Perfusion at the SP5 microscope

The SP5 microscope is equipped with a perfusion system that was implemented in cooperation with the Department of Physiology. The available system offers a number of potential applications that are not only relevant for physiologists but also for general live cell imaging involving the addition or removal of substances, media and growth factors during microscopic imaging. This protocol is intended as a reminder, only. **Everyone interested in using the system should contact me for a personal introduction.**

Potential applications:

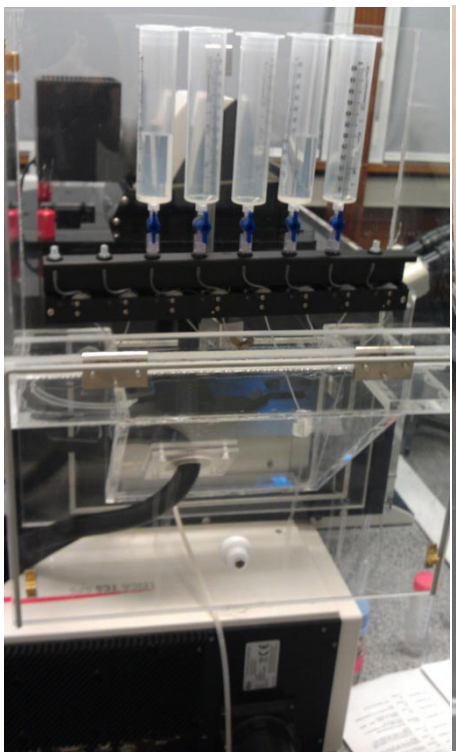
- **Addition of growth factors, inhibitors, etc… avoiding "on scope pipetting" which is very tricky.**
- **Medium change**
- **Real perfusion**
  - **bath perfusion**
  - **local applications (per cell)**

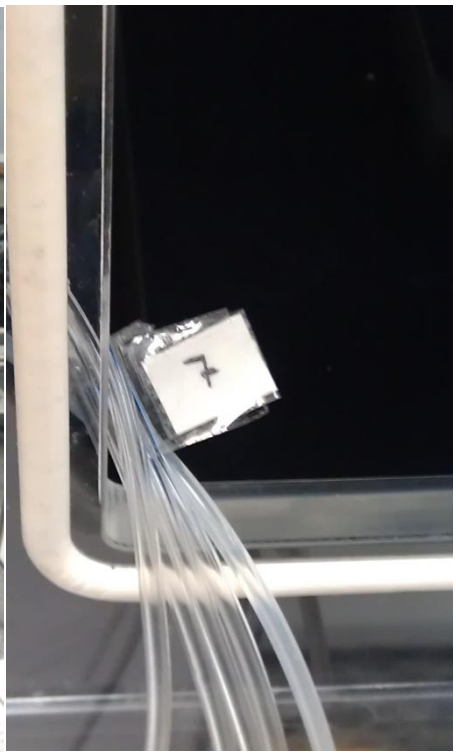
1. Setting up the system for local perfusion (most cases)

- Switch on the main switch (black box).
- Control unit will start.

- Use the arrow buttons to select “Manual” on the display. The syringes 1 -8 will appear as “1C”, 2C, 3C… 8C, C standing for “closed”
- C can be changed to O (=open) by pressing the number keys 1- 8 for each syringe. 9 will open ALL, 0 will close all.
- Make sure that all are closed and fill the syringes with the required liquids.

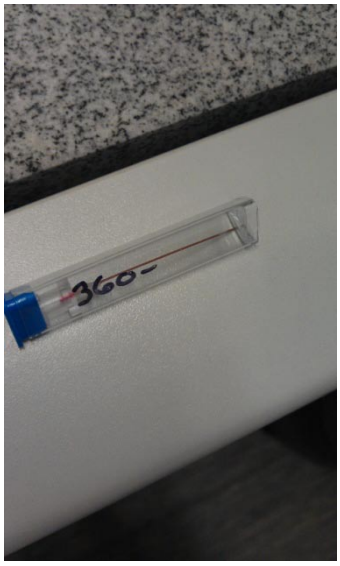
- Syringes are labeled from 1 to 8.
- Take the tubing out of the incubation box and put the ends into a beaker.
- The tubing is labeled according to the syringes. Fill the tubing without air-bubbles (gravity only!) by pressing one number after the other to get to the O(pen) position.

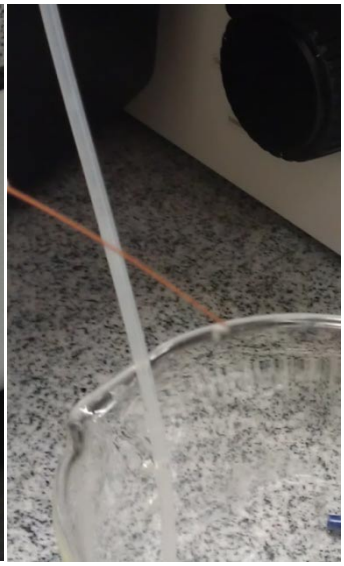




- Take the “pencil” out of the box (local application, everything except bath perfusion).
- Connect the inlets to the tubing again observe the labeling.
- There are several inlets but only one outlet!!!!!

- Fill the pencil by repeating the procedure outlined above (open the valves one by one).
- Connect the tip to pencil and fill as above.
- Check for leakage- the connections can leak (or not) individually and need to be tested one by one by opening and closing, close all at the end.

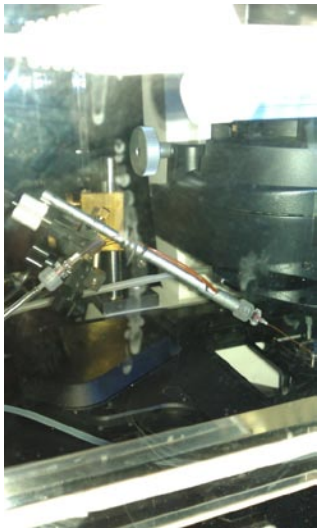




NOW the difficult part:

- Insert the pencil with the mounted tip into the holder inside the chamber (yellow metal screws).
- All the other screws are needed to position the pencil on top of the sample.
- In the optimal case the tip should be close to the objective at a not too flat angle. Now either try to dip the tip into the medium for local application or leave it close to the surface in case you just want to add a small volume to the whole dish.

Ready to go: 4 different liquids can be applied now by pressing the correct numer (1 to 8) on the control unit.



BE AWARE THAT FORGETTING TO CLOSE THE VALVES WILL LEAD TO FLOODING OF THE DISH and the MICROSCOPE !!!!

2. CONTINUOUS (BATH) PERFUSION

Can be done with specifically equipped dishes (such as a Ludin chamber).There, an inlet tube is connected directly (without tip and pencil) to the inlet of the chamber. Liquid supply is as for the local perfusion/application via the syringes mounted on the left side of the microscope.

Second a pump is needed to remove the liquid from the surface of the chamber (floor). Thus it is possible to continuously exchange the medium generating a flow inside the chamber. Bath perfusion and local perfusion can be combined for physiological experiments. Here it is again very important that the **suction is sufficiently effective in order to remove the excess of liquid- > NO FLOODING OF THE MICROSCOPE, PLEASE!**

CLEANING

After use the system must be cleaned by first flushing all tubing with bidest. water (MilliQ or equivalent) and ethanol (p.A.) in order to enhance drying.