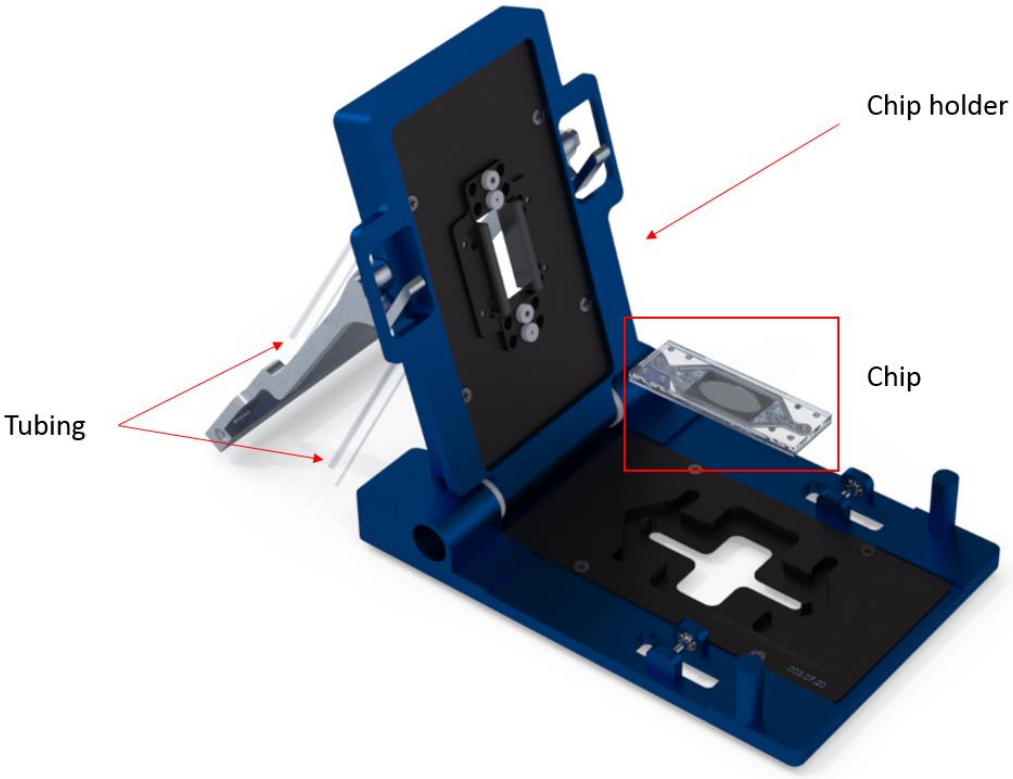
micronit microtechnologies

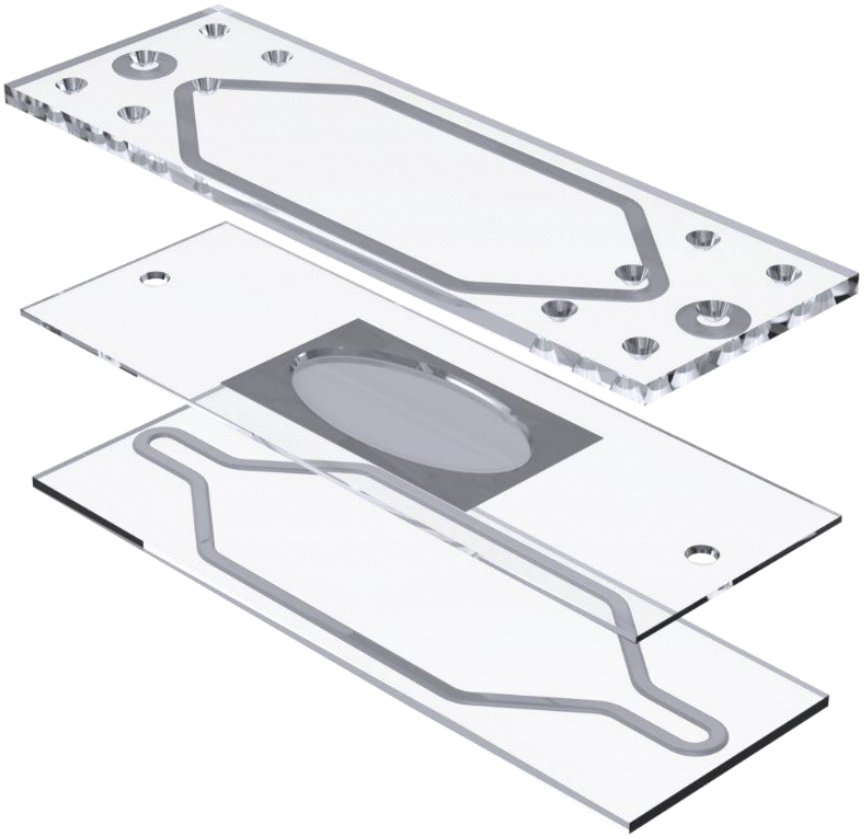
# Organ-on-a-chip

Quick Start Guide v0.2



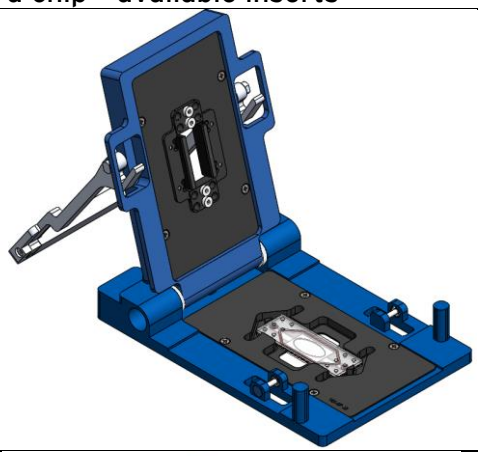
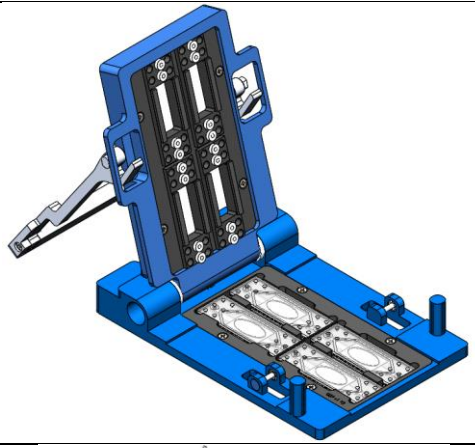
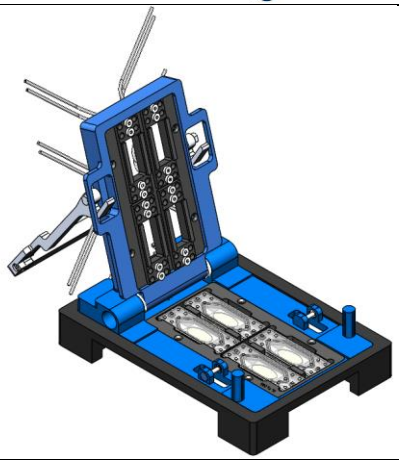

Single slot inserts

4-slot inserts



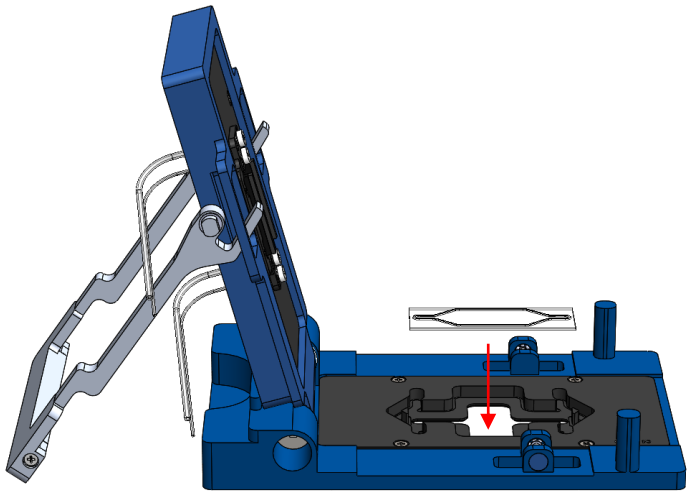
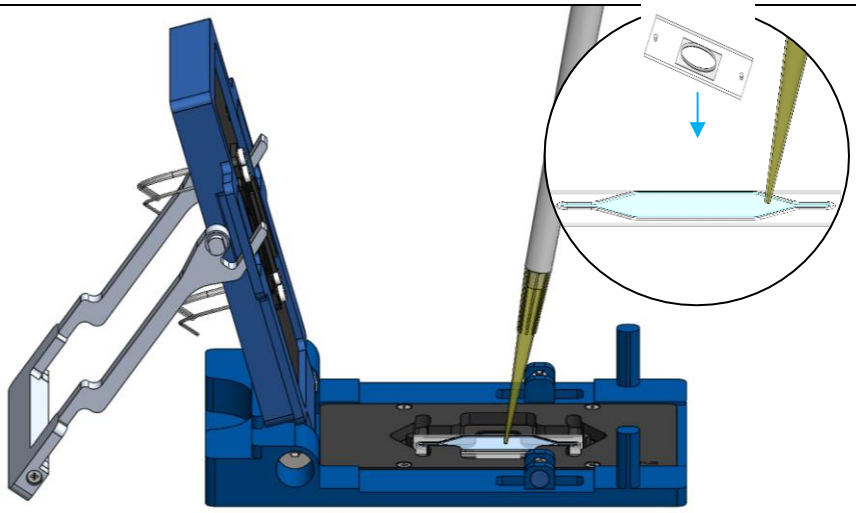
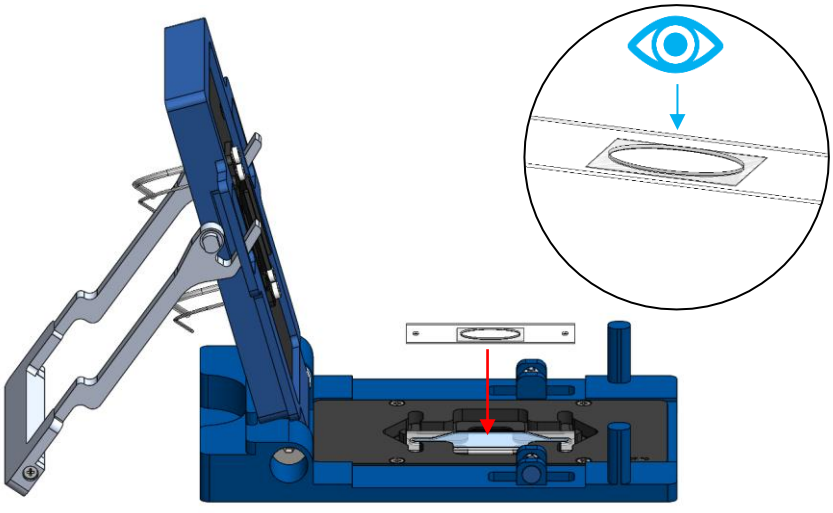

**Chip structure**

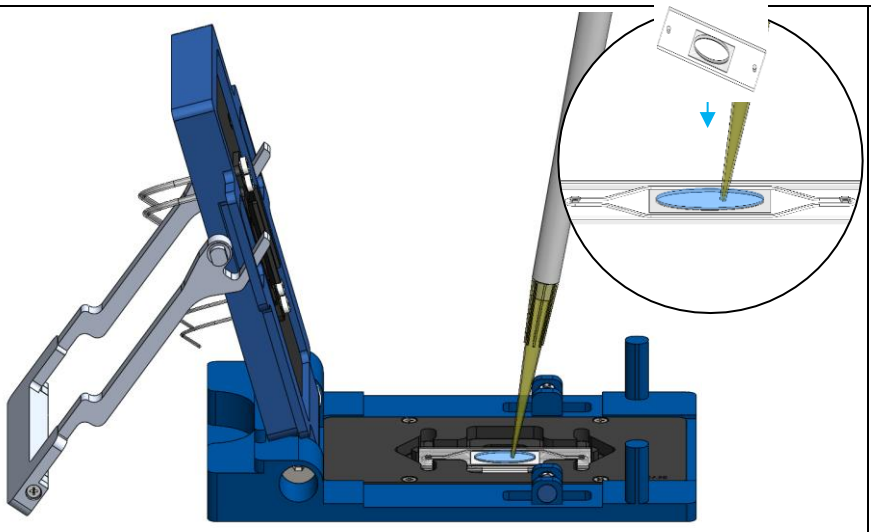
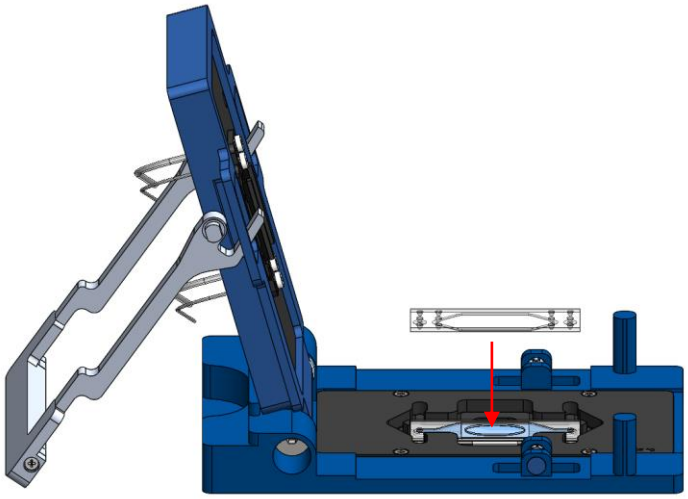
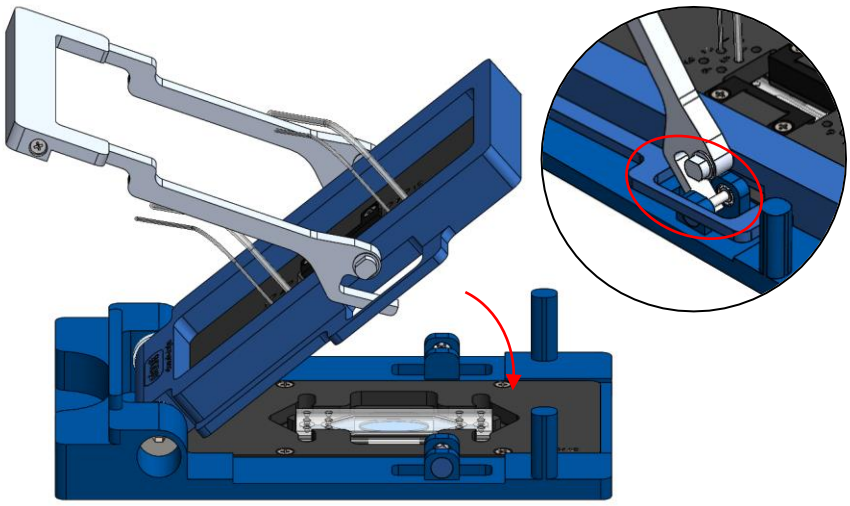
Organ on a chip – available inserts

| | |
|---|---|
|  | Single slot OOC 45x15 with 0.4mm middle layer:<br>Top insert 001.04.xx<br>bottom insert 003.07.xx |
|  | Single slot OOC 45x15 with 2.0mm middle layer: Top insert 003.08.xx<br>Bottom insert 003.09 |
|  | 4-slot OOC 45x15 with 0.4mm middle layer:<br>Top insert 003.10.xx<br>Bottom insert 003.11.xx |
|  | 4-slot OOC 45x15 with 2.0mm middle layer:<br>Top insert 003.10.xx<br>Bottom insert 003.12.xx |

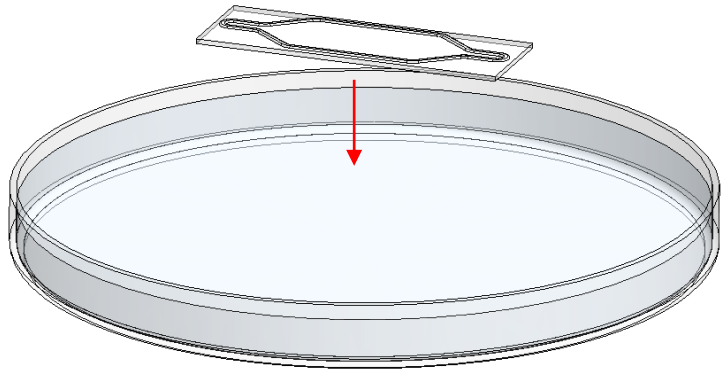
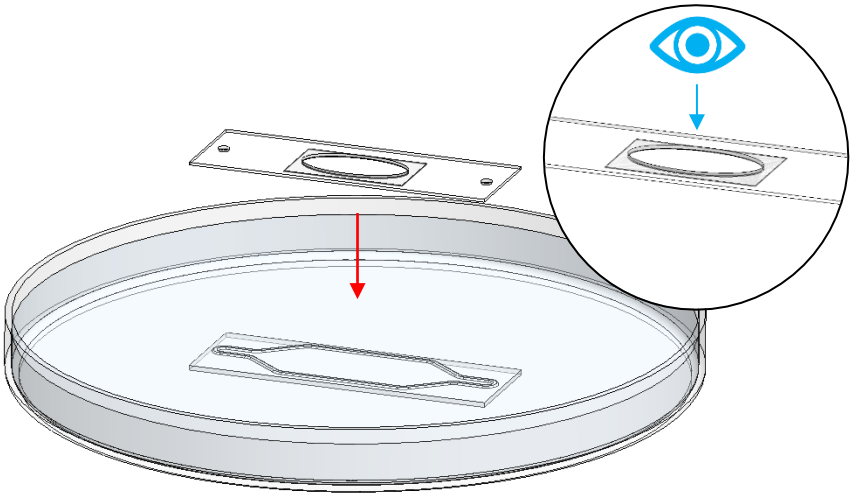
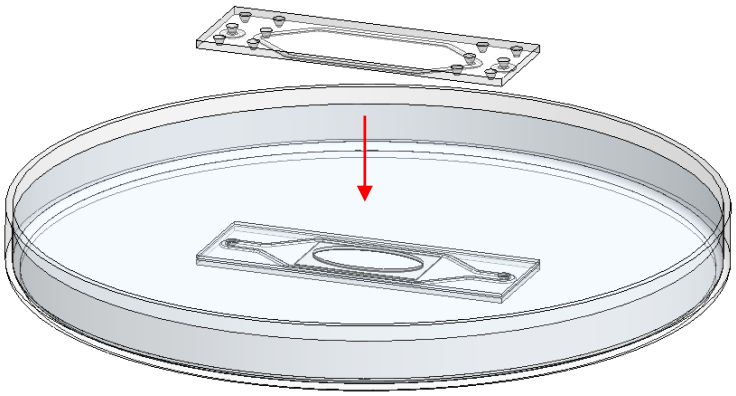
# Chip assembly – In Fluidic Connect Pro
(recommended for single-slot inserts)

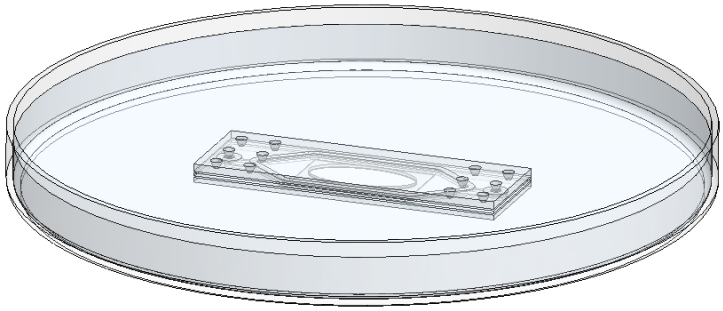
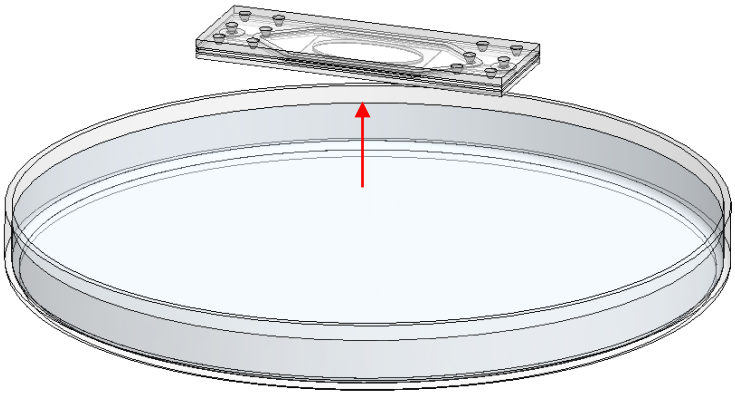
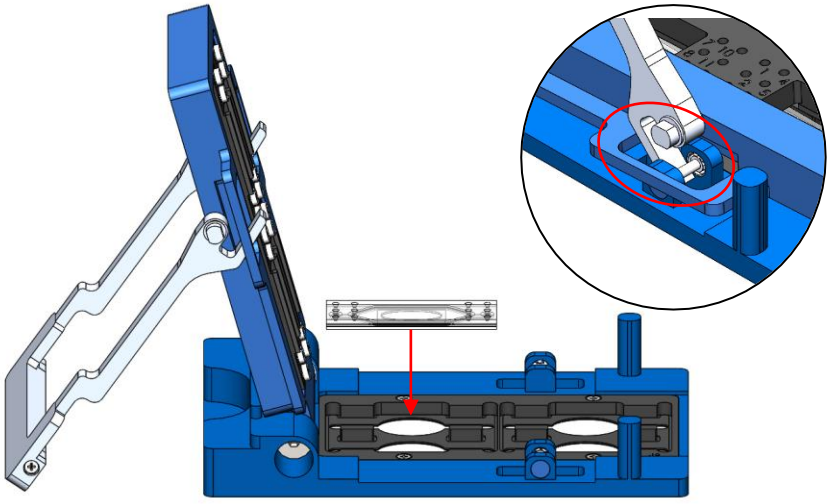
Note: tubing can be assembled before placing the chip(s) or afterwards. See pages 11-15

| | |
|---|---|
|  | Place the bottom layer in the bottom insert of the chip holder, with the gasket up. |
|  | Fill the gasket of the chip with water, until it is completely filled. |
|  | Place the middle layer with seeded cells (for cell seeding, see p.9) on the bottom layer of the chip in the chipholder, with the membrane down. |

| | |
|---|---|
|  | Fill the middle layer of the chip with water, untill the cavity is completely filled. |
|  | Place the top layer of the chip on the middle layer in the chipholder, with the gasket down. |
|  | Close the chipholder. Fix the assembly, using the gripper. |

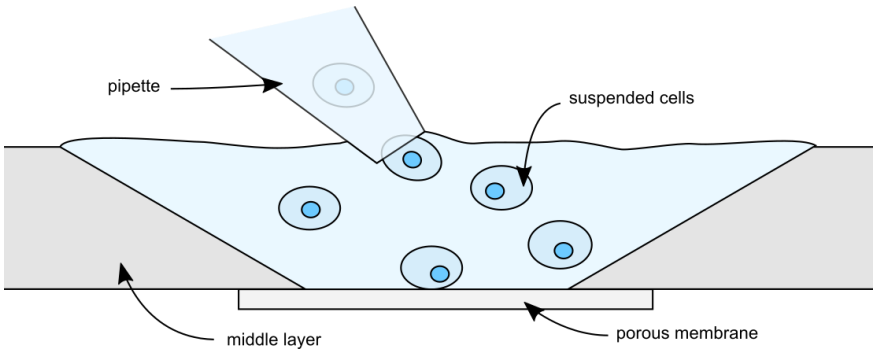
## Chip assembly – submerged
(recommended for 4-slot inserts)

| | |
|---|---|
|  | Place the bottom layer of the chip in a water-filled petri-dish filled with the gasket on top. |
|  | Place the middle layer with seeded cells (for cell seeding, see p.9) on the bottom layer of the chip in the petri-dish, with the membrane down. |
|  | Place the top layer of the chip on the middle layer in the petri-dish, with the gasket down. |

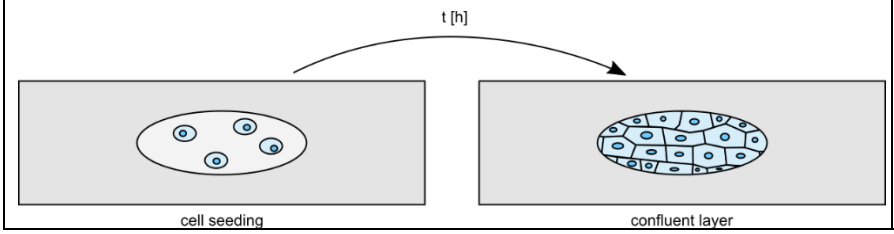
| | |
|---|---|
|  | Make sure the entire chip is submerged. |
|  | Carfully pick the chip assembly from the dish. |
|  | Place the chip-assembly in one of the slots in the bottom insert of the chipholder and close the chipholder. |

# Cell culture

## Cell seeding



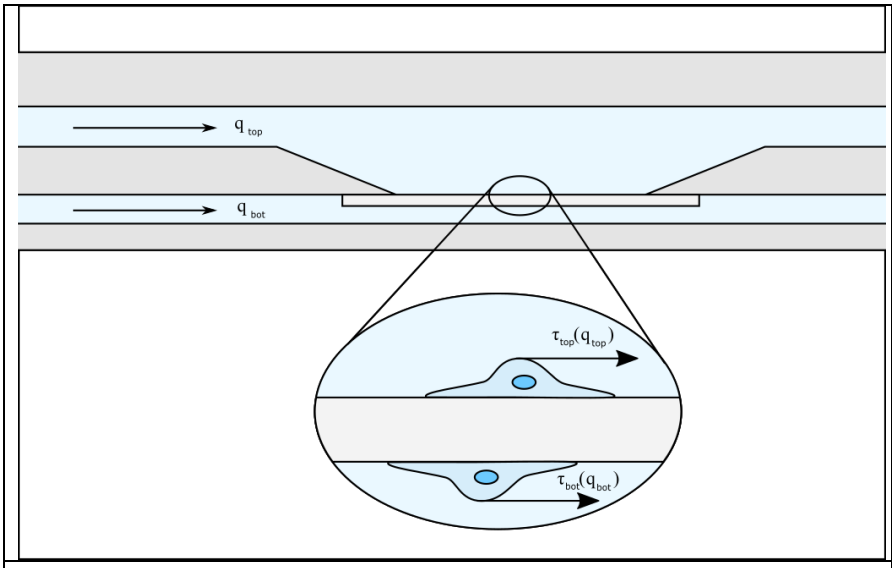

Cells can be seeded directly by pipetting the cell suspension on the middle layer. Recommended seeding densities and membrane coatings are listed in the table below.




Seeding densities reported in the table are meant for reaching confluent cell layers in 72 hours.

| Cell type | Seeding density [cells/cm²] | Time for confluency [h] | Coating | Density [µg/cm²] | Time before assembly [h] |
|---|---|---|---|---|---|
| HUVEC | 140-350 x10³ | 72 | Fibronectin | 10-100 | 2 |
| A549 | 35 x10³ | 72 | Fibronectin | 10-100 | 2 |
| Calu-3 | 300 x10³ | 72 | No coating | - | - |

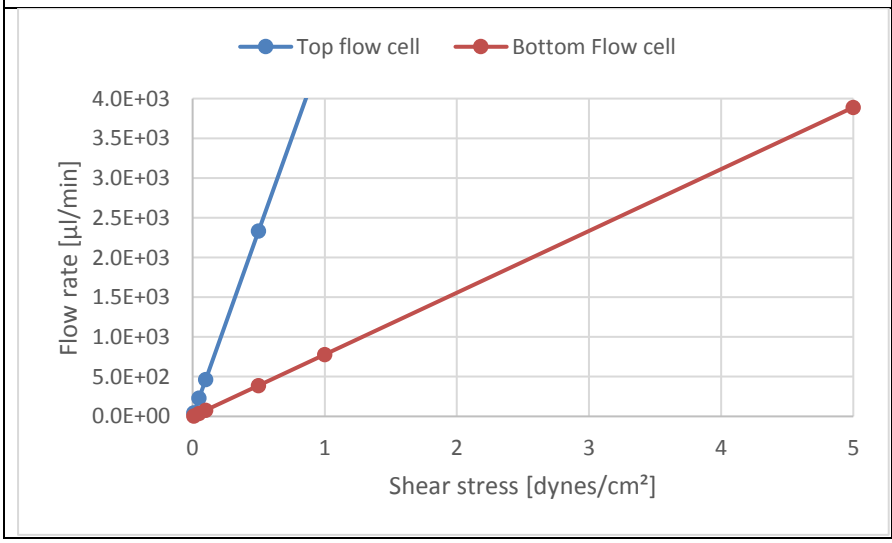
## Perfusion




Cells cultured on the membrane are mechanically stimulated by the fluid flow.
The different height of the channels results in different stress on the cells (for the same flow rates).

| Shear stress τ [dyne/cm2] | Flow rate q [µl/min] | |
|---|---|---|
| | TOP | BOTTOM |
| 0.01 | 47 | 8 |
| 0.05 | 233 | 39 |
| 0.1 | 467 | 78 |
| 0.5 | 2335 | 389 |
| 1 | - | 778 |
| 5 | - | 3890 |

An indication on the flow rates needed to achieve specific shear stresses can be found in this table.
Flow rates higher than 4 ml/min are highly not recommended.



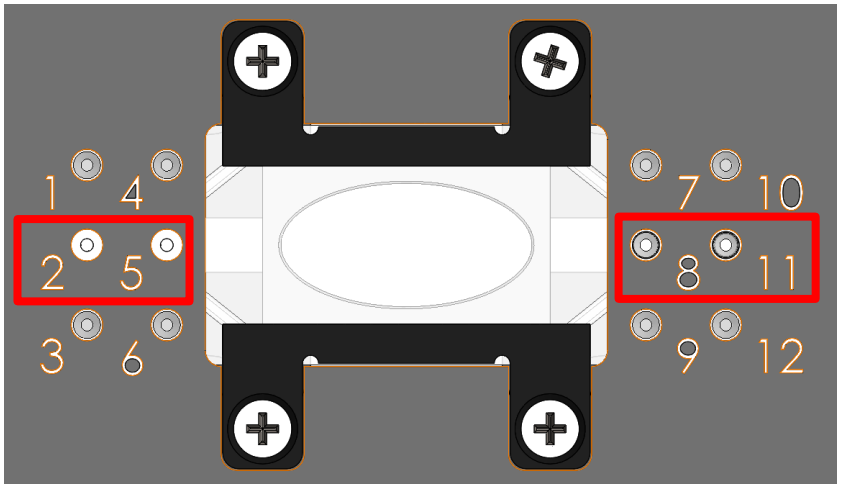
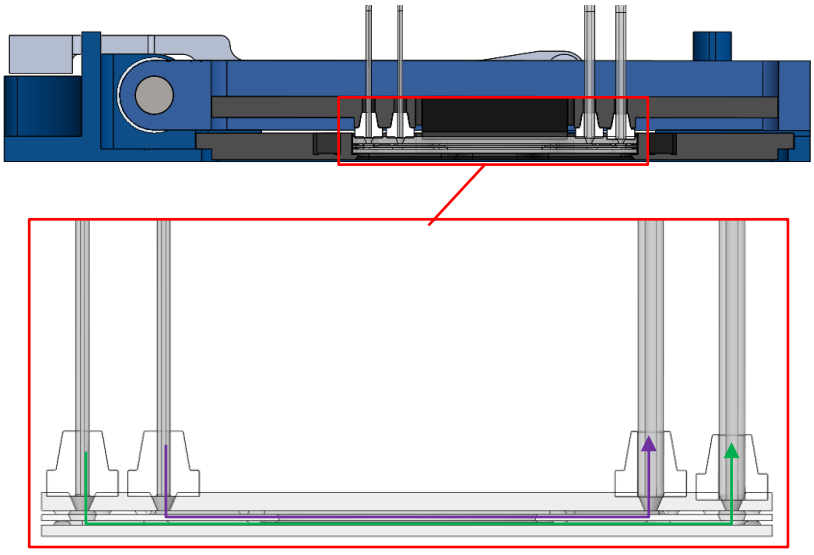

The curves representing the flow rate as a function of shear stress on the membrane in the two channels is reported in this graph.

# Tubing

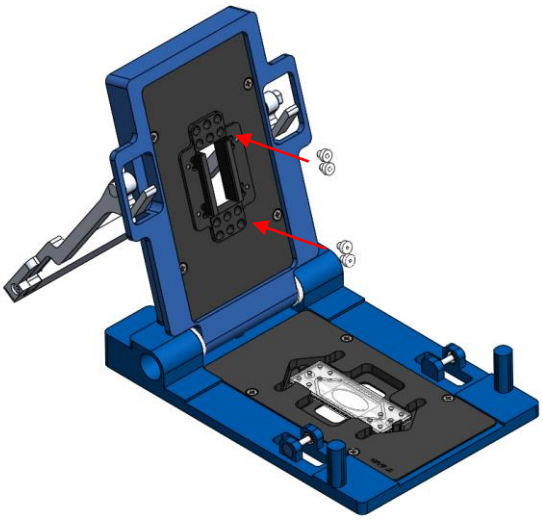
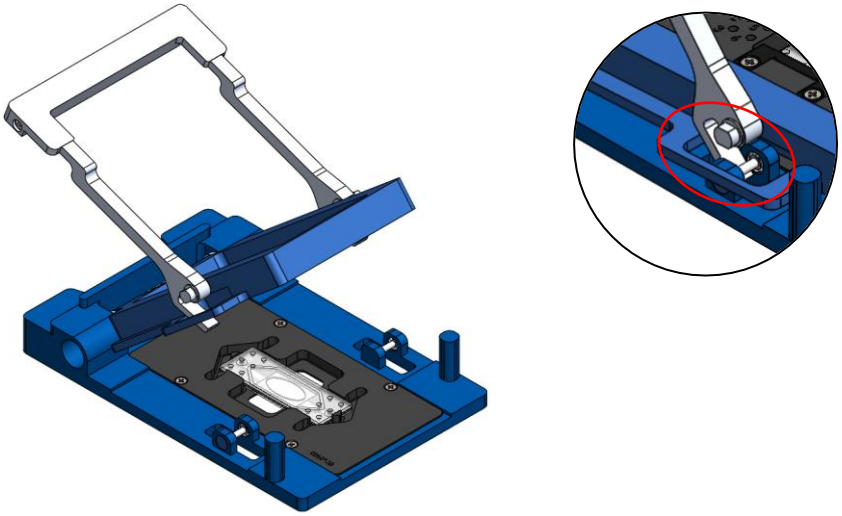
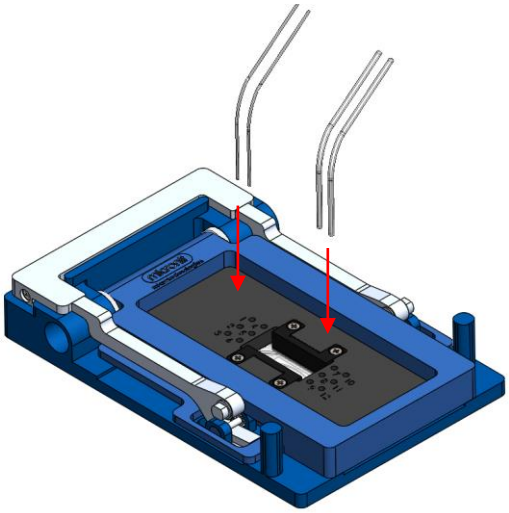
## Tube preparation

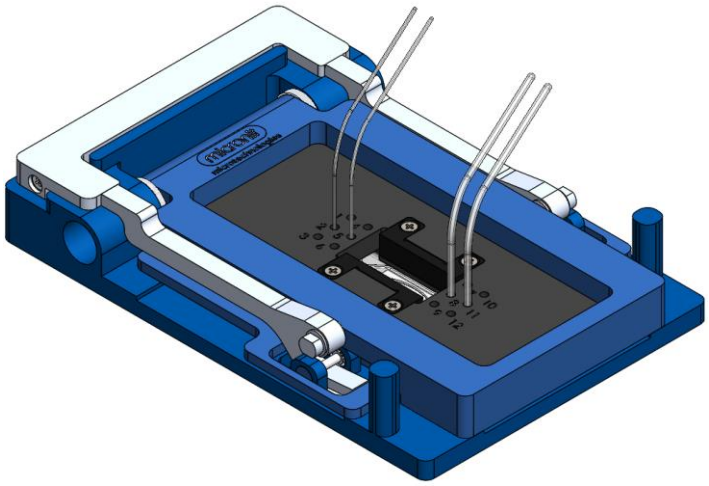
To prepare the tubing before use, use a tube cutter. Make sure a sharp blade is used and cut tubes according to the instructions of the tube cutter
Cut all tubes at the same length.

## Tubing assembly – tubing and ferrule location

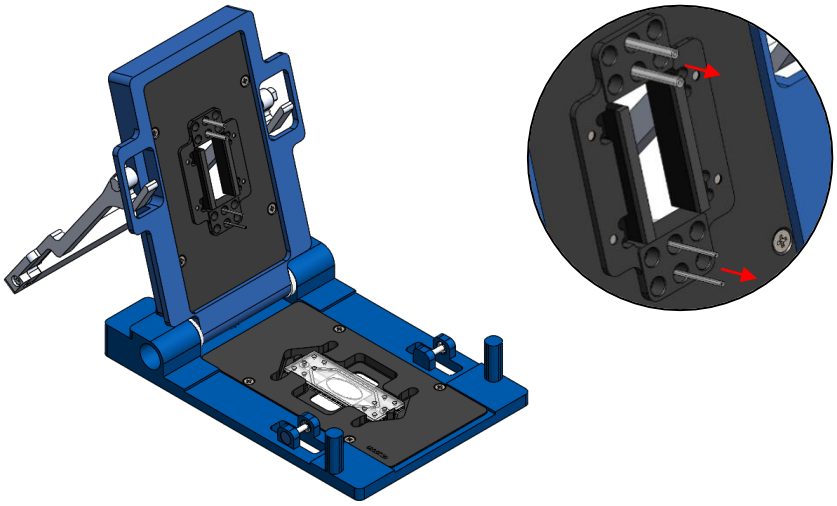
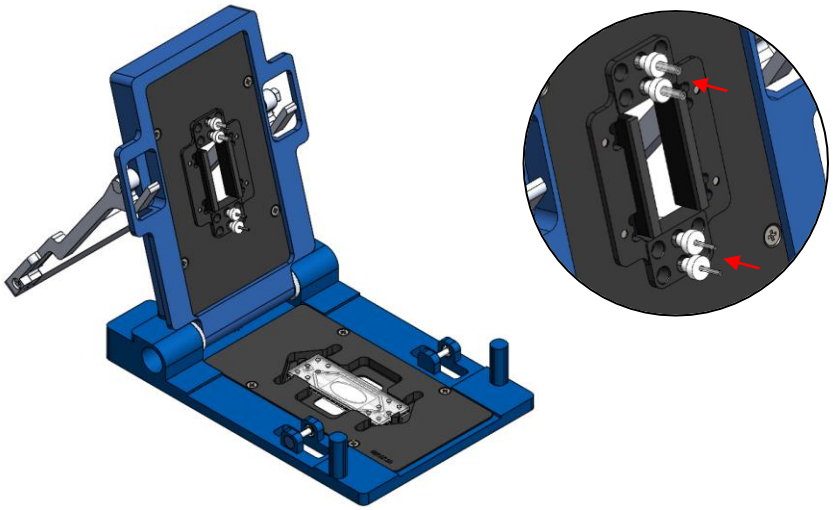
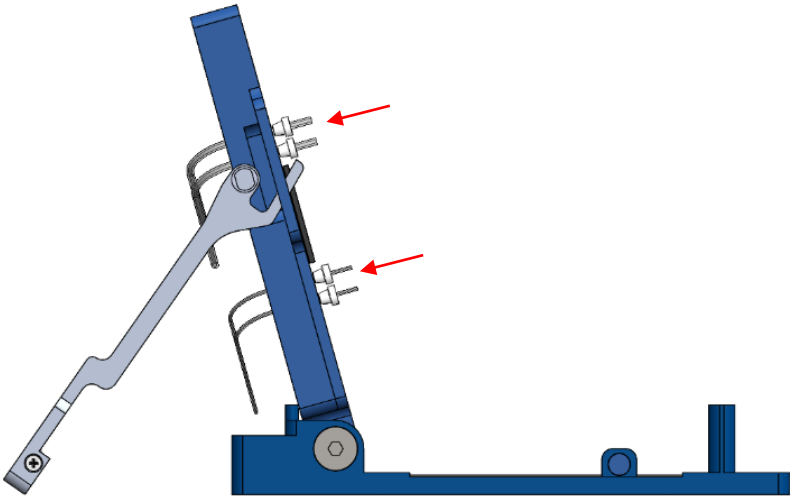
| | |
|---|---|
|  | There are 6 connection holes in the top inserts at both ends of the chip. Only the holes (2, 5 and 8, 11) in the middle at each side need to be used |
|  | For assembly information, see p. 12-15. |

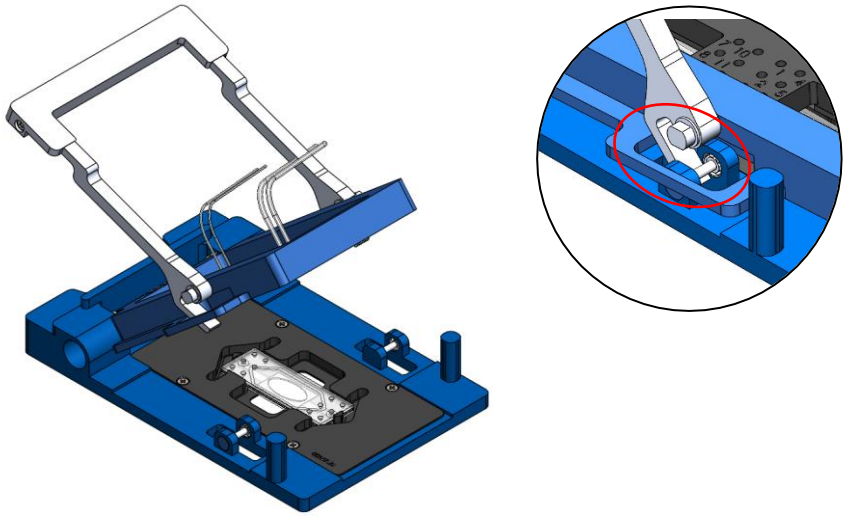
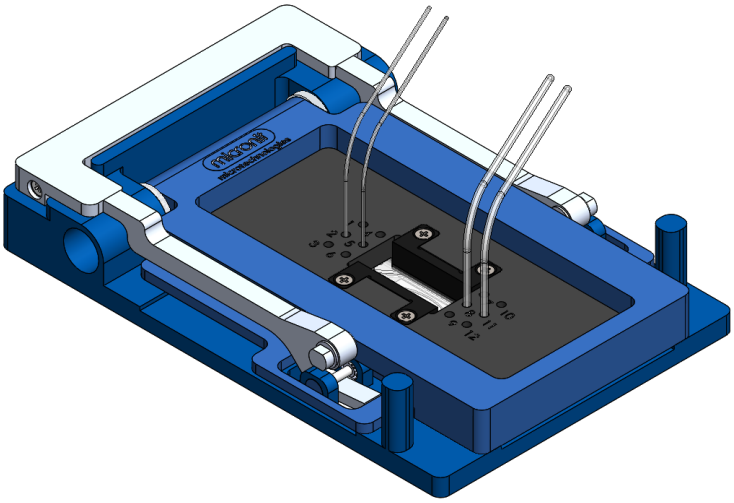
## Tubing assembly – after closing the chip holder

| | |
|---|---|
|  | Add ferrules in top insert at the locations of the in- and outlet-channels. Preferably use ferrules with 1/16" inner diameter for outlet channels and ferrules with an inner diameter of 1/32" for inlet channels. |
|  | Close and fix the chipholder using the gripper. Make sure the ferrules stay in place. |
|  | Press tubing tight in the openings with ferrules. Make sure the outer diameter of the tubing correspondends with the inner diameter. |

| | |
|---|---|
|  | Your setup is ready to use. |

## Tubing assembly – before closing the chipholder

| | |
|---|---|
|  | Put tubing through the holes of the top insert which would connect to the in- and outlet-channels of the chip if the chipholder is closed. Preferably use tubing with 1/32" outer diameter for inlet channels and tubing with 1/16" outer diameter for outlet channels. |
|  | Slide ferrules over the tubing (make sure the inner diameter of the ferrules corresponds with the outer diameter of the tubing)<br>Make sure the tubing sticks out of the ferrules. |
|  | Press the tubing and ferrules back into the top insert. Make sure the end of the tubing is on the same level as the end of the ferrule. The best way is to first press the tapered part of the ferrule in the insert-opening, then press the tubing in the ferrule. |

| | |
|---|---|
|  | If the chip is not yet in the holder, place the chip in the chipholder.<br>Close and fix the chipholder using the gripper. Make sure the ferrules stay in place. |
|  | Your setup is ready to use. |

Note: After opening the chipholder, the top layer of the chip(s) may stick to the ferrules. Please be cautious when opening the chipholder.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Phone +31 53 850 6 850 Fax +31 83 850 6 851 E-mail info@micronit.com Internet www.micronit.com