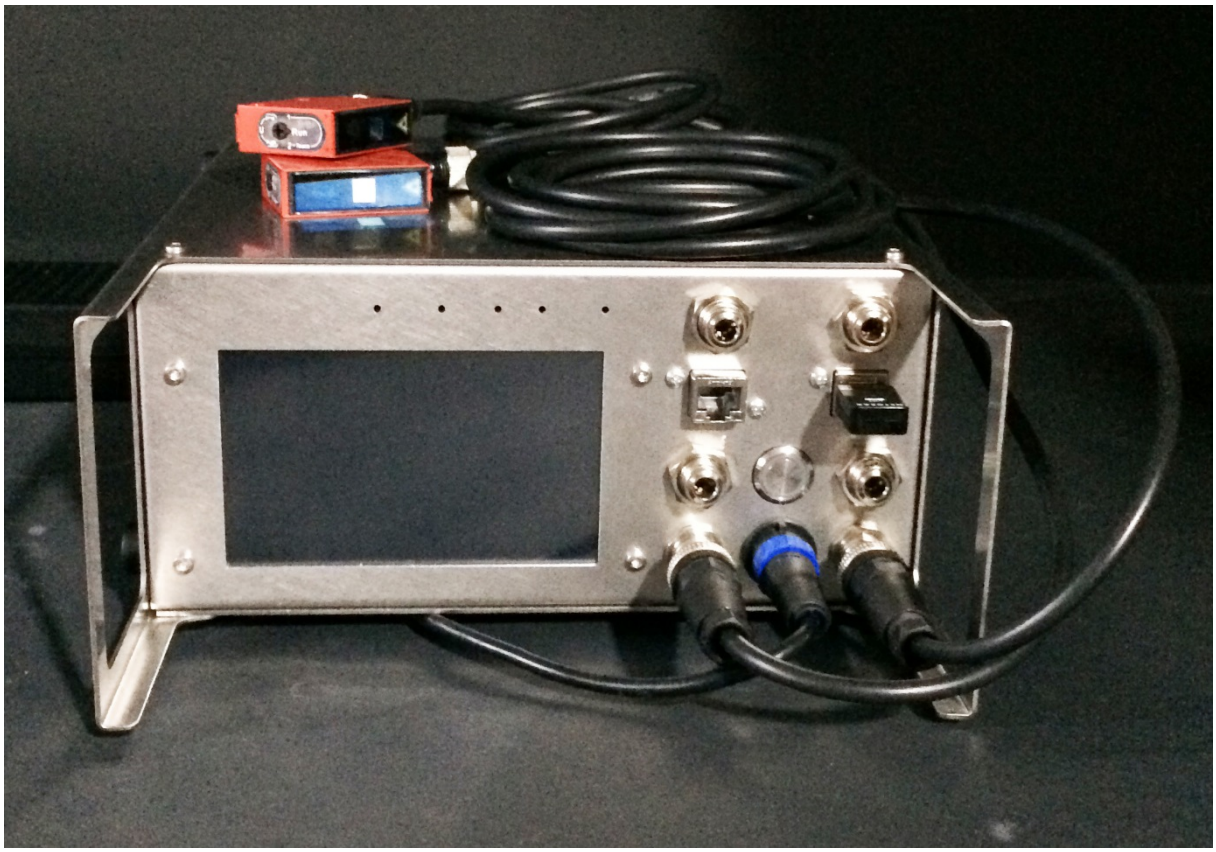
Operating Instruction & Manual
Lachesis drive unit's
covers both the single and dual channel models



PerfuseCell

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Contents

1. Concept overview

Lachesis is a family of Single-Use-Pump (SUP) drive units designed for operation of CellMembra Perfusion-Single-Use-Bioreactor integrating the Clio SUP. Lachesis is available is both mono/single channels and stereo / dual channels models.

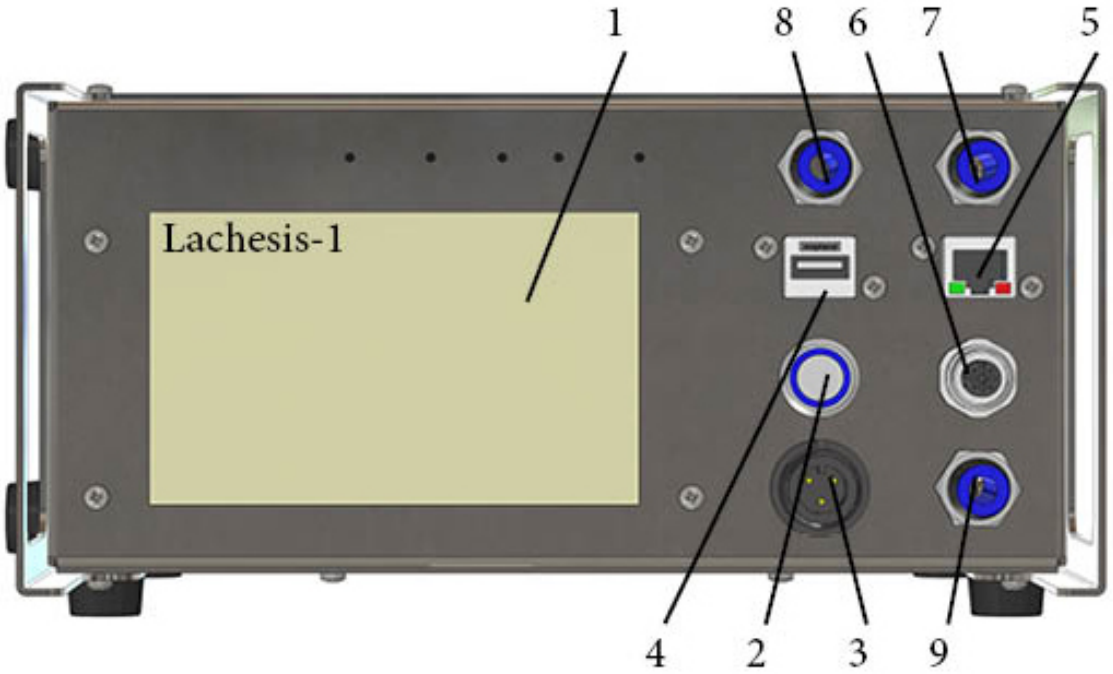
1.1 - Product purpose

CellMembra products is a range of Perfusion-Single-Use-Bioreactor´s (P-SUB´s) for high cell density cultivation operating in perfusion mode. Such as cell retention system for upstream Continuous Processing, cultivation of mammalian cell lines as supplied by www.perfusecell.com. The P-SUB integrates both Clio Single-Use-Pump (SUP) and stacked hollow fibre module / Cross-Flow-Filter (CFF) - all in one package. The process is also named PTF – Pulsating-Tangential-Flow.

Driving the diaphragm Clio SUP requires drive gas both below and above atmospheric pressure. The drive gas is constantly adjusted via a set of proportional valves inside Lachesis. Control is based on input from sensors (distance and pressure) given to the build-in CPU. Stroke and number of strokes determine the pumped broth volume and velocity of the broth passing through the CFF.

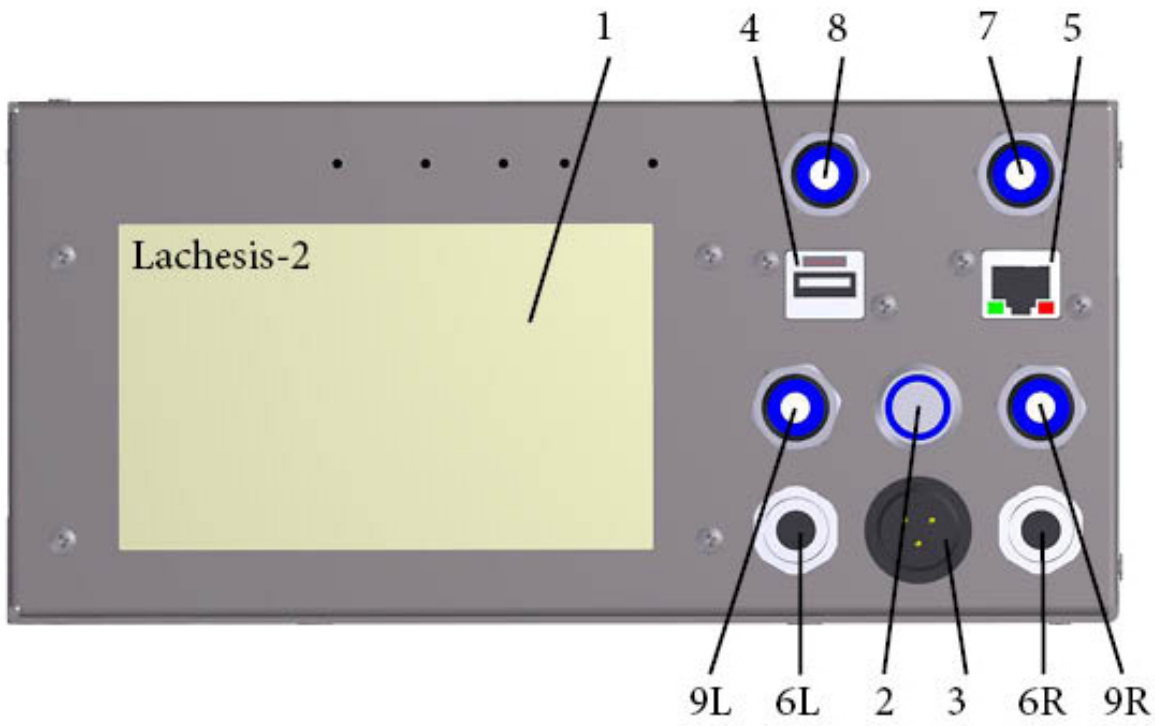
2. - Device Overview

The Lachesis unit is a product marketed by www.perfusecell.com and housed in a Hephaestus U2 cabinet manufactured from AISI 304 stainless steel.



For single channel Lachesis-1 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
6. SUP laser sensor input with M12, RKF5, 5P socket
7. Supply pressure 6 mm one-touch connection
8. Supply vacuum 6 mm on-touch connection
9. SUP drive via 6 mm hose and on-touch connection



For dual channel Lachesis-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 6 mm on-touch connection

9L. Left channel SUP drive via 6 mm hose and on-touch connection

9R. Right channel SUP drive via 6 mm hose and on-touch connection

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

2.1 - Lachesis internal design

Internal sensors and actuators:

- Pressure sensor for supply, >1 - <4 bar atmospheric (will tolerate up to 8 bar supply)
- Vacuum sensor for supply, <0,1 bar absolute (will tolerate up to 8 bar supply)
- 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.

Two identical Leuze Laser sensor's 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.

2.3 - Specification

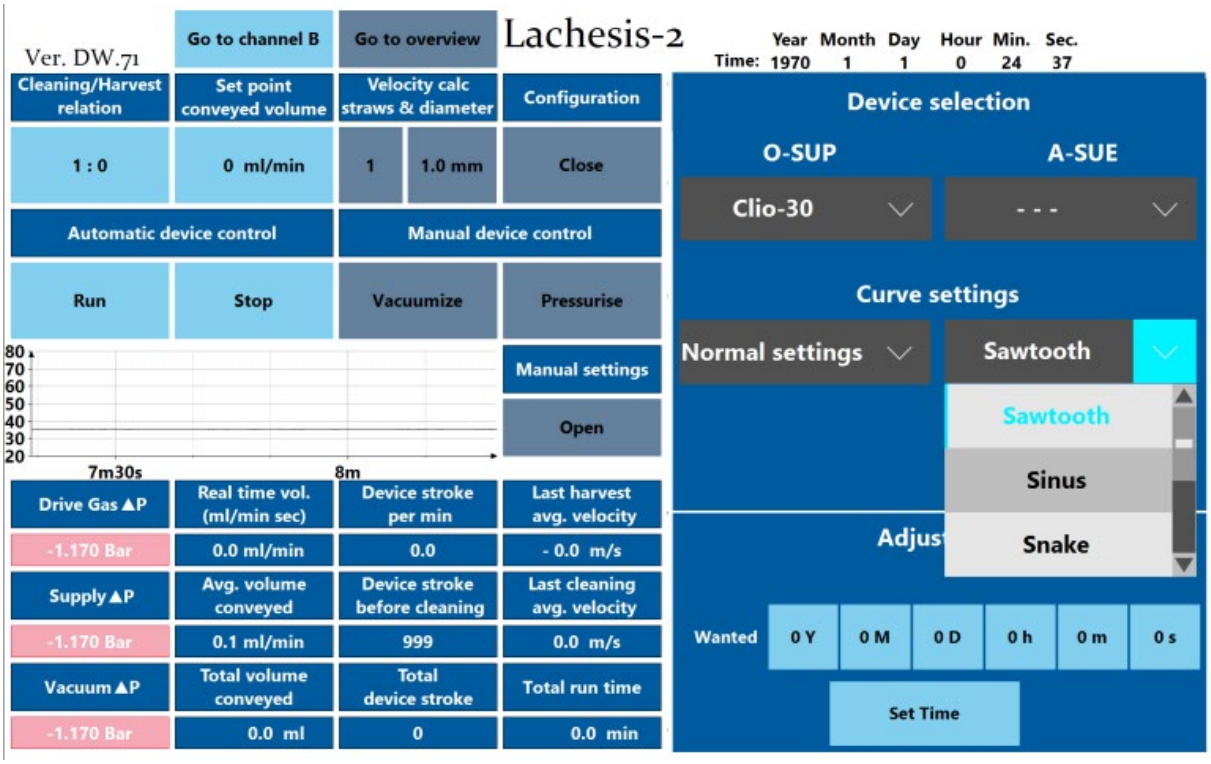
When Lachesis is connected to:

- a suitable supply of pressurised air with sufficient capacity
- a suitable supply of vacuum with sufficient capacity
- Leuze Laser sensor(s) for each either one or two channels
- Clio Single-Use-Pump(s) for at least one channel
- 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.pumpcell.com
Cardiac Output (CO)	Check latest info on www.pumpcell.com
Stroke volume (SV)	Check latest info on www.pumpcell.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
Weight, kilo	3,5

3. Software setup



Graphical-User-Interphase (GUI) for Lachesis drive unit operate with different colours. Light red is warning info from pressure sensors. Dark blue lower rectangle is Apollon PLC information.

3.1 – Basics is the human brain and heart

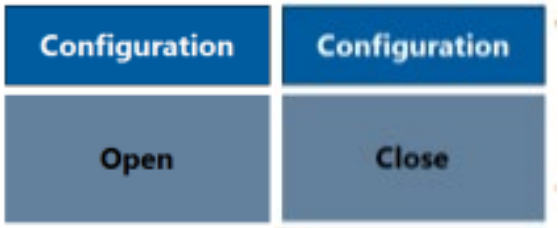
Lachesis control the cardiac cycle of the Euterpe pump. The parameters we work with are:

- Cardiac Output (CO) = total pumped volume, 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

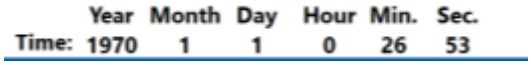
Lachesis copies the purpose of Medulla in the brain and the heart's Sinoatrial Node and combines the features with the ability for interaction, programming, and functionality. Lachesis take advantage of the build-in Apollon brain and receives real-time signals from pressure, temperature, and displacement/Laser sensor(s). Apollon regulate by 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 SUP.

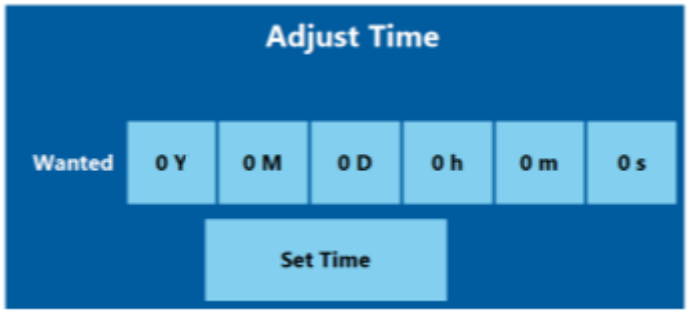
3.2 – You can program various parameters

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



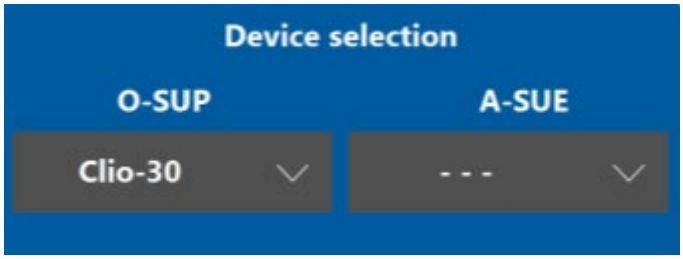
The window "Open" allow clock modification and SUP selection. If clock time is OK or you have modified and chosen the correct size SUP, you have finished - then press "Close".





Adjusting time to alter the pre-set time.

Initial at start-up no SUP is chosen. Press Configuration "Open" and the window for clock adjustment and SUP selection pops up.



If the black bar shows "- - -" = no SUP is chosen. Press the "- - -" black bar under O-SUP or A-SUP and a scroll down window appear, only one can be active at a time. Choose the correct size SUP and the black bar show the SUP chosen. Press then "Close" in the yellow window.

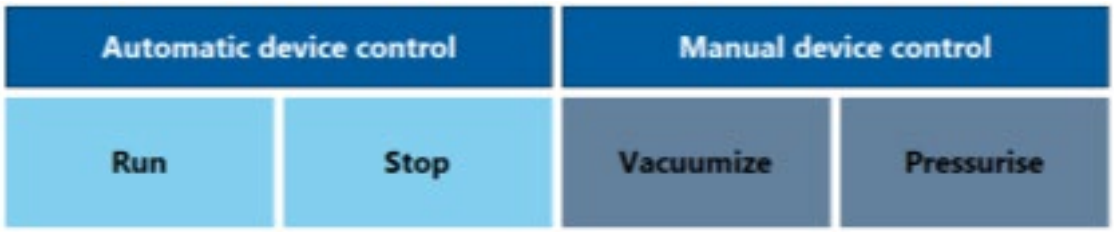
3.3 – How to program Lachesis

You can determine:

1. Cardiac Output (CO) = total pumped volume in ml/min (CO = BpM x SV)
2. Stroke Volume (SV) = programmable in step of 10% from 50 - 100%
3. Velocity Calculation m/sec = through the CFF ranging 1-99 straw and straw diameter

There is a relation between SV and BpM. If you select a low SV the maximum BpM depending on the Clio size determines the CO. Maximum CO occurs with SV = 100%.

3.4 – How to start and stop the process software



Automatic device control and Manual device control buttons

Run
Light green bottom - start up the process based on latest program.

Stop
Light green bottom - terminates the process based on latest program.

Vacuumize
Yellow button for manual operation. Use for system priming and testing functionality.

Pressurize
Yellow button for manual operation. Use for system priming and testing functionality.

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 with will pump double the programmed volume as the pump stroke is 50% of the time. The display shows programmed curve and the actual curves. The pumped volume, CO cannot exceed the SUP specific capacity multiplied with the SV in percent.

Stroke Volume, SV, 10 - 100%
Potentially shear stresses could be of interest. Find the smallest part in the fluid pass and measure the diameter to be inserted in red window under “Velocity Calc:

CFF velocity, m/s: 0.1 – 10
Show actual, on-line velocities in meters per second as an average based on the number of straws you have programmed into the software.

Drive Gas ▲P	Real time vol. (ml/min sec)	Device stroke per min	Last harvest avg. velocity
-1.170 Bar	0.9 ml/min	0.0	- 0.0 m/s
Supply ▲P	Avg. volume conveyed	Device stroke before cleaning	Last cleaning avg. velocity
-1.170 Bar	0.1 ml/min	999	0.0 m/s
Vacuum ▲P	Total volume conveyed	Total device stroke	Total run time
-1.170 Bar	0.0 ml	0	0.0 min

Data acquisition and real-time read out windows.

It's good to know that "Real-time flow" is approximately the double of "Average-flow" of the Clio SUP. This because the SUP is a reciprocating pump and fluid is moved only at every second stroke.

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

3.5 - Alarms

Data from drive gas is shown online so it the actual pressure above the Myocardium diaphragm. Jumping between vacuum and pressure.

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

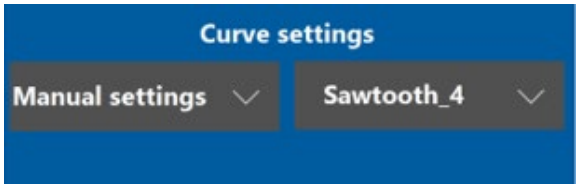
- Pump pressure range, Bar: ÷0.5 to 0.9
- Supply pressure range, Bar: 0.5 to 1.3
- Vacuum pressure range, Bar: ÷0.8 to ÷1.0

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

3.6 – Advanced software with manual settings

3.6.1 - Curve setting

Curve settings do have 2 different dropdown menus:

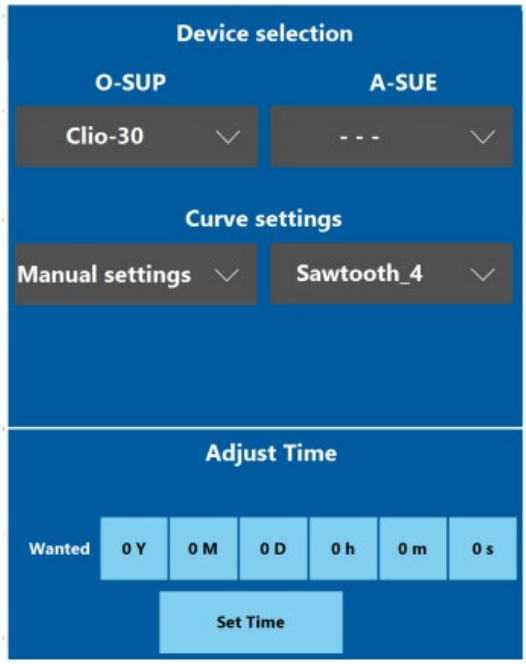


Curve settings scroll down menus

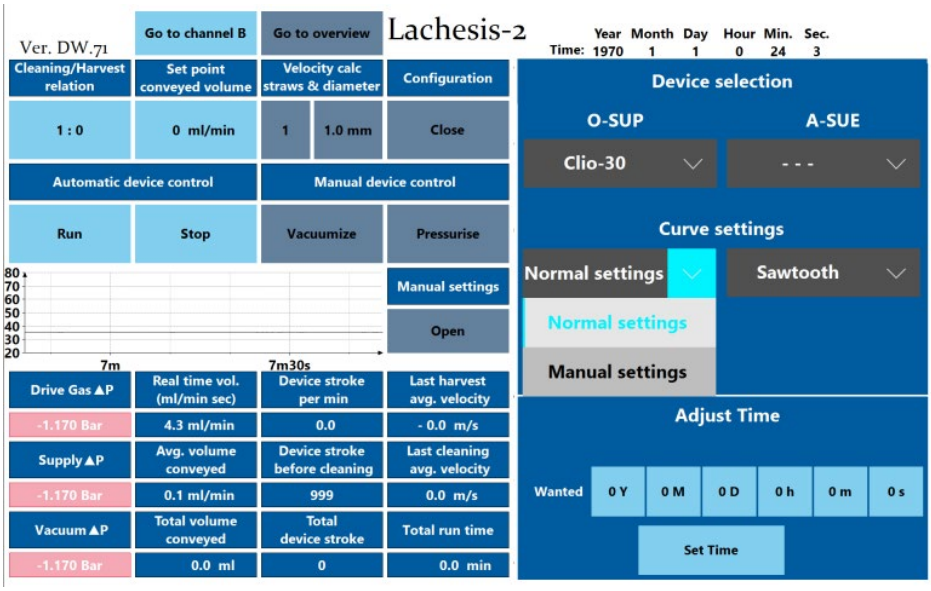
On the left side it is possible to choose between normal settings and manual settings.
The normal settings are default and will allow the former settings.
The manual settings will open for advanced settings that is explained further down.

On the right side it will be possible to choose from 4 different curve formats:

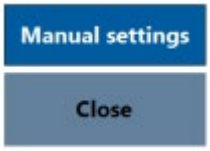
1. Sawtooth
2. Sinus
3. Snake
4. Sawtooth_4



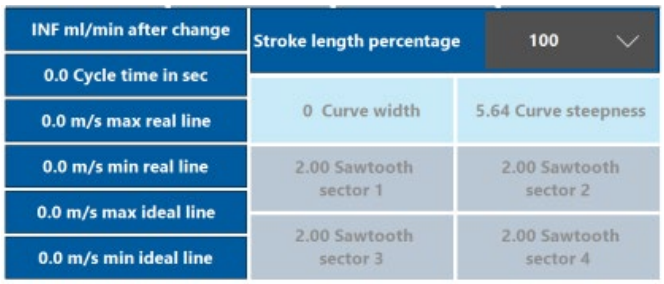
Configuration panel



Lachesis start screen with Curve settings left scroll down menu open

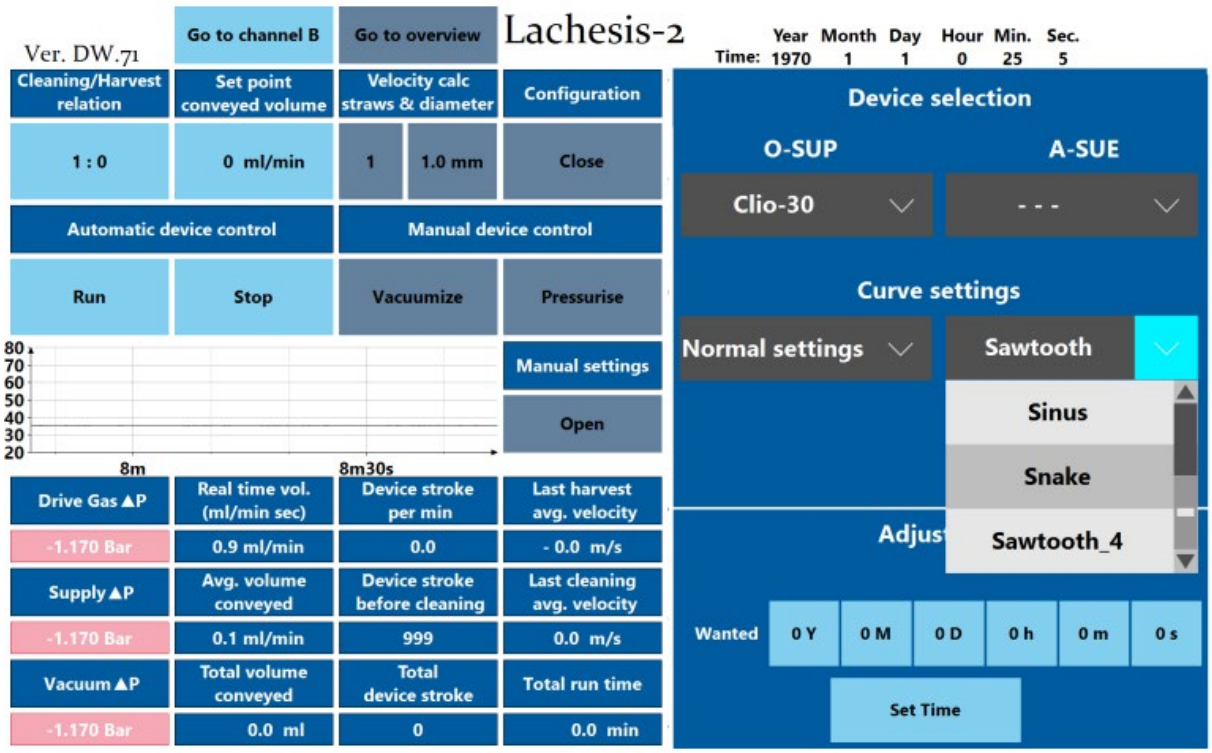


Manual setting button to open adjustment rectangle



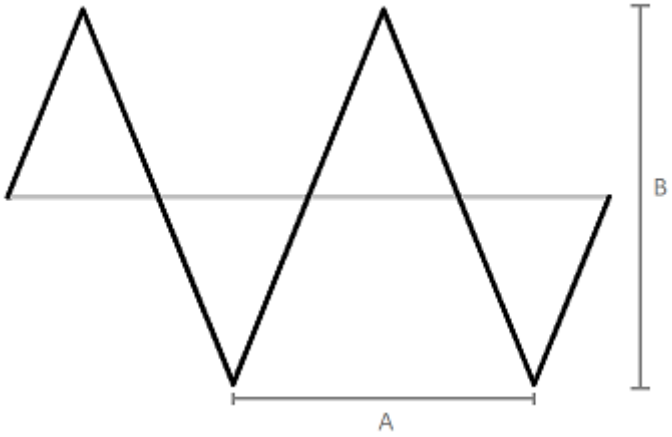
Adjustment screen

- Sawtooth, sinus, snake and sawtooth_4

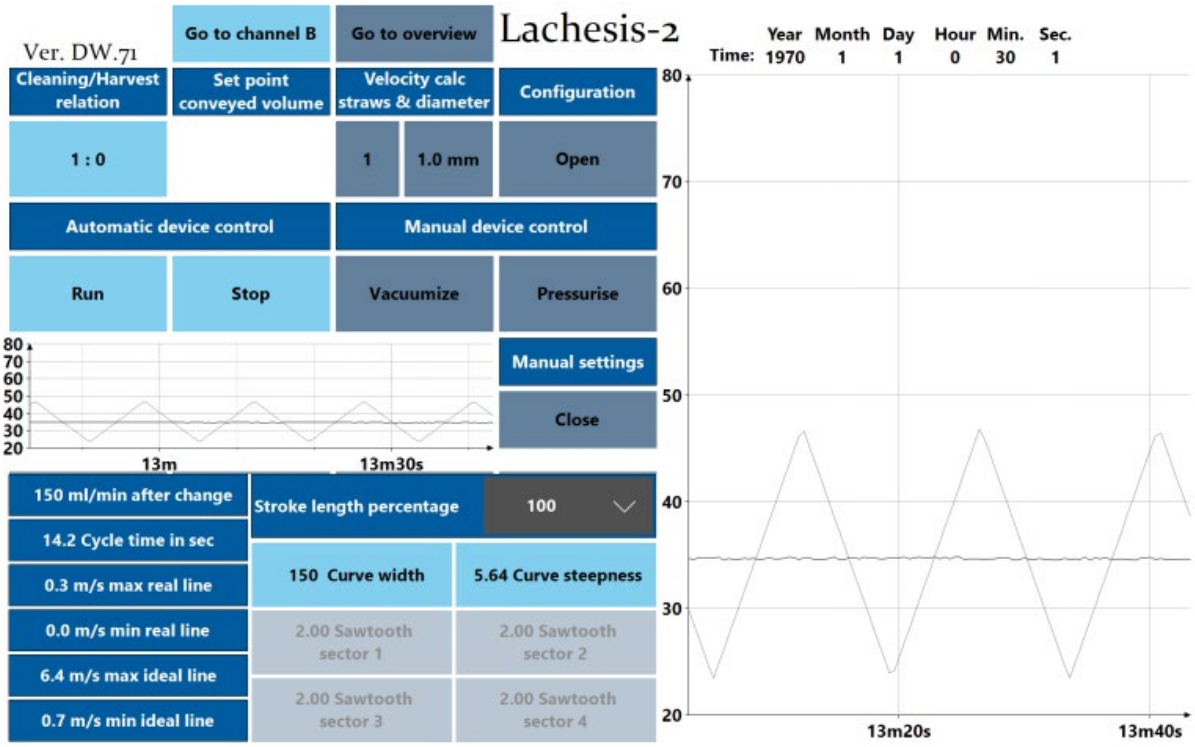


Lachesis start screen with Curve settings right scroll down menu open

Sawtooth curve is the most used curve and is also the curve set for use at the star up of the Lachesis unit. For this curve it is only possible to change the stroke length in percent and the ml/min or curve width, cause the steepness is controlled by the cycle each min, so for a steeper curve then turn up the value of the set point ml/min or curve width.



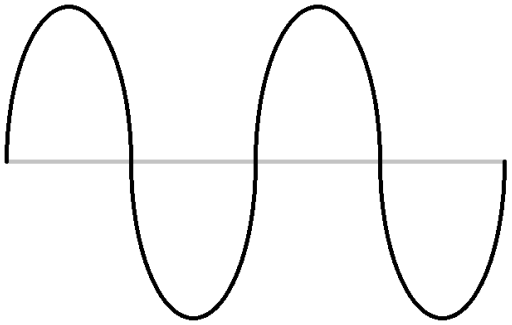
Sawtooth curve, A is equal to one pump cycle and B is equal to stroke length



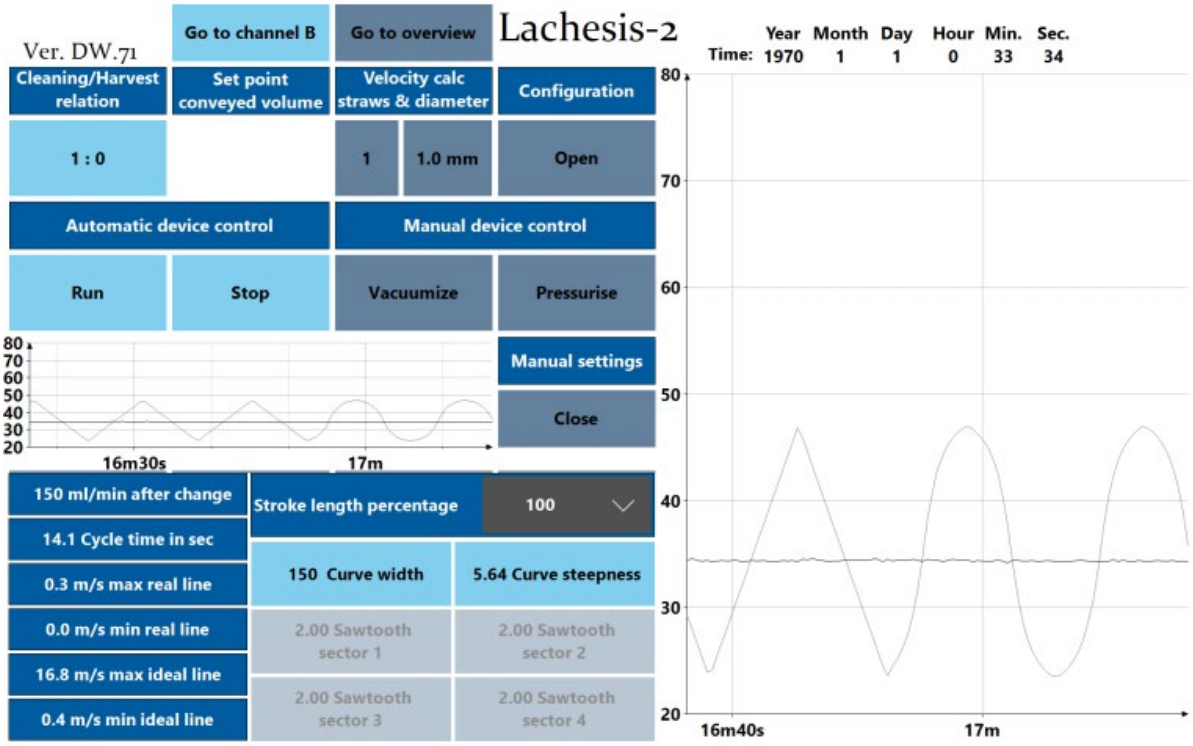
Lachesis screen with adjustment panel open and configuration panel closed and sawtooth curve running

Sinus curve can be adjusted by changing both the curve width, stoke length and curve steepness.

Curve width – higher number = higher ml/min, higher count cycle each min and lower cycle time.
Stroke length – lower % = more cycle each min to compensate for the smaller amount pumped.
Curve steepness – lower value = more straight line, the value 5.64 is the one matched with the normal setting to reach the same amount of ml/min as the sawtooth.

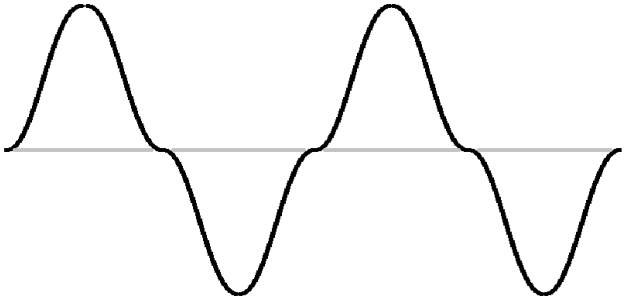


Sinus curve

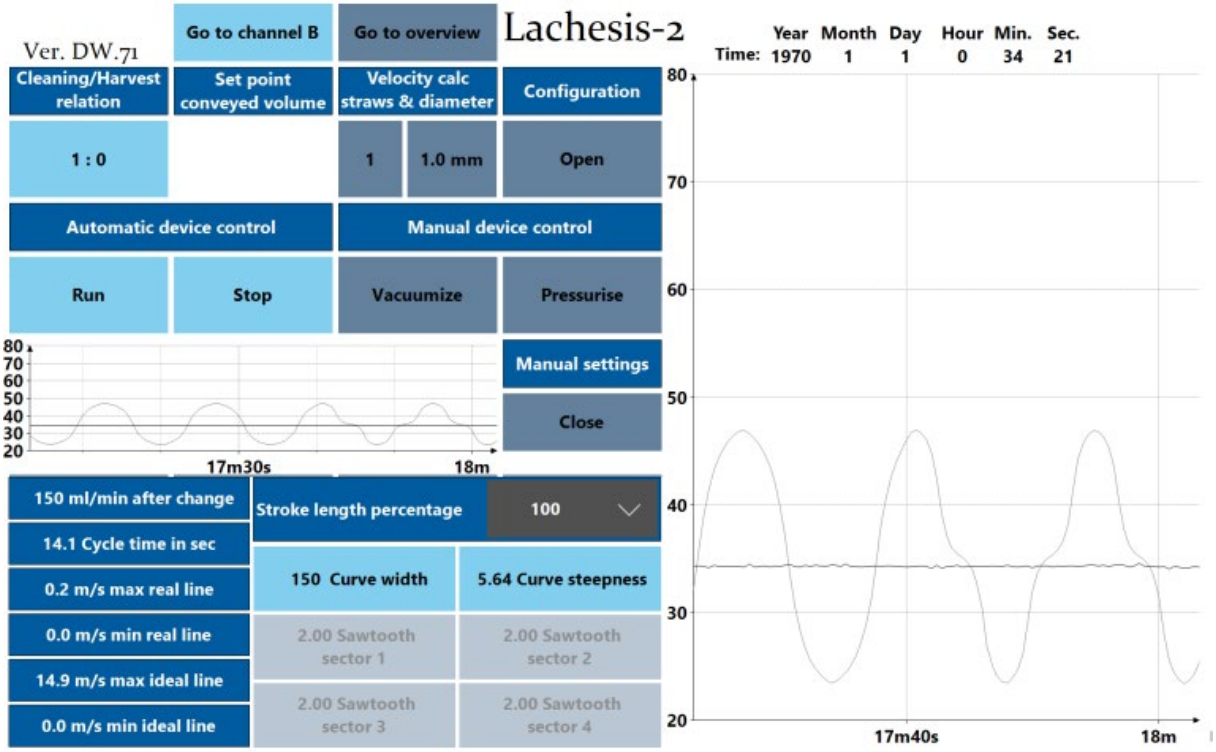


Lachesis screen with adjustment panel open, configuration panel closed, manual setting picked and sinus curve running

Snake curve do have the same change options as the sinus curve.



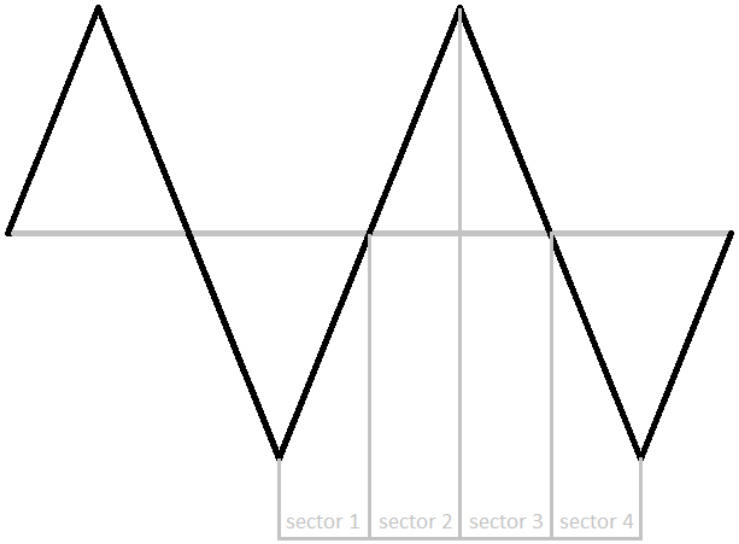
Snake curve



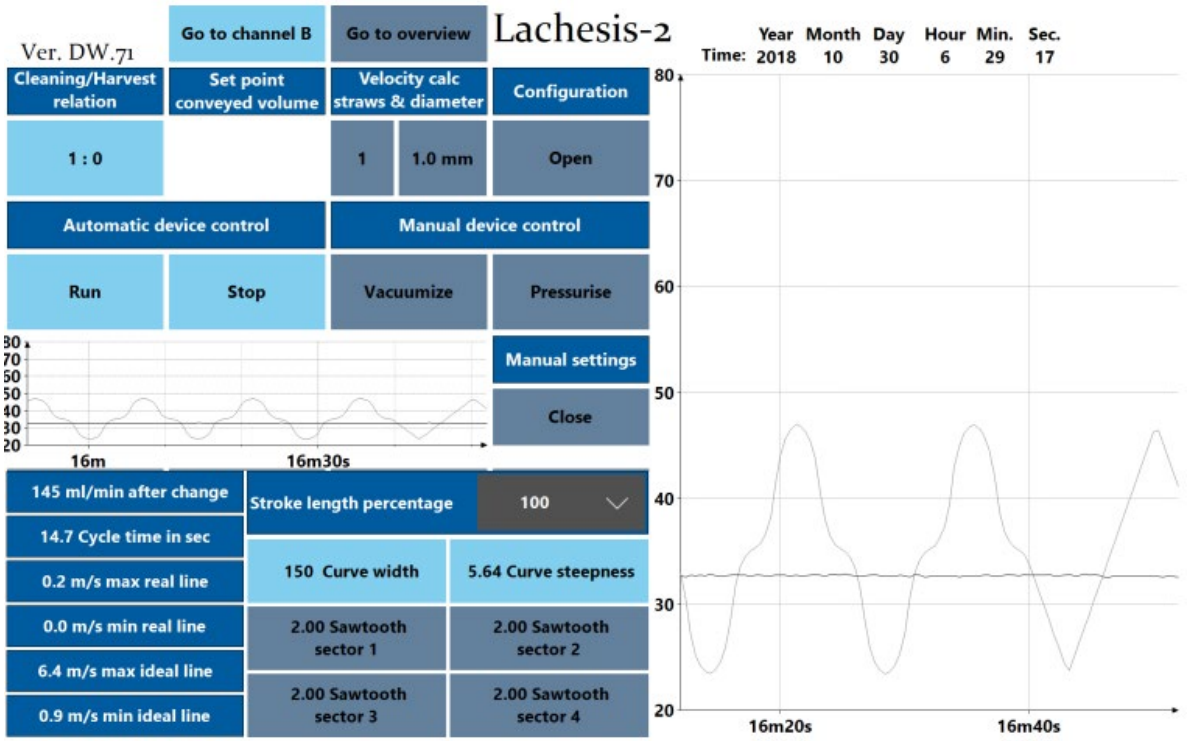
Lachesis screen with adjustment panel open, configuration panel closed, manual setting picked and snake curve running

Sawtooth_4 curve is basically the same structure as the normal sawtooth that is used as default setting, the only difference is that this curve can have 4 different steepness in sector 1, 2, 3 and 4.

For each sector it is possible to set the value between 1,0 and 6,0 where 2 is default, where the higher value you choose the faster/steeper the sector will be.



Sawtooth_4 curve



Lachesis screen with adjustment panel open, configuration panel closed, manual setting picked and sawtooth_4 curve running

3.6.2 - Stroke length 50 to 100 %

Stroke length is available for every setting you have chosen and can be from 50% to 100% in 10 % intervals so 50%, 60%, 70%, 80%, 90% and 100%.

This will start at 100 % and can be changed to the wished stroke length wanted, this will hake the peak to peak length of the curve smaller of larger according to the wanted output.

Stroke length settings

3.6.3 - Curve with (ml/min setting)

Curve width I basically the same as the ml/min that I used for normal settings, but when the steepness is now also to change the real ml/min is showed on top left corner of the adjustment rectangle, so the higher the number of the Curve width is set, the smaller the actual curve width is(compared to the more ml/min you set the more cycle per min is needed, equals faster cycles/smaller curve widths).

Curve width settings

3.6.4 - Curve steepness

Curve steepness do only influence sinus and snake curve, the default setting is 5,64, but can have the value from 1,00 to 5,99 where the lower value that is picked the faster/steeper is the curve.

Curve steepness settings

3.6.5 - Actual ideal line ml/min calculation after manual change.

The ml/min calculation after change is the one to look for after making a manual change cause it can make

Field that shows the ml per minute after manual changes

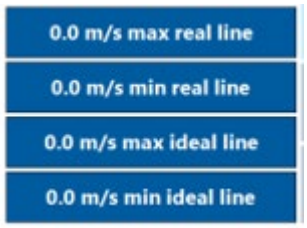
3.6.6 - Cycle time in sec

It shows how many seconds 1 cycle takes.

Field that shows the cycle time in seconds

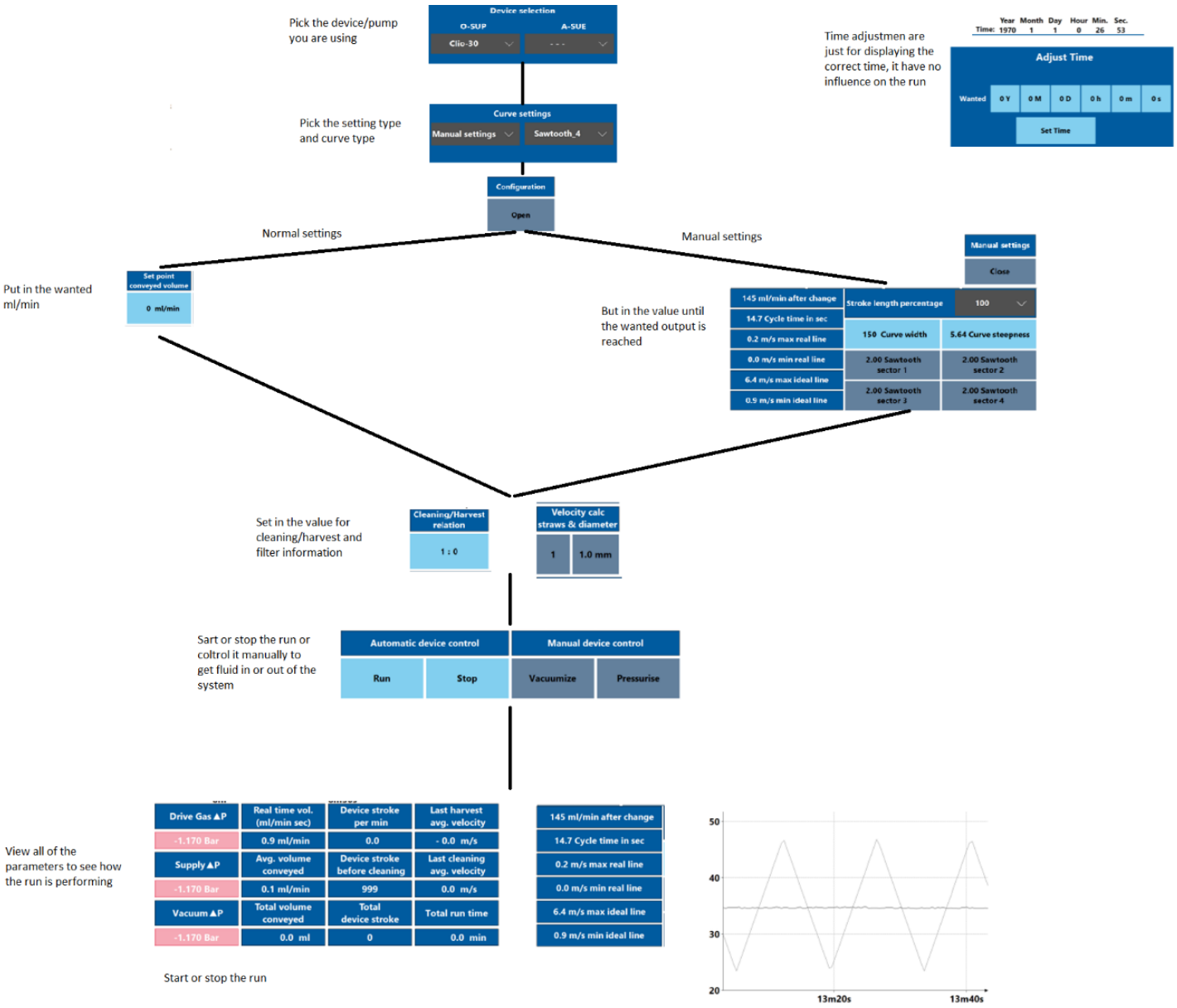
3.6.7 - Minimum and maximum value

Minimum and maximum fluid velocity is showed both in real and ideal values/real and ideal curves.



Fields that shows the speed of the fluid

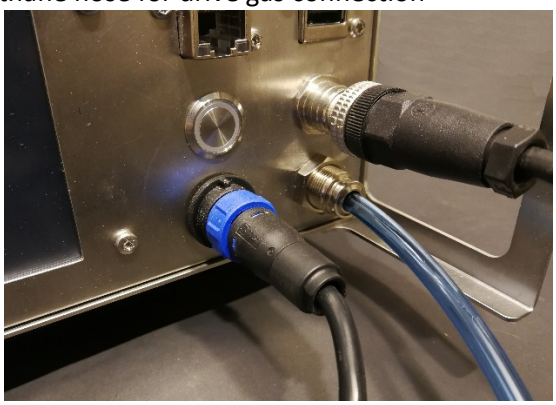
3.6.8 – Step by step run through with advanced software



4. Hardware installation

The scope of the Lachesis product is to drive the Euterpe SUP's. Unpack and disposing the shipping material properly. Check out that you have all the parts shown here.

1. Lachesis(s)
2. Laser sensor(s) – red little box with black cable
3. Power Supply model Zephyros – one per Lachesis
4. NO power input cable to Zephyros – you have tons of these standard cables anyway and with the correct wall plug
5. Transparent blue soft 1,5 meter long polyurethane hose for drive gas connection



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 is via the transparent blue soft polyurethane hose. Laser sensor input via the black M12 cable connector.

Refer to section "2. Device Overview" and be sure you connect vacuum to the left and pressurizes air to the right.

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

1. Connect a pressurised drive gas supply of max 1 Bar (could be Sarpedon / Alagonia) with 6 mm rigid hose.
2. Connect a vacuum source (such as Sarpedon / Alagonia) with 6 mm rigid hose.
3. Connect the 6 mm drive gas hose from the SUP to Lachesis.
4. Mount the level sensor onto the SUP foot.
5. Connect 24 VDC from one of the Eos family of power supplies.

6. Start-up Lachesis 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 Lachesis is ready

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.pumpcell.com/support/safety-instructions

Clock
Lachesis unit need no up-front clock programming and is ready for installation. The unit is pre-programmed with the CET time. If you need different time look under section 3.2

4.1 - Fault information

The build-in display of Lachesis will inform about possible faults whenever detected.

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

5. Communication

Lachesis contain a webserver displaying online information on the build-in display.
Each Lachesis 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 Lachesis unit.

5.1 - Programming

It is of importance ……
……

5.2 - PID routines

Many hours have been invested in setting up the PID routines and the intelligence for auto-tuning. Lachesis will initiate auto-tuning at each start-up for best possible accuracy.

6. Operation Manual

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

- The operating system software (Linux) is loaded
- The system configuration is loaded.
- The operating software is loaded and actual information shown on display

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

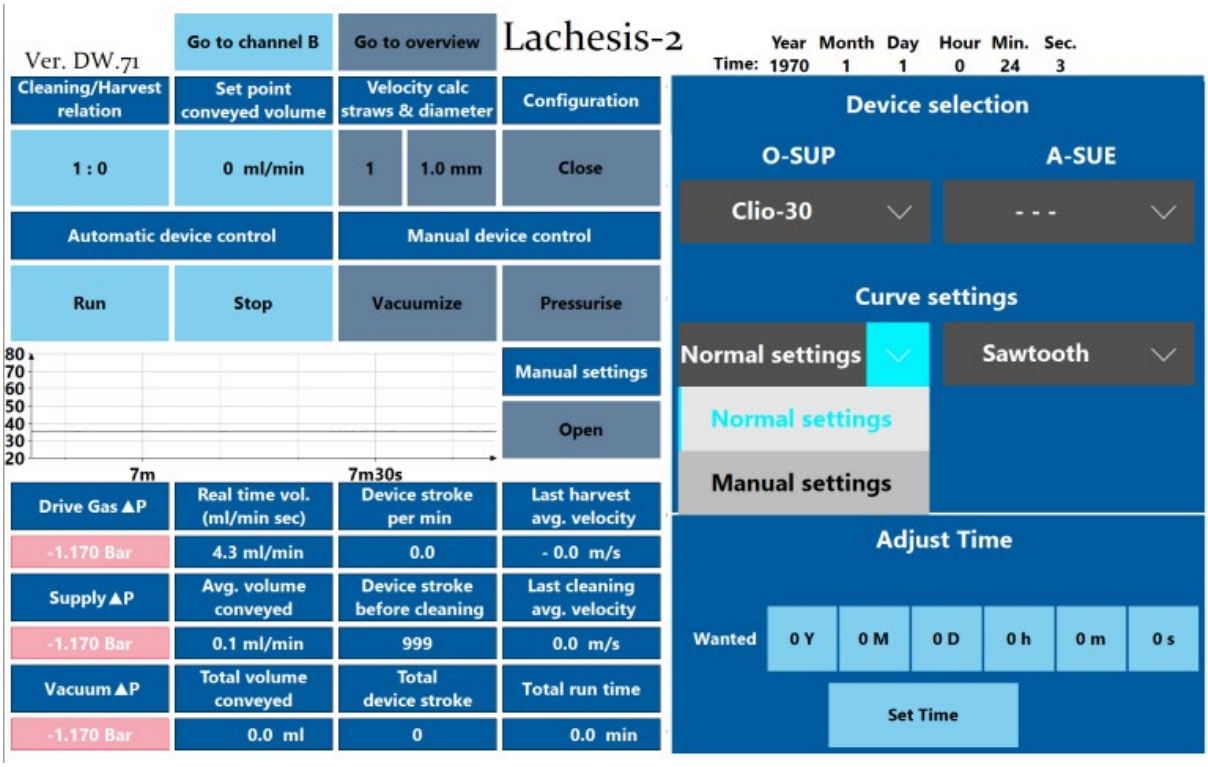


Illustration of the GUI shown on the build-in 5" display.

6.1 - Operation principles

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

6.2 - Wi-Fi connection

When Lachesis is equipped with the NetGear Wi-Fi access point the Apache webhost will be accessible from a browser. Go into "Settings"/ Wi-FI NETWORKS" and check if you can see Lachesis and select. Return to a browser and write anything in the address line for access to Apollon.

6.3 – LAN IP/TCP connection

Note ready yet.

7. Safety precautions

Various component require individual attention. Operator must also have gained familiarity with the Safety Instructions to be found separately on PerfuseCell/Support.

7.1 - Documentation

Lachesis functionality must be checked on a regular basis and data of such testing kept recorded.