CYTOCYPHER

Perfusion system

User's Guide
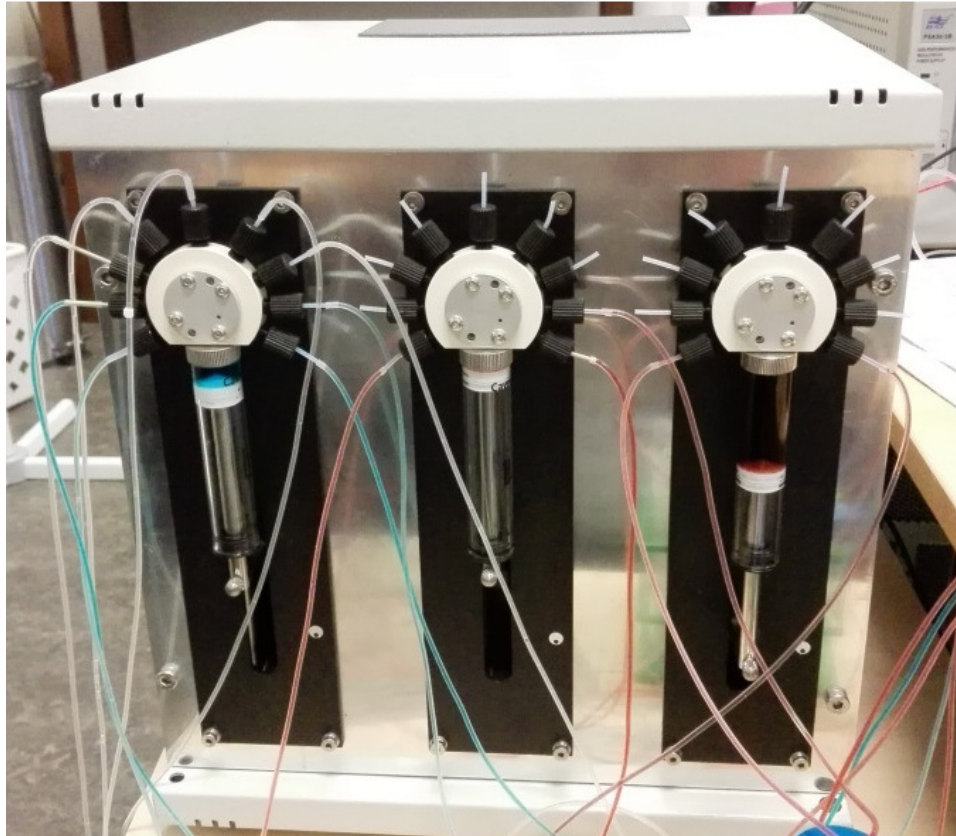
Manual version 1
December 2017

Address:	Cytocypher BV
	15 Eekmolenweg
	Wageningen, 6703 AL
	Netherlands
Phone:	+31 317 728 104
Fax:	+31 317 728 107
Email:	info@cytocypher.com
Web:	www.cytocypher.com

Contents

1 Introduction

1.1 Overview

The Perfusion system is a simple but precise pump system designed to perfuse well dishes with experimental solutions in which cultured myocytes can be simultaneously paced and measured with the Cytocypher High Throughput system. Each perfusion system features 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 Perfusion 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.
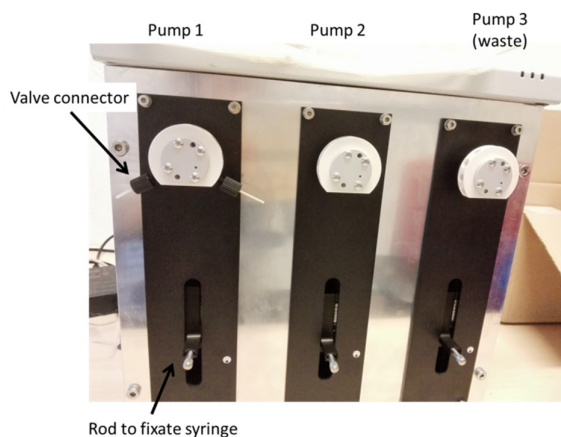
2.1 Pump setup

Figure 1 Overview of the pump system without connections to tubes and syringe
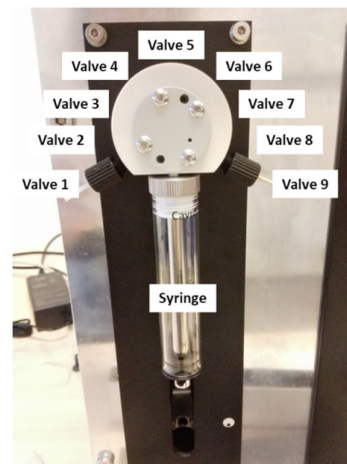

Figure 2 Components on each pump

The Cytocypher perfusion system consists of 3 pumps with 9 valves numbered from left to right (Figure 2). The settings of the pumps are as follows (see also *section 3.1.2,* page 7):

Table 1 Pump settings

	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

2.1.1 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 Replace syringe*, page 15.

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

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)*.

3 Software

The perfusion system is controlled by Multipumpcontroller software (Pump Control v1.0.5). The core program can be installed, configured and removed using a Microsoft Installer (MSI) file. To run the installer simply double-click on the *MultiPumpControllerSetup_1.0.5.0.msi* file.
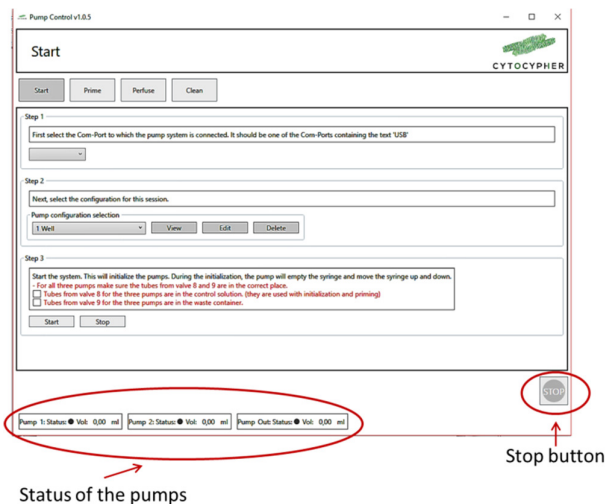
When opening the program it shows 4 tabs `[Start]`, `[Prime]`, `[Perfuse]` and `[Clean]`.

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 3). The pumps can only be used when the status is 'yellow'.

After pressing the `[stop]` button, a pop up appears asking whether you want to empty the pump. If pressed `[yes]` the syringe of the pump will empty through valve 9.

3.1 Start

The `[start]` tab is split into 3 steps:

Figure 3. Start screen Pump Control

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. N.B. The first time it may take a while before it is recognized.

3.1.2 Step 2: Pump configuration selection

Two standard configuration settings are implemented. 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.

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.

  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 mm^3 = 0.785 ml.

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.

The simplest configuration is a 1 well system with one inlet and one outlet (i.e. pump 2 is not used), see **Figure 4.** 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 a used as a cleaning solution intake.
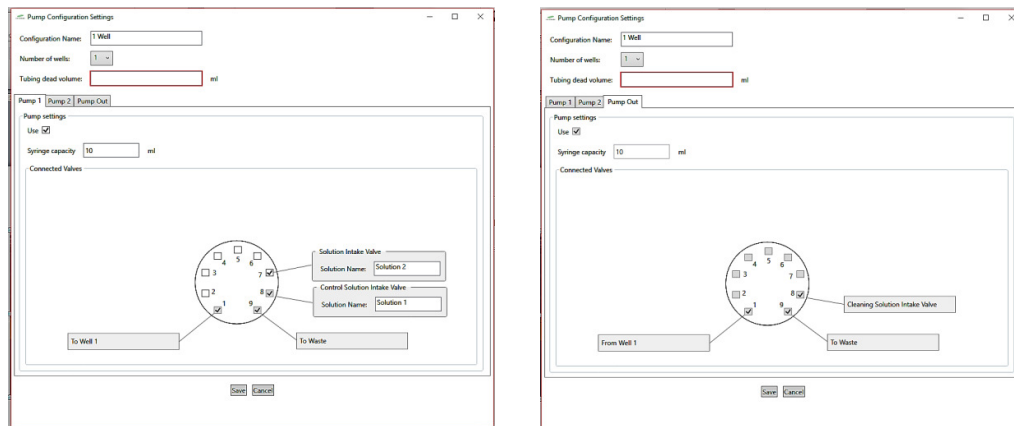

Figure 4. Standard configuration setting of pump 1 and pump out (waste pump 3) for a 1 well system.

For a 6 well system the standard setting is depicted in **Figure 5.** The settings of pump 1 and 2 are identical. With a 6 well system maximally 2 intake solutions can be connected to the pumps.
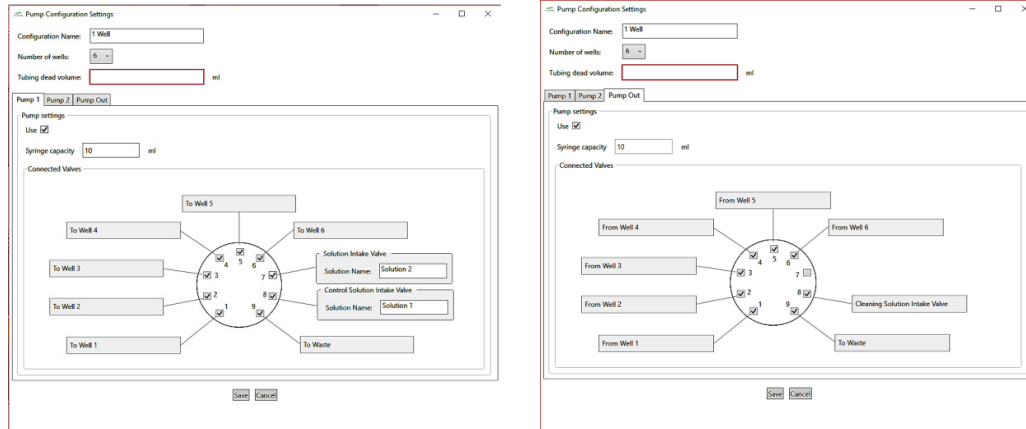
Figure 5. Standard configuration setting of pump 1 and pump out for a 6 well system

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

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 a cleaning solution) 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 <u>control</u> 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.
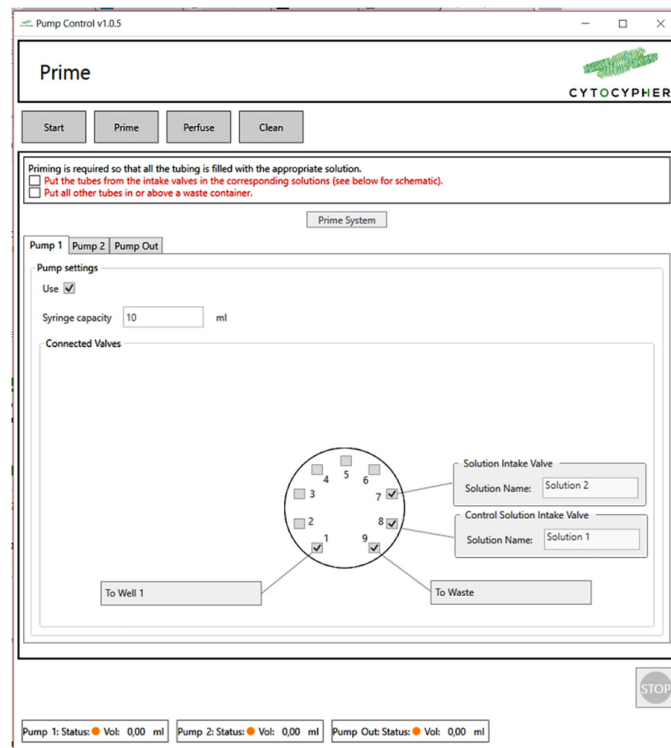

Figure 6. Prime window Pump Control

- 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.
- Now the tubes and the perfusion block are primed and the perfusion block or tubes can be placed into the wells for measurements.

3.3 Perfuse

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 7** for examples of a 1 well or 6 well configuration.
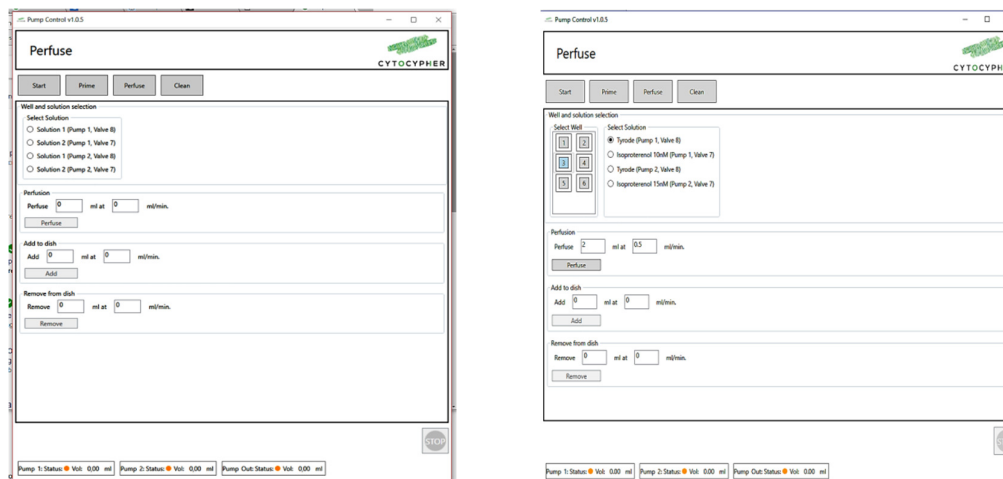

Figure 7. Perfuse window showing the options for a 1 well and 6 well configuration

! 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.

3.3.2 Add to dish

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

3.3.3 Remove from dish

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

3.4   Clean

The tubes and syringes of the pump system must be 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.
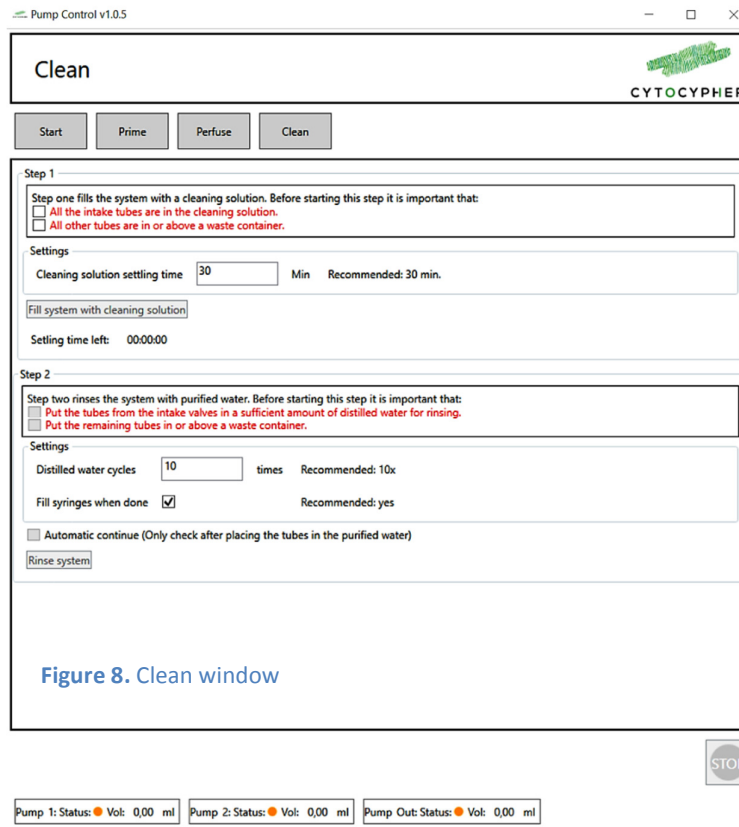

Figure 8. Clean window

3.4.1   Step 1: Filling the system with a cleaning solution

- 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.
- 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].

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

During the settling time of the cleaning solution in step 1, place all intake valves into a container with distilled or de-ionized water. Fill in the number of flushing cycles and tick the

"Automatic continue" box. When the settling time has elapsed it will automatically move to step 2.

It is recommended to keep the tubes and syringes filled with distilled or de-ionized water if the system is used frequently.
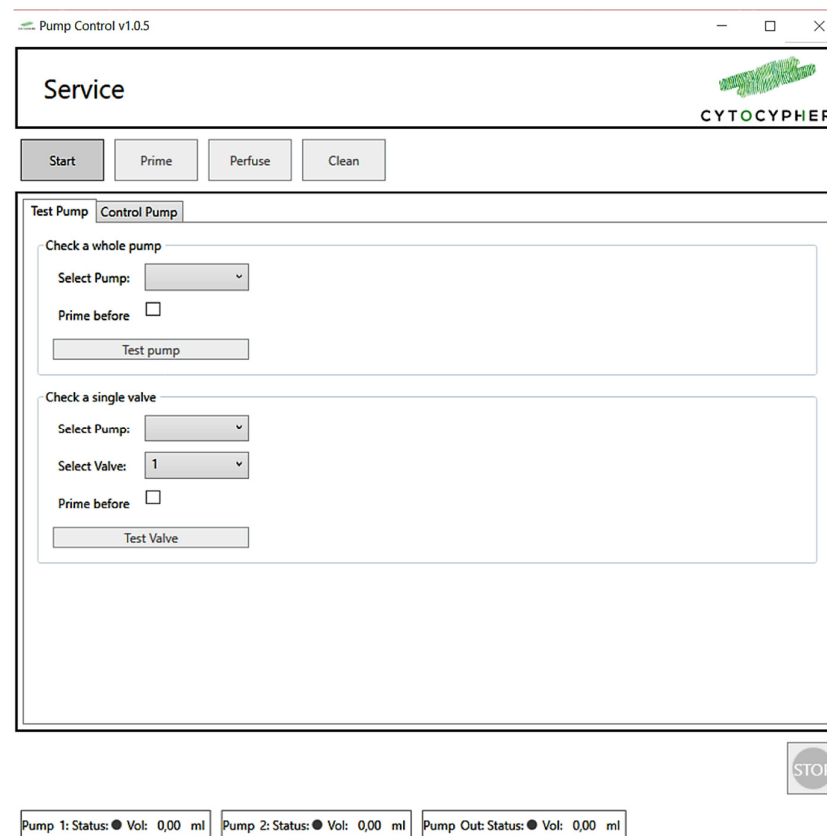
3.5 Service

A hidden tab `[Service]` can be found by pressing `[ctrl]+[alt]+[s]`, this opens a new tab `[Service]` with two tabs `[Test Pump]` and `[Control Pump]`.
N.B. Pumps can only be controlled when step 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) or 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.

3.5.1 Replace syringe

To replace or attach a new syringe you want to move the rod to a lower position. The fastest way to do this is:

- Go through step 1 till 3 of the `[start]` window (see page 7).
- Open the `[service]` window by pressing ctrl+alt+s.
- Select the pump and press `[Test pump]`.
- Press `[stop]` when the rod is in the desired position
- Press `[No]` in the pop up window when it asks whether you want to empty the pump.