channel. Avoid trapping air bubbles.

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Here we **compare the properties** of the µ-Slide I (cell culture channel) with the µ-Slide 8 well (open cell culture format).

µ-Slide I   µ-Slide 8 well
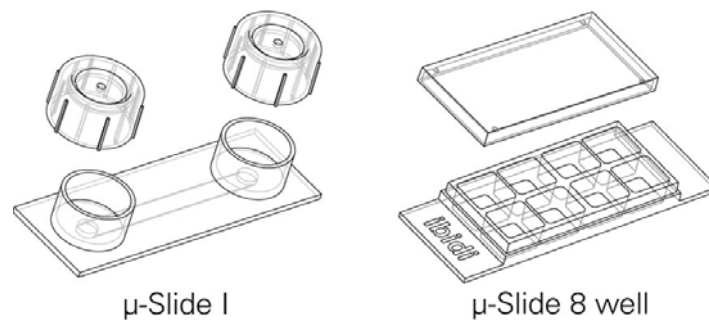
For seeding cells the height of the liquid inside the structure is the main difference. A small area section of both systems is shown below.

Geometry based different heights of liquid inside µ-Slide I and µ-Slide 8 well.

0.4 mm

~3 mm
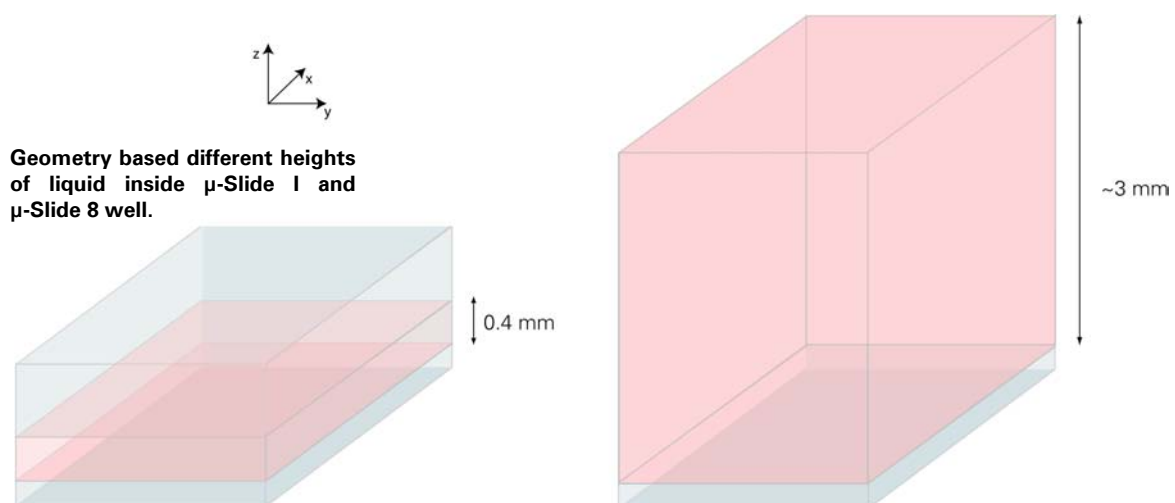
The distance between bottom and ceiling inside the µ-Slide I is 400 µm. A filled µ-Slide 8 well doesn't have a ceiling. Here, the culture medium stands approx. 3 mm. Effectively, the cell culture channel is 7.5 times thinner than the open format.

Application Note 03

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 µ-Slide 8 well is 7.5 times bigger the applied cell concentration has to be lower with the same factor. After cell seeding it looks like shown below.
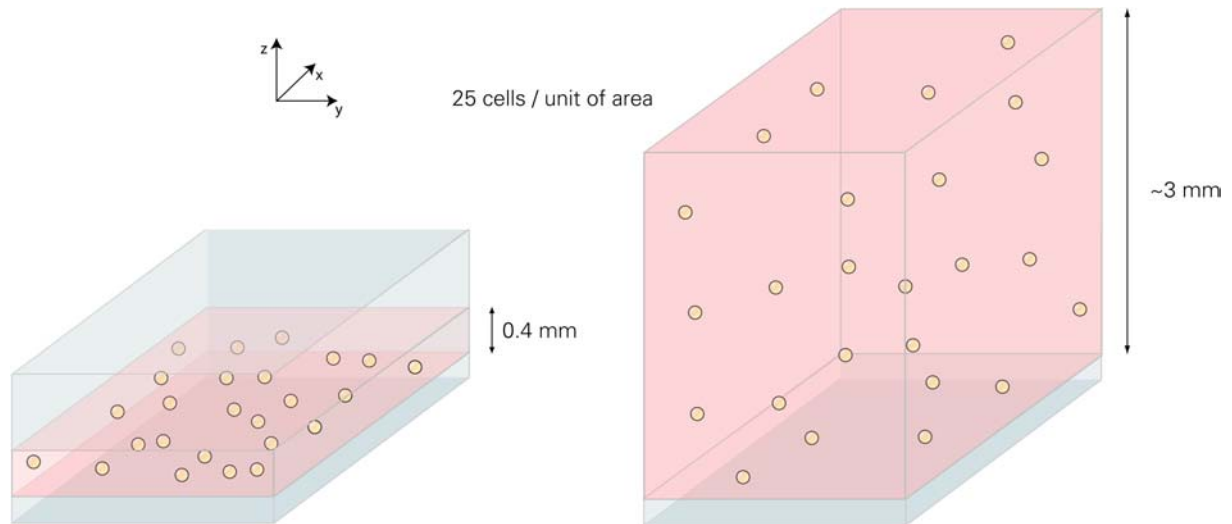
After cell adhesion the number of cells per unit of area is identical.

Different cell densities result in the same grade of confluency due to different heights.
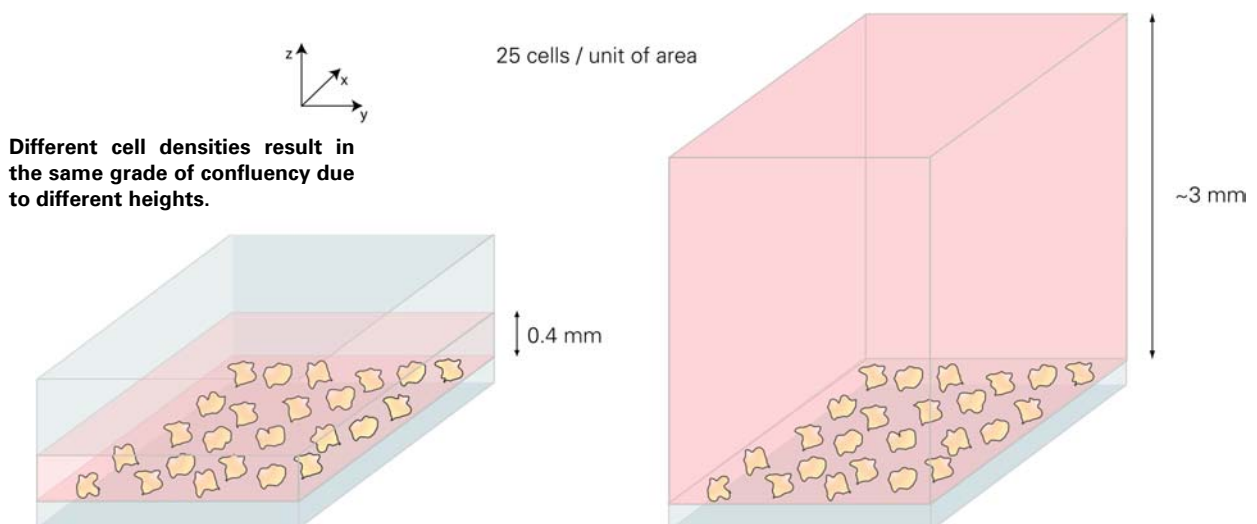
To get comparable degrees of confluency we recommend the use of

$3 \dots 7 \times 10^5$ cells/ml   and $4 \dots 9 \times 10^4$ cells/ml
(µ-Slide I)   (µ-Slide 8 well)

which is the same factor of ~7.5 between µ-Slide I and µ-Slide 8 well.
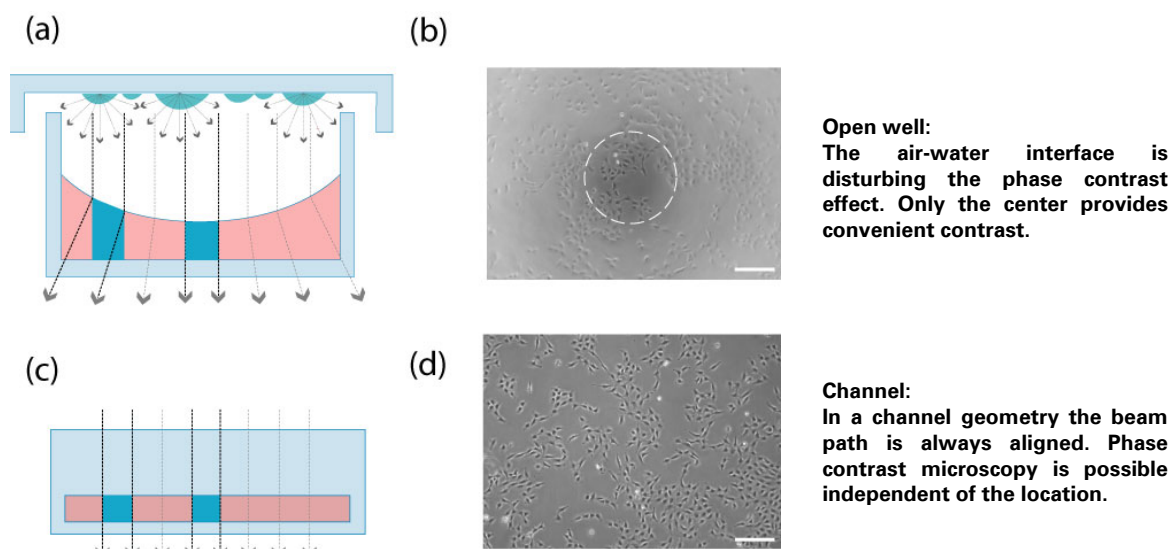
Application Note 03

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

1. Undisturbed phase contrast microscopy possible over the entire cell lawn

The channel geometry enables unconfined use of phase contrast microscopy. The hole growth area can be visualized with the phase contrast technique.
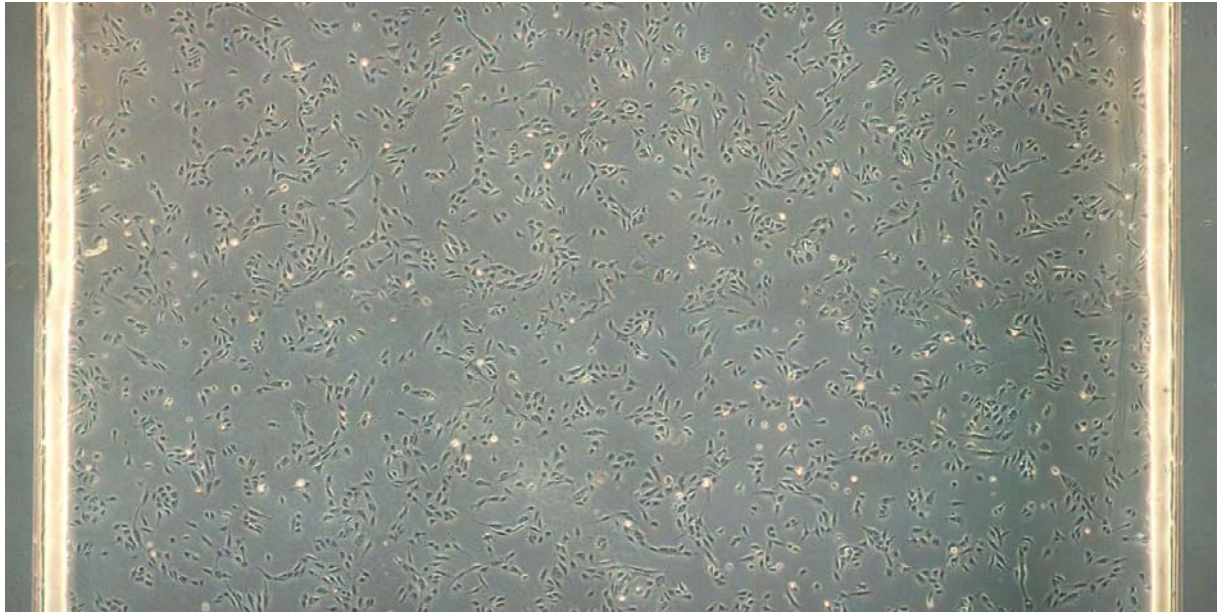

Unlike in open wells the channel does not disturb the beam path of the phase contrast microscope.

(a)

(c)

(b)

Open well:
The air-water interface is disturbing the phase contrast effect. Only the center provides convenient contrast.

(d)

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

Application Note 03

2. Homegenous cell distribution

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).
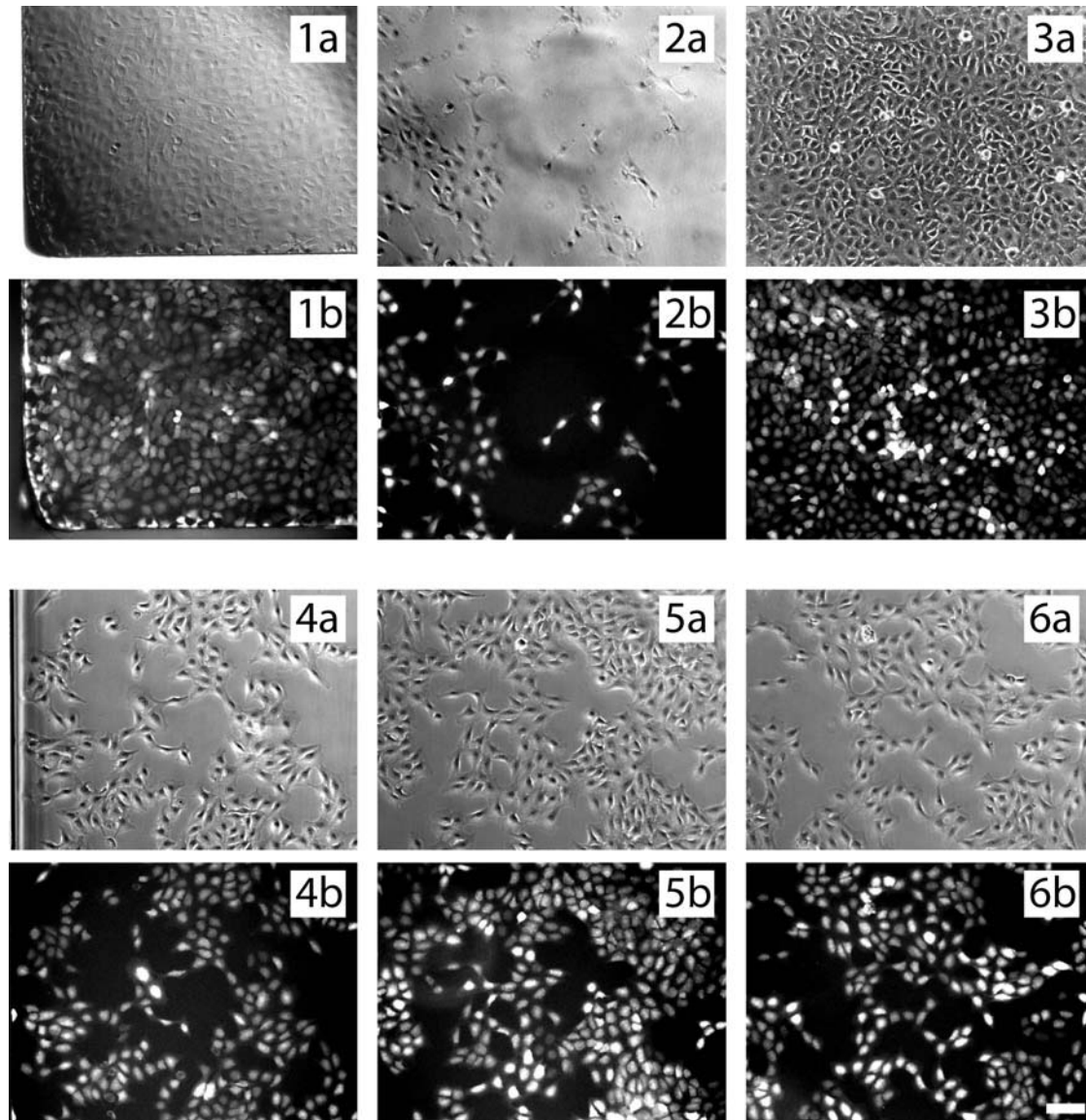
1 open well, edge
2 open well, random location
3 open well, center part
4 channel, edge
5 channel, random location
6 channel, center part