

# BioZone 6<sup>TM</sup>

## **Biozone 6 Setup Guide**

Start-up Manual for the Biozone 6<sup>TM</sup> System

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## System components

The Biozone 6™ System is composed of a precision pressure controller (PPC-1-6CH), a pipette holder, a disposable pipette tip, connector USB cable and a power cable.



Biozone 6 PPC-1-6CH



Biozone 6 PPC-1-6CH



Disposable pipette tip



USB cable



Adaptor and power supply cable

## PPC-1-6CH Setup

Connecting the Biozone® PPC-1-6CH:

- Position the PPC-1-6CH unit stable surface close to, but not connected to the microscope.
- Plug the 5V power cable into the wall socket, then into the PPC-1-6CH.
- Connect the USB cable first to the computer, then to the PPC-1-6CH.
- Turn on the PPC-1-6CH by pressing the power switch which is located at the back of the unit. A blue light should be visible on the front panel.

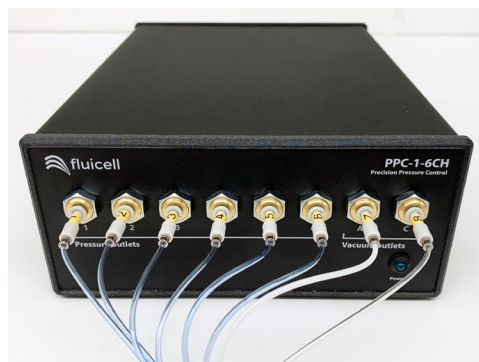
⚠ Only use the 5V cable provided with the PPC-1-6CH unit.



## Connecting the tubing

The pipette holder connects to the PPC-1-6CH via pneumatic tubing. The tubes are connected via quick release connectors and are color coded and numbered for easy identification.


- Connect the tubes by inserting the plugs into the connectors. The tubes are color coded as in blue for solution, white for internal vacuum and clear for external vacuum. The tubes should be inserted in the order indicated by the number on stated on the plug.



- When inserting the tubing plugs, take care to ensure proper insertion reaching the back of the socket. The plug should not be removable without depressing the plastic collar.



- To remove the tubing from each socket, push in the collar to release its grip from the plug and gently remove the tube while holding the plug.

 There should be no need to apply force when removing the plug and it should never be removed by pulling on the tubing. Forceful removal could damage the tubing and/or the socket.

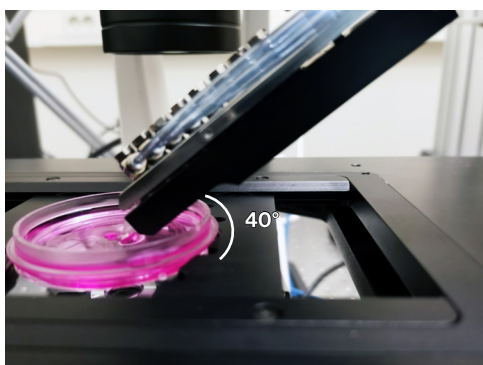


## Biozone 6 positioning

### The stand

The pipette holder is compatible with almost any micromanipulator and it should be positioned to allow the holder to be placed close to the sample.

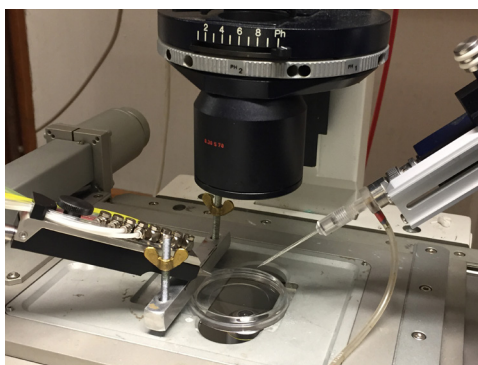
The typical positioning of the pipette holder is shown in the picture below with an angle of 40° to the horizontal of the sample plane.



### Setup example, electrophysiology

Because Biozone 6 can be freely positioned, it can be used in conjunction with almost any experimental setup.

The picture below shows a typical setup for electrophysiology, displaying Biozone 6 together with a standard patch clamp pipette and headstage.



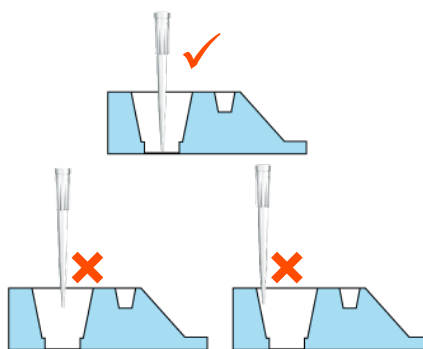


## Using Biozone 6

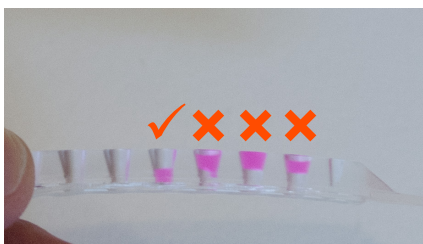
### Filling the consumable pipette tips

The pipette tip can be filled using standard transfer pipette tips. The transfer pipette has to reach the bottom of the well as shown in the picture below. Each well can hold between 10-30  $\mu$ L of solution.

All wells 1-6 need to contain solution, ideally all with the same volume. If fewer wells are needed, remaining wells can be filled with water or buffer.



- It is important to make sure that there is no air trapped in the wells. Aspirated the solution up and down to remove any bubbles. This can be visualized by looking at the side profile of the wells.

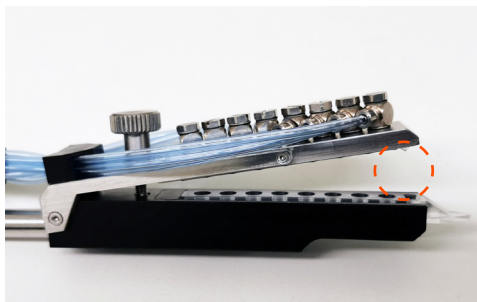



## Inserting the tip into the holder

Insert the pipette tip into holder by partly unscrewing the thumb screw, lifting the top plate and sliding in the pipette tip from the front to the back.

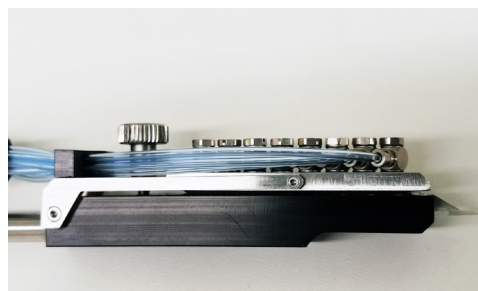
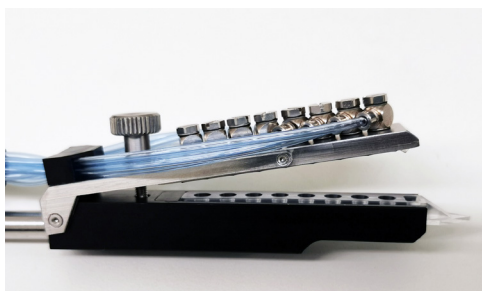
There are two alignment junctions in the pipette setup:

- A back stop, preventing the pipette tip from being slid back too far. The tip must be pushed all the way into the back of the pipette holder.
- A front wedge on the manifold which locks into the rectangular slot at the front of the pipette tip.




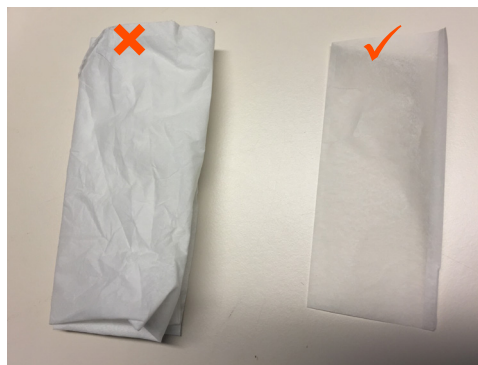
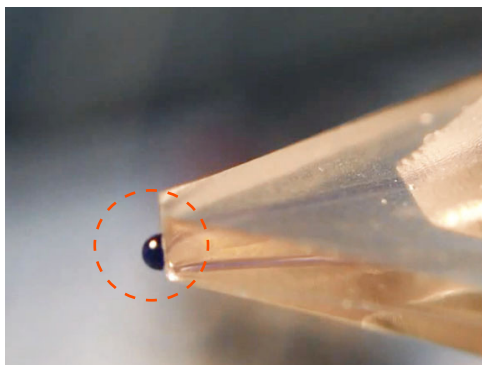
 Incorrect positioning will not allow the pipette holder to close properly. Faulty positioning of the pipette tip is easily recognized due to the need to apply excessive force to close the pipette holder. If incorrectly positioned, Biozone 6 will not function properly and likely will give leak warnings on the PPC-1-6CH.

Correct positioning is shown in the images below.



After initializing the Biozone 6, a droplet should appear at the tip of the pipette. Remove the droplet with a lens tissue.

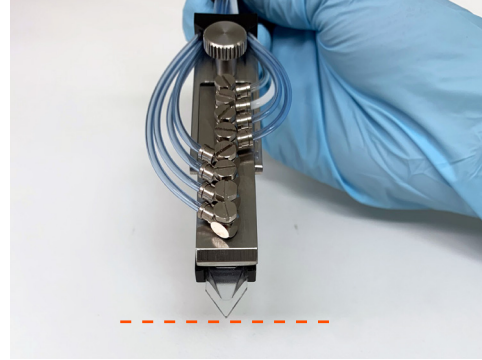
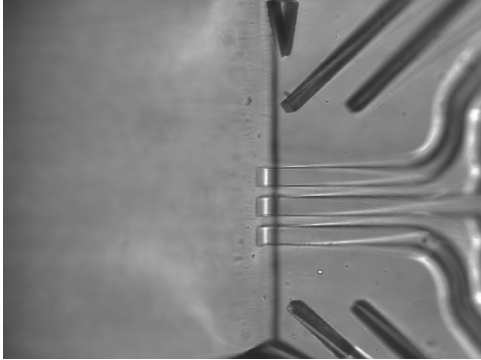
 Use a lint and particulate free tissue needs to remove the droplet. Normal tissue paper can contaminate the tip.



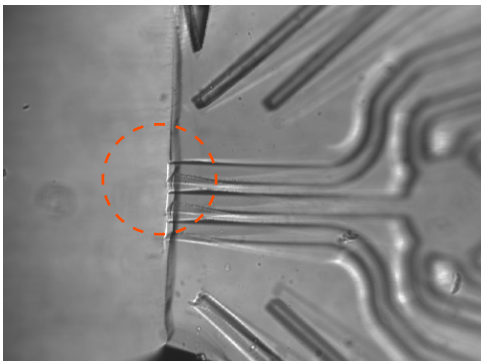


## Pipette positioning guide

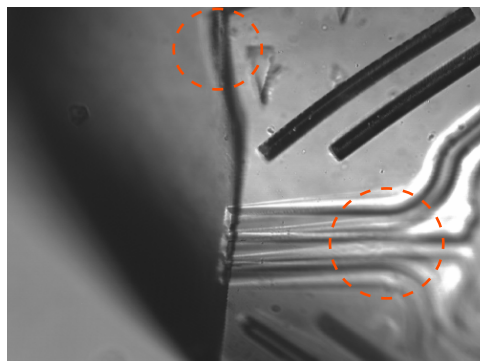
- Ideal positioning of the pipette tip is close to the sample surface. Observation under the microscope should present square channels in equal focus with a sharp front edge, as shown below.



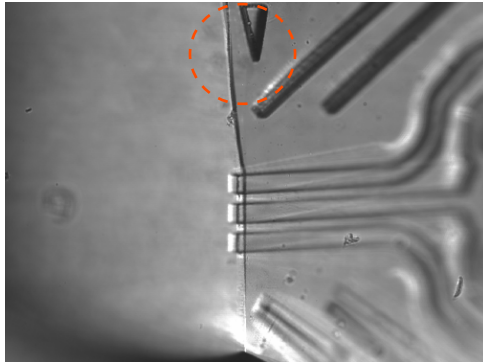
- Pushing the tip too far against the sample surface will provoke bending at the front as shown below, resulting in a visible deformation.



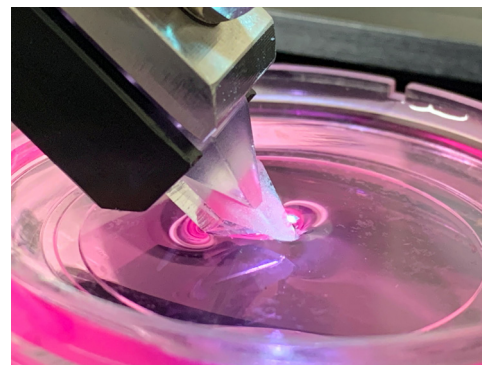
- Translating the tip when in contact with the surface will bend and twist the channels. This can be observed as a variance in focus across the tip and a wavy front edge, demonstrated below.



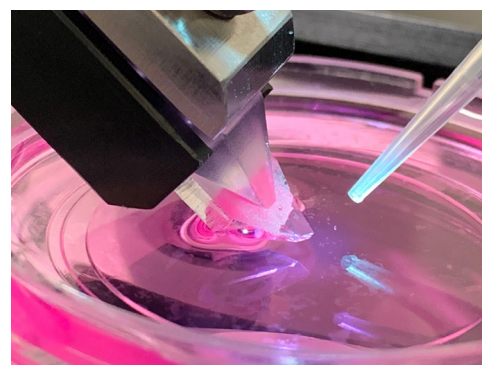
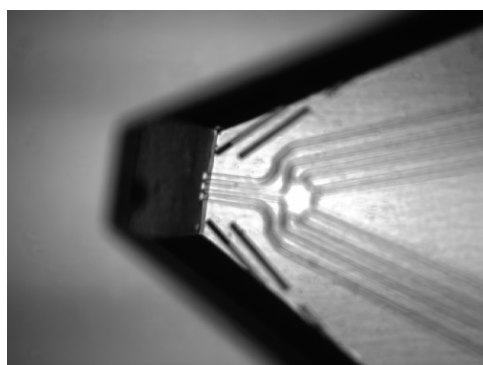
- Mounting the tip on an angle to the sample plane, will result in one side contacting the sample surface. As shown below, there is a variance in focus across the tip, ending in a wavy front corner.



- Due to the hydrophobic nature of the disposable tips, upon insertion into the sample solution, a meniscus around the tip can be formed. This will result in a blurry bright-field microscopy image, and may impair visibility.



- By using a transfer pipette tip, you can break about the tip, resulting in a clear microscopy image.



# Cleaning

When it is suspected that the tubing contains any liquid, the liquid needs to be removed.


## Air cleaning

Air cleaning is a convenient way of driving out liquid from the tubing using air pulses.

1. Remove the pipette tip. Dry and clean the pipette holder with a lint free tissue soaked with ethanol. Pay special attention to the manifold underside which is the closest to the tip wells. Remove all the tubes from the PPC-1-6CH.
2. Connect tubes 5 to 8 into the sockets on the unit for tubes 1 to 4.

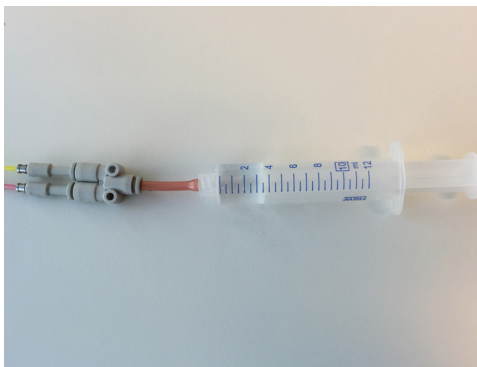


3. Using the software, (Fluicell® Touch Wizard), set the  $P_{on}$  pressure to 200 mbar and set the timer below each solution to 5 seconds.
4. Switch to each solution 1 to 4 in turn, allowing the timed air pulse to conclude and the pressure to re-stabilize to 200 mbar, between each solution.


 The Biozone 6 PPC-1-6CH has to regain a pressure of 200 mbar between each air pulse to ensure uniform pressure delivery. This way of cleaning has to be chosen first, and can be repeated several times.

## Thorough cleaning

If there is any persistent liquid or residues in the tubing after air cleaning process, it can be flushed with distilled water followed by ethanol. In the cleaning kit provided by Fluicell, there is a syringe and a double-ended connector, to attach the pipette holder.



- The flushing should be done over a sink to ensure correct disposal of the flushing liquid.
- As soon as the manual cleaning process is completed, the process as described above (Air cleaning) needs to be performed at least three times to dry out the tubing.

 During the manual cleaning process, the pipette holder and the tubing must not be connected in any way to the PPC-1-6CH