Operating Instruction & Manual
For Clotho Drive Unit

PerfuseCell

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1. Concept overview

Clotho is a family of Single-Use-Pump (SUP) Drive Units designed for operation of **CellMembra** or **CellRetention** Perfusion-Single-Use-Bioreactor. Integrating either the Clio One-way-SUP (O-SUP) or Thalia Alternating-SUP (A-SUP) diaphragm pump. Clotho is available is both mono / single channels and stereo / dual channels models.

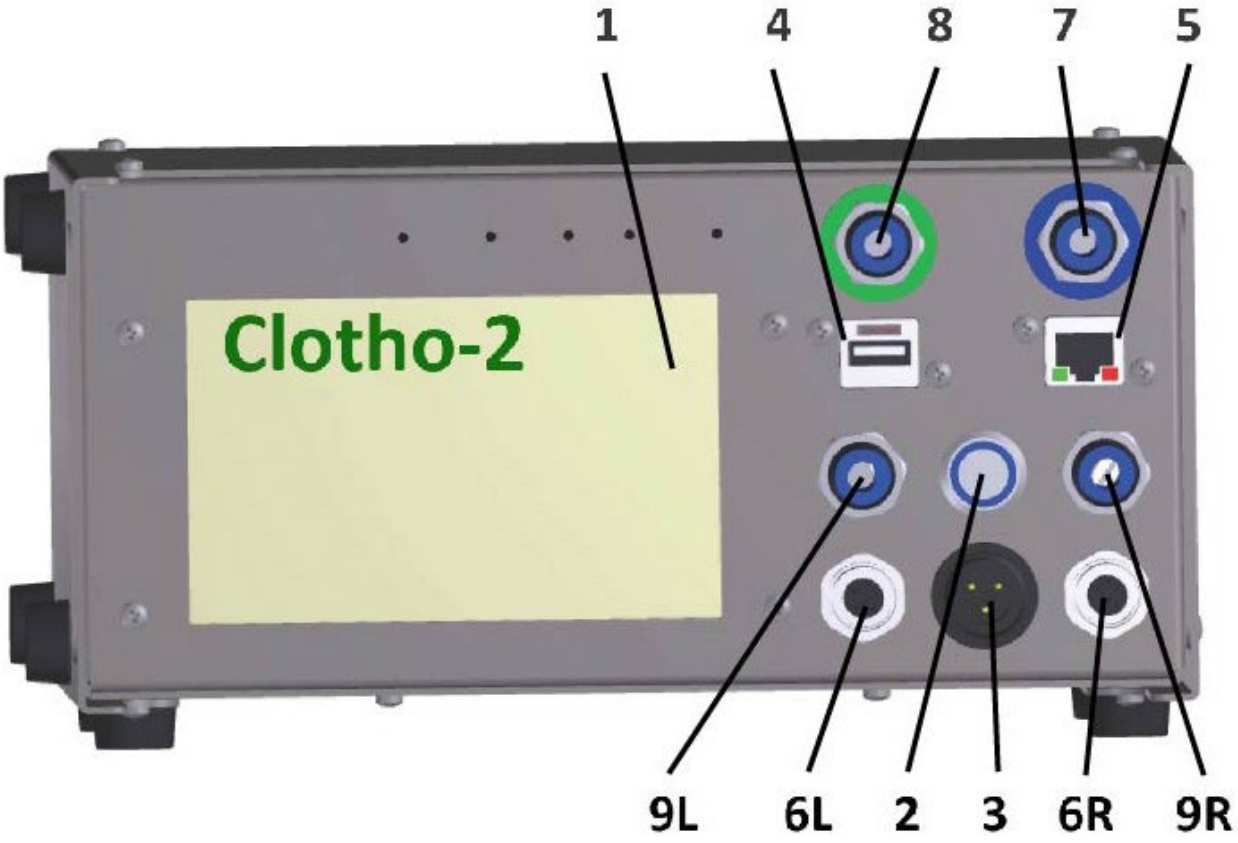
1.1 - Product purpose

www.perfusecell.com manufacturer the products CellMembra and CellRetention. Both products are Single-Use-Bioreactor´s for mammalian high cell density cultivation operating in perfusion mode - Continuous Processing. Both SUBs integrate a diaphragm Single-Use-Pump. Either a O-SUP or A-SUP and one or more stacked hollow fibre module(s) / Cross-Flow-Filter (CFF) - all in one package. The process concept to choose from is either: PTF – Pulsating-Tangential-Flow (Clio O-SUP) or ATF – Alternating-Tangential-Flow (Thalia A-SUP).

Driving the diaphragm SUPs requires supply gas both below and above atmospheric pressure. The drive gas is then constantly adjusted via a set of proportional valves inside Clotho. Control is based on input from sensors (distance and pressure) given to the build-in CPU. Stroke Volume (SV) and number of strokes, Beats-per-Minute (BpM) determine the pumped broth volume, Cardiac Output (CO) and velocity (m/s) of the broth passing through the CFF.

2. - Device Overview

The Clotho Drive Unit is a product manufactured by sister company www.pumpcell.com for PerfuseCell. Clotho is housed in the compact Hephaestus U2 cabinet manufactured from AISI 304 stainless steel.



For dual channel, Clotho-2 front panel is equipped with:

1. 5” touch sensitive TFT display
2. Main power breaker
3. Buccaneer 24 VDC power input
4. USB socket for Wi-Fi access and charging
5. RJ45 socket for Local-Area-Network (LAN) connection

6L. Left channel laser sensor input with M12, RKF5, 5P socket

6R. Right channel laser sensor input with M12, RKF5, 5P socket

7. Supply pressure 6 mm one-touch connection
8. Supply vacuum 8 mm on-touch connection

9L. Left channel SUP drive via 8 mm hose and on-touch connection

9R. Right channel SUP drive via 8 mm hose and on-touch connection

There are two fan openings on the rear panel of Clotho. Avoid pushing the cabinet close to a wall prohibiting ventilation and internal cooling.

2.1 - Clotho internal design

Internal sensors and actuators:

- Pressure sensor for supply, >1 - <4 bar atmospheric (will tolerate up to 8 bar supply pressure)
- Vacuum sensor for supply, <0,1 bar absolute (will tolerate up to 8 bar supply pressure)
- First channel drive gas pressure / vacuum sensor outlet to SUP, ± 1 bar
- Second channel drive gas pressure / vacuum sensor outlet to SUP, ± 1 bar
- Proportional valve for positive drive gas pressure regulation
- Proportional valve for negative drive gas pressure regulation
- Fan for cabinet temperature control

External sensor(s):

- For each channel, a re-usable tri-angular red Leuze Laser sensor mounts on the re-usable bracket, foot on the SUP for on-line measurement of the actual position of the free-floating silicone membrane / diaphragm inside the Clio SUP and Thalia SUP.

Two identical red Leuze Laser sensors shown with 1.5 meter wire and M12 connectors.

2.2 - Requirement

The Drive Unit must be properly installed according to fluid diagram and general recommendations. The drive gas inlet ports are marked. So, for vacuum a green disc suggest green 8 mm hose and a blue disc for pressurized air suggests using a blue 6 mm hose.

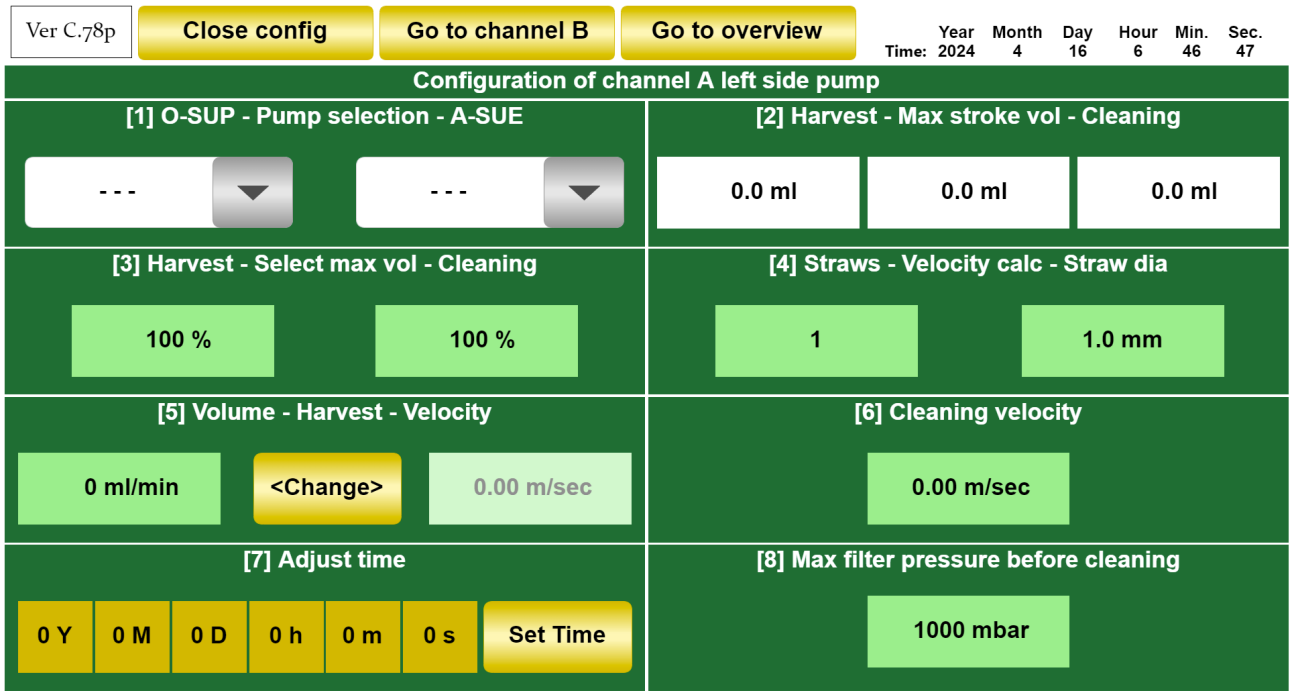
2.3 - Specification

When Clotho is connected to:

- a suitable supply of pressurized air with sufficient capacity - above 1,2 Bar
- a suitable supply of vacuum with sufficient capacity – lower than 900 mBar
- Leuze Laser sensor(s) for each either one or two channels
- Clio and/or Thalia Single-Use-Pump(s) for channel A or A + B
- 24 VDC supply then the specification for both single and dual channels models is:

Beats-per-Minute (BpM), strokes per minute	Check latest info on www.perfusecell.com
Cardiac Output (CO)	Check latest info on www.perfusecell.com
Stroke volume (SV)	Check latest info on www.perfusecell.com
GUI	5” touch sensitive TFT display
Computer power	900 MHz quad-core ARM Cortex-A7 CPU
USB socket	Wi-Fi like NetGear N300 Mini Adaptor
RJ45 socket	IP/TCP via LAN (ModBus protocol)
Power supply	24 VDC, <30 watt via Buccaneer socket
Build in fan, noise level, dBa	<65
Duty cycle	100%
Orientation	any
Operating conditions	10°C to 40°C, <80% relative humidity, non condensing
Life time, estimated, hours	<50,000
MTBM, battery must be changes, hours	10,000
Cabinet size and material	Hephaestus U2 – AISI304
Drive Unit weight, kilo	4.5

3. Software setup



Graphical-User-Interphase (GUI) for Clotho Drive Unit.

3.1 – Basics is the human brain and heart for understanding

Clotho controls the cardiac cycle of the Clio and Thalia pump. The parameters we work with are:

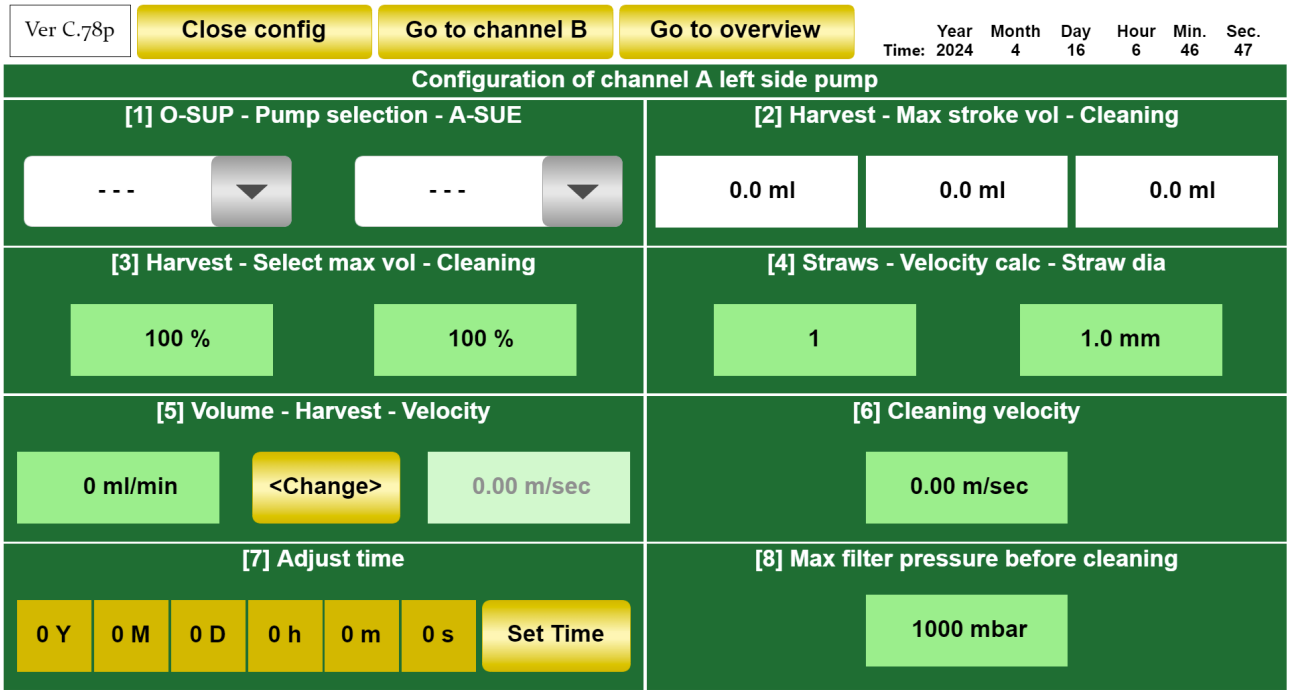
- Cardiac Output (CO) = total volume pumped, SUP capacity, ml/min (CO = BpM x SV)
- Beats-per-Minute (BpM) = determined by the diaphragm diameter
- Stroke Volume (SV) = programmable from 10-100% in 10% section

Clotho Drive Unit copies the purpose of Medulla in the human brain and the heart's Sinoatrial Node and combines the features with the ability for interaction, programming, and functionality. Clotho take advantage of the build-in Apollon brain and receives real-time signals from pressure, temperature, and displacement / Laser sensor(s). Apollon regulates positive feedback control a set of proportional valves for the BpM, and the driving force and movement of the elastic Myocardium wall inside the Clio and Thalia SUP.

3.2 – You can program various parameters

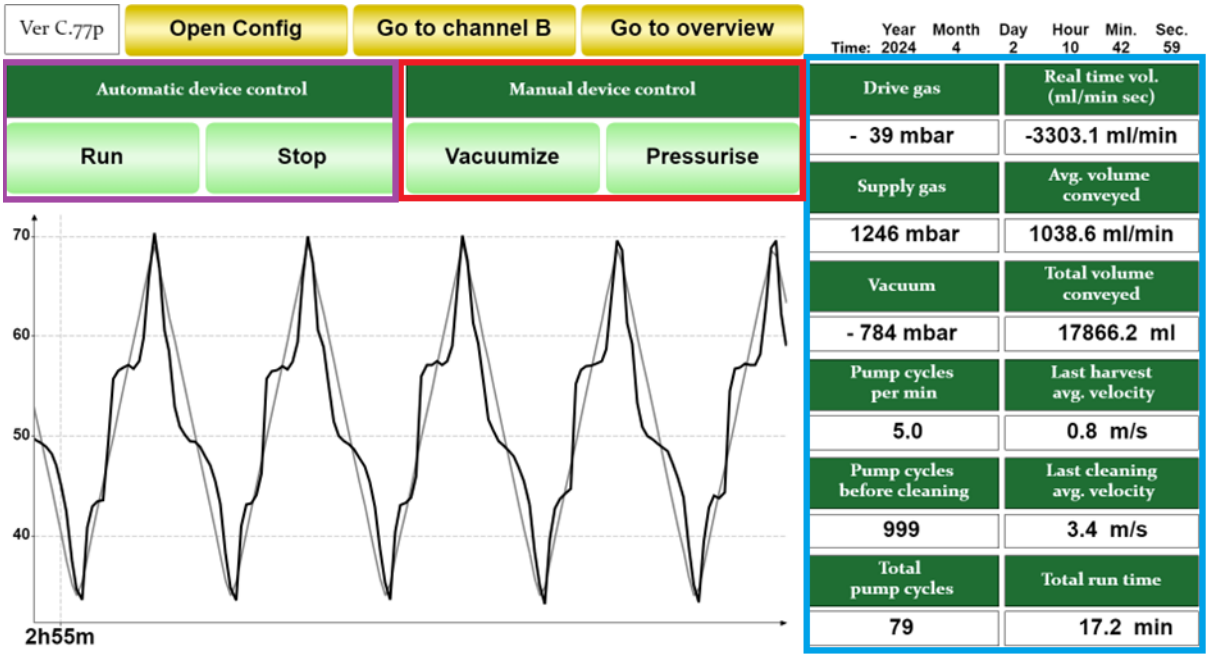
The internal clock is set from factory to CET, Central-European-Time. You should check if the clock is relevant for your geographic area. Clotho is equipped with a battery driving internal clock for accurate time measuring. If you need another time, it's easy to alter by pressing "Open" under "config". Remember to "Set" the time after changes.

3.3 – How to program Clotho

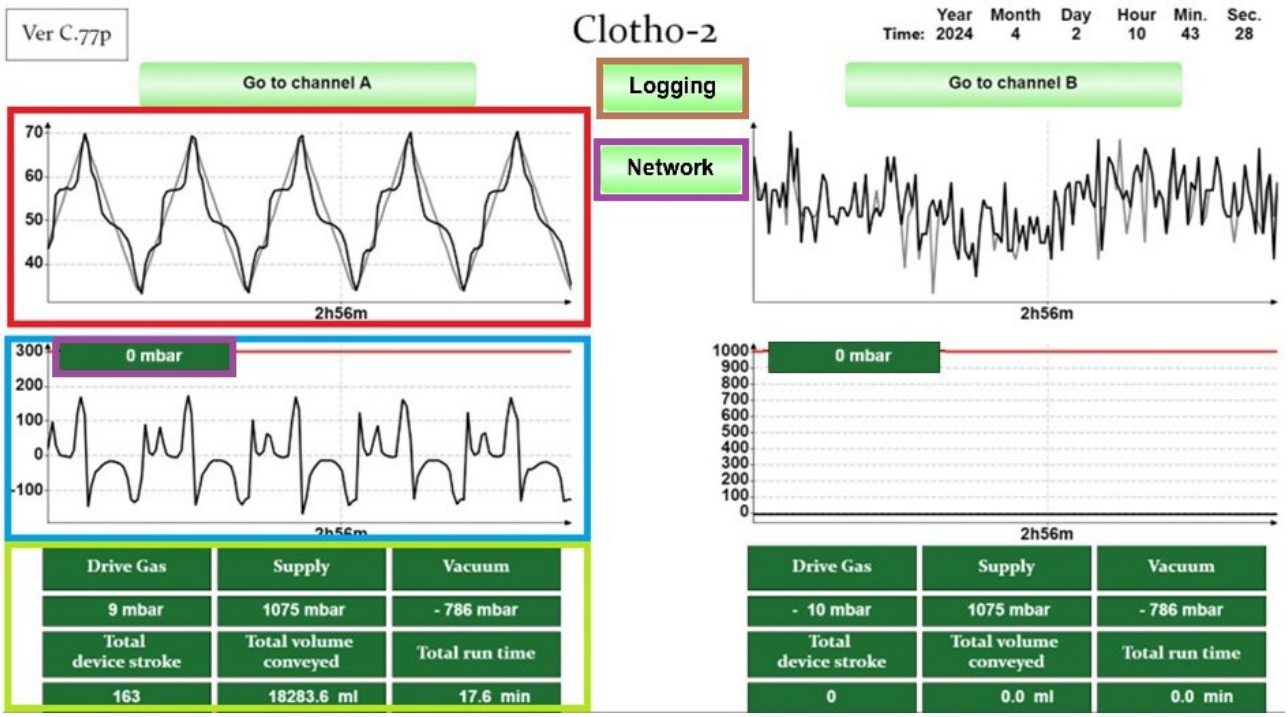


Start interface and follow carefully sequence:
DO following to start a pump with self-controlling cleaning
[1] – Select pump
[2] – Info field with volume size
[3] – How many percentage is in use when Harvest or Cleaning
[4] – Pcs of straws inside the Hollow-Fiber-Filter and the diameter of straws
[5] – Choose speed/velocity of the Harvest stroke of the pump
[6] – Set velocity of the cleaning stroke(Higher m/sec than then Harvest velocity)
[7] – The time can be set in this field
[8] – To set the max pressure trigger point inside the pump(only lowered after watching the system run Harvest cycles for some minutes to determine the trigger point)

→ Press **Close Config** to stay on channel and start the process
→ Press **Go to channel B** to configure channel B (Right channel)
→ Press **Go to overview** to monitoring both channel at once



Blue Area – Info of the ongoing process
Red Area – perform manually pump control if needed to fill in liquid
Purple Area – start or stop the process.
Normally after the Config is filled out (not [8] yet) go start the process in purple area.
→ Press **Go to overview**



Red area shows the distance from the laser sensor to the membrane from channel A

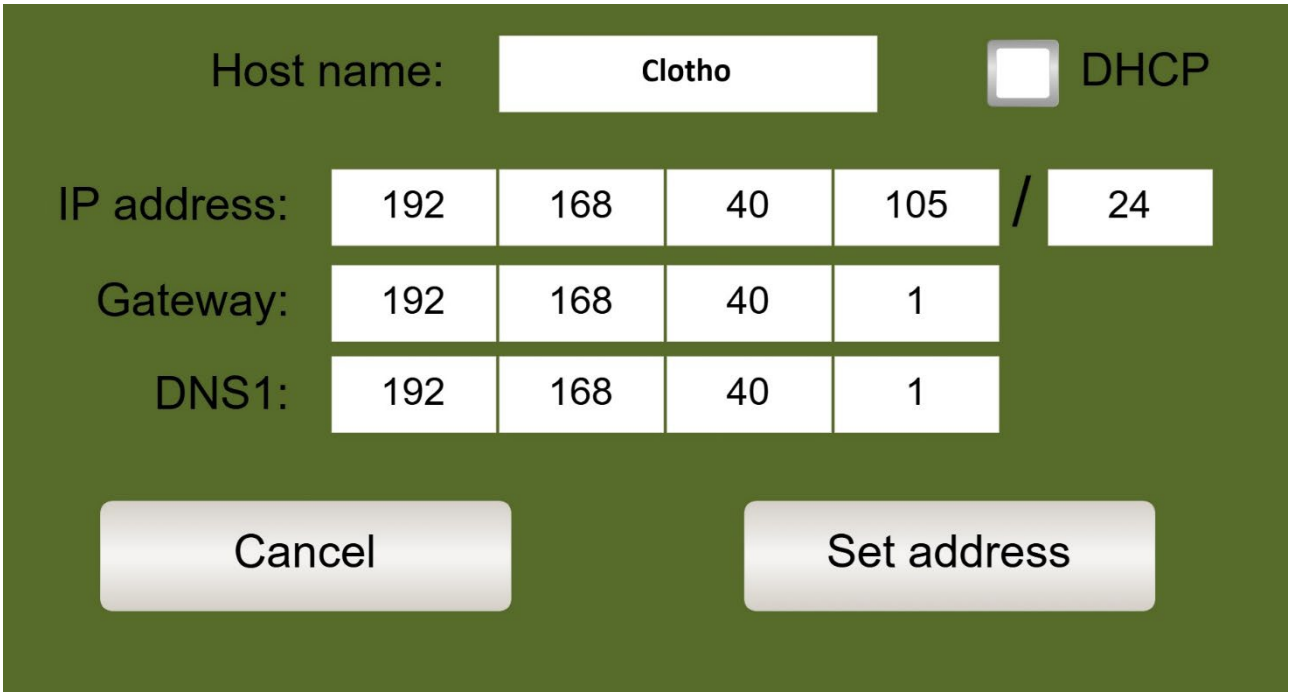
Blue area show the drive gas pressure inside the pump from channel A

Purple area show the max mbar drive gas pressure inside the pump/filter from channel A, it will reset every 2 minutes, it will be a good indicator of where to set the [8] Max Pump Pressure on the **channel A Config,** to a start 100-150 mbar above the normal Harvest run you experience now. And it will be possible to determine more precisely when expertise is gained in each setup, when to clean the filter.

Green area is a copy of info from channel A.

Brown area, Logging is explained on our website - https://cronus-pcs.com/support/communication/data-acquisition/

Purple area, Network takes you to network (below)



Here you can choose to use static or DHCP IP address to secure that Clotho will fit into you network setup with different SCADA software.

If DHCP or manual (static) IP address is set then press Set address and restart (off/on) Clotho, to make the change have effect.

3.5 – Details to how measurements are done

Pumped volume, Cardiac Output (CO mL per minute)
SUP displacement and stroke frequency determines the total volume over time. The SUP will pump double the programmed volume as the pump stroke is 50 % of the time. The display shows programmed curves and the actual curves. The pumped volume, CO cannot exceed the SUP specific capacity multiplied with the SV in percent.

Stroke Volume, SV, 0 - 100 %
Potentially shear stresses could be of interest.

CFF velocity, m/s
Show actual, on-line velocities in meters per second as an average based on the number of straws you have programmed into the software.

Time:	Year	Month	Day	Hour	Min.	Sec.
	2024	1	15	10	17	0

Drive gas	Real time vol. (ml/min sec)
-0.009 Bar	NaN ml/min
Supply gas	**Avg. volume conveyed**
-0.010 Bar	0.0 ml/min
Vacuum	**Total volume conveyed**
-0.011 Bar	0.0 ml
Device stroke per min	**Last harvest avg. velocity**
0.0	0.0 m/s
Device stroke before cleaning	**Last cleaning avg. velocity**
999	0.0 m/s
Total device stroke	**Total run time**
0	0.0 min

Data acquisition and real-time read out windows.

It's good to know that "Real-time flow" is approximately double of "Average-flow" of the Clio RSUP. This is because the SUP is a reciprocating pump and fluid is moved only at every second stroke. Different for the Thalia A-SUP which do not really pump but move a SV forth and back.

"Total volume conveyed" can only be from process start after pressing Run button.

3.5 - Alarms

Data from driving gas is shown online. So, it the actual drive gas pressure on the gas side of the Myocardium diaphragm jumping between vacuum and pressure.

Alarms for:
The alarm area will show if the supply hose for pressure and vacuum is not mounted correctly.

- Pump pressure range, Bar: ÷0.5 to 0.9
- Supply pressure range, Bar: 0.5 to 1.1
- Vacuum pressure range, Bar: ÷0.8 to ÷1.0

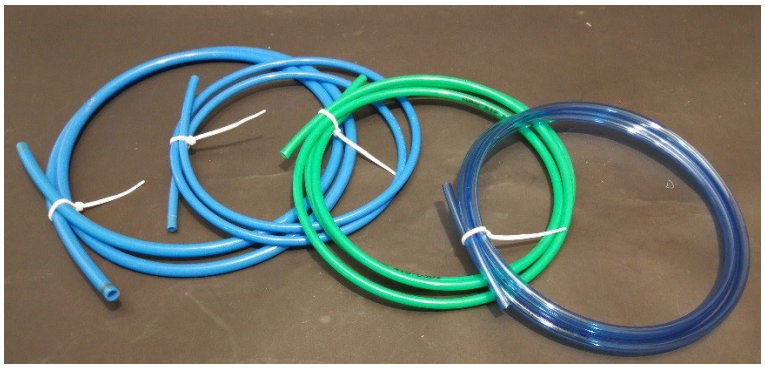
If no pressure or vacuum is available, drops out or insufficient – then an alarm appears as red and the process stops.

4. Hardware installation

The scope of the Clotho product is to drive the CellMembra integrating the Clio O-SUP or CellRetention integrating the Thalia A-SUP. Unpack and dispose in a nice way or store the shipping material properly. Check out that you have all the parts shown here.

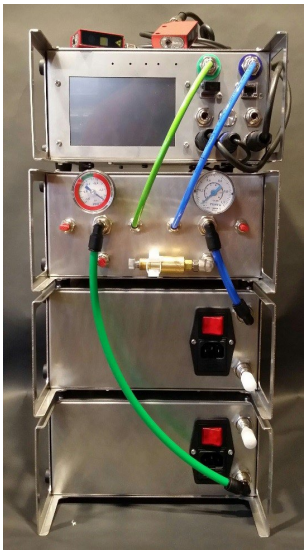
1. Clotho in U2 cabinet
2. Laser sensor(s) – little red box with black cable
3. Power Supply model Zephyros – one per Clotho – both mono and stereo models
4. NO power input cable to Zephyros supplied – you have tons of these standard cables anyway and with the correct wall plug for your application.
5. Transparent blue soft 2-meter-long polyurethane hose for drive gas connection

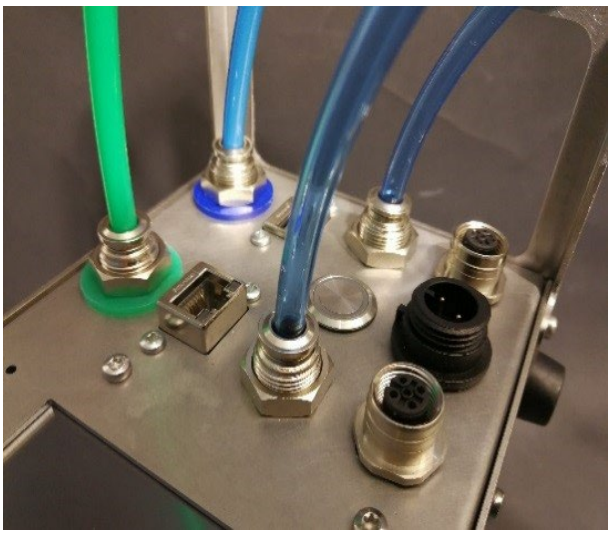
Depending on how much gear you received there will be or not included hoses in different colors.



We recommend using these colours and follow our recommendations. Light blue 6 mm nylon hose for pressurized air. Green 8 or 10 nylon hoses for vacuum depending on Clotho size. Transparent dark blue very flexible polyurethane for drive gas connection from Clotho to SUP.

Focus with these photos is how SUP and Power Supply (PS) is connected. PS connection is simple with the round bright blue finger nut on the black Buccaneer cable connector. Drive gas to the SUP is via the transparent blue soft polyurethane hose. Laser sensor input via the black M12 cable connector.





Be sure you connect vacuum to the left / green one-tough fitting and pressurizes air to the right / blue one-touch fitting. Find more information on how to take advantage of the Sarpedon unit here – www.perfusecell.com/download/drive-unit-manuals

Procedure to follow for model Clotho-1 and Clotho-2:

1. Connect a pressurized drive gas supply of max 1 Bar (could be Sarpedon / Alagonia) with 6 mm rigid blue hose.
2. Connect a vacuum source (such as Sarpedon / Alagonia) with an 8 mm rigid green hose.
3. Connect the 8 mm soft transparent blue drive gas hose from the SUP to Clotho.
4. Mount the red Laser sensor on/in the support foot.
5. Connect 24 VDC from one of the Eos family of power supplies.
6. Start-up Clotho and watch the unit get alive.
7. Spend some minutes and use the two manual buttons to remove air and fill SUP with liquid.
8. Program according to you SOP and planning.
9. The Clotho is ready for action.

Requirement
The system must be properly installed and connected in accordance with the specifications and previous information. Operator must also have gained familiarity with the Safety Instructions to be found separately on www.perfusecell.com/support/safety-instructions

Clock
Clotho unit need no up-front clock programming and is ready for installation. The Drive Unit is preprogrammed with the CET time. If you need different time look under section 3.2

4.1 - Fault information
The built-in display of Clotho will inform you about possible faults whenever detected.

- If no air pressure or vacuum source is detected
- If no movement is detected by level sensor

5. Communication
Clotho contains a webserver displaying online information on the built-in display.

Each Clotho product has an IP address shown at the Manufacturer´s Identification Label under the product. If you hook up via the Wi-Fi connection to a touch sensitive PC, smartphone, or PAD you should see the GUI from the actual Clotho Drive Unit in a browser.

5.1 - PID routines
Many hours have been invested in setting up the PID routines and the intelligence for continues auto-tuning. Clotho will initiate auto-tuning at each start-up for the best possible accuracy. Be patient for like the first half an hour operation with a new P-SUB installed.

6. Operation Manual

At activation of power button then Clotho (or restoration of voltage after a power outage) starts up and the display will show:

- Firmware / operating system software (Linux) is loaded.
- System configuration is loaded from memory.
- Operating software (Clotho) is loaded, and actual information is displayed.

Any user-defined parameters from a previous process are stored in a battery-buffered memory and can be used for the next process. Clotho is pre-programmed from PerfuseCell with simple routines. If there are no faults found by the software Clotho is ready to operate.

6.1 - Operation principles

Assuming a correct assembly of all systems, connections, etc. according to your Fluid Diagram and this manual – check all connections are tight.

6.2 - Wi-Fi connection

When Clotho is equipped with the included NetGear Wi-Fi access point the Apache webhost will be accessible from a browser. Go into “Settings”/ Wi-FI NETWORKS” on your PAD or smartphone and check if you can see Clotho and select. Return to a browser and write “anything” in the address line for access to Clotho.

Netgear mini Wi-Fi USB adaptor.

Software upgrade - http://perfusecell.com/support/communication/software-upgrade/

6.3 – LAN IP/TCP connection

For programming purposes and communication with PCS.

7. Safety precautions

Various component requires individual attention. Operator must also have gained familiarity with the Safety Instructions to be found separately on – www.perfusecell.com/support/safety-instructions

7.1 - Documentation

Clotho functionality must be checked on a regular basis and data of such testing kept recorded. As found here – www.perfusecell.com/support/documentation

Pumping capacities found here
https://perfusecell.com/perfusion-bioreactor/diaphragm-pumps-32

Hollow-Fiber-Filter information found here

https://perfusecell.com/perfusion-support/membrane-filter-35/seprapor-hff-info

7.2 – Definitions

Find here a glossary – www.perfusecell.com/support/glossary