

Pump system

User's Guide



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1 Introduction

1.1 Overview

The Pump system is a simple but precise pump system designed to perfuse well dishes (0.1 – 20 ml/min) with experimental solutions in which cultured myocytes can be simultaneously paced and measured. Each perfusion system features 2 or 3 pumps, 2 designated for input solutions and 1 pump for waste. The pumps can be connected to 6 wells at a time. Adding, removing and perfusion of solutions is controlled via the Multipumpcontroller Software for accurate and precise perfusion

This manual is intended to describe the basic connections and operations of the Pump system.

1.2 User's Guide Conventions

The following stylistic conventions are used throughout this and other CytoCypher manuals.

[Courier New text within brackets] refers to specific named items in button selections

Bold Italic text refers to other manual sections.



2 Hardware

In this Chapter, the hardware related aspects of the Perfusion system will be discussed. The discussion is broken into two parts, the pump setup and the connection to the wells.

2.1 Pump setup

The pumps are fragile equipment. To maintain precision it is important to prevent debris clotting up the pumps. Therefore, use filters for the intake solutions and/or keep them covered. Also, it is important to clean the system at the end of each day.

2.1.1 Parts

The pump system contains the following items: 2 or 3 Pumps including syringes, valve connectors, tubing (Silicone tubing 1.0 x 0.4 x 1.8 mm).
Optional: filters and 35 mm well connectors

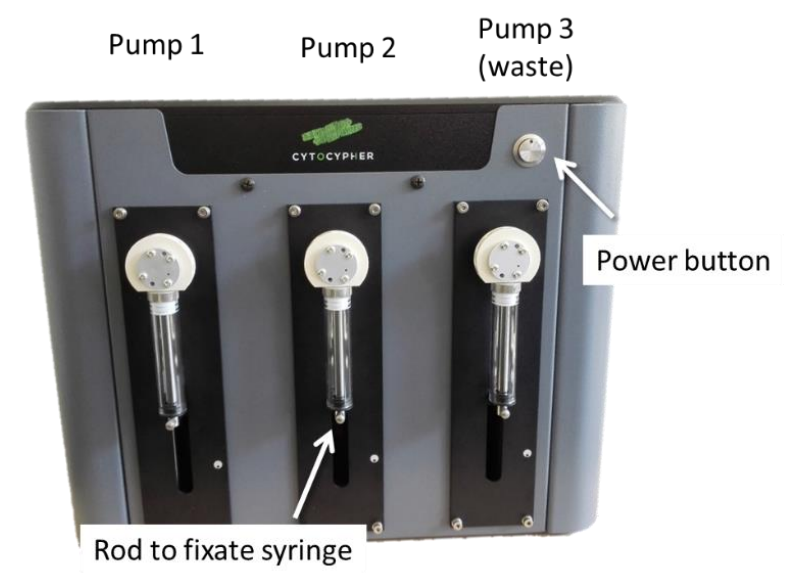


Figure 1 Overview of the pump system



Silicone tubing



Valve connector



Filter



35 mm well connector

Figure 2 Additional components

The CytoCypher pump system consists of 2 or 3 pumps with 9 valves numbered from left to right (Figure 3). The settings of the pumps are as follows (see also *section 3.1.2, page 9*):

	Pump 1	Pump 2	Pump 3
Valves #1 – 6	Output to wells 1 – 6	Output to wells 1 – 6	Waste valves from wells 1 – 6
Valve #7	Intake valve solution 2	Intake valve solution 2	
Valve #8	Intake valve solution 1 (control solution)	Intake valve solution 1 (control solution)	Intake valve cleaning solution
Valve #9	Valve to waste	Valve to waste	Valve to waste

Table 1 Pump settings

2.1.2 Syringes

A syringe should be attached to each pump. To attach or replace a new syringe unscrew the rod indicated in **figure 1** and the syringe can be unscrewed from the pump. This is easier to do when the syringe is partly ‘filled’ i.e. the rod is in a lower position. See *section Attach or replace syringe, page 20*.

The 10ml syringes which are included with the system can be replaced by larger syringes up to 50ml (Cavro® Syringe Tecan Inc.) when desired.

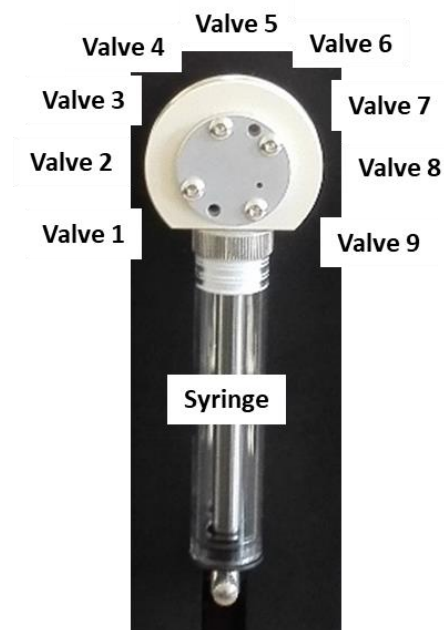


Figure 3 Components on each pump

2.2 Connection to the wells

With the included valve connectors tubing can be attached from the valves to the wells. Standard configuration settings are implemented in the software; valves 1 of each pump should be connected to well 1, valves 2 should be connected to well 2 and so on.

As indicated in table 1, up to 6 wells can be connected to the perfusion system, which can be perfused with 2 different experimental solutions. If less wells are used the free valves can be used as additional intake valves. It is important that the connections of the tubing match the configuration of the software (*section 3.1.2, page 9*).

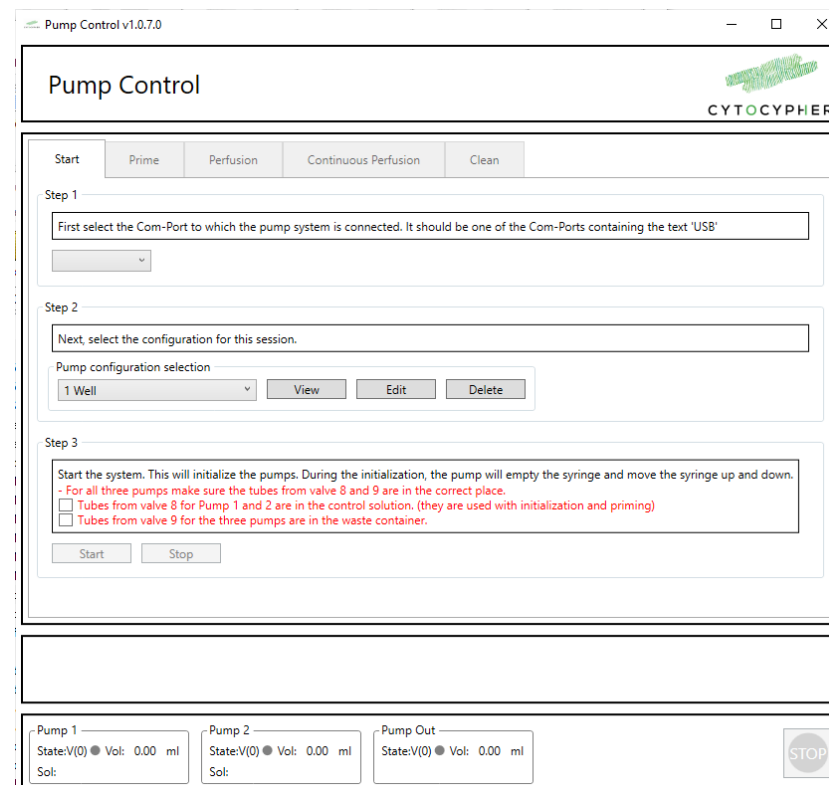
3 Software

The pump system is controlled by *MultiPumpController* software. The core program can be installed, configured and removed using a Microsoft Installer (MSI) file. To run the installer simply double-click on the latest release.

When opening the program it shows 5 tabs [Start], [Prime], [Perfusion], [Continuous Perfusion] and [Clean].

At the bottom of each tab the status of the pumps and a [stop] button which can be pressed at any time is shown (Figure 4). The pumps can only be used when the status is 'yellow'.

If there are no syringes attached to the pumps go to pages 19-20



3.1 Start

The [start] tab is split into 3 steps:

3.1.1 Step 1: USB connection

Connecting to the pump system with the USB cable. Select the USB port the pump is connected to, the first time it may take a while before it is recognized. If it can not be detected at all a *Prolific Driver* is missing. An installer can be found at www.prolific.com

3.1.2 Step 2: Pump configuration selection

Two standard configuration settings are implemented; a 1 well or 6 well configuration using 3 pumps. These configurations can be edited or new standard configurations can be added. Press [edit] and the configuration becomes visible. It is important that this configuration matches the connections made with the tubing.

If your system only contains 2 pumps, it is important to deselect Pump 2 in the configuration setting.

Pump configuration setting

- The name of the configuration can be adjusted.
- Select the number of wells (1 to maximal 6), this automatically updates the configuration of the output valves.
- The tubing dead volume needs to be adjusted depending on the length of the tubing to the wells and should be calculated for the longest tube.

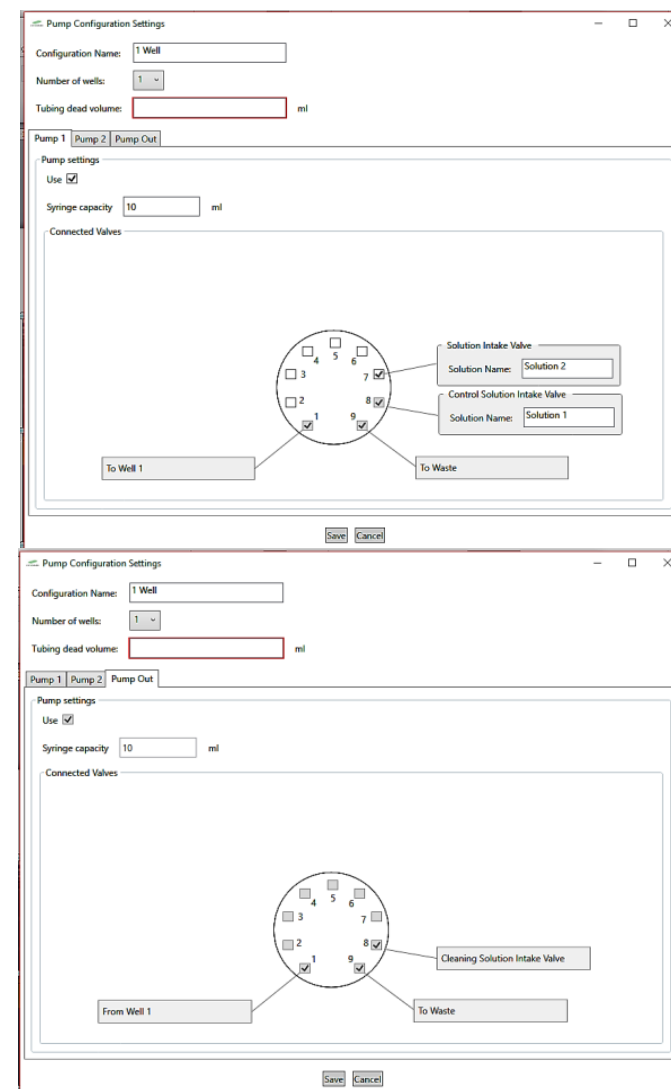


Figure 5. Standard configuration setting of pump 1 and pump out (waste pump 3) for a 1 well system.

Dead volume of the tubing can be calculated by the following formula:

$$Volume (mm^3) = \pi \times r^2 \times l$$

with r inner radius of the tubes (i.e. 0.5 mm for the included tubes) and l the length of the tube. Thus, for a tube of 1 meter (1000 mm) in length, the dead volume is $785 \text{ mm}^3 = 0.785 \text{ ml}$.

Best to overestimate rather than underestimate the dead volume to prevent any air in the system during perfusion

Each pump has a separate tab.

- Pump setting: select if you want to use the pump (default is selected)
- Syringe capacity: Default is 10ml, **but can be changed when using another size syringe**
- Connected valves: Depending on the number of wells selected in the top panel you can select remaining valves and use them as intake valves. Rename the intake solutions. N.B. This is not possible for Pump Out.

For continuous perfusion it is essential to have matching names for your intake solutions

The simplest configuration is a 1 well system with one inlet and one outlet (i.e. pump 2 is not used), see **Figure 5**. For each pump, valve 9 is specified as a waste valve to empty the syringe when necessary. For the waste pump, valve 8 is specified to be used as cleaning solution intake.

For a 6 well system the standard setting is depicted in **Figure 6**. The settings of pump 1 and 2 are identical. With a 6 well system maximally 2 intake solutions can be connected to the pumps.

Press [save] or [cancel] to return back to the [start] window.

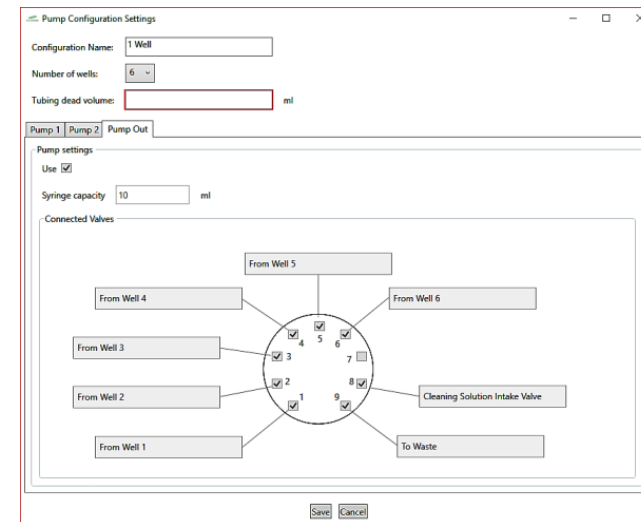
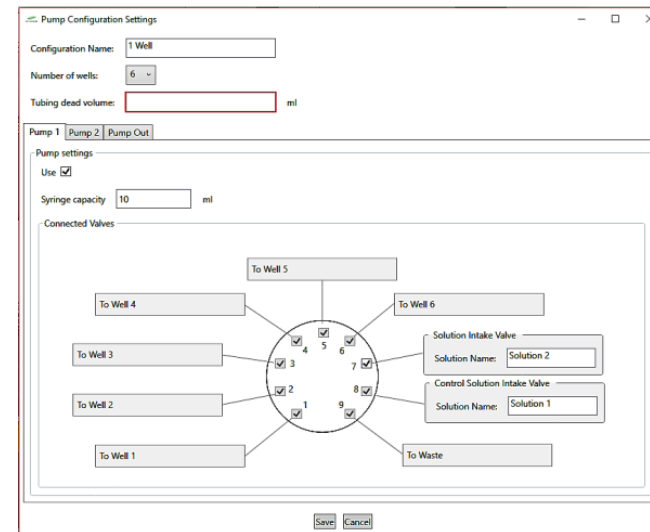


Figure 6. Standard configuration setting of pump 1 and pump out (waste pump 3) for a 6 well system.

3.1.3 Step 3: Initialize the system

Follow step 3 and check both boxes to start/initialize the system. If the syringes are filled (i.e. with distilled water) they immediately start to empty through the waste valve. Hence, the waste tubes should be placed in a waste container.

If you want to adjust your configuration setting, you first need to press [stop] in step 3. After adjustments in step 2, you need to perform step 3 again before perfusion can start.

The status of the pumps used in the current configuration at the bottom of the window will change from 'grey' to 'yellow'.

3.2 Prime

Before starting to perfuse your wells, it is important to prime the tubes with the control and experimental solutions so any air bubbles are expelled from the system. The protocol for priming of 6 wells would be as follow:

- intake solution from valve 8 → extrude via valve 9
- intake solution from valve 8 → extrude via valve 1
- intake solution from valve 8 → extrude via valve 2
- intake solution from valve 8 → extrude via valve 3
- intake solution from valve 8 → extrude via valve 4
- intake solution from valve 8 → extrude via valve 5
- intake solution from valve 8 → extrude via valve 6
- intake solution from valve 7 → extrude via valve 9

Thus, all tubing to the wells are primed with the intake solution of valve 8, for both pump 1 and 2. Therefore, the intake solution of valve 8 for both pumps should be a control solution (i.e. tyrode) and not an experimental solution as this may lead to contamination of the well(s) when placing the perfusion block or tubes in the wells.

- When priming a multi-well system, first connect all tubing to the designated wells and place the perfusion block above a waste container during priming.
- The priming volume pumped to the wells depends on the dead volume input in the [start] window.
- Check both boxes and press [Prime System]
- Check if there is no air in the tubing or syringes. If you still see some air, repeat this step and check all your connections.
- Now the tubes and the perfusion block are primed and the perfusion block or tubes can be placed into the wells.

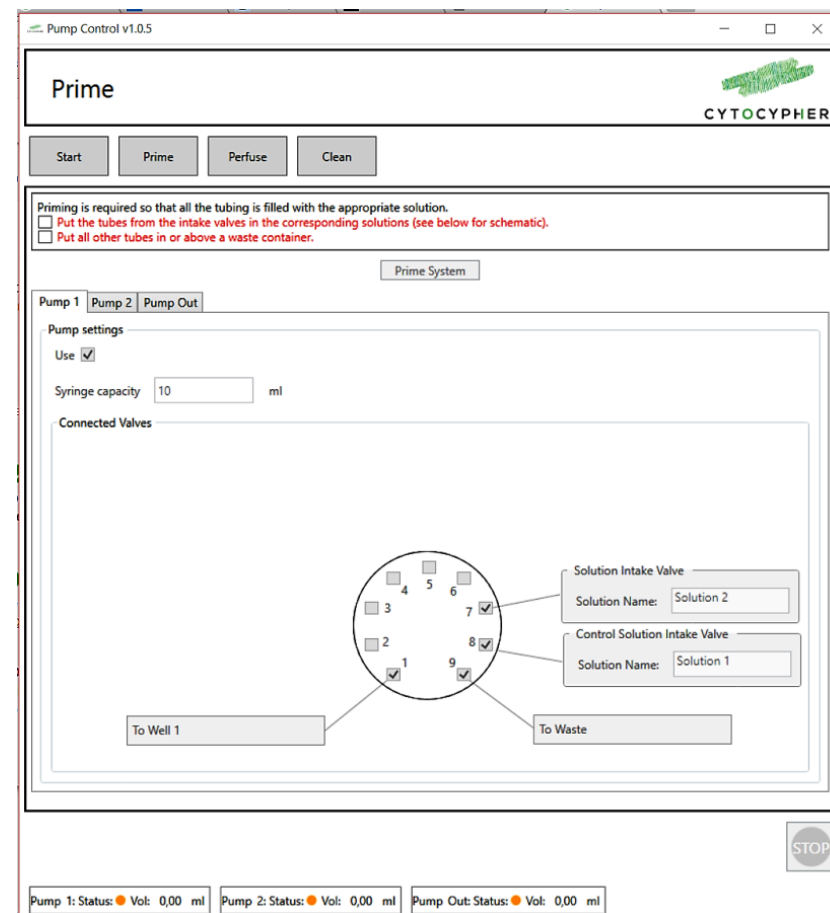


Figure 7. Prime window Pump Control

3.3 Perfusion

Depending on the configuration setting you can select a specific well to perfuse with a specific solution. For a 1 well system it automatically selects the well, for a multi-well configuration select the well you want to perfuse by clicking on the well number. The highlighted well will be perfused. See **Figure 8** for examples of a 1 well or 6 well configuration.

When switching solutions from one pump (valve 8 to valve 7), the first part of perfusion starts with the solution still present in the tube (i.e. dead volume).

There are 3 options:

3.3.1 Perfuse

Select a well and a solution in the top of the window. Insert the total volume you want to perfuse and the speed of perfusion and press [perfuse]. The designated syringe will take up the total volume and will then start to pump it into the well while the same volume is removed from that well by pump out.

With the included tubing the maximum speed is 20 ml/min. Recommended speed for myocyte perfusion is between 0.2 and 4 ml/min.

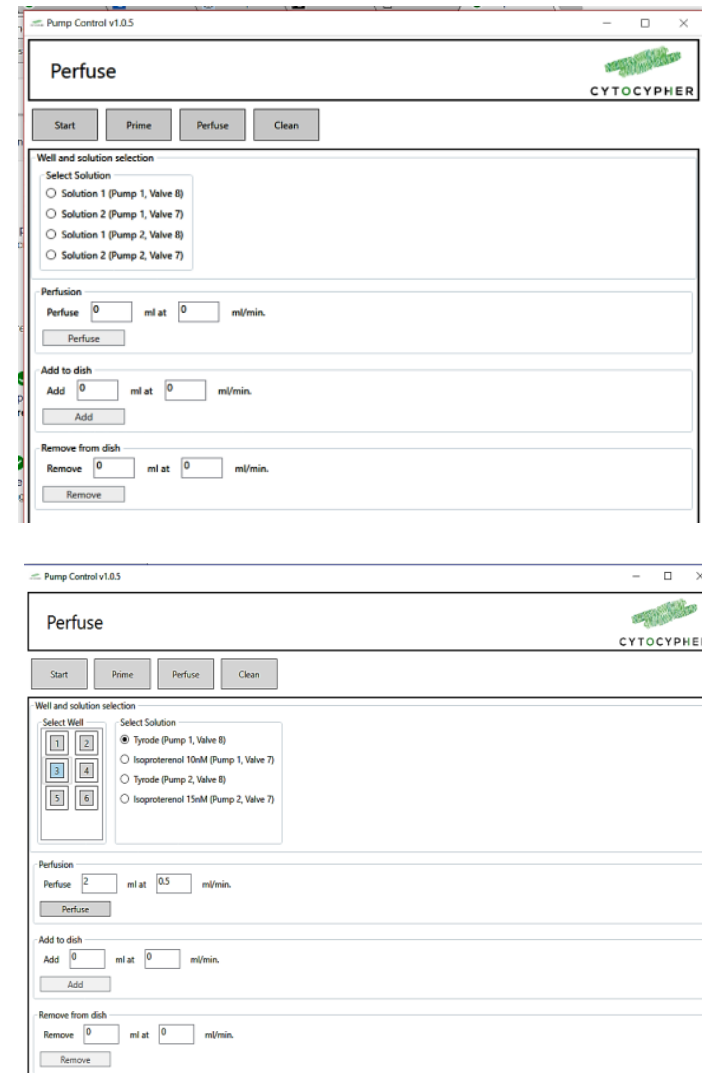


Figure 8. Perfuse window showing the options for a 1 well and 6 well configuration

3.3.2 **Add to dish**

Add the specified volume with the specified speed to a dish and press [Add].

3.3.3 **Remove from dish**

Remove the specified volume with the specified speed from a dish and press [Remove]. This action is performed by the pump out.

3.4 Continuous perfusion

Continuous perfusion is designed to be used with a 3 pump system. The tubes from pump 1 and pump 2 to each well should be combined using Y-tube connectors.

Solutions to select: Only solutions names that are found in both Pump 1 and Pump 2 can be used for continuous perfusion. Hence, it is essential to give them the same name in the Pump Configuration in the [Start] window.

Depending on the configuration setting you can select a specific well to perfuse continuously with a specific solution. For a 1 well system it automatically selects the well, for a multi-well configuration select the well you want to perfuse by clicking on the well number. The highlighted well will be perfused.

Speed: specify desired speed to perfuse continuously.

Press [Perfuse Continuously] to start. At the bottom of the window you can see the steps the pumps are taking to start perfusing.

3.4.1 Switch solutions

To switch to a different solution during continuous perfusion requires 3 steps:

1. Select different solution on top of the window
2. Press [Prepare to Switch]
3. Press [Switch Solution]. This button only becomes active when Prepare to switch is finished.

3.4.2 Reload Inactive Pump

This button can be used if for example air was taken up by the pump due to low level of solution.

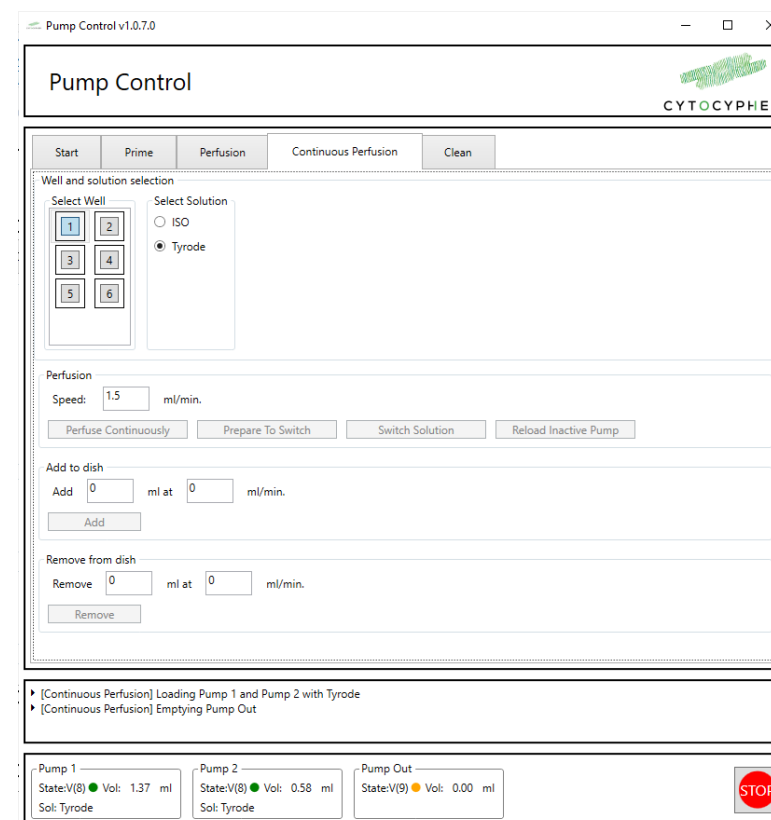


Figure 9. Continuous Perfusion window. Showing the start of loading the Pumps with Tyrode.

- Check the tubing of the intake valves and the level of solution
- Press [Reload Inactive Pump].
 - o It will take up the dead volume and extrude it through the waste valve (i.e. to remove any trapped air in the tubing)
 - o Then fill up the complete syringe.

3.4.3 Add to dish

Add the specified volume with the specified speed to a dish and press [Add].

3.4.4 Remove from dish

Remove the specified volume with the specified speed from a dish and press [Remove]. This action is performed by the Pump Out.

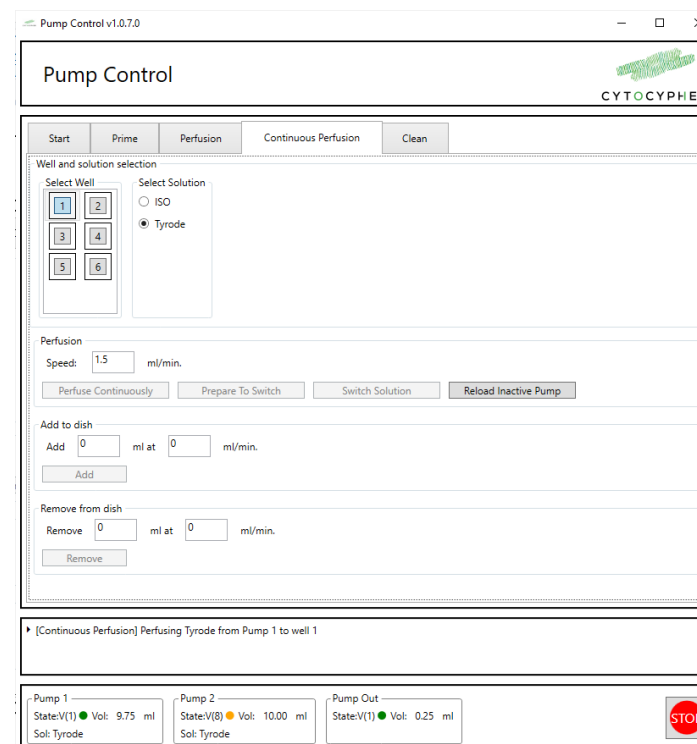


Figure 10. Continuous Perfusion window during perfusing to well 1. Reload Inactive Pump button activated

3.5 Clean

It is important that the tubes and syringes of the pump system are cleaned weekly/daily (depending on the frequency of using the pump system) to remove precipitates such as salts, eliminate bacterial growth, and so on.

A standard cleaning protocol is implemented in the software and consists of 2 steps. This procedure should be performed with the tubing or perfusion block above a waste container.

Recommended cleaning solutions are ethanol or weak detergents

3.5.1 Step 1: Filling the system with a cleaning solution

- Start by placing all intake tubes of each pump in the cleaning solution. For the waste pump, valve 8 is designated for this. All other tubing, and if applicable perfusion block, should be placed above a waste container.
- First, the tubes to the wells that were used are filled with the cleaning solution. For this the syringe will take up cleaning solution from valves 7 and 8 and move this to each tube/well. Thus depending on the amount of wells used this step is repeated.
- Secondly, the syringe will fill completely with the cleaning solution. It is recommended to keep the cleaning solution in the system for 30 minutes.
- Check both boxes and adjust the settling time if necessary, then press [Fill system with cleaning solution].

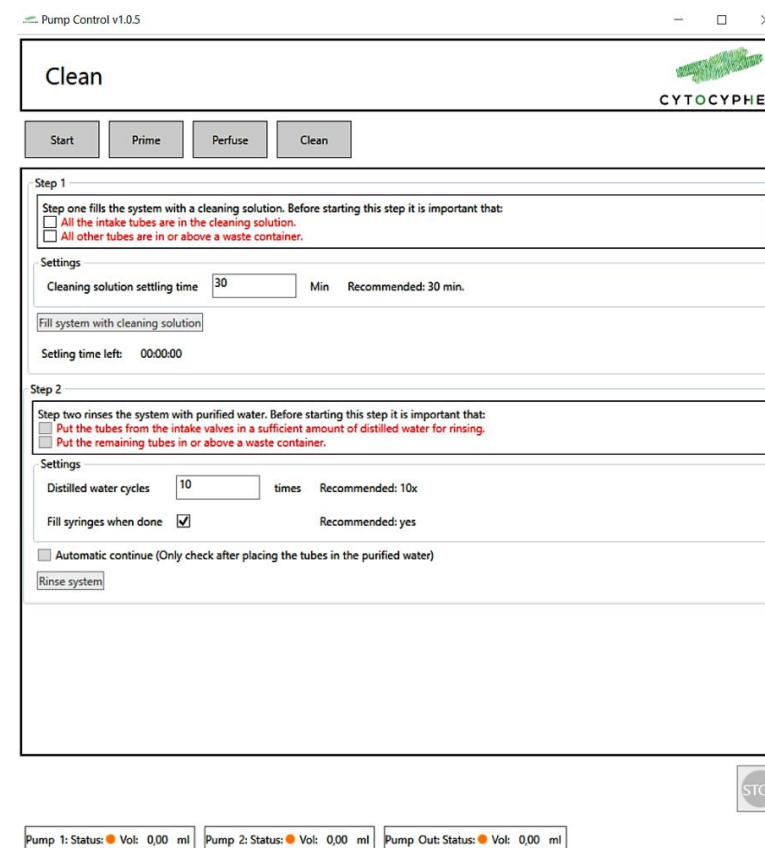


Figure 11. Clean window

3.5.2 Step 2: Flush the system with distilled or de-ionized water

- Place all intake tubes of each pump in a container filled with sufficient amount of distilled or de-ionized water i.e. for a 3 pump system and 10 cycles you need ~350ml of distilled or de-ionized water. (*you can already perform this step when in step 1 all syringes are completed filled with the cleaning solution*)
- Keep the remaining tubes and perfusion block above a waste container.
- If you check both boxes and also the “Automatic continue” box, this rinsing step will be automatically started after the settling time has elapsed.
- The tubes and syringes used will be rinsed with distilled or de-ionized water for a number of times. It is recommended to use 10 cycles.
- It is recommended to keep the tubes and syringes filled with distilled or de-ionized water if the system is used frequently.

3.6 Service

A hidden tab [Service] can be found by pressing [ctrl]+[alt]+[s], this opens a new window [Service] with three tabs [Test Pump], [Control Pump] and [Replace Syringe]

Pumps can only be controlled when step 1 to 3 of the [start] window is completed.

[Test Pump]: This window can be used to specifically test the pumps, valves and tubing for leakage (i.e. when air bubbles remain in the syringes and/or tubing after multiple priming cycles).

[Control Pump]: This window can be used to empty all pumps (i.e. syringes) of a specific pump. Emptying of syringes will always be through valves 9 of the pumps.

N.B. To leave the [Service] window simply click on [Start], [Prime], [Perfuse] or [Clean]. Do not press [x] as this will close the program completely.

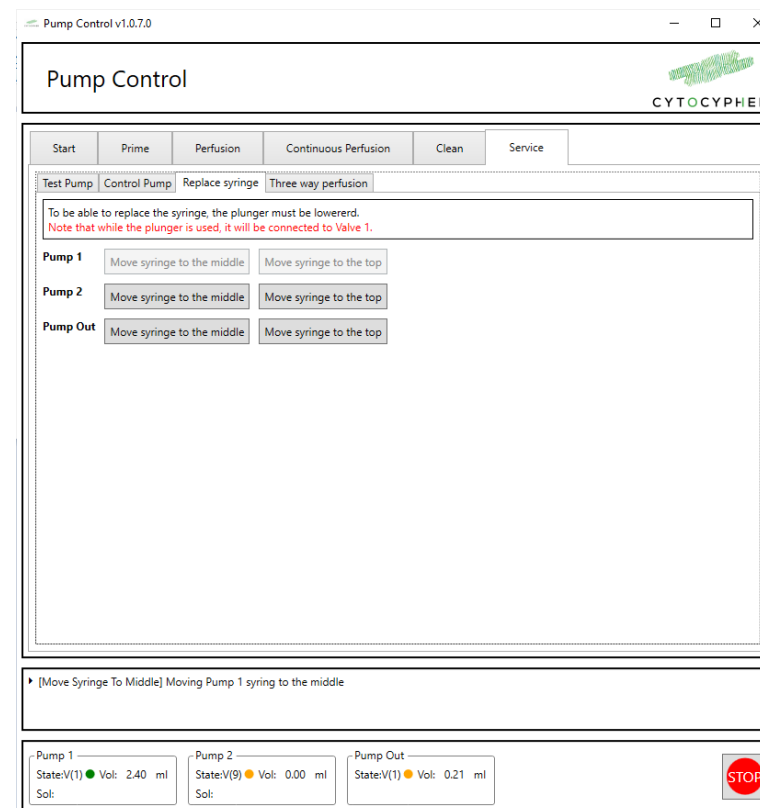


Figure 12. Service window

3.7 Attach or replace syringe

To attach or replace a syringe you need to perform the following steps to lower the rod to fixate the syringe:

1. Start system by pressing power button and open the pump software
2. Initialize the pumps in the [Start] window:
 - Step 1: USB connection: Connecting to the pump system with the USB cable. Select the USB port the pump is connected to, if it cannot be detected at a *Prolific Driver* is missing. An installer can be found at www.prolific.com
 - Step 2: Pump configuration → [edit] deselect pump 2 if your system only consists of 2 pumps. All other settings do not need to be adjusted
 - Step 3: check both boxes → press [start] to initialize the system
3. Open the [Service] window by pressing ctrl+alt+s.
4. Go the [Replace Syringe] tab
5. Press [Move syringe to the middle] of the desired Pump
6. Unscrew the rod and attach or detach the syringe by turning it clockwise or counterclockwise.
7. Repeat step 5 and 6 for each pump
8. Close [Service] window by pressing the [Start] tab

