aquila biolabs

CGQ user guide

A handbook for cell density monitoring in shake flask and bioreactor applications

aquila biolabs

aquila biolabs

Table of contents

aquila biolabs

aquila biolabs

aquila biolabs

aquila biolabs

Introduction and general considerations

Welcome to the CGQ user guide and congratulations for your decision of choosing the CGQ system to monitor your cell cultivation applications. The idea of this user guide is to give you access to all information required to install, integrate and use the CGQ within your laboratory everyday life. Generally, it is strongly recommended to read this user guide prior to any installation or operation works with the CGQ system.

> **Important aspect:**
>
> This kind of grey box will be used throughout the complete document for the indication of important aspects, hints or summaries.

Caveats are indicated by yellow warning signs.

Dangers and risks are indicated by red danger signs.

In order to ensure that this user guide provides you all information you need during your work with the CGQ, we as aquila biolabs are reliant on your feedback. Do not hesitate to contact us to share your ideas regarding errors, missing information or incomprehensibilities so that we can improve this document and keep it up to date with your requirements.

This user guide is under continuous development and should be recognized as preliminary. This user guide, the CGQ device and the CGQuant software may be subjected to changes and improvements without further notice.

aquila biolabs

In case of any question that might arise during the work with CGQ and CGQuant, do not hesitate to contact us (Contact details on pp. 130).

CGQ user guide revisions:

Revision 0	22.09.2015	- initial document
Revision 1	08.06.2016	- minor corrections
Revision 2	24.03.2017	- major changes regarding CGQuant features - major changes regarding firmware updates - disinfection
Revision 3	30.06.2017	- addition of statistical analysis feature - minor changes and corrections
Revision 4	30.11.2017	- addition of experiment restoration - addition of CGQ Monitor application - minor changes and corrections

Revision 5	08.10.2018	- introduction of CGQ BioR, CGQ 2.0 and
		  CGQuant 8.0
		- addition of:
		    - environmental light compensation
		    - multiple base stations
		    - event detection system
		- minor changes and corrections

Revision 6 (current)	22.07.2019	- CGQuant 8.2 with Experiment configurator - minor changes and corrections

This user guide (revision 6) applies only to CGQ devices sold after 01.10.2018 together with CGQuant 8.x. Users of previously sold CGQ devices together with CGQuant 7.4 should consider CGQ user guide revision 4.

aquila biolabs

aquila biolabs

The CGQ system – an overview

The CGQ is an analytical laboratory device that is primarily intended to be used for noninvasive cell density monitoring in real-time, especially in shake flasks (CGQ) and bioreactor (CGQ BioR) applications. However, even completely different applications requiring the real-time analysis of particle concentrations can be addressed by the CGQ system.

Figure 1: The CGQ system – Cell density monitoring for shake flasks and bioreactors.

The CGQ system consists of three different main components, the sensor plate, the base station and the CGQuant software. Each component serves a different purpose. The sensor plate is the physical heart of the CGQ-system, as it performs the measurements and subsequent initial data processing tasks. Several sensor plates are connected to the base station, which organizes all the communication traffic between the CGQuant software and the sensor plate. Furthermore, the base station acts as the central power supply for all the connected sensor plates. Finally, the CGQuant software is the user's window to the world of high precision real-time analysis of cell densities in a broad variety of cultivations processes and applications. CGQuant does all the visualization, data analysis, post processing, exporting, reporting and controlling that is required in order to provide all CGQ users with an intuitive and comfortable solution to watch, analyze and store their experimental data.

aquila biolabs

Absolute maximum ratings

Power supply input

voltage	90 – 264 VAC (47 – 63 Hz)
current	1 – 2 A

Power supply output

voltage	5 VDC
current	6 A

Power supply operating temperature   -30 – 60 °C

CGQ base station input

voltage / ripple	5.0 VDC / 0.1 VDC

CGQ base station output

voltage	5.5 VDC
current	3 A

CGQ operating temperature (tested)   10 – 50 (75)[1] °C

[1]   The CGQ has been operated for weeks at 75°C without any noticeable damaging; however there is currently not enough experience with such high temperatures to specify them as typical operation condition. While it is not guaranteed to work at 75°C, the CGQ will do that in most expectable cases. Operating the CGQ above 50°C requires special darkening covers, which can withstand elevated temperatures and which are not part of the standard device.

aquila biolabs

CGQuant hardware requirements

CPU	current dual core @ 2 GHz/core or better
RAM / HDD	≥8 GB / ≥10 GB
Operating system	Microsoft Windows 7 or newer with .NET 4.7
Screen resolution	1920 x 1080 pixels

For a good user experience, it is strongly recommended to run CGQuant on processors not older than from 2012.

The CGQuant performance requirements scale with the number of flasks being monitored in parallel. For highly parallelized systems it is therefore recommended to use processors that are optimized for performance and not for energy efficiency (as many mobile processors).

The CGQuant performance and user experience can be adjusted for low performance systems in the preferences window by:

- decreasing the number of visible points per chart
- increasing the general sampling period
- increasing the auto-backup interval.

It is strongly recommended to disable automated updates in the Windows operation system settings, to prevent undesired system restarts upon Windows Update events. You should however regularly check for Windows updates and install them manually to keep your operating system up-to-date.

It is recommended to use the "High performance" power plan in the Windows power options.

You can also contact us to verify your chosen system settings.

aquila biolabs

Recommended operating conditions

Temperature	10 – 50 °C
	(Ensure to let the sensor plates adjust to the operating temperature for 30 min.)
Humidity (relative)	0 – 80% (non-condensing)

Shake flask filling volume

optimal range	10 – 15%
good range[1]	5 – 25%
applicable range[2]	2 – 30%
extended range[3]	0 – 50%

Shaking speed

optimal range[4]	150 – 350 rpm
using screws	
shaking diameter ≤ 2.5 cm	0 – 350 rpm
	0 – 250 rpm
shaking diameter ≤ 5.0 cm using sticky pads[5]	0 – 200 rpm

[1]   Measurement quality should be as good as for the optimal range, in few cases slightly reduced precision or weak artifacts might be observed.

[2]   Measurement quality should be acceptable, in some cases reduced precision or artifacts might be observed.

[3]   Measurement quality can be acceptable, in many cases reduced precision or artifacts might be observed, filling volumes above 50% shouldn't be used to avoid spilling of the liquid during shaking.

[4]   Use these speeds for optimal measurement results, for other shaking speeds within the general specification range, in few cases slightly reduced precision or weak artifacts might be observed.

[5]   To ensure safe shaking condition, always refer to the user guides of your sticky pad manufacturer, if recommended shaking speed limits in the sticky pad user guides are lower than those denoted here, use only those shaking speeds specified in the sticky pad user guides

aquila biolabs

Environmental light[1]

	optimal CGQ performance[2]	darkened by CGQ covers
- with shake flasks[3]		coverless
- with bioreactors[4]		coverless
- phototrophic cultivations[5]		

[1] The CGQ actively compensates environmental light. Depending on the application specific environmental light and cultivation conditions, this compensation may be incomplete. Constant environmental light can be compensated efficiently by the CGQ. Strong changes of the environmental light intensity (e.g. from absolute darkness at night to bright daylight at a sunny mounting positon) may be visible as step-like artifacts in the measurement data.

The susceptibility for environmental light induced measurement artifacts may be increased for environmental light transitions, which include periods of absolute darkness with pronounced artifacts especially in shaken cultivation systems.

[2] Optimal measurement performance with regard to sensitivity at low cell densities, optimal signal-to-noise ratio and minimized number and size of measurement artifacts.

[3] In accordance with the above remarks, shake flask cultivations should be performed with CGQ covers, when operated under environmental light conditions with periods of absolute darkness.

[4] In accordance with the above remarks, bioreactors as non-shaken systems are less susceptible to strong changes of environmental light intensities.

[5] In accordance with the above remarks, phototrophic cultivations provide constantly defined environmental light conditions, which can be efficiently compensated by the CGQ. The limit of environmental light compensation is the CGQ's saturation intensity, which may be reached under certain phototrophic lighting conditions.

Recommended operating conditions

aquila biolabs

Do not use the CGQ system or any of its components in water bath shakers! This might result in electric shocks, which could damage your health, the CGQ and any other electric device around.

Do not look into the beam of any of the CGQ sensor plate LEDs! Their emitted light is of high intensity and might damage your eye or retina. Wear protective eye wear. Avoid direct eye and skin exposure to the CGQ sensor plate LEDs! Always keep a safety distance of >1m to any active CGQ sensor plate LED. Always pause or stop running measurements before operating within this safety distance.

CGQ sensor plates (e.g. CGQ BioR) may contain IR-LEDs emitting invisible high intensity light. Do not look into any CGQ sensor plate LED to avoid the exposure of your eyes or skin to dangerous invisible infrared light. Sensor plates carrying IR-LEDs are labeled with the following warning sign:

Do not touch, wet or electrically bridge the base station connectors, especially when the base station is connected to a power supply! This might result in damage to your health and/or the CGQ device.

Do not connect any sensor plate via its USB connector, while the sensor plate is connected to the base station at the same time! This might result in damage to the CGQ and any other device connected to it via USB.

Be careful when mounting CGQ sensor plates into spring clamps to avoid injuries such as contusions, cuts or bruises.

Be careful when handling the shake flask covers. They might have sharp edges that might cause injuries such as cuts.

aquila biolabs

Always stop the shaker before handling the CGQ device or the shake flasks. For mounting and dismounting purposes, the shaking should be turned off to avoid damage to your health and to the devices and flasks on the shaker.

Do not spill liquids over any of the CGQ components. Especially the sensor plates might get damaged or the windows above the sensors might become dirty, thus negatively influencing the following measurements.

Do not use inorganic or organic acids and bases, organic solvents or detergents to clean the CGQ! Some solvents or detergents might be allowed for cleaning, but you should only use those being mentioned in the user guide.

Any kind of opening, manipulating or copying CGQ devices as well as decompiling, reverse-engineering, copying or distributing CGQuant or the CGQ firmware components is strictly prohibited in accordance with German and international law and may lead to compensation claims.

Warnings

aquila biolabs

Declarations and certificates

CE conformity

The aquila biolabs GmbH, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany, herewith declares under its sole responsibility that all devices and equipment being part of the CGQ system and being manufactured by the aquila biolabs GmbH are in conformity with the Council Directives regarding

- Low Voltage Equipment Safety (2014/35/EU)
- → evaluated on the basis of EN 61326-1:2013 and EN 62471:2009
- Electromagnetic Compatibility (2014/30/EU)
- → evaluated on the basis of EN 61010-1:2010
- Restriction of certain Hazardous Substances (2011/65/EU)
- → evaluated on the basis of EN 50581:2012.

This declaration applies to all products with the following identifiers:

CGQ sensor plates:

- CGQ-SP-100-BV   Revisions: 1.00
- CGQ-SP-250-BV   Revisions: 1.00
- CGQ-SP-500-BV   Revisions: 1.00
- CGQ-SP-1000-BV   Revisions: 1.00
- CGQ-SP-2000-BV   Revisions: 1.00
- CGQ-SP-F   Revisions 1.00, 1.10
- CGQ-SP-B   Revisions 1.00, 1.10

aquila biolabs

CGQ base stations:

- CGQ-BS-8-BV   Revisions: 1.00, 1.10
- CGQ-BS-16-BV   Revisions: 1.00, 1.10

Technical documentation is maintained at the aquila biolabs GmbH headquarter in Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany.

Date of declaration: 14.09.2018
Name, position of the undersigned: Konrad Herzog, Managing Director
                                     Research & Development

aquila biolabs

WEEE conformity

WEEE-Registration-No.: 61144888

The aquila biolabs GmbH, Arnold-Sommerfeld-Ring 2, 52499 Baesweiler, Germany, herewith declares compliance of all electronic components of the CGQ system with the Council Directive 2012/19/EU.

Electronic components may contain various hazardous substances that could possibly exhibit negative impacts on your health and the environment. In order to avoid those effects aquila biolabs encourages you to make use of the appropriate local take-back and recycling systems for disposing electrical and electronic equipment. By doing this you are furthermore significantly reducing the pressure on natural resources and thus preserve our planet for subsequent generations.

Components that are affected by this declaration carry the following pictogram:

Date of declaration: 02.11.2015
Name, position of the undersigned: Konrad Herzog, Managing Director
Research & Development

aquila biolabs

FCC compliance

This is a Class A product.

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions:

(1) this device may not cause harmful interference, and

(2) this device must accept any interference received, including interference that may cause undesired operation.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

aquila biolabs

Installation, disinfection and quick start

Installing the CGQ components on your shaker

Using screws

Typically, the CGQ device is installed on a shaker tray that contains screwing threads. However, as the tray and screwing thread geometries vary between different shaker manufacturers, there are some things to keep in mind when planning to install the CGQ using screws.

The base station's bottom plate exhibits three elongated holes, enabling its installation on each kind of shaking tray using screws. When installing the base station, just move it over the tray until at least two screwing threads are visible through the elongated base station bottom plate holes. Then use appropriate screws that fit your trays screw threads to fix the base station on the tray.

> ⚠️ **Avoid the usage of countersunk head screws, as they might destroy the base station bottom plate when being screwed too tightly.**

> ⚠️ **In many cases, it might be reasonable to use a washer per screw for the base station installation. Doing this will reduce the mechanical load at the edges of the elongated holes and might thus increase the stability and lifetime of the base station bottom plate.**

The CGQ sensor plates (standalone or in an adapter, see Figure 2) require certain types of metal clamps to be mounted properly. If your shaker is already equipped with appropriate clamps, you can directly go to page 25, where the CGQ sensor plate installation is described.

If your shaker has a screwing thread geometry and arrangement that fits the clamps being optionally delivered with CGQ sensor plates, you can directly

aquila biolabs

install these clamps according to your usual clamp installation process. Otherwise you have to use the CGQ sticky plate adapters (STCA, see Figure 2). First remove the protection foil and position the adapter in a way that at least two tray screwing threads are fully visible through the adapter's elongated holes. Now fix the adapter on the tray using appropriate screws.

Figure 2: A sticky plate adapter STCA250 (left) and a CGQ sensor plate mounted into an adapter ADAP250 (right).

Avoid the usage of countersunk head screws, as they might destroy the adapter when being screwed too tightly.

In many cases, it might be reasonable to use a washer per screw for the clamp mounting adapter installation. Doing this will reduce the mechanical load at the edges of the elongated holes and might thus increase the adapters stability and lifetime.

Using sticky pads

In order to install the CGQ base station on a sticky pad, just place it on the pad with its black bottom plate towards the shaking tray. Push down the base station to make sure that it is in good contact with the sticky pad. At higher shaking frequencies, it is recommended to request an alternative bottom plate from aquila biolabs, which allows to mount the base station on its backside.

aquila biolabs

Avoid the accumulation of dust or any other kind of dirt at the base station's bottom plate or backside, otherwise the adhesive strength between sticky pad and base station might be weakened. The base station can be cleaned using a soft, damp cloth with water. Persistent dirt can be removed using ethanol or isopropanol instead of water. Always ensure that the base station is completely dry before powering it.

In order to install the CGQ sticky plate adapter (ASTC) on a sticky plate, first remove the protection foil from the adapter bottom. Then place the adapter on the pad and push it down to make sure that it is in good contact with the sticky pad. Premounted sensor plates can stay inside the sticky plate adapters during attachment to the sticky pad.

Avoid the accumulation of dust or any other kind of dirt at the sticky plate adapter's bottom, otherwise the adhesive strength between sticky pad and adapter might be weakened. The sticky plate adapter can be cleaned using a soft, damp cloth with water. Persistent dirt can be removed using ethanol or isopropanol instead of water. Please wipe away any access ethanol or isopropanol to avoid chemically induced cracks of the adapter.

For both, the base station and the sticky plate adapter, please check the following points before starting the shaker.

Ensure that all sticky plate adapters and base stations are fully placed on the sticky pad, otherwise the adhesive strength between sticky pad and component might be weakened, imposing a risk for detachment during the shaking.

Ensure that all components are properly placed and pressed onto the sticky pads to ensure safe shaking conditions. Otherwise there is a risk for detachment during the shaking.

aquila biolabs

⚠ All components to be installed on a sticky pad should be absolutely dry and free of any liquids on the surfaces. Otherwise there is a risk for detachment during the shaking.

⚠ After installation of all components check the adhesive strength and proper installation of the components at the desired shaking speed without any shake flasks mounted into the sticky plate adapters.

To remove the CGQ from a sticky pad shaker, follow the recommendations of the sticky pad manufacturer. Prior to removal disconnect all cables. Generally, you can remove both, the base station and the sticky plate adapter by pulling constantly until the component detaches from the sticky pad.

⚠ Do not use liquids for the removal of the base station from a sticky pad shaker. The liquid might damage the base station electronics and, if connected to the power supply, your health.

aquila biolabs

Mounting a sensor plate
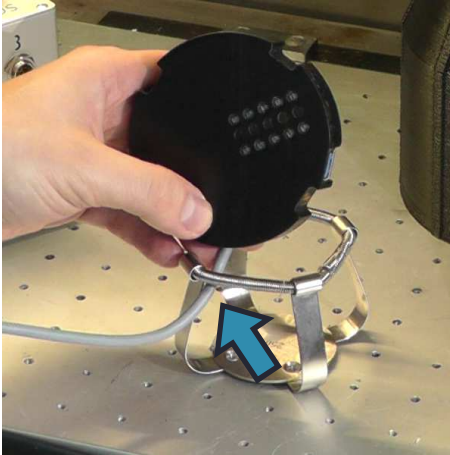
Take the sensor plate (disconnected from the base station) and hold it above the spring clamp with the sensor array facing you. Then pull the sensor plate cable from inside through the foremost clamp opening to outside and move the cable end to the left.

Now turn the sensor plate anticlockwise towards the cable end, until the fixing notch between USB-port and cable is located above the right spring clamp metal clip.

aquila biolabs
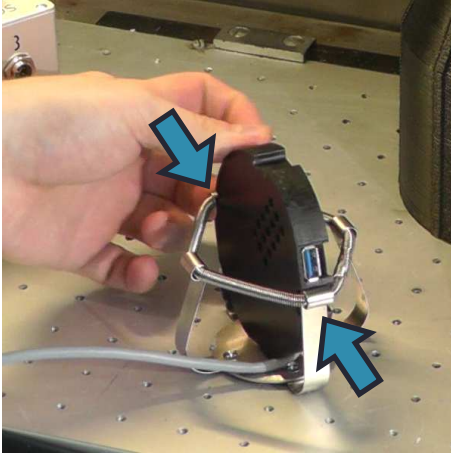
Move down the sensor plate until the spring clamp metal clip touches the notch between USB-port and cable. Then move down the opposite side of the sensor plate accordingly. This will slightly expand the spring clamp.
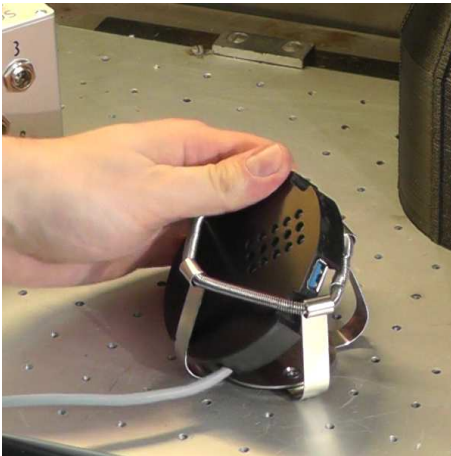
Now turn the sensor plate clockwise around an imaginary axis between the two contacted spring clamp metal clips.

aquila biolabs
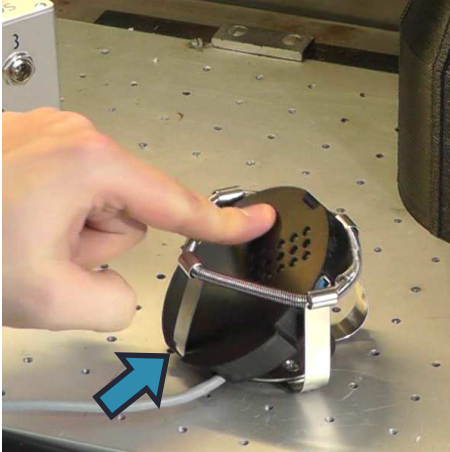
Make sure that the fixing notch at the left side of the sensor plate cable fits the position of the according spring clamp metal clip. Then push down the sensor plate.
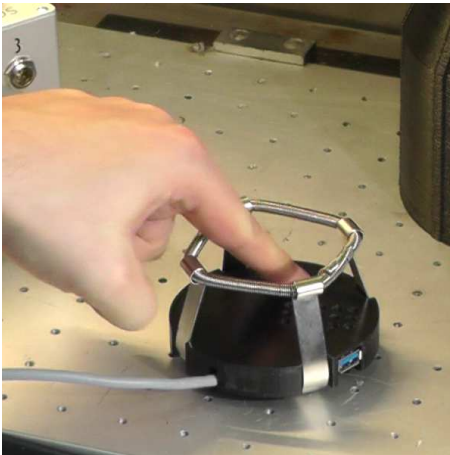
The sensor plate finally snaps into the spring clamp and is correctly mounted for further usage of the CGQ device.

Be careful when mounting or removing sensor plates into or from spring clamps to avoid injuries such as contusions, cuts or bruises.

aquila biolabs

It is strongly recommended to bend the single spring clamp clips inwards after mounting the CGQ sensor plate. Doing this will improve the shake flask fixation in the clamp, thus preventing unwanted detachment of shake flasks during operation.

Make sure that the sensor plate cable is not pinched between sensor plate and spring clamp. Otherwise the device might be damaged during shaking.

Before starting a new fermentation at a temperature different to the current sensor plate temperature, ensure that the sensor plates have adjusted their temperature to the desired fermentation temperature. Usually 30 minutes of temperature adjustment prior to fermentation should be sufficient to avoid temperature induced artifacts in the cell density data.

aquila biolabs

Mounting the shake flask and the flask cover

⚠️ Before starting a culture, please ensure that the utilized cultivation medium is roughly at the same temperature as the cultivation temperature in the shaker. If a cold liquid is used and put into a warmer shaker, condensation might occur at the flask bottom and over the sensor array. Condensation droplets in the optical path will cause strong measurement artifacts, rendering the cell density measurements useless.

⚠️ Do not inoculate your flask by throwing a pipette tip or anything similar into the shake flask. For an optimal measurement result, ensure that there are no objects (e.g. pipette tips, feeding capsules, etc.) floating in the liquid in the shake flask.
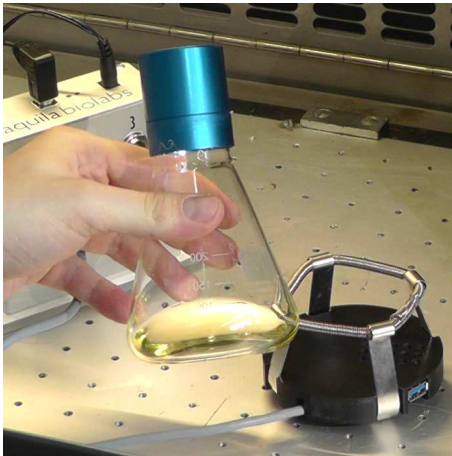
Prepare your shake flask with cultivation medium and inoculate it.

29
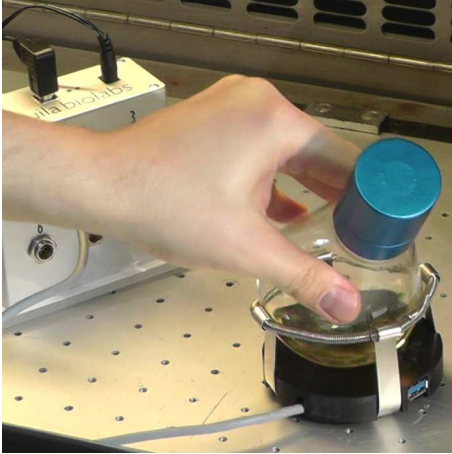
aquila biolabs

Mount the shake flask into the spring clamp as usual.
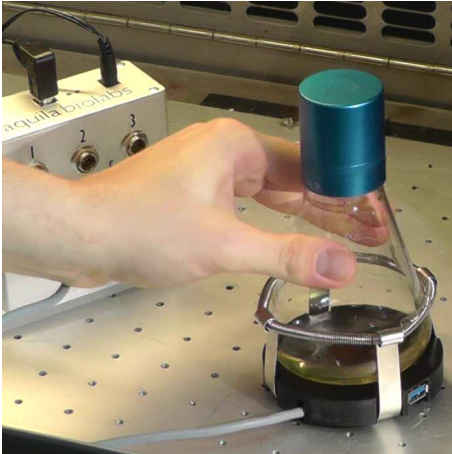
Turn the shake flask around its vertical axis until neither the white labeling area nor potentially present baffles are located above the sensor array.

aquila biolabs
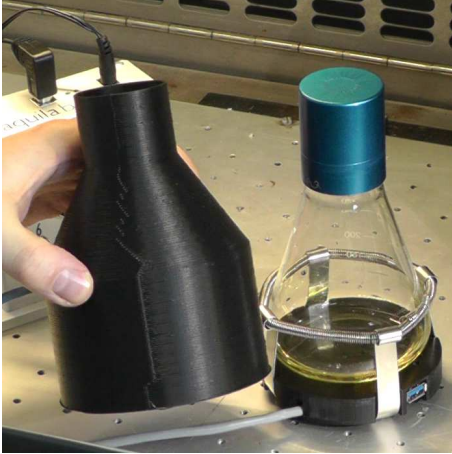
Put the flask cover onto the shake flask and make sure that the sensor plate cable is going through the respective small opening at the bottom of the flask cover.
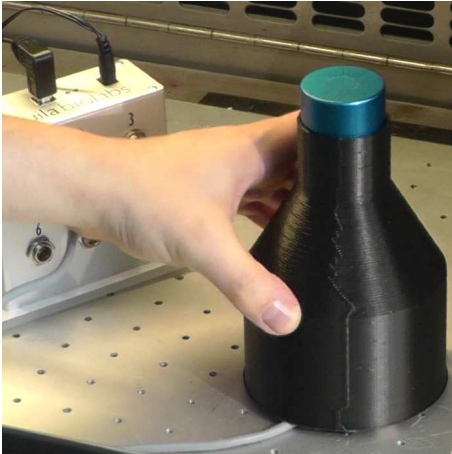
Now you are ready with the hardware related works and you can start setting up the measurement in the CGQuant software.

aquila biolabs

Cable management within the shaker

Shaking movements create a constant mechanical load for the CGQ USB and power supply cables, which must be minimized by suitable cable routing inside the shaker.

⚠ **Always mount CGQ cables with sufficient play, to reduce the mechanical load of the CGQ base station connectors.**

⚠ **Always mount and route the CGQ cables inside the shaker in a way that prevents strain on the cables at any time during the shaking movement.**

⚠ **Always ensure that the CGQ USB and power cables are routed with sufficient play before starting the shaking movement.**

⚠ **CGQ USB and power cables should be fixed to the inner wall of the shaker to maintain the state of sufficient play during shaking.**

⚠ **Proper mounting and cable routing within the shaker should be evaluated at low speeds, before starting the intended shaking movement.**

Figure 3: Routing of Power elongation and USB cable.

Power elongation and USB cable should be routed through the front door of your shaker or through side wall openings, as depicted in Figure 3. Use the cable clamp array from your CGQ package as shown in Figure 4 and resize it according to your requirements. It can either be screwed or glued to position.

aquila biolabs
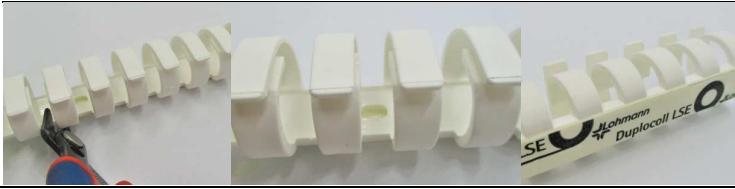
Figure 4: Cable clamp array customization (left), screw mounting hole (center) and sticky back (right).

Use the cable clamp array to guide the cables as depicted below. Both cables must have enough slack between the point where they are held by the clamp and the base station. Both cables must be fastened to the base station tightly.
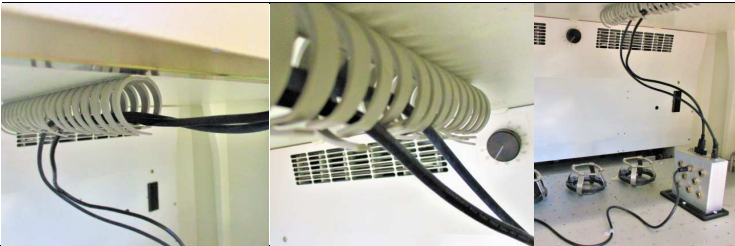
Figure 5: Cable management inside the shaker.

The sensor plate cables should be routed properly on the shaker tray to not hit the wall of the shaker while shaking and should be fixed on the tray using the screwable cable clamps as shown in Figure 6. Ensure that the sensor plate cables have enough slack before connecting them to the base station.
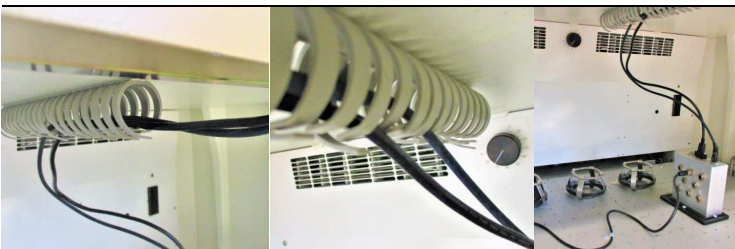
Figure 6: Sensor plate cable routing on the shaking tray.

aquila biolabs

Installing the CGQ on a bioreactor

The CGQ BioR is equipped with a thick layer of elastic foam and with a mounting belt. Both together allow you to install the CGQ BioR at almost any bioreactor in your lab. Initially you need to find a free glass or transparent plastics spot on your bioreactor. Then hold the CGQ BioR with its sensing window onto this transparent spot and fix it there by putting the belt around your bioreactor (or parts of your bioreactor). Finally ensure that the belt is closed firmly. An exemplary setup is shown in Figure 7. If the Velcro belt is too short, please use a larger one or combine multiple belts to get a sufficiently long belt for CGQ BioR mounting. In case of mounting problems, do not hesitate to contact us for technical support.

Figure 7: CGQ BioR with mounting belt - unmounted (left) and mounted (right).

⚠️ Do not mount the CGQ BioR on top of marks or labels on the vessel, as this may negatively influence the data quality.

⚠️ Try to avoid a mounting position, where steel reflective steel components are directly in front of and in short distance (<20 mm) to the CGQ BioR.

⚠️ Always ensure that the CGQ BioR sensor window is completely covered by liquid inside the vessel during your complete process. Foaming and high gas hold-up can significantly interfere with the desired light scattering from growing cells.

34

aquila biolabs

Installing the CGQuant software

On your own PC or laptop

In order to install CGQuant and all required drivers on your PC or laptop, please execute the CGQuant setup executable with administration privileges. Follow the installation guidance provided by the installation routine. You must accept aquila biolabs' End User License Agreement to successfully install all software components of the CGQ system.

On a PC or laptop delivered together with your CGQ system

All PCs or laptops that are delivered by aquila biolabs GmbH in combination with at least one CGQ system have CGQuant preinstalled. There is no additional CGQuant installation required. Everything is prepared for you, so you can directly start your work with the CGQ and CGQuant.

aquila biolabs

Disinfection

The base station (except the bottom plate), spring clamps, ADAP adapters and cables can be disinfected by wiping them softly with 70% ethanol wipes.

The CGQ sensor plates CGQ-SP-100-BV, CGQ-SP-F and CGQ-SP-B (see backside label) can be disinfected by wiping them softly with 70% ethanol wipes.

The CGQ sensor plates

- CGQ-SP-250-BV (see backside label)
- CGQ-SP-500-BV (see backside label)
- CGQ-SP-1000-BV (see backside label)
- CGQ-SP-2000-BV (see backside label)

must not be disinfected using organic alcohols, such as ethanol, propanol, or iso-propanol. These sensor plates have optical windows made of PMMA, which tends to break under the influence of high concentrations of such compounds. You can disinfect these sensor plates using special PMMA disinfectants, e.g. Bacillol® 30 Tissues. Also the bottom plates of base station and sticky plate adapter (ASTC) are made of PMMA and should be disinfected using special PMMA disinfectants.

aquila biolabs

Quick start tutorial

After having successfully installed the CGQ hardware on your shaker or bioreactor and the CGQuant software on your computer, you are now prepared to start using the CGQ system.

1. Connect the base station

- Connect the power supply elongation cable to the base station and to the power supply.
- Connect the power supply to the power supply elongation cable and to the electrical outlet.
- Tighten the cap nut of the power supply plug on top of the base station
- Connect the USB cable to the base station and to the computer.
- Tighten the USB plug screws to fix the USB cable.
- As soon as all connections are established, the green LED should start glowing and the CGQ should enumerate as COM device on your computer.

Try to find a good way to guide the cables out of the shaker. The goal must be to reduce the cables' shearing forces during shaking as much as possible, thus ensuring an optimal contact between cable plug and base station socket. Often it is a good practice to take some duct tape for fixing the cables inside the shaker on the shaker wall and then to guide them e.g. through a small slit in the shaker door or through a hole in the shaker wall towards the outside.

aquila biolabs

2. Mount and connect the sensor plate

- Mount the sensor plate into a suitable spring clamp (pp. 25) or at the side wall of your bioreactor (pp. 34).
- After mounting the sensor plate, connect it to a free base station connector.
- Remember the input number of the connection; you will need it when setting up the measurement in the CGQuant software.

3. Mount the shake flask and cover or inoculate your bioreactor

- Mount your inoculated shake flask on top of the sensor plate into the spring clamp (pp. 29).
- Now put the flask cover onto the shake flask and make sure that the sensor plate cable is going through the respective small opening at the bottom of the flask cover.

- If you are working with a CGQ BioR, inoculate and prepare your bioreactor.

-

The shake flask should be oriented in a way that neither the white labeling area nor potentially present baffles are located above the sensor array. You can simply rotate the flask until the labeling area is pointing towards the side where the cable is leaving the sensor plate. Wrong positioning might cause measurement artifacts (pp. 52).

Do not mount the CGQ BioR on top of marks or labels on the vessel, as this may negatively influence the data quality.

38

aquila biolabs

4. Start your cultivation

- All hardware related works have been done right now, so you can close and start your shaker.

- If you are working with a CGQ BioR, you can now start your bioreactor.

-

5. Start the CGQuant software

- Turn on the PC or notebook, which is connected to the CGQ base station via USB.
- Double-click the CGQuant icon on your desktop to start the CGQuant software.

cgQuant

6. Wait for the connection between CGQuant and CGQ

- Upon startup, CGQuant will try to establish a connection with the CGQ base station. This procedure may take some seconds.
- After successful connection the "Connection"-icon of one base station port starts glowing and in the overview screen, all CGQ device information are displayed.

aquila biolabs
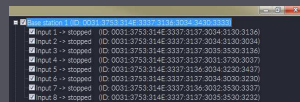
7. Open a new experiment window

- Start a new experiment by clicking the "New Experiment" button. From the dropdown menu select the base station, which is connected to your sensor plate of interest.
- A new experiment window will appear together with an empty process information form.

8. Select an input

- Select an input according to the connector number on the base station, where the sensor plate of your current experiment is connected.
- The numbers that are shown in the list represent all available inputs, which are currently not used by any running measurement.

aquila biolabs
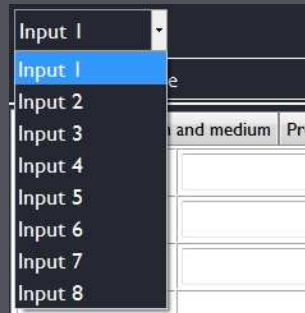
9. Select a category for the filename

- Select a category from the
  process information window to
  be used as filename of the .cgqxf
  - file holding all measurement
  and documentation data of your
  current experiment. Default
  selection is "Experiment name".
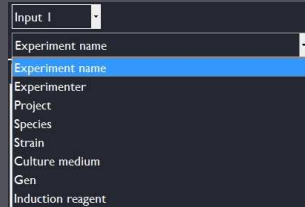
10. Save process information

- Save the process information by
  clicking the "Save and exit"-
  button in the right lower corner
  of the process information
  window.
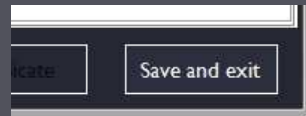
11. Start the CGQ measurement

- Start the measurement by
  clicking the "Start"-button in
  the toolbar on the right. Upon
  measurement start, the icon of
  this button will turn into a
  "Pause"-icon, a current OD will
  be displayed, the experiment
  time is running and data points
  appear in the chart.

aquila biolabs
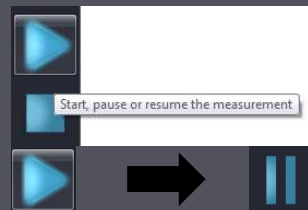
12. Stop the CGQ measurement

- As soon as your cultivation is finished, stop the CGQ experiment by clicking the "Stop"-button.
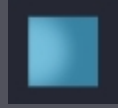
If you decide to stop the measurement you cannot restart it in the same experiment window. If you just want to pause the measurement, click the pause button instead.

13. Export the data to an .xlsx file

- Export your last measurement to an xlsx-file by clicking the "xlsx"-button. The button will start to blink until the export is done. The exported file will be automatically opened afterwards by your default xlsx-opening program (e.g. MS Excel, LibreOffice Calc, etc.)

14. Close the experiment window

- Close the current experiment window by clicking the small blue cross in the upper left corner.

aquila biolabs

CGQ's basic measurement principles

Optical cell density measurements

The CGQ's technique for noninvasive cell density monitoring in shake flasks is based on the principle of light scattering. Basically, each measurement consists of a sequence of three major steps. (1) Light is irradiated by a LED into the fermentation broth through the transparent flask bottom. While most of the photons go straight through the liquid, some interact with the cells and are scattered towards different directions. (2) The backscattered photons are then detected by a photodiode, which converts the scattered light intensity into a weak electric current.
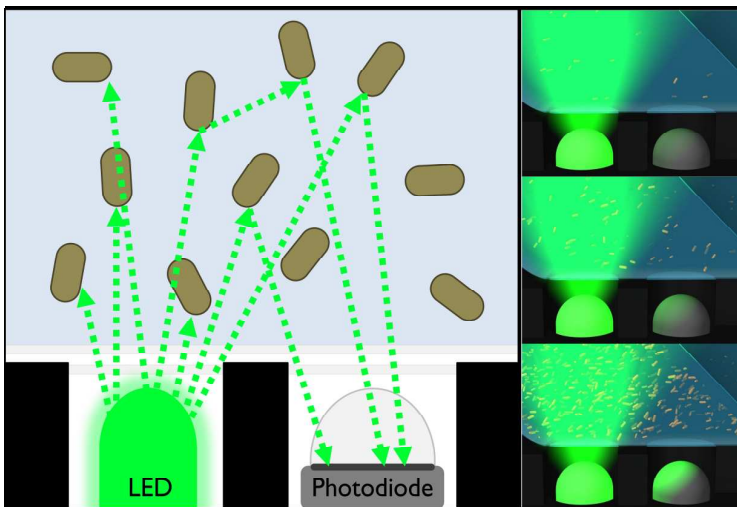
Figure 8: Optical cell density measurement using light scattering techniques.

(3) This initial raw signal is subsequently amplified and subjected to various analytical algorithms on the CGQ sensor plate, finally yielding the current cell density. The higher the cell density in the fermentation broth, the higher is the

43

aquila biolabs

probability of an incident photon to interact with a cell, so that with increasing cell density more light is scattered towards the photodiode.

Each CGQ sensor plate comprises highly sensitive sensorics implemented around an array of LEDs and photodiodes. The CGQ measurements of backscattered light allow for cell density measurements in the range of 0.1 OD_{600} and on the other hand enable you to monitor fermentations that grow beyond 150 OD_{600}. In contrast to typical transmission-based spectrophotometers, the light scattering analysis implemented in the CGQ guarantees cell density measurements over three orders of magnitude without any requirements for diluting or concentrating steps. The CGQ system consequently provides you a simple, efficient and accurate opportunity to monitor biomass concentrations of your shake flask fermentations noninvasively and without the need for additional devices.

With the newly introduced sensor plates for shake flasks (CGQ-SP-F) and bioreactors (CGQ-SP-B, BioR), the CGQ is now equipped with technology and algorithms to distinguish between environmental light and the backscatter signal, thus allowing for environmental light compensation. This is essential for cell density monitoring at bioreactors, which cannot be covered and darkened as easily as shake flasks. Furthermore, the CGQ is now applicable for phototrophic cultivations, as long as the photosynthetic light is not saturating the CGQ sensors. The environmental light compensation is intended for operating conditions with constant or intermediately changing environmental light intensities. Strong intensity changes, especially with periods of absolute darkness, may cause step-like artifacts in the measurement curves. For optimal measurement performance, the use of CGQ covers to darken the shake flasks is still highly recommended. Please also refer to the recommended operating conditions (pp. 13)

aquila biolabs

While the standard CGQ peak wavelength is 521 nm, the CGQ BioR plates contain an additional infrared LED (940 nm). The probability for infrared photons to be scattered by cells is lower than for the standard green light photons, which provides you with a unique opportunity to monitor the growth of high cell density fermentations in the bioreactor with achievable cell densities of 250 OD_{600} (150 g/L) and beyond.

Furthermore, the wavelength of each CGQ sensor plate is customizable during the ordering process according to Figure 9. Depending on application specific requirements, you can choose your preferred wavelength together with our application specialists to avoid an interference of backscatter measurements with secreted or cellular chromophores or fluorophores, such as chlorophyll, heme proteins, GFP, etc.

Position 3: CGQ & BioR – 521 nm
Position 2: CGQ – 521 nm, BioR – 940 nm
Position 1: CGQ & BioR – 521 nm
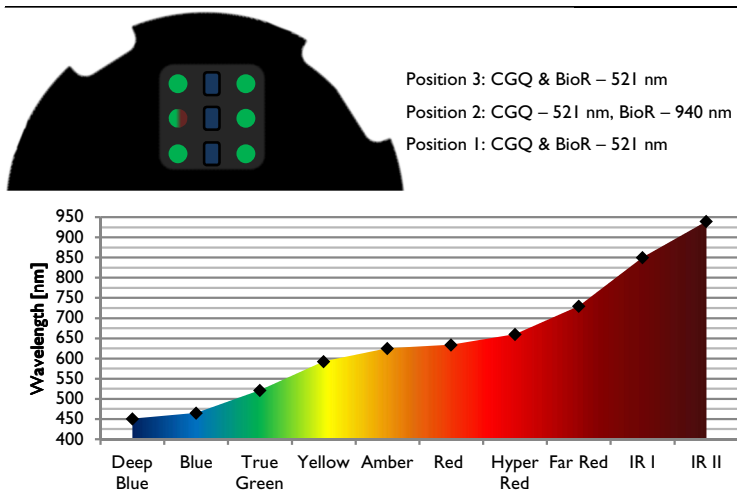
Figure 9: Standard LED colors at the different CGQ measurement positions and available LED colors for wavelength customization of CGQ sensor plates.

aquila biolabs

Challenges in shaken and stirred environments

Dynamic and heterogeneous liquid distributions are a key characteristic of shake flask fermentations. Performing highly accurate optical measurements under conditions of continuous shaking is therefore not trivial. Especially the huge variance of observable liquid distributions (for examples see Figure 10), which depend on a variety of different factors (e.g. flask size, flask shape, liquid volume, shaking frequency, shaking radius, liquid viscosity, temperature, etc.), requires adaptive sensor systems and measuring methods. The CGQ combines these two aspects with extensive data analysis to allow cell density measurements under almost every imaginable shaking condition. Using an array of LEDs and photodiodes, each CGQ sensor-plate can adaptively measure the backscattering intensity at different radial positions, thus accounting for a broad variety of liquid distributions in shake flask fermentations.
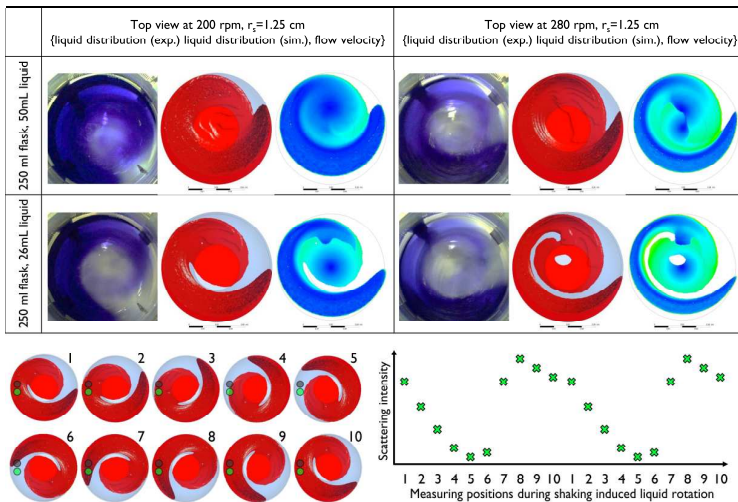
Figure 10: Liquid distribution and CGQ's cell density monitoring principle in continuously shaken systems.

aquila biolabs

The CGQ does not treat the moving-liquid-induced backscattering signal fluctuations as noise, but as a source of valuable information. For determining one cell density value, the CGQ sensor plate collects about one million of single backscattering intensities per second; thereby creating a series of data points that combines information regarding cell density and liquid distribution on a microseconds-resolution. This basic CGQ measurement principle is schematically depicted in the lower parts of Figure 10. Intensive data analysis is performed on the resulting raw data series, finally yielding a single cell density value. Featuring ultrafast data collection, adaptive data analysis and measuring positions, the CGQ provides you with the unique opportunity of accurate real-time cell density monitoring in continuously shaken systems.

While dynamic liquid distribution is a negligible issue in stirred tank bioreactors, the CGQ BioR is subjected to other bioreactor specific challenges. Due to active aeration and stirring, bioreactors tend to foam much heavier than shake flasks, thus requiring the addition of antifoam. Both, foam and antifoam act as additional scatterers to the CGQ BioR and need to be taken into consideration during data interpretation. Depending on the aeration rate and stirrer speed, the bubble size and distribution change over time and can create a considerable background noise in CGQ BioR measurements. The increase in fermentation system complexity from shake flask to bioreactor also increases the number of factors that may influence the CGQ backscatter measurements. The implications of these factors on data acquisition and interpretation are discussed in a separate chapter on pp. 52.

Challenges in shaken and stirred environments

aquila biolabs

Correlation between OD_{600} and the CGQ signal

A typical measure for cell density is the optical density, often referred to as OD_{600} at a measurement wavelength of 600 nm and given by Lambert-Beer's law as

$$c = \frac{\log_{10}\left(\frac{I_o}{I_1}\right)}{\varepsilon_\lambda \cdot d} \qquad \{I\}$$

with I_0 the incident light intensity, I_1 the transmitted light intensity, ε_λ the extinction coefficient at a given wavelength λ, c the concentration of the substance to be measured and d the transmitted path length. As widely known, Lambert-Beer's-law holds true only for homogenous dilute solutions, so that concentrations above 0.5 OD_{600} need to be diluted prior to the transmission measurements and scaled accordingly after the measurement. The background of this range limitation is an increasing contribution of other optical effects, especially of multiple scattering, to the transmissivity, which is not described by Lambert-Beer's law.

As described above, the CGQ measures backscattered light intensities. While scattering signals can be used to measure particle concentrations over several orders of magnitude, it must be noticed that there is no physical law to describe the correlation between cell density (OD_{600}) and scattering (CGQ signals) as simple as done for the transmission measurements with the Lambert-Beer law. Nevertheless, it is possible to describe this correlation using more elaborate empirical models to account for effects such as multiple scattering.

At higher concentrations (usually above ca. 10 OD_{600}) the CGQ signal exhibits a linear correlation with the actual cell density in the shake flask. Lower concentrations (usually below ca. 5 OD_{600}) give a CGQ signal that increases exponentially with increasing cell density. Between these two zones there is a

aquila biolabs

transition zone, where the exponential correlation changes into a linear correlation.
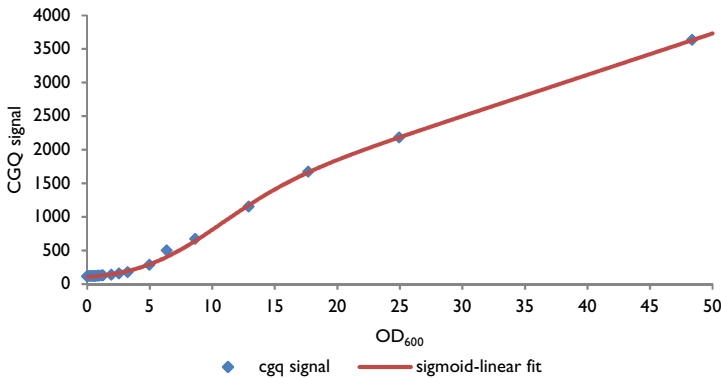
Figure 11: CGQ signal vs. OD$_{600}$ for S. cerevisiae in YPD medium.

As exemplarily shown in Figure 11, this correlation can be described mathematically as a combination of an exponentially sigmoid and a linear function, in its complete form given by

$$I_s = (m \cdot OD_{600} + n) \cdot \frac{p_1}{\left(1 + p_2 \cdot e^{-p_3 \cdot OD_{600}^{p_4} - p_5}\right)^{p_6}} \qquad \{2\}$$

with I_s the scattered light intensity measured by the CGQ, m the slope and n the y-axis intercept of the linear correlation at higher cell densities and p_{1-6} the parameters of the exponential sigmoid function describing the low and intermediate cell densities. However, in many applications it is not required to use this complete function, especially when working at concentrations in the low and intermediate cell density range. Figure 12 depicts exemplarily for *E. coli* on LB medium the correlation of CGQ signal and OD$_{600}$, described using the above complete sigmoid-linear correlation and using a simple four-parametric exponential function, given by

$$I_s = p_1 \cdot e^{p_2 \cdot OD_{600} - p_3} + p_4 \qquad\qquad \{3\}$$

with I_s the scattered light intensity measured by the CGQ and p_{1-4} the parameters of the exponential function.
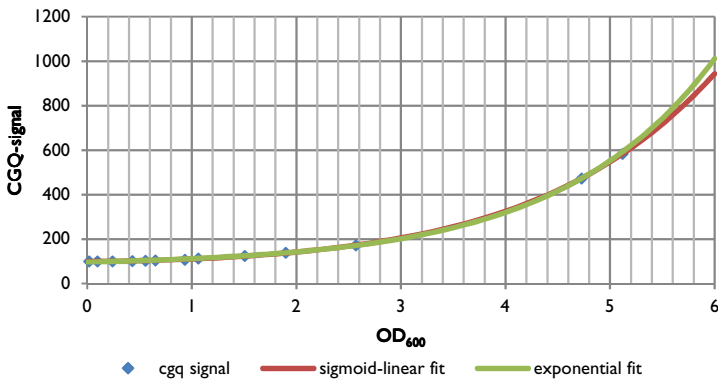
Figure 12: CGQ signal vs. OD_{600} for E. coli in LB medium.

Obviously, the simple exponential function allows a satisfying correlation of the CGQ-signal and the OD_{600} in the range of lower cell densities. Even in the intermediate cell density range up to 10-15 OD_{600}, the simple exponential correlation can be used with some limitations regarding accuracy.

The most important thing about correlation of CGQ signals and real cell densities is, to keep in mind that the parameters of the correlating functions depend on some cultivation system properties influencing the scattering behavior of the fermentation broth, such as cell size, cell shape, medium composition, flask type and material, etc. If you want to derive absolute real cell density values from the CGQ (e.g. OD_{600}, cell dry weight, etc.), you have to calibrate your cultivation system as described in detail on pp. 105.

However, using cell densities in the low and intermediate density range (i.e. between 0.3 and 15 OD_{600} for the above described *E. coli* example) it is possible

aquila biolabs

to work with relative cell density values, which do not require a prior calibration procedure. This concept is based on the valid region of the four-parametric exponential correlation model. Without any calibration and known data pairs of OD_{600} and CGQ signal, you can get relative cell densities as a sufficiently accurate measure of cell density that is proportional to the real cell density, simply by calculating the natural logarithm of the measured CGQ signal.
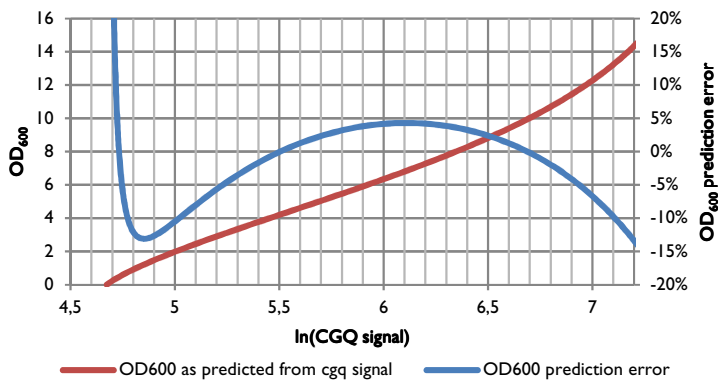
Figure 13: Relative cell density correlation with OD_{600}.

Within the above-mentioned range example for *E. coli* on LB, the nonlinearity of the relative cell density is about -3% on average, +20% at 0.3 OD_{600} (absolute error of 0.06) and -15.5% at 15 OD_{600} (absolute error of 2.3). As soon as at least two real cell density values are known (e.g. initial inoculation OD_{600} and one additionally sampled offline OD_{600}), it is possible to calculate real cell density values on the basis of the relative cell density. For further information regarding the implementation and usage of relative cell densities in CGQuant, refer to pp. 82.

aquila biolabs

Parameters that influence the CGQ measurements

Each CGQ sensor plate is an optical measuring platform designed to provide highest sensitivity and precision in the detection of scattered light, even at lowest particle concentrations. However, this sensitivity means that also changes in the optical environment will be detected by the CGQ. With the new environmental light compensating CGQ and CGQ BioR plates it is possible to monitor growth without the use of shake flask covers. To provide a reproducible optical environment for optimal measurements in shake flask applications, it is still recommended to use the shake flask covers, as they create an absolutely constant environmental light zone above the CGQ sensor plates, comparable to the dark measurement chamber of a spectrophotometer.

The general comparability of different sensor plates is ensured by factory calibration of the sensor arrays. Comparability of factory calibrated sensor plates means that with an identical scatterer positioned above the sensor array the CGQ signal maximally differs by 5% (typically 1-2%) between two sensor plates.

When comparing the real operating conditions of the CGQ system with those of a typical spectrophotometer, some important differences should be noted.

Shake flasks and bioreactors are not a cuvette!

While cuvettes typically exhibit polished and perfect surfaces, a shake flask bottom or bioreactor side wall usually contains refraction anomalies from the production process and might be furthermore scratched to different extends. Such anomalies or scratches in the transparent vessel wall are not generally disturbing the CGQ measurements. However, they might be an additional source of scattered light, influencing the comparability within a single and between several cultivations.

1. Within a single cultivation there is generally no influence on the data quality as long as the sensor-to-vessel position or orientation is not

aquilabiolabs

changed. As soon as the flask or sensor is removed and remounted in a different position/orientation, there might be a different set of anomalies or scratches in front of the sensors, which might cause jumps in the cell density curve.

> ⚠️ **Try to avoid removing and remounting of the shake flask or sensor plate during cultivation. If this cannot be avoided, try to remount the system always in the same position/orientation as before to avoid jumps in the cell density curve.**

2. Between several cultivations, it usually appears that different flasks are used. As each flask is individual regarding the number, size and distribution of anomalies or scratches, the scattering intensities at equal cell densities differ between these flasks. An exemplary distribution of such differences is shown in Figure 14 for a set of 24 similar shake flasks, which were all measured on the same sensor plate.
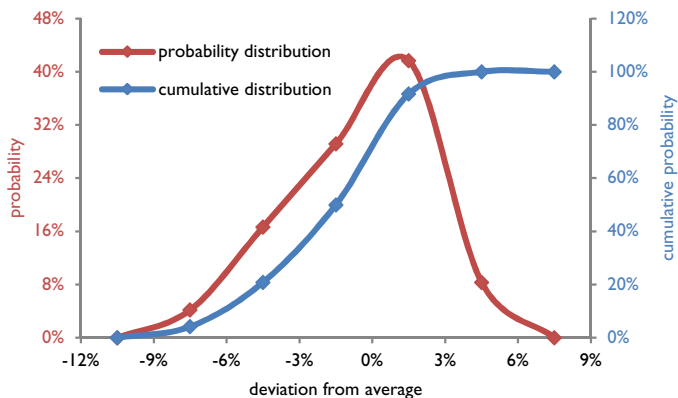
Figure 14: Exemplary CGQ signal differences of a set of 24 shake flasks (aluminum cap, 250 ml), measured at OD_{600} = 4.2, 250 rpm, 25mm shaking diameter, 25 ml filling volume.

aquila biolabs

As long as the flasks' positions or orientations are not changed during the cultivation, these individual flask differences will (in most cases) only cause offsets in the CGQ signal, which can be removed using the CGQuant software (refer to pp. 82). Flask position/orientation changes during cultivation have been described above.

> **If a high comparability between several shake flasks is required, try to use flasks with similar degrees and distributions of scratching. In some cases, it may be favorable to preselect a set of highly similar flask, based on measuring their scattering signal on the same sensor plate, at the same cell density and at equal shaking conditions.**

Shake flasks and bioreactors are not a blank cuvette!

While cuvettes typically exhibit blank surfaces, shake flasks and bioreactors are usually covered by volume markings, engravings and labeling areas. Even if inscriptions are not located directly in front of the sensor window, they can act as an additional source of scattering if located in the sensor array's field of view. This scattering might interfere with the desired scattering of cells in the fermentation broth and may cause artifacts in the cell density curves.

> **Always ensure that the CGQ or CGQ BioR sensor plate is positioned and oriented in a way that no markings, labeling areas or engravings are located in the sensor array's field of view.**

Shake flasks and bioreactors are not an even blank cuvette!

While cuvettes typically exhibit even surfaces, shake flasks and bioreactors can be equipped with baffles, emanating either from the side walls or from the bottom of the vessel. Baffles have a curvy geometry, which might act as an additional source of scattering or as a lens if located in front of the sensor array. This scattering might interfere with the desired scattering of cells in the

aquila biolabs

fermentation broth, thus causing artifacts in the cell density curves. Furthermore, it should be noted that remounting the flask during a running cultivation might result in jumps in the cell density curve, similarly as described above for the scratches. Calibration data, if required, should always be collected "per flask type" (refer to pp. 105).

> **Always ensure the CGQ or CGQ BioR to be positioned and oriented in a way that no baffles are located in front of the sensor array. If the vessel and baffle geometry permits such an orientation, then try to find a position/orientation so that the outer parts of the sensor array are not covered by the baffles.**

Shake flasks and bioreactors are <u>not</u> an even blank <u>quartz</u> cuvette!

While precision cuvettes are typically made of quartz or high quality glass, a shake flask or bioreactor vessel can be made of glass or plastics, being clear, turbid or colored, single- or double-walled. Generally, this is not problematic for the cell density measurement; however different materials might have different effects on the CGQ signal. As long as the material is at least partially transparent for visible light, the CGQ measurement principle will work, but care must be taken about some limitations.

1. Turbid materials reduce the amount of light going into the flask from the LEDs and leaving the flask or vessel towards the photodiodes, which results in reduced sensitivity in the range of lower cell densities, depending on the degree of turbidity. Turbid materials furthermore act as a strong additional scattering source, thus causing a much stronger background signal.

2. Colored materials might reduce the transmission of light into and out of the shake flask or vessel, similarly to turbid materials. However, the transmissivity of transparent colored materials is usually much higher than that of turbid materials, so that the sensitivity reduction is not as

strong as for turbid materials. Nevertheless, applying colored materials or cultivation media with strong absorption in the range of 500 – 550 nm might be difficult and a LED customization should be considered (pp. 45).

3. Some typical plastics being used in single use flasks exhibit anisotropic structures on a molecular level, which might act as additional scattering source, resulting in increased background signal and cell density curve artifacts.

Generally, it is recommended to be careful when interpreting and comparing results from shake flasks or bioreactor vessels made of different materials, as they might exhibit different background signals, sensitivities or artifacts. Background correction can be done in the CGQuant software (refer to pp. 82). Calibration data, if required, should always be collected "per material" (for details see pp. 105).

> ⚠️ **Always be careful when interpreting and comparing non-calibrated results from shake flasks or bioreactor vessels made of different materials, as they might exhibit different background signals, sensitivities or artifacts.**

Shake flasks and bioreactors are <u>not</u> an <u>unagitated</u> even blank quartz cuvette! While cuvettes are typically mounted in an unagitated environment, a shake flask is continuously shaken and a bioreactor is continuously stirred during the measurement, resulting in a continuously moving fluid. All fluidic parameters of those agitated systems, such as filling level, flask or vessel size and shape, shaking or stirring speed, aeration rate, flask cap, inline probes or sampling ports, fermentation broth viscosity, etc. might therefor influence the dynamically measured CGQ signal (refer to pp. 46). The CGQ's data analysis algorithms are made and continuously improved (via firmware updates) to eliminate as many of these signal influencing fluidic parameters as possible. However, you should

aquila biolabs

not assume that the data from a 2000 ml flask, filled with 400 ml of broth and shaken at 150 rpm can be compared directly to a 250 ml flask, filled with 12.5 ml of broth and shaken at 350 rpm. If you want to do direct and especially quantitative comparisons, calibration measurements are required as described at pp. 105. Calibration data, if required, should always be collected "per fluidic system" (for details see pp. 43).

> ⚠️ **Always be careful when interpreting and comparing non-calibrated results from shake flasks or bioreactors with different fluidic parameters (e.g. filling level, size, shape, agitation speed, cap, fermentation broth viscosity, etc.), as they might exhibit different background signals, sensitivities or artifacts.**

> ⚠️ **Always be careful when interpreting and comparing results from shake flasks or bioreactors, which have been subjected to filling volume changes (e.g. by sampling, feeding or induction). As long as the volume changes are smaller than 20% of the initial filling volume, the effects on cell density curves should be negligible, but larger volumetric changes might result in different background signals, sensitivities or artifacts.**

Shake flasks and bioreactors are <u>not</u> an unagitated even blank quartz cuvette representing a <u>steady state system</u>!

While cuvettes are typically filled with a steady state solution as a kind of "current culture snapshot", a shake flask or bioreactor under cultivation is a highly dynamic and complex biological system, which changes continuously over time. These changes can include aspects influencing the fermentation broth's scattering behavior, e.g. filamentously growing organisms can change the viscosity, lipid secreting cells can create biphasic systems, strong morphologic cell modifications like sporulation can change the scattering diameter and secretion of colored substances, secretion or generation of aggregates, cell

57

aquila biolabs

clumping, biofilm formation, etc. can furthermore contribute to the measured scattering signal.

⚠️ **Always be careful when interpreting and comparing results from cultivations, which are subjected to growth associated changes of parameters that influence the fermentation broth's scattering behavior. Those cultures might exhibit different background signals, sensitivities or artifacts, depending on their respective growth behavior.**

Shake flasks are <u>not</u> an unagitated even blank quartz cuvette <u>mounted into a static cuvette holder</u>!

While cuvettes are typically mounted into static cuvette holders, shake flask mounting is done with the help of a flexible clamp. This flexibility may lead to different degrees of deformation of clamps, often depending on their age and position in the shaker. Even slight changes in the clamp deformation can introduce considerable differences in the relative positioning of shake flask and sensor plate, thus influencing the effective scattering liquid volume in the sensor's field of view.
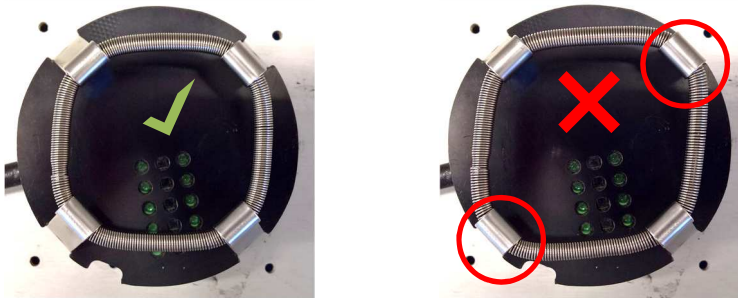
Figure 15: Clamp symmetry and deformation. (left - correct, right - wrong)

Positioning differences due to differently deformed clamps may introduce considerable differences in the CGQ's scattering signal and may therefor negatively influence the flask-to-flask reproducibility and comparability of similar

58

aquila biolabs

CGQ measurements. In order to avoid this effect, it is strongly recommended to check the symmetry of all clamps each time before mounting a shake flask for CGQ measurements.

⚠️ **Always make sure that clamps are centrosymmetrically aligned and not deformed to avoid flask mispositioning and to improve overall reproducibility.**

aquila biolabs

The CGQuant software

Introduction to CGQuant

CGQuant is your interface to real time cell density monitoring of shake flasks and bioreactors using the CGQ. It bundles different intuitive functionalities that are required for typical online monitoring tasks in life science laboratories, including

- components for controlling, visualizing and comparing measurements
- a fully customizable electronic lab-journal
- an event detection system
- data processing and annotation tools
- a calibration and a data export tool.

The parallelization of the CGQ device is continued in CGQuant's graphical user interface, as shown exemplarily in Figure 16. Detailed descriptions of each functionality can be found in the following chapters.

Figure 16: The CGQuant main window.

60

aquila biolabs

CGQuant files and dependencies

CGQuant is a typical .NET application and thus requires the .NET-framework (4.7 or newer) running on your computer with Microsoft's Windows (Windows 7 or newer) installed as operating system. Currently there are no CGQuant implementations available for other operating systems, such as MacOS, Linux or BSD distributions. Some dynamic link libraries (.dll) are required for CGQuant's execution. Most of them are available from typical .NET framework distributions and usually preinstalled on your Microsoft Windows operating system. However, some additional libraries are included in the CGQuant package:

- DocumentFormat.OpenXml.dll
- ClosedXML.dll
- MathNet.Numerics.dll
- PdfSharp.dll
- PdfSharp.Charting.dll
- MigraDoc.RtfRendering.dll
- MigraDoc.Rendering.dll
- MigraDoc.DocumentObjectModel.dll
- RJCP.SerialPortStream.dll
- MimeKit.dll
- MailKit.dll
- BouncyCastle.Crypto.dll

CGQuant uses specific file extensions for saving different data types. Measurement data are written to .cgqfx-files. Old cgqf files are still supported for reading. Calibration data are saved as .cgqc-files. CGQ event descriptions are stored as .cgqed-files. SMTP-client configurations are stored as .smtpconfig-files. Several configuration as well as logging functionalities use classical .txt-files. Measurement data can be exported to the OpenXML file-type .xlsx.

aquila biolabs

CGQuant up to version 6.3 saved data into cgqf-files. Starting from CGQuant 7.0, cgqf-files are only supported for reading. Essentially upon opening a cgqf-file, CGQuant converts it to a .cgqfx-file and saves the old original file into a separate folder on the same directory level as the old file. Environmental data, cannot be shown for cgqf-files.

For reasons of portability and data accessibility on systems, where no CGQuant installation resides, the CGQuant files are basically .txt-files or XML files with a different extension. The cgqfx file wraps different data files into a zip-archive. This also allows skilled users to edit these files manually, e.g. to repair files or to make corrupted files readable for CGQuant again. However, it is strongly recommended to refrain from manual editing CGQuant associated files.

Any changes being introduced into CGQuant files by manual editing bear the risk of file corruption and may result in unreadability or data loss. Manipulating configuration files may lead to serious malfunctions or instabilities of CGQuant execution.

Generally, it is strongly recommended to open all CGQuant associated files (except the exported files) only using CGQuant. Manual editing should be avoided to prevent file corruption and subsequent data loss or CGQuant malfunctions.

After starting an experiment as well as after changes to previously recorded files, CGQuant takes care of saving any kind of relevant changes automatically. Except for the cases of starting an experiment, changing preferences and settings or finalizing calibration measurements, there is no need for you to care about saving files.

All running measurements as well as all currently opened .cgqfx files are automatically saved as backup according to the preset backup interval in the preferences window.

aquila biolabs

CGQuant performs automated status and error logging on CGQuant errors as well as on CGQ errors transmitted via USB. The log files are available under …\CurrentUser\Documents\CGQuant\data\logs and should be always sent together with bug descriptions or other support requests.

aquila biolabs

The main window

CGQuant's main window serves as a frame to access, analyze and collect data. It is organized into a top control toolbar and a tabbed viewer panel for the visualization of measurement and calibration data.
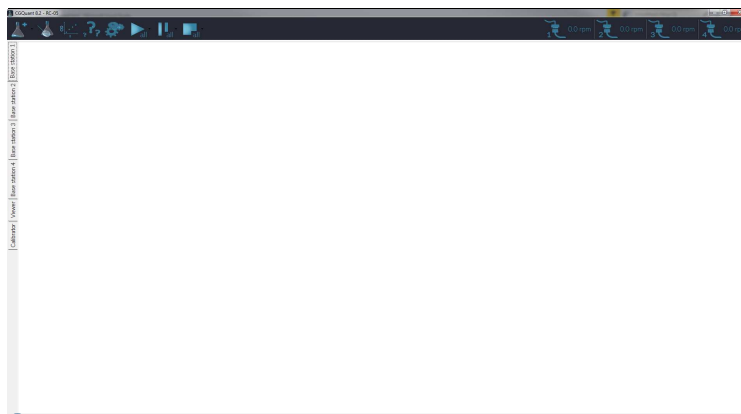
Figure 17: The CGQuant main window.

The main window contains the following control elements:
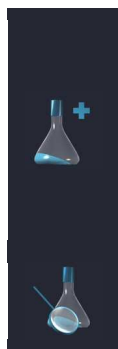
	New experiment	Click this button to start a new experiment. Upon clicking, a dropdown menu appears, displaying all available base stations. Click the base station where your target sensor plate is connected and a new experiment is created. Click the CGQ Experiment Configurator to create a batch of experiments in parallel.
	View data	Click this button to view data from previous measurements.

64

	Calibrator	Click this button to start a new calibration procedure to correlate your CGQ intensities with offline cell density data.
	Help	Click this button to get help.
	Options	Click this button to get access to the CGQuant options and preferences.
	Start all	Click this button to start or resume all running experiments together. Upon clicking, a dropdown menu appears, displaying all available base stations. Click the base station where you want to start or resume all connected sensor plates with an associated running experiment.
	Pause all	Click this button to pause all running experiments together. Upon clicking, a dropdown menu appears, displaying all available base stations. Click the base station where you want to pause all connected sensor plates with an associated running experiment.
	Stop all	Click this button to stop all running experiments together. Upon clicking, a dropdown menu appears, displaying all available base stations. Click the base station where you want to stop all connected sensor plates with an associated running experiment.
	Connection status	Look at this label to check, whether a CGQ base station is connected (lower icon, glowing) or disconnected (upper icon, not glowing). For each of the four base station ports, one label is visible in the main toolbar.

The main window

aquila biolabs

The overview window

The CGQuant overview window opens in the background. It displays an overview chart of all running measurements and a list of all connected base stations with their associated CGQ sensor plates and base station inputs together with their current states and unique identification numbers.
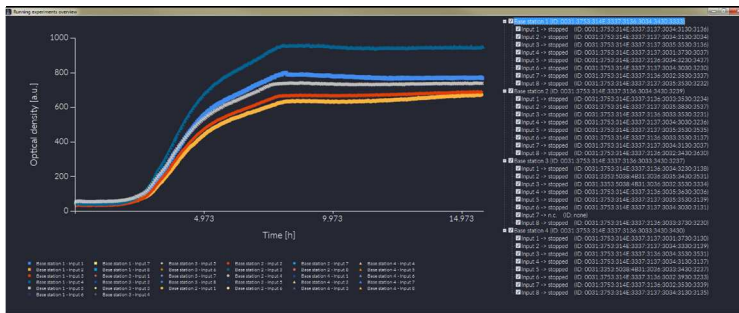
Figure 18: The CGQuant overview window.

The checkboxes enable or disable the visibility of the associated running measurement data in the overview chart. Enabling or disabling the visibility of a base station will also enable or disable the visibility of all data originating from sensor plates connected to this base station.

aquila biolabs

The experiment window

The experiment window is the core component of data visualization and control of the CGQ device. Running experiment windows are displayed in the main tab of their associated base station. Experiment windows for files collected in the past are displayed in the main tab "Viewer"
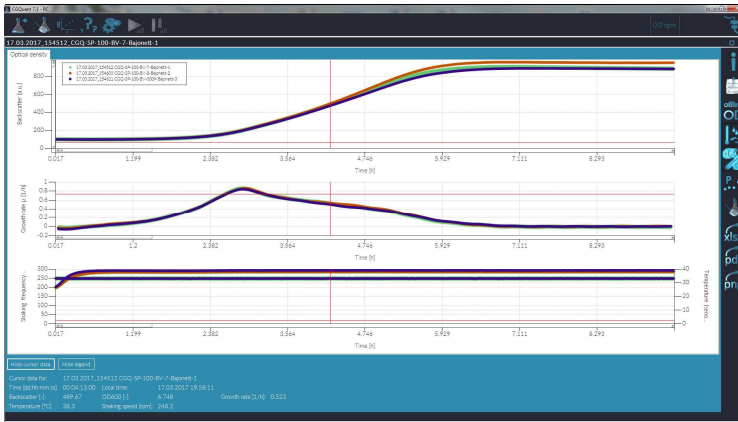
Figure 19: The CGQuant experiment window.

The experiment window contains the following control elements:

I2	Connection number	This label indicates the connection number where to currently used sensor plate is plugged into the base station. It is only available for running measurements.
	Process information	Click this button to open the process information window for the selected experiment.
	Annotations	Click this button to toggle the annotation chart visibility.

aquila biolabs
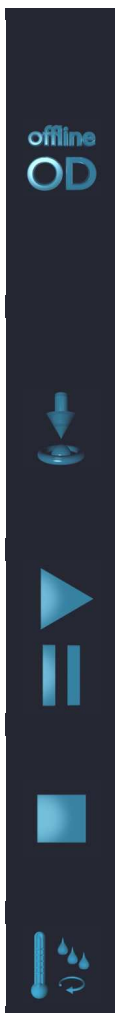
Offline OD

Left-click this button to switch between backscatter data and calculated OD-values. Right-click this button to specify the initial offline OD value of your culture and to select or exchange calibration files as required for calibrated data collection. Additional offline OD values can be saved here, to refine the accuracy of the applied calibration file or to derive a totally new calibration file from a live measurement.

Show connected sensor plate

Click this button to find the connected sensor plate on the shaker tray. Upon clicking this button, a running measurement will be paused and the connected sensor plate will start blinking two LEDs at 1 Hz. Click this button again to resume the measurement. This button is available only for running experiments.

Start/resume experiment

Pause experiment

This button behaves similarly to a media player. By clicking it, you can start, pause or resume the data collection of your current experiment. This button is available only for running experiments.

Stop experiment

Click this button to stop the data collection of your running experiments. When the data collection is stopped, you cannot restart it in this experiment window. This button is available only for running experiments.

Environmental data

Click this button to toggle the environmental data visibility with sensor plate temperature and shaking frequency.

aquila biolabs

dX/dt	Growth rates	Click this button to calculate the growth rates of all experiments shown in the central chart. Click this button again to remove the growth curves from the chart. If the button is switched on, the growth curve of the currently running experiment will be recalculated each time a new data point is added to the chart.
P	Data post-processing	Click this button to get access to the data post processing features. A separate window will be opened where you can e.g. offset multiple curves, change the smoothing level, define events or create and compare consensus curves.
	Load data	Click this button to add additional data to the chart. A separate window will open where you can choose the .cgqfx file to be opened.
xlsx	Export to .xlsx	Click this button to export all the data loaded in the current experiment window into an .xlsx-file. This button is blinking during the exporting process. Afterwards the .xlsx-file will be opened automatically in your default xlsx-program.
pdf	Export to .pdf	Click this button to export data from the current experiment window into a .pdf-file. Details on how to configure your pdf-report can be found on pp. 102.
png	Export to .png	Click this button to export data from the current experiment window into a .png-file. Details on how to configure your pdf-report can be found on pp. 104.

The experiment window

aquila biolabs

The process information window

All bioprocess related information can be written into the fully customizable CGQuant lab journal via the process information window. Upon starting a new experiment, the process information window is opened automatically. You have to select at least the input number, where your target sensor plate is connected to the base station. All other values are defaults as defined in the preferences window (Figure 24), which may be adjusted according to your application. Press Save and exit, then you can start the measurements. You can later access the process information of each data set via the ''i''- button in the experiment window or via the data set context menu.
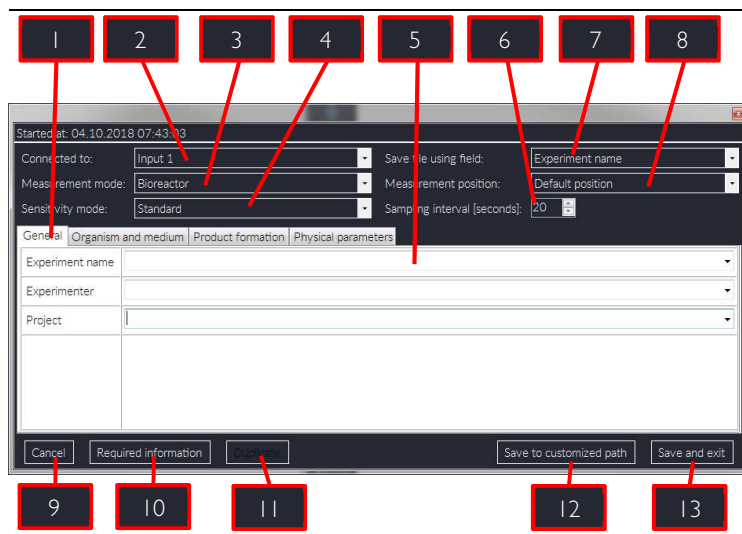
Figure 20: The CGQuant process information window.

The process information window contains the following control elements:

1	Lab journal tabs	The lab journal information is categorized into tabs. Switch between the tabs to access all the information.

aquila biolabs

2	Input number	Use this drop-down menu to select the input number of your current sensor plate at the base station. Only input numbers with connected sensor plates currently not measuring are displayed.
3	Measurement mode	Use this dropdown menu to select your desired measurement mode. Currently available modes are: - Shake flask (may be used with or without covers) - Bioreactor
4	Sensitivity mode	Use this drop-down menu to select the sensitivity mode in CGQ BioR applications. This option is only evaluated for the "Bioreactor" measurement mode and ignored for all others.
5	Lab journal fields	Each lab journal category (e.g. "Experiment name") has an empty field, which accepts the according information, either as words or as numbers. Fields that have been set to be required have a grey background.
6	Sampling interval	Use this field to set the desired CGQ measurement interval (in seconds) for the specified experiment.
7	Filename category	Use this dropdown menu to select, which lab journal category should be used for generating the .cgqfx-filename.
8	Measurement position	Use this dropdown menu to specify the measurement position on the sensor plate. In most cases, this setting should be set to default. However, in some special cases and with some special adapters, it may be required to manually set the measurement position of the CGQ sensor plate. See also Figure 9.

The process information window

aquila biolabs

9	Cancel	Click the cancel button to close the process information window. If you have started a new experiment and click cancel in the first appearing process information window, the complete experiment window will be closed.
10	Required information	By clicking this button, you can jump to the next required field.
11	Duplicate	By clicking this button, you can start a new experiment on the basis of the process information in the current process information window. This option is useful when you perform highly similar experiments with only a few parameters changed. This field is disabled for the initial process information window during experiment setup.
12	Save to customized path	By clicking this button all currently provided process information are saved to the cgqfx-file path as specified by the user and the process information window is closed.
12	Save and exit	By clicking this button all currently provided process information are saved to the current or default .cgqfx-file path and the process information window is closed.

The lab journal is customizable via the CGQuant preferences window, as depicted in Figure 21. You can add new process information fields, either as property or as numeric value, with or without a default value into existing process information groups by clicking the button "Add process information". The "New group" button allows you to introduce additional process information groups.

aquila biolabs
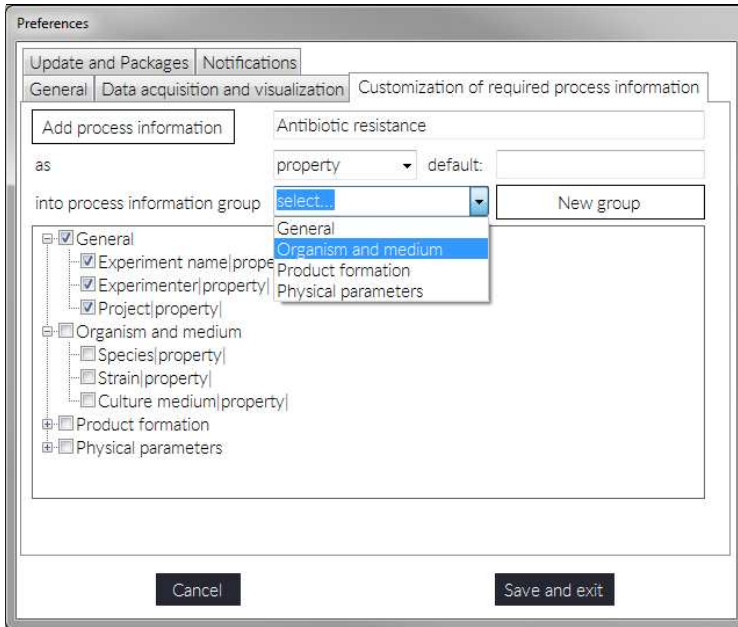
Figure 21: Customizing the CGQuant process information window.

You may also modify existing entries after clicking them in the tree-view. Existing entries may be removed by clicking them, followed by pressing the "Delete" button on your keyboard. Furthermore, you can set selected process information fields or groups to be mandatory by checking the respective checkboxes. Doing so will set these fields in the process information window as mandatory and users can only finish the process information acquisition by filling these required fields.

73

aquila biolabs

The CGQ Experiment Configurator

A batch of experiments can be created and configured in parallel using the CGQ Experiment Configurator.
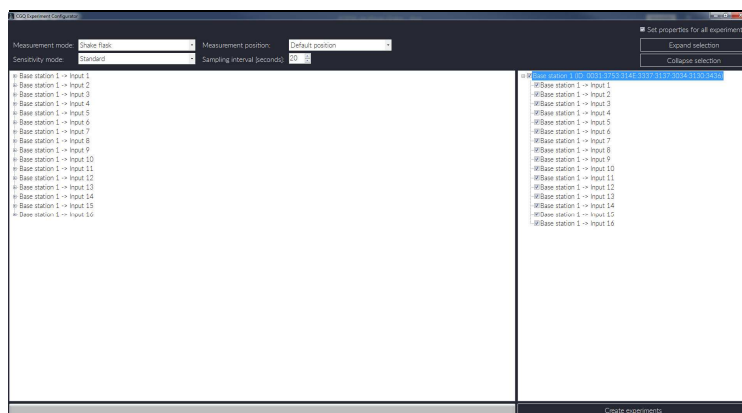
Figure 22: The CGQ Experiment Configurator.

Select the sensor plates to be used in the list at the right side by checking the respective checkboxes. On the left side, a list off all process information is displayed, where you can document each single experiment. Having the "Set properties for all experiments" box in the right upper corner checked will automatically copy the changes of an entry (e.g. "Experimenter") to all currently selected sensor plate associated process information. Navigate through the tree of process information and then click the field below the process information category to change it. Then press the enter or return key to apply your changes. The measurement settings, such as measurement mode and sensitivity, position and sampling interval are always set for all experiments of one batch.

Finally clicking the "Create experiments" button will automatically prepare and create experiments for all selected sensor plates. This process may take some time, depending on the number of experiments to be generated, and may render CGQuant to appear unresponsive. Just wait a few moments and then

aquila biolabs

start all the generated experiments using the start all button for each base station according to your sensor plate selection.
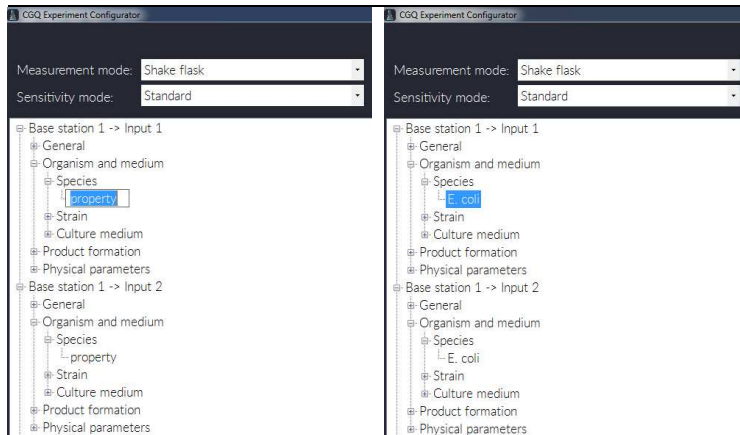
Figure 23: Parallel documentation of properties for all selected experiments (sensor plates).

75

aquila biolabs

Controlling the measurement

There are two ways of starting, pausing and resuming a measurement. A single measurement can be controlled from the experiment window's buttons as described above. Additionally, all measurements connected to the same base station can be paused or started together using the "Start-all" and "Pause-all" buttons in the toolbar of the main CGQuant window. Similar to the "New experiment" button, a dropdown menu is shown, displaying all available CGQ base stations. From this dropdown you select, which base station's sensor plates should be started, resumed or paused all together.

> ⚠️ **The execution of start-all, pause-all, resume-all and stop-all commands may take a few seconds. Please check the sensor plate status in the overview window.**

Controlling all measurements of one base station at the same time can be useful in any case where you want to stop or start the shaker. Clicking the dropdown under "Pause-all" before stopping the shaking movement will avoid the occurrence of measurement outliers due to the decelerating liquid movement. After restarting the shaker, please wait until the final shaking speed is reached, then you can resume all measurements with a single click of the dropdown under the "Start-all" button.

The shaking state of each CGQ sensor plate for shake flasks (CGQ-SP-F) is monitored. This allows for automated measurement control, which can be enabled in the preferences window as depicted in Figure 24. With automated measurement control enabled, the sensor plates wait for stable shaking conditions, before they start a data acquisition.

> ⚠️ **Using the CGQ with shaking frequencies below 150 rpm requires the automated measurement control feature to be disabled.**

aquila biolabs
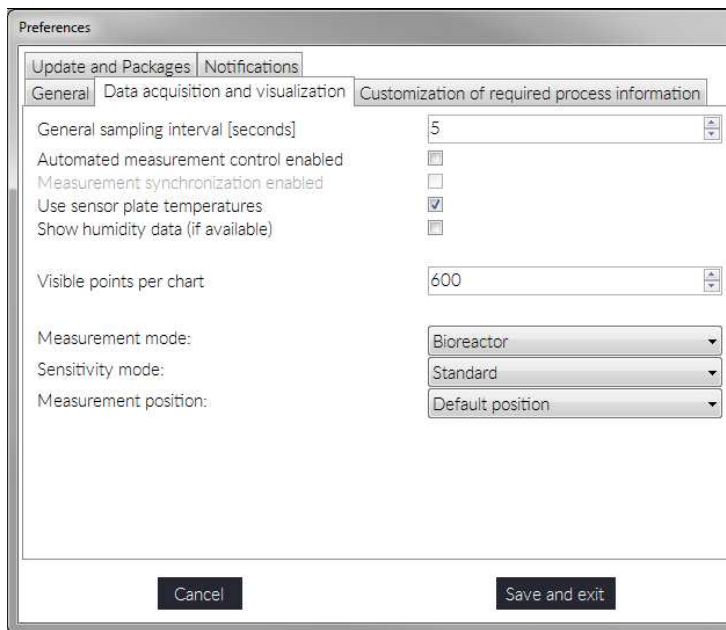
Figure 24: Measurement control options and default settings.

Furthermore, you can set the default general sampling period (time between two data acquisitions) and the number of visible points per chart. Both settings influence the performance of CGQuant and may be changed according to your requirements. The general sampling period sets the data acquisition density. By setting the visible points per chart, you define how many points will be shown in the experiment window chart per dataset. This value may be decreased to improve the general CGQuant performance. As long as there are enough data points available, CGQuant will always keep the point count per chart constant, e.g. data will be reloaded upon zooming.

By setting the measurement and sensitivity modes ad well as the measurement position in the preferences window, you decide, which default values are sued during the setup of a new experiment.

aquila biolabs

Parameters, which directly influence the data acquisition, must be set before starting an experiment (e.g. the automated measurement control). Earlier started experiments will not be influenced by such reparameterizations unless their sensor plate is reset (e.g. by power cycling).

Running measurements can be stopped by clicking the stop buttons, or by closing the experiment window. The stopping procedure may take some seconds, which is notified at the bottom of the respective experiment window as depicted in Figure 25.

Figure 25: Notification upon measurement stop request.

aquila biolabs

Annotations

CGQuant features an annotation tool allowing you to add comments or data files to running or previously recorded measurements. Annotation points are displayed in a separate chart, which can be toggled using the annotation-button in the experiment window's right toolbar. Available annotations are displayed at the bottom of the chart and can be opened with a left-click. Alternatively, you can access an old annotation or add a new annotation by right-clicking on the respective biomass curve. A context menu opens, where you can select, how to proceed (Figure 26).
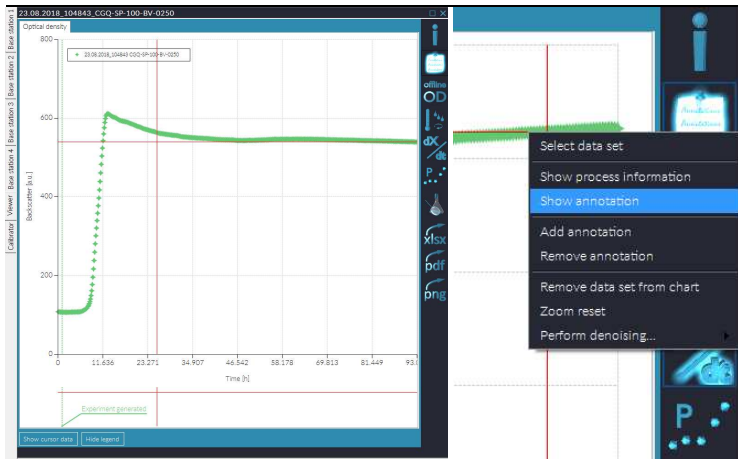
Figure 26: Accessing an annotation via the annotation chart(left) or the context menu (right).

Add/Show annotation will open the annotation window, where you can enter annotation title and annotation text. When opening a new annotation, CGQuant automatically adds a timestamp for the current cursor position. This timestamp can be edited according to your requirements.

You can furthermore attach data files or images to the opened annotation, as exemplarily depicted in Figure 27. Images will be directly previewed in the annotation window, while other data files will be previewed with their standard

aquila biolabs

file-type icon. Files or images that have been attached to an annotation are copied by CGQuant and saved into the .cgqfx file. This allows you to keep all your experiment related data together in one file.
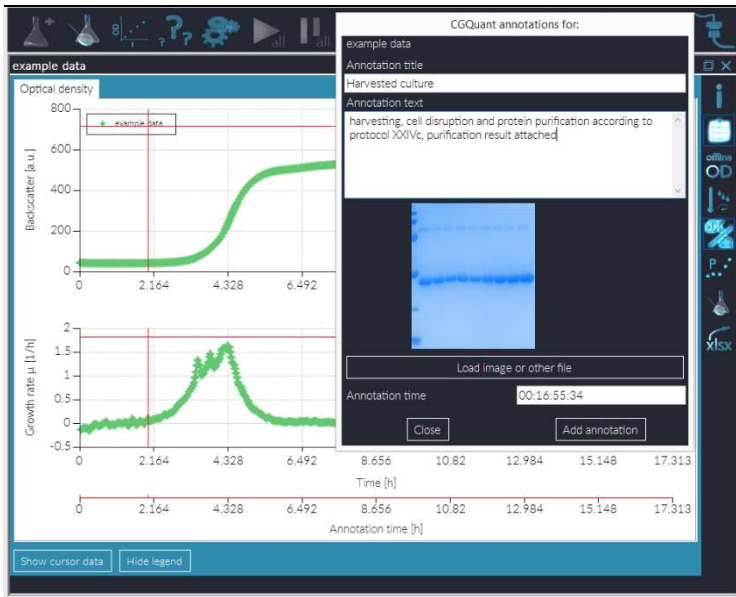
Figure 27: Creating an annotation

CGQuant automatically creates an initial annotation ("Experiment generated") to store general information such as hardware IDs and measurement modes. This initial annotation cannot be edited or deleted.

aquila biolabs

Comparing multiple measurements

You can compare multiple measurements by opening them in the same window. This can be achieved either by clicking the respective button in the experiment window or CGQuant main toolbar or by dragging and dropping .cgqfx-files into an existing experiment window (data will open in that window) or into CGQuant (data will open in a new experiment window).
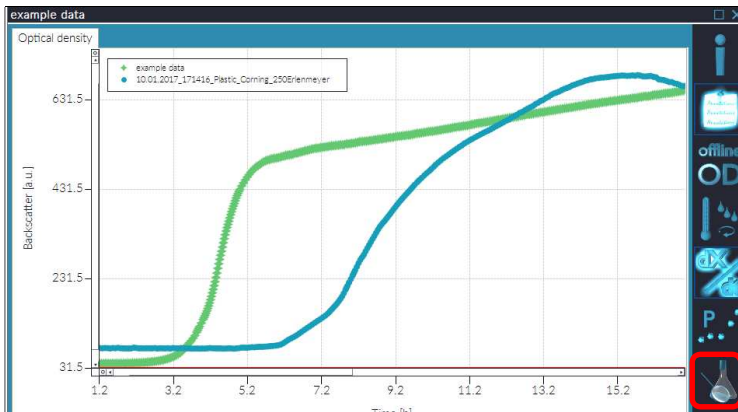
Figure 28: Adding data to an opened experiment window.

aquila biolabs

Using the central chart

You can zoom into the central chart by scrolling the mouse wheel or using the cursor. Left-click into the chart, hold the left mouse button and move the mouse. A grey area will appear, indicating the region to be zoomed. To reset the zooming, click the small button with the circle icon at the end of the scroll-bars or use the chart context menu.

By right-clicking a cell density curve, you can open the chart context menu, as depicted in Figure 29. Here you can add, show or remove annotations, remove data sets, reset the zoom level and change the denoising level.
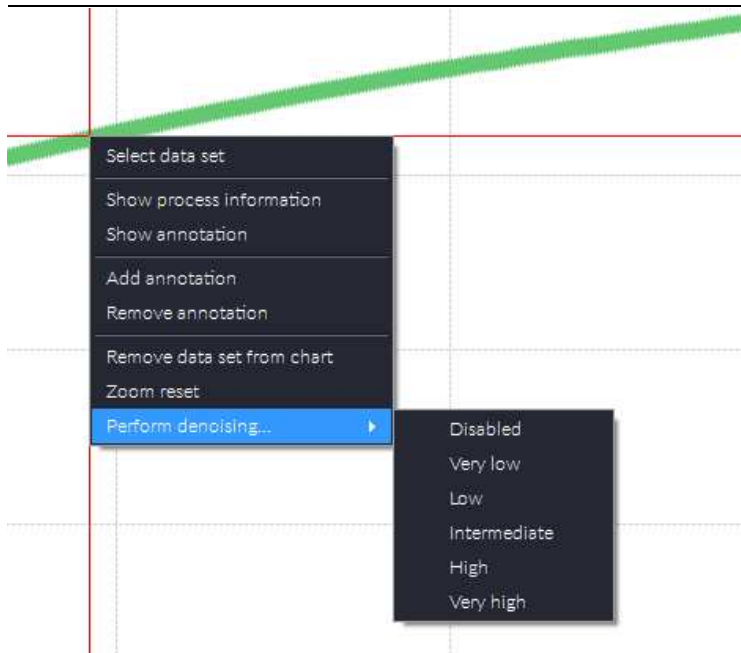
Figure 29: CGQuant chart context menu.

You can also select the current data set. CGQuant will then list all available data at the cursor position time as exemplarily shown in Figure 30.
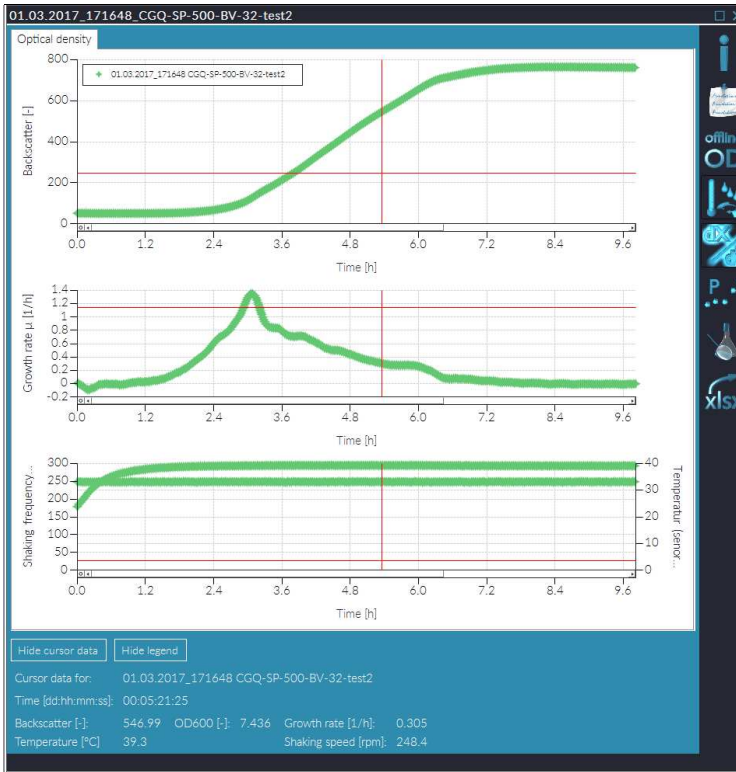
aquila biolabs

Figure 30: CGQuant cursor values.

In order to get the maximally available chart area, you can hide the cursor data and the legend by clicking the respective buttons below the chart.

aquila biolabs

Data post-processing – Curve adjustment

By clicking the "Data post-processing"-button in the right toolbar of the experiment window, you get access to the data post-processing options in CGQuant. These options are accessible from different tabs with the first one being the basic curve adjustment tab.
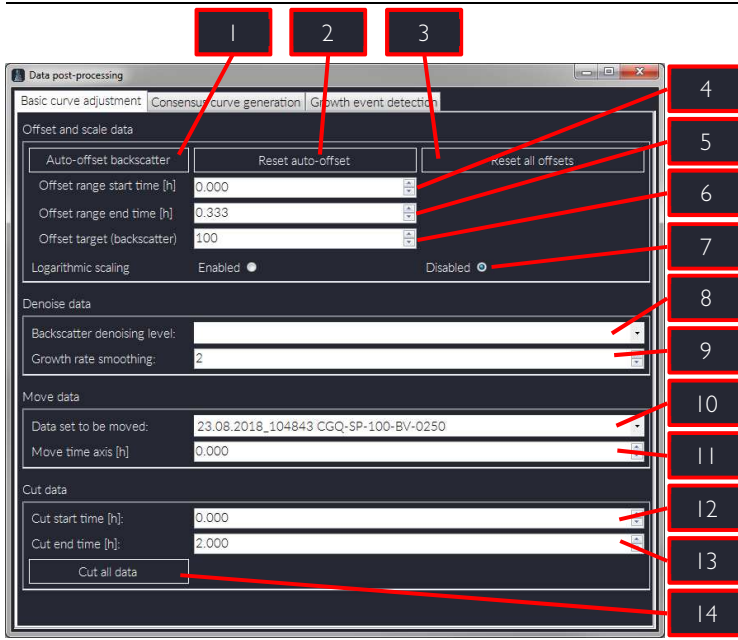
Figure 31: Curve adjustment in the CGQuant data post-processing window.

The basic curve adjustment tab contains the following control elements:
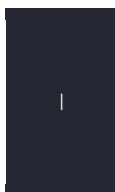
1	Auto-offset	Clicking this button will automatically offset all backscatter data in the current chart according to the offset settings below. You should use this function only for curves that have been inoculated to the same initial cell density.

aquila biolabs

2	Reset auto-offset	Clicking this button will reset all backscatter data in the current chart to their original values.
3	Reset all offsets	Clicking this button will reset all backscatter data and all data times in the current chart to their original values.
4	Offset start time	This field sets the start time of the data range being used for offset calculation.
5	Offset end time	This field sets the end time of the data range being used for offset calculation.
6	Offset target	This field sets the backscatter value, to which all data series are automatically offset.
7	Enable/disable the logarithmic scaling	Here you can enable or disable the logarithmic scaling as described at pp. 47 to get a relative cell density that is proportional to the OD_{600} in many cases.
8	Denoising	Select here the denoising level that is applied to all cell density curves in the current chart. This setting also influences the growth rate smoothness. You can set the denoising also via the experiment chart context menu.
9	Growth rate smoothing	Increasing this number will automatically increase the smoothing level of all displayed growth rate curves as described below (pp. 88)
10	Data set to be moved	Here you can select the data set to be moved on the time axis.
11	Move time axis	Using this field you can move the selected data set along the time axis. This can be of interested when comparing different data sets or when there is need for lag-time

compensation. All offsets will be exported to .xlsx-files as well.

12	Cut start time	This field sets the start time of the data range to be cut away from all loaded data sets. To cut data from the beginning, use a value lower than the first time value in the chart.
13	Cut end time	This field sets the end time of the data range to be cut away from all loaded data sets. To cut data to the end, use a value higher than the last time value in the chart.
14	Cut all data	Click this button to cut all currently loaded data according to the settings above. The cut data will be saved as separate files with similar file names extended by "_cut".
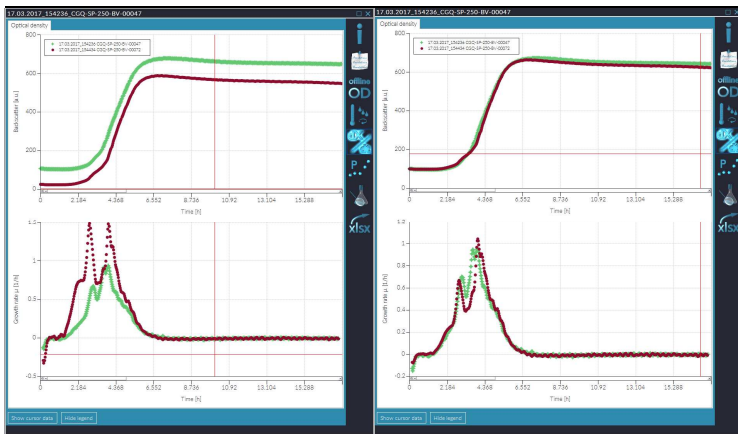
Figure 32: CGQuant data before and after backscatter auto-offset.
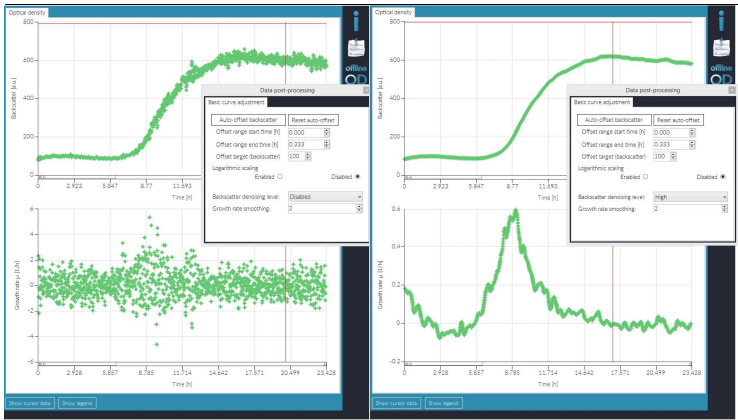
Figure 33: CGQuant data before and after backscatter denoising.

aquila biolabs

Data post-processing – Growth rate calculation

Growth rates indicate, where the cultivated organisms grow faster or slower and where metabolic changes occur. They can furthermore help to compare different experiments more adequately. CGQuant calculates growth rates in the background and displays them, if you enable the "Growth rate"-button in the experiment window toolbar (refer to pp. 66). The growth rates are calculated as μ according to the differential equation of microbial growth

$$\frac{\partial X}{\partial t} = \mu \cdot X \qquad \{4\}$$

with X the cell density, t the time and μ the growth rate. Solving this differential equation with respect to time t yields the exponential formulation of microbial growth, given by

$$X_t = X_0 \cdot e^{\mu \cdot t} \qquad \{5\}$$

with X_t the cell density at time t and X_0 the initial cell density. However, even the growth rate μ is a function of time t, as it changes throughout the complete process of growth due to the reaction of cells to their environment (e.g. substrate or oxygen limitations, metabolic shifts, toxins or toxic metabolites and products, pH, stress, etc.). Thus, the assumption of exponential growth according to equation {5} with an almost constant growth rate μ holds true only for very small time spans. Combining equation {5} for two time points t yields

$$\frac{X_{t_1}}{e^{\mu \cdot t_1}} = \frac{X_{t_2}}{e^{\mu \cdot t_2}} \qquad \{6\}$$

which can be rewritten to

$$\mu = \frac{\ln \frac{X_{t_2}}{X_{t_1}}}{(t_2 - t_1)} \qquad \{7\}$$

88

aquila biolabs

with μ being the average exponential growth rate according to equation {5} over the time interval from t_1 to t_2.

CGQuant-calculated growth rates can be smoothed in the data post-processing window as described above. Basically, the smoothing is carried out by increasing the time interval from t_1 to t_2. The resulting growth rate curves are smoother, but exhibit slightly decreasing growth rates with increasing smoothing level. This is caused by the time interval expansion, which, as mentioned above, might lead to the calculation of growth rates for intervals that cannot be assumed to have constant growth rates.
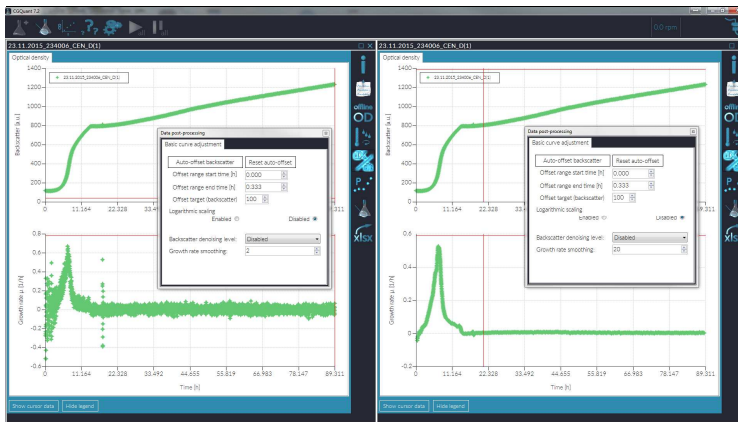
Figure 34: CGQuant data with growth rates at different smoothing levels.

Precise growth rates being comparable to those found in the literature can be calculated only for calibrated data. Growth rate curves for non-calibrated data sets will exhibit a comparable shape regarding peak positions and peak height ratios, but different absolute growth rate values compared to calibrated data sets. However, even non-calibrated growth rate curves contain valuable information, especially regarding the time course of metabolic shifts, induction related growth rate changes or lag-, acceleration and deceleration phases. All growth rates will be exported upon clicking the "xlsx-export"-button.

aquila biolabs

Data post-processing – Growth event detection

Growth events can be defined and monitored from the "Growth event detection" tab of the data post-processing window. This allows you to automatically get notifications as soon as predefined thresholds, shapes or threshold and shape together are detected within the monitored data set.
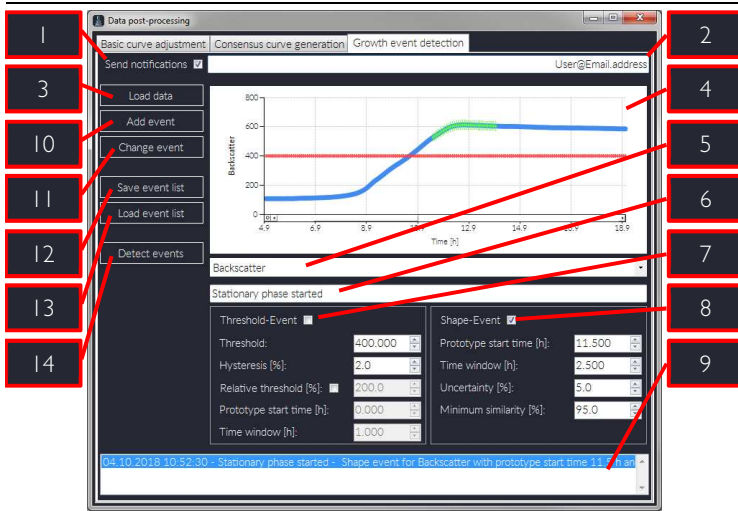
Figure 35: Definition of growth events in the data post-processing window.

The growth event detection tab contains the following control elements:

1	Send notifications	Enable this checkbox to get email notifications via the SMTP-Server configured in the CGQuant preferences (see Figure 37).
2	Recipient email address	In case you enabled the notification sending, please provide an email address of the notification recipient. Each time a new event has been detected, an email notification will be sent to this address.

90

aquila biolabs

3	Load data	Click here to load a .cgqfx-file as prototype data set for the definition of events.
4	Prototype chart	The loaded prototype data set is shown in this chart.
5	Event data type	The selected data type is evaluated for the detection of the associated event.
6	Event name	Please provide a name for the event. This name will be shown as a chart annotation upon detection.
7	Threshold-Event	Enable this checkbox to add a threshold to your current event description. You can set an absolute threshold or a relative threshold. The relative threshold is computed as a multiple of the average value within the defined time window of the monitored data set with the time window being defined by its start time and time window width. Regardless of the threshold type, a hysteresis value must be defined, to prevent noise-induced triggering of multiple events. For rising curves, the threshold + hysteresis must be crossed to trigger an event, for falling curves the threshold - hysteresis must be crossed to trigger an event. The hysteresis limits are visualized in the prototype chart as error bars of the red threshold points.
8	Shape-Event	Enable this checkbox to add a shape to your current event description. The shape definition is done on the basis of the prototype data set and visualized by green data points. The prototype start time and time window define the time width of the shape event.

aquila biolabs

The shape itself is defined by the prototype data set. Using the uncertainty parameter, you can relax the shape definition to allow event detections in different growth curves. Additionally, you can set a minimum similarity between the prototype shape and the monitored data set.

The prototype shape is normalized to its maximum and minimum values to make the shape scale invariant.

The shape event will be triggered whenever a curve section of time window length in the monitored data set is found, which is at least as similar as required (by minimum similarity) to the normalized prototype shape within the defined range of uncertainty (green error bars in the prototype chart).

9	Event list box	Defined events are listed in this list box to after clicking the "Add event" button. Clicking a list entry loads the selected event, displays it on the loaded prototype data and allows for event modification.
10	Add event	Add the current event definition to the event list by clicking this button.
11	Change event	Modify the currently selected and loaded event from the event list.
12	Save event list	Click this button to save the currently defined event list to a .cgqed file. This step is required prior to starting the event detection.
13	Load event list	Click this button to load a previously defined event list from a .cgqed file. This can be done without loading a prototype data set.

aquila biolabs

| 14 | Detect events | Click this button to start the event detection. In running measurements, the event detection is then automatically performed whenever new data are available. In previously recorded data sets, the event detection is performed only once per click. |

The detected events are displayed together with the CGQuant annotations in the experiment window chart, whenever the "Annotation"-button is enabled. You can move the mouse over the events to see their definition information. An event detection example is depicted in Figure 36 for two different organisms, *E. coli* and *S. cerevisiae*. The shape event describing the entry of a stationary growth phase as shown in Figure 35 was used for both growth curves.

During a running measurement it may be favourbale to get instant notifications whenever a new event has been detected. CGQuant can send such notifications if an SMTP client is properly configured in the CGQuant options and preferences and if the email notifications are enabled together with a valid exipient address in the event detection tab of the data post-processing window.
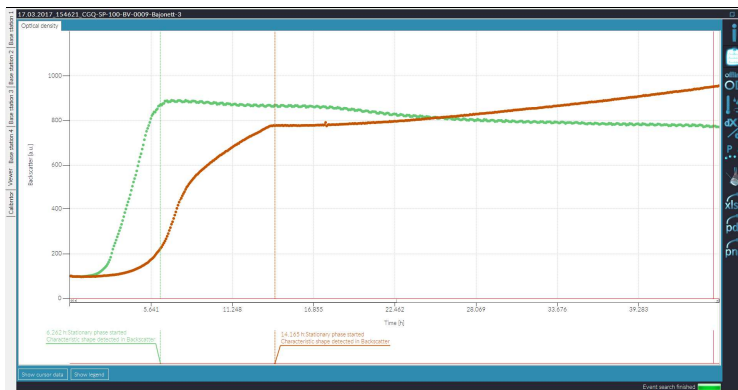
Figure 36: Event detection of the above defined stationary phase shape event in data sets from E. coli (green) and S. cerevisiae (brown).

aquila biolabs

In order to configure CGQuant as SMTP client, open the CGQuant preferences window and navigate to the "Notifications" tab as depicted in Figure 37. Enable the email notifications and provide an email address, from which the notifications will be sent. Then specify a sender name, e.g. "CGQuant", which will be displayed later in your email program.

The SMTP settings (server name, port, TLS/SSL and authentication requirement) can be obtained from your email provider, if you do not know them. It is strongly recommended to use encrypted message transfer via SSL or TLS in combination with the suitable port (usually port 465 or 587).

If your email server requires authentication, enable the corresponding checkbox and provide your credentials (user name and password). The credentials are saved together and encrypted using the Windows OS data protection API with current user data protection scope.

aquila biolabs
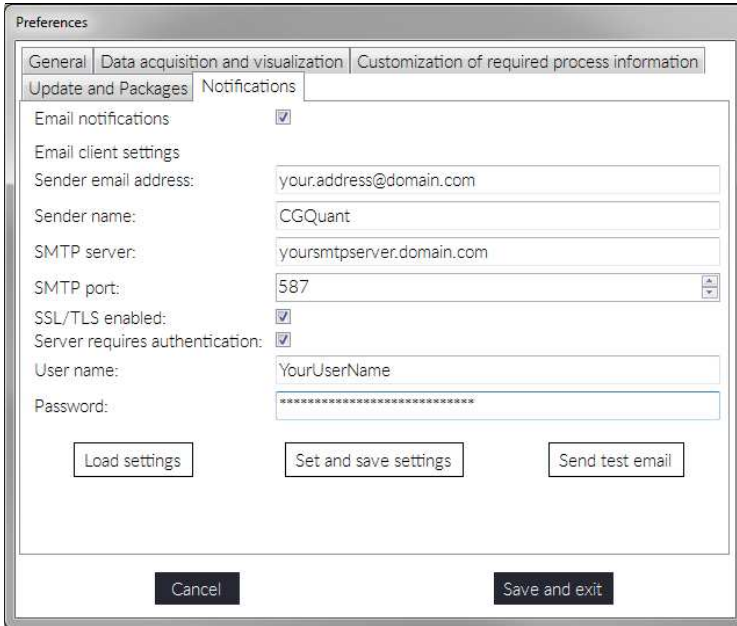
Figure 37: Configuring an SMTP client to allow CGQuant the sending of email notifications.

Finally activate and save the SMTP configuration by clicking the "Set and save settings" button. It is recommended to test the SMTP configuration by sending a test email.

> Be aware that your email credentials may be accessible by any user having access to the PC running CGQuant with your user account. Even though the password is not shown in CGQuant, it may be retrieved from memory by a skilled person.

95

aquila biolabs

Data post-processing – Consensus data

Good scientific practice requires the collection of replicates as well as their statistical analysis. CGQuant supports you with this task by providing you with tools for the generation and analysis of consensus data. Replicate data sets having been collected under similar experimental conditions and technical setups are thereby merged into a single .cgqcd file and represented as an average growth curve together with user-selectable confidence intervals.

In order to generate consensus data, all replicate data should be opened together in the same experiment window. As soon as all your data are loaded, please open the data post processing window. Perform the required post-processing (e.g. Auto-offset) and then navigate to the tab "Consensus curve generation". Select all experiments that should be merged into one consensus data set and move them to the consensus curve experiments, by clicking the move-button or by single drag-and-drop operations.
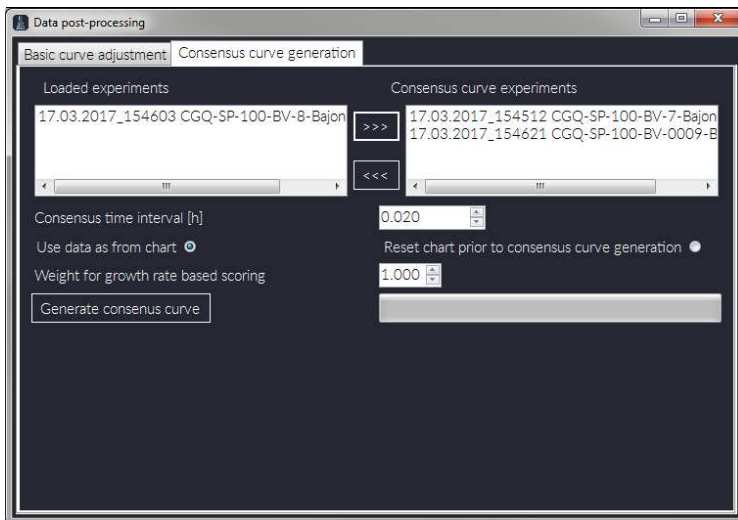
Figure 38: Consensus data generation.

aquila biolabs

Before generating the consensus curve, you can customize this process.

According to the chosen consensus time interval, all data sets will be unified with respect to their measurement time points, resulting in equal point-to-point distances for all relevant replicates. During the unifying process for each replicate, whenever there is no data point exactly on the multiples of the chosen interval, the unified data point will be calculated by linear interpolation between the two closest measurement points with respect to the measurement times.

Depending on your selection, the consensus curve generation will either act on the processed data as available from the chart, including all offsets, or the chart and all offsets will be reset prior to the consensus curve generation. Generally it is recommended, to perform the auto-offset processing before consensus curve generation.

In the process of consensus data generation, all replicates will be aligned automatically by moving them on the time axis. This alignment ensures that different lag-times are not negatively influencing the consensus data quality. The alignment uses a scoring function, which scores backscatter differences, growth rate differences or both. By default, the scoring is performed only on the basis of growth rates. While growth rate based scoring usually performs best, cases may arise, where you also want to score against the backscatter data. In order to do that, lower the weight for growth rate based scoring, e.g. a value of 0.5 will score growth rate differences and backscatter differences equally.

Finally start the consensus curve generation by clicking the respective button. You will be asked to provide a filename for saving the generated .cgqcd file. Upon successfully finishing this process, the new consensus curve will be opened in a new experiment window automatically (Figure 39).

aquila biolabs
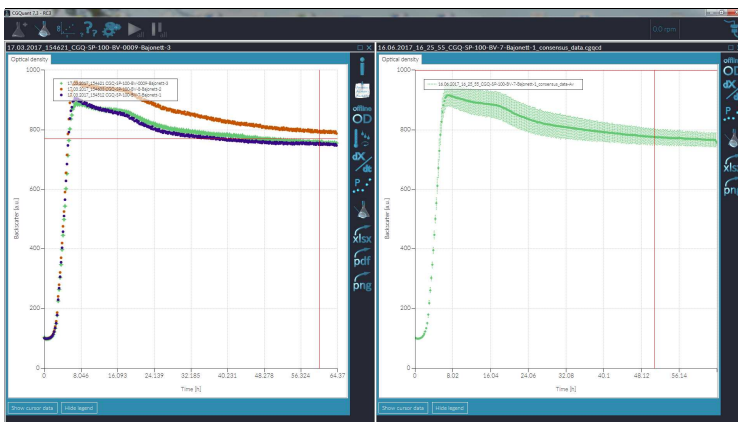
Figure 39: Triplicate and consensus data set.

In order to analyze and compare consensus data sets, open the post-processing window and navigate to the tab "Curve analysis" (Figure 41). There you can select the confidence intervals being used for data visualization (Figure 40).
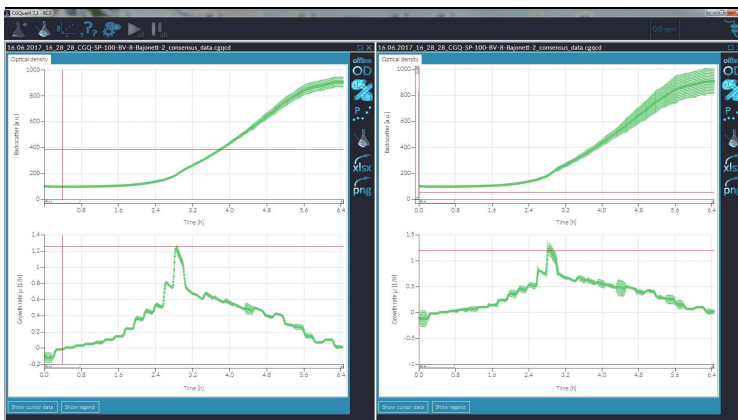
Figure 40: The same triplicate consensus data set with standard deviation (left) and 95% confidence interval (right).

Furthermore you can select a reference data set and perform a statistical evaluation of all loaded consensus curves against the chosen reference. The comparison results are displayed as averages in a table and you can visualize

98

aquila biolabs

them graphically by selecting one of the following items in the analysis result dropdown menu:

- Absolute measurement uncertainty

- Relative measurement uncertainty (compared to average)

- Absolute difference to reference data

- Relative difference to reference data (compared to reference data average)

- Probability for each data set to be different from the reference according to a t-Test

- Probability for each data set to be different from the reference according to a Welch-Test

Each of these results is computed as a per-point-comparison between the loaded consensus data sets.
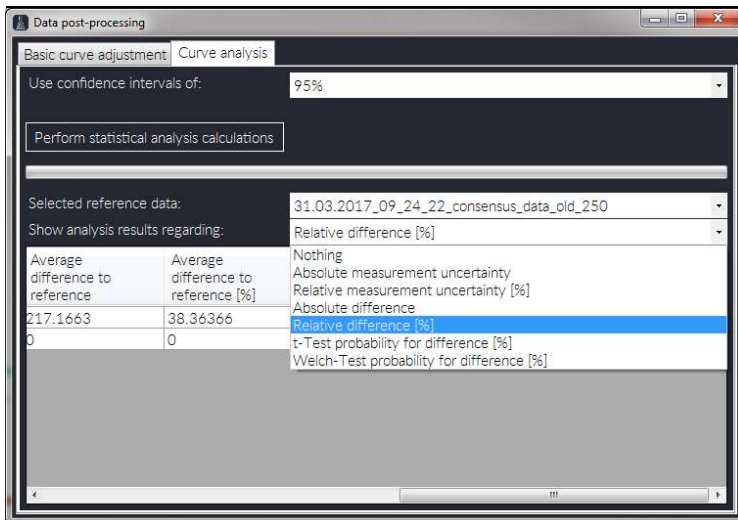
Figure 41: Consensus curve analysis.

aquila biolabs

Exporting the data to .xlsx files

Data from an experiment window can be exported to an .xlsx-file using the xlsx-export button in the right toolbar (refer to Figure 19 on page 67). Clicking this button will export all data shown in the chart into one file, including the cell density curves, process information, growth rates, calibration data, environmental data, annotations and offline OD-values. Each dataset loaded and displayed in the chart is exported into one tab-page of the .xlsx-file.

All data series will be exported according to their current state and as drawn in the chart, i.e. growth rates will always be exported according to their current smoothing level. To facilitate the handling of big datasets on less powerful computers, a data reduction window (see Figure 42) that is opening upon clicking the xlsx-export button allows you to reduce the data point density.
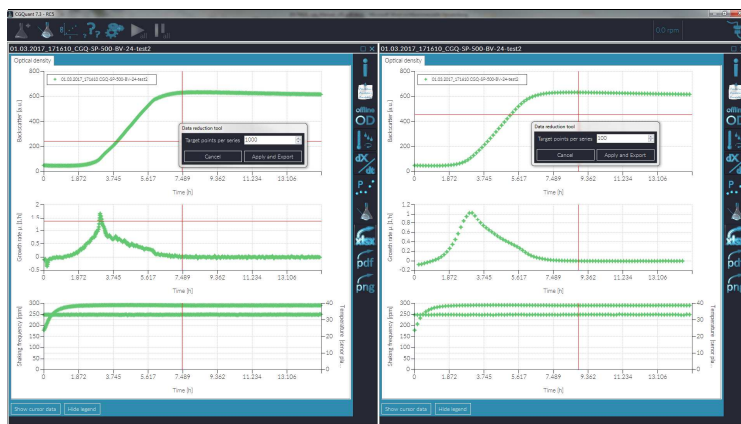
Figure 42: Data reduction prior to .xlsx-export.

You can adjust the data density using the up-down-control. The data to be exported will be previewed in the chart. Clicking "Apply and export" starts the exporting process, while clicking "Cancel" interrupts the export and brings you back to the experiment window.

aquila biolabs

A running export process is indicated by a blinking .xlsx-export button. After successfully finishing the export, the blinking stops and the generated file is automatically opened with your default program for .xlsx-extension files (e.g. Microsoft Excel or LibreOffice Calc). Finally all the data are redrawn in the chart with the original data density settings.

Exporting the data to .xlsx files

aquila biolabs

Exporting the data to .pdf files

Clicking the .pdf-button allows you to build a PDF report either from a single experiment or from all loaded experiments in the current experiment window. You can customize the report using the CGQuant PDF Report Builder window as depicted below. Once you have defined your own report style, you can save these settings or use them as default values.
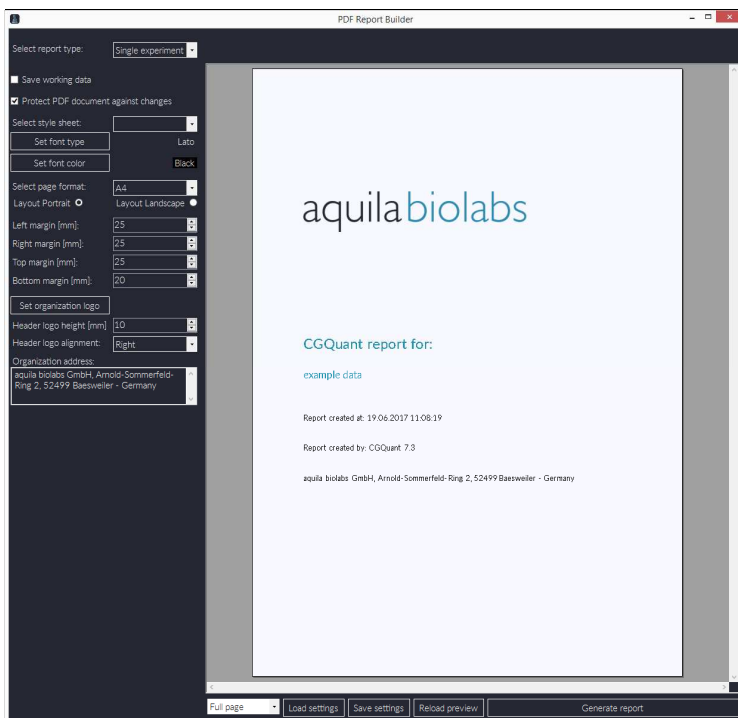
Figure 43: PDF Report builder.

A CGQuant report contains figures of all charts, all process information and the currently loaded calibration file. Furthermore, you can optionally save the current working data to the report.

102

aquila biolabs

The PDF document to be generated can also be protected against changes. Enabling this option will generate a random key composed of 64 characters, including letters, capital letters, numbers and additional characters. This key is then used to encrypt the PDF file using RC4 (128-bit).

Use the "Reload preview" button to update the preview in case some changes are not automatically incorporated into the preview.

Finally, click the "Generate report" button and choose a path for the PDF file to be saved. After successfully finishing the .pdf-export, the newly generated PDF file will open up automatically in you default PDF-viewer.

aquila biolabs

Exporting the data to .png files

Clicking the .png-button allows you to export data from the chart to a PNG image file. You can customize the image using the CGQuant Chart Export window as depicted in Figure 44. The customization process includes:

- Axes spans, intervals and titles
- Chart title
- Data types, marker styles and size
- Legend style and position
- Grid and axes lines
- Image size
- Fonts of all text components

Data are directly taken from the experiment chart, including zooming levels and smoothing. This should be considered when preparing data for export.
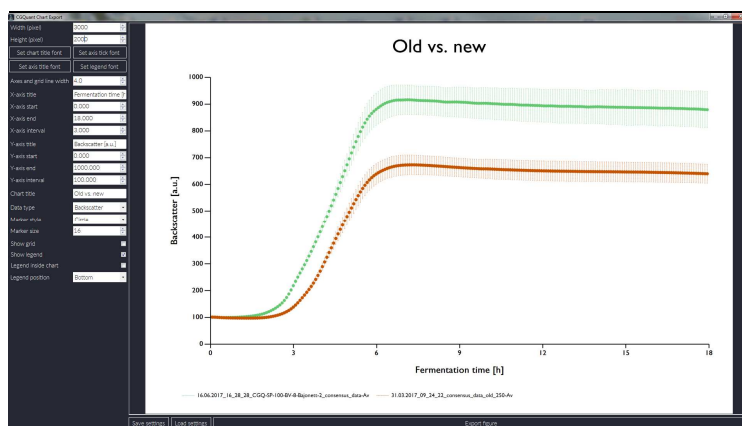
Figure 44: Chart export to .png image.

Settings can be saved as defaults or to separate preset files, which can be loaded for easier image export.

Click "Export figure" to specify a filename and save the PNG image.

104

aquila biolabs

Collecting calibration data

Calibration is required in all applications where true cell density values, typically OD_{600} or cell dry weight (CDW), are needed and the native CGQ derived backscatter data are not sufficient to meet your requirements. The CGQuant software contains an elaborate calibration module (the CGQuant calibrator) allowing you to get accurate calibration curves quickly, intuitively and with minimal hands-on time. Based on your bioprocess parameters CGQuant generates an optimized calibration protocol and guides you through the complete calibration procedure without the requirement for any external calculations or additional third-party software.

CGQuant's calibration protocol is based on a multi-step procedure with repeated cycles of measuring a well-defined cell suspension once using the CGQ and once using an appropriate offline device (e.g. UV-VIS spectrometer for OD_{600}-readings). Only a few things need to be prepared before starting the calibration:

1. A highly concentrated master stock solution of cells in the stationary phase.
    a. At first, you should grow the cells on the medium to be used during calibration until they have reached the stationary phase.
    b. Then spin them down to create a highly concentrated master stock and store it on ice to prevent further growth or damaging of the cells.
2. At least one shake flask of appropriate size.
3. Enough medium for the preparation of substocks and for diluting the offline OD_{600} samples.
4. A CGQ device without any running measurements. Connect the CGQ to your PC running CGQuant.

aquila biolabs

5. A shaker prepared to provide the shaking conditions for your calibration.

6. Other stuff, such as CGQ darkening covers, cuvettes, pipettes, etc.

When performing calibrations in a bioreactor, the same steps apply as for shake flasks; just think of your bioreactor being a shake flask. Furthermore, all shaking related issues may be ignored or replaced by stirring speed instead of shaking speed.

To start the CGQuant calibrator, just click the calibration-button in the main window's toolbar (see pp. 64). The start screen will open up as shown in Figure 45. CGQuant's calibrator is organized into two parts, the left part contains user interaction controls, while the right part is used to guide you through the calibration procedure and to visualize the measurements. Just follow the instructions provided by the CGQuant calibrator and navigate straight through your calibration tasks using the "Next-step-Button"
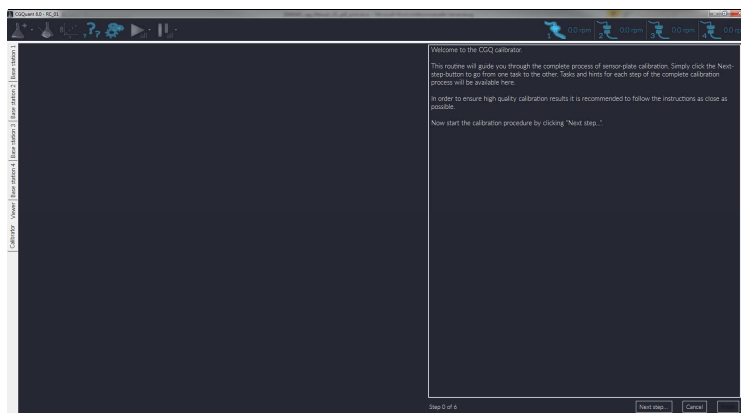
Figure 45: CGQuant calibrator start screen.

The first calibration task is the calculation of calibration points and of the stock solution concentrations being required to create the well-defined solutions for each point. As depicted in Figure 46, a table needs to be filled with different

aquila biolabs

information and parameters regarding your bioprocess to be calibrated and regarding the design of the calibration procedure. Based on these parameters, the CGQuant calibrator will calculate an optimal calibration procedure for your specific application.
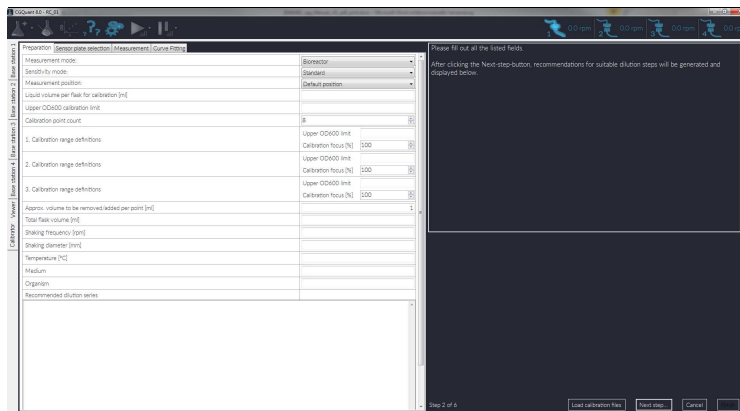
Figure 46: CGQuant calibrator overview on dilution series parametrization and calculation.

The calculation of optimal calibration points is primarily based onto two parameters, the upper OD_{600} calibration limit and the calibration point count. You should choose the upper calibration limit to be as high as the typically observed highest cell density in your bioprocess. Depending on this limit, the CGQuant calibrator will automatically set a recommended calibration point count. You can change it with respect to your special requirements, but at least 8 points need to be collected, otherwise the reliability of your calibration would be decreased, resulting in questionable cell density readings during normal CGQ measurements on the basis of such a weak calibration.

You can fine-tune the calibration protocol by changing the three focus windows regarding upper local cell density limit and the calibration point count percentage. The basic idea behind these focus windows originates from the curve that describes the relationship of CGQ backscatter intensities and real

aquila biolabs

cell densities (e.g. OD_{600}). As described above (pp. 47) this relationship can be formulated as a sigmoid function with an exponential beginning (1st window), a transition zone (2nd window) and finally a linear part (3rd window). If you would like to focus the calibration on a certain part of that curve, you can change each window's location and width using the calibration range definitions. However, in most cases the automatically suggested calibration ranges should be sufficient.

Preparation	Sensor plate selection	Measurement	Curve Fitting		
Measurement mode:					Shake flask
Sensitivity mode:					Standard
Measurement position:					Default position
Liquid volume per flask for calibration [ml]					25
Upper OD600 calibration limit					10
Calibration point count					14
1. Calibration range definitions			Upper OD600 limit		1
			Calibration focus [%]	43	
2. Calibration range definitions			Upper OD600 limit		5
			Calibration focus [%]	29	
3. Calibration range definitions			Upper OD600 limit		10
			Calibration focus [%]	29	
Approx. volume to be removed/added per point [ml]					1
Total flask volume [ml]					250
Shaking frequency [rpm]					250
Shaking diameter [mm]					25
Temperature [°C]					37
Medium					M9
Organism					E. coli
Recommended dilution series					

Point	OD600 calc.	Added stock volume [mL]	Substock OD600
0	0	0	6
1	0.2	0.833	6
2	0.4	0.862	6
3	0.6	0.893	6
4	0.8	0.926	6
5	1	0.962	6
6	2	0.862	30
7	3	0.893	30
8	4	0.926	30
9	5	0.962	30
10	6.25	0.862	41.25
11	7.5	0.893	41.25
12	8.75	0.926	41.25
13	10	0.962	41.25

Figure 47: CGQuant calibrator example for dilution series parametrization and calculation.

To calculate or recalculate the dilution series after having filled the table, just click the "Next step…"-button. An exemplary calibrator configuration with automatically generated dilution series is shown in Figure 47. The dilution series list is accessible throughout the whole calibration procedure. As for any CGQ

aquila biolabs

measurement, the measurement mode, sensitivity mode for bioreactors and the measurement position must be defined. These parameters are initially loaded according to their defaults from the CGQuant preferences window.

The next step is the selection of the base station, sensor plates and filenames to be used (exemplarily shown in Figure 48).
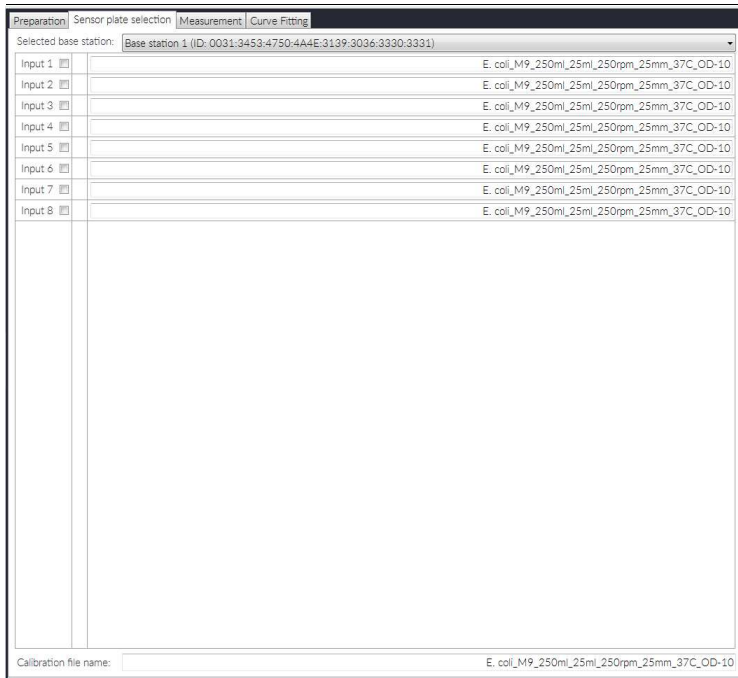
Figure 48: CGQuant calibrator plate and filename selection.

Usually you will need only one sensor plate, however, if you e.g. want to perform triplicates, you can select several inputs. The filenames can be changed globally in the lowermost text box. Please specify a unique filename for each sensor plate input that is to be used. Sensor plate inputs to be used need to be checked.

aquila biolabs

After having finished the sensor plate and filename selection, click the "Next step…"-button to proceed with the calibration measurements (see Figure 49 and Figure 50).
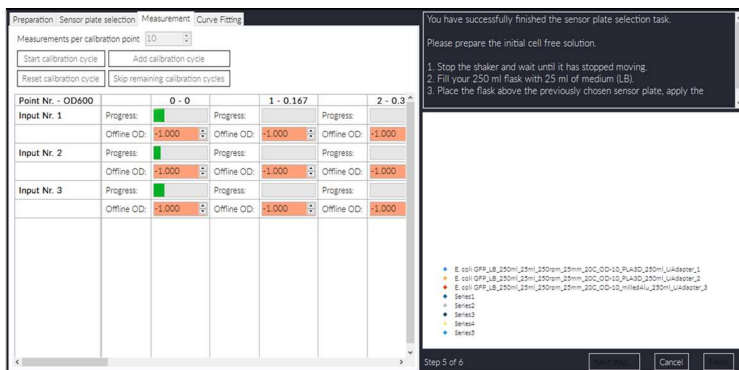
Figure 49: CGQuant calibrator measurement step overview.

Each calibration point is calculated from several measurements. You can adjust the default value of 10 according to your requirements. Higher values will reduce noise, but increase the measurement time, but 10 is usually a sufficient value. Read and follow the instructions in the calibrator's right part. Then, as soon as your shaker has reached the target shaking frequency start a calibration cycle. The measurement progress will be shown in the table. After one cycle is finished, you can either redo the calibration cycle (e.g. if something went wrong during the measurement) or proceed with the next cycle. All collected calibration points will be shown in the chart to the right.

> ⚠️ Keep in mind to use each sample removed from the currently calibrating liquid for precise offline cell density measurements (e.g. OD_{600}). Write all offline cell density values to the appropriate fields. (refer to Figure 50)

> ⚠️ In calibration mode, the automated measurement control is always turned off by CGQuant. It is therefore crucial to wait, until the shaker has reached its target speed before starting a new data acquisition phase.

110

aquila biolabs

You can skip remaining calibration cycles or add further calibration cycles by clicking the respective buttons.

If you want to test the calibration tool without doing real measurements, you can check the "Calibrator evaluation enabled"-box. An exemplary set of calibration data will be used instead of real calibration data.
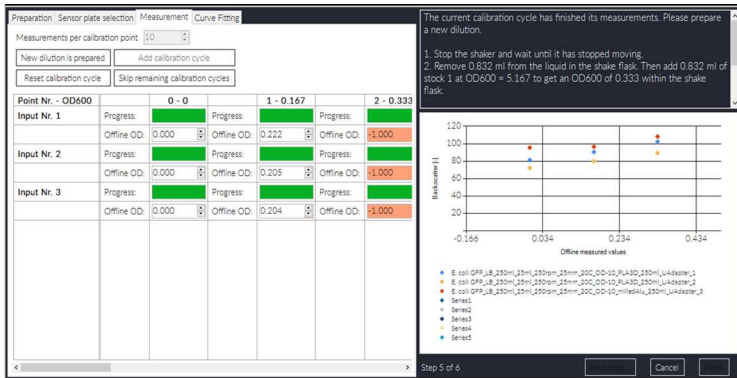
Figure 50: CGQuant calibrator measurement progress.

After having finished the calibration data collection, you can step to the last calibration task, the curve fitting and file saving (Figure 51). Here, the collected calibration data will be fitted automatically to equation {2} introduced on page 49. You can influence the fitting procedure by defining the convergence limit and the local iteration limit. While the default local iteration limit of 500 is usually a good compromise between performance and fitting quality, the convergence limit might be adjusted according to the calibration data quality.

Before starting the fitting procedure, select the data to be fitted in the leftmost list. Upon selection, they will be displayed in the chart. You can select whether to use calculated or measured offline cell density values using the according check-box. Usually the measured OD_{600}-values should be used, but in some cases, where measured data are not available, you can also use the calculated data from the initial dilution series list.

111

aquila biolabs
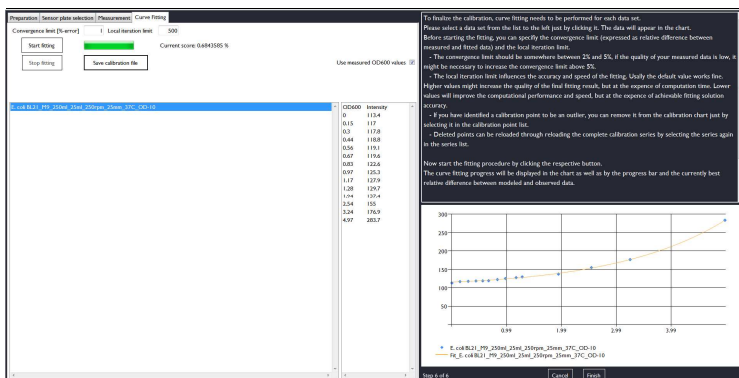
Figure 51: CGQuant calibrator curve fitting.

Start the fitting procedure by clicking the "Start fitting"-button. CGQuant will automatically perform the curve fitting until either the convergence limit or an internal iteration limit is reached. CGQuant will always display the currently best fitting solution in the chart accompanied by its current score. If a solution is found that meets your requirements, but without having reached the convergence limit yet, you can manually stop the fitting by clicking the "Stop fitting" button. In some cases there might be outliers in the calibration data that prevent CGQuant from finding a good fit. You can delete these outliers from the fitting chart by clicking them in the data point list and then you will be asked whether to delete the selected point. All measured points can be restored by clicking the calibration data series in the leftmost list again. If you have collected multiple calibration data sets for the purpose of statistical robustness, you can merge all loaded calibration data into a single calibration data set, which then can be used for fitting. Finally, after having obtained a fit of sufficient quality, save the calibration data by clicking the "Save calibration file"-button.

Calibration data can be generated alternatively on the basis of growth associated offline samples as described in the following chapter.

aquila biolabs

Using calibration data

The calibration files can be used to calculate real cell densities from the CGQ backscatter data. Starting from CGQuant 7, all newly generated cgqfx-files are equipped with a default calibration file. This allows you, in combination with at least two offline values, to estimate your offline cell densities in real-time. All data collected during a measurement can be displayed according to the calibration file cell densities by toggling the cell density view via the "OD"-button in the experiment window.

Be extremely careful when using the default calibration file. It is only intended for very rough offline cell density estimation and it requires at least two offline values to be entered by the user.

It is strongly recommended to replace the default calibration file with a suitable calibration file for the strain, medium, flask type and shaking environment of the target experiment.

Deriving offline cell density values (e.g. OD_{600}) from online backscatter readings as provided by the CGQ is challenging. This is caused by the different measurement approaches as well as by the factors that influence backscatter readings (see pp. 52). Thus it is required to provide at least two offline support data points to CGQuant, which will be used for calibration file refinement.

All actions regarding calibration file usage and offline data are carried out in the offline support data window (Figure 52), which can be opened by performing a right-click on the experiment window's "OD"-button.

This window allows you, to change the calibration files for those datasets, which have been selected in the upper right list box. You can display the content of a loaded calibration file by selecting the desired data in the middle right dropdown list.

aquila biolabs
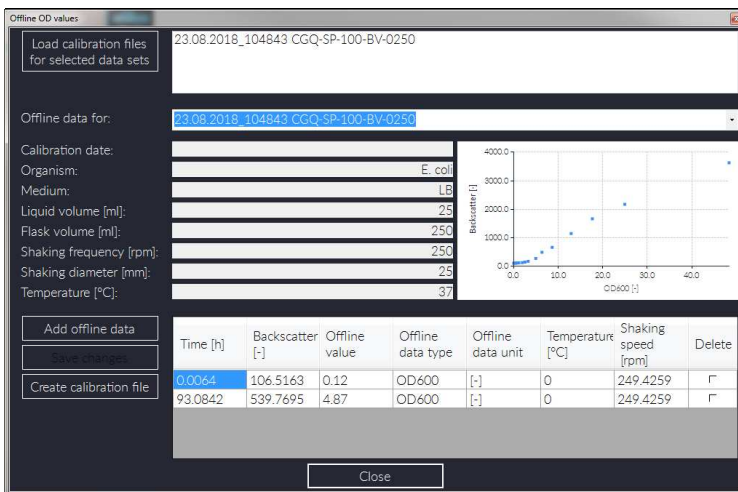
Figure 52: CGQuant offline support data window.

Offline data can be added by clicking the "Add offline data"-button. CGQuant will then automatically fill the time entry with the latest experiment time. Alternatively, you can change that time according to your requirements. CGQuant will automatically search for the backscatter intensity at that time point. Then enter your offline cell density value and click the "Save changes"-button. You can delete an offline support data point by checking the "Delete"-checkbox, followed by saving the introduced changes.

Upon clicking the "Close"-button CGQuant refines all loaded calibration files with the available offline data and updates the chart accordingly.

On the basis of saved offline data you can create a calibration file. Click the "Create calibration file" button and follow the instructions. You will need to provide calibration descriptions and then you will need to perform the fitting and file saving in the CGQuant calibrator as for usual calibration files.

114

aquila biolabs

Updating the sensor plate firmware

From CGQuant 8 on, all CGQ sensor plates sold after 01.10.2018 can be updated in place via the base station. Updates over USB are not supported for these new sensor plates.

1. Stop the measurement of all sensor plates to be updated.
2. Open CGQuant on your computer.
3. Open the "Preferences" window in the CGQuant main menu.
4. In the "Preferences" window, navigate to the "Update and Packages" tab.
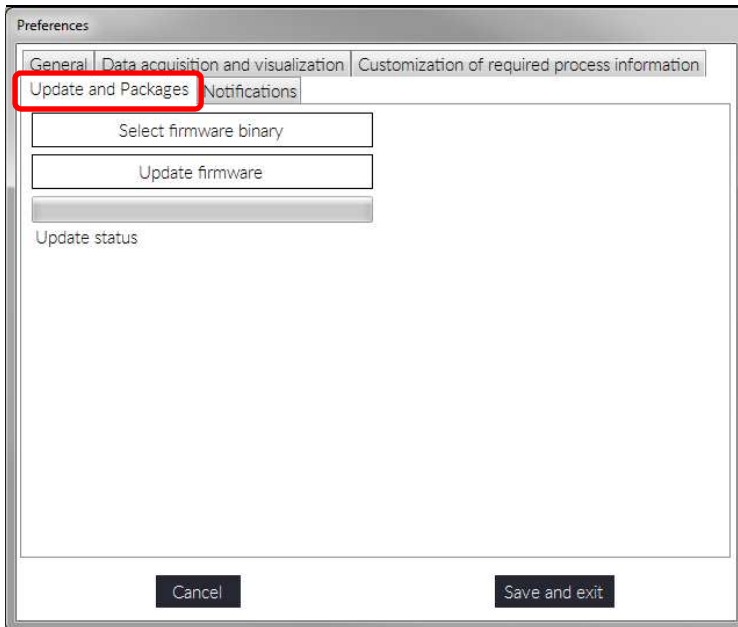
Figure 53: Navigating to "Update and Packages".

5. Click the "Select firmware binary" button, navigate to the folder "…\CGQuant\firmware binaries\CGQ sensor plates" and load a suitable .cgqfin file.

aquila biolabs

6. Start the firmware update procedure and wait for it to be finished. The firmware update progress is reported in the preferences window and in the overview window, as exemplarily shown in Figure 54 and Figure 55. If there is no observable progress with the firmware update, please power-cycle the complete CGQ system by disconnection the base station from the power supply, waiting for some seconds and reconnecting it again.
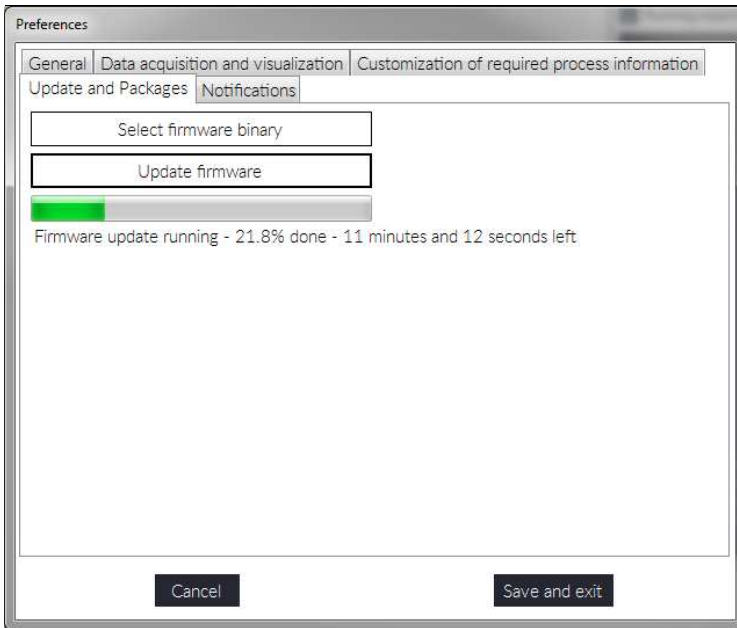
Figure 54: CGQuant preferences window during firmware update.

aquila biolabs
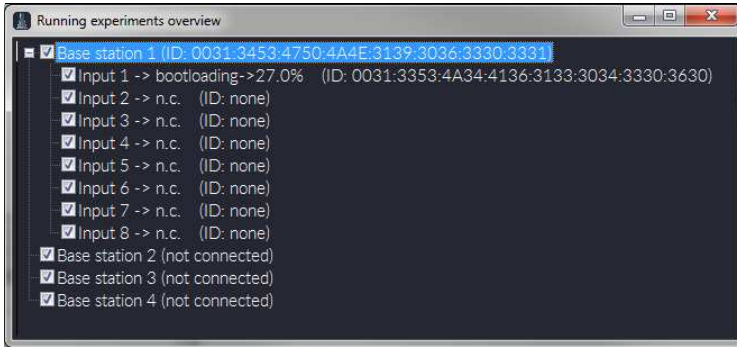
Figure 55: CGQuant overview window after successful firmware update.

7. As soon as a successful firmware update is reported in the "Preferences" window, your sensor plate is updated.

8. Finally, close the "preferences" window by clicking the "Cancel" button.

⚠️ **Remember that CGQuant 8 usually requires both, sensor plates and base station, to be updated with the respective firmware versions.**

⚠️ **Be patient with the firmware update of sensor plates. The improved usability comes at the cost of lower data rates through the sensor plate to base station communication. The update of many sensor plates in parallel may under certain circumstances take several hours.**

Updating the sensor plate firmware

117

aquila biolabs

Updating the base station firmware

Before starting the firmware update of your base stations, please ensure that you have installed CGQuant 8 on your computer and that you have the base station USB cable, the base station power supply and the power elongation cable available. Now you can start to update the CGQ base stations as described in the following.

1. Disconnect all sensor plates before updating the base station by unplugging them from the base station front panel.
2. Disconnect the base station from your computer (if connected before) by unplugging the USB cable on either side.
3. Disconnect the base station's power supply by unplugging the power supply elongation cable and the power supply cable.
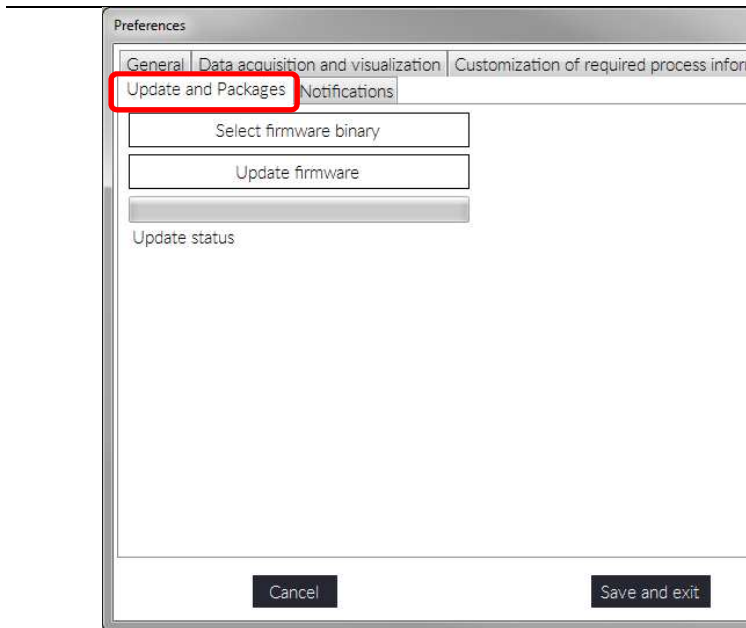
Figure 56: Navigating to "Update and Packages".

aquila biolabs

4. Open CGQuant 8 on your computer.

5. Open the "Preferences" window by clicking the respective button in the CGQuant main menu.

6. In the "Preferences" window, navigate to the "Update and Packages" tab.

7. Click the "Select firmware binary" button, navigate to the folder "…\CGQuant\firmware binaries\CGQ base stations" and load a suitable .cgqfi file.

8. Plug in the base station power supply into the right port of the base station top panel and connect power elongation cable and power supply.

9. Plug in the USB cable at the base station top panel and at a free USB port of your computer and wait for CGQuant to connect to the base station.

10. Start the firmware update procedure and wait for it to be finished. The firmware update process is a series of different steps, which are reported in the "Preferences" window, as exemplarily shown in Figure 57 and Figure 58. If the update process fails or stalls, please disconnect the base station power supply, reconnect it and try to upload the firmware again.

Updating the base station firmware
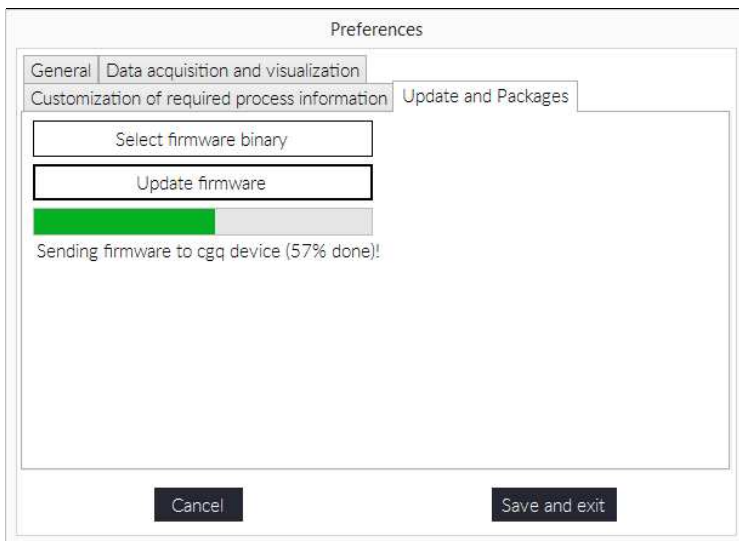
Figure 57: CGQuant "Preferences" window during firmware update.
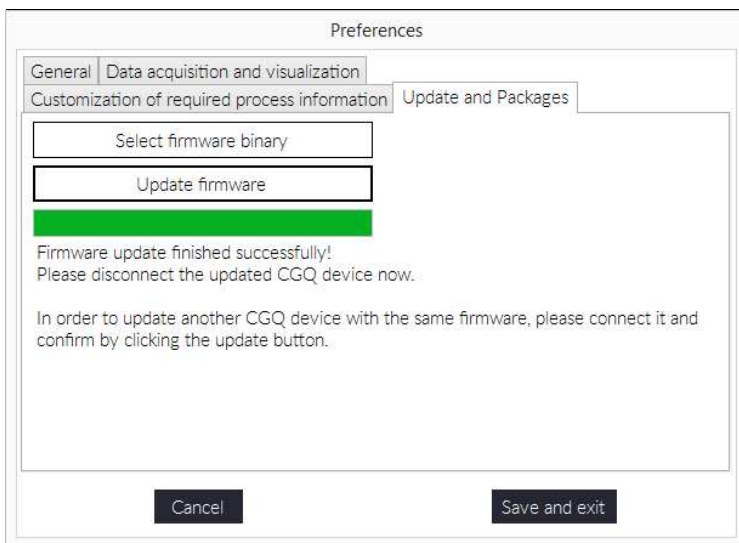
Figure 58: CGQuant "Preferences" window after successful firmware update.

120

aquila biolabs

11. As soon as a successful firmware update is reported in the "Preferences" window, your base station is updated and you can disconnect it.

12. In order to update additional base stations with the same firmware binary, proceed with step 8 and iterate through the subsequent steps until all your base stations are updated.

13. Finally, close the "Preferences" window by clicking the "Cancel" button.

⚠️ **Remember that CGQuant 8 usually requires both, sensor plates and base station, to be updated with the respective firmware versions.**

Updating the base station firmware

aquila biolabs

The CGQ Monitor

The CGQ Monitor is a small tray application, which is co-installed with CGQuant. It monitors and restarts CGQuant automatically in case it has crashed or has become unresponsive for any reason. Upon CGQuant restart, all previously running experiments are automatically restored and restarted, in order to prevent data loss or incomplete measurements.

The CGQ Monitor can be closed via its content menu, which appears upon right-clicking the CGQ Monitor tray icon. It is strongly recommended to not close CGQ Monitor during CGQuant operation.

In order to ensure optimal cooperation of CGQuant and CGQ Monitor, the latter is installed to the auto-start programs and opens automatically upon Windows user login. Furthermore, CGQuant checks on startup, whether the CGQ Monitor is already running and opens it, if required.

aquila biolabs

Help section

Troubleshooting

The following chapter provides hints to solve problems that might arise during the usage of CGQ and CGQuant. Please select one of the issue types and go along the trouble shooting flow charts.

- Solving connection issues
- Solving data acquisition issues
- Solving calibrator issues
- Solving calculated offline value issues
- Solving data interpretation issues

Please read the manual carefully to obtain information that is required for answering the troubleshooting flow chart questions.

In case they do not yield the desired result, please contact our service (refer to page 130) and remember the number of the trouble shooting end point.

aquila biolabs

Solving connection issues

```
                            ┌─────────┐
                            │  Start  │◄───────────────┐
                            └─────────┘                │
                                 │                     │
                                 ▼                     │
                      ╱─────────────────╲    No    ┌──────────────────────┐
                     ╱  Is the base       ╲───────►│ Connect the base     │
                     ╲  station connected  ╱        │ station to the       │
                      ╲ to the power       ╱        │ power supply.        │
                       ╲  supply?         ╱         └──────────────────────┘
                         ╲──────────────╱
                              │ Yes
                              ▼
                      ╱─────────────────╲    No    ╭──────────────────────╮
                     ╱  Is the green base ╲───────►│ Contact aquila       │
                     ╲  station LED glowing?╱       │ biolabs' support (1).│
                       ╲──────────────────╱        ╰──────────────────────╯
                              │ Yes
                              ▼
                      ╱─────────────────╲    No    ┌──────────────────────┐
                     ╱  Is the base       ╲───────►│ Connect the base     │
                     ╲  station connected  ╱        │ station to the       │
                       ╲ to the PC via USB?╱        │ PC via USB.          │
                         ╲──────────────╱          └──────────────────────┘
                              │ Yes
                              ▼
                      ╱─────────────────╲    No    ┌──────────────────────┐
                     ╱  Is CGQuant 8      ╲───────►│ Open CGQuant 8       │
                     ╲  opened on your PC? ╱        │ on your PC.          │
                       ╲──────────────────╱        └──────────────────────┘
                              │ Yes
                              ▼
                      ╱─────────────────╲   Yes    ╭──────────────────────╮
                     ╱  Is a CGQuant 8    ╲───────►│ Connection           │
                     ╲  connection label   ╱        │ established.         │
                       ╲ glowing?         ╱         │ Congratulations -    │
                         ╲──────────────╱          │ issue solved.        │
                          │                        ╰──────────────────────╯
                   No     │   <3x No    ┌──────────────────────┐
                ┌─────────┤────────────►│ Wait 30 seconds for  │
                │         │              │ connection           │
            ≥3x No       │              │ establishment between│
                │        │              │ CGQuant and CGQ      │
                ▼        │              │ base station.        │
                         │              └──────────────────────┘
      ╱─────────────────╲
     ╱  Do you hear the   ╲    No    ╭──────────────────────╮
    ╱   USB enumeration    ╲───────►│ Contact aquila       │
    ╲   sound or do you     ╱        │ biolabs' support (2).│
    ╲   see the base        ╱        ╰──────────────────────╯
     ╲  station as CGQ      ╱
      ╲ in the Windows    ╱
       ╲ device manager? ╱
         ╲──────────────╱
              │
              │ Yes, proceed on next page.
              ▼
```

aquila biolabs

Yes, from previous page.

Have you updated your base station and sensor plates to firmware revision 3?

No → Update your base station and sensor plates according to pp. 118

Yes

Start (pp. 124) ← Restart your computer, disconnect the CGQ power supply and go to Start

Did the CGQ driver install successfully when you installed CGQuant 8?

No → Execute the CGQuant 8.0 Installer with administrator privileges and observe the driver installation success.

Yes

Is the CGQuant 8 connection label glowing?

No → Contact aquila biolabs' support (3).

Yes

Connection established. Congratulations - issue solved.

Troubleshooting

aquila biolabs

Solving data acquisition issues

Start a new experiment in CGQuant connected to the CGQ.

Is a sensor plate connected to the base station port selected in CGQuant?
— No → Connect a sensor plate to the selected port or restart the experiment with another port.

Yes ↓

Is the sensor plate status in the CGQuant overview window "n.c."?
— <3x Yes → Disconnect the sensor plate from the base station and wait 10 seconds. Reconnect the sensor plate to the same port and wait 20 seconds.
— ≥3x Yes →

Have you updated your sensor plates to firmware revision 3?
— Yes →
— No → Update your base station and sensor plates according to pp. 115.

No ↓

Did you start the measurement in CGQuant?
— No →

Yes ↓

Contact aquila biolabs' support (4).

Start the measurement (pp. 67).

When watching the sensor plate state over some minutes, is it jumping between "idle" and "measuring"?
— Yes →

No ↓

Are data points displayed in the experiment window?
— Yes → Data acquisition running. Congratulations - issue solved.
— No → Contact aquila biolabs' support (5).

Is your shaker shaking at >150 rpm?

Yes, proceed on next page.

No, proceed on next page.

126

aquila biolabs

Is the automated measurement control checkbox (pp. 76) in the CGQuant preferences checked?

Is the automated measurement control checkbox (pp. 76) in the CGQuant preferences checked?

Yes

Yes

No

No

Uncheck the automated measurement control checkbox (pp. 76) in the CGQuant preferences and save the settings. Then stop and close your experiment.

Contact aquila biolabs' support (6).

Contact aquila biolabs' support (7).

Start a new experiment in CGQuant connected to the CGQ.

Troubleshooting

aquila biolabs

Solving calibrator issues

Start a new calibration with CGQuant connected to a CGQ.

Is the usage of stock solutions with OD_{600} >100 required?

Yes → Increase calibration point count or volume to be removed/added per point. Then click "Next step".

No ↓

Are all required sensor plates running their measurements (progress bar moving)?

<3x No → Disconnect the responsible sensor plate from the base station and wait 10 seconds. Reconnect the sensor plate to the same port and wait 20 seconds.

≥3x No → Disconnect the power supply from the electrical outlet and wait 10 seconds. Reconnect the power supply to the electrical outlet and wait 60 seconds.

≥6x No → Contact aquila biolabs' support (8).

Yes ↓

Does the fitting procedure find an appropriate solution?

No → Stop the fitting process and save the calibration file. Reopen the calibration file later, try to refit with different convergence parameters and save as new file.

Yes ↓

Calibration done. Congratulations - issue solved.

Did you finish the refitting successfully?

Yes → Calibration done. Congratulations - issue solved.

No → Contact aquila biolabs' support (9).

128

aquila biolabs

Solving data interpretation issues

Start with opening at least one existing .cgqfx-file

Could all files be opened successfully?

No → Contact aquila biolabs' support (10).

Yes

Are there curves that considerably drop in the beginning?

Yes → Please ensure that your experimental setup is in agreement with the recommended operation conditions (pp. 13), the sensor plate (pp. 25) and flask (pp. 29) mounting guidelines and the CGQ experiment checklist (pp. 136).

No

Do you still observe backscatter drops in the beginning?

Yes → Contact aquila biolabs' support (11).

No

Are there curves with considerable noise present in the data?

Yes → Please increase the backscatter denoising level (pp.84 and pp. 82).

No

Are there curves with a strong signal increase in the beginning?

Yes → This may indicate foaming. Please try to use antifoaming agents in your next experiments.

No

Are there curves with different initial backscatter values, even though all flasks were inoculated to the same density?

Yes → This may be attributed to individual flask or positioning differences (pp. 52). Try to use the Auto-offset function (84). Furthermore, please ensure that your experimental setup is in agreement with the recommended operation conditions (pp. 13), the sensor plate (pp. 25) and flask (pp. 29) mounting guidelines and the CGQ experiment checklist (pp. 136). In some case it may be additionally required to preselect a set of equal shake flasks.

No

No artifacts observed. Congratulations - issue solved.

If your issue persists after considering all hints, contact aquila biolabs' support (12).

Troubleshooting

129

aquila biolabs

Contact

aquila biolabs GmbH

Arnold-Sommerfeld-Ring 2

52499 Baesweiler

Germany

www.aquila-biolabs.de

telephone: +491632922615
email: info@aquila-biolabs.de

aquila biolabs

Notes

aquila biolabs

Notes

aquila biolabs

Notes

aquila biolabs

aquila biolabs

Notes

aquila biolabs

CGQ experiment checklist

Is the CGQ base station connected to the power supply (green LED glowing)? ☐

Is the CGQ base station connected to PC and CGQuant via USB (connection label glowing)? ☐

Are the CGQ USB and power cables routed with sufficient play within your shaker? ☐

Is an available CGQ sensor plate connected to the CGQ base station (check also sensor plate status in CGQuant overview window)? ☐

Is the shake flask filled with uncooled, noncondensing cultivation medium? ☐

Is the medium inoculated without leaving any tips or toothpick inside the flask? ☐

Is the shake flask mounted centrosymmetrically above the CGQ sensor plate (check clamp deformation symmetry)? ☐

Is the shake flask mounted in a way that no labels or labeling fields are located above the CGQ sensor array? ☐

Are shake flask and CGQ sensor plate correctly covered for applications with increased sensitivity requirements? ☐

Is the automated measurement control feature suitable for your shaking conditions (deactivate at <150 rpm)? ☐

Have you generated, documented and started a new experiment in CGQuant? ☐

Have you added antifoam to your culture in applications with baffled shake flasks? ☐