Manual of the omnistat

Continuous culture system for experimental evolution

written by

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Components description of the bioreactor system

Overview of the system

The bioreactor system consists of 24 individually digitally controlled and logged bioreactors. The bioreactors are grouped into 6 modules of four bioreactors that sit together in an autoclavable stainless steel frame. The system is operated via a real time Labview application that is run from a cRIO (NI compact RIO) computer. An emergency power supply in combination with the UPS (uninterruptable power supply) system ensures a continuous power supply to the bioreactor when an experiment is running (Fig 1). The pH in the bioreactors is kept constant with additions of base (NaOH) initiated by pH sensor data from the bioreactors. It is also possible to measure the DO (dissolved oxygen). Bioreactors can be connected via tubes to create corridors between the bioreactor to induce migration between bioreactors (patches). Additionally computer controlled pinch valves provide the possibility to vary the medium components that are added to the bioreactors (Fig. 2).

The bioreactors

The bioreactor

The bioreactors primarily consist of a metal head plate and a 100 ml (standard volume), 150 or 250 ml glass reactor vessel (*duran GL45*) (fig. 3). These two components are secured with a hollow screwcap that clamps the glass reactor vessel and the metal head plate with a silicone ring-seal that sits between the two (fig. 4). The head plate has 9 ports (fig. 4): 2 sensor ports (pH, Oxygen or turbation 12 mm Ø), 3 static ports (2x 2 mm Ø, 1x 3 mm Ø) for (chimney, nutrient, nitrogen gas en base additions) and 4 height adjustable ports (efflux, sampling, migration and aeration 2 mm Ø) (fig. 4). The sensors are secured with a clamping nut and a silicon O-ring (fig. 4). If one of the two sensor-ports is not used one can be closed off with a closing nut and a silicone O-ring (fig. 4). The height adjustable ports consist of a stainless steel tube, that is secured into the port with a PTFE ferrule and a stainless steel clamping nut. If a port is not used the port can be closed off by inserting a solid stainless steel closing rod inside the port instead of the stainless steel tube (fig. 4). For aerobic growth a special aeration rod can also be fitted to the bioreactor to maximize oxygen input in the culture (fig. 4). For homogenization of the culture each bioreactor contains a PTFE coated stirring rod (VWR *38 mm 442-0398*).

Module frame

The bioreactors sit in a stainless-steel frame that holds four individual bioreactors in total (6 modules in total). This frame allows the bioreactors to be autoclaved more easily and secures the bioreactors when they are running. The frame consists of a top-plate and a height adjustable bottom-plate which are both supported by four legs. A bioreactor centralizing disk that sits between the reactor vessel and the screwcap allows for the firm and centralized placement of the bioreactors in the top-plate of the frame (fig. 5+6). The top plate also features cutaways that function as bottle holders (8X), tube guides (16X) and tube-holders for calibration of the sensors (10X). If necessary the bottle holders and tube guides can be closed off to prevent bottles or tubing sliding out of the frame by securing the closing rail with two pins on the module frame (Fig. 5). Beneath the bioreactors the stirring magnet

holders are located on the bottom-plate. The bottom-plate is height adjustable with hex-nuts that allow the stirring magnets to be placed at different heights depending on the size of the reactor vessel (100, 150 or 250 ml). A set of tools are available to unscrew nuts for various modifications to the bioreactor system (fig. 7).

Pumps

All the influxes and effluxes of liquids that take place inside the bioreactor are controlled via peristaltic pumps. These pumps can all be controlled either manually or via the real-time Labview application to set their speed and direction. More intricate control of the pumps can be programmed with the add-on software (*Dizanta-Compass*). This will be more extensively discussed in the software section of the manual. Each pump uses plastic cassettes to secure the tubes in the pump. These cassettes keep the correct amount of tension on the peristaltic tubing to maximize pumping accuracy and minimize wear on the tubing. The pumps use special 2-stops (IPCN-24) or 3-stops (Reglo ICC and Reglo Digital) tubing. The stops on the tubing serve as guides that allow the tube to be securely fitted in the cassettes. The tubes necessary for the pumps are made of PharMed. This material is resistant to chemicals, autoclaving and physical wear by the peristaltic pumps. The tubes either come with glued non-autoclavable stoppers (PharMed Ismaprene) or welded autoclavable stoppers (PharMed-BPT).

Ipcn24

The IPCN-24 is a 24 channel peristaltic pump with a planetary drive with 8 actively driven stainless steel rollers for extremely high accuracy with flowrates between 0,0004-11 ml/min (*Ismatec IPCN-24*) (fig. 8 and 9) . The pump can either operated offline by putting the flow settings in by hand, with an analog signal (not operational) or serial communication via RS232. The IPCN-24 usually controlled by the Labview interface via the RS232-port. In this application the speed and direction of the pump can be set. There are 3 ipcn-24 pumps that each operate 2 modules (8 bioreactors). The pump is primarily used for medium influx as well as efflux. To generate the required dual flow directions, the efflux tube is put in reverse orientation compared to the influx tube. Furthermore the efflux tube always has a bigger inside diameter (usually one or two sizes bigger) than the influx tube to prevent the influx to become greater than the efflux. The influx and efflux take up 16 pump-channels (8x influx + 8x efflux) of the 24 available channels of the pump. Up to 8 additional inputs (1 per bioreactor) can be used for things such as anti-foam, antibiotics, sugars, signal molecules etc. For these additional channels, the pumping speed can only be changed in respect to the dilution rate by the chosen ID of the peristaltic tubing.

Ismatec Reglo ICC

The reglo ICC is a 8 roller 4 channel digitally controlled peristaltic pump with independent control of all four channels (*Ismatec REGLO ICC MS-4/8*) (fig. 10 and 11). In the usual setup this pump dynamically adds NaOH solution into each bioreactor to keep the culture pH constant. The pump is connected via a USB to the cRIO and controlled by the Labview application through a PID (proportional-integral-derivative) loop. This loop proportionally activates the pump channels based

on the deviation of the measured pH-value in the bioreactor compared to the set-point value. The three parameters (proportion, integral and derivative) can be adjusted in Labview to optimize the pH control. Each module has one 4-channel pump making a total of 6 pumps for the entire system.

Ismatec Reglo digital

The reglo digital is a 12 roller 4 channel digitally controlled (RS232) peristaltic pump (*Ismatec REGLO Digital MS-4/12*) (fig. 12). It functions as the migration pump continuously transfers culture back and forward between bioreactors at a low rate to create a corridor. To do this the pump alternates the pump direction in time. At what interval the direction is switched and what speed the pump rotates can be programmed with Dizanta Compass. The pump can also be used for dynamical additions of chemicals to the bioreactor such as antifoam, nutrient components, stressors(eg antibiotics,) or signal molecules.

Masterflex Easy Load L/S

The Masterflex Easy Load L/S is an offline pump that can be manually set for direction and speed. The pump is used to filter-sterilize media by transferring is through a filter-unit into a sterile bottle. There are two pumps in the bioreactor lab to allow to make two bottles of media in parallel. These pumps do not require any tubing-cassettes or stoppers on the tubing, instead tubing can be directly fed into the pump.

Ismatec MS-CA 4/840

Ismatec MS-CA 4/840 Is an offline pump with four channels, 8 rollers that has one pump rate in which only the directional of rotation that can be controlled. The pump can be used in emergency situation where additional pumping is temporally needed. The pump works with the same cassettes and 3-stops tubing as the Reglo pumps.

Bottles

All bottles used for the bioreactor system use four-port GL45 screwcaps. Using these caps allows for great flexibility in setting up any experiment. These caps can be screwed on 100 ml up to 20 L bottles. The caps consist of a screwcap with four extruding channels with external thread, four plastic nuts with internal, four silicone ring-seals which sit between screwcap channel and the screw nut to create an air tight seal between the tubing and the bottle. Three stainless steel nuts are secured to the tubing that goes into the bottle to keep it at the bottom. The four ports on the screwcaps are usually used for 3x input or output, 1X air-filter (to either release pressure or vacuum that is built up into the bottle). The bioreactor system uses 20 L medium and waste medium bottles (Fig. 14 and 15). For the medium, brown glass bottles are used to reduce photic reactions in the medium, while the transparent bottles are used for waste medium and storing autoclaved water. The tubing can be set up in a way that one bottle of medium or waste medium supplies either one or a multitude of bioreactors. For other additions such as acid, base or antifoam smaller bottles (≤2L) are used.

Tubing

The tubing that is used for the bioreactor systems can be divided into two types. Firstly PharMed peristaltic tubing which is used in the peristaltic pumps. This beige tubing is made from a material that allows for prolonged accurate pumping and is very temperature and chemically resistant. Secondly silicone transparent transfer-tubing, this type of tubing is used for all other connections made in the bioreactor system.

Influx

The influx tubing starts with a quick-connector that attaches to the medium bottle. From here silicone tubing (ID 2 mm) is attached connecting to the peristaltic tubing (ID 1.30 or 1.02 mm PharMed BPT), the diameter of the peristatic tubing might vary based on the desired dilution rate of the bioreactor. The other side of the peristaltic tubing is connected to another section of 2 mm silicone tubing that connects to the top end of the liquid-entry-system. The side-input of the liquid-entry-system is connected with a 6 mm silicone tubing to a gas filter which has another piece of 6 mm silicone tubing ending in a luyer-fitting that will connect to one of the output tubes from the N_2-gasflowmeter. This gas-input functions both as anaerobic ventilation of the bioreactor headspace as well as forcing down the droplets of medium through the liquid-entry-system. The liquid entry-system is a glass drip-feed system for the medium. It has a top-input where the media is fed in, a side-input which connected to a N_2-gas output that pressure forces the media down into the bioreactor via the bottom-output into the medium-port on the bioreactors. The primary function of drip-feed system is to eliminate the chance of the culture to grow back upstream into the medium input when running the bioreactor. This is achieved by dripping in the medium instead of using an uninterrupted medium feed. The gas-input additionally makes sure that the droplets that fall down the drip-feed are pushed into the bioreactor directly. This is important because otherwise medium will accumulate in the bottom of the liquid-entry-system until the weight of the medium overcomes the capillary force within the bottom output, causing a sudden flush of medium in down into the culture causing a more irregular feed into the bioreactor.

Efflux and sampling

The efflux tubing starts from the efflux-port on the bioreactor with a short 1 mm silicone tubing that splits up into two tubes, one 1 mm tube ending with a female luyer-fitting from where sampling can be done. The other tube changes to a 2 mm silicone tubing that is subsequently connected to a peristaltic tubing (ID 1,52 mm PharMed BPT) that goes into the ipcn-24 pump. This tube is then connected to another piece 2mm silicone tubing that ends in a quick-connect that attaches to the waste medium bottle.

pH control

This tubing starts with a connector that attaches to a connector on the base/acid bottle. From this connector 1 mm silicone tubing is used that splits up into four 1 mm silicone tubes. Each of the four tubes is connected to a peristaltic tube (ID 0.89 mm PharMed BPT) which goes into the pH-pump (Reglo ICC). The peristaltic tubing is then again connected to 1 mm silicone tubing that goes all the

way to the pH-port on the bioreactor. Great care should be taken when connecting the connector to the base/acid bottle after autoclaving. Clean both connector ends with ethanol and purge the base/acid bottle to remove all the possible air in the tubing. Then attach the tubes sterilely at the flame.

Chimney

The chimney is attached to chimney-port on the bioreactor with a 2 mm silicone tubing , this is then converted to a 6 mm tube. This 6 mm tube is filled with a twisted piece of aluminum foil which prevents condensation water in the chimney to be pushed up into the gas filter. From this tube the chimney spits up into two outputs each with a gas filter attached. The one of the two filters serves as a backup filter in case one of the two filters fails.

Aeration

The aeration tubing starts with luyer-fitting that is attached to one of the gas outputs of the air flowmeter. From there a 6 mm tube is attached to a gas filter, from the gas filter a 6 mm silicone tube is converted to a 1 mm silicone tube that attaches to aeration-port on the bioreactor.

Migration

The migration tubing consists of a 1 mm tube attached to the migration-port on one bioreactor. This tube is then attached to a peristaltic tube (ID 0.89 mm PharMed BPT) which connects to another piece of 1 mm silicone tubing that attaches to the migration-port on the other bioreactor.

Antifoam tubing

The antifoam tubing or tubing meant to add any other type of liquid to the bioreactor, starts with a luyer-fitting that attaches to the output of the antifoam bottle from there a peristaltic tube (ID 0.89 mm PharMed BPT) is attached. This is then connected again to a 1 mm silicone tubing that attaches to the antifoam-port on the bioreactor.

Filter sterilization tubing

This piece of tubing is used to filter-sterilize medium. It does so by submerging one end of the tubing in a bottle of freshly prepared unsterilized medium and pumping (Masterflex easy load) this medium through a filter into a sterile medium bottle. The tubing starts with a long piece of 6 mm silicone tubing that is weight down on the upstream end by two stainless steel bolts that are slipped on the tubing. On the downstream side, the tubing is attached to the input side of a medium filter(). From the output of the medium filter another piece of 6 mm silicone tubing is attached that converts to a 3 mm silicone tubing that attached to the input port on the medium bottle.

PH-sensor

To measure the pH in the bioreactors during experiments each bioreactor can be equipped with a pH sensor which is connected to a controller. For each bioreactor module (four bioreactors) one controller is used (Fig. 15 and 16). The controller receives the signals from each pH-sensor which is then displayed real-time on the screen of the controller and subsequently send using 0-20 mA signal to the bioreactor computer. This pH value is then logged and used to control the pH pump via a PID-loop.

The pH-sensor is a solid gel pressurized sensor with a silver/silver chloride reference suitable autoclaving (*Applisens pH sensor for mini bioreactor 8 mm 15 cm*) (Fig. 17). The sensor typically withstands around 20 autoclaving cycles before it should be replaced. After autoclaving and calibration a sensor will remain accurate for up to 3-4 weeks if it cannot be recalibrated during its use.

O_2-sensor

To measure the DO (dissolved oxygen) in the bioreactors during experiments each bioreactor can be equipped with a DO-sensor which is connected to a controller (Fig. 18). The controller receives the signal from the DO-sensor which is then displayed real-time on the screen as either a percentage or amount (mg/l). The sensor signal is subsequently send using 0-20 mA signal to the bioreactor computer. This value is then logged and can also be used for controlling other systems such as pinch valves to change gas mixtures.

The DO sensors used for the bioreactors are polarographic (*Applisens 8 mm 15 cm polarographic DO_2 sensor for mini bioreactor*) (Fig. 19). The sensors measure partial pressure of dissolved oxygen in liquid. The sensor is equipped with a gas-permeable PTFE membrane that allows oxygen to pass to be measured by the sensor. The sensor operates by the Clark-cell principle: a fixed polarization voltage of 675mV across the sensors anode/cathode reduces O_2 molecules. This reduction results in a current that is linearly dependent on the amount of reduced O_2 molecules. The sensor has a protective cap covering the membrane to avoid mechanical damage and to limit evaporation of electrolyte.

Air and N_2 input

The air and N_2 input system consists of a (1) N_2-supply and an air-supply with both a reduction valve with a bar meter(Fig. 21 and 23), (2) distribution tubing from the reduction valve (Fig. 20) and (3) 4-channel flowmeters with a main open/close valve and accurately adjustable needle valves for each specific channel to regulate gas input into the bioreactors (Fig. 22 and 24). The range for the air-flowmeters is 0-100 ml/min and for the N_2-flowmeters 0-25 ml/min.

Stirrers

The bioreactor system uses magnetic submersible stirrers (*Cimarec micro*) that are placed inside of the module frame stirrer holders (fig. 26) after autoclaving the bioreactors. The stirrers are then

submerged into the water bath together with the bioreactors. The magnetic stirrers propel stirring rods inside the bioreactors to agitate and homogenize the culture. The magnets are offline and operated per four stirrers via a hand operated tuning-knob located above the bioreactor (fig 25).

Waterbaths

The bioreactor are being kept at a constant temperature by submerging the bioreactor module frame into a temperature controlled waterbath (*Julabo MB*) (Fig. 27). The waterbaths are hooked up to the bioreactor computer via a RS232 connection and can be either controlled via the bioreactor or manually.

Pinch valve system

The pinch valve system is designed to be able to change liquid or gas input or output in time within the bioreactors. This is achieved by an array of computer controlled pinch valves that can open or close tube channels connected to the bioreactor. Examples for the use of such a system can be varying aerobic versus anaerobic conditions in time by changing gas input in time. Adding stress molecules to the bioreactor to create evolutionary bottlenecks in time. Changing media components during a run to induce metabolic switching. Because the pinch valve system is hooked up to the labview application pinch valve activation can also be made dependent on the output parameters of the bacterial culture such as acidification rate or turbidity. The pinch valve system consists of three switch boxes (one for every two modules) each containing an array of 8 double channel pinch valves (*Sirai 3/2 NC-NO solenoid pinch valve*) (Fig. 30). The pinch valves on the switch boxes can be controlled manually or from the cRIO. From the cRIO the pinch valves can either be operated directly from labview application or indirectly via the add-on software Dizanta Compass. The latter one allows the pinch valves to programmed with more complex logic behind them. The control signals from the cRIO are send from an i/o card (Scheme 1) that it translates into 0-5 V TTL signals that go to three control boxes located on the wiring guide above each two bioreactor modules (Fig. 28 and 29). The signals are then amplified and transmitted to the pinch valves. In an ‘deactivated state’ a spring in each pinch valve always keeps one of the channels closed while the other channel is open. When the valve is activated, the coil inside the valve opens up the formerly closed channel and at the same time closes off the other formerly open channel.

Computer system

NI CRIO

The operating system of the bioreactor is controlled by a real time target, a cRIO (*National instruments compact RIO-9067, 8 slot integrated dual-core controller and artix-7 FPGA*) computer that is mounted via a DIN-rail to the wall. The computer is powered by a 24V DC output converter (*NI PS-15 power supply*) that is connected to the standard 220V AC (Fig. 31 and 32). Sensor data from the pH and DO (dissolved oxygen) sensors is converted by the sensor controllers into a standard 0-20 mA signal. These signals enter the computer via two current input modules (*NI 9208 24-bit current input module with D-sub*). Pinch valves are operated from the computer via TTL digital output module (*NI*

9403 with D-sub 32 channel TTL digital input/output module). The pumps controlling the pH (*REGLO ICC*) inside the bioreactors are controlled via a serial signal send from the USB port from the cRIO that is branched into 6 channels via a USB hub located on the wiring guide above the first bioreactor module (Fig. 33). The migration pumps(*REGLO digital*), flux pump (*IPCN-24*) and the water baths are connected to the computer via three serial RS232 modules with 4 channels each (*NI 9870 4-port RS232 serial module W/4 10P10C-DE9*). For an overview of all connections see the wiring scheme (Scheme 1).

PC

The cRIO is linked to a PC. During the running of the bioreactor system the software is always operated by the cRIO which holds a database with the most recent data. The cRIO updates the PC four times every second with the latest data by messages. The PC updates the tags in the database to the controls available on the GUI (graphical user interface). The cRIO data that is logged is transferred to the desktop computer (D:ProControl/data/).

Security

The whole computer system is connected to an emergency power supply that will kick in a matter of 1-10 sec when there is a power failure. Furthermore all the other systems except for water bath 4, 5 and 6 are connected to and uninterruptible power supply (UPS) that can buffer a power dip of up to 40 sec long. If for some reason the desktop computer is turned off or cashes while the bioreactor system is operating the cRIO automatically logs the data on to an external hard disk that is connected to the usb port via a hub (Fig. 33). When the desktop computer is operational again the data will be saved on the computer again.

Software

The bioreactor system uses a Labview based GUI (graphical user interface). The first tab of this interface allows the user to set the input values of the pumps (activity, speed and direction), waterbaths(temperature), pH (set point and PID) and pinch valves (Fig. 34 and 35). The second tab of the GUI plots all logged parameters such as, pH, DO, temperature, pinch valve activity, pump speed, pump directionality and turbidity (not operational) per module or bioreactor (Fig. 36). This allows for monitoring of the bioreactors during running of an experiment as well as adjustment of its parameters. Although the Labview GUI allows easy reliable bioreactor use it does not allow the user to easily program more complex logic between or on top of the input and output parameters.

Software framework

The software framework is a database subscription framework. The compact RIO (cRIO) from National Instruments (NI), holds the database with the most recent data. The cRIO updates the PC with the latest data four times per second by messages. The PC updates the tags in the database to the controls available on the graphical user interface (GUI). The tag configuration sets which tags are available in the system for data acquisition and data generation. Tags are clusters, which hold information about the connection type, ranges, alarm ranges, scaling etcetera.

To minimize system failure the compact RIO runs independently from the PC application. Therefore the cRIO application is running while powered. When a working LAN connection has been established, data will be transferred and the GUI will be updated. By default the received databases are stored in the format TDMS (Technical Data Management Streaming) on the host PC. The PC application can run and function while the cRIO is not connected or turned off, though updates are delayed until the cRIO is reconnected or switched on.

The GUI shows the latest version of the database to the user. There are indicators to visualize data and controls which both visualizes the data and can change values. The controls (and indicators) can be of the type Boolean which allows for switching between TRUE and FALSE, which represents for example an ON and OFF state or OPEN and CLOSE state. Each control represents a tag from the database and the value is updated on the GUI. The tag contains properties described in the tag configuration. This can e.g. limit a control to a range of input values. In order to access the other tag properties, the context menu shows the information stored. By selecting “View parameters Tag”, the popup windows allows for visualization of the tag properties and editing of writable tag properties. The same holds true for editing and viewing the properties of the PID settings. When editing, pressing ‘Cancel’ will revert the changes and ‘Ok’ will store the new parameters.

PID loops

Any set of parameters can be turned into a PID loop. A PID loop has a setpoint (SP), process variable (PV), output parameter (OP), control switch (CTR), output rate, output range and a default value when switched off. The P stands for proportional gain, I stands for the integral time in minutes and the D stands for the derivative time in minutes. The concept is that the system changes the output action in order to minimize the error between the set point and the process variable. The error at time point t, e(t), is calculated from

$$e(t)=SP(t)-PV(t) \qquad \text{eq. 1}$$

The controller action at timepoint t is as following

$$u(t)=K_c\,(u_p\,(t)+u_i\,(t)+u_d\,(t)) \qquad \text{eq. 2}$$

where Kc stands for the controller gain (P). The proportional action up is calculated by

$$u_p\,(t)=e(t) \qquad \text{eq. 4}$$

The integral action is calculated by

$$u_i(t) = 1/T_i \int_0^t e\,dt \qquad \text{eq. 4}$$

where Ti stands for the integral time (I) in minutes. The derivative action is calculated by

$$u_d(t) = T_d \, de/dt \qquad \text{eq. 5}$$

where Td stands for the derivative time (D) in minutes.

Configuration

The configuration tool allows the administrator to modify the default tag values and default PID settings. For the tags:

- Tag name: tag name
- Units: units of tag
- IO Type: type of connection
- Access: read only (R), write only (W), read and write (RW)
- Alarm LL, L, H, HH: alarms values for low low, low, high and high high alarms
- Auto ack: auto acknowledgeable
- Raw: range of raw values
- Eng: scaling of raw values to engineering values
- Connection: connection address
- Description: extra information for user or device
- Design: hard limits for range of setpoints
- Default: start up value

For the PID settings:

- P: proportional gain
- I: integral time in minutes

D: derivative time in minutes

Output rate: rate for the output in engineering units per minute

Range output: range of the output

Default value: default value when switched off

Setpoint: setpoint

Process variable: process variable tag name

Output parameter: output parameter tag name

Control: control tag name

Dizanta Compass

For more complex programming the add-on software Dizanta Compass is used. Dizanta Compass allows you to take any of the input or output parameters from the bioreactor system and program them into a logic scheme that produces input values for the GUI of bioreactor system (Fig. 37). When the program is run, it simply acts as a virtual user changing input values in the labview GUI according to what is programmed in Dizanta. This software allows for a very flexible and variable use of the bioreactor system. An example of logic on top of the parameters could be a preprogrammed temporal variation of chemical additions to the bioreactor, by preprogramming the pinch valves and pumps to add certain type and amount of chemicals through time to the bioreactor. An example of logic between parameters could be making the dilution rate of the bioreactor dependent on the growth speed of the culture, by making the bioreactor flux-rate dependent on acidification rate or turbidity of the culture.

Protocols

Making vitamin and metal 1000x stock solutions for CDMPC

1. Start by adding 50% of the end volume of demi water to a flask and adding a clean stirring rod.
2. Next weight and add all vitamins or metals to the solution while it is stirring. See scheme 2 for all components.
3. Then add water until the 100% volume is reached.
4. For the vitamin solution the pH must be adjusted in order to dissolve all vitamins. Add NaOH solution until a pH of 7 is reached. Now the components will dissolve in a matter of minutes. As the vitamin solution dissolves it will acidify the solution, so the pH must be adjusted several times until all vitamins are dissolved.
5. Then the 1000x vitamin or metal solution is filter sterilized by passing it through a 0,2 µm filter. Aliquot the solution into 20 ml portions for making 20 L medium at once and aliquot smaller volumes for making smaller amount of medium. The aliquots stock solution should then be frozen at -20 C. The stock solutions should only be defrosted once when it is used!

Making filter-sterilized media

1. To make for a filter-sterilized medium such as CDMPC (chemically defined medium for prolonged cultivation) you start with a 20 L medium bottle and a 20 L transparent medium-preparation bottle.
2. The medium bottle should have already the filter-sterilization tubing attached to the input channel before autoclaving. This tubing is roiled up into a packed of aluminum foil and taped against the side of the medium bottle to make it easy to autoclave the whole thing. This setup makes sure that the tubing downstream of the medium filter is completely sterile without risk of contamination because there is no need for connecting any tubes. Make sure that the medium bottle is height marked at the 20 L mark with a piece of tape.
3. The medium-preparation bottle should be filled up with ±18 L of demi water and a big stirring rod should be added to the bottle. The water is autoclaved in one of the big autoclaves on the fourth floor.
4. Now weight the medium components (see scheme for CDMPC recipe). Add them to the medium-preparation bottle with 18 L water while the liquid is stirred with a stirring rod. The components dissolve fasted when the liquid is stirred at the highest possible speed.
5. In the case of making CDMPC the amino acids should be added first, wait until everything is dissolved, this can take up to 10-45 min depending on the temperature of the water.
6. Next you add the buffers and carbon sources, again wait until everything is dissolved.
7. The vitamins and metals can be added from a 1000X or 500x frozen stock solution.
8. When all components are dissolved in the medium, the pH should be adjusted (pH 6.5 in the case of CDMPC). To do so the pH sensor is hanged into the medium while stirring at a low speed (make sure to regularly calibrate the sensor!). Then NaOH or HCl solution is added until the pH is stably set at the desired value.

9. Now the media is ready to be sterilized. The medium-preparation bottle is put on the bench next to the Masterflex pump. The empty and sterile medium bottle is put on the ground below the pump.
10. Now the packet containing the filter-sterilization tubing is opened and the upstream part of the tubing is lowered into the medium preparation bottle. Use a piece of tape to stick the auto-going tubing to the side of the medium-preparation bottle, this will prevent the tube from falling out of the bottle during pumping. From the medium preparation bottle the tubing is led through the Masterflex pump and clamed into the rollers. Make sure that the medium filter is always hanging in an upright position under the Masterflex pump. This is important to effectively purge any trapped air from the filter via the degassing valve.
11. Before activating the pump check if the tubing is set up correctly, check for any places where the tubing might be folded. Then open up the degassing valve on the medium filter and activate the pump.
12. During the first minute of pumping make sure that all the air is purged from the filter. Air tends to get stuck in between the lamella of the filter, firmly tap the filter to release these air bubbles.
13. When almost all of the ±18 L of medium is pumped into the medium bottle more water should be added. Make sure to stop the pump before air is sucked into the tubing. Then add 1L of demiwater and swirl the water around the bottle to make sure a much of the medium components are added to the medium. After the 1 L is pumped through add another 1 L of demi water until the 20 L mark on the medium bottle is reached. Now clamp off the 3 mm silicone tubing, that is attached to the medium bottle input with two clamps. Cut the tubing just upstream of the two clamps to de-attach the medium filter from the medium bottle. Write down the preparation date on a tape on the medium bottle. The medium bottle is now ready to use. CDMPC stays good for at least a month.
14. Make sure to immediately clean the medium preparation bottle and the filter-sterilization tubing with NaOH solution and demiwater directly after use to prevent any kind of microbial growth!!! After cleaning, autoclave a new batch of water in the medium-preparation bottles. These can then be stored ready to be used for preparing the next batch of media. The filter-sterilization tubing should be straight away fitted with a new medium filter, repackaged into aluminum foil and autoclaved with a cleaned medium bottle. If this is not possible the pieces of tubing should be hanged to dry.

Building up and running the bioreactor

Handling and calibrating pH sensors

The sensors should be calibrated before autoclaving the bioreactors in buffer solutions of pH-7 (green) and pH-4 (red) .

1. The tubes with calibration solution should be placed inside the bioreactor frame so that they are submerged into the waterbath. Make sure the waterbath has the same temperature as the running temperature of the experiment (normally 30 °C).
2. Remove the pH sensor cap and clean the sensor tip with demi water (always handle the sensors with gloves!).

3. Put the sensor inside of the red 4 pH buffer solution.
4. Wait for 15 min for the buffer solution and sensor to attain the same temperature as the waterbath. Then rinse the sensor with demi water, make sure you keep the sensor in the upright position to prevent movement of sensor gel upward in the sensor and bubbles from forming inside the sensor tip.
5. Enter the menu of the sensor controller by pressing the menu key from the main measurement screen and select 'calibration'.
6. Select the pH channel that you want to calibrate and press enter.
7. Enter again and check if the pH value of the sensor is stable and adjust the value to the pH of the buffer solution (ph4 for the red solution) if needed.
8. Then transfer the sensor to the second buffer solution, make sure to first rinse the sensor with demi water before putting it in the second solution. Again press enter when the signal is stable and adjust the value to the buffer value (pH 7 for the green solution).
9. Slope and offset value are now displayed and saved in the calibration log.

A calibrated sensor remains usable for 3-4 weeks depending on the required accuracy. After use in an experiment the sensor is again autoclaved with the bioreactor and subsequently removed from the sensor ports and rinsed thoroughly and stored in sensor solution until the next use. Sensors can be autoclaved around 20 times before they tend to fail. Sensor failure will we indicated by the failure to calibrate.

Handling and calibrating oxygen sensors

The sensor can be best calibrated after sterilization because the autoclaving can alter the sensitivity of the sensor. After sterilization the sensor should at least be polarized for 6 hours before calibration. The DO sensors are mounted inside the bioreactor with a sensor clamping nut.

1. Remove the O2 sensor cap and clean the sensor tip with demi water (always handle the sensor with gloves!).
2. Visually inspect the sensor 's PTFE membrane. I should be undamaged and clean.
3. Carefully slide the sensor through the sensor port of the bioreactor. Make sure that the clamping nut is sufficiently loose but still attached to the bioreactor cap and make sure that there is a undamaged silicone O-ring the sensor port.
4. Gently lower the sensor in it correct position.
5. Clamp the nut tight with a nr. 11 wrench until the sensor is secure, don't tighten to strongly!
6. Make sure that the protective cap on the top is properly screwed on to the sensor before autoclaving.
7. When the autoclaved bioreactor is back into the waterbath remove the protective cap and connect the sensor cable to the sensor, store the protective in the designated container. Now the sensor should be polarized for at least 6 hours.
8. After six hours of polarization calibration should be done inside the sterile demiwater in the bioreactor. Make sure that the bioreactor is already stabilized at process conditions (stirring speed, temperature and gas flow).
9. Calibration can either be done via a one point (100%) or two points (100% and 0%). If you do not need very high accuracy, for instance when you grow aerobic, a one point calibration will

595 do fine. However when you grow anaerobically or semi-anaerobically it is a good idea to do a
596 two-point calibration. For the two point calibration check the manual of the DO control box.
597 10. One-point calibration: saturate the bioreactor at process conditions for approx. 30-40 min
598 with air (100 ml/min). When a stable DO signal is reached perform the 100% calibration on
599 the DO controller by pressing the '**sens**' button for 3 seconds. Now the message '**sens. Cal'**
600 appears followed by the actual value. Now changes the value of the sample concentration to
601 100% with the '**sens**' and '**set1**' button. Confirm and end the calibration by pressing the '**set2**
602 **ent**' button. If everything has gone alright the message '**update**' will appear.

603

604 ## Handling the waterbath

605 For a good operation of the waterbaths they should be filled with 7 L of demi water(always use
606 demi-water to prevent formation of calciumcarbonate!). Then disinfectant (*aqua resist*) should be
607 added (1.5 ml/L) to prevent microbial growth inside of the waterbath. This antimicrobial solution
608 contains a blue indicator colorant. New antimicrobial solution must be added to the waterbath water
609 whenever the blue color is almost invisible in the water. Make sure to wear gloves when handling the
610 antimicrobial solution or the waterbath water, since the solution is toxic! Because the heaters of the
611 waterbath use a lot of power, care should be taken not to overload the fuse box. When running more
612 than 3 modules make sure not to activate all the waterbaths at once, but instead turn them on three
613 or less at a time, wait for them to reach their target temperature and turn on the remaining
614 waterbaths. When running the system the waterbath need to be topped up with water every other
615 day to prevent the water level to go below minimum. However it is advised to top up the waterbaths
616 every day as good practice. This will prevent problems if one day you happen to forget to top up the
617 water.

618

619 ## Setting up gas flowmeters

620 The gas systems are fed from the air-supply and N_2-supply (5-6 bar) that can be opened or closed
621 with a valve located next to the door of the lab. These valves should normally always be open. From
622 here the gas can be tapped off from the reduction valves located underneath the shelf midway on
623 the bioreactor lab table (Fig. 21 and 23). The gas pressure should be set at 1 bar for a proper
624 operation of the flowmeters. This can be achieved by first opening all open/close valves of the
625 flowmeters that you intent to use during the experiment. Next you gently open the general gas valve
626 located on the side of the reduction valve (Fig. 21 and 23). If you see that the pressure meter already
627 goes up beyond 2 bar while opening the valve, you have to twist the big nob to reduce the pressure
628 because too much pressure can damage the flowmeters. You can now read the current pressure that
629 comes out of the reduction valve into the system. Then adjust the barrage of the output to 1 bar by
630 gently twisting the big nob until pressure meter shows exactly one bar. Finally the amount of added
631 gas can then be adjusted on the channel valves on the flowmeter (Fig. 22 and 24).

632

633 ## Setting up the switch system

634 The pinch valves can be operated manually or via the computer. Each pinch valve has its own
635 tumbler switch that can be put is three positions: the 'middle position' means that the pinch valves

are operated via the bioreactor software, switch in the 'left position' is a manual inactivation of the pinch valve(cannot be overwritten by the computer) and switch in the 'right position' will manually energize the pinch valve. Additionally an orange indicator light is located next to the switch indicating whether the valve is energized. To use the pinch valves the control boxes should be turned on by pushing the green switch on the box (Fig. 28). Make sure that all the switches on the switchbox are put into the 'middle' position (software controlled). The tumbler switches on the switch box have a safety lock to prevented unwanted movement of the switches when running. To move the tumbler switch the nob should first be lifted up to unlock. When a pinch valve is activated (manually or software) it first receives a high energetic pulse (activation current) to make the switch movement, after this initial switch a lower current (hold current) will keep running through the activated switch to keep the valves in its position for as long as the switch is on. This feature will prevent the pinch valves to overheat when they are activated for long periods of time. The amount of 'hold current' that is run through the pinch valve can be set with a tuning knob on the control box (Fig. 28). Each pinch valve has two channels: a passively open channel that is closed when the pinch valve is activated and a passively closed channel that is open when the pinch valve activated. Whenever there is asymmetry in the switch regime you want to induce, always put the more frequently used input in the passively open channel to minimize activation time and thus overheating of the pinch valves. For example, if you would add solution-A for 30 minutes and solution-B for 5 minutes, the input of solution-A should be in the passively open channel of the pinch valve and the input of solution-B should be in the passively closed channel of the pinch valve.

A special type of silicone tubing that has relatively soft walls should be used for the pinch valves: HelixMark silicone platina treated tubing with a 1,98 ID and 3,18 mm OD with a Shore-strength of 50 A (VWR 228-1067) or a tubing with similar properties. Never use a type of tubing in the pinch valves which does not have these specifications, because the tube might not close completely or the pinch valves might be damaged! Since this type of tubing is not very rigid it is not recommended to use more than a little piece in the place where the tubing runs through the pinch valves. Using this tubing for longer parts of the tubing might run the risk of tubing folding in on itself and blocking the flow.

Depending if you what to switch between gases or liquids, different pinch channel setups are required. If you want to switch between two liquids, you put each tube corresponding to the respective liquid in one of the two channels of the pinch valve. The valve will always open up one channel and close-off the other, enabling you to switch between two liquids with one pinch valve. However if you switch between two gases you will need two pinch valves. This is because gas is always flowing from each channel regardless whether it is closed or open. This means that if a channel to the bioreactor is closed, a venting channel should be opened allowing the unused gas to escape until it is connected to the bioreactor again. This is achieved by splitting up each gas input into two channels that each are fitted inside a channel of the pinch valve. One channel exiting the pinch valve goes to the bioreactor (input channel) and one channel exiting the pinch valve is open to the air (the venting channel). In conclusion whenever you are running the bioreactor, one gas will be connected to their input channel, while the other gas will be connected to its venting channel. This is reversed for both pinch valves when the gas input is switched to the other gas. So two pinch valves are needed to switch between two gasses.

Measuring dilution rate

If flow measurements are desired during the running of the bioreactor a Y-section can be attached just upstream of the peristaltic tubing. A piece of 50 cm 2 mm silicone tubing is attached to the Y-section connection and clamped off at the other end. After autoclaving, the bottom of a sterile 2 ml disposable pipet should be sterilely connected to the clamped of piece of silicone tubing. This is best done at the flame by cutting the tube with a sterile scissor. Make sure to additionally clamp off the tube just after the Y-junction. Additionally a 2 µm syringe filter should be attached to the top end of the pipet (also sterilely!). To measure the dilution rate of a bioreactor the pipet should be secured in a vertical position with one of the rod holding systems. Then the tube attached to the pipet should be opened to the influx channel by removing the clamp. Subsequently medium should be drawn all the way up the pipet to the level just below the little cotton air filter inside the pipet (make sure not to make the cotton wet because it will render the pipet useless for measuring!). Then the medium input attached to the medium bottle close to the y-section should be clamped off. As a result the medium pump will start pump just the medium that is in the pipet. The dilution rate can now be measured by measuring how much time it takes before the pump has pumped away a certain amount (usually timing 1.5 ml is easiest) of medium from the pipet. When finished, the tube attached to the pipet should be clamped off again and the clamp on the media input should be removed.

Graphical user interface

To start the operating software on the bioreactor click on the labview icon “PC281 RUG Labview” which is located on the desktop of the PC. You will now see the ‘control tab’ of labview GUI with all the set point controls visible and ready to use. If somehow the system is not responding as expected you can open NI MAX in order to check the status of cRIO. Within NI MAX you open “remote systems” and choose “NI cRIO 9067-030703de” (Fig. 40). If all seems well in NI MAX, restarting the cRIO often solves the problem. You can do this by pressing the restart button on the top of the screen, a restart takes approximately 10 min to complete.

To set the pH for the bioreactors you type in the desired set point and click on the ‘play-button’ (Fig. 35). Make sure that the directionality of the pump is set correctly in respect to the tubing setup. Clicking on the arrow will change the pump direction. The default direction is set at clockwise. When the play button is pressed the PID loop will start to activated the pH pump based on the deviation of the measured pH from the set point. The resulting activity is based on the values that are given to P, I and D, which can be retuned whenever a new type of experiment is started with different acidification or alkalization characteristics. To set the PID value you should right click on the pH window and choose “View parameter Tag”. In some cases it might be preferred to use the pH pump for some other function. In this case the play button should not be pressed but the a speed value (%) should be typed in directly in the GUI.

Both the medium pump (ipcn-24) and the migration pump (Reglo digital) can only set for speed and direction from the Labview application. When the migration pump is used to create connectivity between bioreactors this is programmed by pumping culture back and forward between two bioreactors. This is achieved by programming the pump to change direction at a set interval using Dizanta Compass.

From the GUI the pinch valves can only activated by clicking on them and again clicking to deactivate. However in most cases pinch valves will be used either in a preprogrammed temporal scheme or be made dependent on other variables from the bioreactor. To program such behavior Dizanta Compass will be needed which will be further explained in the following section.

Using Dizanta Compass

Dizanta Compass is a licensed add-on software kit that allows for complex logic to be programmed on top of, and between the input and output parameters from the bioreactor. Dizanta compass has a limit of 350 program lines within the current license. To start the program first you need to log in Dizanta Gears. To make a new program you open the editing screen. On the right bottom you can see the amount of available lines. Any program is build up out a number of steps that contain a certain number of actions (Fig. 37). A program is made by selecting one of the 26 preprogrammed input elements (Fig. 38). The input elements are either stand-alone (step, wait, repeat, jump to step, etc.) or relate to input and output parameters from the GUI (activate/deactivate, increase/decrease, set point, not, and, or, etc.) (Fig. 39). When the type of input element is selected the relevant characteristics can be edited (name, input parameter, set point value, time etc.) on the left side and subsequently added to the program (Fig. 38 and 39). After you finished a program for the bioreactor you should test run it within the program to check for bugs. Be aware that sometimes steps with a lot of input elements should be repeated a few times or have additional wait steps of a few seconds to avoid the program to skip certain actions because it runs to quickly through them. If all seems fine you can save the program and open it in the "control" window to run it.

Programming and setting up migration pump

To run an experiment with spatial structure, the migration pumps are used to provide connectivity between spatial patches (the bioreactors). The rate of migration within the bioreactor system is expressed as % flux compared to the bioreactor flux. For example a bioreactor with a flux (dilution) rate of 500 µl/min with a 5% migration rate would transfer 25 µl/min of culture to the other bioreactor and vice versa in the case bidirectional migration. In order to induce any migration rate in the bioreactor system two values should be calculated: the migration pump rate (R_{mp}) and the migration switch time (T_s) (the time interval at which the pump changes the pump direction). These values depend on four parameters, corridor tube volume (V_c), bioreactor dilution rate (R_d), migration rate (R_m) and switch interval based on growth rate percentage (P_d). To calculate these values see the formula below this section.

The volume of the tubing that comprises the corridor between bioreactors should be as small as possible. This is important because selection within this tubing is expected to be different from those in the patches (bioreactors), so you want the smallest possible fraction of cells experiencing these 'corridor conditions' compared to the bioreactor conditions. 0,89 mm peristaltic tubing is used because it is the smallest possible PharMed BPT tubing available which is still compatible with the smallest connectors. 1 mm silicone tubing is used for the rest of the tubing length. When making migration tubing it is important to keep the tubing as short as possible to reduce the volume and to make each 'corridor' tubing exactly the same so that experiments will induce all the same conditions. When this tubing is finished it is important to accurately calculate the total volume of corridor tubing

using the formula stated below. This is important because you need to know the amount of 'dead volume' that must be pumped every time the direction of the pump is switched before effective migration is possible. This dead volume is added to the desired 'netto' migration volume to calculate the total migration pump rate.

To achieve bidirectional migration, the migration pump needs to alternate the pump direction at a certain interval. The switch interval is optimized at 50% of the growth rate in the bioreactors, meaning that within one generation the pump must have pumped two intervals in each direction. This value is derived from a tradeoff between switching as little as possible to reduce the impact of the 'dead volume' on the total pumping activity and on the other hand switching often enough for cells in each patch (bioreactor) to experience migrated cells within each generation. Based on the equations both the switch time as well as the migration pump rate can be calculated. It is important to note that if the flux rate of the bioreactor is adjusted during an experiment both the pump rate as well as the switch rate should be recalculated in order to ensure a constant representative migration rate.

V_{pt} = Peristaltic tubing (µl)
V_{st} = Silicone tubing (µl)
V_{mr} = Migration rod (µl)
V_c = Corridor volume (µl)
L_{pt} = Peristaltic tubing length (mm)
L_{st} = Silicone tubing length (mm)
L_{mr} = Migration rod length (mm)
D_{pt} = Peristaltic tubing diameter (mm)
D_{st} = Silicone tubing diameter (mm)
D_{mr} = Migration rod diameter (mm)
T_s = Switch time (min)
R_d = Dilution rate (µl/min)
R_m = Migration rate (µl/min)
R_{mp} = Migration pump rate (µl/min)
P_m = Migration percentage (%)
P_d = Switch interval based on growth rate percentage (%)

$$Vpt = Lpt \times \left(((Dpt/2)^2) \times \pi\right)$$

$$Vst = Lst \times \left(((Dst/2)^2) \times \pi\right)$$

$$Vmr = Lmr \times \left(((Dmr/2)^2) \times \pi\right)$$

$$Vc = Vpt + Vst + Vmr$$

$$Rm = Rd \times Pm$$

$$\boldsymbol{Ts = 60/\left((Rd/1000) \times Pd \times \frac{1}{2}\right)}$$

$$\boldsymbol{Rmp = (Rm \times 2) + (Vc/Ts)}$$

Closing down the bioreactor

At the end of an experiment the module with bioreactors is first disconnected from all in and outputs and then autoclaved in same way as at the start of the experiment. The autoclave module should then be first thoroughly cleaned taking apart the entire bioreactor. Peristaltic tubing that can potentially be used another time should be soaked in 1% NaOH solution overnight and thoroughly rinsed with demiwater. After six weeks all peristaltic tubing should be discarded except for the pH pump tubing this can be used for approx. 18 weeks before replacing. Any dirty silicone tubing should also be replaced. Larger parts of the bioreactor such bioreactor, head-plate, module frame, screwcap etc, can be cleaned in one of the washing machines on the fourth floor. After thoroughly cleaning all parts of the bioreactor they should be immediately dried with pressurized air and then be put in the drying oven on the fourth floor. The bioreactor and modules should then be reassembled and put back in their position ready for use.

Building up the bioreactor:

1. Before building the bioreactor it should be clear how many inputs and outputs are needed and which sensors are required for the specific application. The most important question for the setup of the bioreactors is if you want to run anaerobically or aerobically. If you want to run aerobically extra functions should be added: an air-input, a DO sensor (optional) and possibly an extra input for additions of antifoam solution to prevent clogging up of the chimney.
2. When the setup is clear, add all necessary rods into the reactor cap using a number 8 wrench to unscrew the nuts of the height adjustable ports and screw them tight by hand. For exact locations of the ports see Fig. 3. The relative positions of the various ports are important in view of homogeneity, reproducibility and reliability of sensor readings. Make sure to use the tubes that are cut at an angle for inputs and for outputs the straight cut tubes. This is important because small additions of solution are more easily released from the tubes that are cut at an angle.
3. Now you can screw the cap on to the bottle and put a stirring rod in the reactor, don't forget to also add the centralizing disk between the reactor vessel and the screwcap. Then add demineralized water to the exact reactor volume with which you intend to run. Usually 60 ml is a handy volume to run with for the 100 ml reactor vessels, because it is easy volume to convert dilution rates. For example, if one runs at a flux-rate of 1 ml/per minute (with a working volume of 60 ml) this corresponds to a dilution rate of 1 v\h.
4. Now the sensors should be calibrated (see section pH-sensor and O_2-sensor).
5. Next you carefully insert the sensor(s). Crudely adjust their height so that they are deep enough that they sit into the liquid yet do not touch the stirring magnet on the bottom!
6. Then the bioreactor is placed on the magnet stirrer and the magnet stirrer is set at the speed of running (usually at 2/3 of the capacity). Now you will see that the volume of the water goes a bit down at the center and up at the sides. Also temporarily attach the gas input to the bioreactor so that gas is coming into the reactor and you can see that it works.
7. Next it is time to finely adjust the height of both sensors and the inputs and outputs. Sensors should be set at a depth of more or less 3 mm above the stirring rod when it is stirring. Now the efflux port can be adjusted. Unscrew the efflux port a little bit and adjust the height so that the tube just touches the water then screw the port tight (not too tight !). This will make sure that the volume of the reactor will always be at 60 ml. Now the bioreactor is ready for Autoclaving.

After connecting all of the tubing

1. The bioreactors should be autoclaved in the big autoclave on the 4th floor lab kitchen. The system is autoclaved for 20 min at 120˚C. Make sure that enough water is added to the autoclave before starting the autoclave. An entire autoclaving session approximately takes 3-4 hours.
2. After removing the bioreactor frame from the autoclave add the stirring magnets into the frame sockets and put the frame into the preheated (30˚C) waterbath. The tubing can then be placed back into the tubing guides and the peristaltic pumps, while the bioreactors further cool down. Do not yet secure the tubing cassettes into the pump.
3. For anaerobic running of the bioreactor the speed should be set at 1/3 of the way. (four o'clock position).

4. Connect the pH and possibly the DO sensor to the controllers. If a DO sensor is used it should be calibrated after autoclaving (see protocol).
5. Connect the gas inputs to the flowmeter. Check if the flowmeter indicates the correct gas flow 20 ml/min N_2 and additionally 100 ml/min air if the bioreactor is used for aerobic growth and adjust if necessary.
6. If you intent to use the pinch valves, check if the pinch valve power box is on.
7. Now connect the medium bottle, the efflux (waste) bottle, the acid/base and if necessary the migration tubing between bioreactors.
8. Now the tubing cassettes can be clicked into the pumps.
9. If necessary now perform the calibration of the DO sensor.
10. Start up the Labview application on the pc.
11. Activate the pump at high speed to wash out the demiwater from the bioreactors for four full dilutions.
12. Turn off the medium pump now and inoculate each bioreactor with 5 ml overnight culture via the sample port on the efflux tube.
13. Activate the pH control on the Labview application. Then grow the culture overnight in batch modus and sample the culture via the efflux sampling port.
14. If the culture is dense enough and all parameters (gas flow temperature, pH) are constant the experiment can start in continuous culture(bioreactor) modus.

Gluing stoppers after autoclaving

Because the welded-stopper-tubes are more than 2 times the price of the glued-stopper-tubes, it might sometimes be more economic to use glued stoppers and re-glue the stoppers after autoclaving using glue (*ERGO 5861*) and a primer (*ERGO 5150*). For gluing instructions see the protocol.

1. After the autoclaving the old glue should have turned from transparent to somewhat opaque and yellowish, this indicates that the old glue can be removed. All old glue should be removed carefully without damaging the tubing or the stopper and this area should be cleaned with a bit of ethanol and subsequently dried.
2. Then apply the primer (*Ergo 5150*) with the brush to both the tube and the stopper. This primer is based on an additive which is dissolved in an organic solvent. It serves to prepare non-polar and hard-to-glue polymers such as PharMed or silicone tubing. Let the primer dry before you start gluing.
3. Apply the adhesive (*Ergo 5861*) directly from the bottle on the stopper and glue the stopper at the exact location where it was before. This is to make sure that the tensioning of the tube will now deviate from the original specifications. Work fast because the glue dries fast! After 30 min the glue will be completely dry and the tubes are ready to use.

channel	NI 9208 I	*NI 9208 II*	*NI 9403*	*NI 9870 I*	*NI 9870 II*	*NI 9870 III*	*USB hub*
1	ph 1	ph 13	pinchvalve 1	julabo 1	julabo 5	reglo digital 3	reglo ICC 1
2	ph 2	ph 14	pinchvalve 2	julabo 2	julabo 6	ipcn-24 1	reglo ICC 2
3	ph 3	ph 15	pinchvalve 3	julabo 3	reglo digital 1	ipcn-24 2	reglo ICC 3
4	ph 4	ph 16	pinchvalve 4	julabo 4	reglo digital 2	ipcn-24 3	reglo ICC 4
5	ph 5	ph 17	pinchvalve 5				reglo ICC 5
6	ph 6	ph 18	pinchvalve 6				reglo ICC 6
7	ph 7	ph 19	pinchvalve 7				extrenal hard disk
8	ph 8	ph 20	pinchvalve 8				
9	shield	shield	shield				
10	shield	shield	shield				
11	ph 9	ph 21	pinchvalve 9				
12	ph 10	ph 22	pinchvalve 10				
13	ph 11	ph 23	pinchvalve 11				
14	ph 12	ph 24	pinchvalve 12				
15	DO 17		pinchvalve 13				
16	DO 18		pinchvalve 14				
17	DO 19		pinchvalve 15				
18	DO 20		pinchvalve 16				
19			RSVD				
20			pinchvalve 17				
21			pinchvalve 18				
22			pinchvalve 19				
23			pinchvalve 20				
24			pinchvalve 21				
25			pinchvalve 22				
26			pinchvalve 23				
27			pinchvalve 24				
28	shield	shield	shield				
29	shield	shield	shield				
30							
31							
32							
33							
34							
35							
36							
37							

Scheme 1.

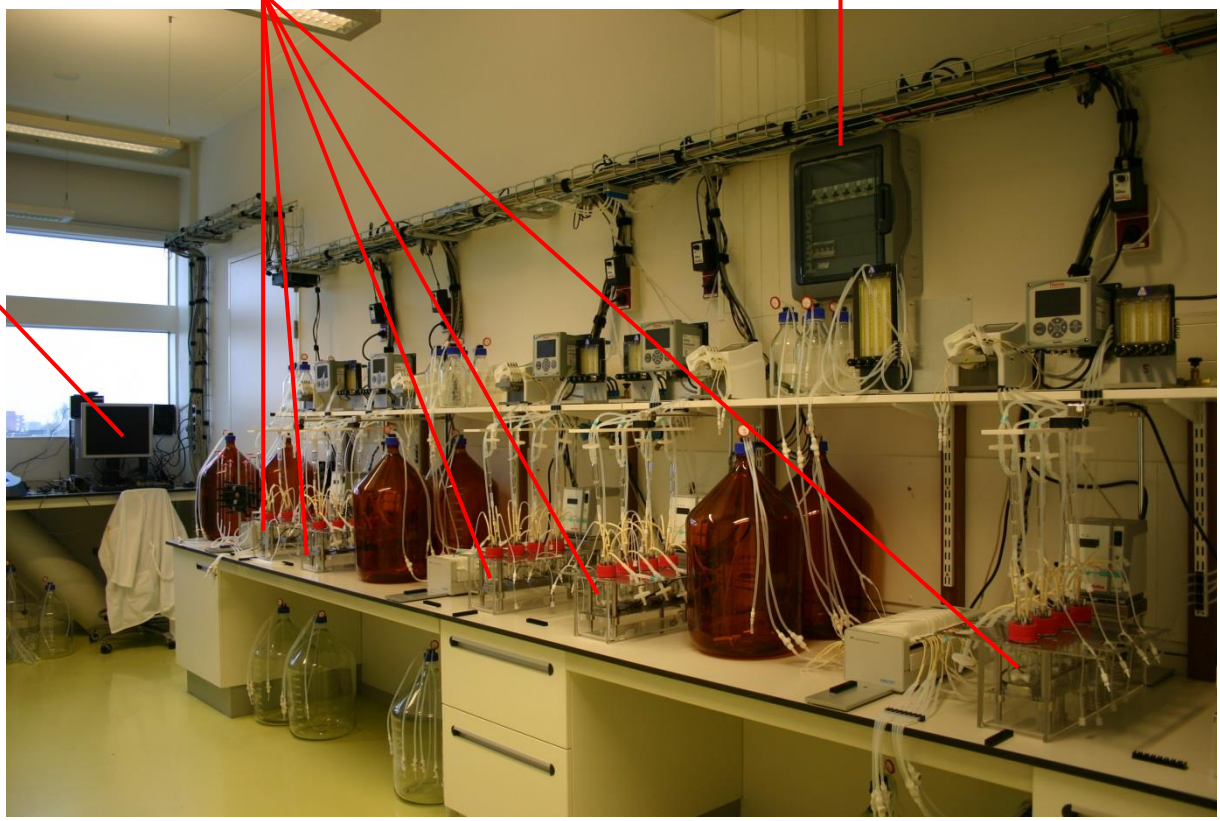
Chemostat modules
Fuse box and UPS
Chemostat computer

Fig. 1

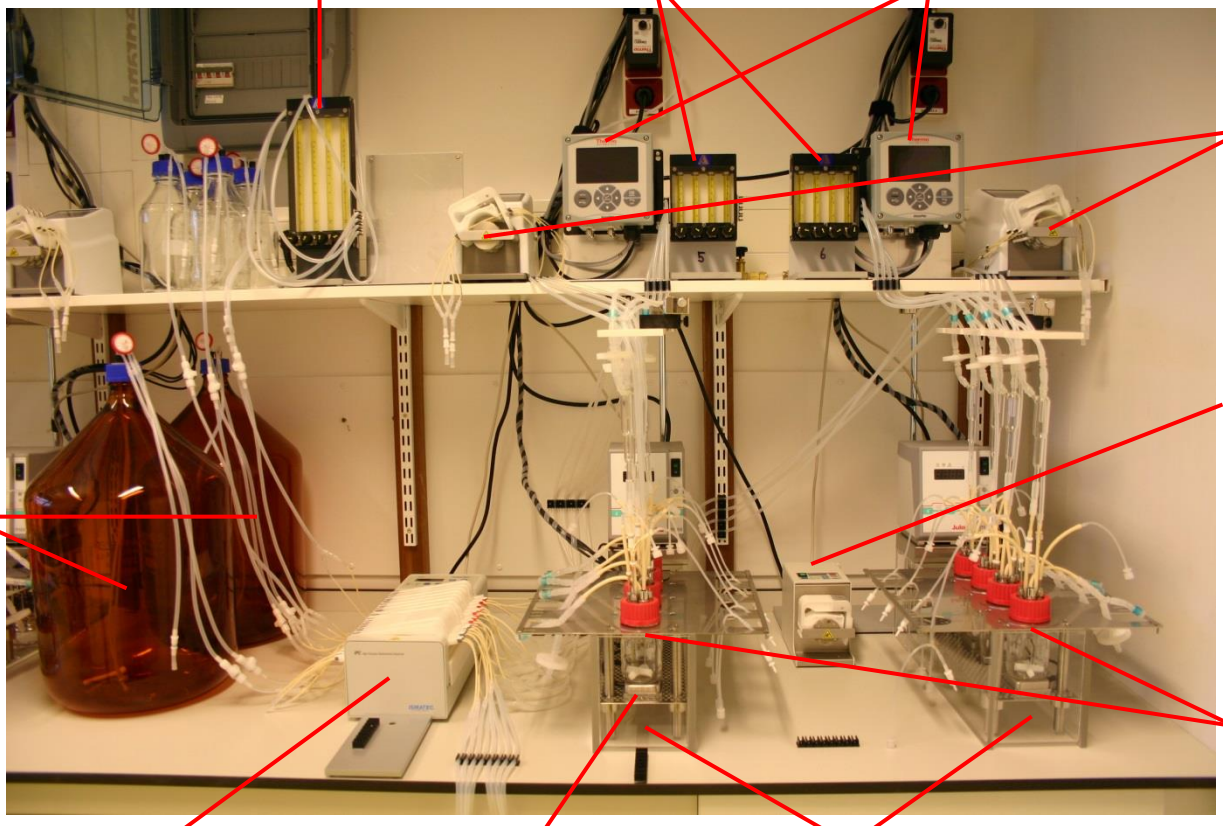
air-input
N_2-input
pH-controllers
pH pump
Migration pump
Medium bottles
Chemostat modules
Medium pump
Magnet stirrer
Waterbaths

Fig. 2

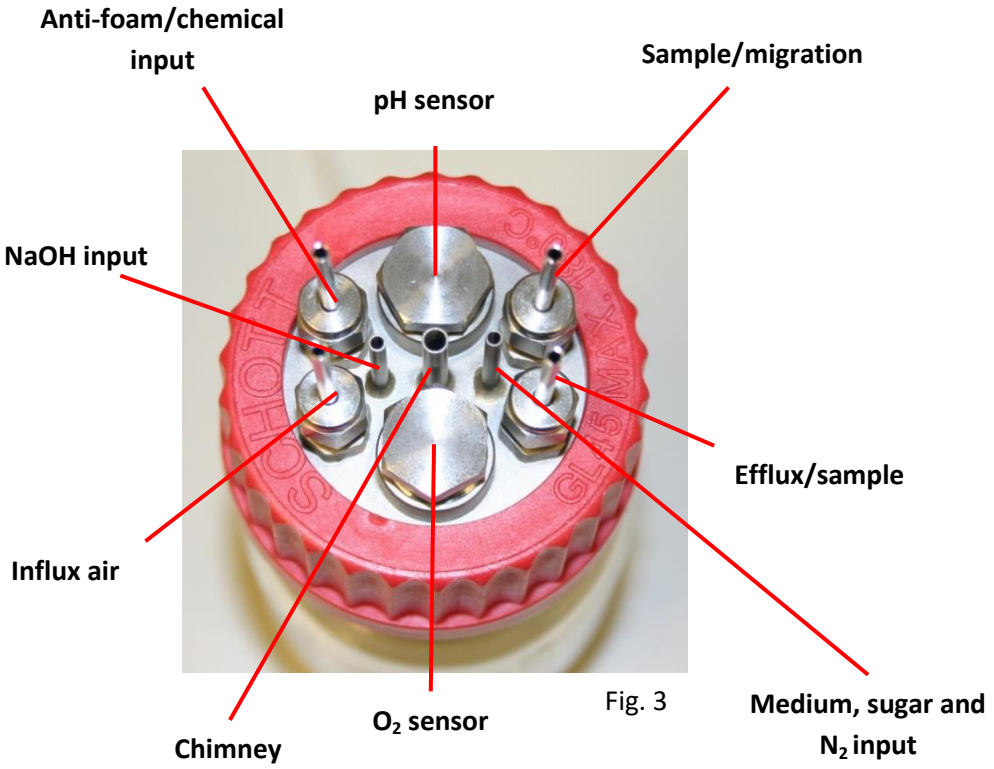
Anti-foam/chemical input
pH sensor
Sample/migration
NaOH input
Efflux/sample
Influx air
Chimney
O_2 sensor
Medium, sugar and N_2 input

Fig. 3

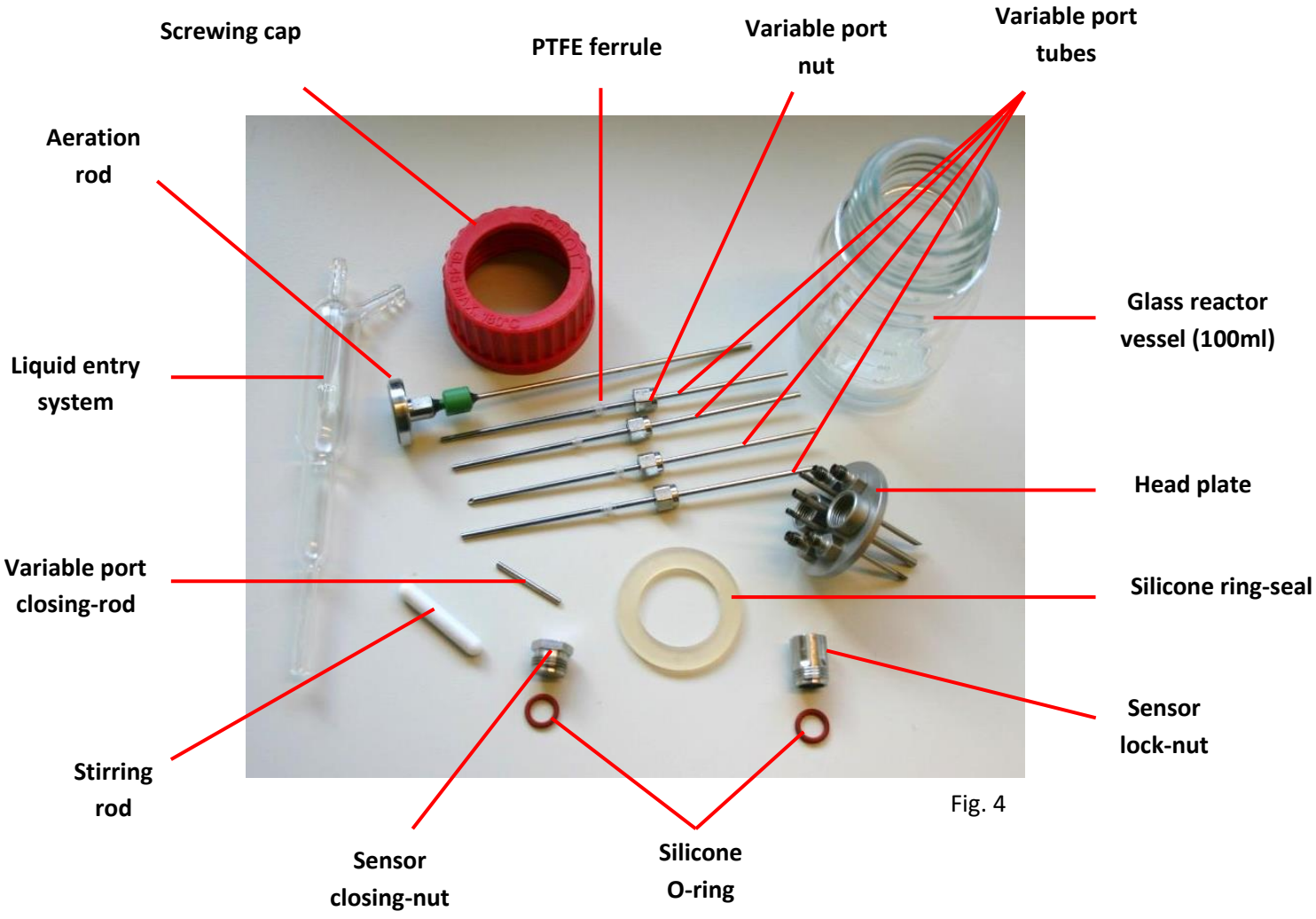
Screwing cap
PTFE ferrule
Variable port nut
Variable port tubes
Aeration rod
Glass reactor vessel (100ml)
Liquid entry system
Head plate
Variable port closing-rod
Silicone ring-seal
Sensor lock-nut
Stirring rod
Sensor closing-nut
Silicone O-ring

Fig. 4

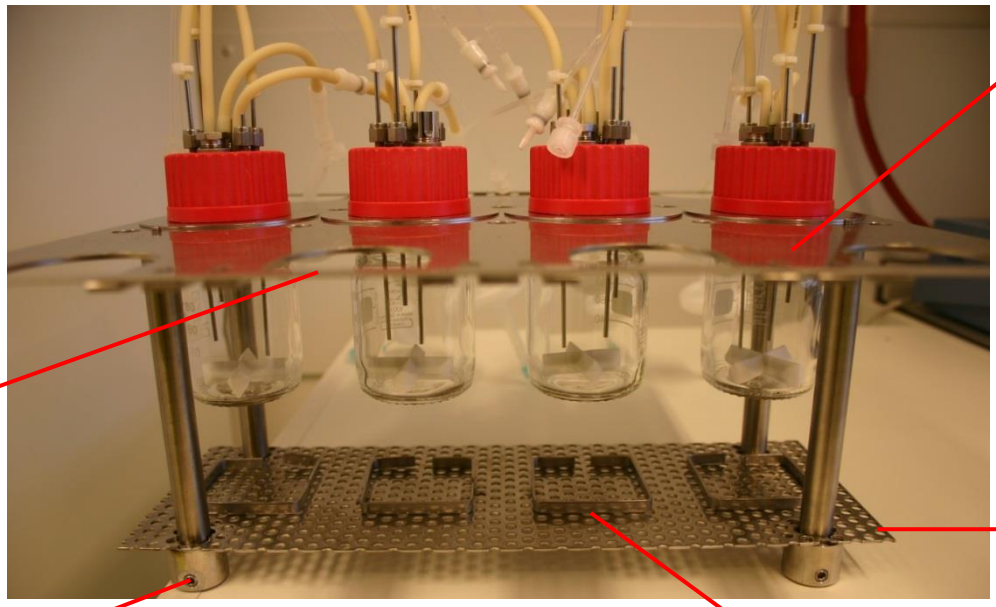
Top-plate
Closing rail
Bottom plate
Height adjustment hex-nut
Stirring magnet holder

Fig. 5

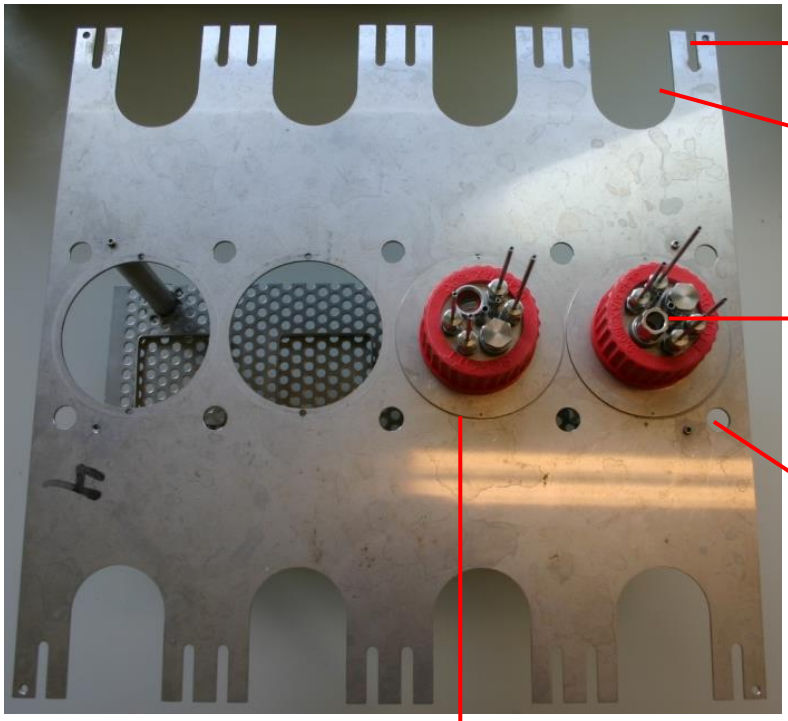
Tube guide
Bottle holder
Chemostat
Calibration solution tube holder
Chemostat centralizing disk

Fig. 6

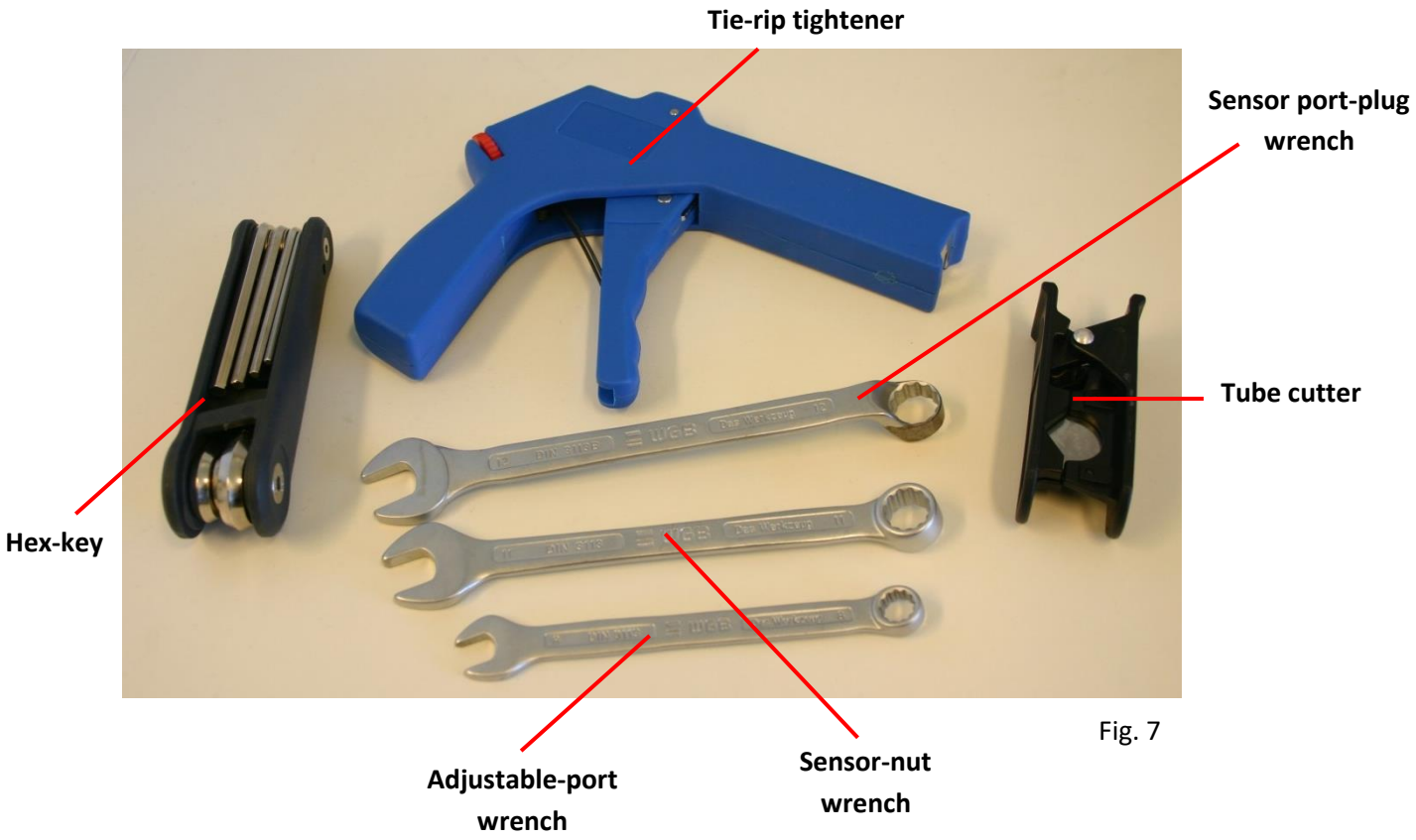
Tie-rip tightener
Sensor port-plug wrench
Tube cutter
Hex-key
Adjustable-port wrench
Sensor-nut wrench

Fig. 7


RS232 control
cRIO
Analog control
(not operational)
On/off
button
Power input
RS 232 IN
ANALOG
ISMATEC
ETL LISTED
CONFORMS TO
UL 61010A-1
3024922
CERTIFIED TO
CAN/CSA STD C22.2 NO. 1010-1

Fig. 8

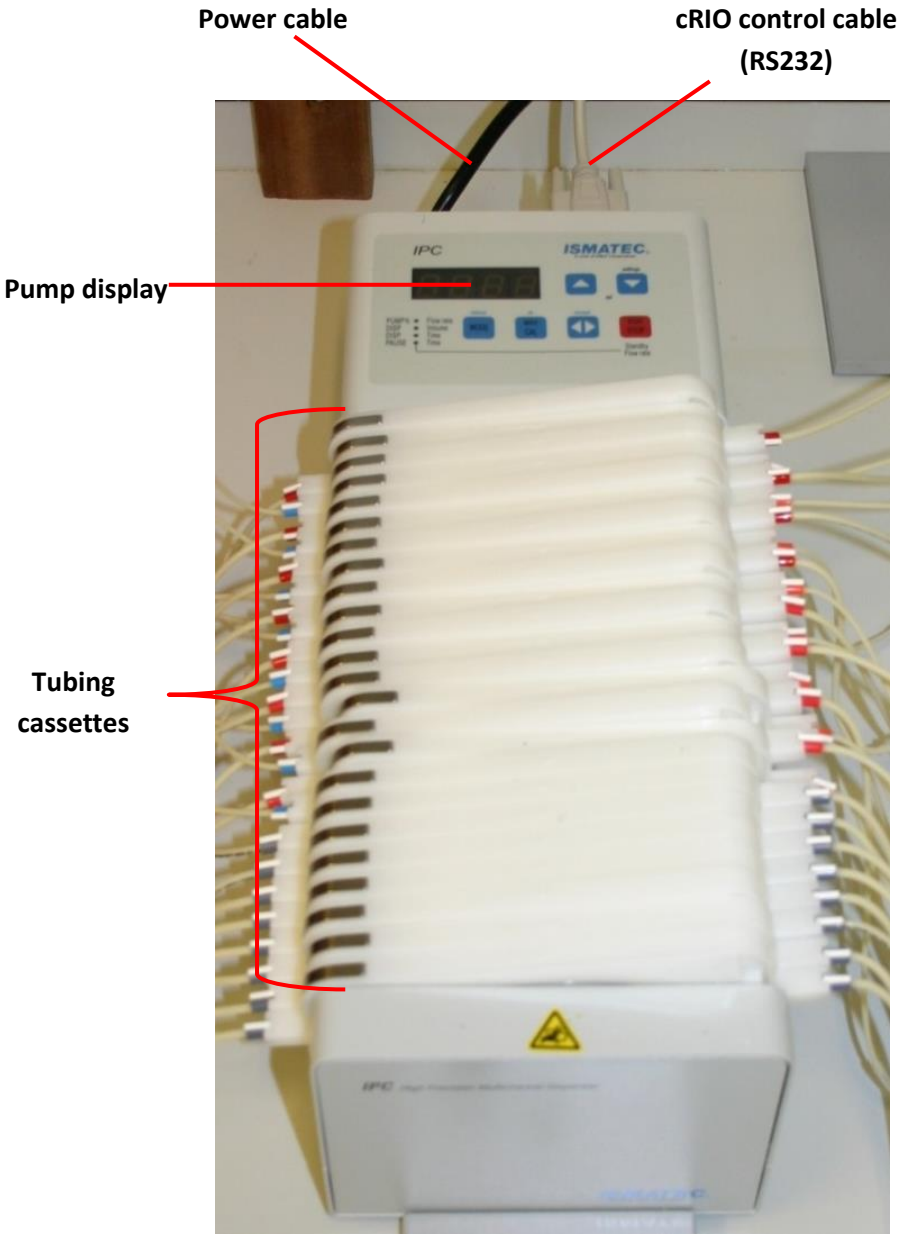
Power cable
cRIO control cable
(RS232)
Pump display
Tubing
cassettes
IPC
ISMATEC

Fig. 9

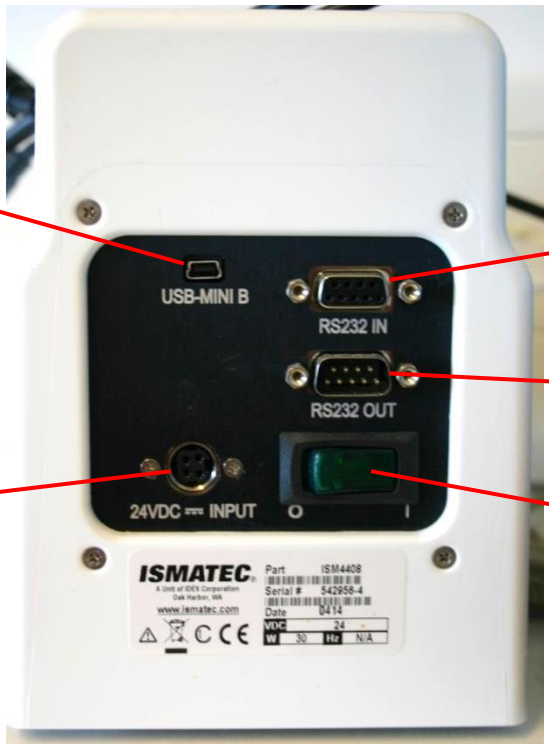
USB control cRIO
RS232 IN (not operational)
RS232 OUT (not operational)
Power input
On/off button
USB-MINI B
RS232 IN
RS232 OUT
24VDC INPUT
O
I
ISMATEC

Fig. 10


Power cable
cRIO control cable (USB)
Pump display
Tubing cassettes
Remote-Modus
ISMATEC
REGLO ICC

Fig. 11

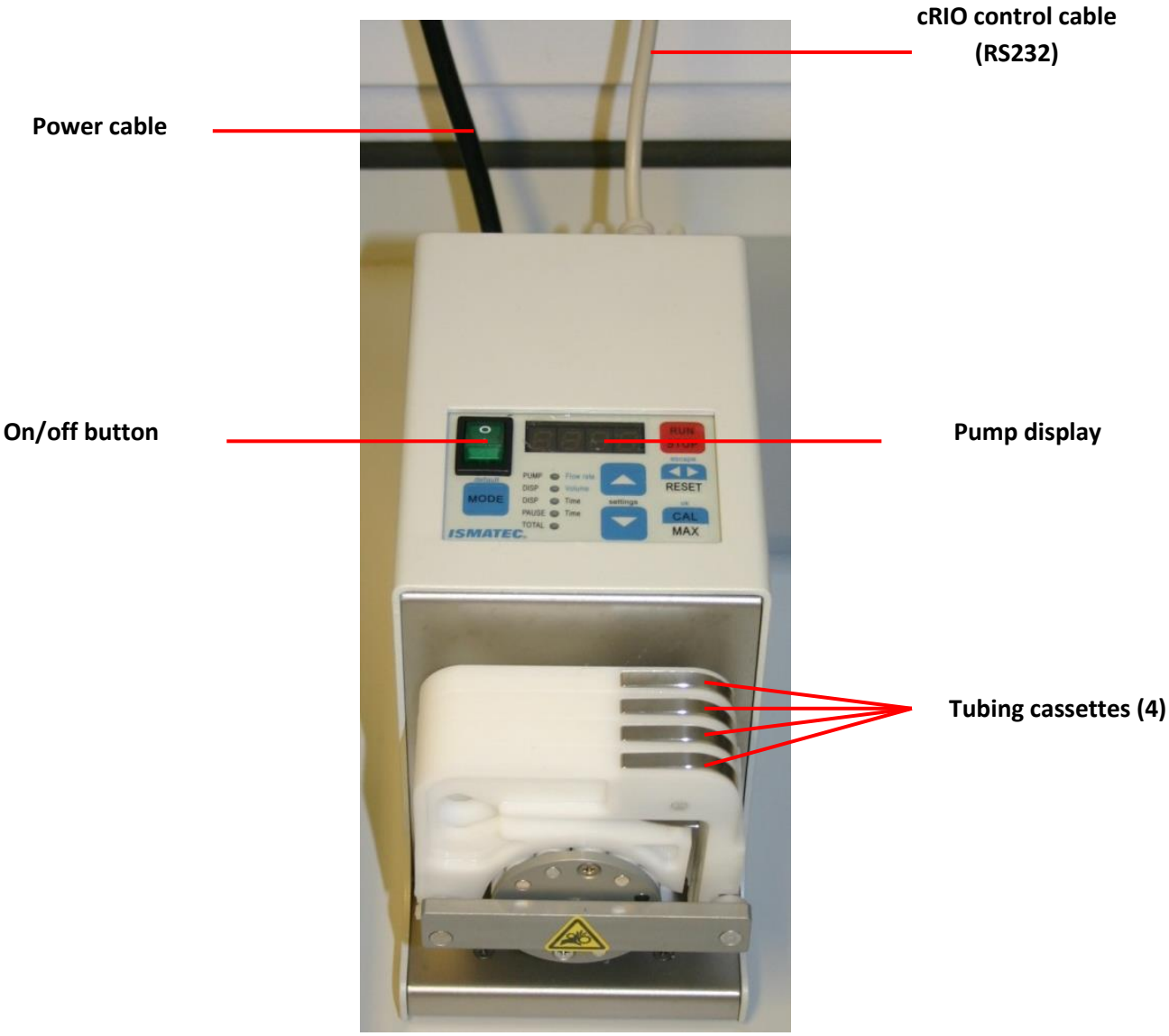
cRIO control cable
(RS232)
Power cable
On/off button
Pump display
Tubing cassettes (4)
MODE
RESET
CAL
MAX
ISMATEC

Fig. 12

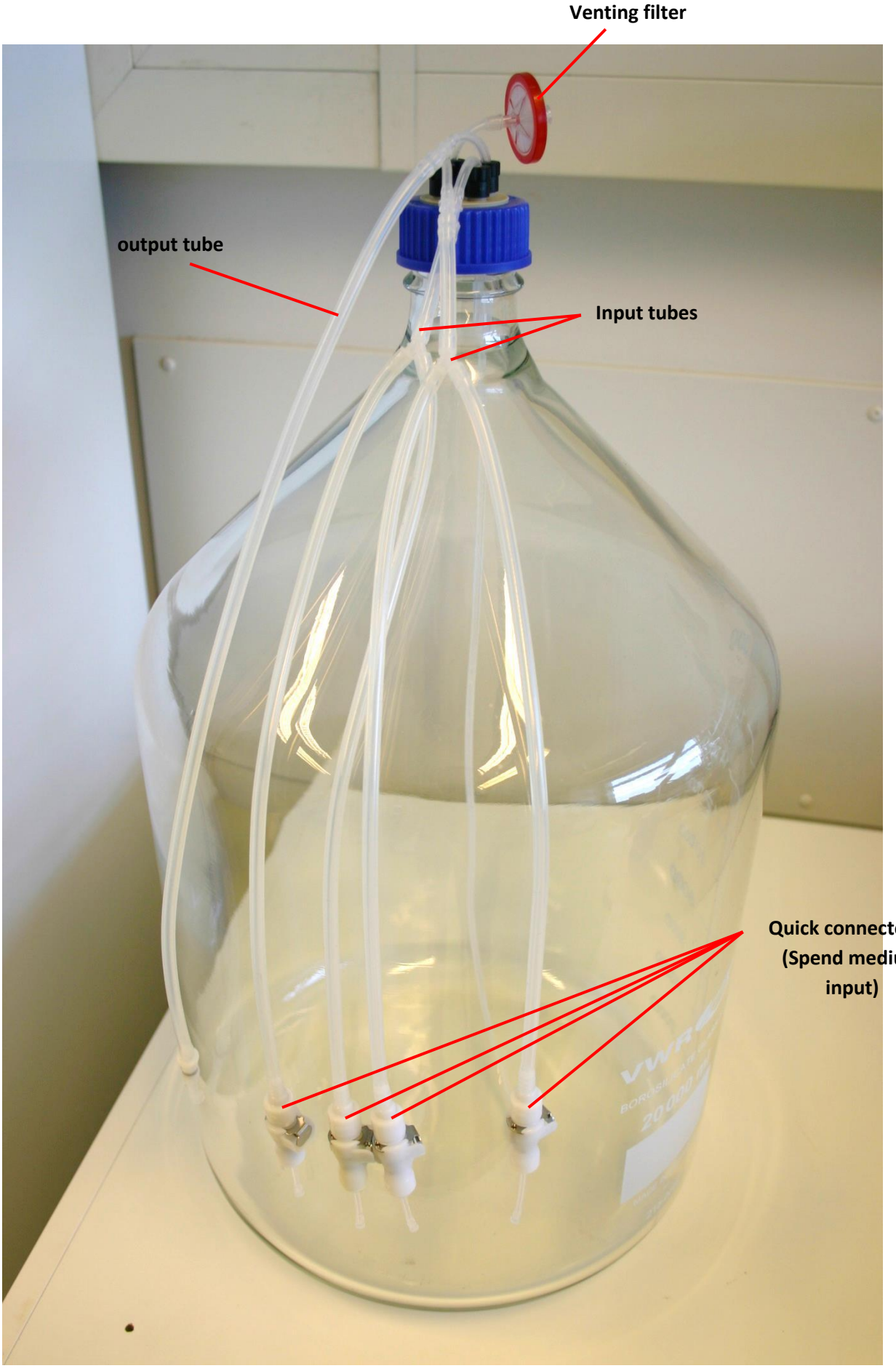
Venting filter
output tube
Input tubes
Quick connectoren
(Spend medium
input)

Fig. 13


Venting filter
Quick connectoren
(medium output)
Medium input

Fig. 14

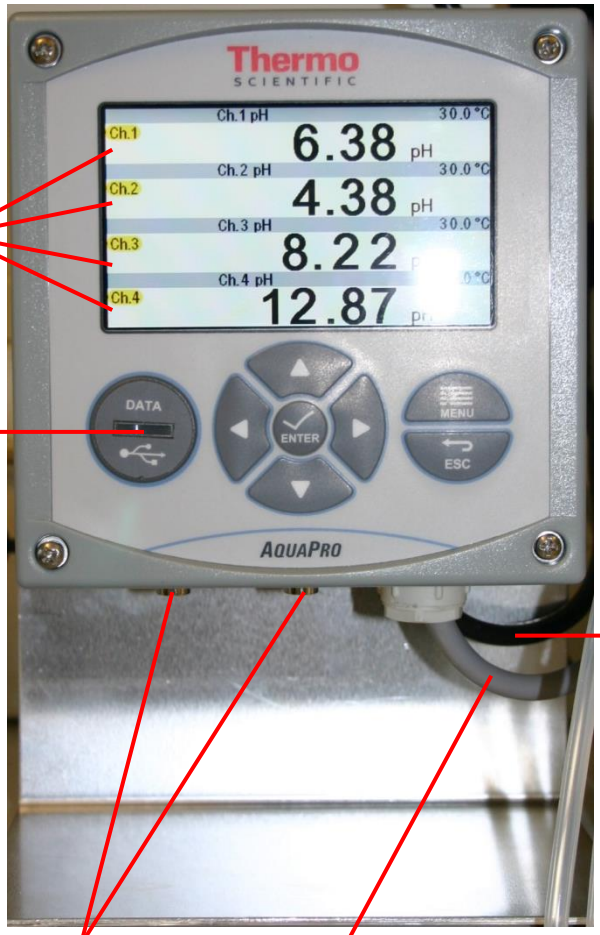
Thermo
SCIENTIFIC
Ch.1 pH
6.38 pH
Ch.2 pH
4.38 pH
Ch.3 pH
8.22
Ch.4 pH
12.87
DATA
ENTER
MENU
ESC
AQUAPRO
Display pH-sensor values chemostats
Optional usb storage port
Power cable
pH sensor connectors
Sensor output data cRIO (mA)

Fig. 15

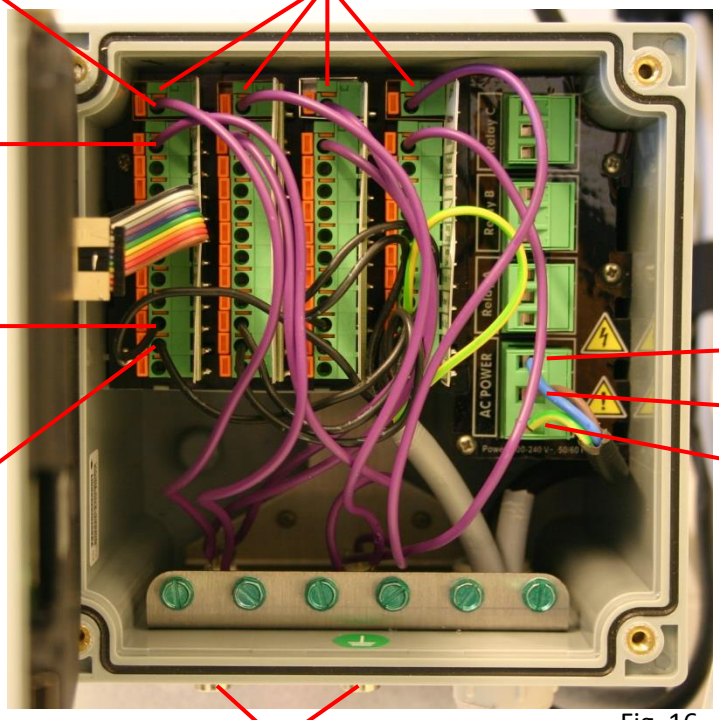
Input pH-sensor signal
pH channels
Input pH-sensor shield
output pH-value (mA)
output ground
Power +
Power –
Ground
Sensor connectors

Fig. 16

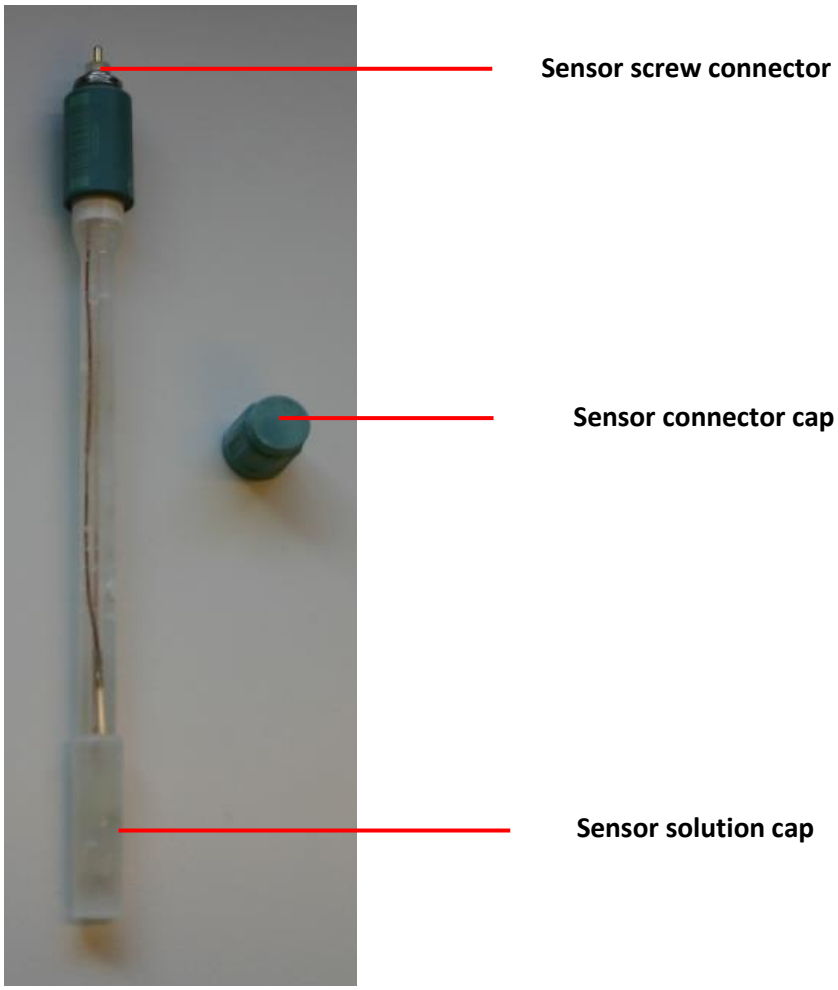
Sensor screw connector
Sensor connector cap
Sensor solution cap

Fig. 17

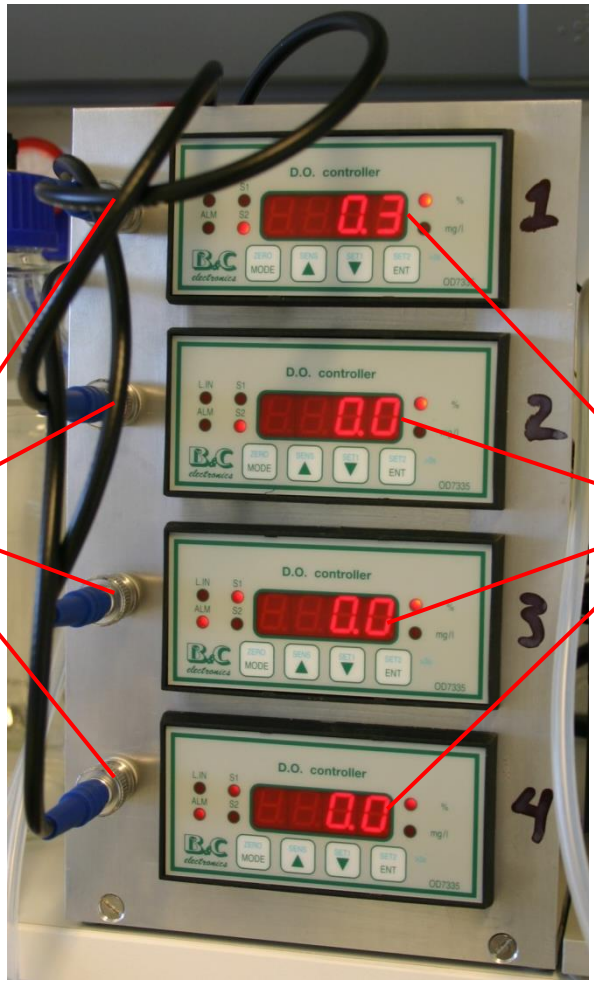
D.O. controller
1
2
3
4
O2-sensor connectors
Display O2-sensor values chemostats

Fig. 18

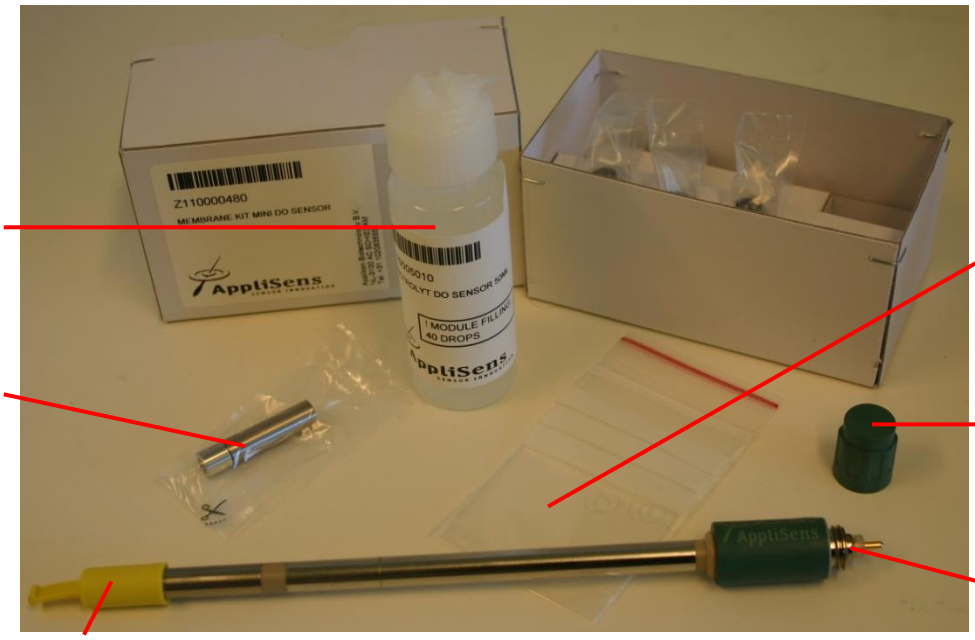
sensor storage solution
Spare sensor membrane
sensor membrane cap
Silicone seal
Sensor connector cap
Sensor screw connector

Fig. 19

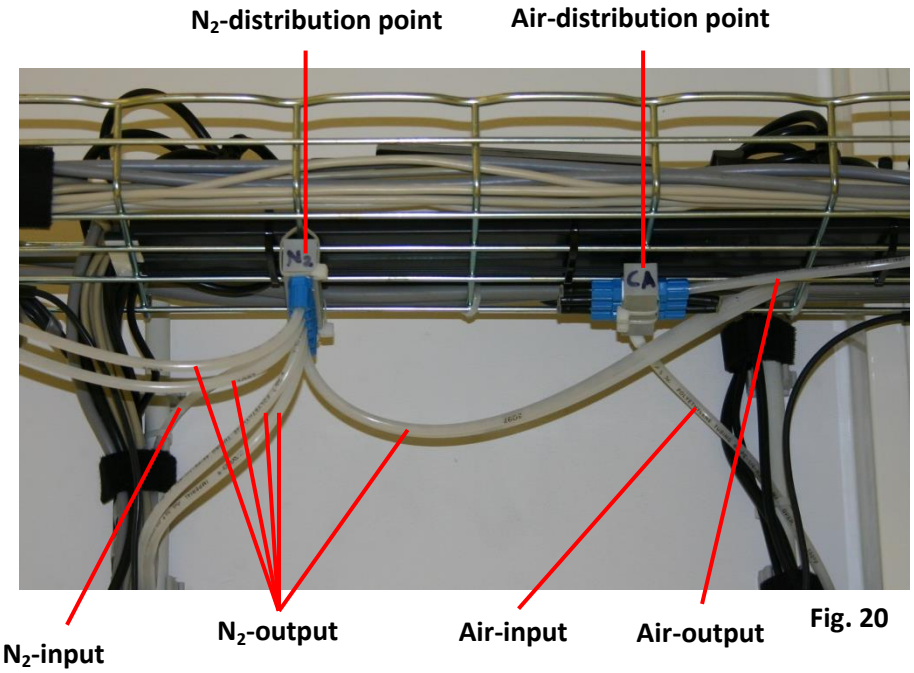
N_2-distribution point
Air-distribution point
N_2-input
N_2-output
Air-input
Air-output

Fig. 20

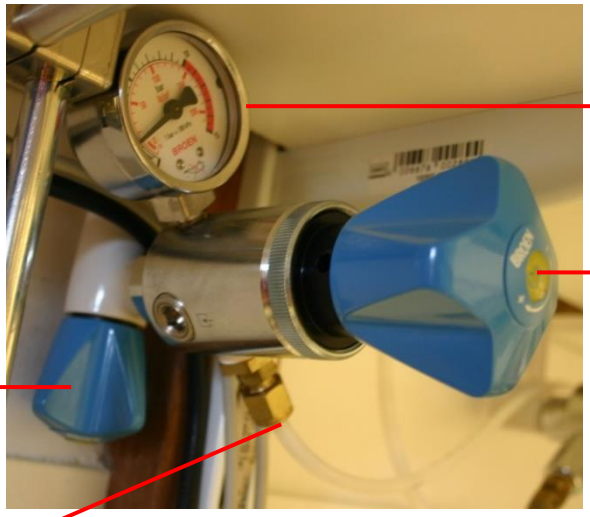
Output pressure (bar)
Reduction valve
Main valve
Air-output

Fig. 21

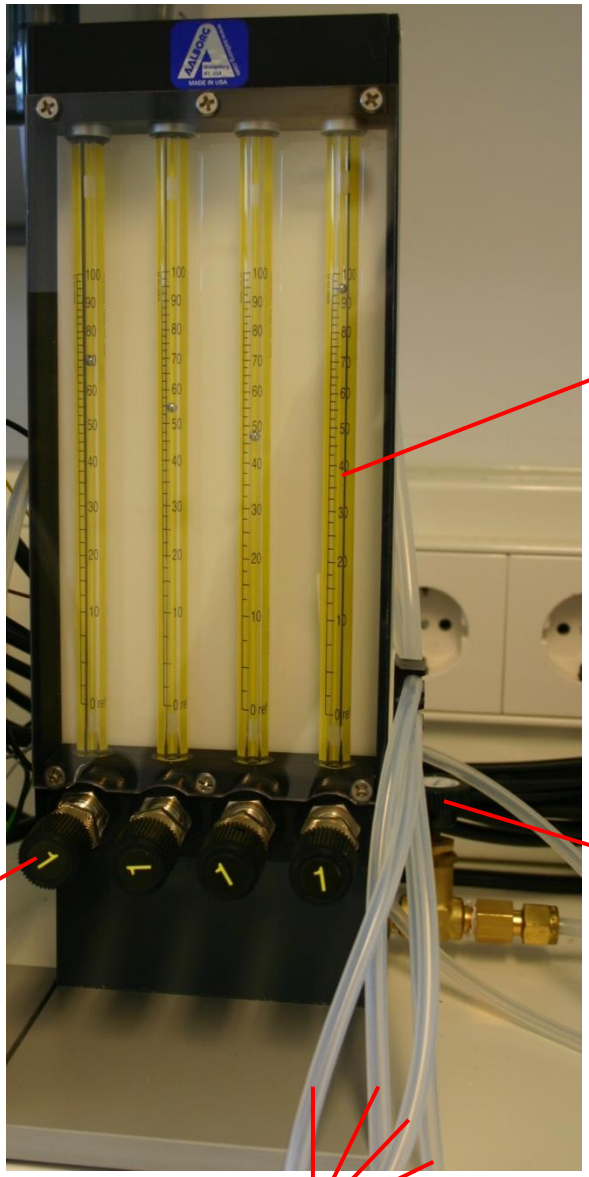
Gas input scale (ml/min)
Main input valve (1 bar)
High accuracy channel valve
Output channels

Fig. 22

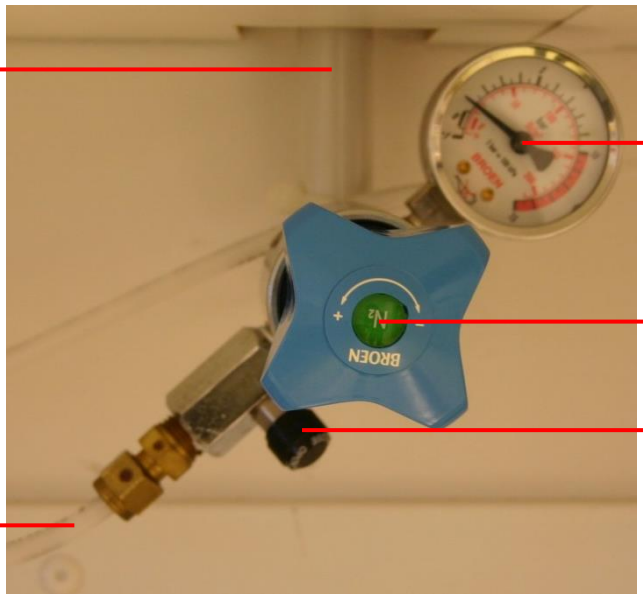
N_2-input
Output pressure (bar)
Reduction valve
Main valve
N_2-output

Fig. 23

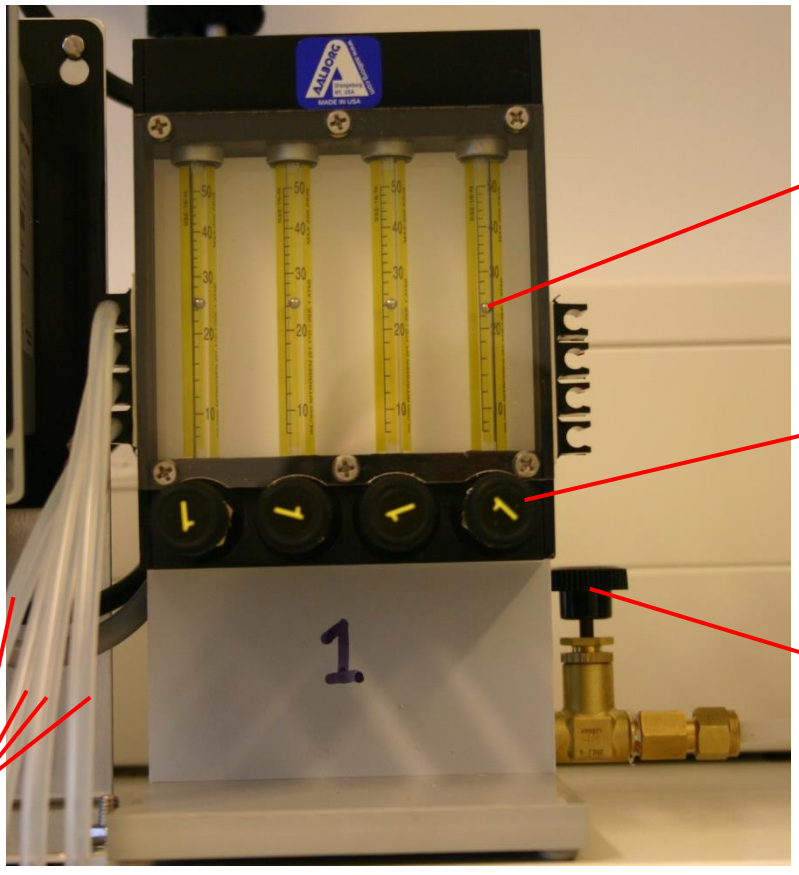
Gas input scale (ml/min)
High accuracy channel valve
Main input valve (1bar)
Output channels

Fig. 24

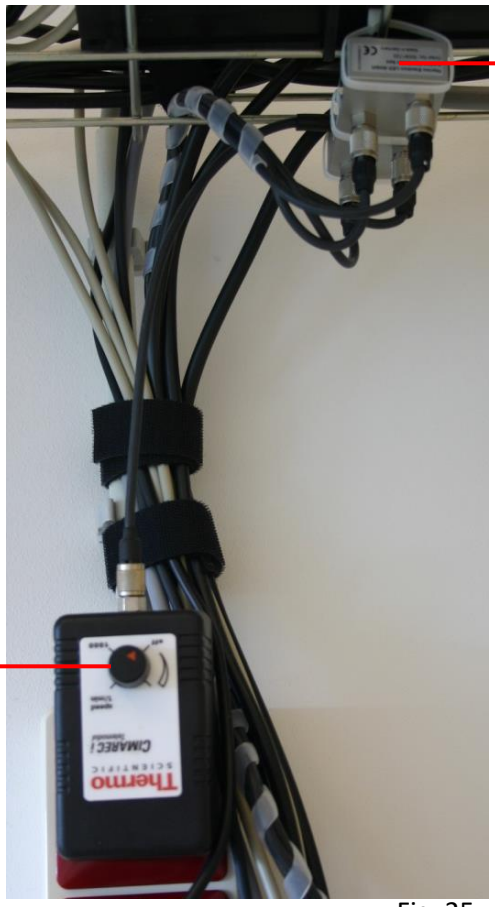

Fig. 25

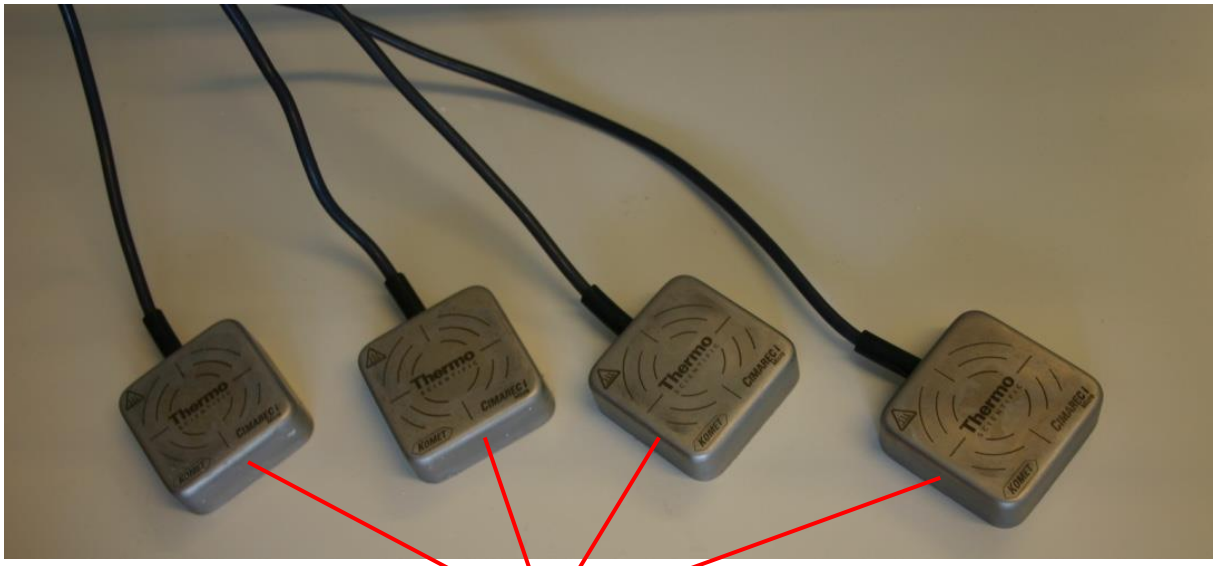

Fig. 26

Submersible stirrers

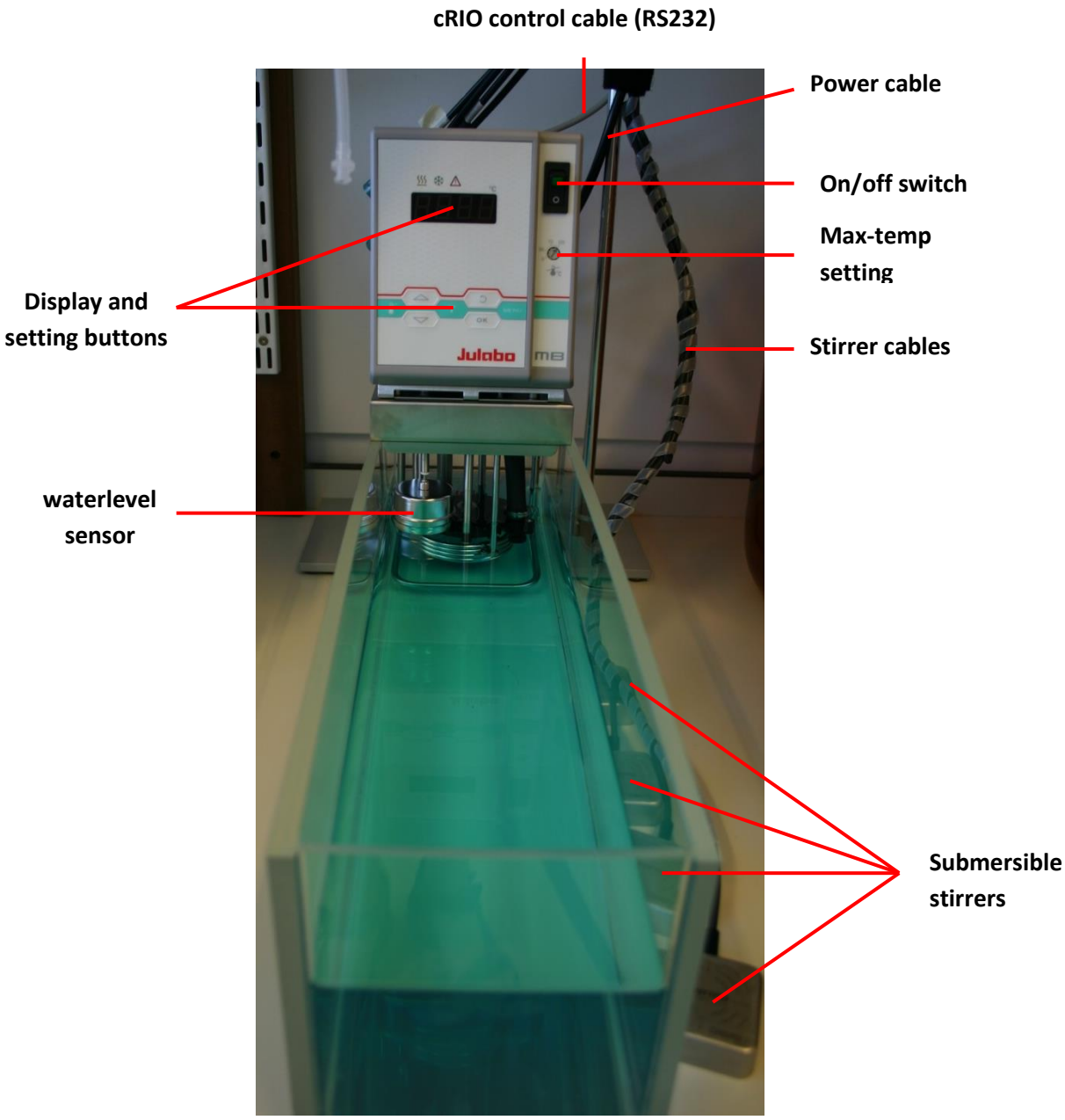
cRIO control cable (RS232)
Power cable
On/off switch
Max-temp setting
Display and setting buttons
Stirrer cables
waterlevel sensor
Submersible stirrers
Julabo
MB

Fig. 27

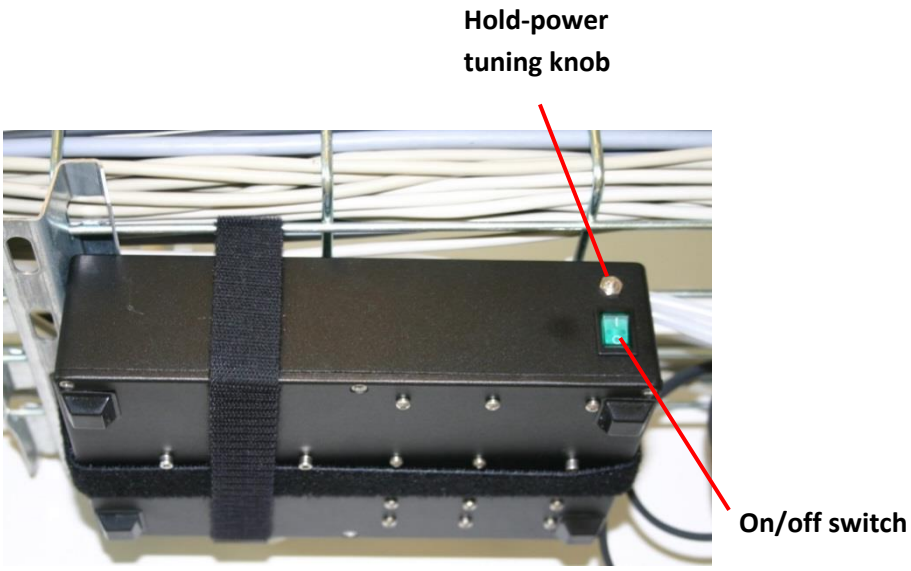

Fig. 28

Fig. 29

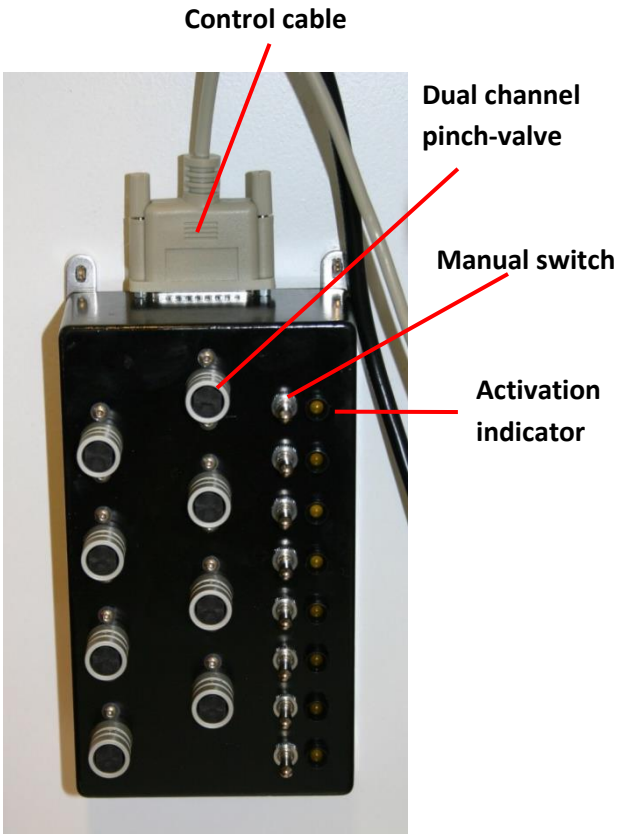

Fig. 30

Fig. 31

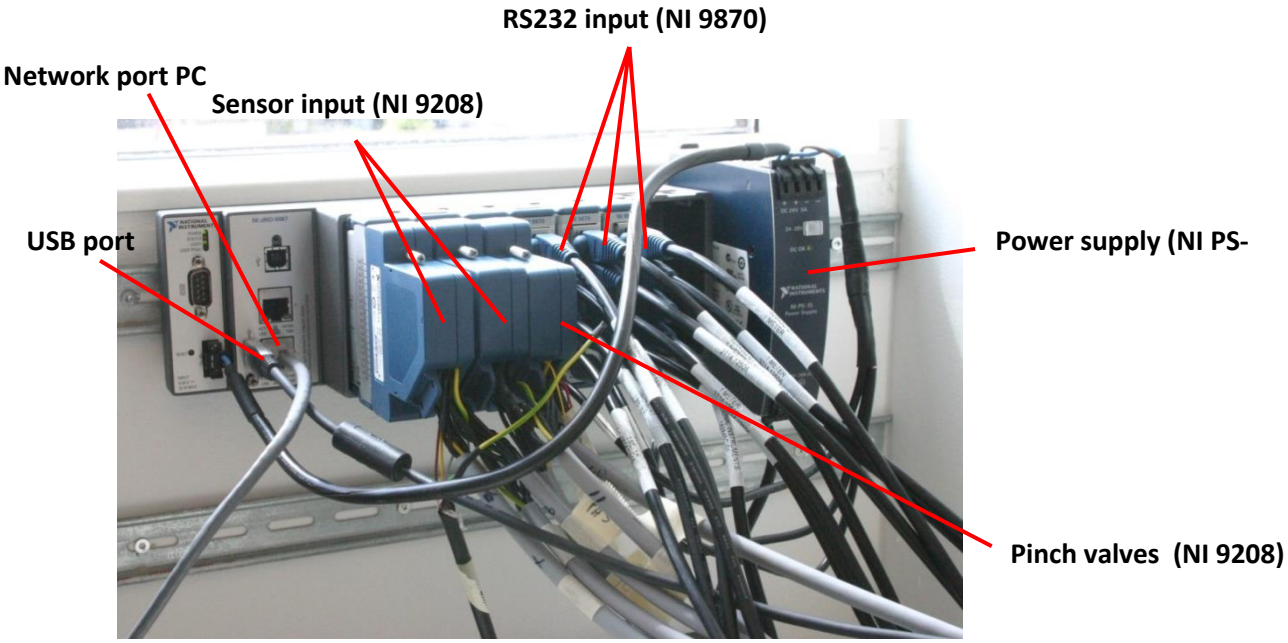
RS232 input (NI 9870)
Network port PC
Sensor input (NI 9208)
USB port
Power supply (NI PS-
Pinch valves (NI 9208)

Fig. 32

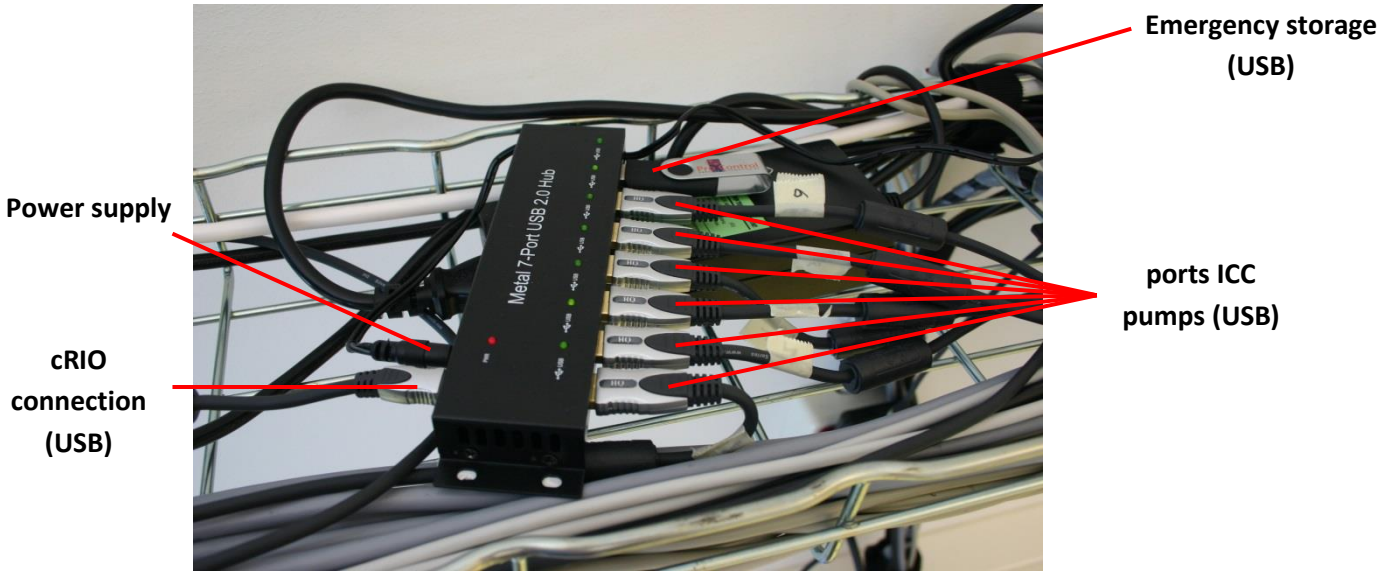
Emergency storage (USB)
Power supply
ports ICC pumps (USB)
cRIO connection (USB)
Metal 7-Port USB 2.0 Hub

Fig. 33

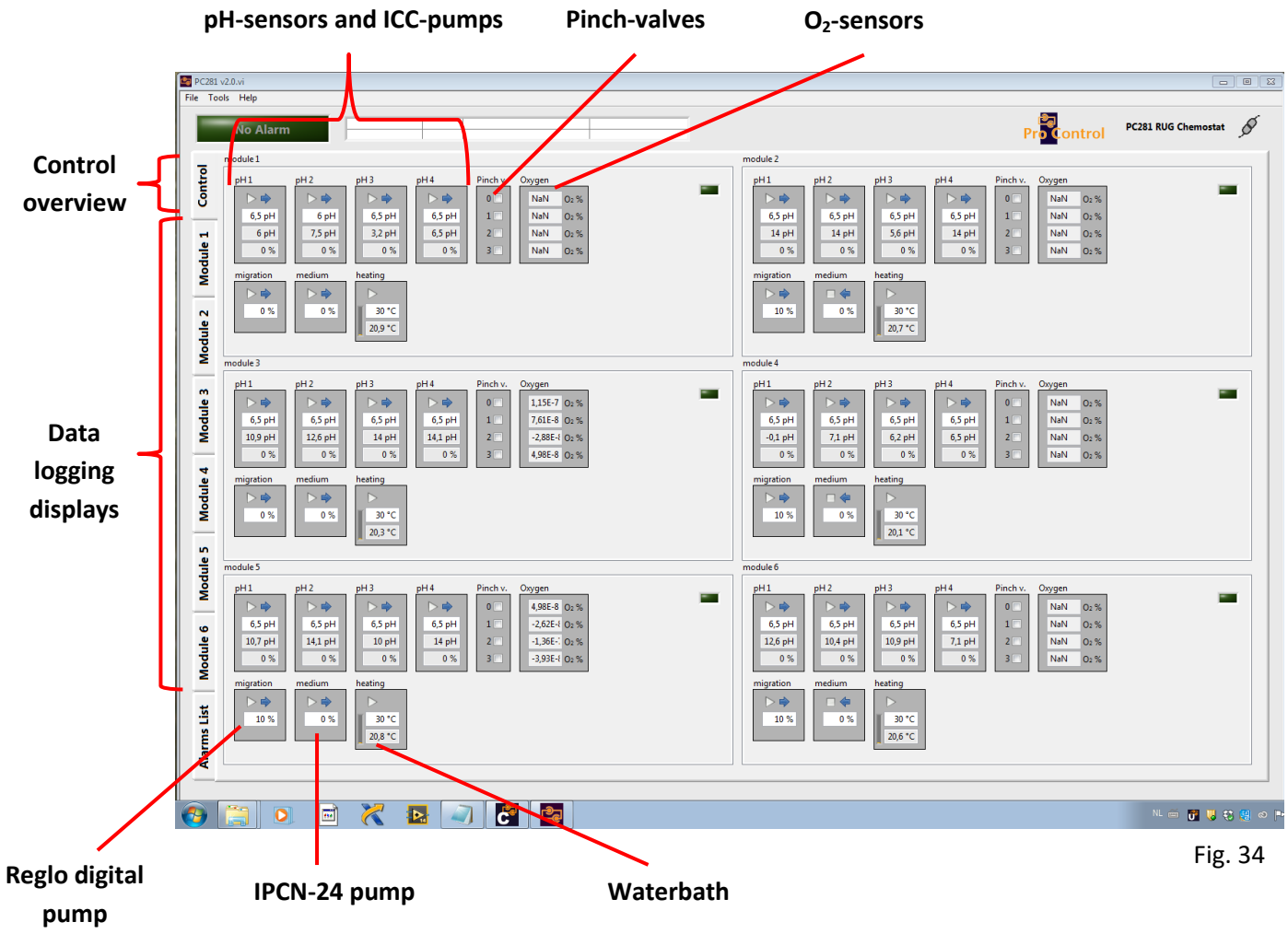
pH-sensors and ICC-pumps
Pinch-valves
O2-sensors
Control overview
Data logging displays
Reglo digital pump
IPCN-24 pump
Waterbath
PC281 v2.0.vi
File Tools Help
No Alarm
PC281 RUG Chemostat
Control
Module 1
Module 2
Module 3
Module 4
Module 5
Module 6
Alarms List
module 1
module 2
module 3
module 4
module 5
module 6
pH 1
pH 2
pH 3
pH 4
Pinch v.
Oxygen
migration
medium
heating

Fig. 34

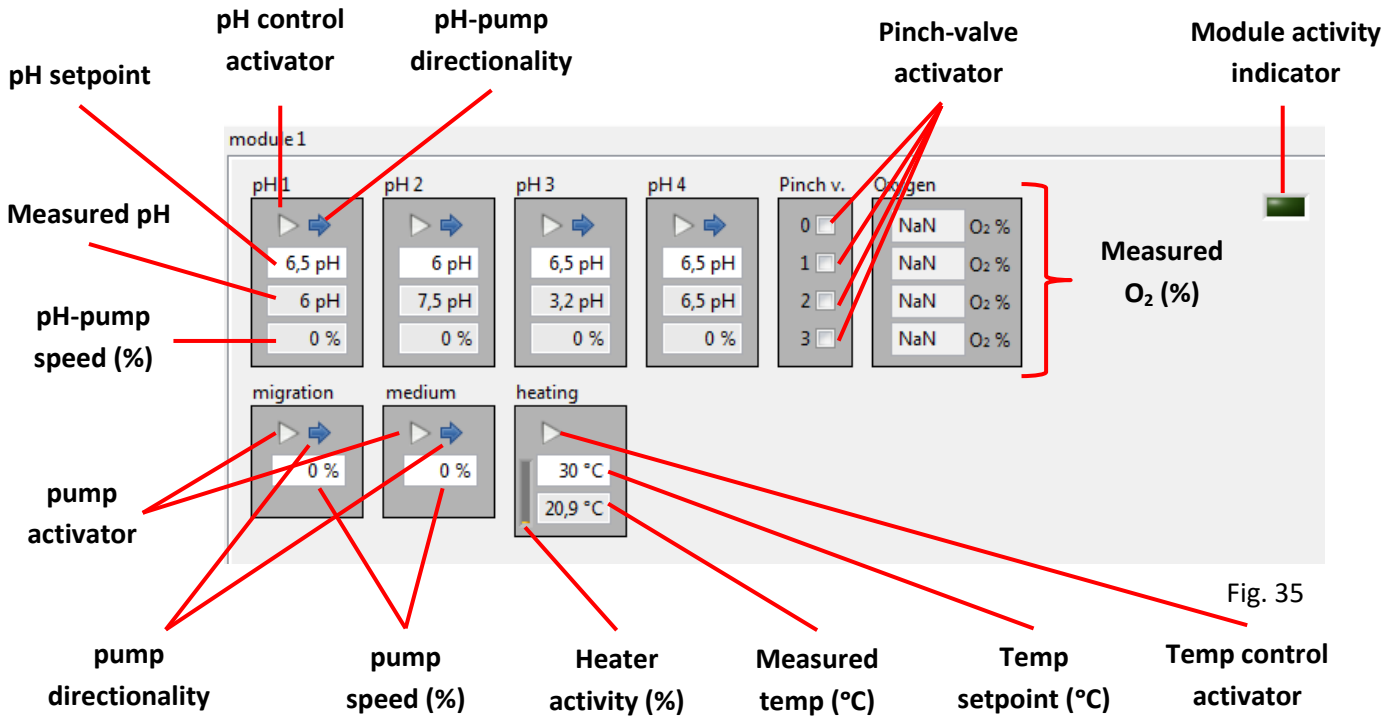
pH setpoint
pH control activator
pH-pump directionality
Pinch-valve activator
Module activity indicator
Measured pH
pH-pump speed (%)
Measured O2 (%)
pump activator
pump directionality
pump speed (%)
Heater activity (%)
Measured temp (°C)
Temp setpoint (°C)
Temp control activator
module 1
pH 1
pH 2
pH 3
pH 4
Pinch v.
Oxygen
migration
medium
heating

Fig. 35

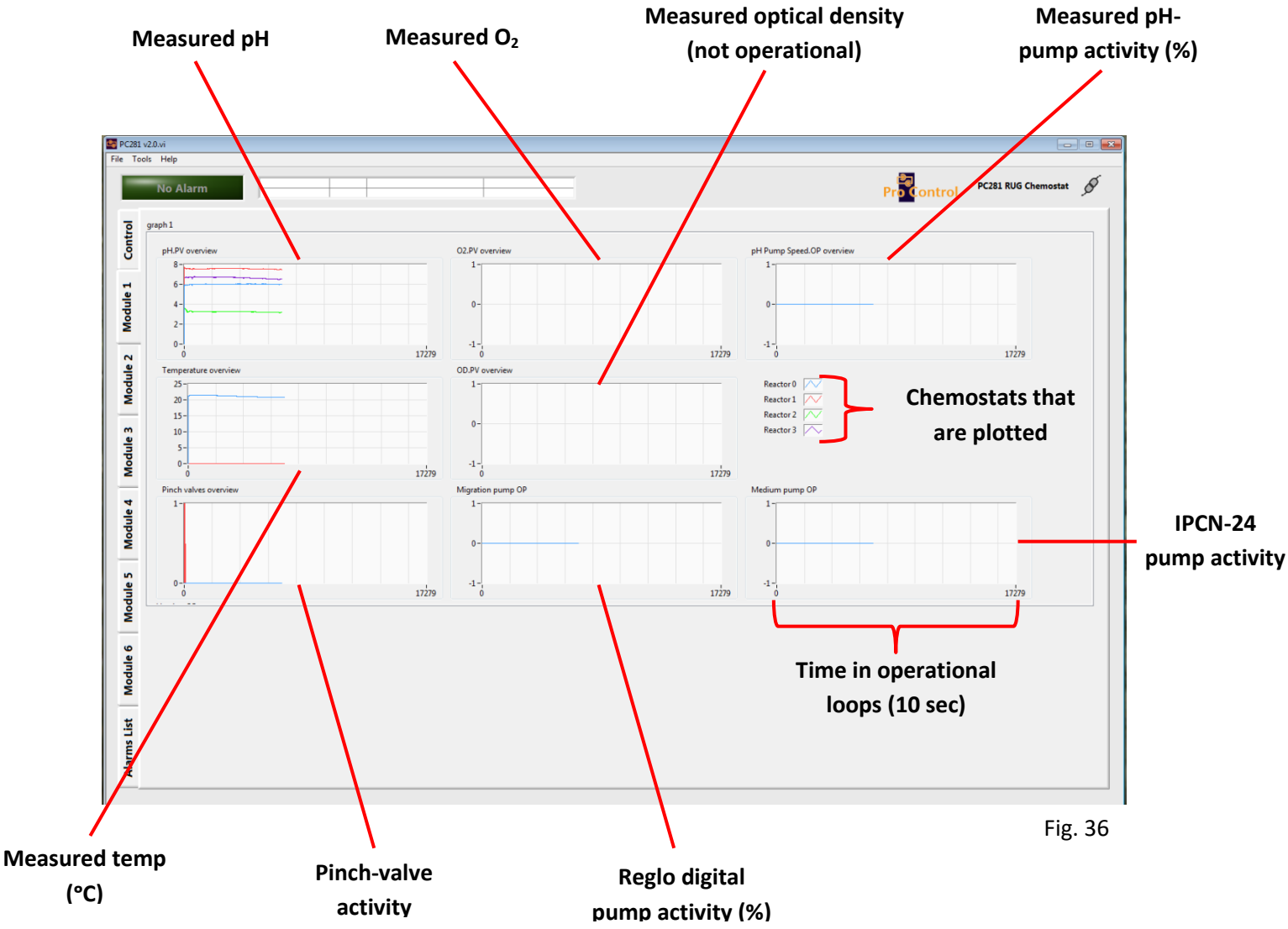
Measured pH
Measured O_2
Measured optical density (not operational)
Measured pH-pump activity (%)
PC281 v2.0.vi
File Tools Help
No Alarm
PC281 RUG Chemostat
graph 1
pH.PV overview
O2.PV overview
pH Pump Speed.OP overview
Temperature overview
OD.PV overview
Reactor 0
Reactor 1
Reactor 2
Reactor 3
Chemostats that are plotted
Pinch valves overview
Migration pump OP
Medium pump OP
IPCN-24 pump activity
Time in operational loops (10 sec)
Control
Module 1
Module 2
Module 3
Module 4
Module 5
Module 6
Alarms List
Measured temp (°C)
Pinch-valve activity
Reglo digital pump activity (%)

Fig. 36

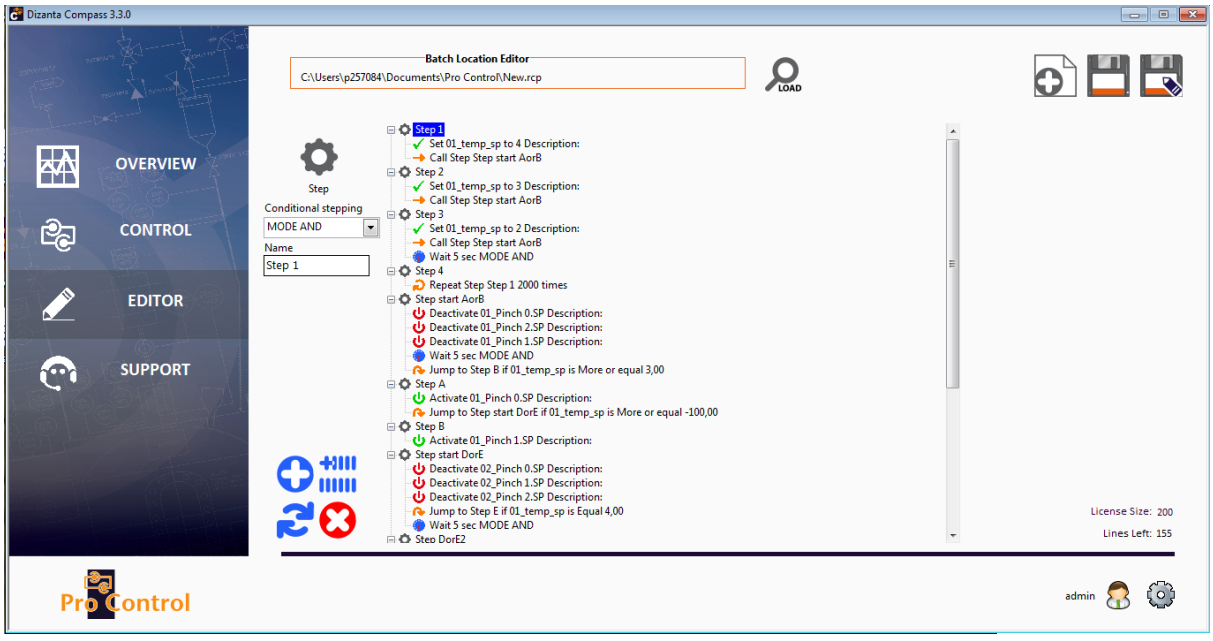
Dizanta Compass 3.3.0
Batch Location Editor
C:\Users\p257084\Documents\Pro Control\New.rcp
LOAD
OVERVIEW
CONTROL
EDITOR
SUPPORT
Step
Conditional stepping
MODE AND
Name
Step 1
Step 1
Set 01_temp_sp to 4 Description:
Call Step Step start AorB
Step 2
Set 01_temp_sp to 3 Description:
Call Step Step start AorB
Step 3
Set 01_temp_sp to 2 Description:
Call Step Step start AorB
Wait 5 sec MODE AND
Step 4
Repeat Step Step 1 2000 times
Step start AorB
Deactivate 01_Pinch 0.SP Description:
Deactivate 01_Pinch 2.SP Description:
Deactivate 01_Pinch 1.SP Description:
Wait 5 sec MODE AND
Jump to Step B if 01_temp_sp is More or equal 3,00
Step A
Activate 01_Pinch 0.SP Description:
Jump to Step start DorE if 01_temp_sp is More or equal -100,00
Step B
Activate 01_Pinch 1.SP Description:
Step start DorE
Deactivate 02_Pinch 0.SP Description:
Deactivate 02_Pinch 1.SP Description:
Deactivate 02_Pinch 2.SP Description:
Jump to Step E if 01_temp_sp is Equal 4,00
Wait 5 sec MODE AND
Step DorE2
License Size: 200
Lines Left: 155
Pro Control
admin

Fig. 37

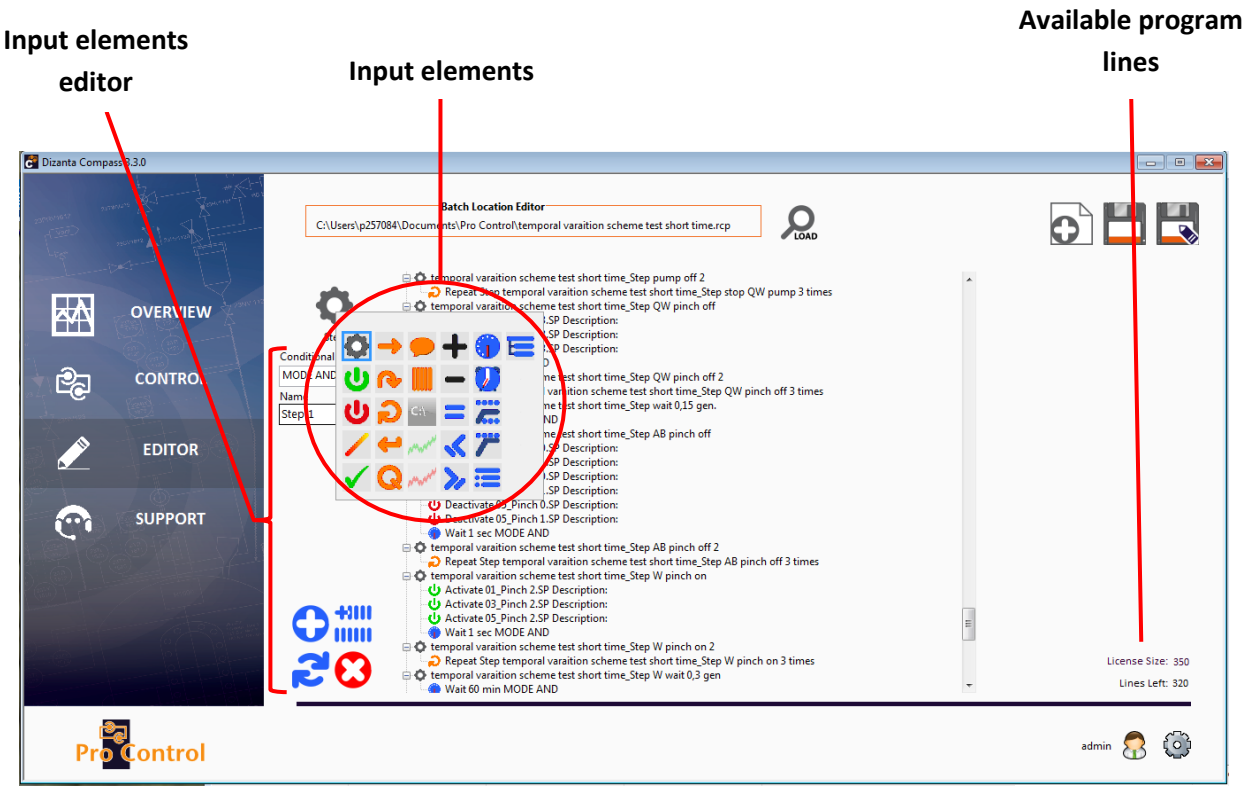
Input elements editor
Input elements
Available program lines
Dizanta Compass 3.3.0
Batch Location Editor
C:\Users\p257084\Documents\Pro Control\temporal varaition scheme test short time.rcp
LOAD
OVERVIEW
CONTROL
EDITOR
SUPPORT
Pro Control
admin
License Size: 350
Lines Left: 320

Fig. 38

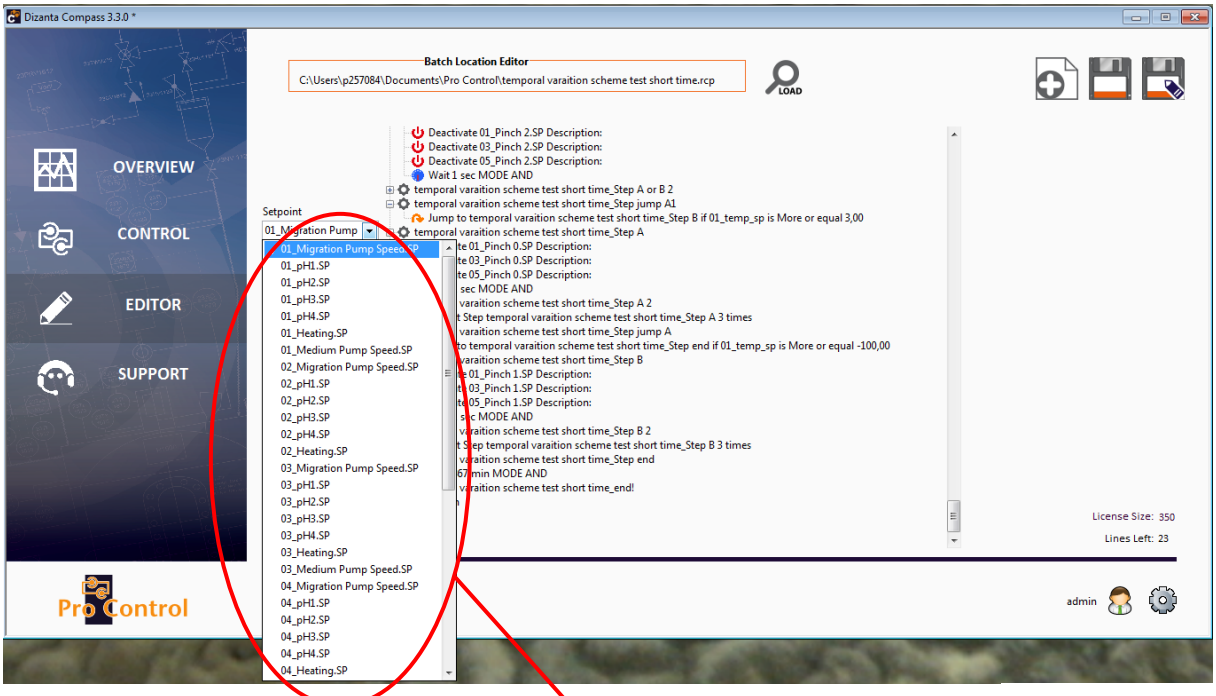
Dizanta Compass 3.3.0 *
Batch Location Editor
C:\Users\p257084\Documents\Pro Control\temporal varaition scheme test short time.rcp
LOAD
OVERVIEW
CONTROL
EDITOR
SUPPORT
Pro Control
admin
Setpoint
01_Migration Pump
01_pH1.SP
01_pH2.SP
01_pH3.SP
01_pH4.SP
01_Heating.SP
01_Medium Pump Speed.SP
02_Migration Pump Speed.SP
02_pH1.SP
02_pH2.SP
02_pH3.SP
02_pH4.SP
02_Heating.SP
03_Migration Pump Speed.SP
03_pH1.SP
03_pH2.SP
03_pH3.SP
03_pH4.SP
03_Heating.SP
03_Medium Pump Speed.SP
04_Migration Pump Speed.SP
04_pH1.SP
04_pH2.SP
04_pH3.SP
04_pH4.SP
04_Heating.SP
License Size: 350
Lines Left: 23
Input and output parameters

Fig. 39

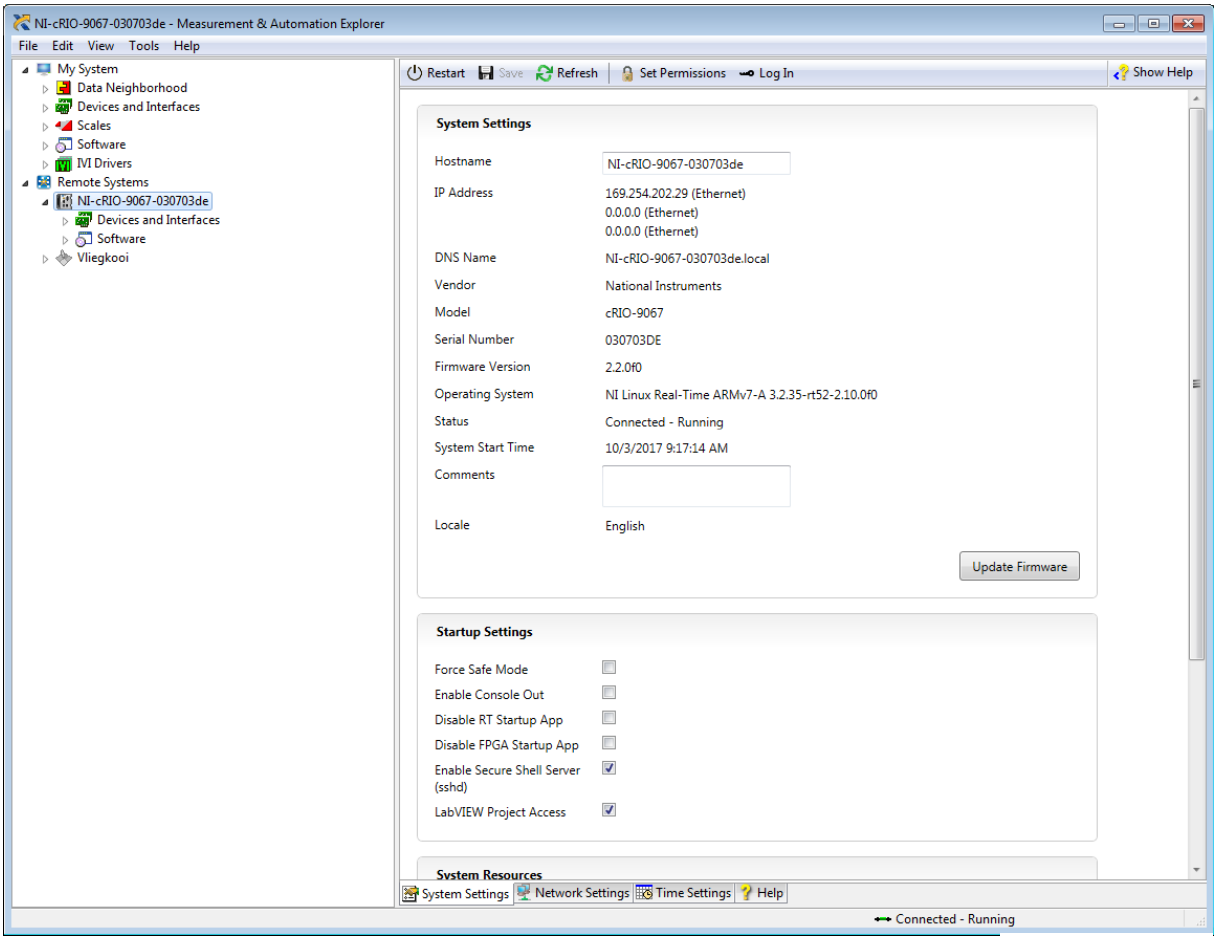
NI-cRIO-9067-030703de - Measurement & Automation Explorer
File Edit View Tools Help
My System
Data Neighborhood
Devices and Interfaces
Scales
Software
IVI Drivers
Remote Systems
NI-cRIO-9067-030703de
Devices and Interfaces
Software
Vliegkooi
Restart
Save
Refresh
Set Permissions
Log In
Show Help
System Settings
Hostname
NI-cRIO-9067-030703de
IP Address
169.254.202.29 (Ethernet)
0.0.0.0 (Ethernet)
0.0.0.0 (Ethernet)
DNS Name
NI-cRIO-9067-030703de.local
Vendor
National Instruments
Model
cRIO-9067
Serial Number
030703DE
Firmware Version
2.2.0f0
Operating System
NI Linux Real-Time ARMv7-A 3.2.35-rt52-2.10.0f0
Status
Connected - Running
System Start Time
10/3/2017 9:17:14 AM
Comments
Locale
English
Update Firmware
Startup Settings
Force Safe Mode
Enable Console Out
Disable RT Startup App
Disable FPGA Startup App
Enable Secure Shell Server (sshd)
LabVIEW Project Access
System Resources
System Settings
Network Settings
Time Settings
Help
Connected - Running

Fig. 40