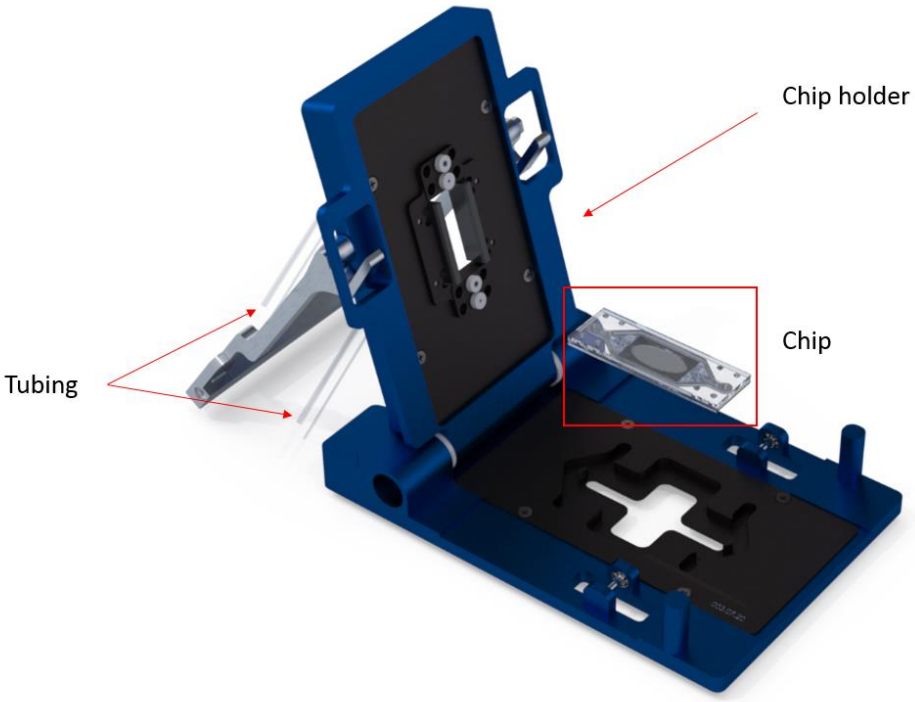
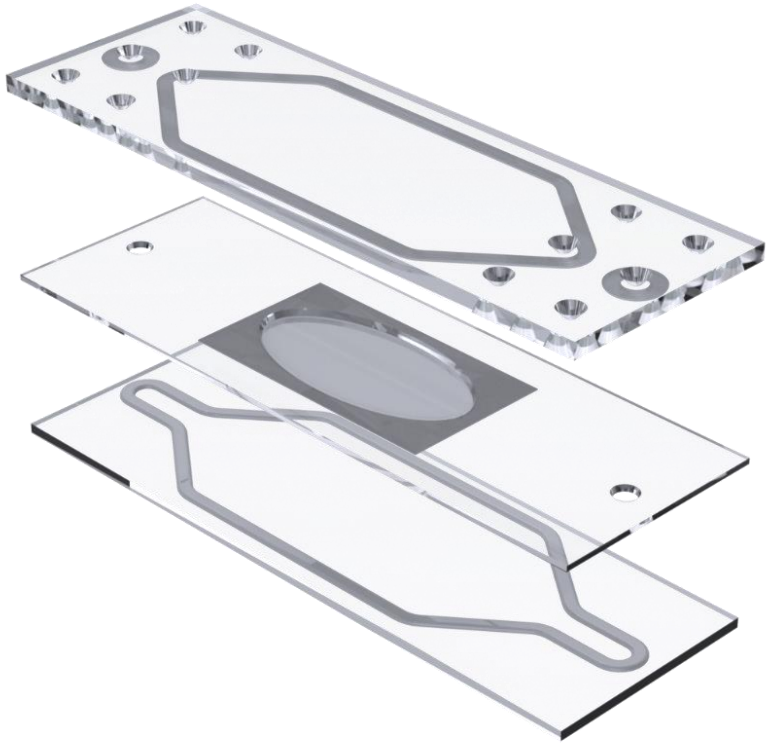
Organ-on-a-chip

Quick Start Guide

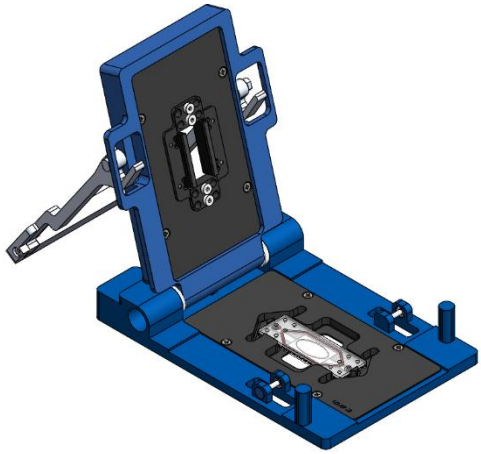
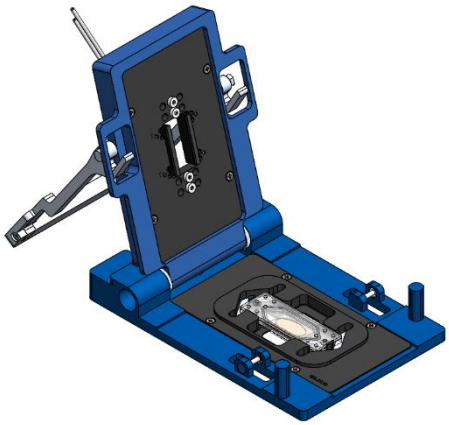


Single slot inserts



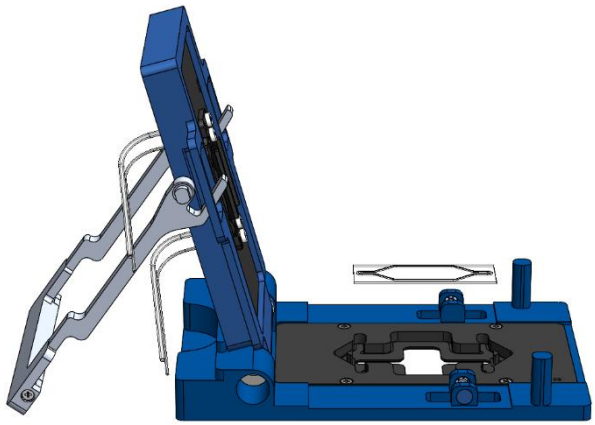
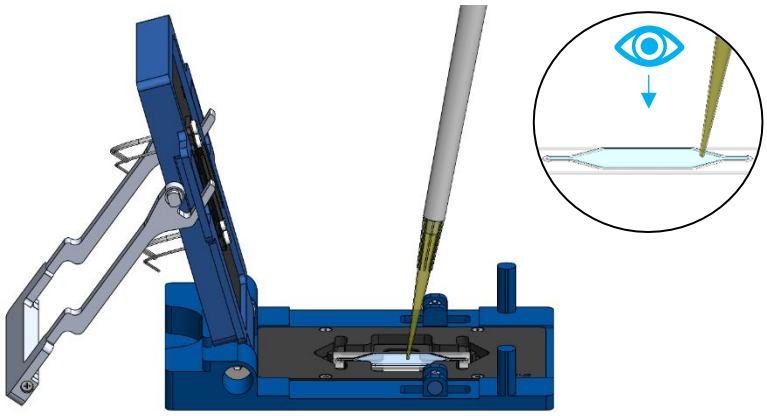
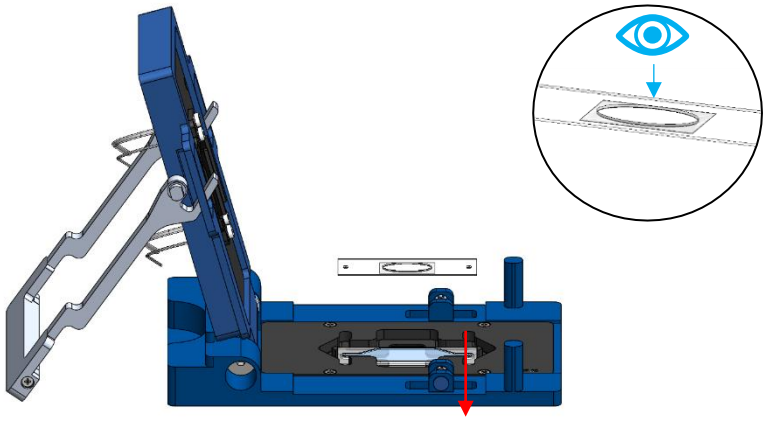
Chip structure

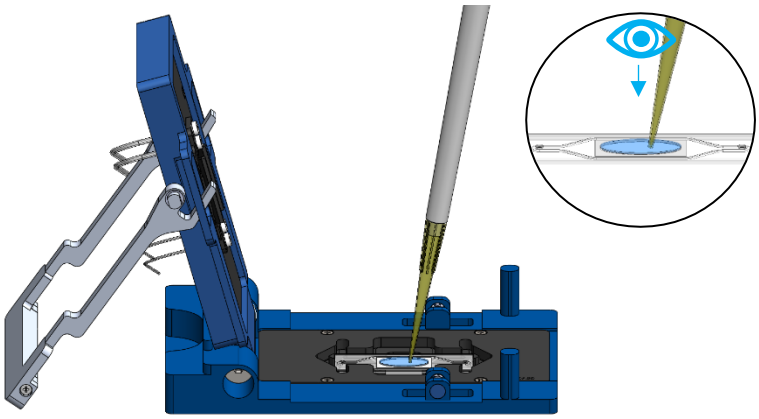
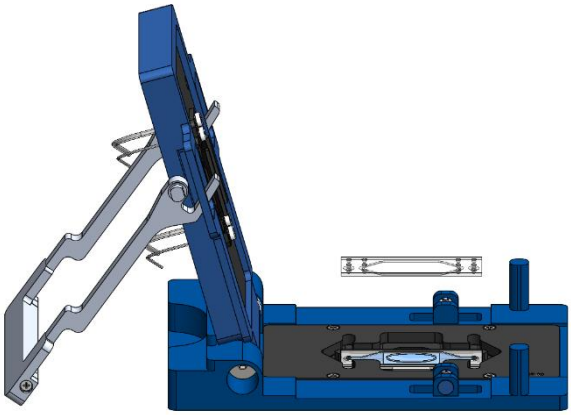
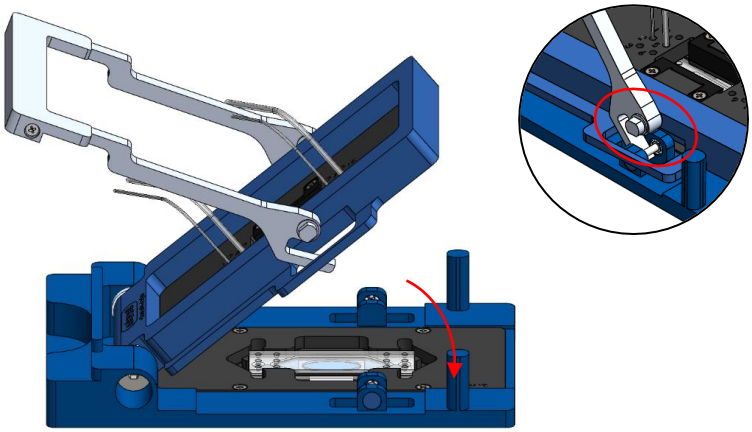
Organ on a chip – available inserts

	Single slot OOC 45x15 with 0.4mm middle layer: top insert 001.04.xx bottom insert 003.07.xx
	Single slot OOC 45x15 with 2.0mm middle layer: top insert 003.08.xx bottom insert 003.09

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

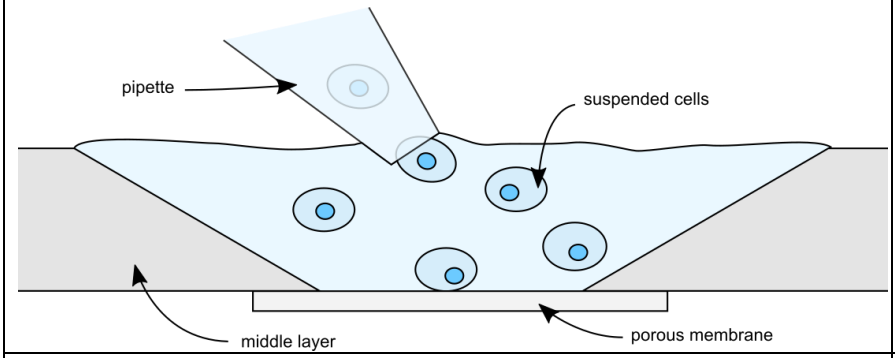
Note: tubing can be assembled before placing the chip(s) or afterwards. See pages 11-15.

	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 page 6) on the bottom layer of the chip in the chipholder, with the membrane down.

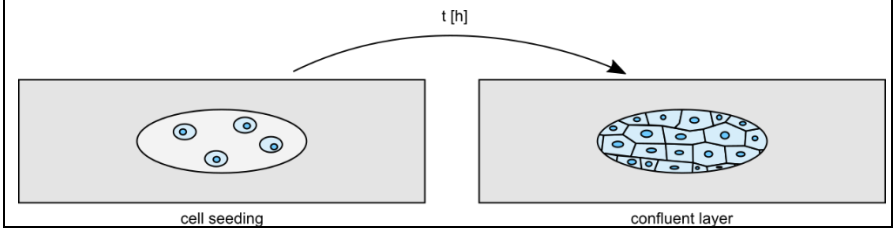
	Fill the middle layer of the chip with water, until the cavity is completely filled.
	Place the top layer of the chip on the middle layer in the chipholder, with the gasket down.
	Close the chipholder. Fix the assembly, using the gripper.

Cell culture

Cell seeding



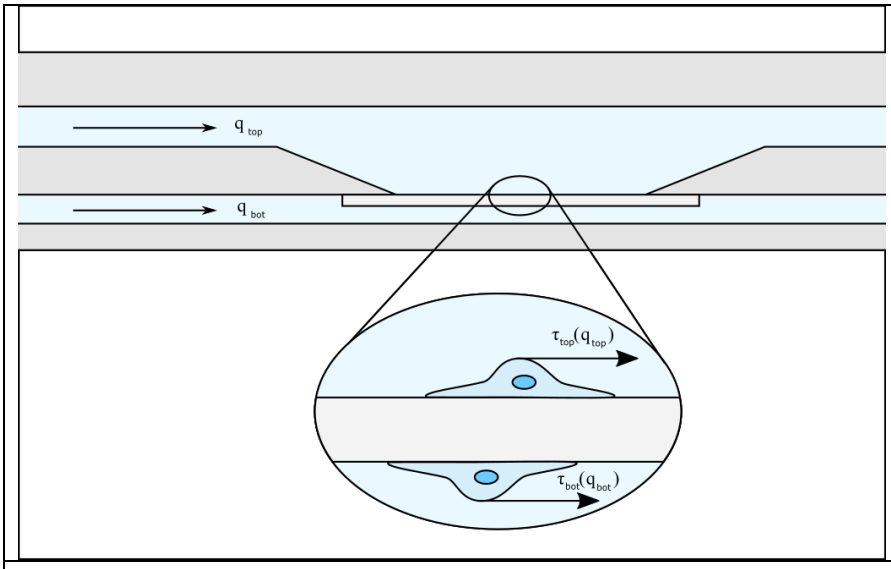
Cells can be seeded directly by pipetting the cell suspension on the middle layer. Recommended seeding densities and membrane coatings are listed in the table below.



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

Cell type	Seeding density [cells/cm^2]	Time for confluency [h]	Coating	Density [µg/cm^2]	Time before assembly [h]
HUVEC	140-350 x10^3	72	Fibronectin	10-100	2
A549	35 x10^3	72	Fibronectin	10-100	2
Calu-3	300 x10^3	72	No coating	-	-

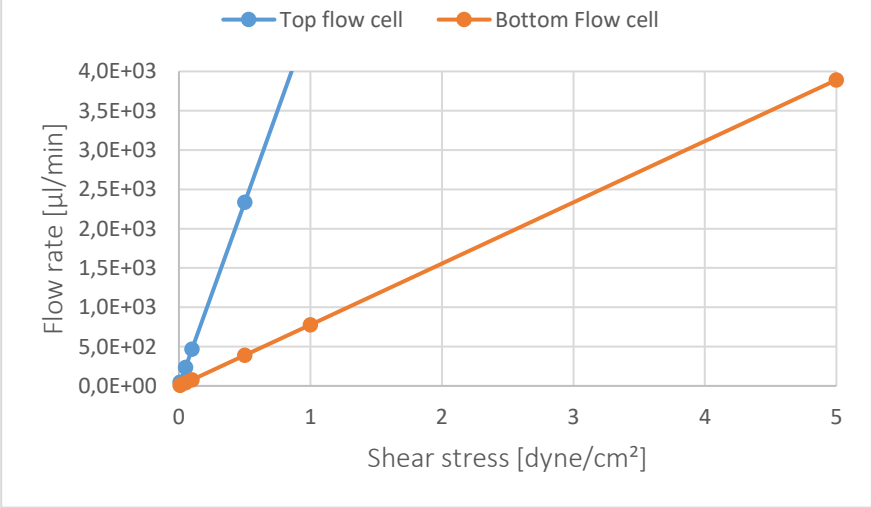
Perfusion



Cells cultured on the membrane are mechanically stimulated by the fluid flow.
The different heights of the channels result in varying 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

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



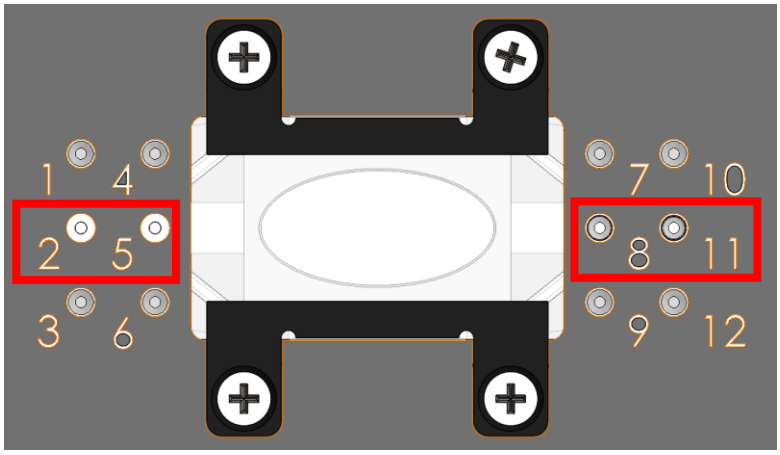
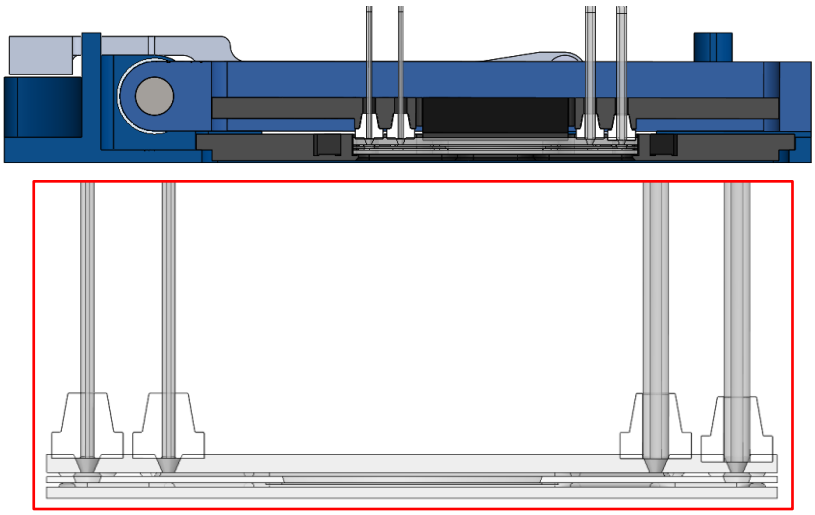
The curves representing the flow rate as a function of shear stress on the membrane in the two channels is reported in this graph.

Tubing

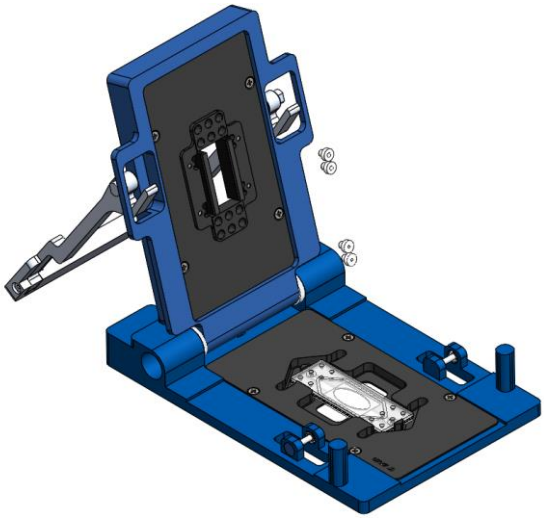
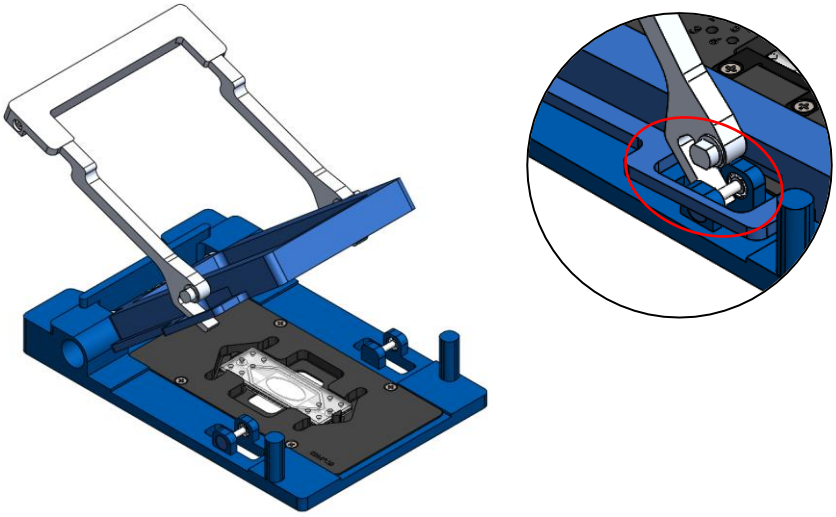
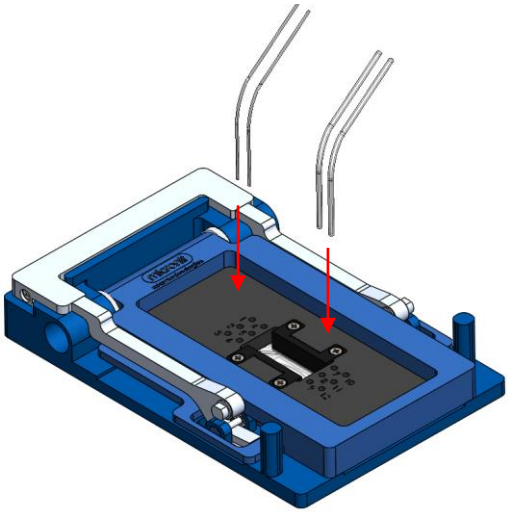
Tube preparation

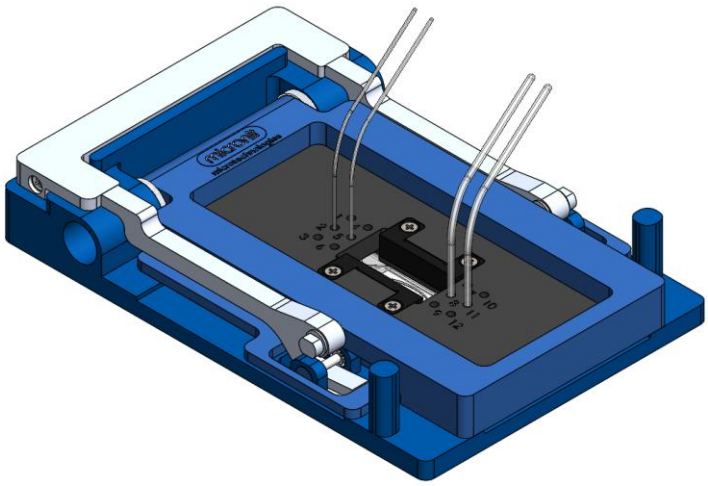
To prepare the tubing before use, use a tube cutter. Make sure a sharp blade is used and cut tubes according to the instructions of the tube cutter. Cut all tubes at the same length.

Tubing assembly – tubing and ferrule location

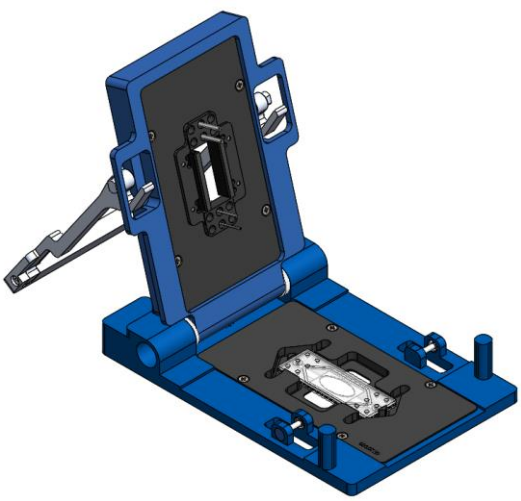
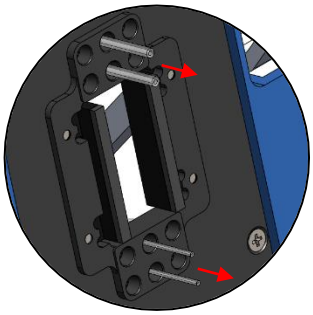
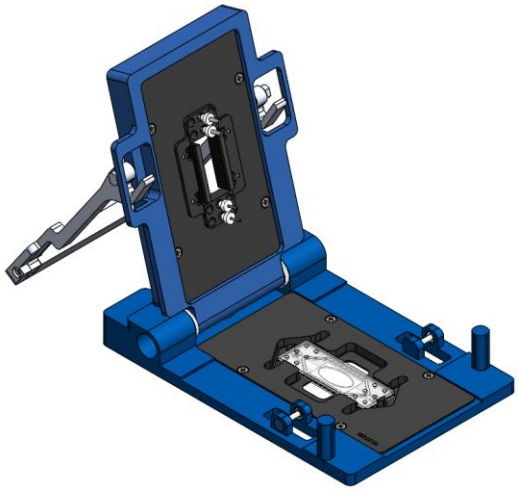
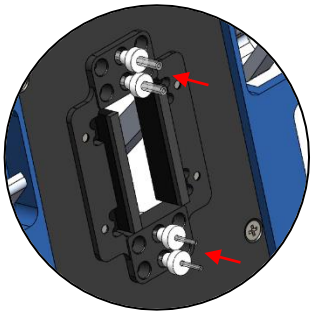
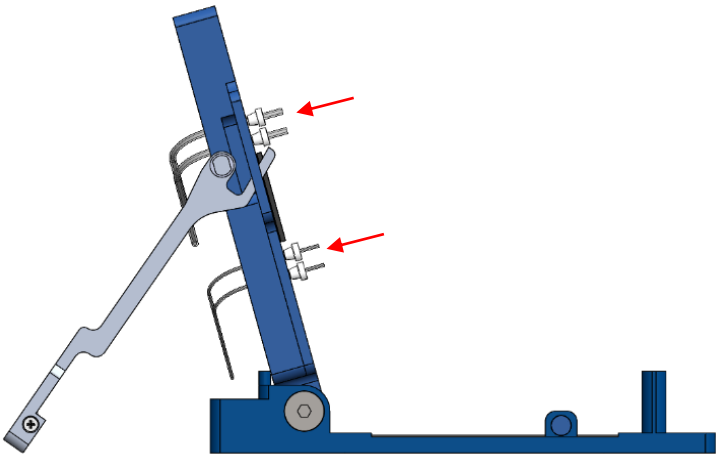
<table>
<tr>
<td> 
</td>
<td>There are six connection holes in the top inserts at both ends of the chip. Only the holes in the middle at each side need to be used (2, 5 and 8, 11).</td>
</tr>
<tr>
<td>  </td>
<td>For assembly information, see pages 11-12.</td>
</tr>
</table>

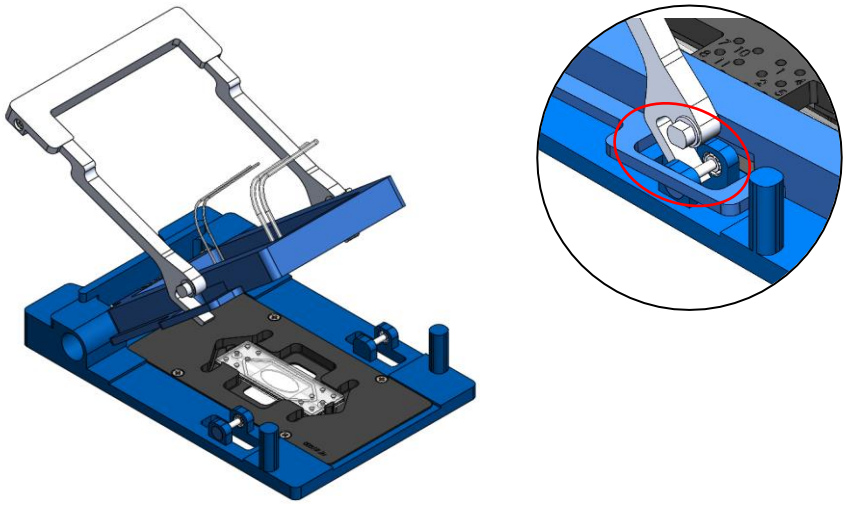
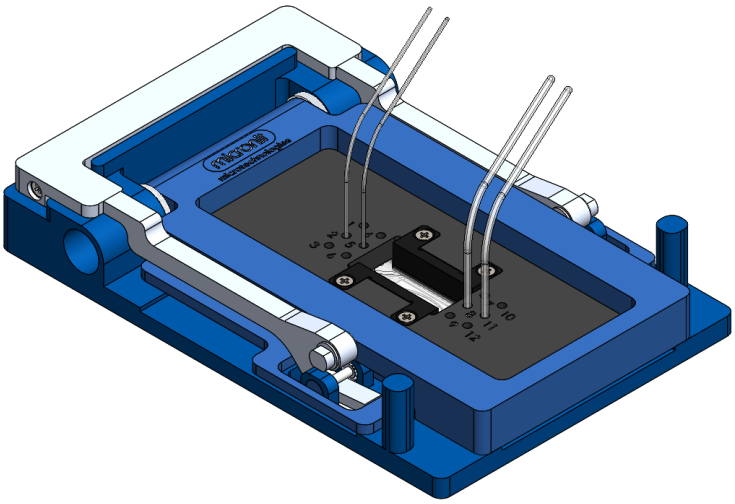
Tubing assembly – after closing the chip holder

	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 that the tubing outer diameter is 1/16” for the milk white ferrules and 1/32” for black ferrules.

	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). 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 into the ferrule.

	If the chip is not yet in the holder, place the chip in the chipholder. 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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