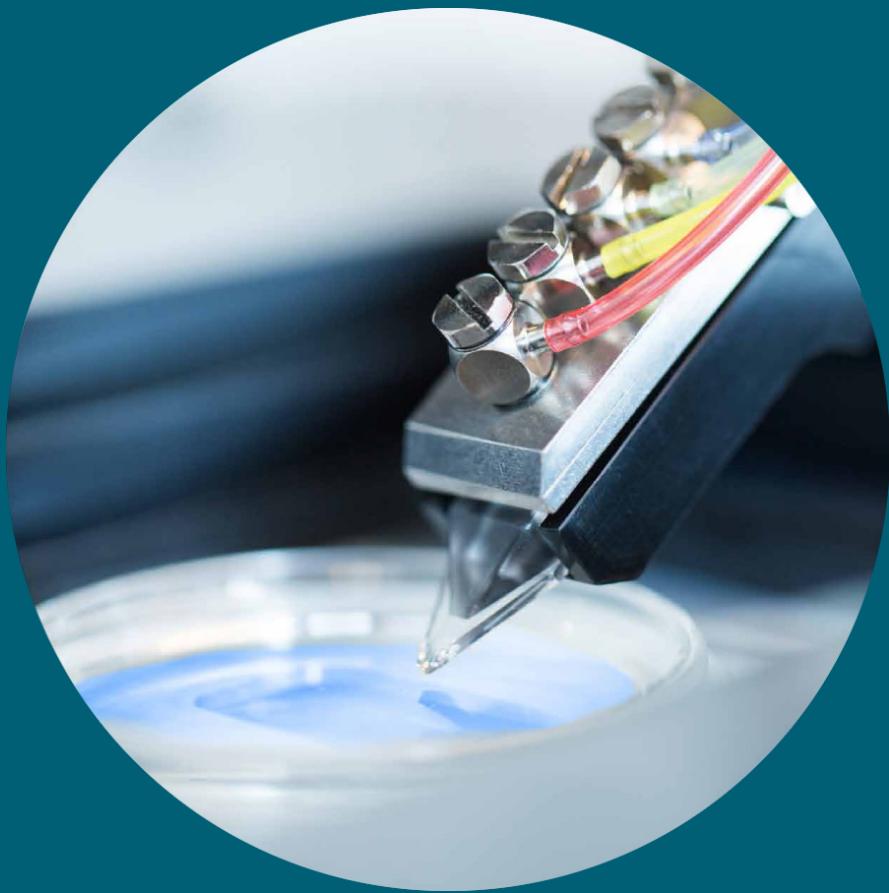




BioPen® System

Quick Start Manual



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Contents

Setup guide / Startup manual

1. Biopen system components	<u>3</u>
2. Biopen PPC-1 setup	<u>4</u>
3. Connecting the tubing	<u>5</u>
4. Positioning the Biopen	<u>6</u>
4.1. The stand	<u>6</u>
4.2. Setup example: electrophysiology	<u>6</u>
5. Using the Biopen	<u>7</u>
5.1. Filling the consumable pipette tip	<u>7</u>
5.2 Inserting the pipette tip into the pipette holder	<u>7</u>
6. Pipette positioning guide	<u>9</u>
7. Cleaning	<u>11</u>
7.1. Air cleaning	<u>11</u>
7.2. Thorough cleaning	<u>11</u>

Biopen Wizard / Basic functions

1. Interface guide Starting Biopen wizard	<u>15</u>
2. Starting Biopen wizard	<u>17</u>
3. Setting up a new tip	<u>18</u>
4. Solution Delivery and switching	<u>21</u>
5. Delivery zone control	<u>24</u>
5.1. Changing the size of the delivery zone	<u>24</u>
5.2. Changing the flow speed.....	<u>26</u>
6. Solution delivery duration.....	<u>27</u>
7. Actions panel.....	<u>29</u>
8. Advanced panel/ Set delivery zone percentages	<u>30</u>
9. Protocol editor.....	<u>32</u>
9.1. Write a protocol.....	<u>33</u>
9.2. Load a protocol	<u>36</u>
9.3. Run a protocol	<u>38</u>
10. Shutdown the instrument	<u>40</u>



Setup guide

Start-up manual for the Biopen® system

Contents

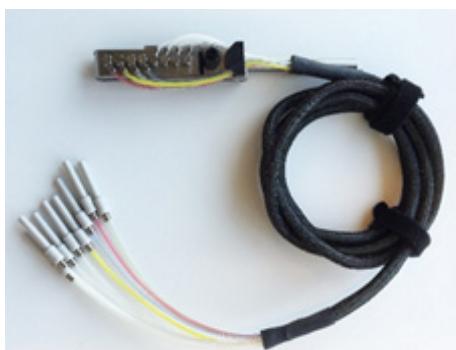
1. Biopen system components	<u>3</u>
2. Biopen PPC-1 setup	<u>4</u>
3. Connecting the tubing	<u>5</u>
4. Positioning the Biopen	<u>6</u>
4.1. The stand	<u>6</u>
4.2. Setup example: electrophysiology	<u>6</u>
5. Using the Biopen	<u>7</u>
5.1. Filling the consumable pipette tip	<u>7</u>
5.2 Inserting the pipette tip into the pipette holder	<u>7</u>
6. Pipette positioning guide	<u>9</u>
7. Cleaning	<u>11</u>
7.1. Air cleaning	<u>11</u>
7.2. Thorough cleaning	<u>11</u>

1. Biopen® system components

The Biopen® system is composed of several components: a Biopen® precision pressure controller PPC-1, a pipette holder, a disposable pipette tip, as well as power and USB cables.



Biopen PPC-1



Pipette holder



Pipette tip



USB cable



Adaptor power supply & cable

2. Biopen PPC-1 setup

Connecting the BioPen® PPC-1:

- The BioPen® PPC-1 needs to be safely positioned on a stable surface close to, but not connected to the microscope.
- Once positioned, the cables have to be plugged into the sockets located at the back of the PPC-1.
- Firstly, the 5V Power cable should be plugged into the wall socket, then into the PPC-1.
- Secondly connect the USB cable first to the computer, then to the PPC-1.
- Turn on the PPC-1 by pressing the power switch which is located at the back of the unit. A blue light should appear at the front.



Only the 5V cable provided with the BioPen® PPC-1 should be used.



3. Connecting the tubing

The pipette holder connects to the PPC-1 via pneumatic tubing. The tubes are connected via quick release connectors color coded for ease of identification.

-  The plugs should be connected following the same color scheme as on the top of the pipette holder; red corresponds to number 1, yellow to number 2, etc.



-  When inserting the tubing plugs, care should be taken 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, the collar must be pushed in, to release its grip from the plug. It can then be removed gently.

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.

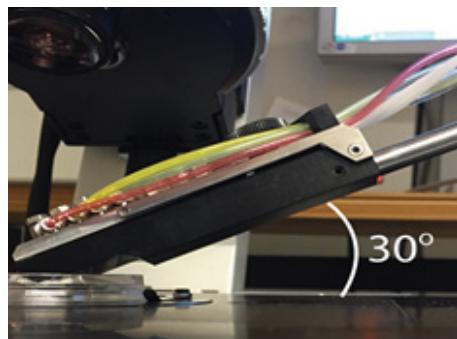


4. Positioning the Biopen

4.1. The stand

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

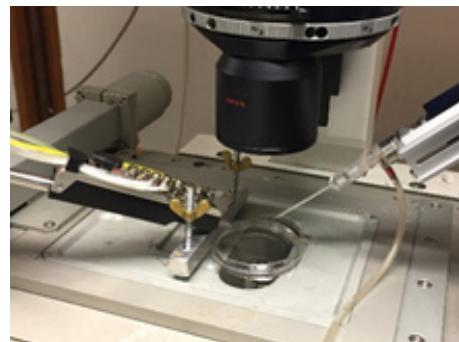
The typical positioning of the pipette holder is shown in the picture below with an angle of 30 degrees to the horizontal of the sample plane.



4.2. Setup example: electrophysiology

Due to its freely positionable nature, the Biopen® System can be used in conjunction with almost any other probes.

The picture below is showing a typical setup for electrophysiology, displaying it in relation to a standard patch clamp pipette and headstage.

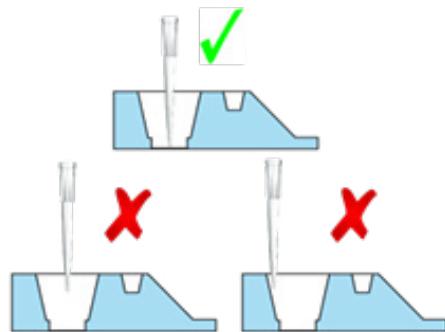


5. Using the Biopen

5.1. Filling the consumable pipette tip

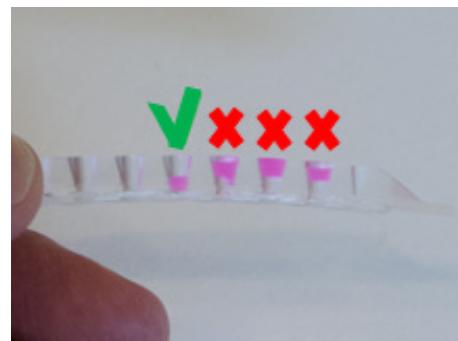
The pipette tip can be filled using standard transfer pipette tips. The transfer pipette has to reach the bottom of the well as shown in the picture below. Each well can hold between 10-30 µl of solution.

- ⚠ All wells 1 to 4 need to contain solution; ideally all with the same volume. If fewer well is 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. The solution can be aspirated up and down to remove any bubbles.

This can be visualized by looking at the side profile of the wells.

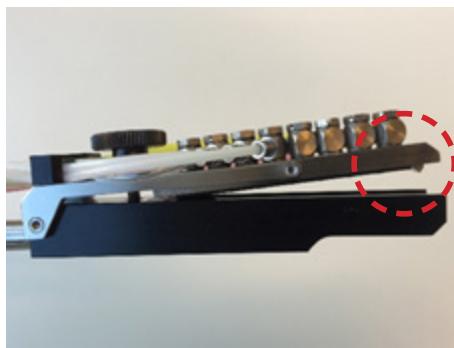


5.1. Inserting the pipette tip into the pipette holder

The pipette tip has to be inserted into the pipette holder by partly unscrewing the thumb screw, lifting the top plate, then, sliding in the pipette tip from the front to the back.

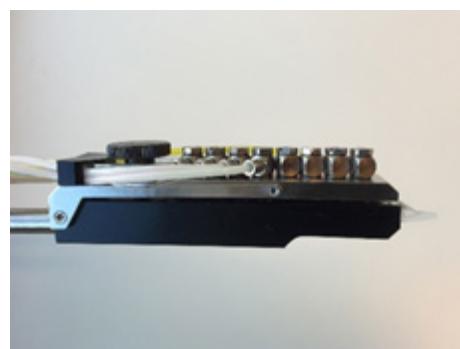
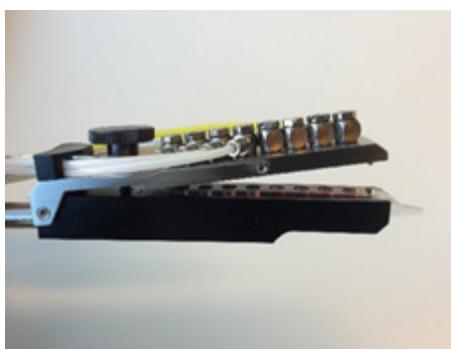
There are 2 alignment junctions in the pipette setup:

- 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 and will be easily recognized due to the need to apply excessive force to close the pipette holder. If incorrectly positioned, the Biopen® will not function properly and likely will give leak warnings on the PPC-1.

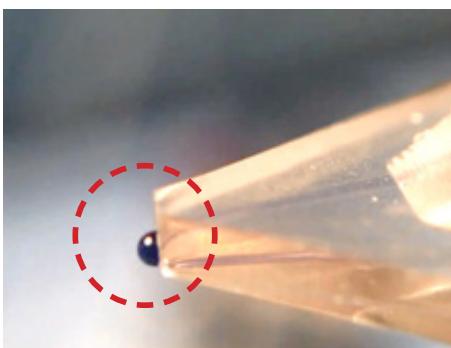
Correct positioning of the pipette tip in the pipette holder is shown in the pictures below.



After setting up a new tip (see [Setting up a new tip](#) paragraph in the Biopen® Wizard section, p18, a droplet should appear at the tip of the pipette which has to be removed with a lens tissue.

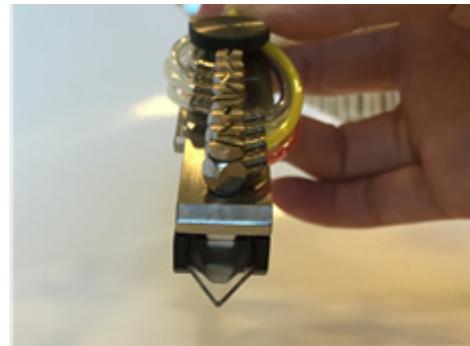
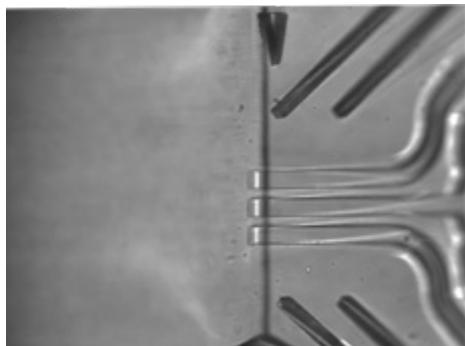


Do not use normal tissue to remove the droplet, it would contaminate the tip. A lint and particulate free tissue needs to be used.

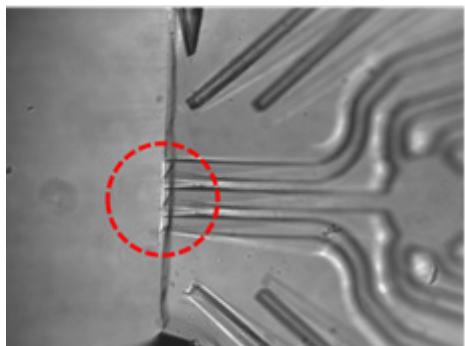


6. Pipette positioning guide

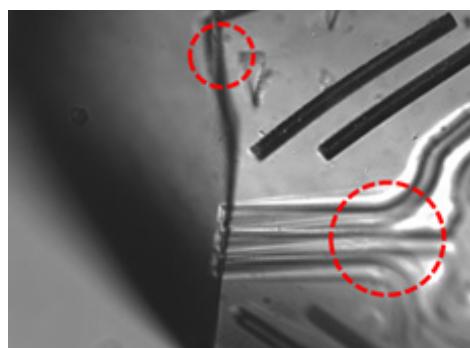
Ideal positioning of the pipette tip is close to the sample surface. Observation under the microscope should present square channels of equal focus, as shown below, with a sharp front edge.



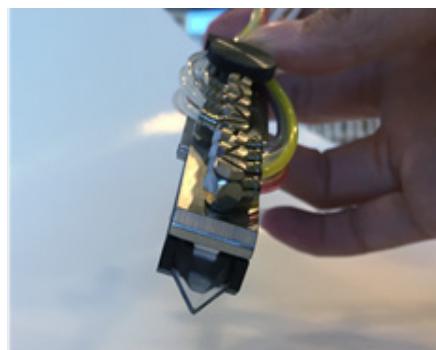
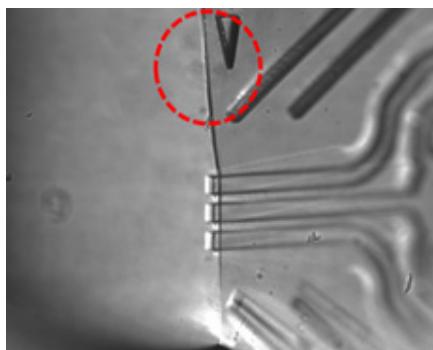
Pushing the tip too far against the sample surface will provoke bending at the front as shown below, resulting in a visible deformation



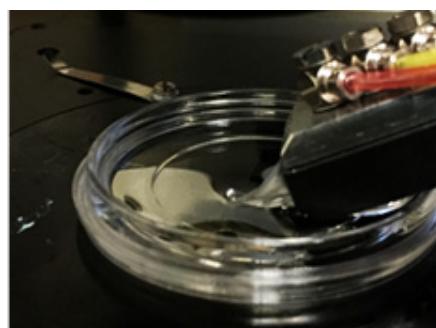
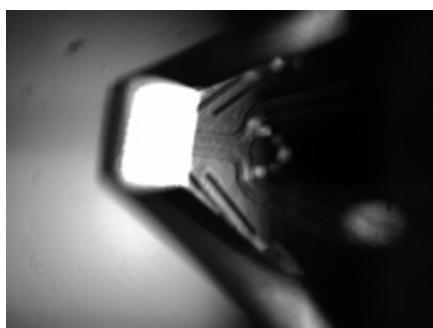
Translating the tip when in contact with the surface will bend and twist the channels and also push the tip forward. As shown, this can be visualized by a variance in focus across the tip and a wavy front edge.



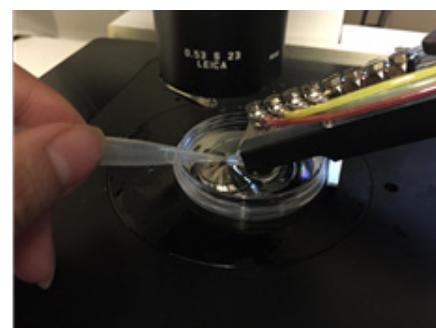
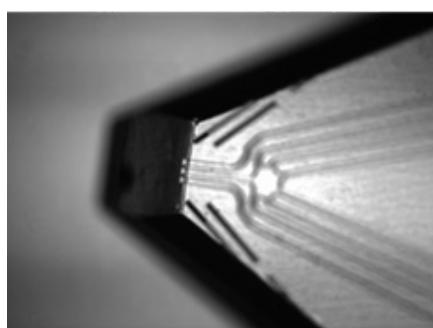
Mounting the tip on an angle to the sample plane, will result in one side contacting the sample surface. As shown below, there is a variance in focus across the tip, ending in a wavy front corner.



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 the miniscus about the tip, resulting in a clear microscopy image.



7. Cleaning

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

7.1. Air cleaning

A convenient way of proceeding is to apply pulses of air to drive out the liquid.

1) First, remove the pipette tip. Dry and clean the pipette holder with a free tissue soaked with ethanol, especially the manifold underside which is the closest to the tip wells. Remove all the tubes from the PPC-1.

2) Connect tubes 5 to 8 into the sockets on the unit for tubes 1 to 4. 



3) After connecting the computer to the Biopen PPC-1 unit via the USB cable, turn on the unit by pressing the power switch localized on the back. Double-click on Biopen icon to launch the program. Click on *Connect* button on the top main bar (see [Starting Biopen wizard](#) paragraph in the Biopen® Wizard section, p17).

4) Click on the *Editor* button on the top main bar and load the *cleaning process* protocol. Run the cleaning process protocol (see [Load a protocol](#) sub-paragraph in the Biopen® Wizard section, p36). This way of cleaning has to be chosen first, and can be repeated several times.

7.2. Thorough cleaning

If there is any persistent liquid or residues in the tubing after process 1, 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, as leaks can occur, in addition to correct disposal of the flushing liquid.



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

As soon as the manual cleaning process is completed, the process as described here above (see [7.1. Air Cleaning](#)) needs to be performed at least **three times** to dry out the tubing.



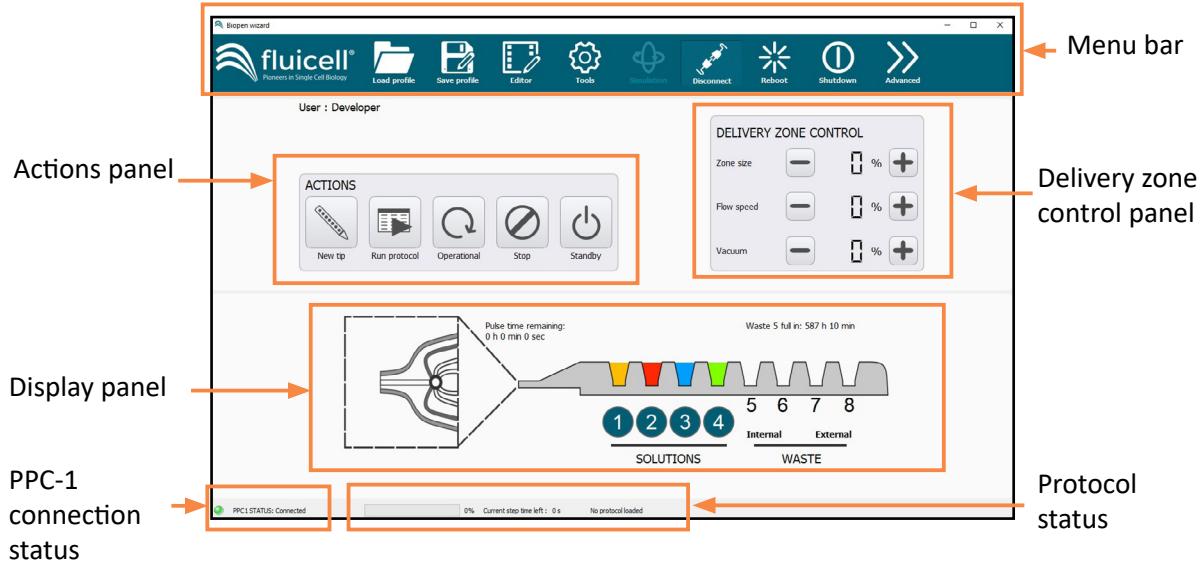
BioPen® Wizard

Quick start / Basic functions

Contents

1. Interface guide	<u>15</u>
2. Starting Biopen wizard.....	<u>17</u>
3. Setting up a new tip	<u>17</u>
4. Solution Delivery and switching.....	<u>21</u>
5. Delivery zone control.....	<u>24</u>
5.1. Changing the size of the delivery zone	<u>24</u>
5.2. Changing the speed of the flow.....	<u>26</u>
6. Solution delivery duration	<u>27</u>
7. Actions panel.....	<u>29</u>
8. Advanced / Set values	<u>30</u>
9. Protocol editor	<u>32</u>
9.1. Write a protocol.....	<u>33</u>
9.2. Load a protocol.....	<u>36</u>
9.3. Run a protocol	<u>38</u>
10. Shutdown the instrument	<u>40</u>

1. Interface guide



The interface is divided into six panels:

MENU BAR: provides access to such functions as Load and Save profile, Tools, Simulation, Connection, Reboot, Shutdown and activation of Advanced interface panel

ACTIONS PANEL: contains basic commands to run the Biopen

DELIVERY ZONE CONTROL PANEL: allows to adjust the delivery zone

DISPLAY PANEL: shows information about the fill level of the solutions and waste wells, the name of the currently delivered solution and an expected approximate size of the solution delivery zone. Within this panel are buttons to individually start or stop the delivery of solutions

PPC-1 STATUS: displays the connection status between the computer and the pressure controller PPC-1

PROTOCOL STATUS: provides information about the loaded protocol and its current status.

The Biopen software displays 9 buttons on the top menu bar: ***Load profile, Save profile, Editor, Tools, Simulation, Connect, Reboot, Shutdown*** and ***Advanced***.

LOAD PROFILE: loads a previously saved profile

SAVE PROFILE: allows to save the current profile to a configuration file

EDITOR: displays the editor panel to write, load or edit protocols

TOOLS: opens a new window to setup your preferences

SIMULATION: aims to help you familiarize yourself with the software or simulate complex protocols without running the pressure controller PPC-1 or being connected to the pipette holder

CONNECT: allows to connect or disconnect the pressure controller PPC-1

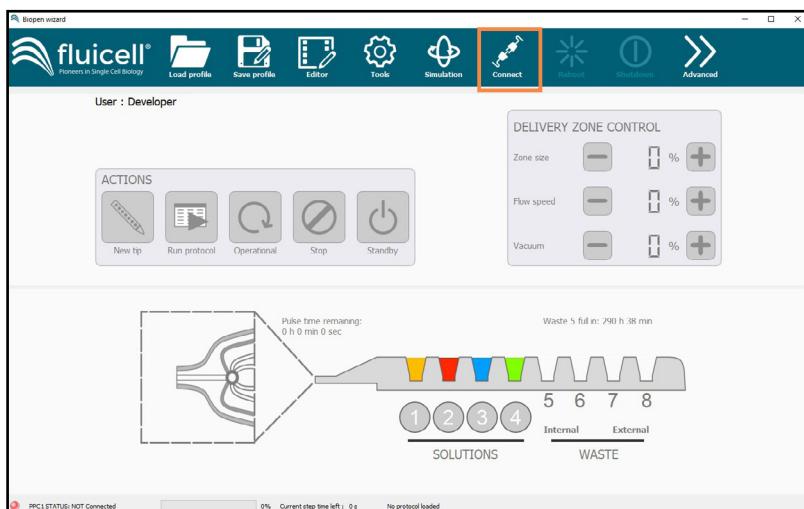
REBOOT: resets the device

SHUTDOWN: initiates the switching off the Biopen system

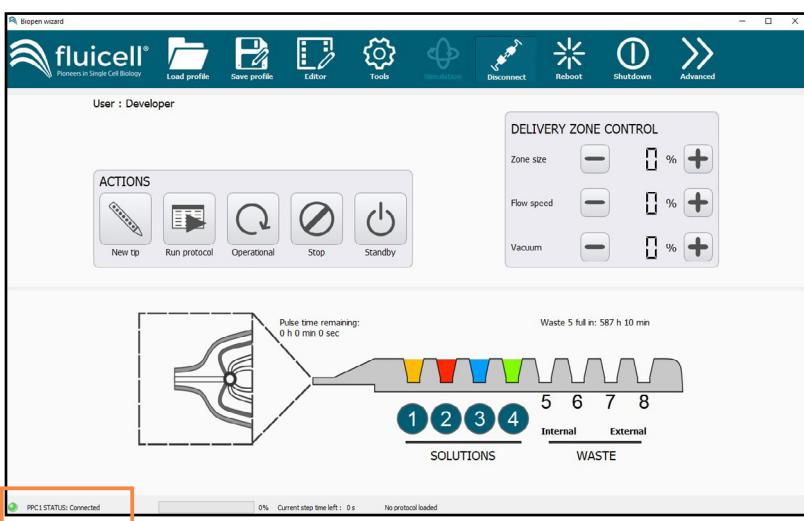
ADVANCED: opens an advanced panel with more options and settings.

2. Starting Biopen wizard

After connecting the computer to the Biopen PPC-1 unit via the USB cable, turn on the unit by pressing the power switch located on the back (see [Biopen PPC-1 setup](#) paragraph in the setup guide section p4). Double-click on Biopen icon to launch the program. Click on *Connect* button on the top main bar (see the orange rectangle in the figure below).



A green circle following by “PPC1 STATUS: Connected” should display at the left bottom of the software panel (see the orange rectangle in the figure below).

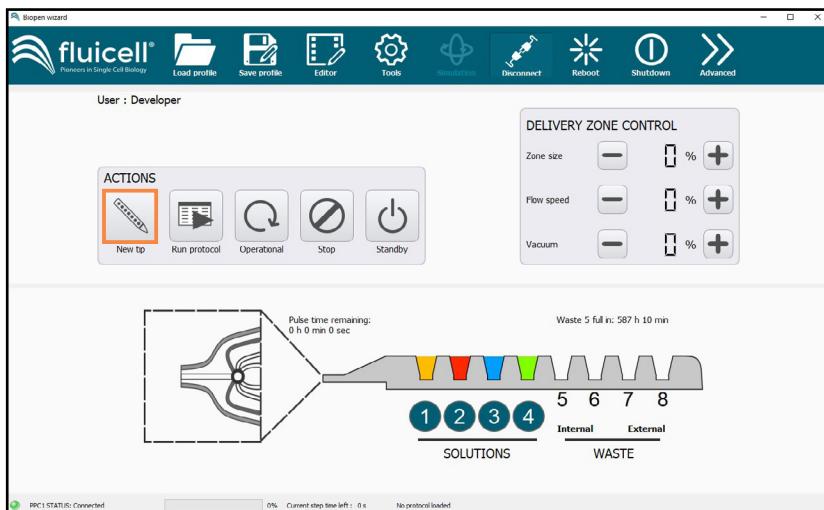


- If the circle stays red, check first that the USB cable is well connected.
- If it's still not working, go to Tools>>Communication and click on *Update available devices*. Then press on the *Apply* button and *OK*. And try to connect again.

3. Setting up a new tip

The software has different protocols preconfigured for setting up the Biopen pipette tip ready for operation. The most used of which is the *New tip*, which prepares a loaded pipette tip for operation. This is a step-by-step procedure, with clear instructions at each step to ensure smooth operation.

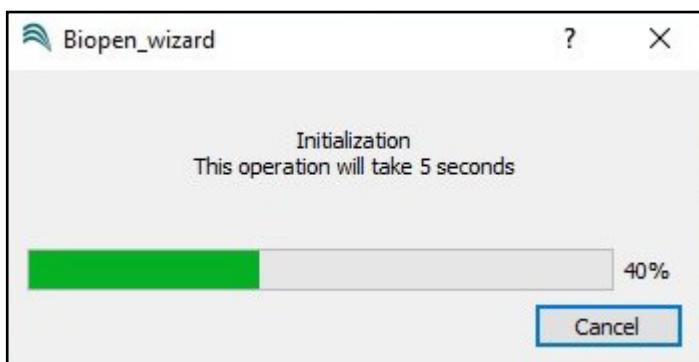
Click on *New tip* button to start (see the orange rectangle in the figure below).



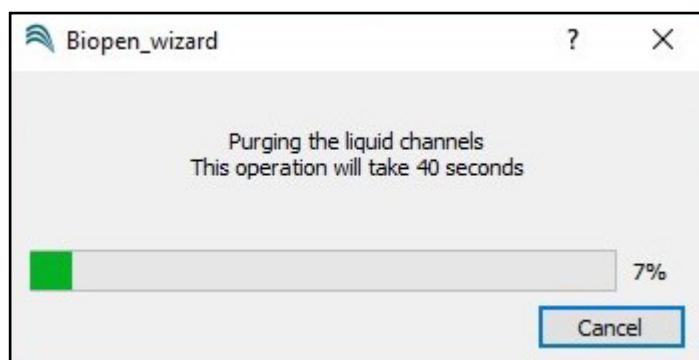
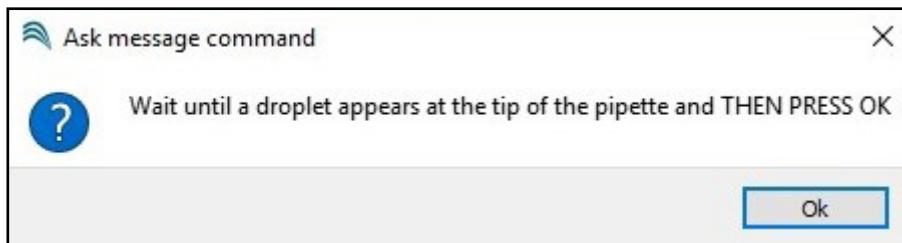
A pop-up notice window displays. Follow the instruction, making sure that the pipette tip is securely placed in the holder and the thumb screw is properly tightened. Pressing *Ok* begins to pressurize the pipette tip.



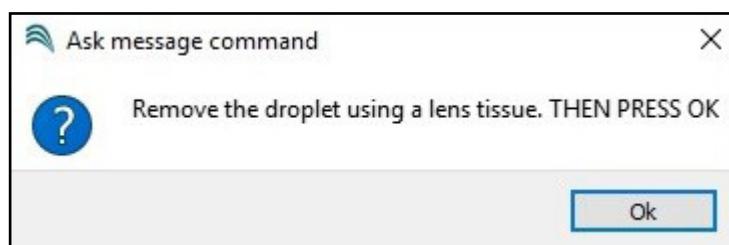
The operation takes 5 seconds.



Wait until a small droplet appears at the tip of the pipette and then press *Ok*. A timed purge then begins.



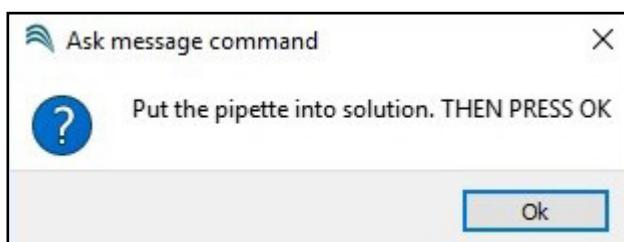
When completed, first remove the droplet using a lens tissue **AND AFTERWARDS** press *Ok*.

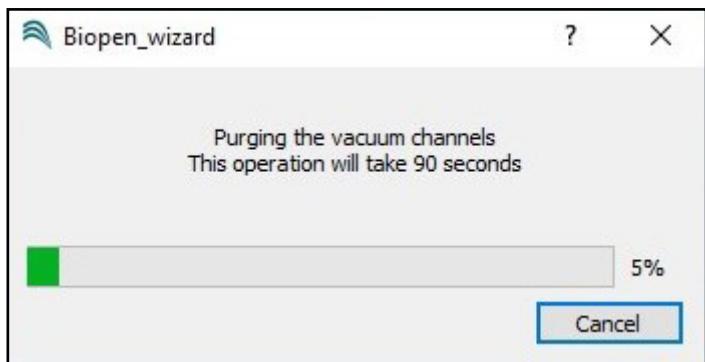


After this the pipette needs to be submerged into your sample solution, ensuring the tip is beneath the liquid surface.

This should not be placed near to the target species, cells or surface, but comfortably submerging the tip of the pipette in the solution within the petri dish. Occasionally an extra drop of solution is needed to be placed on the front of the tip to break the surface tension. This will minimize any shadowing or image distortion (see [Pipette Positioning guide](#) paragraph in the setup guide section p9).

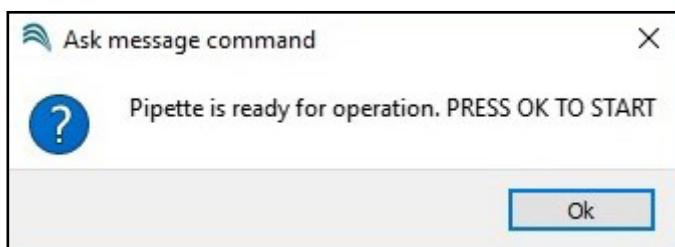
Clicking *Ok* begins the purge of the vacuum channels.





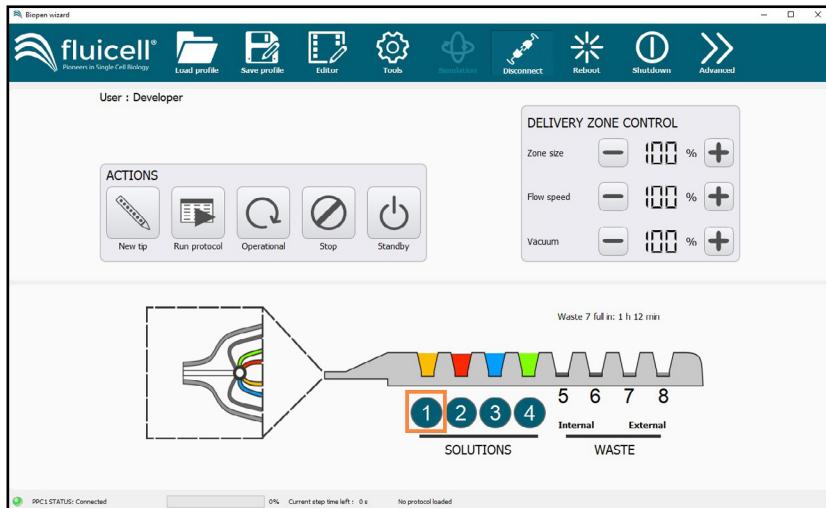
Now the pipette tip is ready for operation.

Click *Ok* to start.

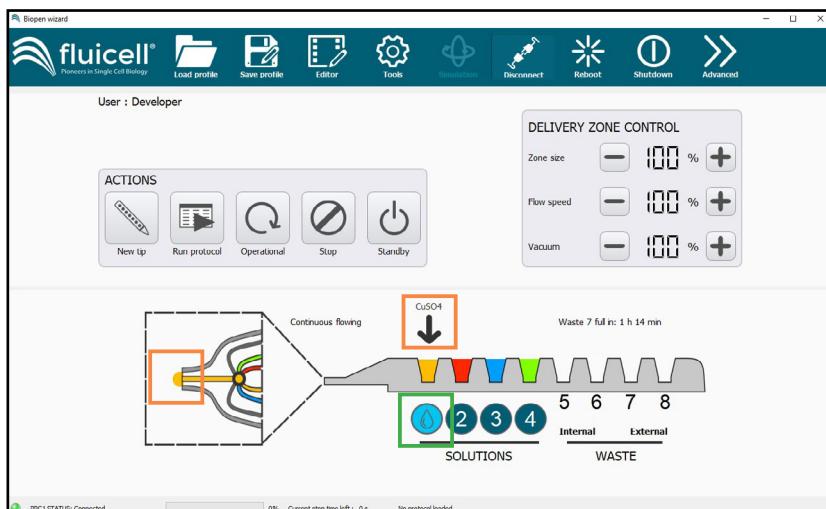


4. Solution delivery and switching

You can deliver independently any of the loaded solutions 1, 2, 3 or 4 **after setting up the tip** (see [Setting up a new tip](#) paragraph of this section, p18). **To deliver continuously solution 1**, click on the solution 1 button (see the orange rectangle in the figure below).

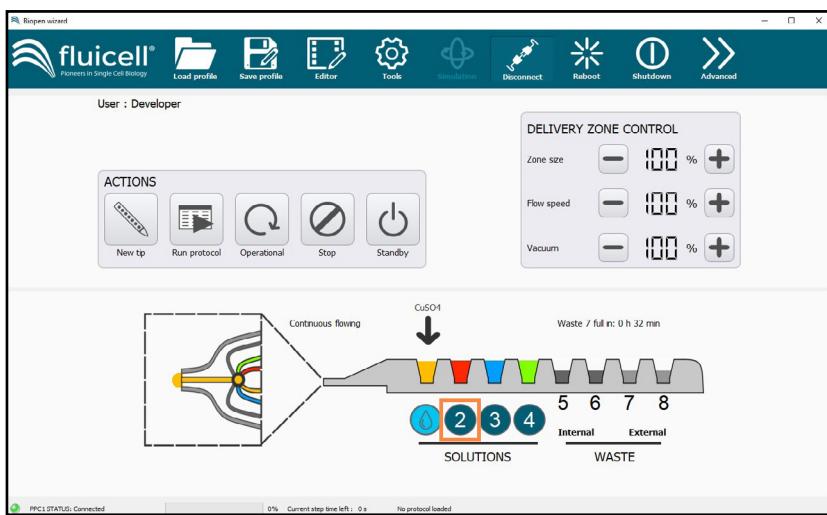


The name of solution 1 appears on the top of the well and the delivery zone appears at the end of the tip (see the two orange rectangles in the figure below). Also the activated solution button changes its appearance to indicate which is the running (see the green rectangle in the figure below).

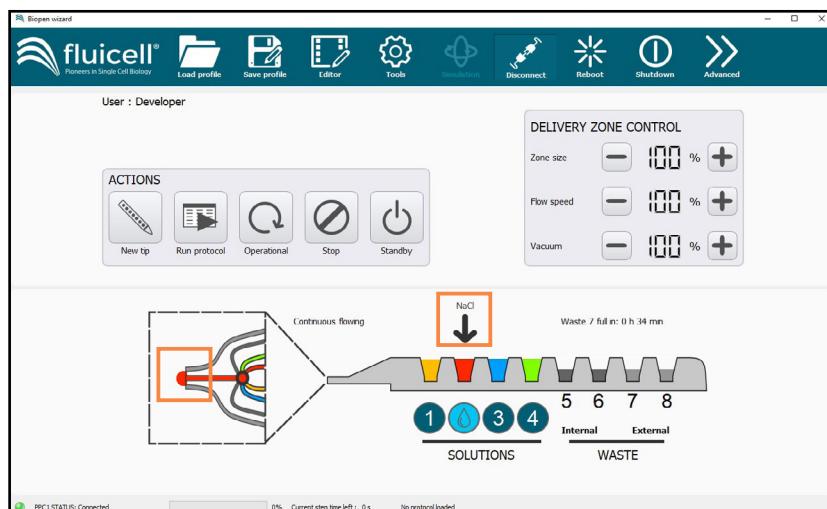


To switch to solution 2, click on the solution 2 button (see the orange rectangle in the next figure).

- When using the solutions buttons in the display panel, there is no need to stop one solution before switching to another, this is done automatically.



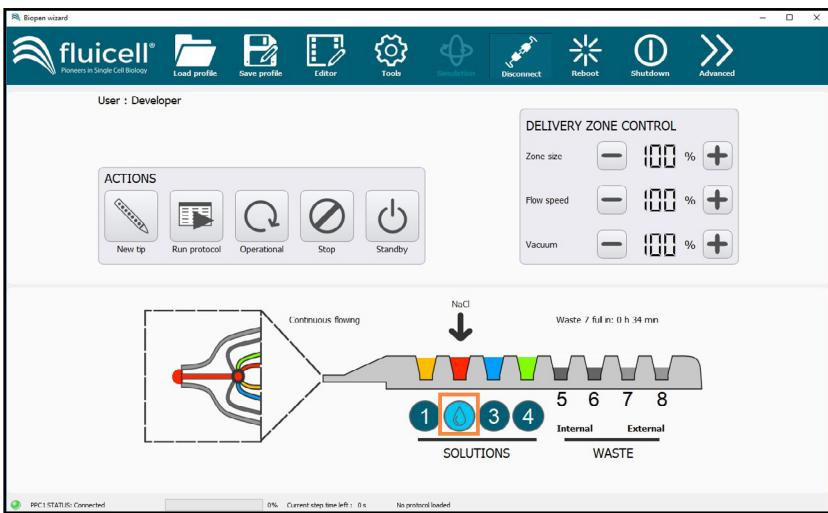
The name of solution 2 appears on the top of the well and the delivery zone displays at the end of the tip (see the two orange rectangles in the figure below).



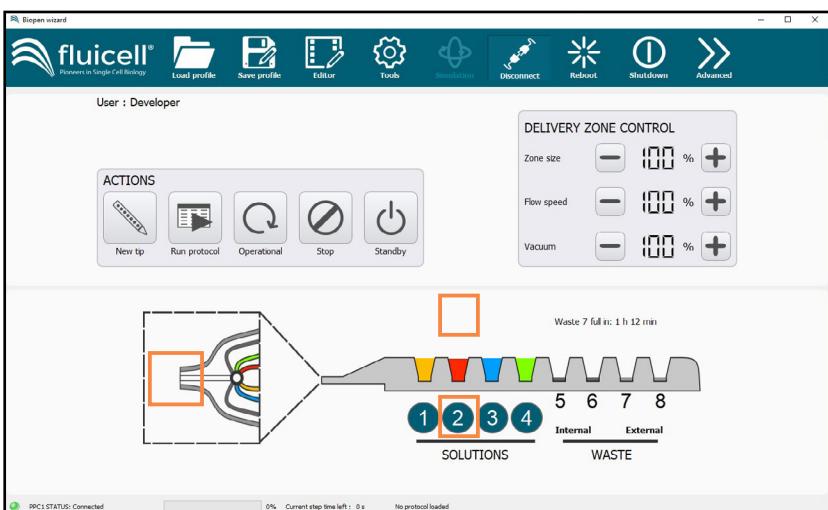
To switch to solution 3, click on the solution 3 button.

To switch to solution 4, click on the solution 4 button.

To STOP delivery, press again the button of the currently delivered solution (see the orange rectangle in the next figure).



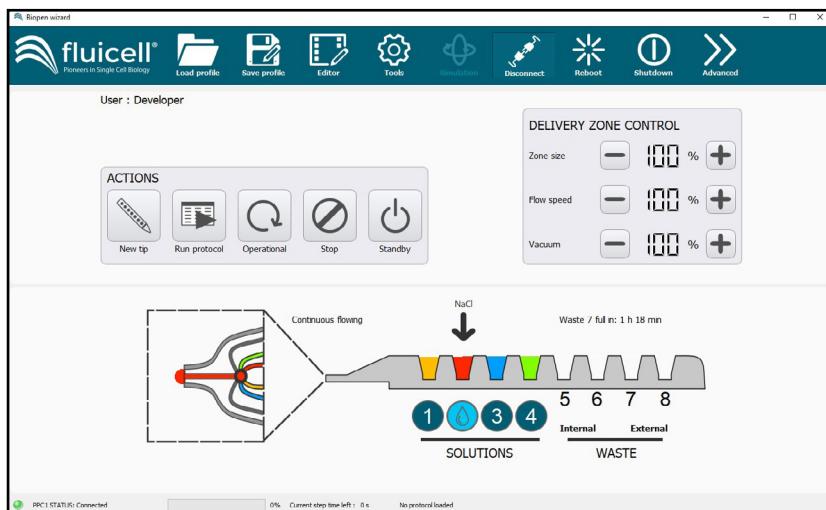
The solution delivery stops, the name of the solution and the delivery zone disappear (see the orange rectangles in the figure below).



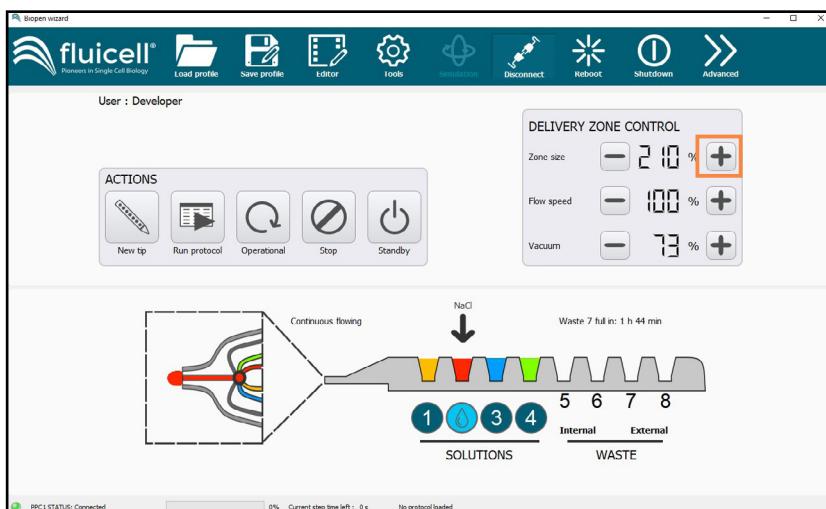
5. Delivery zone control

5.1. Changing the size of the delivery zone

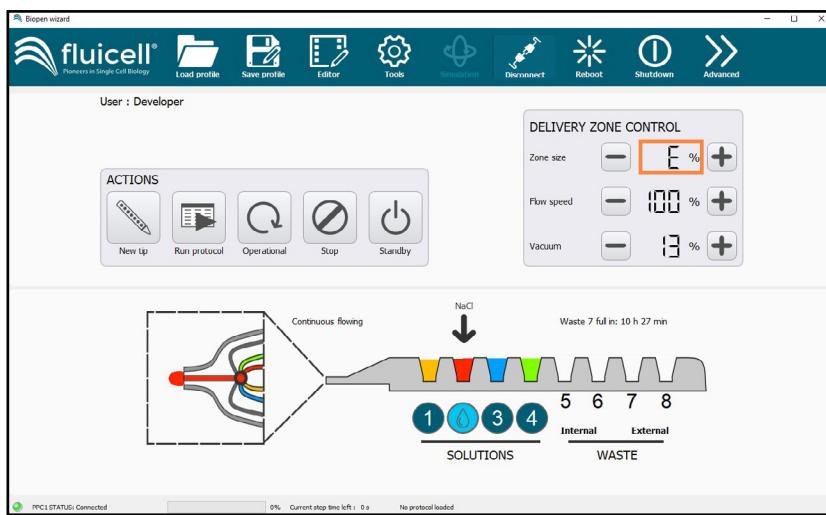
First, select the solution you want to deliver. In the following example, solution 2 is being delivered by clicking the solution 2 button.



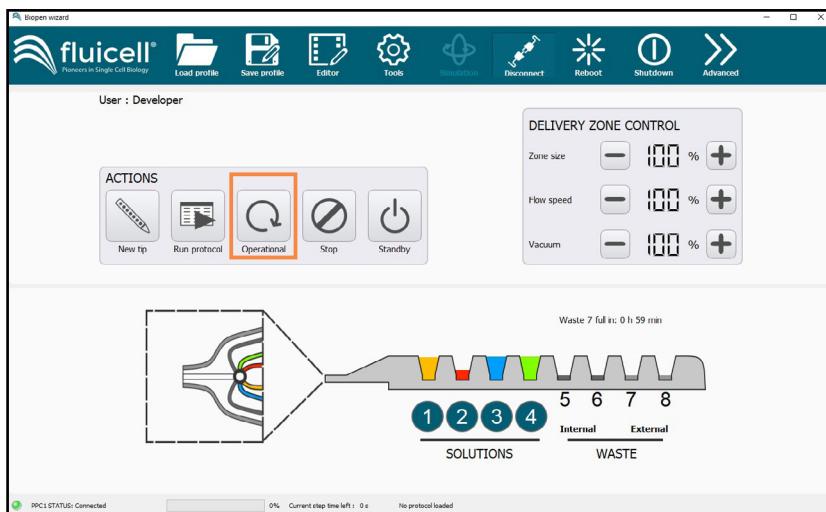
To **INCREASE** the size of the delivery zone, click on the **+** button (see the orange rectangle in the figure below).



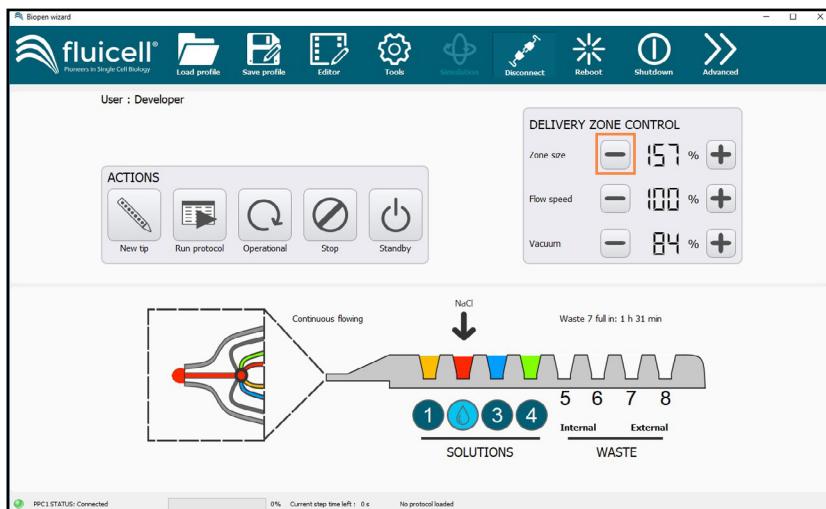
A range between 50 to 200 % is recommended, increasing the size of the delivery zone beyond it, may compromise the localized delivery process and result in **diffusion** of your solution into your sample. It is recommended to always observe under the microscope the size of your delivery zone. An "E" for Error will display (see the orange rectangle in the figure below) if the zone size is set out of the recommended range.



To return to **STANDARD VALUES**, click on the *Operational* button (see the orange rectangle in the figure below). This stops the delivery and returns all the percentages in the *DELIVERY ZONE CONTROL* panel to 100 %.

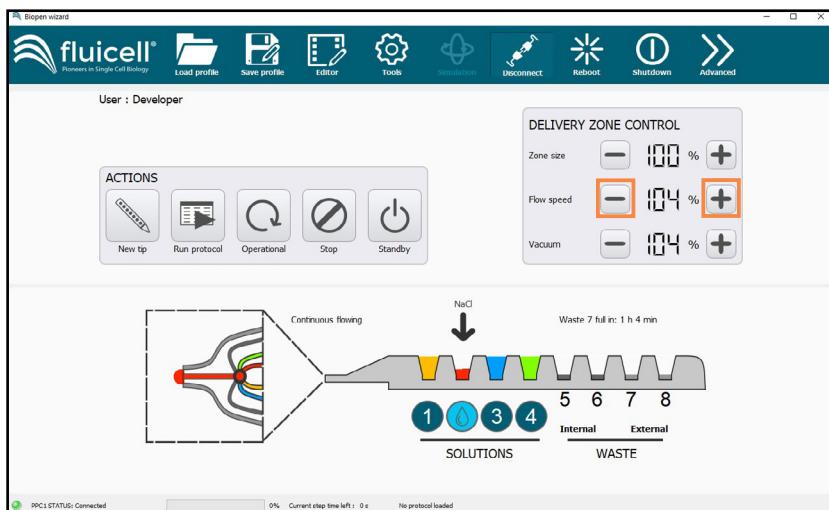


To **DECREASE** the size of the delivery zone, click on the - button (see the orange rectangle in the next figure).



5.2. Changing the flow speed

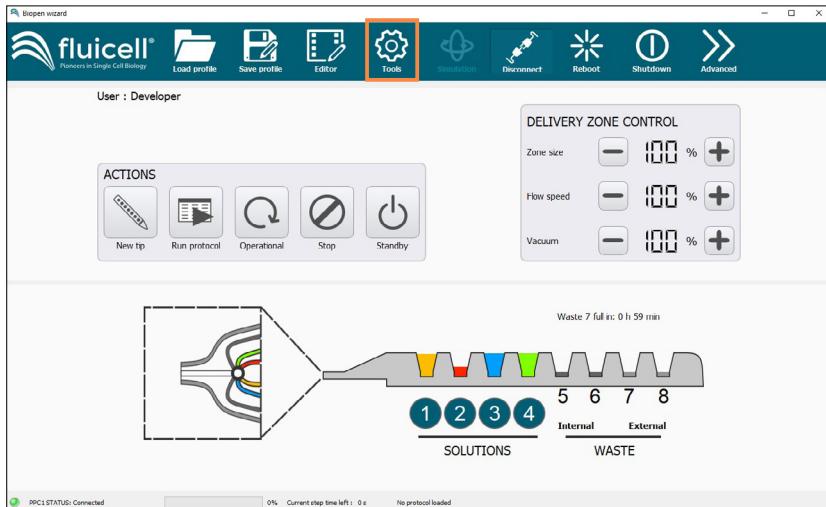
You can **INCREASE** or **DECREASE** the **flow speed** of delivered solution by pressing the **+** and **-** buttons (see the two orange rectangles below).



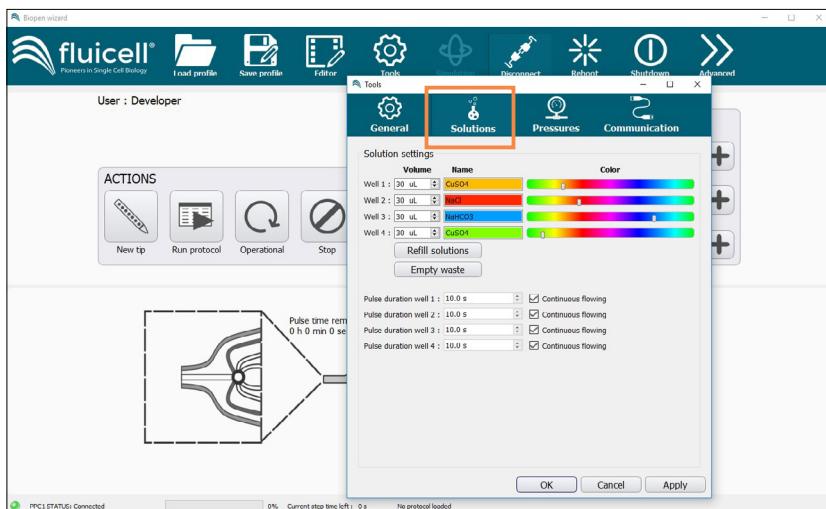
! Be aware that by increasing the flow speed, you will consume your solutions more rapidly and fill your waste wells faster. In contrast, by decreasing the flow speed, you will consume less of your solutions, reduce the waste fill time and slow down the solution exchange speed.

6. Solution delivery duration

You can adjust the delivery duration of each solution by clicking the **Tools** tab on the top Menu bar (see the orange rectangle below).

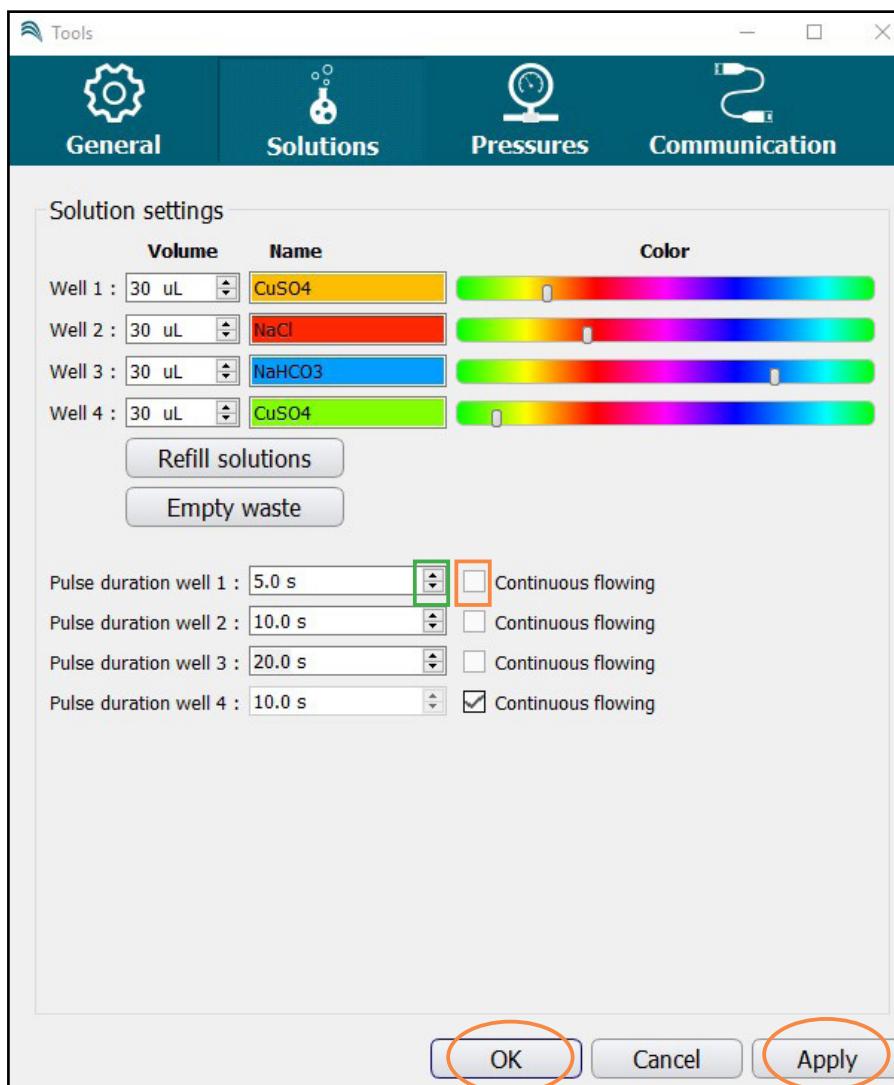


A pop-up window displays. Click on the *Solutions* tab (see the orange rectangle in the figure below).



For each solution, you can choose between **continuous flowing** or **pulse**. To adjust pulse duration, unselect first the *Continuous flowing* box to the right of the chosen solution (see orange rectangle in the figure below). Then increase or decrease the pulse duration by clicking up or down arrows (see green rectangle in the figure below). Notice that the **time unit is second**. To save the changes, click the **Apply** button and **OK** (see the two orange circles in the figure below).

In the example below, solutions 1, 2 and 3 will be delivered respectively 5, 10, 20 seconds and the solution 4 will be delivered continuously.



7. Actions panel

The Actions panel has 5 buttons: **New tip**, **Run protocol**, **Operational**, **Stop** and **Standby**.

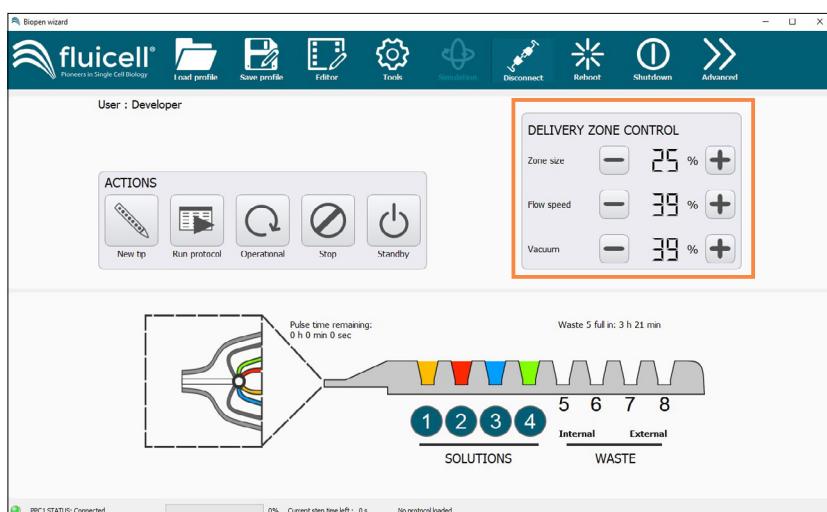
NEW TIP: initiates the preparation of the Biopen system for operation. It is a step-by-step setup with clear instructions at each step to ensure smooth start (see [Setting up a new tip](#) paragraph in this section, p18).

RUN PROTOCOL: starts the last protocol loaded in the *Editor* window.

OPERATIONAL: sets all the values of the delivery zone back to 100 %.

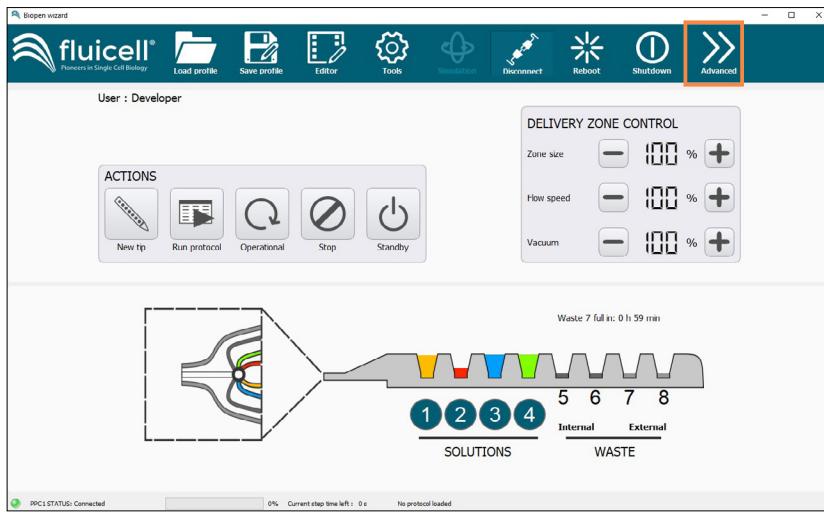
STOP: sets all the delivery zone value to 0 % and depressurizes the system. This function should be used for any actions requiring removal of the pipette holder out of the sample solution (e.g. empty the waste wells, change the tip).

STANDBY: sets all the delivery zone values to a holding level (see the orange rectangle in the figure below). These values are set to balance the flows within the tip for longer periods of waiting (e.g. changing location within the sample or periods away from the instrument).



8. Advanced panel / Set delivery zone percentages

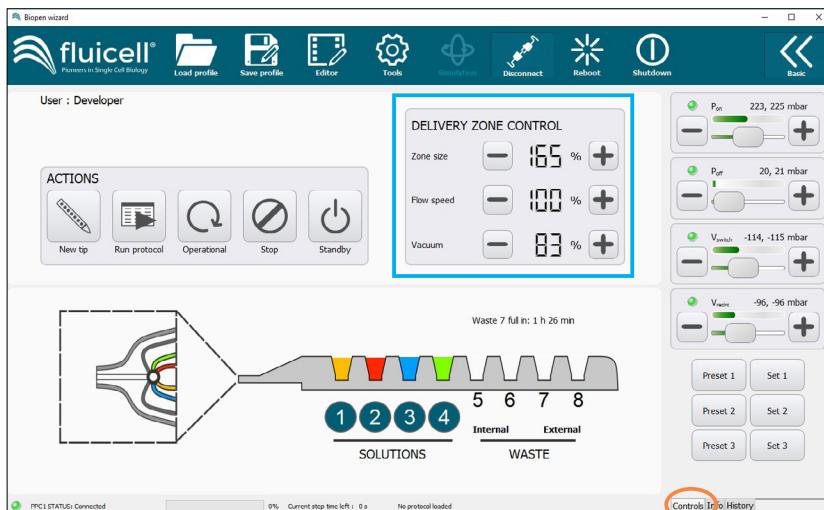
On the top Menu bar, click on the **Advanced** button for more options (see the orange rectangle in the figure below).



An additional panel appears on the right side of the software window. Click the **Controls** tab located at the bottom (see the orange circle in the figure below) to set your values.

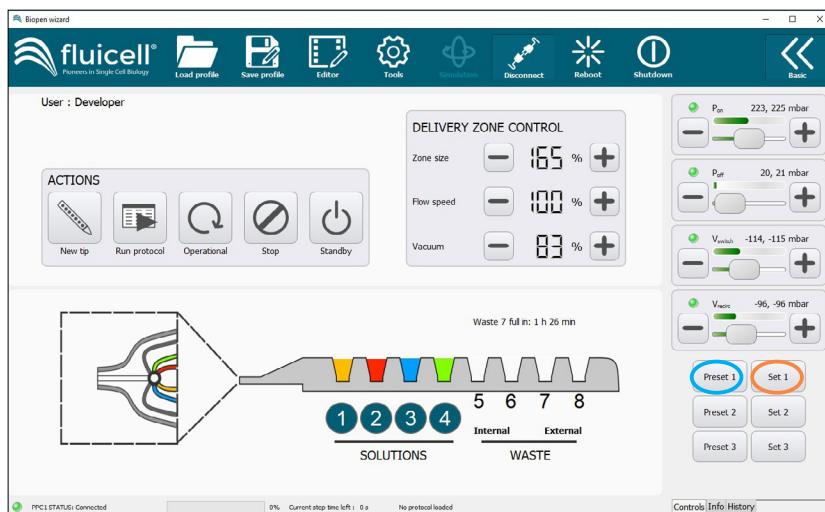
First on the *DELIVERY ZONE CONTROL* panel (see the blue rectangle in the figure below), select the best percentage values for your experiment by clicking the + or - buttons.

In the example below, 165, 100 and 83 % have been selected respectively for the zone size, flow speed and vacuum values.



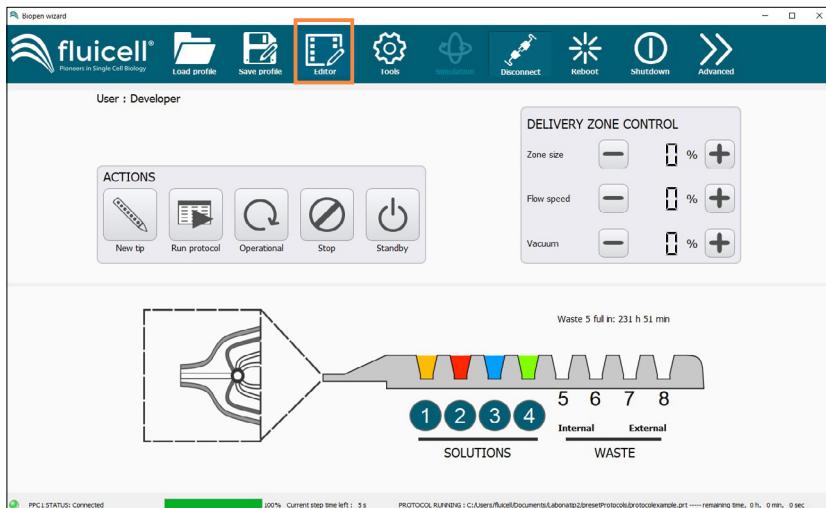
To save this set of values, click on the **Set 1** button (see the orange circle in the figure below). To load this set of values, click on the **Preset 1** button (see the blue circle in the figure below).

Two others set of values can be saved by clicking on *Set 2* and *Set 3* buttons, and they can be loaded by clicking *Preset 2* and *Preset 3* buttons.



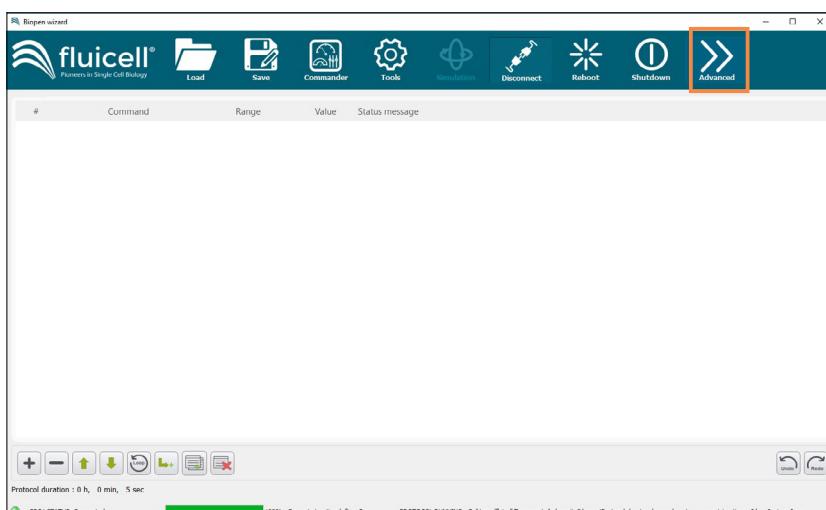
9. Protocol editor

Protocols can be written, loaded or edited by clicking on the *Editor* button on the top Menu bar (see the orange rectangle in the figure below).

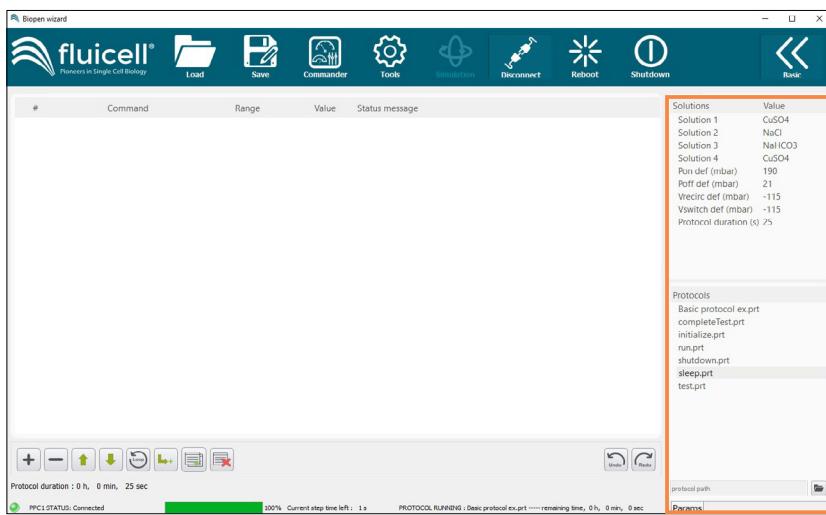


The Editor panel then displays (see the figure below).

Click on the *Advanced* button to access all off the options (see the orange rectangle below).

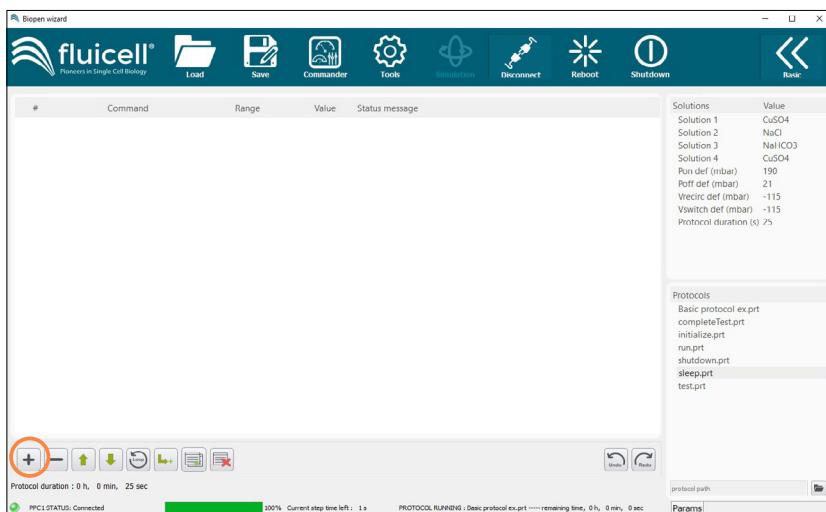


An additional panel appears on the right side of the software window (see the orange rectangle in the next figure).

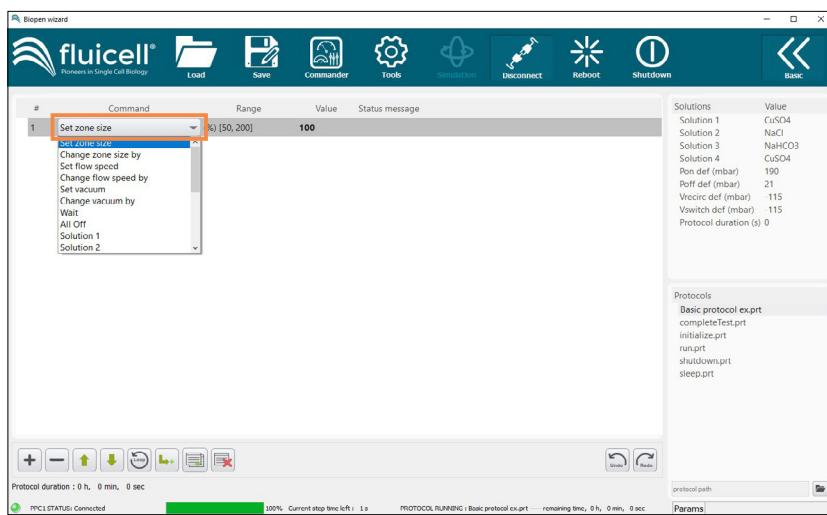


9.1. Write a protocol

To start your own protocol, click on the **+** button (see the orange circle in the figure below) to add a new command.



A *Set zone size* command line displays by default. Double-click on the *Set zone size* box (see the orange rectangle in the figure below) to select in the drop-down menu the command you want to add.



Various commands are available in this drop-down menu. For each of them, unit and range of values are visible in the second column.

Here it is a brief description of the most common ones:

SET ZONE SIZE: percentage of the zone size in relation to default (range between 50 to 200 %)

CHANGE ZONE SIZE BY: increase/decrease the current zone size by a defined percentage (range between -40 to + 40%) (for example if the current zone size is 150 %, increasing by 10% will give a final zone size value of 165 %)

SET FLOW SPEED: percentage of the flow speed in relation to default (range between 50 to 250 %)

CHANGE FLOW SPEED BY: increase/decrease the current flow speed by a defined percentage (range between -40 to + 40%)

SET VACUUM: percentage of the vacuum in relation to default (range between 50 to 250 %)

CHANGE VACUUM BY: increase/decrease the current vacuum by a defined percentage (range between -40 to + 40%)

WAIT: time between commands (unit in second)

ALL OFF: stop delivery of any solution

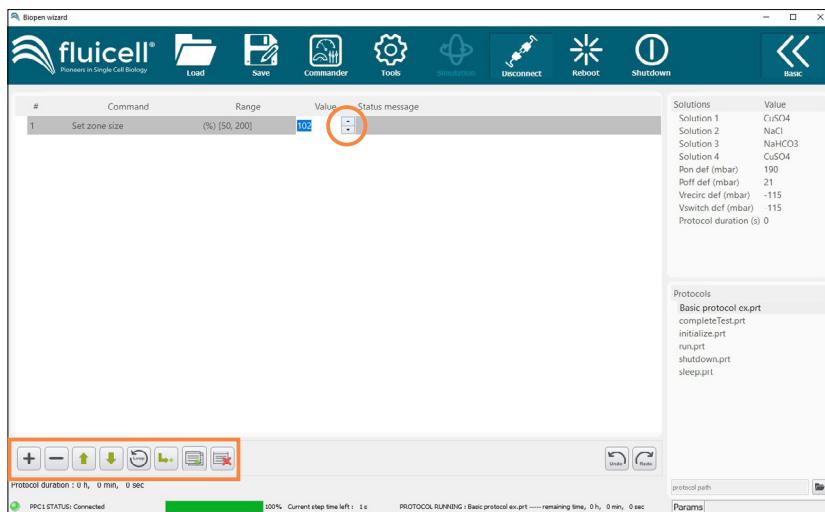
SOLUTION 1: start or stop delivery of solution 1 (0 for stopping, 1 for delivering)

SOLUTION 2: start or stop delivery of solution 2 (0 for stopping, 1 for delivering)

SOLUTION 3: start or stop delivery of solution 3 (0 for stopping, 1 for delivering)

SOLUTION 4: start or stop delivery of solution 4 (0 for stopping, 1 for delivering)

To adjust the value of the selected command, click on up or down arrows (see the orange circle in the figure below). Additional functions are accessible in the bottom left-hand corner of the Editor panel (see the orange rectangle below).

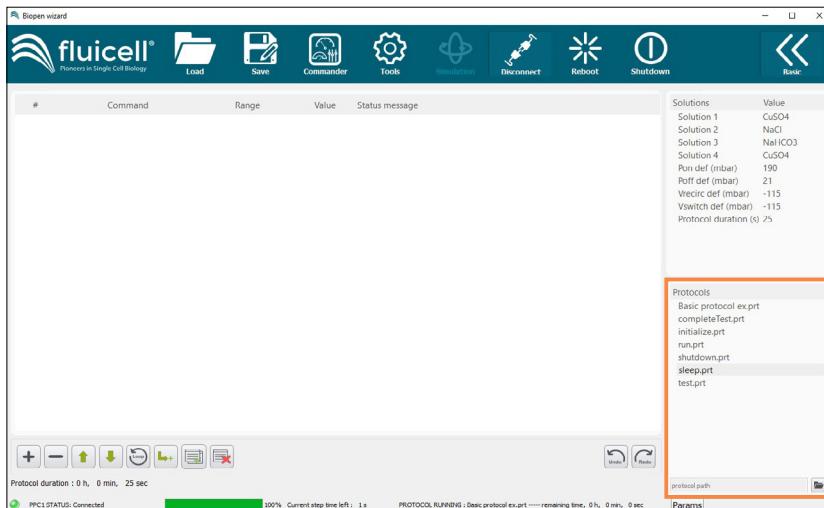


Below a brief description of each button:

-  Add a new command line
-  Delete the select command line
-  Move up the selected line
-  Move down the selected line
-  Create a loop
-  Add a new command in the loop
-  Duplicate the selected command
-  Clear all commands

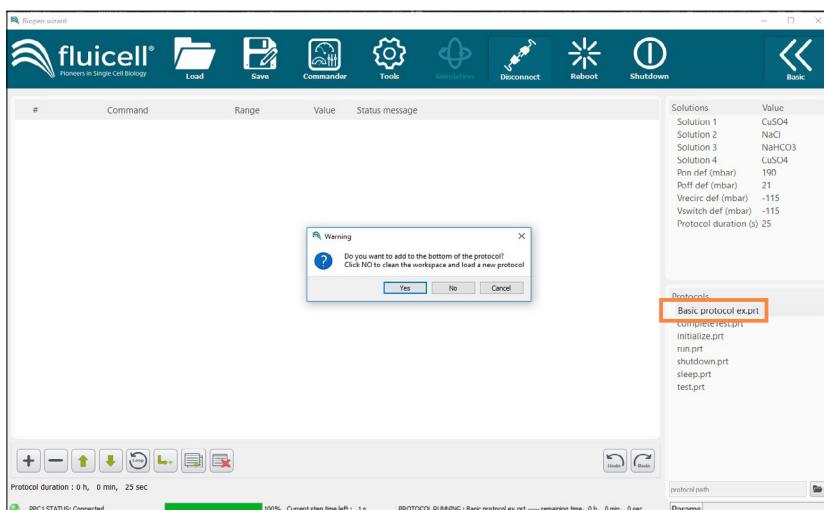
9.2. Load a protocol

A list of existing protocols are accessible in the bottom right-hand corner of the Editor panel (see the orange rectangle in the figure below).



To load an existing protocol, for example *Basic.protocol.exprt* file double-click on it (see the orange rectangle in the figure below). A pop-up notice window appears.

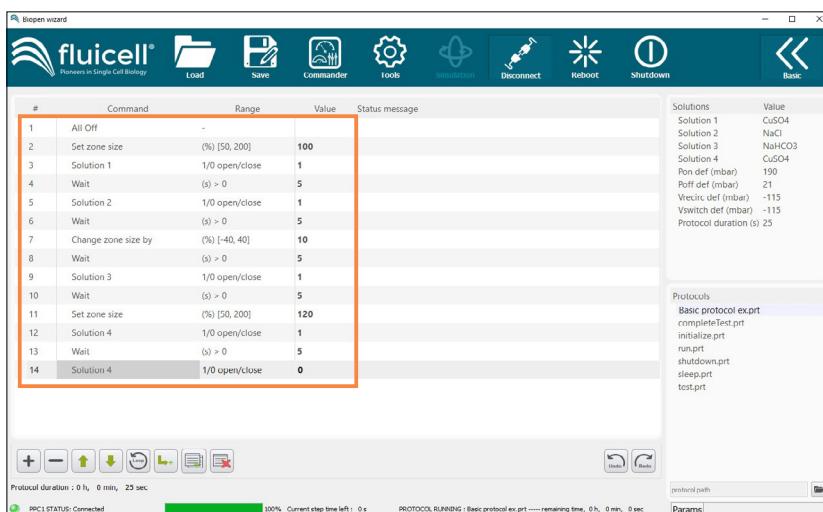
Click **No** if you want to clean the work place and load the protocol. Click **Yes** if you want to add the protocol to the bottom of the current list of commands.



All the command lines of the protocol display in a chronological order (see the orange rectangle in the figure below)

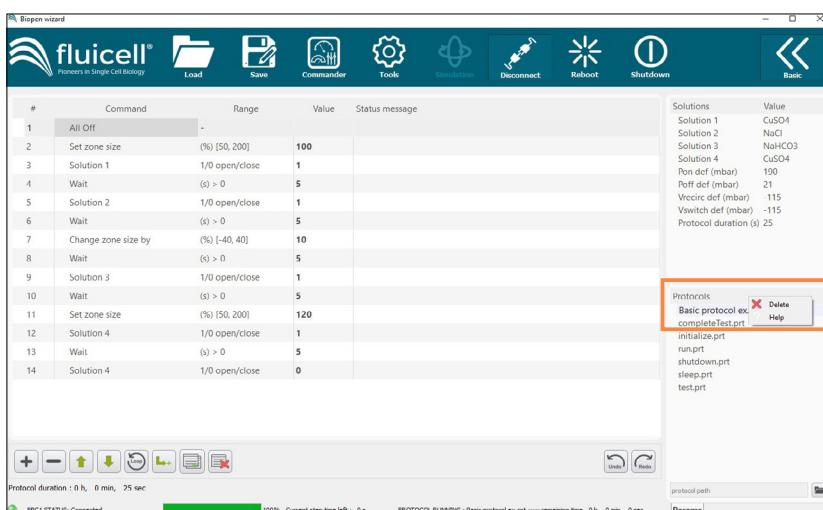
Here they are the following steps of the protocol loaded below:

- any current delivery will be stopped,
- zone size will be set to 100 %,
- solution 1 will be delivered for 5 seconds,
- solution 2 will be delivered for 5 seconds,
- zone size will be increased by 10 %,
- solution 2 will be delivered for 5 seconds,
- solution 3 will delivered for 5 seconds,
- zone size will be set to 120 %,
- solution 4 will be delivered for 5 seconds,
- delivery of solution 4 will stop.

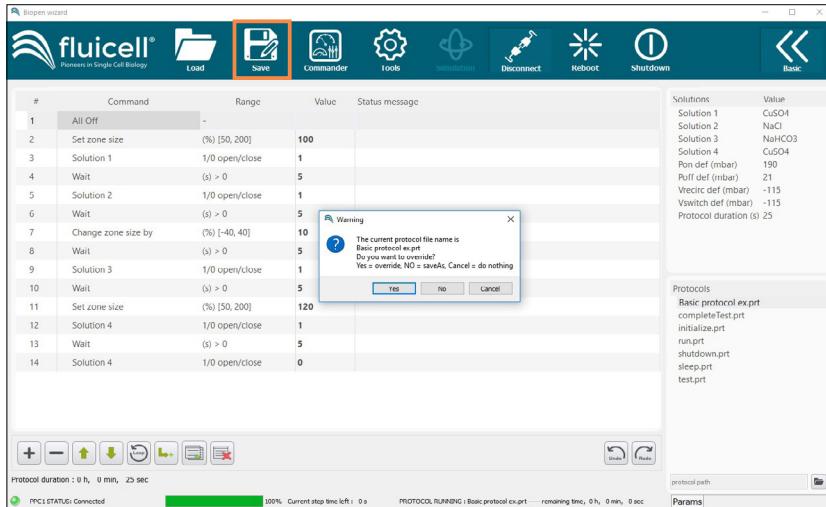


You can edit or delete any command of the protocol by selecting the command line you want to modify or delete.

You can also delete the protocol file by selecting first the file and then right click (see the orange rectangle in the figure below). A pop-up notice window displays. Select *Delete* if you want to delete the file.

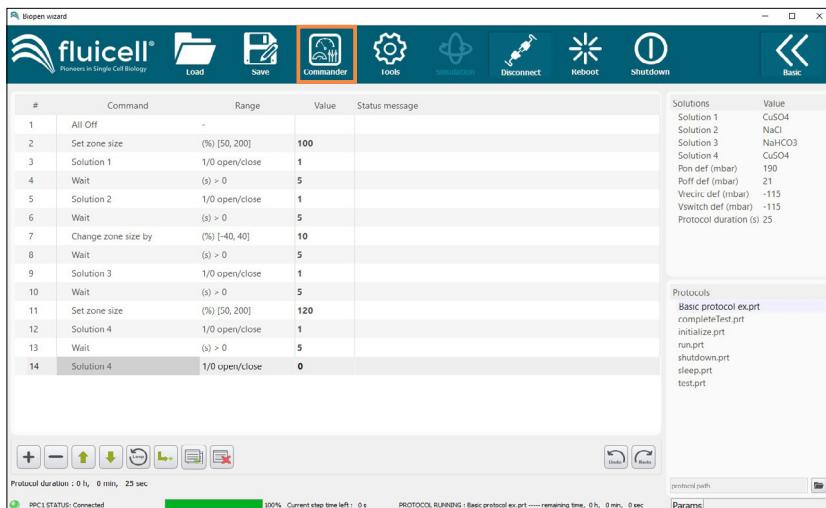


You can **save your protocol** by clicking on the **Save** icon on the top main bar (see the orange rectangle in the figure below). A pop-up notice window displays. Select **Yes** if you want to overwrite the file or **No** if you want to save the file under a different name.



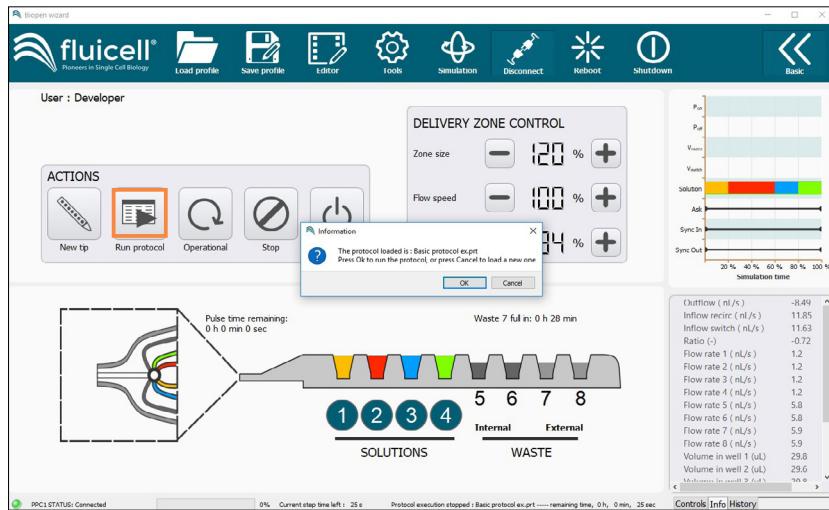
9.3. Run a protocol

To run the loaded protocol, click first on the **Commander** icon on the top main bar to come back to the main panel (see the orange rectangle in the figure below)

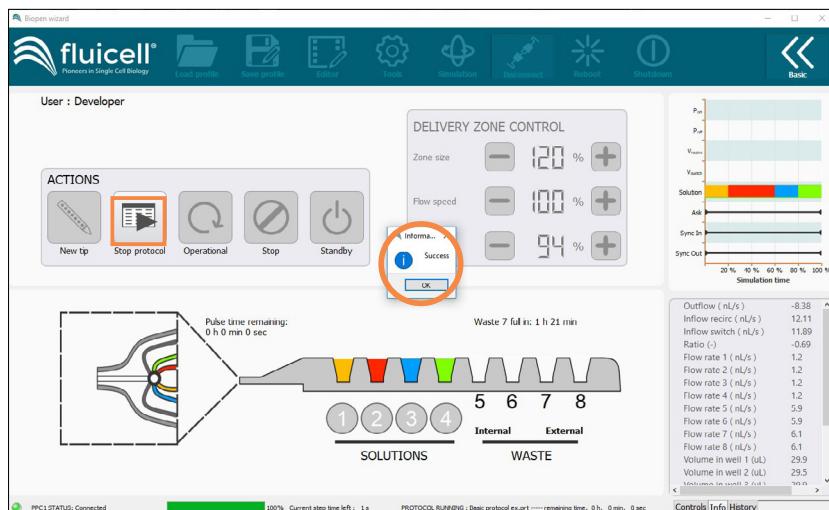


Then click on the Run protocol button (see the orange rectangle in the next figure). A pop-up notice window displays. Click **Ok** to run the last loaded protocol or click **Cancel** if you want to run another one.

Then click on the Run protocol button (see the orange rectangle in the figure below). A pop-up notice window displays. Click *Ok* to run the last loaded protocol or click *Cancel* if you want to run another one.

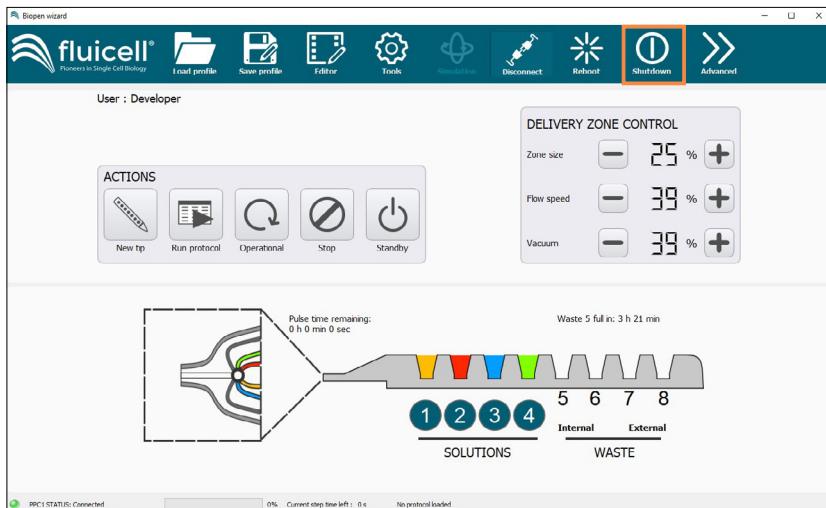


If you need to interrupt the running of the protocol, click on the *Stop protocol* button (see the orange rectangle in the figure below), otherwise wait until a *Success* notice window displays. Press *Ok* to validate the end of the running process (see the orange circle in the figure below).

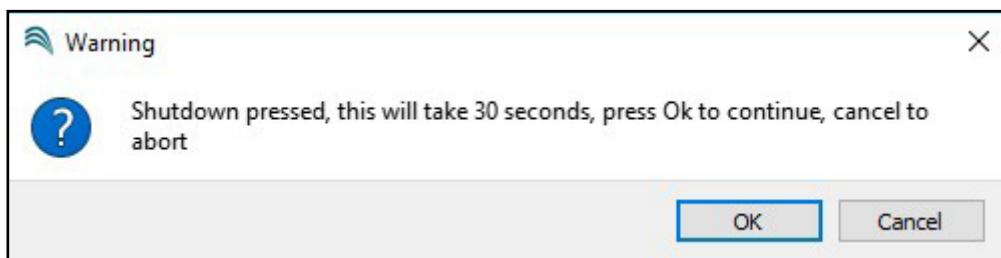


10. Shutdown the instrument

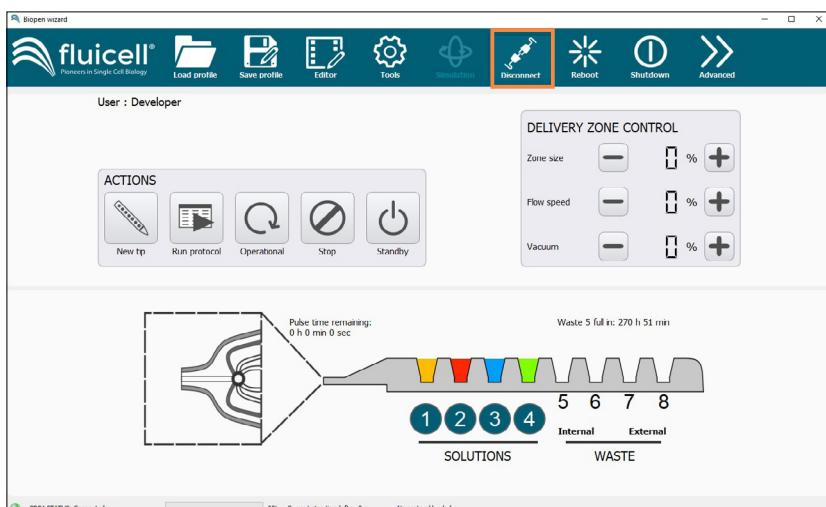
To switch off properly the Biopen system, click the **Shutdown** button on the top menu bar (see orange rectangle below).



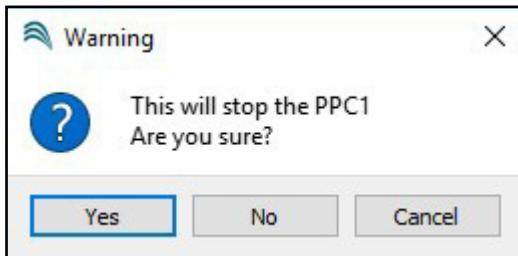
A pop-up notice window displays. Press **OK** if you want to turn off the Biopen system.



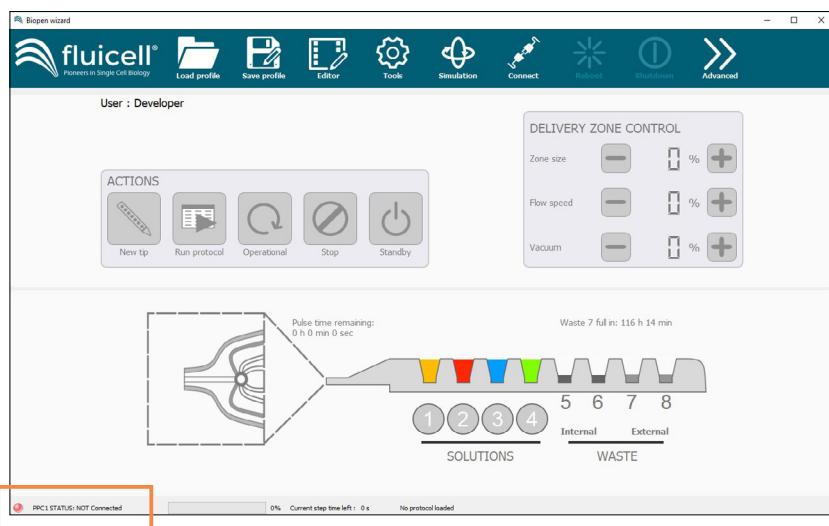
All the delivery control zone values drops to 0 %. Then click on the *Disconnect* button (see orange rectangle in the figure below).



A pop-up notice window displays. Click Yes to stop the pressure controller PPC1.



A red circle following by “PPC1 STATUS: NOT Connected” should display at the left bottom of the software panel (see orange rectangle in the figure below).



Now you can safely **close the Biopen software** and **turn off the pressure controller PPC-1** by pressing the switch on the back of the pressure controller and by **removing the pipette tip** from the holder.