This manual covers the Sophion product named Qube 384

Compliance

The Qube 384 instrument complies with directives and standards as described in the Declaration of Conformity in Appendix F.

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Trademarks

Sophion Bioscience disclaimer

Sophion Bioscience A/S, Sophion K.K. and Sophion Bioscience Inc. are not liable for any damage resulting from the use of the Qube system, related software or this manual. Sophion Bioscience A/S, Sophion K.K. and Sophion Bioscience Inc. make no guarantee or representation, expressed or implied, for the correctness, accuracy, reliability, performance or otherwise of the Qube system, its software or the contents of this manual. The operating entity assumes the risk for the results and the performance of the Qube system after system acceptance. The disclaimer written above may be incomplete and should be considered together with all other agreements made between Sophion Bioscience A/S, Sophion K.K. and Sophion Bioscience Inc. and the users of the system.

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Contents

Introduction to Qube

Scope and purpose

This user manual provides detailed information on using the Qube 384 (Qube), a fully automated screening system for ion-channel drug discovery developed by Sophion Bioscience A/S (Sophion). The Qube system is used for high and medium throughput drug screening on ion channels and for ion channel safety screening. It is intended for use by operators who are trained, are aware and capable of detecting and assessing hazards and of taking appropriate steps to minimize any hazardous situations that may occur

Qube performs 384 parallel and automated high-quality patch clamp experiments and the system comprises a 384-pipette tip robot integrated into the platform, two internal PCs for data acquisition and processing, an on-board cell preparation module, an optional plate stacker with an auto fill reservoir unit, and an external PC controlling all parts of the instrument.

As an integral part of the Qube system, this manual must be easily accessible to users at all times.

Suggestions for improvements are always welcome; please feel free to contact us with your feedback at info@sophion.com.

Warning! Qube is a system with automated moving parts (crushing risk) manipulating potentially hazardous or toxic liquids and genetically engineered cells (biohazard). It can therefore be dangerous if not used correctly. The operators of Qube must be aware of the related risks and consequences. Operators of Qube should read all the chapters in the manual and especially note the content of chapter 2 Safety and Warnings. We recommend that all operators receive training from Sophion employees.

How to read this manual

Operators and persons tasked to work on the Qube must have read and understood the entire manual before using the system, with particular attention on following chapters:

- 0. The Qube System
- 2. Safety and Warnings
- 3. Start-up and shut-down procedures
- 5. ViewPoint

As part of planning local safety procedures, we recommend reviewing Appendix C Safety guide. This describes examples of potential accidents or malfunctions when using the Qube system and suggests what to do if they occur. It is important that this list be considered together with the local safety procedures of the user's laboratory and be integrated into the user site procedures accordingly.

Tips, notes, cautions, and warnings

Throughout the manual, several tips, notes, cautions and warnings are written. These are marked in blue, grey, orange, and red boxes, respectively. The purpose of tips, notes, cautions, and warnings is shown in the following.

Tip! A tip is a small advice that might not be obvious from the other instructions but will help performing and optimizing the task.

Note! A note contains information that should be paid special attention to. Notes are typically information that is important for correct assay setup. Failure to conform to notes may negatively affect performance and data quality.

Caution! A caution contains information that should be followed. Cautions are typically information that is important for correct system setup and use. Failure to conform to cautions may lead to permanent loss of data, cells, reagents, and consumables.

Warning! A warning contains information that has an impact on physical instrument performance and safety considerations. Failure to conform to warnings may lead to system and property damage and pose a safety hazard for the operator.

Additional warning symbols

In addition to the general symbol used in the warnings defined in the previous section, the following symbols may be used for more specific warnings.

Table 1 Overview of specific warning symbols

Symbol	Meaning
	Toxic material
	Electricity
	Biological hazard
	Crushing of hands

Quick Guides

Things to remember (Qube ABC)

A. Make sure that all mechanics are free to move before executing any protocol or utility protocol
B. Choose the correct MTP type in the worktable protocol
C. Make sure that MTPs are correctly mounted in the slots

Warning! Almost all moves start by retracting all vertical axes. Make sure that the 384-robot, including gripper arms and the automated cell preparation unit (ACP) are free to move before starting any protocols or utility protocols

Startup procedure

1 **Wake from standby**

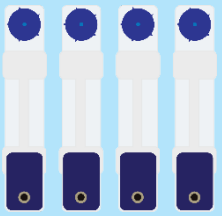
a. Make sure that Qube is powered on
b. Log in to ViewPoint
c. Wake Qube from standby

2 **Place all consumables**

1. Place all the consumables into their relevant positions
    a. Pipette tips
    b. ACP pipette tip in ACP tip ejector
    c. Cell waste bottle and funnel
    d. Centrifuge tube in centrifuge
    e. EC reservoir for the ACP
    f. Cell transfer plate (CTP)
    g. QChip 384
    h. MTPs or reservoirs with barcodes
        i. EC, IC, and pre-wet solution
        ii. Compounds

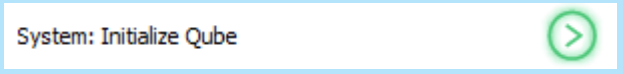
3 **Add liquids**

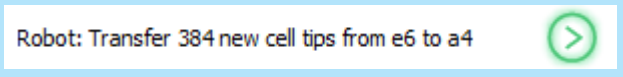
1. Fill LMS source containers
    a. One source container has capacity for approximately seven plate runs
2. Cell cup with cells in solution, minimum 5 mL



4 **Run startup protocols**

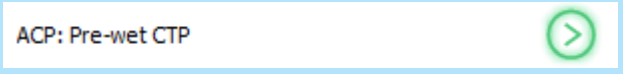
1. Run utility protocol 'System: Initialize Qube'
2. Transfer tips to tip loading tools as needed with the tip transfer utility protocols





5 **Prepare for first plate run**

1. Run utility protocol 'ACP: Pre-wet CTP' just prior to starting the first plate run



Shutdown procedure

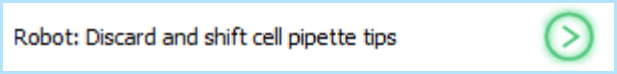
1 **Perform daily care tasks**

1. Follow the 'Daily care' Quick Guide on page 12

Daily care Quick Guide

2 **Shift cell pipette tips**

1. Place an empty pipette tip rack at worktable position e6
2. Run the utility protocol 'Robot: Discard and shift cell pipette tips'



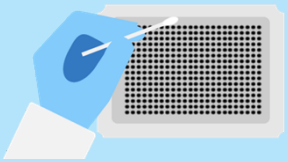
3 **Place Qube into standby**

1. Set Qube to standby

Daily care

1 **Clean the washing station**

a. Use a cotton swab to clean around the chimneys in the washing station

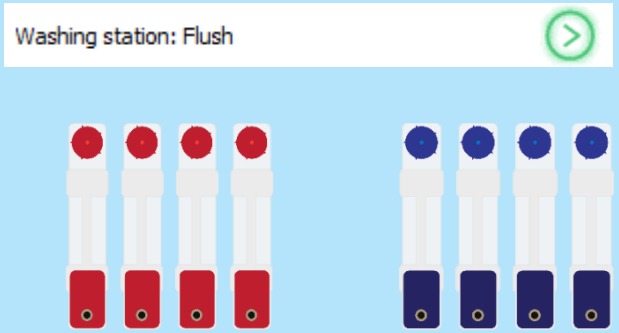


2 **Prepare the LMS for shutdown**

a. Run the utility protocol 'Washing station: Flush'

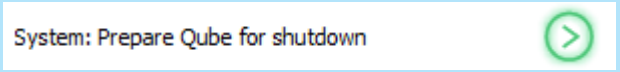
b. Empty all supply containers

c. Empty all waste containers



3 **Prepare Qube for shutdown**

a. Run the utility protocol 'System: Prepare Qube for shutdown'



4 **Clean the Qube workplane**

a. Remove all consumables and dispose of as required for biosafety

b. Discard the cell waste container as required for biosafety

c. Clean the workplane and all parts of spill etc.

5 **Rinse the autofill reservoir**

a. Remove and empty the autofill reservoir

b. Replace and connect the reservoir

c. Connect a bottle with MilliQ water or similar quality

d. Prime the reservoir with water

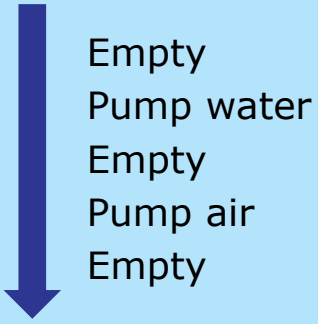
e. Remove and empty the autofill reservoir

f. Replace and connect the reservoir

g. Connect an empty bottle

h. Prime the module with air

i. Remove and empty the autofill reservoir

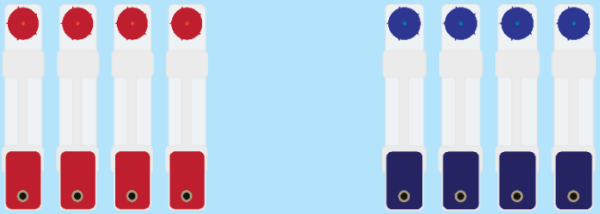


Weekly care of the liquid management system (LMS)

1 **Empty containers**

a. Empty all supply containers

b. Empty all waste containers



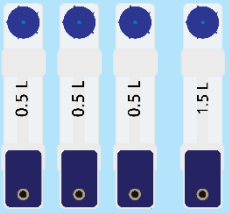
2 **Prepare QClean at a ratio 1:250**

a. Dilute 12 mL QClean in 3 L of system water

b. Add 0.5 L to the first three supply containers. Add the remaining 1.5 L to the fourth supply container

c. Return all containers to the drawer and connect them

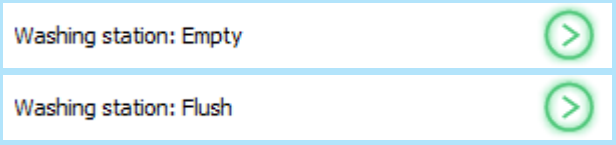
QClean +
System water



3 **Run QClean procedure**

a. Run the utility protocol 'Washing station: Empty'

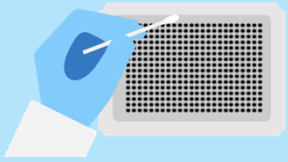
b. Run the utility protocol 'Washing station: Flush'



4 **Wait 15 minutes, meanwhile:**

Clean the washing station

a. Use a cotton swab to clean around the chimneys in the washing station



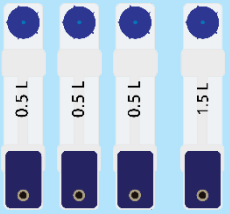
Prepare the rinsing step

a. Empty supply containers and flush them twice with system water

b. Fill three of them with 0.5 L system water and the last with 1.5 L system water

c. Return containers to the drawer and connect them

System water



5 **Rinse system**

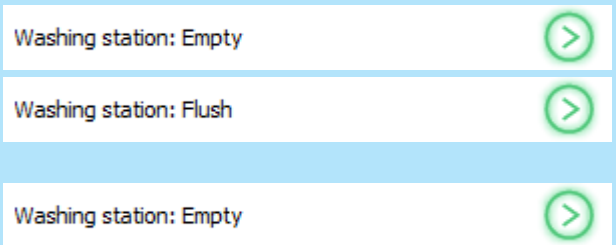
a. Run the utility protocol 'Washing station: Empty'

b. Run the utility protocol 'Washing station: Flush'

c. Empty all supply containers

d. Run the utility protocol 'Washing station: Empty'

e. Empty all waste containers



The Qube System

Qube is a fully automated screening system that uses the planar patch clamp technique for parallel ion-channel measurements on living cells.

The **Qube instrument** consists of:

- 384-channel pipetting robot
- Worktable for placing compounds and consumables
- 384 individual patch clamp amplifiers
- Automated cell preparation module
- Pressure system
- Integrated liquid management system for washing tips
- Internal PC for execution denoted 'Controller PC'
- Internal PC for data handling denoted 'Data PC'
- Optional plate stacker attached to the left side of Qube
- Optional auto fill reservoir included with stacker
- Optional components for temperature control

The **Qube system** consists of:

- Qube instrument
- QChip 384 measurement plates
- User PC connected to Qube
- Sophion Analyzer and Sophion ViewPoint software to setup, control, and analyze Qube operation

All experiments are performed on QChip 384 measurement plates and Qube makes it possible to do ion-channel measurements on 384 sites in parallel. Due to its high level of automation, Qube can be operated by trained laboratory personnel and does not require the presence of an electrophysiologist.

1.1 Setup and connection of the system

The Qube system is delivered and installed by Sophion personnel who will work together with the customer to ensure that everything works to their satisfaction.

The Qube instruments requires connection to an external PC. Prior to the delivery of Qube, a detailed, technical guide 'External Guidelines for Qube IT and Network Setup' has been provided. The following sections contain a short excerpt of the full network guide. Please do not hesitate to contact Sophion if you need a new copy of the aforementioned guide.

1.1.1 Qube network setups

The Qube instrument must be connected to a PC via Ethernet for the user to operate the system. A pre-configured *Qube User PC* dedicated for this purpose is delivered together with the Qube.

The Qube instrument can be connected to the User PC, other client PCs, and a backup system in different network configurations. This section shows examples of five different options for setting up the Qube network.

For the best possible use of the Qube system, it is recommended to employ network options B, C, D, or E, (shown Figure 2 to Figure 5), which give access to the data from multiple PCs and allows for a centralized handing of data backup.

Regardless of the chosen setup, the Qube instrument must be connected to a network fulfilling the general network requirements specified in section 1.1.2 on page 16.

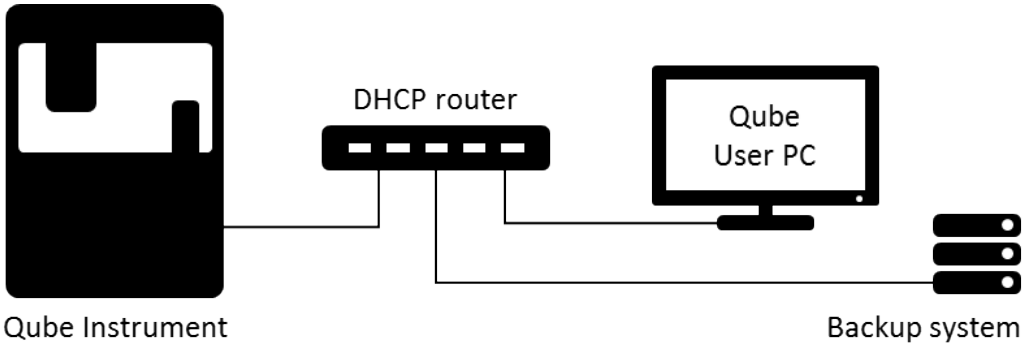


Figure 1 Network Option **A**: Stand-alone setup without connection to any external network. The backup system is supplied and configured by the customer

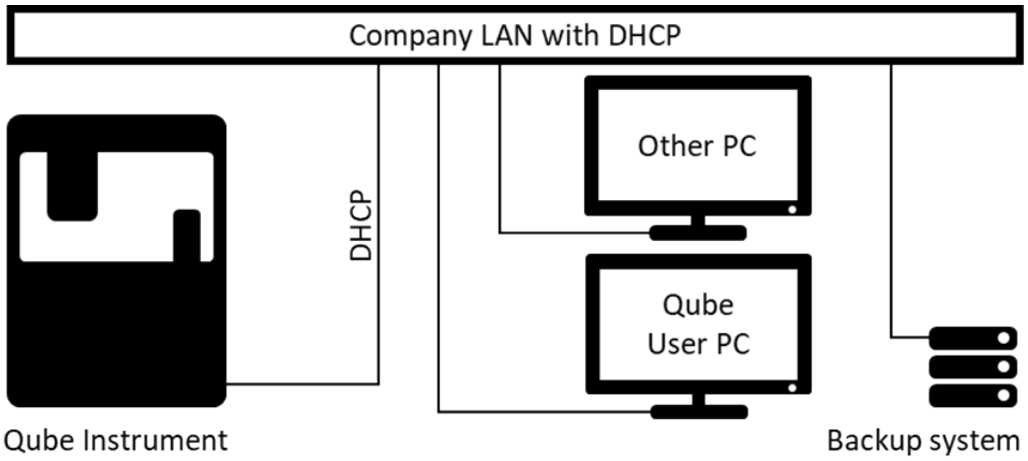


Figure 2 Option **B**: Direct DHCP network integration setup. The Qube system and User PC are directly attached to the company LAN and run on a centrally configured network. The backup system is supplied and configured by the customer

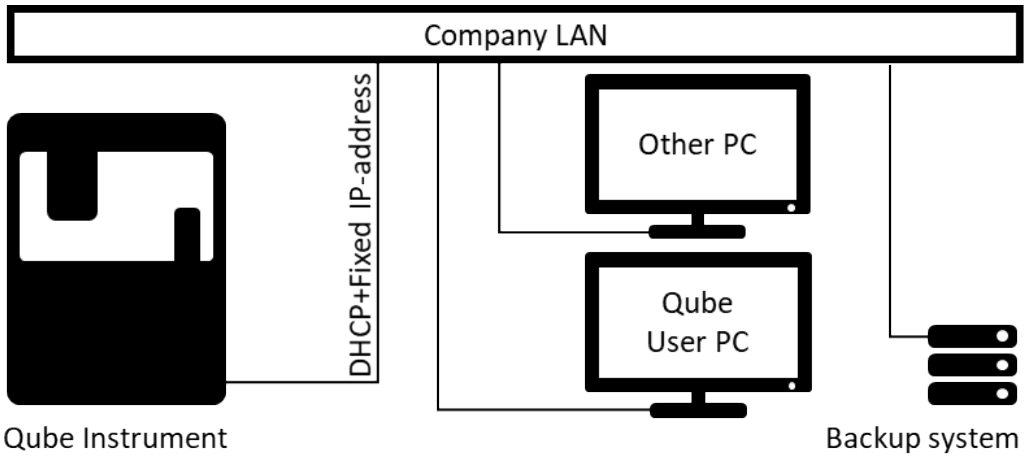


Figure 3 Option **C**: Direct network integration setup without DHCP. The Qube system and User PC are directly attached to the company LAN and running on a centrally configured network. The backup system is supplied and configured by the customer

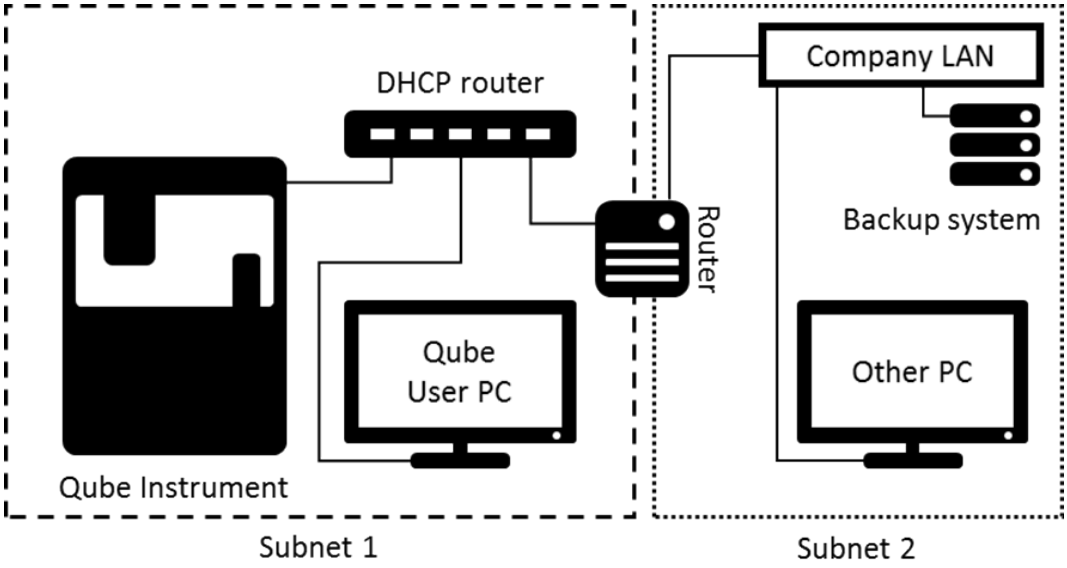


Figure 4 Option **D**: Router setup with separate subnets. The Qube instrument and User PC reside on a subnet separated by a router from the remaining company LAN. IP addresses on the separate subnet 1 are handled by the DHCP router. A DHCP router is supplied by Sophion. The separating router and backup systems are supplied and configured by the customer

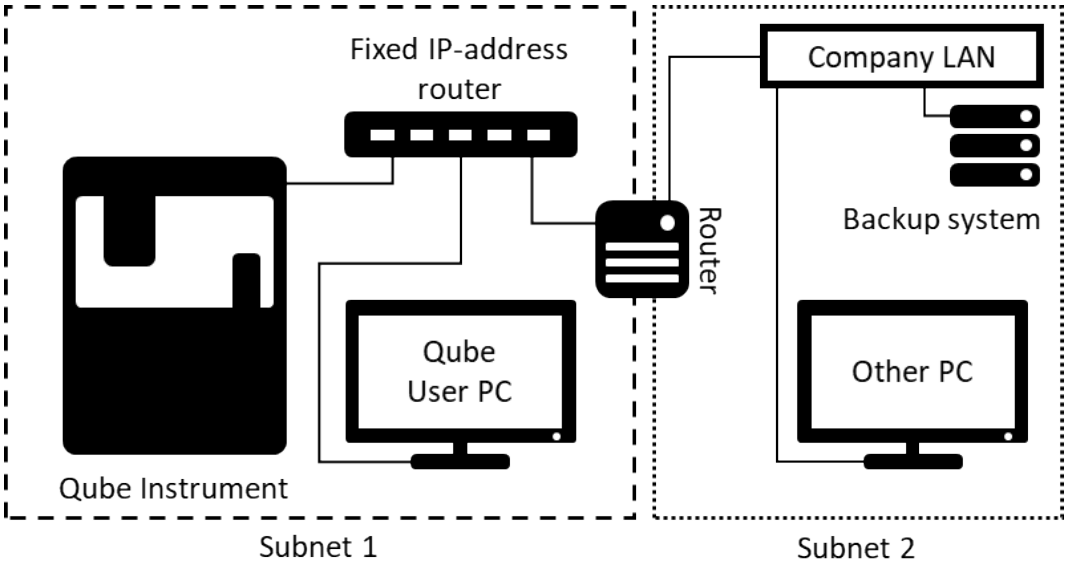


Figure 5 Option **E**: Router setup with separate subnets. The Qube instrument and User PC reside on a subnet separated by a router from the remaining company LAN. IP-addresses on the separate subnet 1 are set locally on the instrument and User PC. The Qube instrument still has DHCP enabled as well. The router, supplied by Sophion, can be configured to work with fixed IP-addresses by disabling the built-in DHCP server. The separating router and backup systems are supplied and configured by the customer

1.1.2 General network requirements

The Qube instrument is by default set up to use DHCP and has a built-in firewall that only allows communication on the ports listed in Table 2. The Qube instrument can be configured with a fixed IP-address while still supporting DHCP. Regardless of the chosen network configuration, the Qube instrument and the Qube User PC must be able to communicate via the listed ports, and the Qube instrument must be connected to a network fulfilling these general requirements:

- IP protocol: DHCP or fixed IP-address
- Network bandwidth:

- 100 Mbit/s minimum
- 1 Gbit/s recommended

- Available network ports according to Table 2

It has a built-in firewall that only allows communication on the ports listed in Table 2. Ports for normal use are needed for communication between the Qube instrument and any PC that needs to connect to Qube. The two last ports, 22 and 5900, are used for service only and do not need to be available for other PCs than the User PC.

Table 2 Overview of ports of the built-in firewall in the Qube instrument.

Port	Use	Communication
3304	Normal	QubeLoader software
3305	Normal	QubeLoader software
3306	Normal	Qube MySQL database
3307	Normal	Qube ViewPoint Broadcast server
3308	Normal	Qube MachineController software
3309	Normal	Qube DataServer software
22	Service	SSH access to Qube internal Data Handling PC
5900	Service	VNC access to Qube internal Control PC

1.1.3 Configure data backup

Qube has built-in features for backing up the data and database to a network location outside Qube. Please make sure to set up and test the backup. See section 4.5 on page 38 for detailed instructions of the backup configuration.

Caution! It is important to set up automatic backup. If something unfortunate happens to the Data PC, the backup can be the only way to restore valuable data. There is no backup unless you set it up in ViewPoint Maintenance.

1.2 The Qube Instrument

The Qube instrument is operated by the ViewPoint software on the user PC, and all the instrument elements are integrated to ensure the correct flow of operation.

The Qube worktable (see Figure 6) has slots for:

- Compound plates
- QChip 384 plates
- Extracellular and intracellular solutions

Furthermore, the worktable also comprises:

- The washing station for pipette tips
- Pipette tip loading tools
- The Automated Cell Preparation module (ACP)
- The Biochip Interface (BCI) where the QChip 384 interfaces with the amplifier and pressure systems during measurements

Depending on the specific configuration of your Qube, the worktable may for instance also contain:

- Conveyor from stacker
- Autofill reservoirs
- Feeders from external equipment

Figure 6 Qube worktable. The use of the available slot plates can be tailored depending on the assay. The mechanical layout may vary depending on the specific Qube configuration

1.2.1 Data PC and Controller PC

A Qube instrument has two internal PCs:

- A Data PC that stores and handles measurement data
- A Controller PC that controls Qube

Both PCs are needed for Qube operation and are turned on by the power switch on the back of the instrument, which also powers all other devices. PCs can be powered off via ViewPoint Maintenance software on the user PC. Qube Data is stored on the Data PC, and therefore it needs to be powered to analyze data. Analysis is done with Sophion Analyzer and while it can be done on the user PC, it is preferable to do this on a more powerful office PC.

1.3 The Qube User PC

The Qube User PC is a standard computer with no custom specifications. The PC comes pre-configured with Sophion Analyzer and ViewPoint applications installed. In contrast to any other PCs connected to the Qube system, it is also configured to control the Qube instrument, e.g. control device power and start and stop experiments. The User PC should be placed next to the Qube instrument.

1.4 QChip 384

QChip 384 is the measurement plate on which all experiments are performed. The QChip 384 contains 384 individual measurement sites, each with: individual flow channels for cells and external solutions, and electrode pairs for voltage control, current control, and data recording.

Each flow channel in the QChip 384 consists of an inlet well and a waste reservoir. The waste reservoir has unlimited capacity, since liquid is removed by the pipettes from the waste throughout an experiment, allowing for multiple liquid additions. Flow through the channel is controlled by the pipettes to ensure rapid liquid exchange.

Ag/AgCl measurement electrodes are placed in the flow channels of the QChip 384. When the QChip 384 is placed in the BCI, the integrated electrodes are connected to the Qube amplifier system via gold pins in the section of the BCI known as the Bed of Nails (BON).

Three standard types of QChip 384 are currently available for the Qube system.

- QChip 384, which has a single patch hole at each measurement site
- QChip 384X, which has 10 patch holes at each measurement site

- QChip 384D, which has varying numbers of patch holes, from 1 to 36, in an array across the QChip

QChips with a specific number of holes per measurement site can be ordered as custom products. Use the assay development QChip 384D to determine the proper number of patch holes.

1.4.1 Handling the QChip 384

The QChip 384 plates are stored at room temperature in the bags they are shipped in.

The QChip 384 (see Figure 7) must be placed in the Qube with the barcode on the left-hand side. This ensures that the QChip 384 is in the correct orientation, and that the barcode reader has access to the barcode.

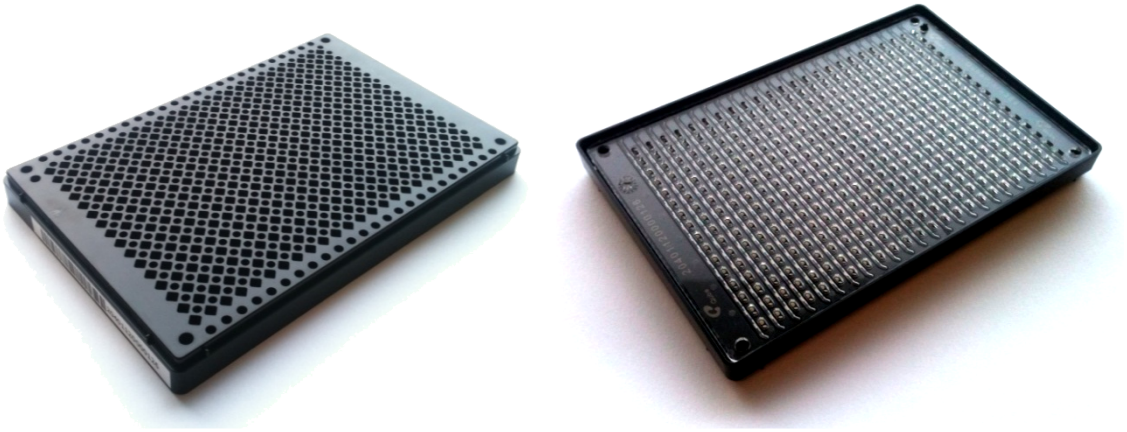


Figure 7 QChip 384. The left image shows the upper side. Note the extra hole in the upper left corner and the barcode that must be facing left when placed on the worktable. The indication 'upper right' is printed in the upper right corner of the plate to further ensure proper QChip placement. The right image shows the QChip 384 from below with 384 pairs of electrodes. There is a common rear side channel for each column which can also be seen

Warning! Used QChip 384 plates should be treated as biohazardous and toxic waste and disposed of according to lab procedures and local and national regulations.

Caution! It is possible to place the QChip incorrectly if it is rotated by 180° in the Qube instrument. In that case the execution will terminate when the barcode reader attempts and fails to read the QChip barcode. Make sure that QChips are always placed correctly in the Qube instrument.

1.5 Qube software

The Qube software consists of three different applications: Sophion ViewPoint, Sophion ViewPoint Maintenance, and Sophion Analyzer. In this manual, they will often be referred to as ViewPoint, ViewPoint Maintenance, and Analyzer. The manual for Sophion Analyzer is a separate document which can be accessed via the question mark button in both ViewPoint and Analyzer. Pressing F1 opens a local help menu in the Analyzer software. Detailed descriptions about ViewPoint Maintenance and ViewPoint can be found in chapters 4 and 5, respectively.

ViewPoint and ViewPoint Maintenance are used for operating the Qube instrument, whereas Sophion Analyzer is used for analyzing the obtained data. ViewPoint is used for setting up and executing protocols, whereas ViewPoint Maintenance is used for administering Qube. Please see chapter 5, page 54 for details on setting up an experiment.

All three programs can be opened from any PC connected to the network provided that Qube is connected to the same network. However, the user PC is the only computer that is

configured to control Qube movements and switching the instrument's internal PCs on and off. Therefore, some parts of the software will appear different when viewed from different types of PC.

1.5.1 Logging in to Qube

ViewPoint, ViewPoint Maintenance, and Analyzer are logged into by providing a username and password when prompted after double clicking on the appropriate icon (see Figure 8). In the login prompt (Figure 9) there is also a field for the Database. Qube will broadcast its name here and the dropdown is active when there is access to more than one Qube (or QPatch in case of Analyzer).

Only users that are stored in the Qube database will be granted access. Sophion personnel will create the first user and you can then create more users as needed. See page 37 for details on user management.

Figure 8 Icons to Sophion software for Qube. From left to right: ViewPoint, Analyzer, and ViewPoint Maintenance icons

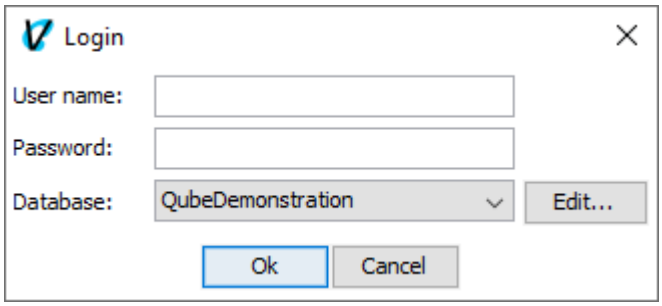


Figure 9 Login prompt. Qube will broadcast its name in the drop down named 'Database'

1.5.1.1 Manual login configuration

The broadcasting feature only works if the Qube instrument is placed on a network with the same subnet as the PCs running ViewPoint, ViewPoint Maintenance, and Analyzer. This means that, depending on your network installation, the Qube may not automatically show up in the dropdown menu of the login dialogs of ViewPoint, ViewPoint Maintenance, and Analyzer.

In that case, details about the Qube instrument must be manually entered in the client software on every PC that needs to connect to the Qube instrument. Follow the instructions below to set up the connection.

1. Start ViewPoint, ViewPoint Maintenance, or Analyzer
2. Press 'Edit…' in the database login dialog, cf. Figure 10
3. Press 'New…' to create a new entry
4. Fill in the following in the available columns, cf. Figure 11 and Figure 12:
    a. Display name: *QubeNNN_Manual,* where *NNN* is the ID of your Qube
    b. Database name: *qubedb*
    c. Server: Qube IP-address or domain name depending on your configuration
    d. Protocol: *mysql* (Only available for Analyzer)
    e. Port: Default

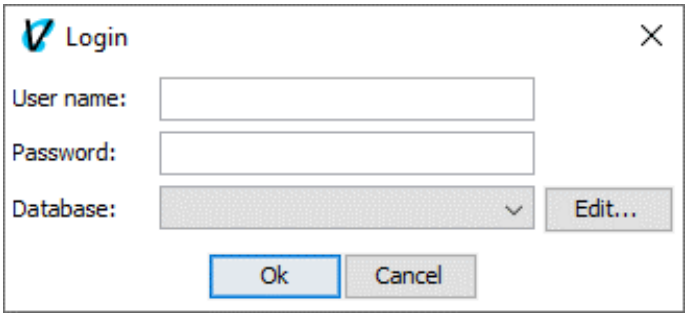


Figure 10 Press Edit to access the manual database configuration window

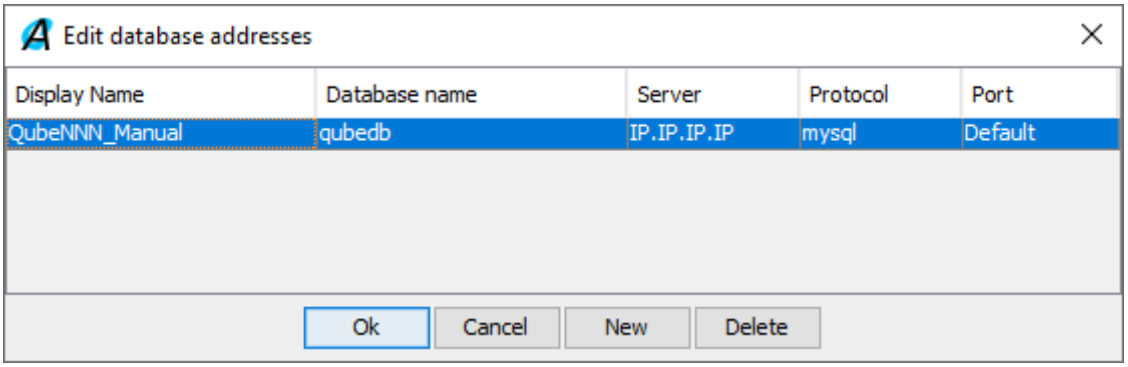


Figure 11 Example of manually configured login to a Qube using a directly entered IP address

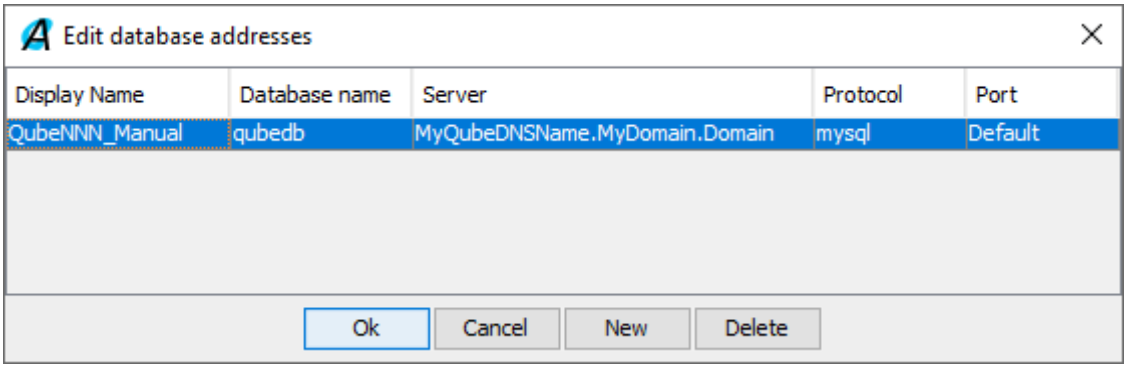


Figure 12 Example of manually configured login to a Qube using the Qube domain name

Consult your local network administrator for details about network configuration.

2. Safety and Warnings

2.1 Introduction

This chapter contains essential information about the Qube system to ensure the personal safety of the operator as well as warnings that must be followed to keep the Qube system in an operating condition. Qube is a safe system to work with if the directions stated in this manual are followed.

2.2 Safety

Qube has been designed and tested in accordance with the International Electrotechnical Commission (IEC) Publication 61010-1 (*Safety requirements for electrical equipment for measurement, control and laboratory use*).

It has left the factory in safe condition. This user manual contains important information and warnings that the user must follow to ensure safe operation and to retain Qube in safe condition.

Warning! The Qube Instrument operates at Class 1 in accordance with IEC safety standard 60601-1: It requires a three-conductor power cord with a protective ground conductor and a plug with ground contact to be safe to operate. The Power cord should be rated for minimum 6 A.

The mains line plug should only be inserted in a socket outlet provided with a protective ground contact. Do not negate this protection by using an extension cord without a protective conductor.

2.3 Intended purpose and operating conditions

2.3.1 Safe conditions

The Qube instrument is designed and approved for operation in commercial and light industry environments (electromagnetically controlled environments). Qube 384 must only be operated in a non-condensing environment.

Warning! If Qube is used in a manner not specified by Sophion, Qube can become unsafe to use.

The instrument must only be used by trained[1] personnel who are aware of the risks of electrical measurements, moving robotic parts, hazardous and toxic liquids, and related biohazards. All users of the instrument must have read and understood this manual.

See also the system requirements in Chapter 9.

2.3.2 Biohazards

Warning! If Qube is used in a manner not specified by Sophion, Qube can become unsafe to use.

The Qube system is designed to perform automated experiments on transfected cell lines. Every user operating the Qube instrument must therefore have sufficient knowledge and training on how to handle biohazardous material.

If the user or the Qube system accidentally causes any spill, or splashes droplets containing transfected mammalian cells, the user must clean up the spill and decontaminate the area

[1] User is trained when having been trained by Sophion personnel

immediately. The user should follow local lab procedures at all times, wear a laboratory coat and protective gloves when cleaning up the spill:

1. Wipe up the spill with paper towels – Preferably wipe towards the center of the spill
2. Clean the affected area with a decontaminant or detergent
3. Clean the area using 70% ethanol
4. All the paper used for decontamination must be placed in appropriate containers for biohazardous waste disposal

If the user determines that a hazardous substance has seeped through the platform of Qube into places where it is not possible to clean by the instructions presented in this manual, then the user must do the following:

1. Place a sign on the machine stating that it is out of order and must not be used or touched
2. Call personnel authorized by Sophion to handle the situation

2.4 Hazardous or toxic compounds

Warning! The effects on humans of the compounds being tested using the Qube system are generally unknown. All compounds should therefore be treated as potentially hazardous or toxic. Any person operating the Qube instrument should have sufficient knowledge on how to handle hazardous and/or toxic compounds and follow local lab procedures.

2.5 Electromagnetic compatibility

The Qube instrument complies with European Commission directive 2004/10/EC on electromagnetic compatibility. The instrument also complies with CENELEC standard EN 61326. All electromagnetic compatibility tests have been performed according to this standard and according to the limits for equipment to be used in 'industrial' environments.

2.6 Emergency stop

The Qube instrument is equipped with an emergency stop button (Figure 13). The button is red and clearly visible on the front of the instrument. The button cuts power to all moving parts of the system that can be a potential hazard to the operator or the system itself.

Warning! Please notice that the gripper arms keep being powered and potentially actively grabbing a plate. Do not open the front cover of the Qube or reach in – risk of crushing

Figure 13 Emergency stop placed on the front in the right-hand side of Qube

The emergency stop button should be pressed if:

- There is any possibility of danger to persons
- Moving parts appear to be malfunctioning (e.g. items get stuck inside moving parts, moving parts collide, or the plate gripper drops plates)

If the emergency stop has been pressed:

1. If there is any spill, handle the spill according to the safety guides in Appendix C
2. If required, open the cabinet door and manually move the pipetting robot and ACP pipettor to a position where they are free to move in all directions, **also up**
3. If required, remove any items from the workplane that may hinder movement of the mechanics
4. Check for any apparent damage to mechanics
5. If there is no apparent damage to mechanics, release the emergency stop by turning the red emergency stop button counterclockwise
6. Restore the instrument by running the appropriate sequence of recovery utility protocols
    a. If Qube has been stopped with a plate in the grippers, the 'Recovery: Open gripper arms' utility protocol should be executed. Remember to manually place the 384-robot and gripper into a safe position for plate release. A position is considered safe where the gripper arms are allowed to fully open and the plate is dropped a distance as short as possible
    b. If Qube has been stopped with pipette tips loaded on the 384-head, the 'Recovery: Eject 384 pipette tips at e6' utility protocol should be executed. Remember to place a suitable container at e6 before executing the protocol
    c. If Qube has been stopped with a QChip 384 in the BCI, the 'Recovery: Remove QChip 384 from BCI' utility protocol should be executed
7. Make sure to manually clean up and prepare Qube for a new run after recovery, including placing new QChip 384 plates, emptying or exchanging centrifuge tube, transferring new pipette tips, etc.

It is not possible to continue or recover an experiment if the emergency stop has been engaged during the run. The QChip 384, related cells, and consumed compound will be lost. All data obtained until the time point for engaging the emergency stop are stored and accessible.

2.7 Power supply

The Qube instrument operates on mains or line voltages between 100 V and 240 V AC nominal, 50 Hz to 60 Hz. There is therefore no need to switch to different input voltages.

The maximum transient overvoltage is Category II. The maximum input current to the instrument is 6 A.

Ensure the Qube instrument is installed so the power switch is easily accessible.

Figure 14 Left photo: Bulkhead plate placed on the rear right of Qube when looking at it from the rear side. The plate has the power inlet, main switch, Ethernet connection, and inlet for pressure and vacuum. Center and right photo: Arrows indicate how to open the main power input fuse compartment by placing a screwdriver in the slot under the compartment and pulling it out

Warning! The main power supply and plug of the equipment used with Qube must comply with any national and local regulations.

2.8 Fuses

Warning! Using patched fuses or short-circuiting the fuse holder is not permitted. Sophion Bioscience is not liable for any damage caused as a result of failure to use fuses and fuse holder correctly, and all warranty claims become null and void.

The power input fuses are externally accessible at the power inlet main switch. To replace them:

- Turn off power
- Unplug the power cable
- Open the fuse compartment with a small screwdriver
- Replace the fuses

Only use the same type of fuses as written on the product label below the power inlet, cf. System specifications, page 128.

Warning! There are also fuses located inside the instrument. The user must not replace these fuses. Users should call a service engineer authorized by Sophion.

2.9 Vacuum and pressure connection

The Qube instrument operates with pressure and vacuum sources, which are connected to the pressure and vacuum inlets on the back of the system, cf. Figure 14. See also the system requirements in chapter 9.

2.10 Instrument ventilation

The Qube instrument must be located so that the holes for ventilation on both sides and on the rear side are not covered or blocked. This means that it should be placed at least 10 cm from walls.

2.11 Cabinet safety

For safety reasons, Qube has been designed so that electrical power is automatically disconnected from all hazardous, moving parts whenever the cabinet is open. The mechanism for detecting cabinet opening is directly wired to a safety relay. The system is therefore safe to use when the user follows the guidelines described in this user manual.

Caution! If the cabinet door is opened during Qube operation, the consequences are the same as engaging the emergency stop (see section 2.6 on page 23). Make sure that Qube is not executing any protocols before opening the door to avoid unintended interruption.

Users have minimal risk of getting hurt by moving parts. The risk is increased if:

- The cabinet is damaged
- The mechanism for detecting cabinet opening is damaged or tampered with

Warning! Never use a Qube where the cabinet glass is broken or the mechanism for detecting cabinet opening is damaged. This will compromise your safety.

If the cabinet glass is broken or if the mechanism for detecting cabinet opening is damaged, then the user must do the following:

1. Disconnect the power supply
2. Disconnect the vacuum and pressure
3. Place a sign on the machine stating that it is out of order and must not be used or touched
4. Call personnel authorized by Sophion to handle the situation

Warning! Never tamper with the mechanism for detecting cabinet opening. This will compromise your safety.

2.12 384-robot pipetting head and gripper

The robot unit in the Qube instrument handles liquids, QChip 384 measurement plates, and microtiter plates (MTPs). The robot is generally safe since the hardware prevents it from moving when the cabinet is open.

Warning! If the robot arms crash into other parts of the system or drops items or liquids in unintended locations, the system may be malfunctioning. Press the red emergency stop button if you discover any unexpected behavior.

2.13 BioChip Interface

The BioChip Interface (BCI) has a lid; a manifold that is operated pneumatically with great force. There is no risk of limbs getting pinched since the manifold is unpressurized once the cabinet door is opened. The user should never disable the safety mechanism or otherwise make access to the BCI Interface while the pressure to the manifold is still on.

2.14 Automated Cell Preparation module

The Automated Cell Preparation (ACP) module is equipped with a stirrer for keeping cells in suspension, a robot-accessible centrifuge, a saline reservoir, and a Cell Transfer Plate (CTP) for delivering cells to the 384-robot. With the integrated single-channel pipettor and waste handling, the ACP can prepare cells simultaneously with the 384-robot preparing MTPs and QChips.

Warning! The plastic test tube and the aluminum holder for the plastic test tube must always be inserted correctly into the centrifuge, and the centrifuge lid must be placed on top. If this is not done, parts might loosen during spinning and shoot out randomly. The centrifuge will never spin while the cabinet is open, and users have little risk of injury. However, a flying centrifuge test tube might seriously damage the equipment.

Tip! After placing the aluminum holder, make a little "swing-out" test of it to verify proper positioning.

2.15 Bed of Nails

The Bed of Nails (BON) is located at the BCI position on the instrument worktable. It constitutes the electrical connection between a QChip 384 plate and the Qube amplifier system.

Warning! Do not touch the Bed of Nails area, the inputs of the amplifiers are very sensitive and can easily be damaged.
Take special care when cleaning the Bed of Nails. See section 7.5 on page 112.

If any liquid is spilled into the unit, quickly turn off the system power on the power switch. Spilling a small amount of liquid does not normally damage the electronic circuits. The BON is constructed to prevent liquids from damaging the circuitry. However, salt residue on the nails may short-circuit the measurement electrodes and prevent the amplifiers from functioning correctly.

This problem and any other type of damage to the BON can be fixed by replacing the BON. We recommend that a service engineer authorized by Sophion does this.

2.16 Disposal of consumables

2.16.1 Disposal of used QChip 384 plates

Warning! Used QChip 384 plates should be treated as biohazardous and toxic waste and disposed of according to lab procedures and local and national regulations.

2.16.2 Disposal of used compound plates

Warning! Used compound plates should be treated as toxic waste and disposed of according to lab procedures and local and national regulations.

2.17 Qube embedded instrument software

The user may not install or run any other software on the Qube instrument than the software supplied by Sophion. Running other software may destroy the equipment and may be hazardous to people.

Embedded software must only be updated using Sophion software update applications.

All claims under the warranty become null and void if other software is installed in the instrument.

2.18 Moving the Qube instrument

The robot systems need to be secured before the Qube instrument is being moved and recalibrated after the Qube instrument has been moved.

Warning! The system should only be moved under supervision of a service engineer authorized by Sophion.

2.19 Calibrating the instrument

Incorrectly modifying the instrument calibration may cause the 384-robot to crash into parts inside the Qube instrument and may seriously damage the equipment.

Warning! The calibration of the instrument can be modified in specific configuration files on the hard-disk drive of the embedded instrument computers. Only Sophion personnel must modify these files.

2.20 Temperature control (if installed)

The circulator has several built-in safety and regulation settings, and these have been pre-configured for optimum safety and performance. Altering any other settings than the temperature set point will void instrument warranty.

Warning! Make sure that the temperature control circulator is always placed on a firm, horizontal, stable surface. If placed on a raised surface, make sure that the circulator is not placed too high. The level of the circulator lid must be below the level of the FEED connector on the Qube instrument side panel. Never attempt to move a liquid filled circulator. Never attempt to move a powered circulator.

3. Start-up and shut-down procedures

This section describes how to turn Qube on and off, and the first steps in preparing Qube for operation, in terms of power, software, and physical preparations. Some of the information in this chapter assumes that you are familiar with the ViewPoint and ViewPoint Maintenance software. See chapters 4 and 5 for software details.

It is not important to do everything in the same order as written here, for instance 'loading tips', 'plates', 'tubes' etc. can be done early or late in the preparation but if you follow the guidelines below, nothing will be missed.

In the sections below, the terms 'utility protocol' or 'utility button' will be used interchangeably and they refer to the green play-buttons found on the lower part of the 'Tools' tab in ViewPoint (Figure 15).

It is worth knowing that the User PC is only a 'remote control' for Qube, so switching it on and off does not affect Qube operation.

In the following sections, some items are referred to with SBXXXX numbers. These are catalogue numbers for Qube consumables and spare parts. A complete list can be found in Appendix B on page 132.

3.1 Start-up procedures and utility protocols

3.1.1 Qube Instrument

Before the Qube Instrument is turned on, ensure that the:

1. Network cable is connected
2. Pressure and vacuum inlets are connected
3. Pressure levels are within the specified range, see System Requirements and Specifications on page 127
4. Daily care tasks have been carried out by consulting the care logbook
5. Worktable is clean and dry by inspecting it

Note! Switch on the Qube Instrument at least 20 minutes before conducting experiments. The electronic measurement components are temperature sensitive and the operating temperature should reach a steady level before conducting any experiments to achieve reproducible measurements.

If Qube has been shut down completely using the main switch (Figure 14 on page 25):

1. Turn on the power for Qube at the main switch on the back of the instrument
2. Wait a few minutes before proceeding. This allows the internal PCs to boot up
3. Turn on the Qube User PC
4. Log in to Qube with ViewPoint using the appropriate username and password
5. Wake Qube from standby by clicking on the 'Machine standby/on' icon in the 'Tools' tab (Figure 15)
6. The standby icon will flash for two minutes before switching to Ready, indicating that the system is powered on and ready to use

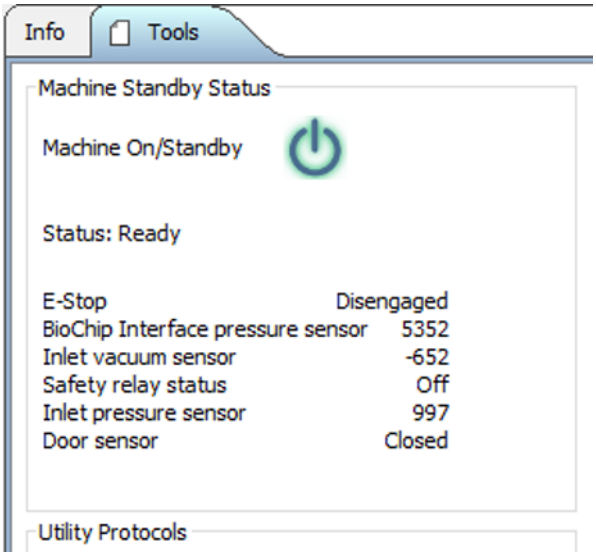


Figure 15 Part of the screen visible under the 'Tools' tab in ViewPoint

3.1.2 Homing the system

It is not possible to operate Qube until the entire system has been homed. Homing can be done with the utility protocols 'System: Clear and home' or 'System: Initialize Qube'. The latter will also load the ACP pipette tip and prime the washing station. Please refer to section 3.1.4 Initialize Qube – ACP and LMS, page 31, if using the latter option for homing.

During homing, the 384-robot will retract the pipetting head and the gripper and move to the corner above the physical worktable position 'e1'. The ACP will home by moving the vertical axis to the upper limit and rotate between front and rear positions.

3.1.3 Self-test

The utility button 'System: Self-test' will take Qube through a run of short tests:

- The robot should move around
- The barcode reader should go down and flash its illumination LEDs
- The BCI manifold should open and close
- The pipettor on the ACP should swing and go up and down
- Different readouts from the amplifier and pressure system should be displayed in the run window (see section 5.3 on page 82 onwards)
- The entire test should complete without failing

Caution! The self-test does not show pop-up messages. Errors are only shown in the 'Errors' tab and the tool tip. Depending on the error type, protocols may possibly be started on Qube anyway, but the error may reoccur during protocol execution thereby affecting data acquisition or execution in general. Check to see if critical errors are displayed in the 'Errors' tab before starting other protocol runs.

3.1.4 Initialize Qube – ACP and LMS

The purpose of initializing Qube is to load the ACP-tip and flush the Liquid Management System (LMS).

1. It is also advisable to place:
    - The tube (SB2251) in the QFuge
    - The cell transfer plate (CTP) in its place on the ACP
    - The bottle for the cell waste (SB2253) with its funnel top **securely** mounted
    - The ACP tip (SB2210) in the ACP tip ejector
        - Please refer to Figure 71 for mounting instructions

Figure 16 ACP elements to be in position for initializing Qube

Caution! It is not advised to reuse the ACP waste bottle. Reusing the ACP waste bottle entails a risk of triggering the ACP overflow sensors if the bottle is not properly cleaned. In this case an ongoing plate run will not be affected but Qube will terminate any further execution after completion of that plate run. The bottle should instead be replaced with a new bottle.

The LMS uses clean water to wash the tips on the 384-robot. The water only needs to be deionized. The LMS is placed in the drawer on the front of Qube and it consists of four supply containers and four waste containers connected to the automatic valve system via eight different spring mounted levers. Each container:

- Has a capacity of 5 L
- Is marked 'Supply' in blue or 'Waste' in red
- Is released by pushing on the small metal plate at the end. To prevent the lever from swinging freely, it is recommended to hold it with a finger

2. Before starting experiments:
    - The supply containers should be filled with clean water
    - The waste containers should be empty

Qube uses approximately 0.75 L of system water in a single QChip 384 run with three compound additions (no extended wash). The containers can be filled and emptied during Qube operation and the LMS will automatically switch between the available containers.

3. When ready, click on 'System: Initialize Qube' and wait until the green window in the 'Run' tab says 'Ready' (see Figure 58 on page 83)

Caution! A failure can occur if an LMS container is disconnected when that specific container is being used. By looking at the center-part of the black valves on the containers, the one currently in use is pushed in compared to black valves on the other containers. The safest way to proceed is to only switch containers when the LMS is not active at all as guided by the sound or lack of sound from the device.

Tip! The steps done by 'System: Initialize Qube' can also be done individually using the utility protocols 'System: Clear and home', 'Washing station: Flush' and 'ACP: Load ACP pipette tip'.

Qube will keep track of whether the ACP pipette tip is loaded so no jamming occurs; it does this by preventing further loading of pipette tips. If Qube has been fully powered off or the Controller PC or Controller Software has been restarted with a pipette tip loaded, the information about pipette tip state is lost and the instrument must be recovered manually. Please refer to Section 8.4 for detailed instructions.

3.1.5 Sophion Analyzer

Sophion Analyzer can be started on one or more PCs. The only requirement is that Qube is connected to a local network that is accessible for the PCs running Sophion Analyzer. Qube can be in standby mode or awaken from standby. It does not influence the availability of the data since Analyzer communicates with the Qube database irrespective of Qube operation. However, if Qube is executing an experiment, the sampling of data during the operation will have priority over Analyzer's access to the database and hence it can decrease the performance of Analyzer on these occasions.

3.2 Shut-down procedure

3.2.1 Qube Instrument

Qube can only be turned on and shut down from the user PC that is set up to control it. To shut down the Qube instrument:

1. Click the 'Machine standby/on' power icon in ViewPoint
2. Shortly after, Qube will be in standby mode and 384-robot and amplifiers will be powered off

It is recommended to leave Qube in this state since that allows access to, and analysis of, data. Also, the database server is normally always left on because backup jobs typically run at night. If you do want to turn off the database server, follow the directions below on how to turn off the database server and controller PC before powering up the instrument.

3.2.2 Database server

If you want to turn off Qube completely:

1. Log out of any ViewPoint and Sophion Analyzer sessions
2. Log in with ViewPoint Maintenance
3. Select the 'Software' tab
4. Click on the 'Wrench' icon
5. Select 'Shut down both PCs'
6. Wait a few minutes to ensure that the internal PCs have shut down properly
7. The main switch on the back of Qube can now be turned off
8. The user PC can be turned off like any other PC. The ViewPoint Maintenance software will automatically prompt you for closing and the user PC can be shut down

Warning! It is advised to never turn off the main switch before shutting down the internal PCs via ViewPoint Maintenance as this poses a risk of accidental interruption of database actions.

Warning! Before you shut down the database server, ensure there are no Qube Software clients running. Any clients using ViewPoint or Sophion Analyzer will be disconnected from the database server when it is powered off. The software gives a warning if somebody is connected to the database and it is recommended to inform these users before turning Qube off. The user PC is among the users in the list if ViewPoint or Analyzer is running on the User PC. Shut down these applications as well before turning off Qube.

3.2.3 Restarting Controller PC/software or Data PC/software

If needed, the Controller or Data software and entire PCs can also be restarted without turning off the power switch on the back:

1. Log in with ViewPoint Maintenance
2. Select the 'Software' tab
3. Click on the 'Wrench' icon next to 'Controller' or 'Data', whichever is appropriate
4. Choose Stop software or restart PC, whichever is appropriate
5. For the data PC, there will be a warning about potentially getting a new IP address and consequently wait time will be required
    - Notice: Restarting the Data PC will result in temporary loss of connection to the Controller PC and applications.

Caution! Ensure that the database files have been successfully backed up according to your company's backup policies. We recommend a daily back up of the database.

4. ViewPoint Maintenance

ViewPoint Maintenance is used for administering Qube, and includes several different functionalities such as:

- Creating users
- Viewing the log
- Viewing lists of protocols and protocol runs
- Importing, viewing, and editing compound list
- Deleting data and protocols
- Viewing the status of internal PCs
- etc.

After ViewPoint Maintenance has been opened, eight tabs with different functionalities can be seen (see Figure 17). The assigned Qube name is seen at the top of the screen together with the version number of ViewPoint Maintenance.

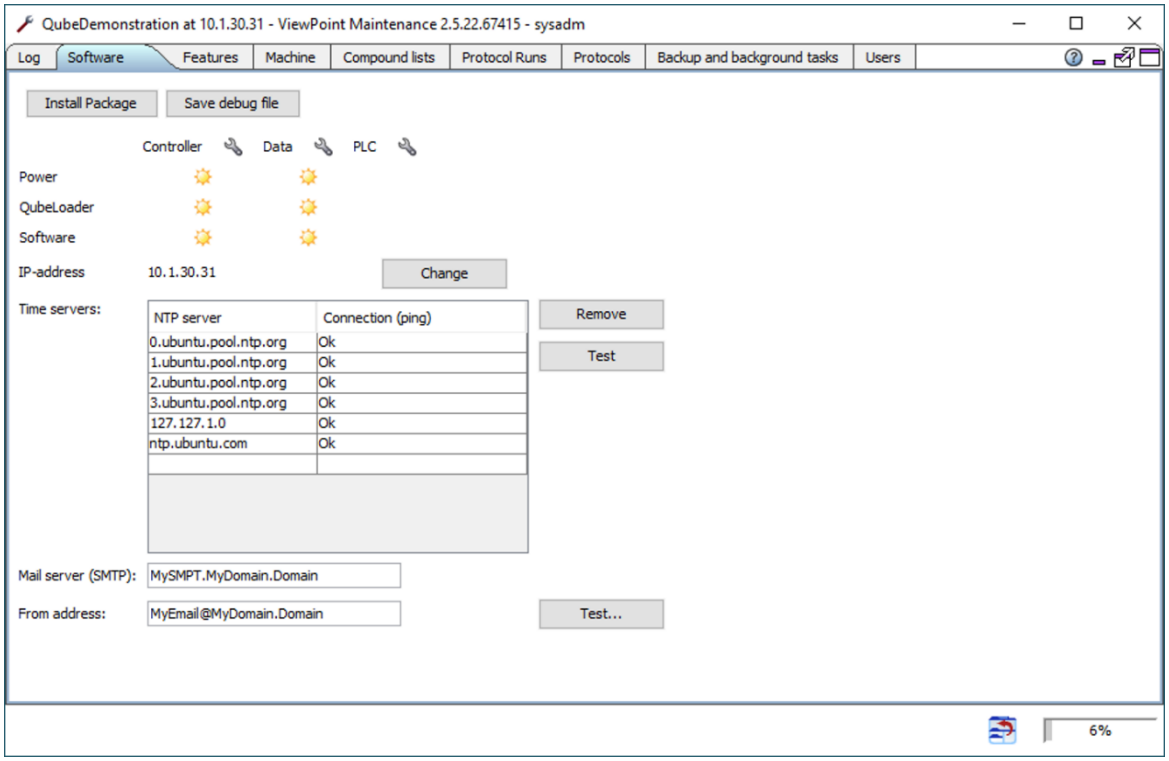


Figure 17 ViewPoint Maintenance. The active tab is 'Software' where the status of both internal PCs are shown together with the connection status for various time servers. The details of this and the other tabs are described in the text

4.1 Log tab

The log contains real time log messages from all devices on Qube.

4.2 Software tab

In this tab (see Figure 17 on page 34), the status of the software and hardware on both internal PCs in Qube is indicated by one of the three icons shown in Table 3.

Table 3 Status icons for internal PCs and software applications

Icon	State	Description
	Constantly lit	The item is running
	Flashing	The item is starting
	Constantly lit	The item is stopped
	Flashing	The item is stopping
	Constantly lit	The item is not responding, and the status is unknown

When ViewPoint Maintenance is opened on the user PC, the software can also be controlled from this tab.

By clicking on the Controller PC 'wrench' icon you can:

- Stop, kill (i.e. stopping 'harder'), or start Controller Software
- Restart Controller PC
- Shut down Controller PC
- Shut down both PCs

By clicking on the Data PC 'wrench' icon you can:

- Stop Data Software and Controller Software
- Start Data Software
- Restart Data PC
- Shut down Data PC
- Shut down both PCs

When Sophion provides an update of the software to Qube, the update package is installed by clicking on 'Install package' button and pointing to the location of the package. The rest of the operation is automatic, and a detailed manual of that operation is given below.

4.2.1 How to update Qube software

In line with Sophion's ongoing commitment to continuously improve Qube performance, new software versions may become available. In most cases the installation of new software is so user friendly that the user can do it following the description below.

Before starting the update procedures, make sure that Qube is awake (not in Standby mode – see chapter 3). No devices should be in 'Error' mode. If a device is in 'Error' mode, this should be resolved first. Please contact your Application Scientist if you need assistance to resolve the error.

To update Qube User PC software (i.e. Sophion ViewPoint, Sophion Viewpoint Maintenance, and Sophion Analyzer):

1. Download installvp.x.x.xxx.exe from the source(s) provided by Sophion and start it
2. Click on Next and Accept all way through the installer wizard
3. After installation is complete, start Viewpoint or ViewPoint Maintenance
4. Verify that the version is correct by checking the version number in the upper left corner of the window. The format is x.x.xxx.xxxxx where the first five digits are what we refer to on a daily basis when referring to version

To update the software and firmware on the internal Qube hardware, please follow the steps below. The update is performed via the User PC:

1. Ensure there are no tips mounted on Qube (384 head and/or ACP)
2. Ensure that ViewPoint Maintenance is up to date (see above)
3. Ensure that Qube is awake and in a 'Ready' state
    - Ensure that all devices are in state STOPPED
4. Download the update package 'Qube-updates-x.x.xxx.zip from your site on the support server and place it where you can find it from the Qube User PC
5. Open Sophion ViewPoint Maintenance
6. Select 'Software' tab – see section 4.2 on page 34
7. Check for 'Yellow suns' for all devices – if other icons are present (see Table 3), the problem should be resolved first.
8. In the Software tab, click the 'Install Package' button
9. Choose the previously downloaded update package
10. The 'Update Qube' window will appear
11. You might click on the 'Details' button to see the log messages and more detailed progress information
12. Press Start
13. Ask other users to log off, see details below
14. Wait for the installation to finish. It will say 'Finished' update in the 'Update Qube' window

As a last step, the installer will show a window for saving a configuration file. Please send the configuration file to your Sophion Application Scientist. To do so:

15. Save the file to a location where you can find it
16. Close the update panel
17. Send the file to your Sophion Application Scientist

Note! If other users are logged on to the system, including the user PC, a warning appears. Logged in users may cause the update to fail and users might experience error messages. Please contact the users and ask them to log off. The list of active users will automatically update when they log off.

Tip! If you missed point 15, you can export a debug file manually. Please click on the 'Export Machine Info' button in the 'Machine' panel and send the resulting file to your Application Scientist.

4.2.2 How to set a fixed IP-address

It is recommended to connect Qube to a network with a DHCP server and use this to reserve an IP-address for the instrument. However, if you need to assign a fixed IP-address to the Qube instrument before network connection, the 'Software' tab contains an entry for setting up a fixed IP-address for the Qube instrument.

Since the Qube instrument is configured to use DHCP when delivered, it must initially be connected to a DHCP network to set up the fixed IP-address via the ViewPoint Maintenance on the User PC. The DHCP router delivered with Qube can be used for this purpose.

Follow these steps to set up a fixed IP-address for the Qube instrument.

1. Connect the User PC and Qube instrument to a network with a DHCP server
    a. The supplied DHCP router can be used for this purpose
2. Ensure the DHCP server is running
3. Power up the Qube instrument and the User PC
4. Log in to the Qube instrument via ViewPoint Maintenance
5. Go to the 'Software' tab
6. Press the 'Change' button next to the IP-address field
7. Select 'Use fixed IP-address'
8. Fill in the three fields, 'IP-address', 'Net mask', 'Gateway', and 'DNS servers'

9. Press ‘Ok’
10. Connect the Qube instrument to the network requiring the fixed IP-address

The IP-address settings are applied immediately, and the instrument therefore does not need a reboot after the reconfiguration.

Tip! Qube supports multiple DNS servers. These can be added as a space separated list of server addresses in the ‘DNS servers’ field.

4.2.3 How to set up email properties

Users can configure an email account with a mail server and email address. This account is then used to send reports or result tables after project calculations have finished. This is especially useful together with automatic projects (see ‘Automatic project’ in section 5.2.8 in this manual and ‘automatic export actions’ in the Analyzer manual on how to use this function).

The mail server must be the name of a standard SMTP server that accepts connections without authentication on port 25.

The ‘From address’ must be a standard email address, e.g. name@domain.tld. Typically, an email address within your company’s domain must be used.

You can use the ‘Test…’ button to send an email via the specified mail server, in order to see if the email properties have been set up correctly.

4.2.4 How to export a debug file

The software tab also contains a ‘Save debug file’ button (see Figure 17). The debug file contains numerous machine configuration files, a screenshot of the desktop, and log files from the time span defined in the dialog. See section 8.2 for more details on debug options.

4.3 Features tab

The ‘Features’ tab contains an overview of available software Add-ons and which of these are installed on Qube.

For Qubes equipped with a temperature control module, the ‘Features’ tab also has an entry to enable and disable the module. See section 7.4.7 for details.

4.4 Users tab

The user-tab shows a list of current users (see Figure 18). An existing user can be edited by highlighting the line and clicking the ‘Edit’ button in the bottom of the page. Users can be deleted by clicking the ‘Delete’ button. It is not possible to delete a user who is registered as an owner of a protocol or a protocol run.

New users can be created by clicking the ‘New’ button and enter initials, name and password. A user can be created as operator, electrophysiologist, super user, or administrator. It is optional to set a date for which the password expires.

Users that are created as operators or electrophysiologists can only view protocols they have created, whereas users created as super users or administrators have access to all protocols on Qube.

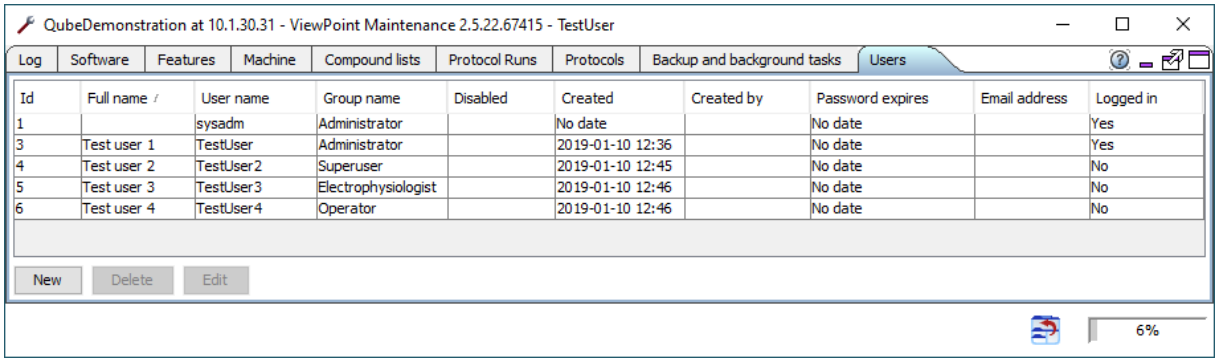


Figure 18 ViewPoint Maintenance. 'Users' tab

4.5 Backup and background tasks tab

In the 'Backup and background tasks' tab, the status of previous backup runs and other background tasks can be seen. The backup is also set up here (see Figure 19).

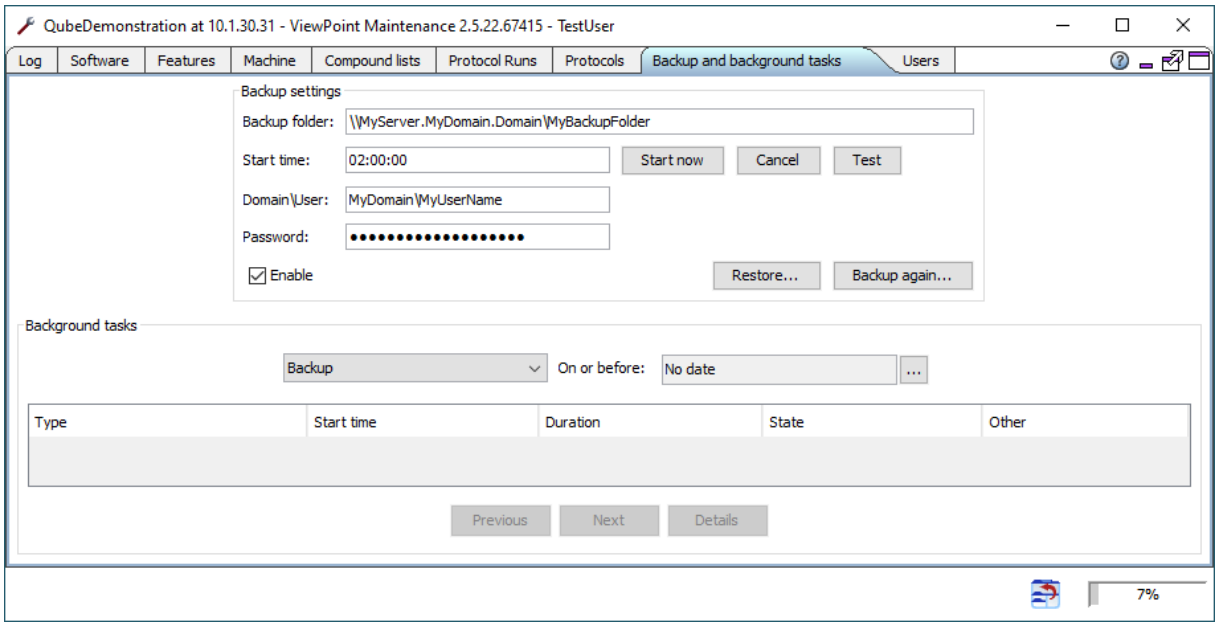


Figure 19 - ViewPoint Maintenance. 'Backup and background tasks' tab showing settings and state of performed backups

In the top panel, a destination folder for backup files and a start time for the backup runs must be set. It is recommended that the time for the backup is set to a time when the Qube is less likely to be running, e.g. at night.

Check that the backup runs correctly via the 'Test' button. When this button is clicked, a file titled '*test[dateandtime].zip*' will be written to the backup folder, and shortly after a message will appear stating whether the file was written correctly.

The backup can be started immediately with the 'Start now' button.

The backup function will only make a backup of each protocol run once. If one or more protocol runs need to be backed up again, the 'Backup again…' button can be used to add specific protocol runs to the next scheduled backup. This can for example be useful if, for some reason, files have been deleted from the backup folder, or if you want to save backup disk space in combination with data reduction.

Caution! It is important to set up automatic backup. If something unfortunate happens to the Data PC, the backup can be the only way to restore valuable data. There is no backup unless you set it up using this tab. Make sure to set up automatic backup and verify that it does back up data.

4.5.1 Backup before or after data reduction

Qube has a data reduction feature to save disk space, see section 4.7.4. If a protocol run has been data reduced before the backup starts, the reduced run will be backed up. If the protocol run has been data reduced after the backup, only the run on the internal Qube Data PC will benefit from data reduction. The run in the backup folder will still contain the unreduced data. With the 'Backup again…' button it is possible to overwrite the full run in the backup folder with the data reduced run.

Note! If data on the internal Qube Data PC has been data reduced but none of the data in the backup folder has been data reduced, there is a risk that the amount of data in the backup folder becomes larger than the capacity of the internal Data PC. In this case it will not be possible to restore the database with all data included. It is, however, still possible to restore the full database and only include a subset of the raw data.

4.5.2 Restoration of database and data from the backup

In case it becomes relevant, the database and all data can be restored from the backup folder.

1. Open the 'Backup and background tasks' panel in ViewPoint Maintenance
2. Click on 'Restore...' button
3. Browse to the backup folder and select the database dump file ('*qubedb.zip*' for standard Qubes and '*databasename.zip'* for external data servers) and click on 'Open'. This will restore the database only
4. When the database restore is finished, click the 'Restore…' button again
5. Select all the '*Run-XXXX.zip*' files (without the '*qubedb.zip*' file) and click on 'Open'. This will restore the actual runs with measurement data

The restoration from backup will also recreate the users, but it will reset the users' passwords and their initials; therefore, users must be notified.

Please note that for external data server solutions, the path for the binary data files is user determined. If a new external server is installed with a different path for the data files than the previous case in the backed-up database, then some minor changes are needed in the database after the restore has been done. We suggest you contact Sophion for further assistance.

Caution! Do not attempt to restore the database and data from the backup without having consulted your Sophion Application Scientist or a service engineer authorized by Sophion.

Tip! Restoration of the run files can take multiple hours, or even days depending on the amount of data and network capacity. You can choose to restore the run files in multiple restore steps to limit the duration of individual steps. The database can be used in between steps of restoration of run files if needed.

4.5.3 Background tasks

The background tasks panel shows the progress and result of various background tasks:

- Backup
- Data reduction
- Data migration
- Automatic export: Exports reports and result tables

- Automatic project: Creates projects automatically when a protocol run has completed
- Add to project: Adds protocol runs to existing projects when a protocol run has completed
- Find protocol run size: Is done when a protocol run has completed or when it has been imported.
- Cleanup of failed migrations: If power is lost during data migration, then a cleanup will be done when the Qube software starts again

4.6 Protocols tab

All protocols that have been created on Qube can be seen in the list in this tab (see Figure 20). If there are more protocols than can be seen on the screen, navigation to the other protocols is done with the 'Next' and 'Previous' buttons.

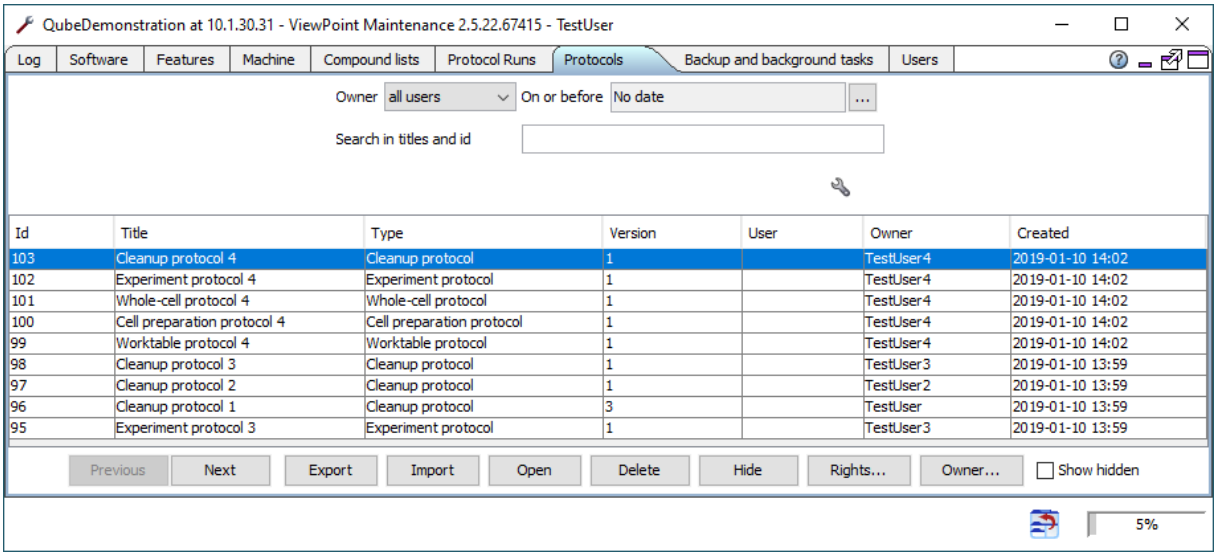


Figure 20 ViewPoint Maintenance. 'Protocols' tab

Each protocol can be viewed in detail when it is opened by clicking on the line. Protocols can be searched for by:

- Entering part of the name of the protocol in the search field
- Selecting the user that created it
- Picking a date on or before which it was created

Protocols that are no longer used can be hidden by clicking on the 'Hide' button. When a protocol is hidden:

- It will no longer be visible in ViewPoint or ViewPoint Maintenance
- It can be seen if 'Show hidden' is checked
- Once shown, it can be unhidden by clicking the 'Unhide' button (the protocol will again be visible in ViewPoint and ViewPoint Maintenance)

Protocols can be exported and imported using the corresponding buttons. Per default, protocols that have been used for generating data in a protocol run cannot be deleted.

Contact your Application Scientist if you would like to enable the capability to delete data.

4.6.1 Protocol owner and rights

The 'Owner' button can be used to select a new owner for a specific protocol. User group rights (Electrophysiologists, Administrators etc.) are set up by default. The 'Rights' button enables you to modify the rights settings of a given protocol (see Figure 21). A specific user or a user group can be given more or fewer rights for a specific protocol. It is possible to remove all user rights for a protocol except e.g. for the creator of the protocol.

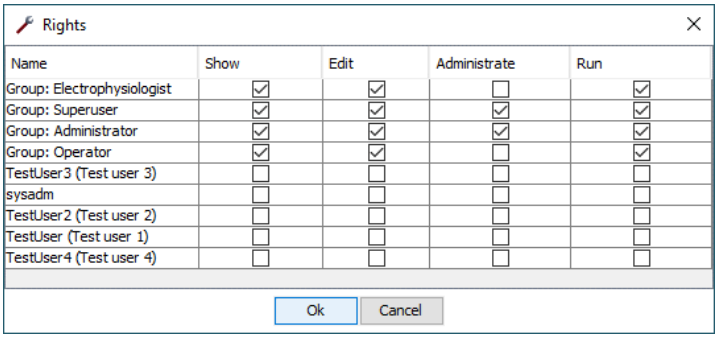


Figure 21 ViewPoint Maintenance. 'Rights' window. Editing the rights for a given protocol when clicking on 'Rights…' in the 'Protocols' tab

4.7 Protocol Runs tab

In this tab, all runs are listed that have been executed on Qube (see Figure 22). Runs executed by a specific user or on a specific date can be seen by using the owner or date fields as described in 'Protocols'. If there are more protocol runs than can be seen on the screen the navigation to the other protocols is done with the 'Next' and 'Previous' buttons.

Export and import of protocols and protocol runs can be done in this tab as well.

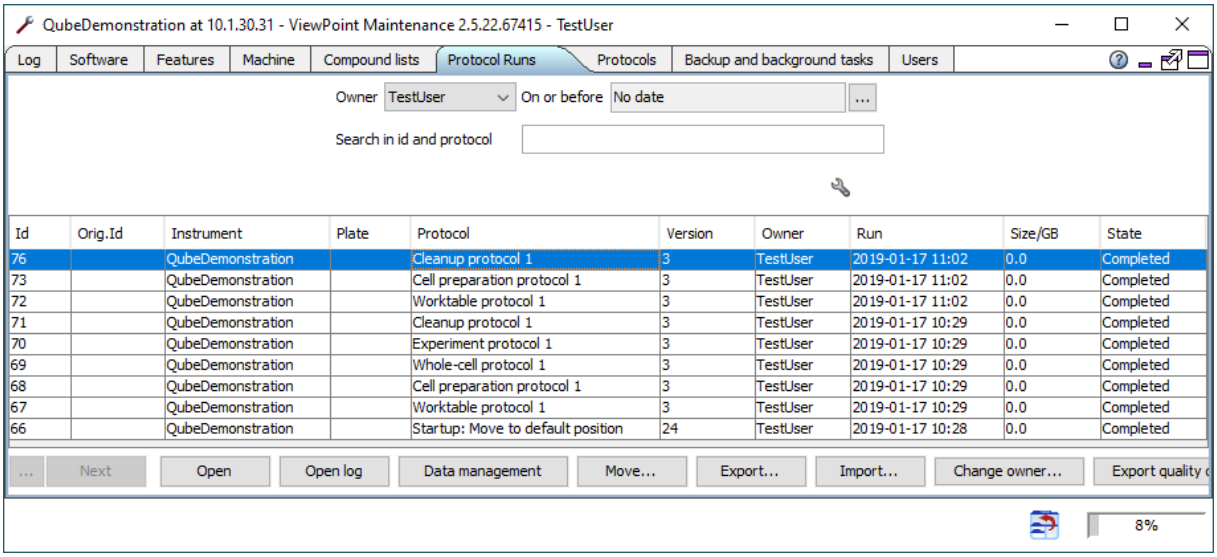


Figure 22 ViewPoint Maintenance. 'Protocol Runs' tab

When opening a run, the data from this run can be seen in a view similar to ViewPoint.

4.7.1 Showing log info

For all runs, the corresponding log can be opened using the 'Open log' button. A timeframe for log import can be selected. By checking the 'Include full log' checkbox, the full log for processes that started running before the selected time period, or ended after the time period, can be included (see Figure 23). This is not recommended because it will usually cover a very high number of log messages. When a protocol run is exported, it is possible to leave out the compound list from the file.

Use of the log system is typically only relevant for advanced debugging and technical protocol run evaluation. Log analysis will typically be done by, or in, collaboration with your Sophion Application Scientist or Service Engineer.

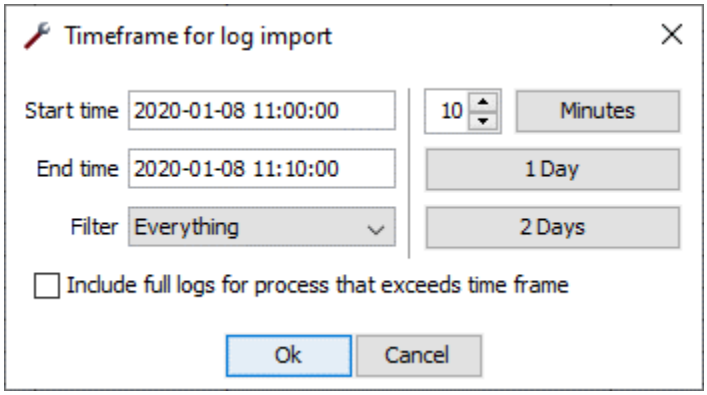


Figure 23 ViewPoint Maintenance. 'Protocol Runs' tab. Choosing the timeframe for the log file when clicking on 'Open log'

4.7.2 Exporting quality data

Quality data can be exported using the 'Export quality data' button. Quality data contains information on priming and sealing rates for each measurement site for all runs in a selected time period. This data can be used by your Application Scientist for monitoring success rates for the system over time, debugging, and general improvement in performance. The time interval for which the data is retrieved can be selected by setting start and end time or choosing the default periods (last day or the last two days).

4.7.3 Data management

The 'Data management' button is used for data reduction, deleting protocol runs, and migration of data to other databases.

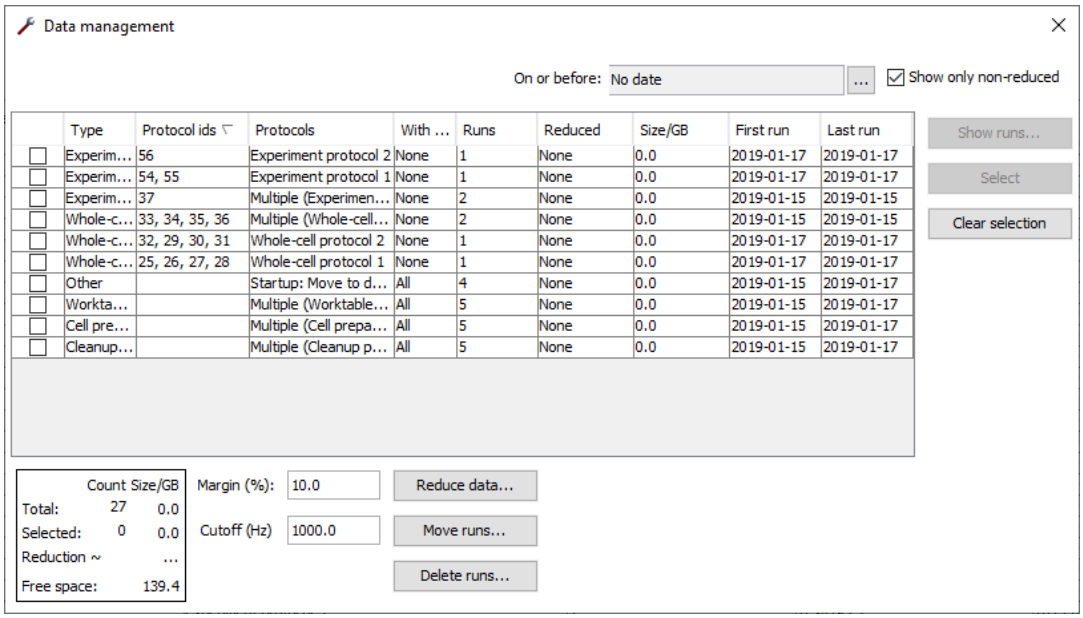


Figure 24 The 'Data management' window is the main entry for applying data reduction and moving protocol runs to other databases with the data migration feature

It will open a window, that shows all protocol runs on the system, grouped by type and protocols, so that it is easy to select a group of similar protocol runs (see Figure 24). It is possible to filter the selection by run date (so that you only see old protocol runs) or by data reduction state.

It takes a while for all the protocols of the runs to be read, so please be patient.

The combined size is also shown, so that it is easy to find groups of protocol runs that use a large part of the disk space.

It is possible to select a whole group of protocol runs or open the group with the "Show runs…" button and select each protocol run individually.

In the bottom left corner, you can see the total size of all protocol runs and of the selected protocol runs. It also shows an estimate of how much space will be freed if data reduction is used on the selection.

4.7.4 Data reduction

The 'Data management' button is used for data reduction or deleting protocol runs. The data reduction function is used to reduce the amount of data that is stored in the database. The section of data that should be removed is selected in Sophion Analyzer and in the 'Data management' option, and in ViewPoint Maintenance the reduction is executed for the selected runs.

Caution! Deleting or reducing data is irreversible. Be aware of this fact before deleting or reducing data.

For data reduction to be available, the configuration file for ViewPoint Maintenance must have a specific setting – ask your Application Scientist for assistance.

Data reduction is based on the cursors placed on the current traces in the analysis. This is done in Sophion Analyzer (See the separate manual for Sophion Analyzer for details).

Data reduction:

- Works for both current cursors and zero cursors
- Is not active on recordings obtained with pulse trains
- Is not active on recordings with less than 2000 samples, e.g. 80 ms x 25 kHz
- Cursors cannot be placed and hence the analysis cannot be done in the reduced part of the current trace
- When data are reduced, there will be blue bars indicating the non-reduced part of the trace in Sophion Analyzer

Data reduction can be done both retrospectively and proactively. The most efficient way to reduce storage usage is by reducing data proactively so the back-up also benefits from less data to back-up, see also Section 4.5. Both methods are described below.

4.7.4.1 Retrospective data reduction

In the Protocol Runs tab, when clicking the 'Data Management' button, a new window opens with all executed protocols. Be aware that it can take some time to read through the entire database, but there is a counter in the upper left corner of the data management window to inform about the progress. The following information can be found:

- 'Protocol IDs'
- Number of protocols in the group that have cursors placed in the resulting current traces (in Analyzer)
- Size of the data generated with that protocol
- etc.

To reduce data:

1. Select a protocol by checking the box to the left of the row
2. The small window in the lower left corner will indicate the reduction that will be obtained
3. Click on 'Reduce data…'
4. A warning window will open (see Figure 25 with both windows open)
5. The *plan run* ID (see Figure 25) in which the selected protocol(s) has been used is displayed
6. There can be multiple plan runs based on the same protocol, so it is possible to deselect if some of the plan runs should not be reduced. By default, all plan runs based on the same protocol are selected
7. Click 'Ok' to execute

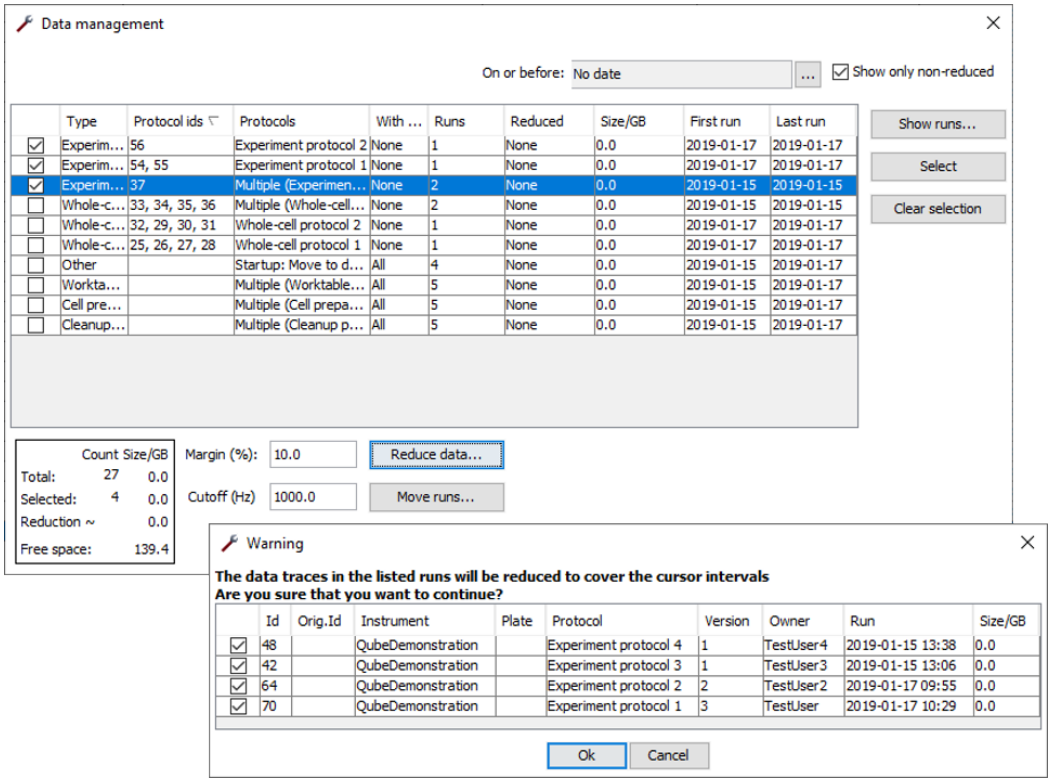


Figure 25 Reducing data. In ViewPoint Maintenance under protocols run tab, selected runs can be data reduced based on cursors placed in Sophion Analyzer. There is a warning window to avoid unintended data reduction - note that data reduction is irreversible

4.7.4.2 Proactive data reduction

It is also possible to reduce data immediately after they have been sampled to save space on the Data PC as well as the back-up disk. This is done in ViewPoint, see chapter 5, and is also described here. This option has been developed for screening campaigns where an assay has been set up and no changes are anticipated so that data reduction can be done most efficiently.

When planning a run in ViewPoint based on an experiment protocol with a waveform where cursors have already been placed via Sophion Analyzer, the way to reduce data is by checking the 'Reduce data' box in the summary tab. A 'Data reduction warning' window will pop up to avoid unintended data reduction (see Figure 26).

Be aware that the setting, i.e. the choice of data reduction, is stored and assigned to the user. The next time the same user logs in to Qube and loads a plan, data reduction is checked. There will, however, also be a warning on that occasion.

Caution! Deleting or reducing data is irreversible. Be aware of this fact before deleting or reducing data.

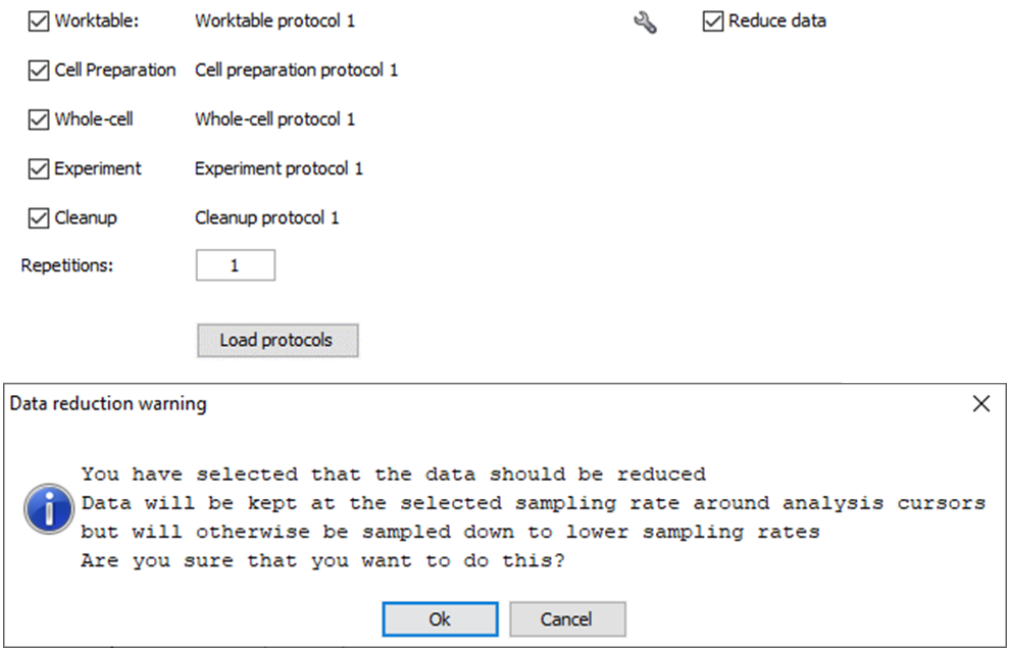


Figure 26 Data reduction activated in ViewPoint when loading a plan based on experiment protocol with cursors placed in Sophion Analyzer. Both the initial dialogue and the warning window are shown

4.7.5 Data migration

Protocol runs can be moved to a standalone database on the same network with one of these buttons:

- 'Move runs…' button in the data management panel
- 'Move…' button in the protocol runs panel

They will open a window that allows you to add or remove protocol runs from the selection (see Figure 27).

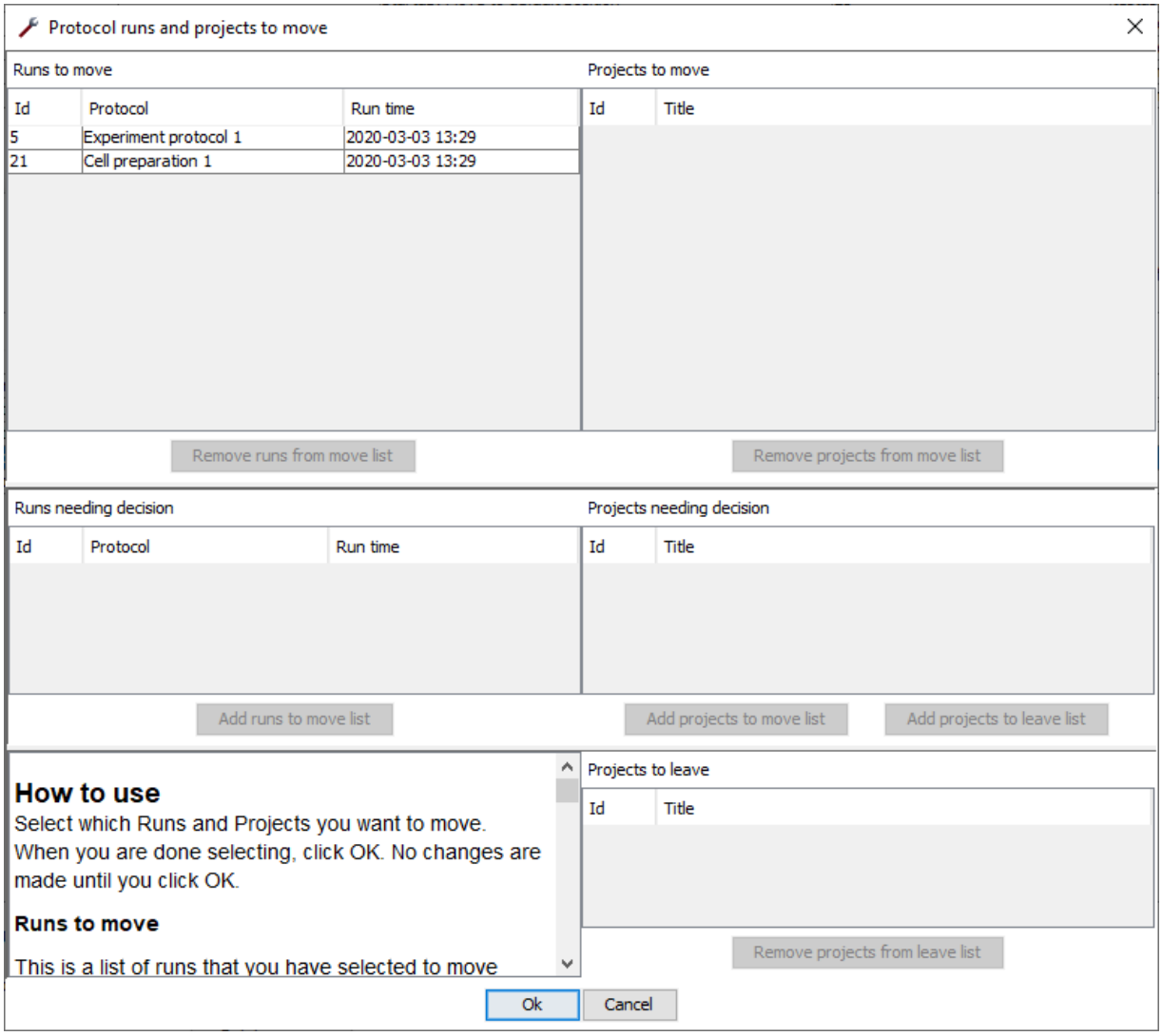


Figure 27 Data migration decision window

When migrating a protocol run, then there are some conditions that must be fulfilled:

- All other protocol runs belonging to the same plate run must also be included (e.g. if you select an experiment protocol run, then the corresponding whole-cell protocol run must also be included
- All projects containing the protocol run will also be migrated
- All other protocol runs in the projects mentioned above must also be migrated

To fulfill these requirements, use the window shown in Figure 27.

The panel 'How to use' shows brief instructions on how to use the panel.

At the top of the window the selected runs and any projects that only contain selected protocol runs are shown.

In the center of the window, projects that contain some of the selected protocol runs, but also contain other protocol runs can be seen.

Click on one of the projects 'needing decision'. Then the protocol runs that it includes will be highlighted.

Now, do one of the following:

- Click on 'Add runs to move list'. This will also include those runs in the migration and thereby move them from the Qube to the standalone database as well
- Alternatively, click on 'Add projects to leave list'. Then the selected project will not be moved, and the selected runs will be removed from those projects, i.e. the runs will be migrated without the project analysis

When the tables in the center are empty, you can proceed with the data migration by clicking 'Ok'.

The prompt for the destination database then appears (see Figure 28):

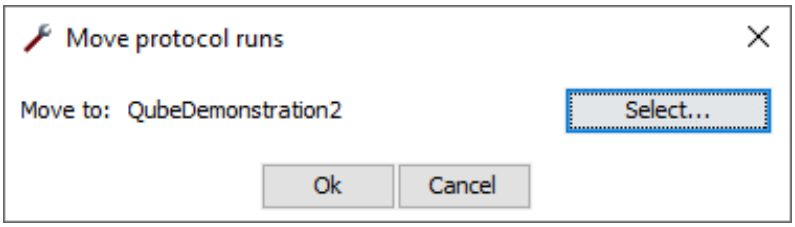


Figure 28 Data migration destination window

After clicking OK, data migration begins. Once it is complete, the data migration result window appears (see Figure 29).

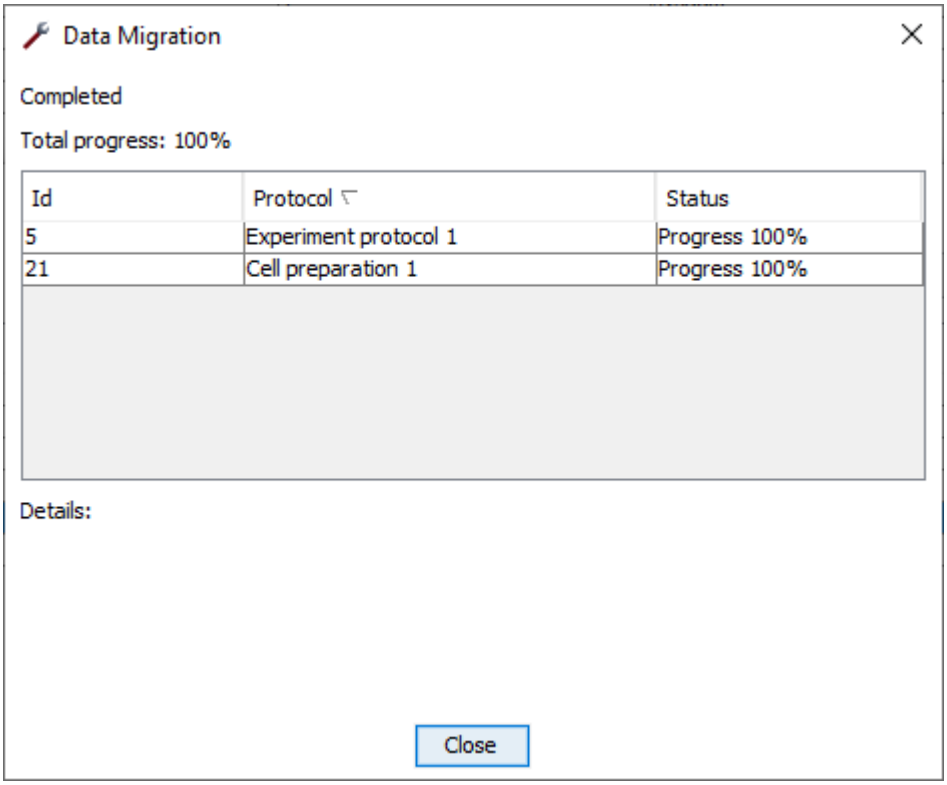


Figure 29 Data migration result window

To see the details of the migration results in the field at the bottom of the window, click on one of the protocol runs.

Note that data migration is performed by the destination database, so it will continue even if you close the result window. However, you cannot return to the result window after it

has been closed, so it is not possible to monitor results unless the result window is kept open.

The protocol runs will be migrated in groups that belong together. These protocol runs belong to the same plate run or are included in the same project. If the network connection between the source of the protocol runs (the Qube) and the destination database is lost while the protocol runs are migrated, then the software will delete any protocol runs that were already copied and leave the original protocol runs intact on the source Qube.

In addition, any immediately preceding Worktable or Cell Preparation runs will be copied (not moved, because they may have been used for multiple experiment runs). This means there is a complete set of Worktable, Cell preparation, Whole-cell, Experiment, and Cleanup protocols on the destination database.

4.7.6 Exporting protocol runs

Protocol runs can be exported using the 'Export…' button in the 'Protocol Runs' panel. Reasons for exporting protocol runs can be to transfer data to a different Qube, or to share the protocol run with your Sophion Application Scientist for assistance with, for instance, data analysis and other run related questions.

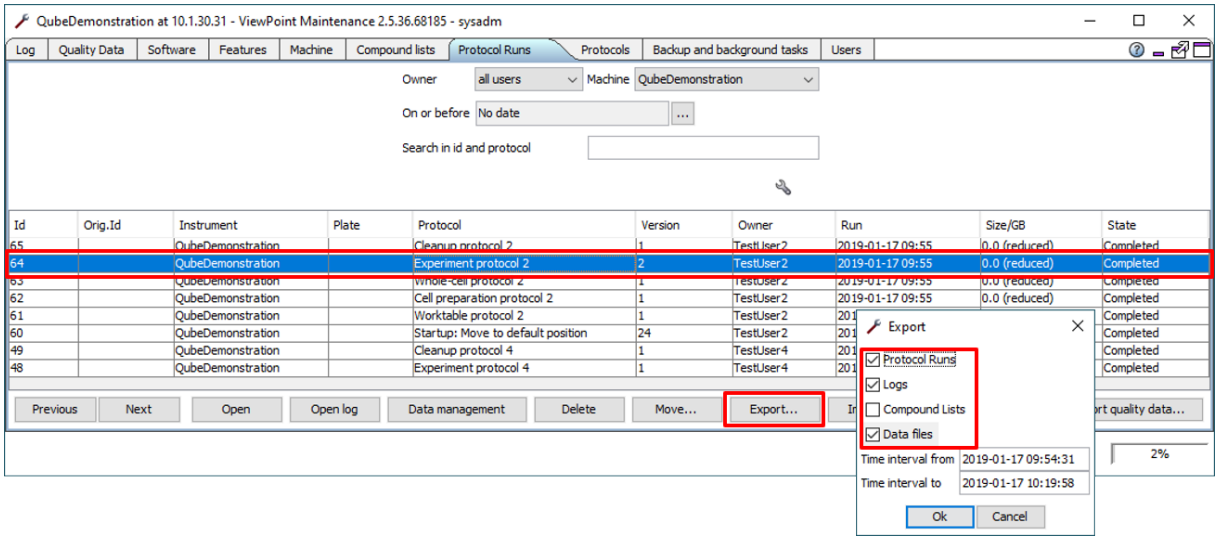


Figure 30 Protocol run export from ViewPoint Maintenance showing the different export options

To export protocol runs, select the run(s) to be exported and press the 'Export…' button (see Figure 30). Multiple runs can be exported in a single step by selecting multiple runs using of Shift or Ctrl keys. It is also possible to export protocol runs and plate runs via ViewPoint, see section 5.3.1.1 on page 84.

The 'Export' dialog has different export options:

- 'All Protocol Runs in one file': If multiple runs are selected for export, this option ensures that they are all exported in the same file. If the option is deselected, a separate file will be created for each run unless the runs belong to the same QChip 384
- 'Protocol Runs': Information of the execution sequence, settings, timestamps etc. This option should always be selected to be able to import the plan run on another instrument
- 'Logs': Full database log from the protocol run. The log time intervals are automatically adapted to the chosen protocol runs
- 'Compound Lists': Compound lists coupled to the run. This option is deselected by default
- 'Data files': All data including raw current traces are exported. Deselect this option to decrease file size if the purpose of the export is not related to the recorded data

Note! There is **no anonymization** of data or other information in exported protocol runs.

Note! Plate runs, i.e. protocol runs belonging to the same QChip 384, will always be exported as bundled runs with all protocols in a single file even if not all of them are selected for export. It is not possible to export individual protocol runs if they belong to a plate run.

Note! Very large runs will be split into zip files of around 4 GB each. The first file has the extension .zip, the second file has the extension .z01, etc. Make sure that all the files are available in the same folder for subsequent import.

4.7.7 Importing protocol runs

To import protocol runs, click the 'Import…' button and navigate to the folder where the exported protocol runs are stored. Select the protocol runs and click the 'Open' button. Multiple runs can be imported in one operation by selecting all the runs before clicking the 'Open' button.

Note! Very large runs will be split into zip files of around 4 GB each. The first file has the extension .zip, the second file has the extension .z01, etc. Make sure that all the files are placed in the same folder before importing and select the .zip file only. The import function will automatically import the associated files with extension .z01, .z02, etc.

Note! Protocol runs imported from another Qube database will get a new Id on the receiving database. Be aware of this when referring to the protocol Id. The original Id and Qube will also be shown in the Id column for traceability.

4.8 Compound lists

In this tab, all existing compound lists can be seen.

If there are more compound lists than can be seen on the screen, navigating to other protocols is done with the 'Next' and 'Previous' buttons (see Figure 31).

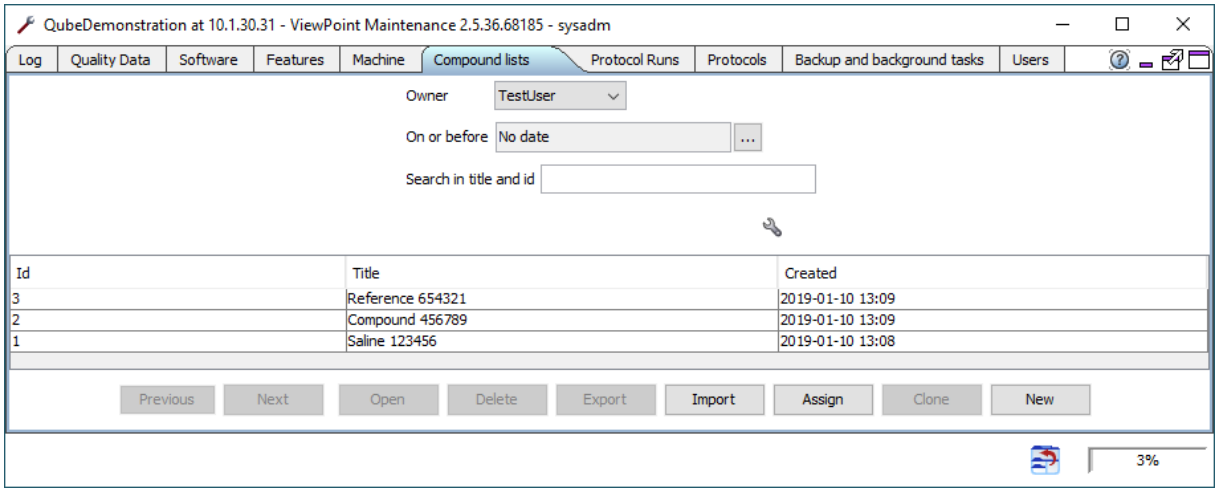


Figure 31 ViewPoint Maintenance. 'Compound lists' tab

Compound lists can be imported and exported, and existing compound lists can be deleted. Only simple compound lists (1x1, 16x1, 1x24) can be edited and cloned.

4.8.1 Compound list import

When using a 384-plate, the compound list is meant to be created in Excel or similar, with columns designating the well, compound name, and concentration including measurement unit. The list is then imported to ViewPoint or ViewPoint Maintenance either by dragging it onto the window or by selecting 'Import'. If your compound management facility creates compound lists that are not readily understood by ViewPoint, please contact your Application Scientist.

An Excel file can contain multiple compound lists if they are placed as individual sheets. The sheet's name is then understood by ViewPoint as the barcode of the physical compound plate. To assign a compound list to a compound plate that has been assayed – see section 5.3.5.

When the compound list is imported, it is automatically matched against all completed protocol runs. If one or more of the MTPs in the compound list match completed protocols runs, then a prompt is shown whether the MTPs should be assigned to the runs.

If a compound list is imported before the corresponding protocol run has completed, then the compound list will be automatically assigned to the protocol run, when the protocol run completes.

If you have old protocol runs and compound lists that have not yet been assigned, then you can use the "assign" button to assign them.

It is not possible to import a compound list that has a barcode, if that barcode is already used by a different compound list in the system. However, for Assay Development, it is possible to import multiple compound lists that have empty barcodes.

4.8.2 Create simple compound list

A new simple compound list can be created:

1. Click the 'New' button
2. Assign the right type of liquid (this is important for the analysis):
   - 'Saline'
   - 'Compound'
   - 'Reference'
   - 'Undefined'
3. Assign the format (i.e. the layouts of the physical compound plates):
   - 1x1
   - 16x1
   - 1x24
4. Type in the 'Compound name' and its 'Concentration' for each row or column.

4.9 Machine tab

The Machine tab has two sub tabs: 'Info' and 'Tools'. The 'Tools' tab is only available on the User PC configured to control the Qube instrument.

4.9.1 Info tab

In the 'Info' tab information on the software and status of the machine can be seen (see Figure 32). In the top panel, the machine information is displayed, including:

- Machine name
- IP-address
- Disk space on the data PC
- State

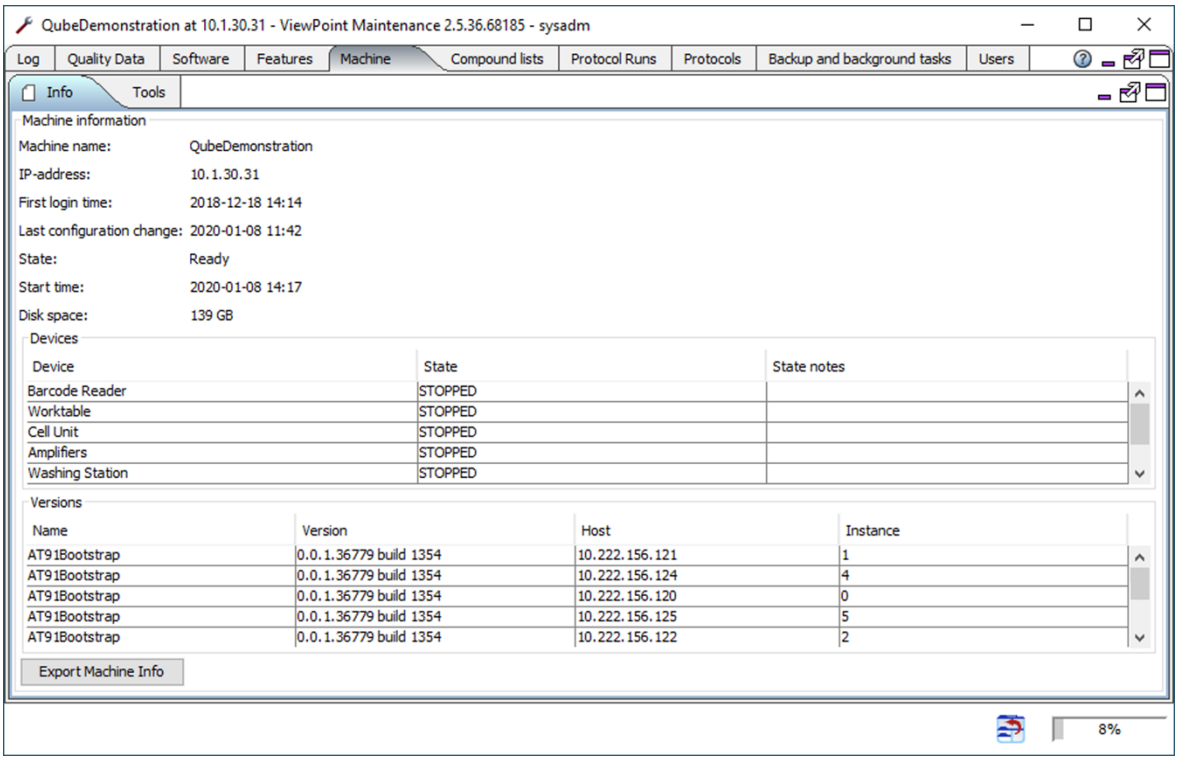


Figure 32 ViewPoint Maintenance. 'Machine' tab

The line 'State' describes the machine state, and it is to be understood as describing the overall instrument. Table 4 gives an overview of different machine states.

Table 4 Overview of available machine states

Machine states	
Standby	Qube is in standby and cannot execute any plans. Devices such as 384-robot, amplifier system, and stacker are not powered. Internal PCs are powered so, for instance, new assays can be set up and data can be analyzed
Waking from standby	Qube is waking from standby. All devices will be powered up and establish connections during this process
Going to standby	Qube is going into standby mode. Device connections will shut down and some devices will power off during this process
Ready	Qube is ready to load and run protocols and utility protocols. There may still be restrictions to which protocols can be run, even with the machine in 'Ready', for instance if Qube has not been homed
Not ready	Qube cannot load or run protocols before certain conditions have been met. This could for instance be closing the cabinet door
Error	Qube will be in an error state if one or more devices are in the device state ERROR
Running	Qube is executing protocol(s). No new protocols can be loaded or started before the ongoing protocol execution has completed
Going to pause	A pause has been requested for protocol execution and Qube is in the process of pausing the run
Paused	Protocol execution is paused. Qube is ready to continue the execution
Stopping	Protocol execution has been aborted and Qube is in the process of stopping the run

In the second panel, the state of each device is displayed. Each device can have the states summarized in Table 5.

Table 5 Overview of available device states

Device states	
STANDBY	The device is in standby
PAUSED	The device is paused and ready to continue
STOPPED	The device is idle and ready to use. All devices need to be ready to use before the entire Qube is ready to load and run protocols
EXECUTING	The device is involved in protocol execution
FAILED	The device execution was aborted in the previous run protocol. The device is otherwise ready to run
ERROR	The device is in an error state and is not ready to run. The error must be addressed and resolved before the device and Qube is ready to run again. The error condition is described in the 'State notes' field

The last panel displays software and firmware versions of all devices.

4.9.2 'Tools' tab

The 'Tools' tab is only visible on the User PC configured to control the Qube instrument. It contains a 'Power supplies' section and a button for toggling between on and standby.

The ‘Power Supplies’ section shows which power supplies are turned on or off. The power supply buttons are controlled automatically via the ‘Machine On/Standby’ button and should, under normal conditions, not be used separately to control device power. Doing so can conflict with the establishment of device communication which is necessary to wake Qube from standby in a proper way.

The entire ‘Tools’ tab in ViewPoint Maintenance is intended for use by service engineers only and should not be used for normal operation.

5. ViewPoint

This chapter will introduce the software that is used to operate and run experiments on Qube. Qube is controlled via the ViewPoint software, and data is subsequently analyzed in Sophion Analyzer.

Sophion ViewPoint and Analyzer, as well as ViewPoint Maintenance, are installed on the user PC and potentially on all other PCs connected to Qube.

In short, ViewPoint is used to setup and execute experiments on Qube, while Sophion Analyzer is used to analyze data. ViewPoint Maintenance is used on more rare occasions for creating users, checking the back-up status, deleting or reducing data, see chapter 4 for more details.

5.1 Starting ViewPoint

Click the icon on the desktop (Figure 33) and login with your credentials. If you do not know your username and password, contact your local Qube administrator.

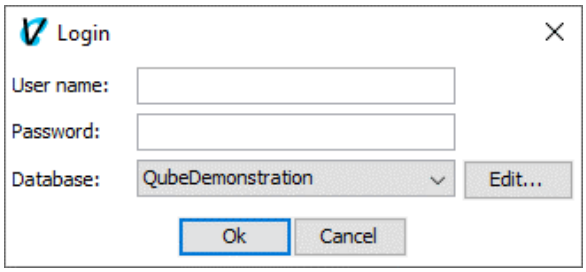


Figure 33 ViewPoint login screen

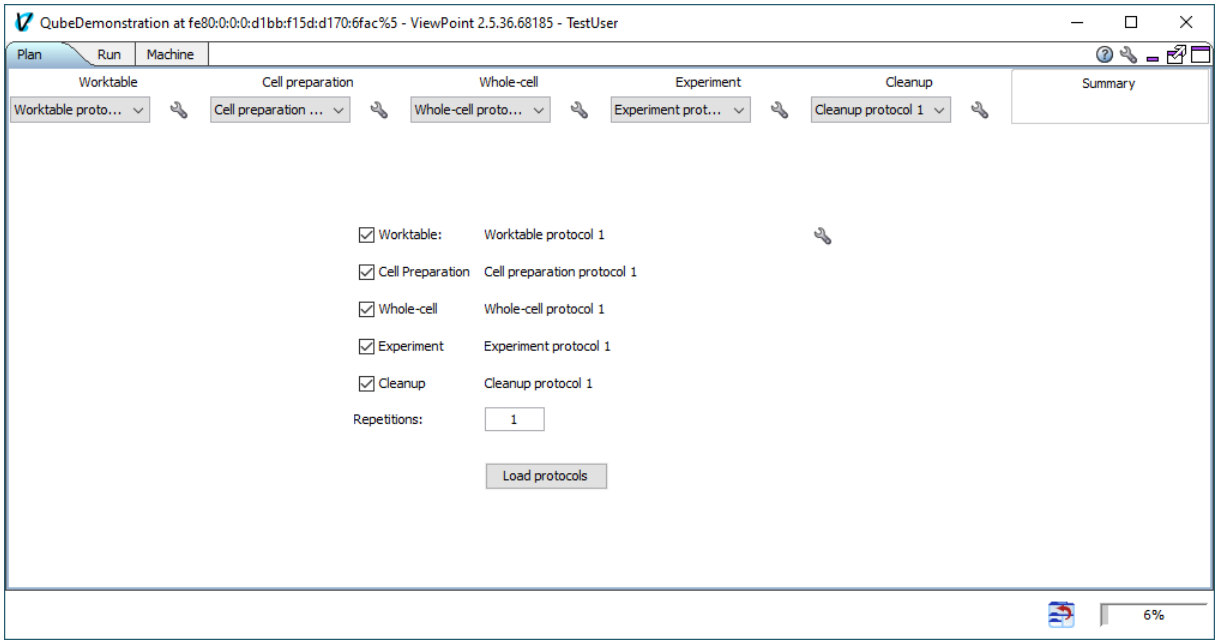


Figure 34 Overview of the 'Plan' tab

After logging in, the screen in Figure 34 will appear. In the top left corner, there are 3 main tabs, namely Plan, Run, and Machine:

- 'Plan' is where the experiments are designed
- 'Run' is where an ongoing experiment is supervised
- 'Machine' is where the system is initialized and monitored

5.2 Plan tab

In the 'Plan' tab, different protocols can be created, edited, and selected to load for one or more plate runs. A plate run consists of 1 to 5 protocols, and a normal run will have all 5 protocols including a(n):

- 'Worktable' protocol
- 'Cell preparation' protocol
- 'Whole-cell' protocol
- actual 'Experiment' protocol
- 'Cleanup' protocol

The following sections will walk through setting up the experiment.

5.2.1 Basic navigation and layout

The layout and navigation of each protocol is similar. The protocol setup for each can be found underneath the top tabs, with a dropdown menu and a wrench for each object (see Figure 35) in the ViewPoint 'Plan' panel.

Figure 35 Dropdown menu and 'Wrench' icon for each protocol

Clicking the dropdown box allows you to:

- Select a recently run protocol
- Search for a protocol

The 'Wrench' icon gives access to:

- Making new protocols
- Renaming the currently selected protocol
- Importing protocols
- Exporting the currently selected protocol
- Saving a copy of the currently selected protocol under a new name to create a new protocol

When a protocol is selected or a new one is created, click on the heading for the protocol (for example 'Worktable') to open the editor window. This applies to all types of protocols (Figure 36).

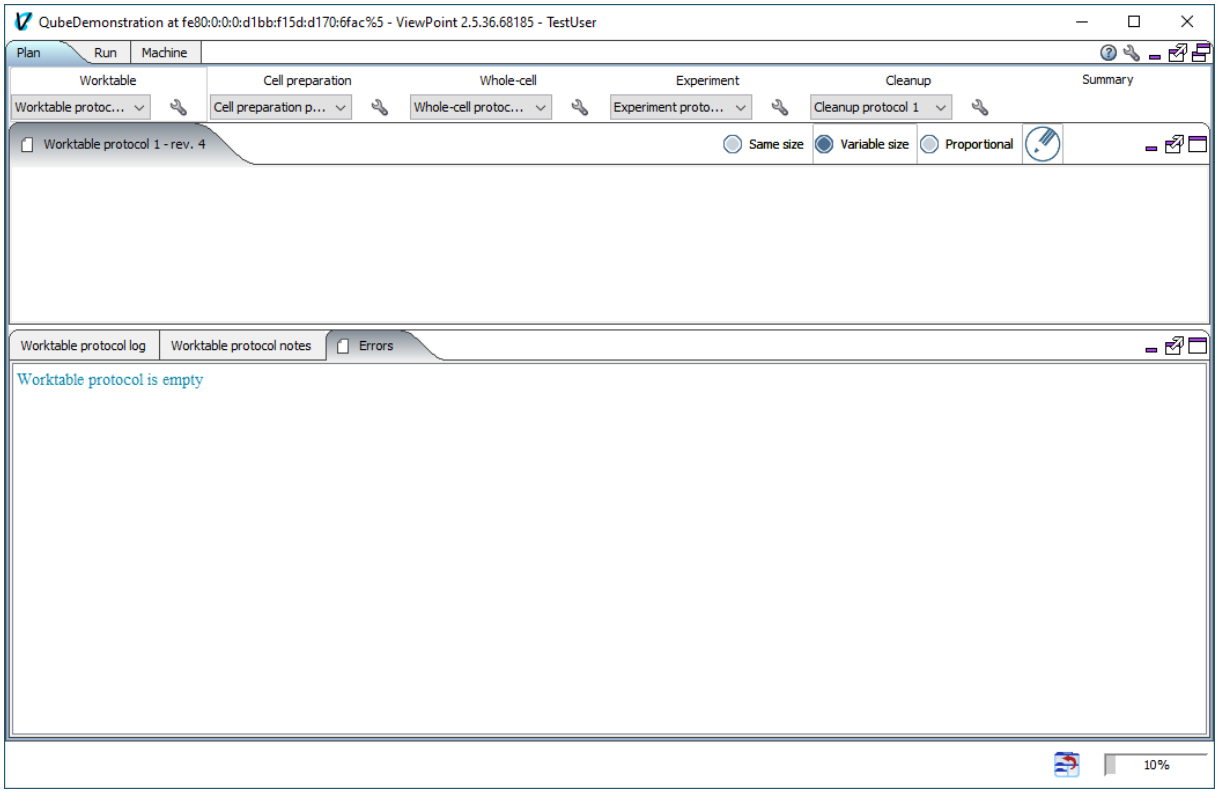


Figure 36 Empty worktable protocol setup

The name and revision number are visible in the tab to the left. To the right there are three radio buttons that can be toggled to change the view of the blocks in the timeline plot. The following buttons are next to the radio buttons:

- The 'Pencil' icon toggles editing mode (on/off)
- The 'Plus' icon is to add the first of extra new block(s)
- The blue arrow is the undo button
- The 3 purple lines and windows are used to alter display settings of the window, see section 5.5

It is important to note, that no changes can be made to the protocol without entering edit mode. **The following sections will assume that edit mode is on**. If an option is missing, it is probably because edit mode is not enabled.

Whenever leaving the protocol editor, it will also exit edit mode to avoid unwanted editing.

5.2.2 General steps for making a new protocol

All protocols are built by inserting one or more blocks based on pre-defined block templates. Once inserted, the block is visualized with the devices involved in the protocol along the left, and time along the top (e.g. Figure 41 on page 64). When a block is selected, some of them will have a parameter field box where changes can be made to the block. These parameters may be holding potential, liquid source position, etc.

Follow these general steps to make a new protocol:

1. Press the 'Wrench' icon next to the protocol drop-down
2. Choose 'Add new […] protocol'
3. Give the protocol a suitable name
4. Press the 'Plus' icon to open the 'Add block' window
5. Select a block and click 'Ok'
6. Edit the parameters available in the block(s)
7. Optional: Add notes in the '[…] Protocol notes' tab

8. Optional: Click the 'Pencil' icon to leave editing mode

Tip! The 'Details' button in the 'Add block' window contains detailed information about the currently selected block. The same information is available in an 'Info' section after the block has been inserted in a protocol.

Tip! '[…] Protocol notes' can be used to add general notes about the protocol, e.g. instructions about which liquids to use, etc. Protocol notes are **not** suitable for noting details about the results and conditions for a specific run. Use the 'Run notes' instead, see section 5.3.4.

Note! The protocol name is not unique. It is possible to have multiple protocols with the same name, so please take care when naming the protocols.

5.2.2.1 Number of blocks per protocol

Caution! The number of available block templates and how many blocks should be inserted is different for the different protocol types. Make sure to use the correct number of blocks per protocol to avoid faulty execution.

The following list gives an overview of the number of blocks to use in each protocol type:

- 'Worktable' – Several block templates are available. Only one block should be inserted
- 'Cell preparation' – one or more block templates are available. Only one block should be inserted
- 'Whole-cell' – one or more block templates are available. Only one block should be inserted
- 'Experiment' – Several block templates are available. Each block can be inserted multiple times in different combinations
- 'Cleanup' – One or more block templates are available. Only one block should be inserted

Tip! It is possible to get 'Customer Specific Block Templates'. Ask your Application Scientist for details.

The following sections will walk through creating a protocol for each protocol type.

5.2.3 Worktable protocols

The 'Worktable' protocol specifies what Qube can expect to find on the different positions on the worktable. It can also include other features such as adding plates from external feeders, diluting stock compound plates, and adding new pipette tips.

5.2.3.1 Worktable layout

The layout defines the names and use of different positions. There are two different nomenclatures for the positions:

- Physical position:
  - The fixed position of a slot plate on the worktable. This name is shown in blue in the worktable protocol interface. Most positions follow a grid layout naming scheme with letters and integers denoting row and column position, respectively
- Logical position:
  - A configurable position used by the 384-robot for navigating. These position names are shown in orange in the worktable protocol interface. When

referring to positions in the manual and the software these are typically logical positions unless stated otherwise

How to physically place the plates in the instrument will be specified in section 6.1 page 93.

1. Make a new protocol by following the steps detailed in Section 5.2.2
2. Double-click on the inserted block
3. There should now be a window similar to Figure 37 showing the layout panel. This reflects the physical layout of Qube

In the lower part of the 'Layout' panel, the 'Type' column should match the plate types used on the individual positions on the worktable. A dropdown menu for choosing a different plate type is available for each position by clicking in the 'Type' column.

There are important parameters that need to match between the software and the physical plate, for example, when choosing the plate type, the plate height and well depth must match. Please refer to Table 7 page 60 for details. If in doubt, ask your Application Scientist.

The number in the square brackets on the QChip 384 positions specifies the order they will be used when loading multiple repetitions of an assay.

The numbers assigned to compound positions are only to identify them while setting up the 'Experiment' protocol. Compounds can be added in any order and repeatedly, provided that there is enough solution in the compound plate.

The four compound plates placed at positions 'Compound 1[x]' will be pipetted into individual QChip 384[x] whereas 'Compound 2' (or 3) will be pipetted into QChip 384 plates if selected in the experiment protocol.

Caution! If the experiment protocol is set up to call for a position that is not defined in the worktable, the execution will stop with an error and the QChip 384 will be lost. Ensure that the worktable and experiment protocols are compatible before starting a plate run.

Tip! Qube comes preconfigured with worktable layouts to handle many applications. It is not possible to reconfigure the worktable layouts. Please contact your Application Scientist if you have an application that is not covered by the available layouts.

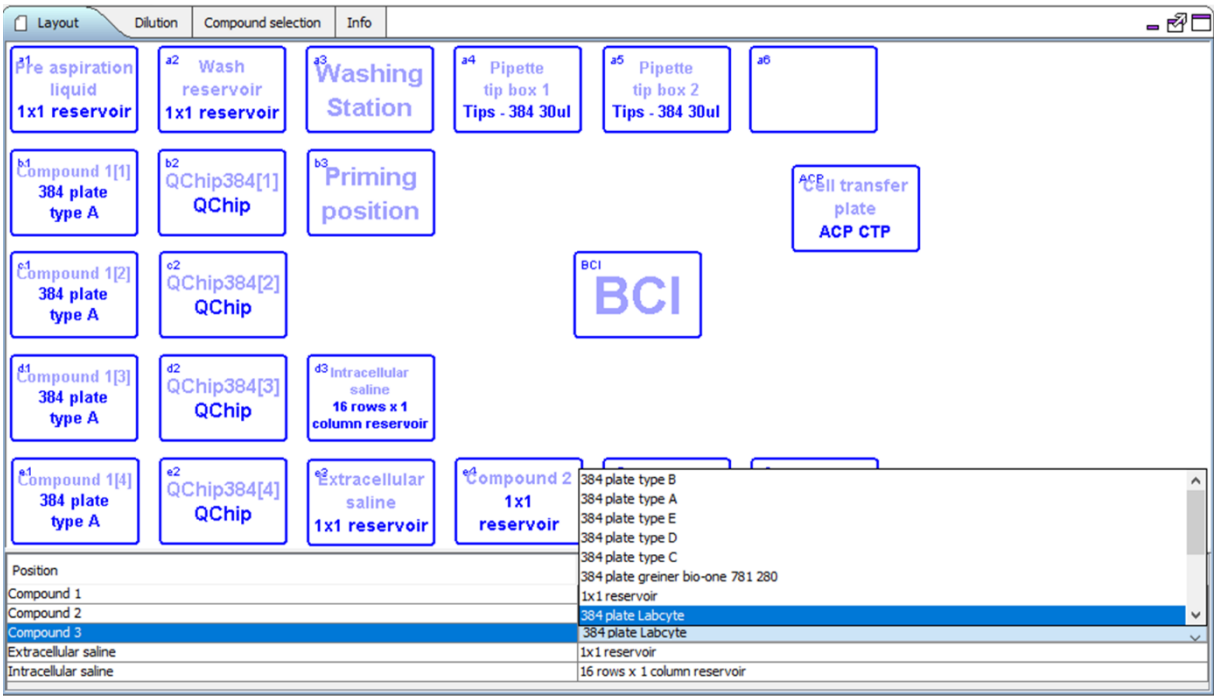


Figure 37 Worktable for screening with two extra compound plates for reference and visible drop-up for the plate types. Position 'Compound 3' is hidden behind the plate type selection menu

A description of the typical use of positions on the worktable is given in Table 6. A description of the different plate types that are available are described in Table 7.

Table 6 Description of different positions on the worktable and their typical use

Typical use of worktable positions	
Pre aspiration liquid	Position for pre aspiration liquid which is used to pre-wet the pipette tips to enhance pipetting precision. The liquid herein should be bio-friendly, e.g. the extracellular liquid used in the experiment. Mandatory for use in the whole-cell protocol
Compound #	Position for test compounds. This can be referred to in the experiment protocol
Compound #[x]	Position for test compound specified for QChip 384[x]. This can be referred to in the experiment protocol
Intracellular saline	Position for saline for intracellular environment. Mandatory for priming the QChip 384
Extracellular saline	Position for saline for extracellular environment. Mandatory for priming the QChip 384. This can also be referred to in the experiment protocol for adding to the cells
Priming position	QChip 384 plates are primed at this position – cannot be accessed by the user
Wash reservoir	Position used for extended wash of pipette tips in e.g. DMSO or ethanol. The use of this is specified in the experiment protocol. The position cannot be changed by user
Washing station	Used for standard tip wash in pure water. The position cannot be changed by user
QChip 384[#]	Positions dedicated to QChip 384 plates – the number of QChip 384 plates placed on the worktable should be equal to the number of repetitions specified in the summary panel
Pipette tip box #	Position where the robot picks up tips from the tip loading tools
Pipette tip rack[#]	Positions where plastic based pipette racks are placed when using automated exchange of pipette tips
Automated tip exchange	Position where Qube automatically locks plastic pipette tip racks in place when using automated exchange of pipette tips
Manual tip exchange	Position where plastic pipette tip racks can be placed manually when automated tip exchange is not used
BCI	Where the QChip 384 is placed for the recordings
Cell transfer plate	Position for the CTP. This is where the 384-robot picks up and transfers cells to the QChip 384

Table 7 Description of the available plate types

Available plate types	
1x1 reservoir	Reservoir plate with one compartment (SB2260)
1 row x 24 columns reservoir	Reservoir plate with 24 columns (SB2262)
16 rows x 1 column reservoir	Reservoir plate with 16 rows (SB2261)
384 plate type A-E	384-well compound plate with dimensions that can be tailored by your Application Scientist

384 Plate greiner	384-well compound plate with plate height 14.4 mm and well depth 11.5 mm
384 plate greiner bio-one 781 201	384-well F-bottom compound plate with plate height 14.4 mm and well depth 11.5 mm
384 plate greiner bio-one 781 280	384-well V-bottom compound plate with plate height 14.4 mm and well depth 11.5 mm
384 plate greiner bio-one 784 201	384-well Deep Well Small Volume™ plate
384 plate Labcyte	384-well compound plate with plate height 14.4 mm and well depth 11.5 mm
Auto refill reservoir	Automation reservoirs for the Qube standalone version

5.2.3.2 Dilution of stock compound plates

Some worktable block templates enable Qube to dilute stock compound plates as an integrated part of the assay. This feature is intended for screening scenarios where Qube can be loaded with compound plates containing stock compounds in for instance DMSO. The compound plates are diluted prior to being screened as a part of the worktable setup for each plate run which minimizes the time that the compounds are dissolved in saline.

If the configuration allows for stock compound dilution, the worktable protocol will have a panel named 'Dilution' similar to the one shown in Figure 38. The interface will automatically adapt its appearance depending on whether dilution is chosen. The dilution feature adds a fixed amount of 25 µL diluent to the stock compound plate. The source for the diluents is chosen in the 'Diluent' dropdown menu. Per default the pipette tips will be washed in the washing station after each compound plate dilution step. It is highly recommended to leave the wash step on in order to avoid cross contamination.

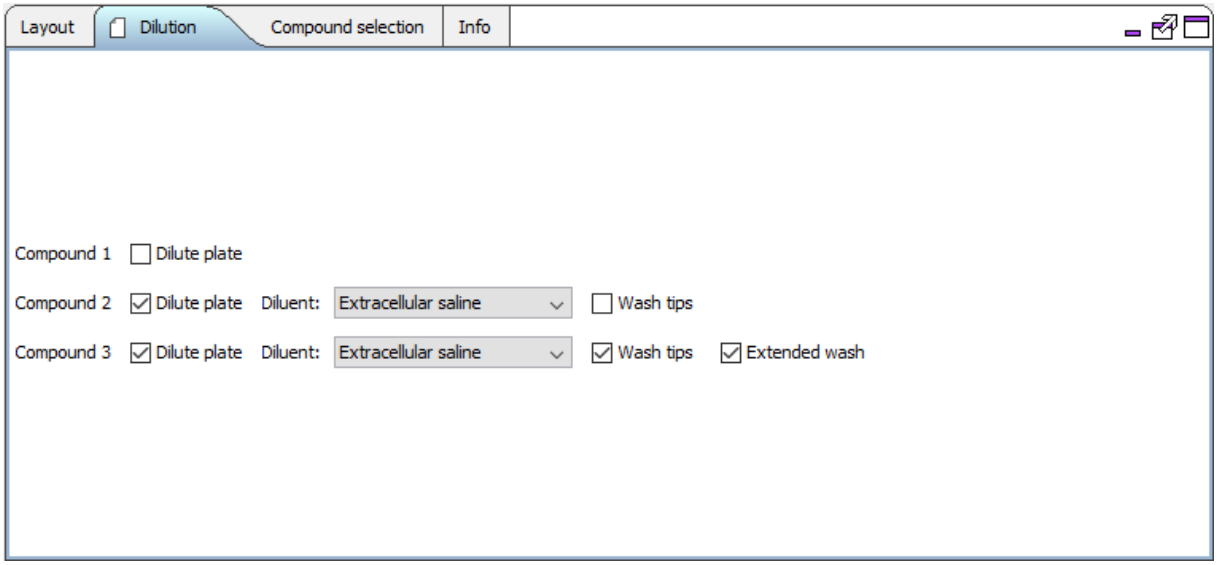


Figure 38 'Dilution' panel for diluting stock compound plates. This example shows a block template with the ability to dilute three different compound plates

5.2.3.3 Selective use of compound positions

Per default all compound positions shown in the 'Layout' panel will be available for the experiment. For some worktable block templates, it is, however, possible to use only a subset of the available positions. The positions to be used are selected in the 'Compound selection' panel. For Qubes without a stacker, the robot will only read barcodes and allow stock compound solution at the selected positions. For Qubes equipped with a stacker, the stacker and robot will only place plates, read barcodes, and allow stock compound dilution at the actively selected positions.

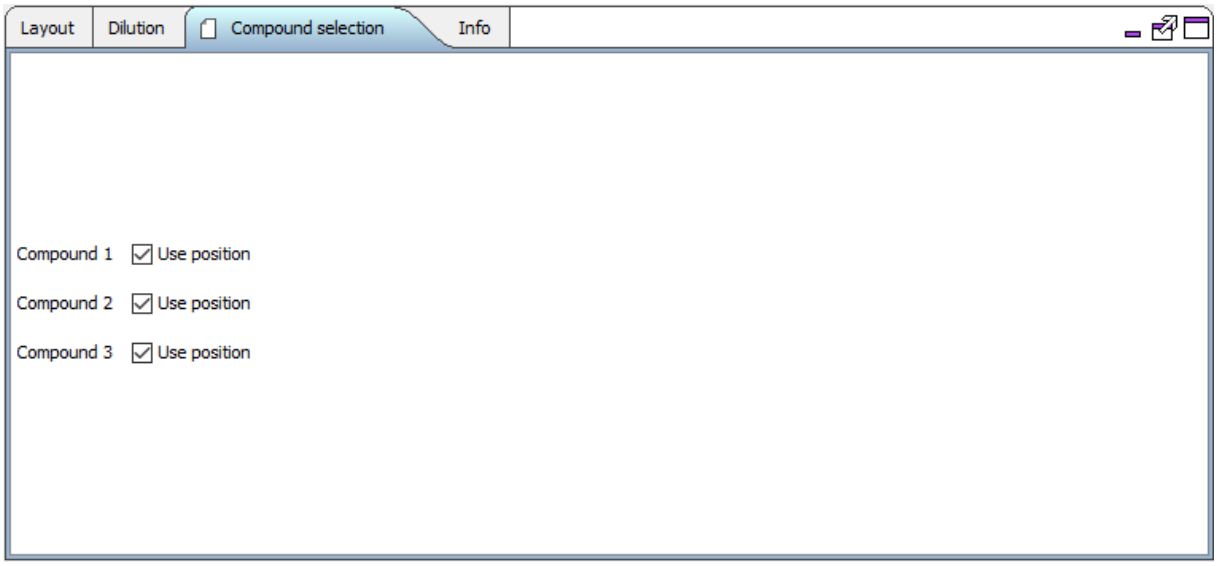


Figure 39 'Compound selection' panel for using a subset of the available positions

Caution! If positions are not selected, Qube will terminate execution during the experiment protocol if any of the non-selected positions are called for. This will result in loss of cells, QChip, and compounds. Ensure that the worktable and experiment protocols are compatible before starting a plate run.

5.2.3.4 Automated exchange of pipette tips

For Qubes equipped with a stacker automation solution, it is possible to set up Qube to exchange pipette tips automatically with a fixed frequency. Hereby, a fresh set of pipette tips can be used for every 'N' QChips that have been screened. Qube has a capacity of up to 6 sets of compound tips. The exchange frequency 'N' can be varied between 1 and 20 and exchange of cell pipette tips can be set up to follow the exchange of compound pipette tips or be exchanged at a reduced frequency. Exchange of pipette tips is set up at the 'Tip exchange' panel in the worktable blocks (see Figure 40).

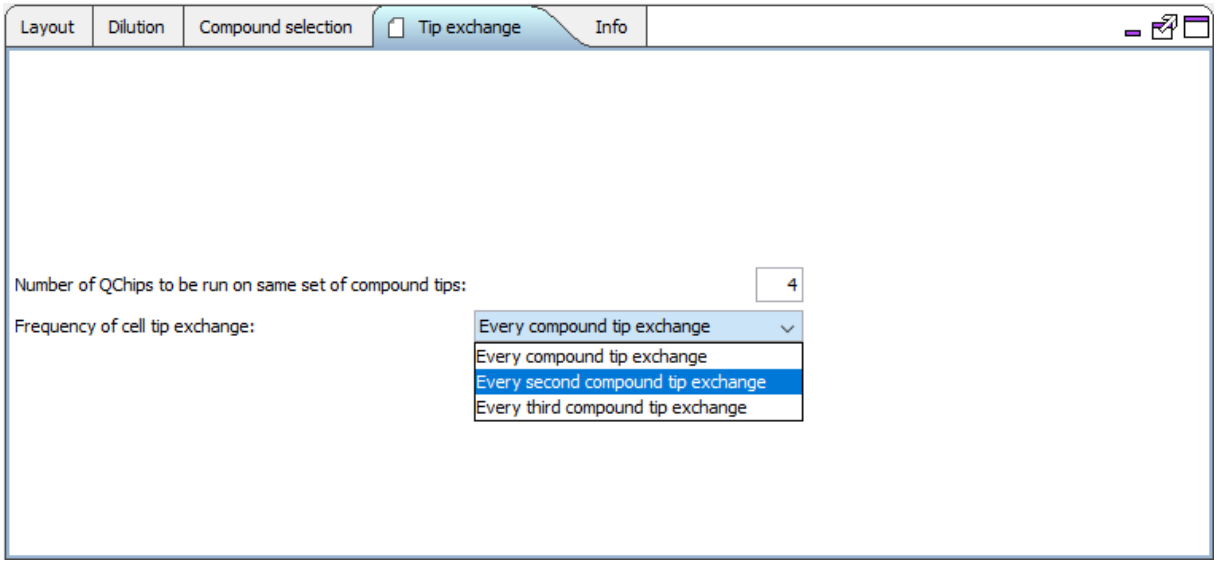


Figure 40 'Tip exchange' panel for automated exchange of pipette tips

5.2.4 Cell preparation protocols

The 'Cell preparation' protocol specifies how Qube prepares cells for an experiment.

Create a 'Cell preparation' protocol by following the steps in Section 5.2.2. This protocol is simpler than the worktable protocol and only has have five parameters, see Table 8.

Table 8 Description of 'Cell preparation' protocol parameters

'Cell preparation' protocol parameters	
Centrifuge G-force	Determines how much force will be applied to the cell suspension during centrifugation
No. of cell washes	Determines number of times, the cells will be spun down and resuspended. For each number of cell-washes the following will be done: 1. Cells are spun down 2. Supernatant is removed 3. Cells are resuspended in extracellular liquid (EC)
Centrifuge time	Determines the time the cells will be spun down. The time includes the acceleration time needed to the reach the desired g-force
Resuspend in Cell Transfer Plate	By default, the cells are resuspended by the 384-robot before being delivered to the QChip 384. Check this option to add an additional resuspension step to be done by the automated cell preparation module prior to the 384-robot resuspension step.
Wait for worktable execution	By default, the cell preparation starts simultaneously with the worktable protocol. Check this option to delay the start of the cell preparation

5.2.5 Whole-cell protocols

The 'Whole-cell' protocol adds intracellular and extracellular saline to the QChip 384. Afterwards, cells are added, positioned and the membrane ruptured using a pressure protocol.

Create a 'Whole-cell' protocol by following the steps in Section 5.2.2. The blocks used for the whole-cell protocol are very comprehensive because there are many steps involved in preparing a QChip for an experiment. The user interface, however, is simple with a few key parameters.

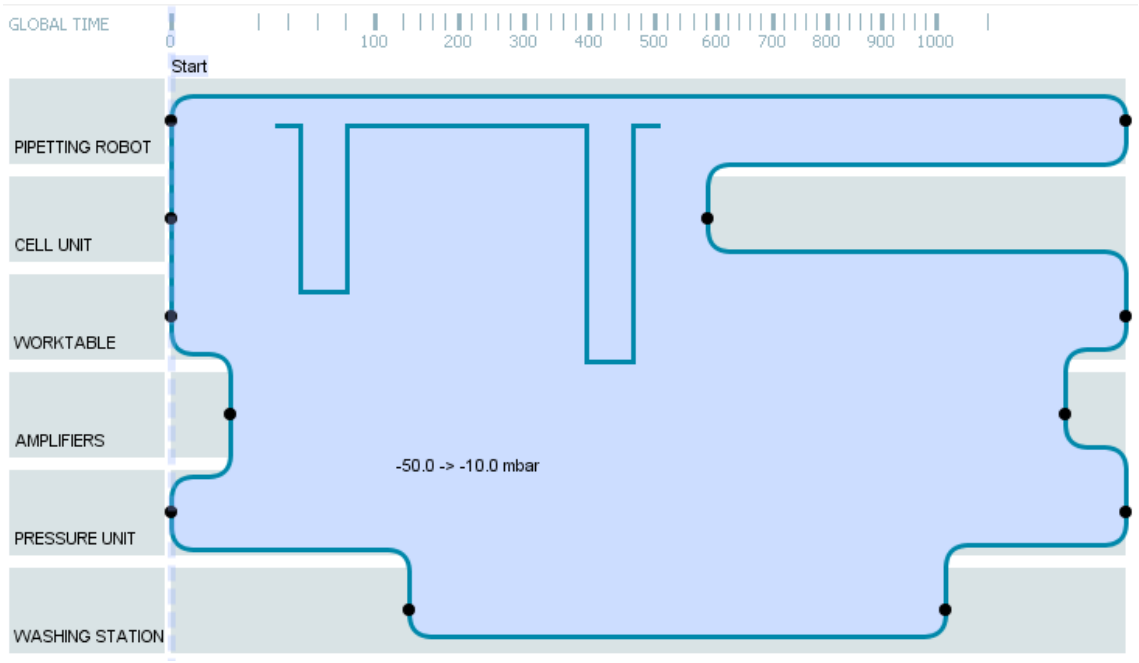


Figure 41 'Whole-cell' block showing all the active devices. Also shown is a graphical representation of the whole-cell pressure waveform. The pressures used during and after positioning are also shown in the block interface

In Figure 41, the 'Whole-cell' block shows a view of the tracks that indicate the different devices (left) with their activity at different times.

Across the block, a chart of the whole-cell pressure waveform for rupturing the cell's membrane and two of the pressure settings are shown. In the 'Parameters' window, the

tab 'Whole-cell pressure' defines this pressure waveform. To change this, read how a waveform is edited in the 'Experiment' protocol (section 5.2.6.7).

Table 9 Description of the 'Whole-cell' protocol parameters

'Whole-cell' protocol parameters	
Holding potential	
During seal formation	Potential until the whole-cell pressure waveform is applied
During whole-cell suction	Potential while the whole-cell pressure waveform is applied
After whole-cell	Potential after the whole-cell pressure waveform has been applied. This is the holding potential during the experiment unless changed in the experiment protocol
Pressure	
During positioning	Pressure applied to position cells
After positioning	Pressure applied after cells are in position and throughout the experiment
Seal formation period	
Before whole-cell suction	The time between setting the steady pressure for the last cells and measurement of the on-cell resistance and capacitance followed by the onset of whole-cell suction
After whole-cell suction	The time between the end of the whole-cell suction and measurement of the whole-cell resistance and capacitance

5.2.6 Experiment protocols

This protocol determines how the actual experiment is executed after the whole-cell configuration has been achieved. The experiment protocol will typically consist of multiple blocks, each with several, adjustable parameters.

5.2.6.1 Adding blocks

Create a new experiment protocol and add a block following the steps in Section 5.2.2. There are several block templates to choose from, including customer specific block templates. All block templates have descriptive names. All block templates can be combined in the same experiment and used multiple times.

Depending on the specific block, a number of different tabs are available for adjusting various settings regarding current or voltage clamp and liquid handling. A typical selection of tabs is shown in Figure 42. Details about individual tabs and settings are given in the coming sections.

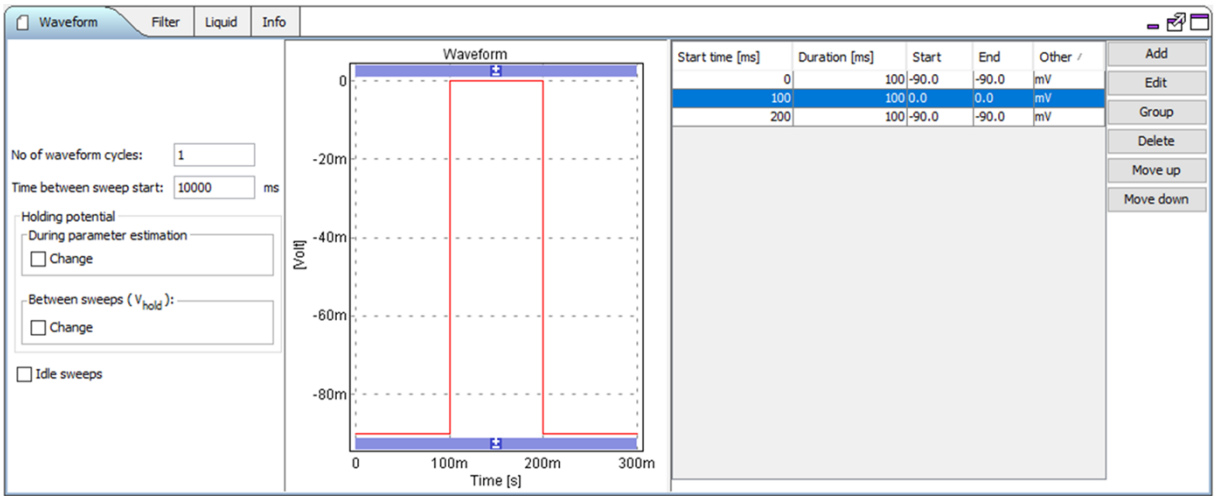


Figure 42 The typically available tabs for an experiment block. In this example, the 'Waveform' tab is active

You can add multiple blocks after sequentially other using the 'Plus' icon. This will insert blocks that are not linked. It is also possible to insert blocks with linked parameters.

5.2.6.2 Linking and unlinking parameters

It is possible to copy and link certain parameters between the blocks in the experiment protocol. This means that changes to the waveform and the filter settings in one block will be updated simultaneously in all the linked blocks. The remaining parameters are not linked and can be adjusted independently between the blocks. An overview of which parameters that can be linked between blocks is given in Table 10:

Table 10 Overview of which parameters that can and cannot be linked between blocks

Tab	Parameter	Linkable
Waveform	'No of waveform cycles'	No
Waveform	'Time between sweep start'	No
Waveform	'Holding potential' 'During parameter estimation'	Yes
Waveform	'Holding potential' 'Between sweeps (V_{hold})'	Yes
Waveform	'Idle sweeps'	Yes
Waveform	All waveform segments	Yes
Filter	All parameters	Yes
Liquid	All parameters	No

During the analysis, Sophion Analyzer also recognizes linked protocols and automatically assigns the same cursors to all sweeps across the protocols if they have been linked. If they have not been linked, it is still possible to handle it in Analyzer, just slightly more work is needed – see the manual for Sophion Analyzer for details.

Blocks can be linked in two different ways; either by inserting a linked copy of a block, or by pasting parameters from one block into an already existing block. The two methods differ slightly, especially regarding the handling of parameters that are not linked.

To insert a linked copy of a block, do the following:

1. Right-click a block to copy
2. Select 'Copy'

3. Right-click again while pointing on a position outside the other blocks where you want the block to be inserted
4. Select 'Paste'
5. Two linked blocks will now be present (Figure 43)
    - All parameters in the pasted block will initially have the same values as in the block they were copied from
    - The parameters that are not linked can subsequently be updated independently in the blocks
6. Repeat paste to insert as many linked blocks as needed

To link already existing blocks, do the following:

1. Right-click the block whose parameters you want to copy
2. Select 'Copy'
3. Right-click an existing block that you want to be linked
4. Select 'Paste parameters'
    - Only the linkable parameters from Table 10 will be updated and linked
    - Parameters that are not linked will have the same values as they had before the linking

Linked blocks can be recognized in the following way: When one block is selected, it turns blue, and the other blocks will also become highlighted in a lighter blue.

A block can be unlinked by right-clicking it and selecting 'Unlink Parameters'.

Note! Only the linkable parameters listed in Table 10 are copied and linked if parameters are pasted into already existing blocks. This means settings 'No of waveform cycles', 'Time between sweep start', and liquid handling parameters are neither copied nor updated in the receiver blocks.

Tip! If identical or almost identical blocks are to be used in a protocol, it is often easier to copy and paste an entire block instead of linking already existing blocks. This initially results in blocks where all parameters have the same values. Thereafter, the required minor changes can be made to the pasted blocks.

Tip! To ease navigation when copying and pasting blocks it is often an advantage to use the 'Same size' view.

5.2.6.3 Rearrange block sequence

If a block is to be inserted in the middle of a sequence, it is important to select the 'Same size' radio button (see Figure 36 on page 56). Insert a block either at the desired position or at the end and drag and drop to the desired place afterwards.

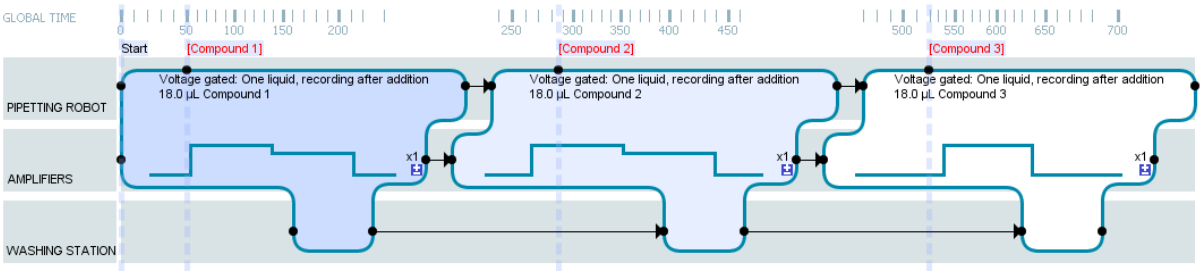


Figure 43 Three experiment blocks, where the middle block has been selected for editing (editing panels are not shown). The two first blocks are linked as indicated by their two blue surface colors. The last block is not linked to the other blocks as indicated by its white surface

5.2.6.4 Editing period and experiment borders

There are four dashed vertical lines in Figure 43, one called 'Start' and three below the red compound names. These lines are 'period borders' and they are necessary to divide the experiment into periods in the Analyzer software.

The default name for period borders in an experiment block follows the position for the liquid source. The name can, however, also be edited manually by right clicking on the name and typing free text. Thereafter, that period border name does not follow the choice of liquid source.

The name used for the period border will appear in the data tree in the Analyzer, and it is therefore very useful for keeping an overview and for tracking additions.

The period borders can also be transformed into experiment borders. Right click on the border name and choose 'Change to experiment border'. Experiment borders are indicated by a solid line instead of the dashed lines used for normal period borders. Experiment borders will make the Analyzer software treat measurements on either side of them as separate experiments.

Experiment borders can be changed back to normal period borders by right clicking on the border name and choosing 'Change to normal period border'.

Caution! Period borders are very important for data handling in the Analyzer software. Data recorded without proper period borders may be impossible to analyze. If you by accident delete a period border, you must ensure to immediately add the border to the 'period.border' contact point again.

5.2.6.5 Experiment block Liquid tab

Table 11 Description of the Liquid tab parameters in the Experiment blocks

Liquid tab parameters	
Source (N)	The worktable position(s) that contains the compound or saline to be dispensed to the QChip
Time between additions	The time between liquid additions to the QChip for blocks with multiple liquid sources. It is defined as the time span between the start of the liquid addition of the first source liquid to the start of the liquid addition of the next source liquid
Resuspend sample(s)	Resuspend the sample(s) at the source position(s) before aspirating and dispensing them to the QChip
Extended wash	Option for adding a second pipette tip wash cycle after the first, standard tip wash. The extended wash will wash the pipette tips in the 'Extended wash' reservoir position followed by another wash in the washing station

5.2.6.6 Experiment block Filter tab

The 'Filter' tab contains settings that are related to the current or voltage clamp waveform. Besides the filter and sampling settings, the tab can also contain settings for capacitance, leak current, and series resistance (R_s) compensation.

Table 12 Description of the Filter tab parameters in the Experiment blocks

Filter tab parameters	
Parameter estimation	Specifies the extent of parameter estimation and associated auxiliary sweeps (see Terms and abbreviations on page 136) before the voltage or current clamp protocol execution. The time allocated for parameter estimation is by default 5 s. It is possible to force the time span down to 4.3 s if needed, however with a small risk of occasionally delaying a sweep. Parameter estimation encompasses determination and compensation of R_{total}, C_{total}, R_s*, $R_{membrane}$*, C_{fast}*§, and C_{slow}*§¤. The following options are available: • 'None': No parameter estimation or compensation is done • 'Before first sweep': Typically used for repeated waveforms which change with each repetition, e.g. for *IV* or *It* protocols (see Figure 48), or for pulse trains (see Figure 50). By using this feature, you ensure usage of the same parameter set for a full protocol, e.g. an *IV* protocol. Furthermore, this feature can be utilized when 'Time between sweep start' needs to be shorter than 4.3 s. This means parameter estimation and compensation are only done at the start of a liquid period • 'Before every sweep': By using this option, a set of parameters is estimated before each sweep. The minimum allocated estimation time is 4.3 s, which increases the lower limit of the 'Time between sweep start' for repeated waveforms. If less than 4.3 s is needed between each sweep, the option 'Before first sweep' should be used. If there is only one repetition of a sweep, parameter estimation and compensation will be done before this single sweep
Leak subtraction (checkbox)	Compensates for leak currents. Cannot be performed without 'Parameter estimation' enabled. After current has been recorded with leak estimation, traces can be visualized in the Sophion Analyzer and ViewPoint software both with and without the leak subtraction applied
Rs compensation (checkbox)	Optional feature (SB3320). Compensates for series resistance, R_s. Cannot be performed without 'Parameter estimation' enabled. Performs series resistance compensation on single hole QChip 384 plates if checked. The compensation is automatically disabled on multi-hole QChip 384 plates
Filter	Specifies filter type – 'No', 'Bessel' or 'Butterworth'. Both filter types are 8th order filters. The Butterworth filter should only be used when the voltage protocol is a pulse train type (see Figure 50)
Cut-off frequency	Specifies cut-off frequency for the filter
Sample rate	Specifies sampling rate from 1 kHz to 50 kHz
Rs compensation (percentage)	Optional feature (SB3320). The fraction of compensation applied. Reducing the percentage can reduce the risk of oscillations. However, if they do occur, automatic clip detection steps in to avoid cell loss
Rs time constant	Optional feature (SB3320). Affects the speed of R_s compensation. Increasing the time constant can reduce the risk of oscillations. However, if they do occur, automatic clip detection steps in to avoid cell loss. Ensure the speed of the R_s compensation matches the speed of the ion channel measured

*Only applies for single-hole QChip 384. §In ViewPoint the parameter C_{fast} is denoted Cchip and the parameter C_{slow} is denoted Ccell. ¤C_{slow} is determined but not compensated.

Note! Leak subtraction and R_s compensation are automatically disabled in experiment blocks with a waveform containing one or more current clamp segments.

Note! R_s compensation is automatically disabled for multi-hole QChip 384 plates.

Note! Setting the 'Parameter estimation' to 'None' will disable all compensation and can increase the risk of clipping. This setting is only recommended if it is of utmost importance to disable all auxiliary sweeps before the voltage clamp protocol is executed.

5.2.6.7 Experiment block Waveform tab

In the 'Waveform' tab, the voltage clamp or current clamp protocol is defined. It is only possible to define a current clamp protocol if the current clamp feature has been enabled (SB3350). The term waveform will be used as a general term independently of the clamp type. The 'Waveform' tab has the overall parameters, shown in Table 13, and several additional settings in the table and editor described in the following.

Table 13 Description of the parameters relating to repetition of waveforms

Waveform tab parameters		
No of waveform cycles		Number of times the whole waveform is repeated
Time between sweep start		Time between the start of one sweep and the start of the next sweep in the waveform
Holding potential	During parameter estimation	If left unchecked, the V_{hold} specified in the 'Whole-cell' protocol or the 'Holding potential During parameter estimation' in a preceding experiment block is used. If checked, the holding potential during parameter estimation is set to the valued entered here. This value is maintained for the rest of the run or until a new value is set in a subsequent experiment block.
	Between sweeps (V_{hold})	If left unchecked, the V_{hold} specified in the 'Whole-cell' protocol or the 'Holding potential Between sweeps (V_{hold})' in a preceding experiment block is used. If checked, V_{hold} can either be set to a specific value or the latest $V_{xx.}$ The V_{xx} value must be within ['Min', 'Max'], otherwise the 'Fallback' value is used. This value is maintained for the rest of the run or until a new value is set in a subsequent experiment block.
Idle sweeps		If checked, the defined waveform will be repeated beyond the number defined in the 'No of waveform cycles'. This can be used to continue recording while the 384-robot is working, i.e. until the instrument is ready for recording in the following block. Idle sweep waveforms will be repeated with the exact same settings for filtering, parameter estimation, timing etc. as defined for the main waveform in the block. Idle sweeps are not available in ligand gated blocks. Idle sweeps are identified by a yellow frame in the ViewPoint 'Results' track and by an asterisk in the Analyzer software

Note! The holding potentials during parameter estimation and between sweeps are completely independent of each other. Setting the value of one of the two holding potentials will not affect the value of the other in either the present or subsequent blocks. Be aware of this when changing holding potential in an experiment protocol.

Note! It is important that the 'Holding potential During parameter estimation' has a value where the ion channels are closed. If not, the ion channels may partly be open during execution of the auxiliary sweeps. This will in turn result in erroneous parameter estimation.

Tip! The auxiliary sweeps for parameter estimation are executed with negative voltage steps from the holding potential during parameter estimation. The step size is either -10 mV or -30 mV depending on the parameter to be estimated. Setting the 'Holding potential During parameter estimation' is thereby used to directly define the starting voltage level for the auxiliary sweeps without affecting V_{hold} between other sweeps.

Note! Idle sweeps are performed while the 384-robot is working. This has a risk of pipetting noise in the idle sweeps.

Tip! Idle sweeps can also be used for stepped protocols. Depending on timing, idle sweep execution may be stopped before all steps have been performed. In that case, the sweeps from the partially executed stepped protocol will not be available for analysis in Analyzer. The sweeps can still be seen in ViewPoint.

A waveform is created from segments of straight lines. To add a voltage protocol or current clamp protocol to an experiment block:

1. Make sure to enter the edit mode by clicking on the 'Pencil' icon
2. Select the block
3. Open the 'Waveform' tab
4. Click 'Add'
5. An editor opens to create a new segment for the waveform
6. A segment has:
    - A clamp type (voltage or current)
    - A duration
    - A start voltage or current value
    - An end voltage or current value
    - There may be changes to some components in terms of time, voltage or current
7. Enter appropriate values for each parameter (e.g. Figure 44)
8. When clicking 'Ok', the segment will be drawn in the waveform window

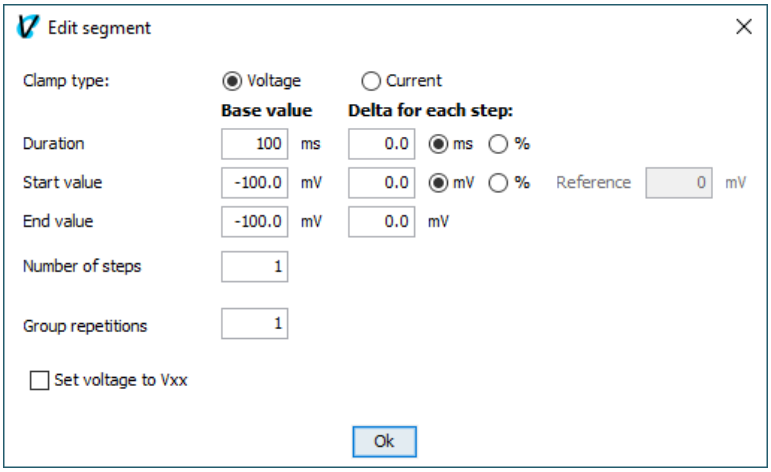


Figure 44 Add segment in voltage or current protocol waveform

9. Repeat to add more segments
10. A basic waveform is created (Figure 45 and Figure 46), which can be either voltage clamped, current clamped, or mixed

Tip! The waveform is shown at the interface of the currently selected block as well as linked blocks in the tracks panel. Use this to get a quick overview of the waveforms set up for the entire experiment.

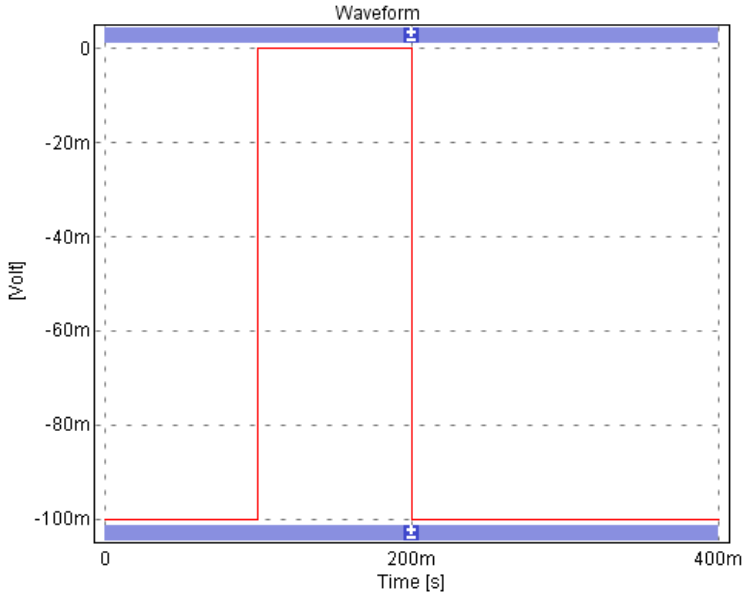


Figure 45 Basic waveform with a voltage clamp protocol

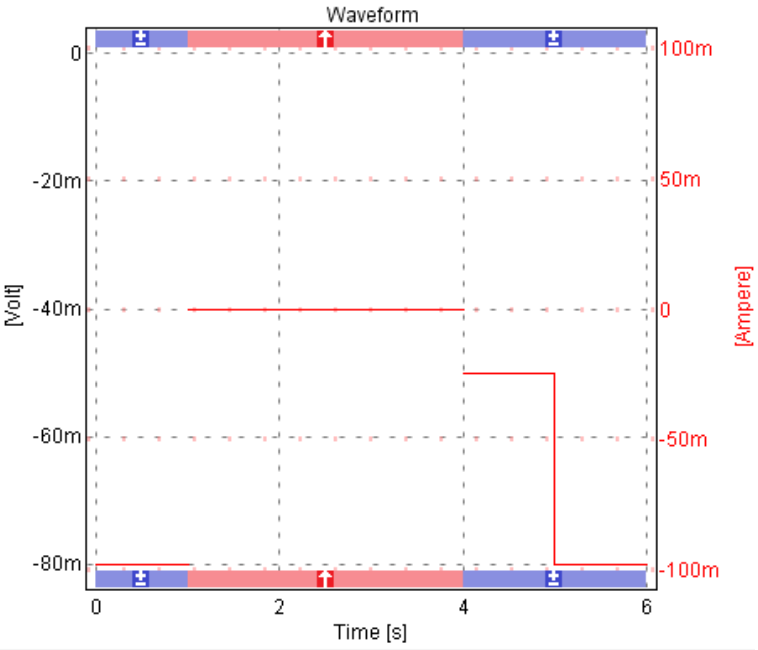


Figure 46 Basic waveform with both voltage clamp and current clamp segments

Tip! The color of the bars at the top and bottom of the waveform plot indicates voltage clamp (blue) or current clamp (red) for each segment.

To create a waveform that changes with each sweep, e.g. for *IV* or *It* protocols:

1. Add a segment, as shown in Figure 47
   - Start value and end value refer to the first sweep
   - Each following sweep will add the value in the 'Delta for each step' column
   - The 'Number of steps' is number of repetitions
2. This will result in a waveform, as illustrated in Figure 48

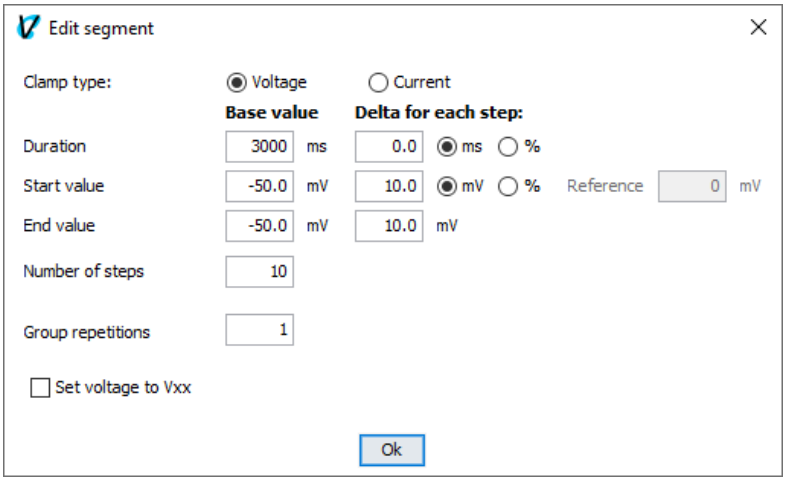


Figure 47 Segment changing with each sweep

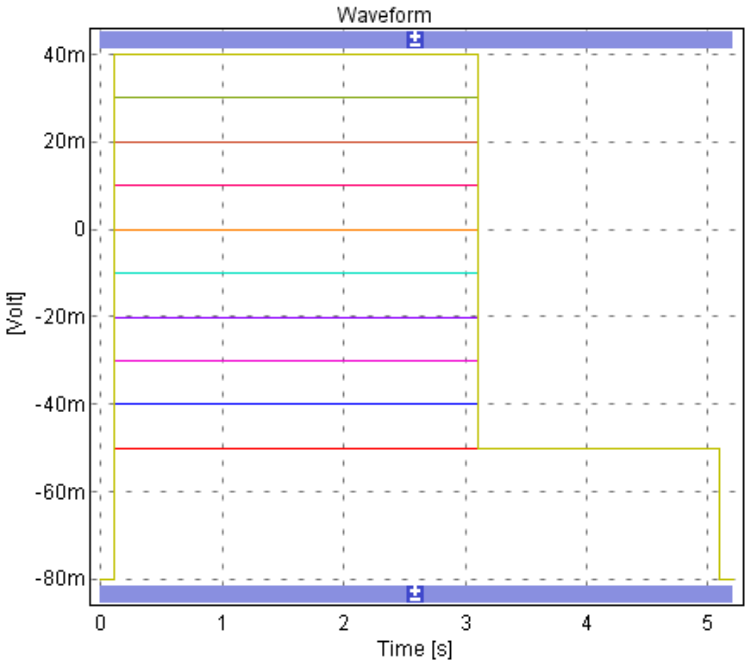


Figure 48 Waveform changing with each sweep

Note! Leak subtraction in combination with *It* protocols require time increments that are absolute values or, if using relative values, integers (e.g. 100%, 200%, etc.). It also requires identical voltage start and end values for the segment. If you need to make *It* protocols where stepped segments differ from this, you must manually disable leak compensation.

To make a pulse train (Figure 50):

- Specify the segments in a pulse
- Select them
- Group them
- Enter number of group repetitions (Figure 49)

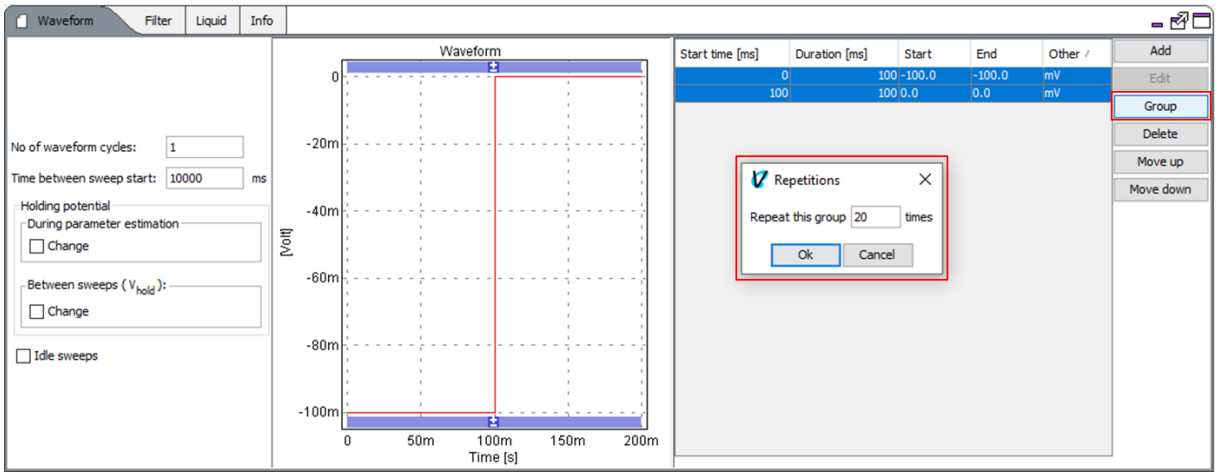


Figure 49 Making a pulse train with 'Group repetition'

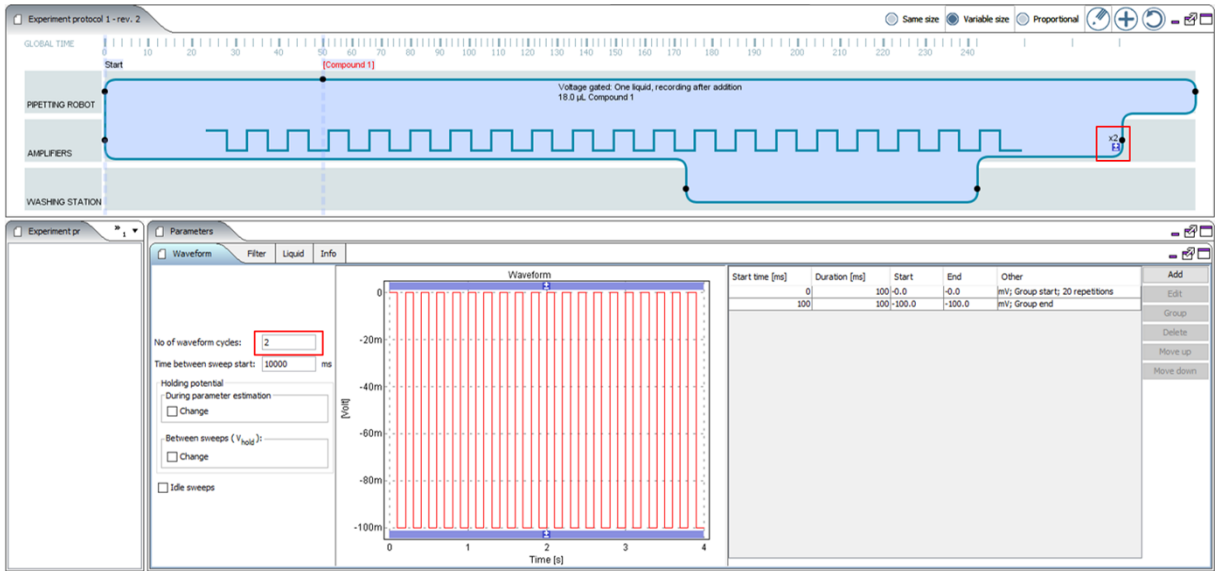


Figure 50 Pulse train depicted in Waveform editor as well as on the experiment block - note that this entire pulse train is repeated as given by the highlighted 'x2' in the block

To create a waveform segment with a value of V_{xx} (Figure 51):

Ensure that the V_{xx} values for individual sites have already been determined in the experiment protocol, see section 5.2.6.8 for more details. After this, it is possible to set one or more segments to clamp at V_{xx}. Follow these steps to clamp at V_{xx}:

1. Create a voltage clamp segment with a default clamping value
2. Enable 'Set voltage to Vxx'
3. Set allowable 'Min' and 'Max' values for V_{xx}

When executing the voltage or current clamp protocol, the chosen segment will be set to voltage clamp at V_{xx} if the previous, automatically determined V_{xx} value is within the chosen ['Min','Max'] range. If the V_{xx} value is not within this range, the segment will instead be clamped at the chosen default 'Start value' and 'End value'.

In the table next to the waveform, all segments that are defined to clamp at V_{xx} are shown with a 'Vxx' annotation in the 'Other' column. In the waveform window, V_{xx} segments are shown with markers at each end of the line and the allowed ['Min','Max'] range is indicated along with the default value (see Figure 52).

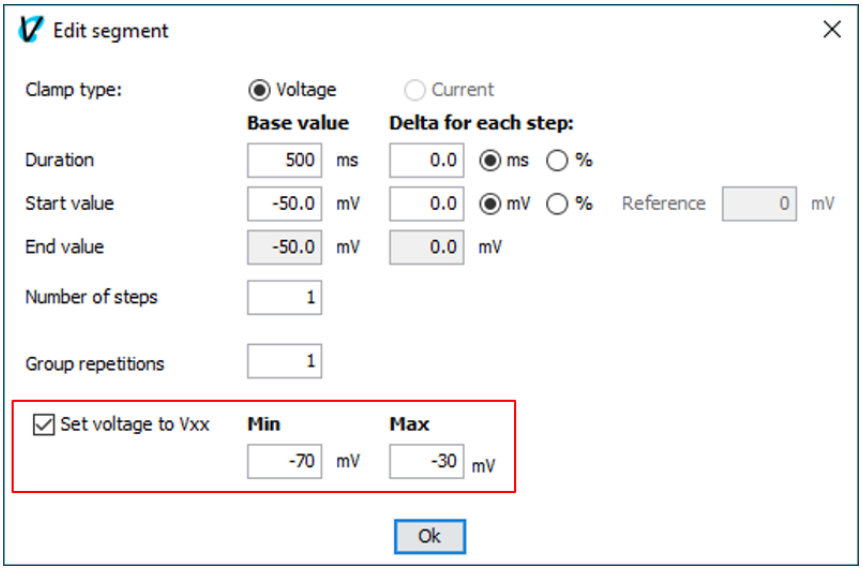


Figure 51 Segment with clamp voltage set to V_{xx} based on measurements made earlier in the experiment protocol. If the measured V_{xx} value falls outside the chosen 'Min' and 'Max' limits, in this example, the segment will instead clamp to a default value of -50 mV

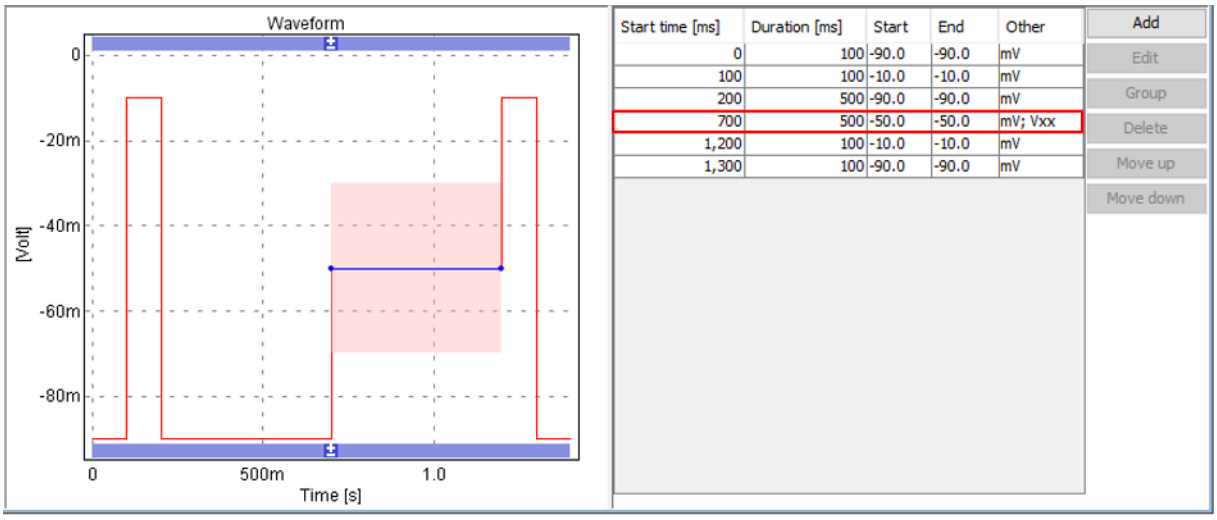


Figure 52 The table next to the waveform shows which segments are defined to clamp at V_{xx} as indicated in the 'Other' column. The waveform shows the segment with the default value and the allowed ['Min','Max'] range

Note! V_{xx} segments must have an identical 'Start value' and 'End value'. The user interface supports this by automatically disabling the 'End value' input fields.

Note! If V_{xx} has not been measured prior to execution of the voltage or current clamp protocol, all V_{xx} segments will be clamped at the chosen default value instead.

Note! If multiple segments are set to V_{xx}, they will all share the same ['Min', 'Max'] range. The range is determined by the latest edited range.

5.2.6.8 Experiment block V_{xx} tab

Some experiment blocks can be used to measure the individual V_{xx} values for each of the 384 sites and thereafter apply these individual results to subsequent experiment blocks. The V_{xx} values are determined by executing a specific, stepped voltage clamp protocol, which automatically generates and analyzes an *IV* curve as soon as the protocol has been executed. Waveforms are sampled at 50 kHz and can be filtered as defined in the 'Filter' tab.

Voltage or current clamp protocols and holding potentials in subsequent experiment blocks can then use the V_{xx} values by enabling the 'Set voltage to Vxx' for one or more segments, see 5.2.6.7 and enable the 'Between sweeps (V_{hold})', see 5.2.6.10. The V_{xx} measurements are set up in the 'Vxx' tab (see Figure 53). Follow these steps to set up the V_{xx} measurement voltage protocol:

1. 'IV curve:'
    a. 'Boltzmann fit with negative slope'
    b. 'Boltzmann fit with positive slope'
2. 'Start voltage:' The voltage for the first step
3. 'Step voltage:' The voltage between each step
4. 'Step number:' The total number of steps
5. 'Step duration:' The duration of the step segment
6. Only available for 'Boltzmann fit with positive slope':
    a. 'Interpulse voltage:' Voltage of segment between first step and test pulse
    b. 'Interpulse duration:' Duration of segment between first step and test pulse
    c. 'Test pulse voltage:' Voltage of the test pulse where the cursor is placed
    d. 'Test pulse duration:' Duration of the test pulse where the cursor is placed
7. 'Time between sweep start:' Time between start of each waveform execution

Set up the 'Online Cursor' as appropriate for the specific ion channel. The cursor is shown graphically in the 'Waveform' panel and updates automatically if the 'Online Cursor' settings are changed.

The 'Holding potential' settings define the holding potential before, between, and after the voltage protocol execution.

Note! The V_{xx} measurement blocks do not support individual holding potentials for parameter estimation or between sweeps. Be aware that both these holding potentials will be affected in subsequent experiment blocks when the 'Holding potential' is enabled in a preceding V_{xx} measurement block.

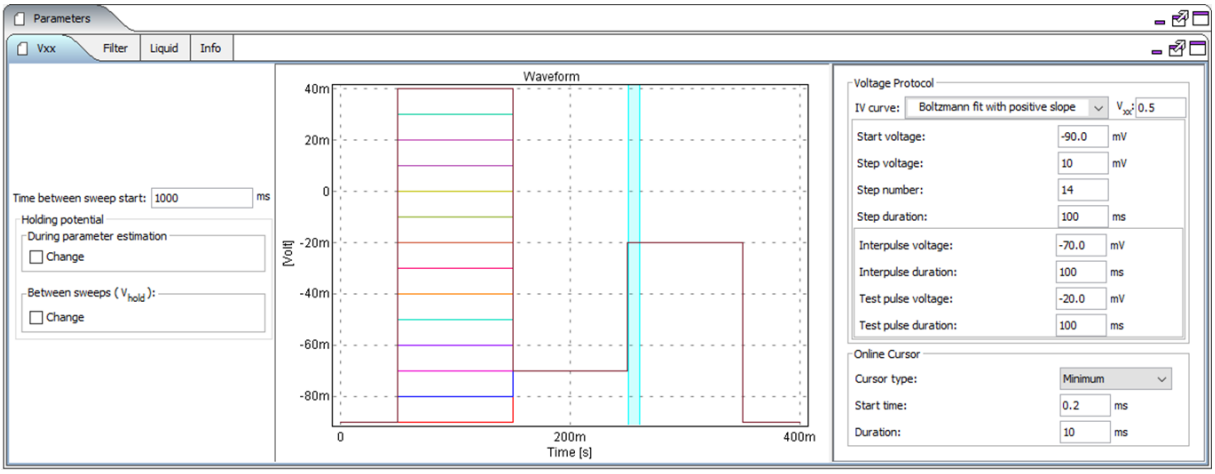


Figure 53 User interface for setting up automatic V_{xx} measurements. The 'Waveform' window will automatically update as the 'Voltage Protocol' and 'Online Cursor' are edited. The cursor is visualized as the light blue section in the 'Waveform' window

Tip! It is recommended to set up the filter settings with the same 'Cut-off frequency' and filter type as will be used in the voltage or current clamp protocols in the subsequent blocks. This minimizes the risk of variations in calculated cursor values due to filter differences.

For some applications, the 'Interpulse' segment may not be wanted. Simply remove that segment by setting the 'Interpulse duration' to 0 ms.

There are several mutual restrictions to ensure, for instance, that the cursor duration cannot exceed the length of the test segment, and that time between sweep start is longer than the total waveform duration and vice versa. For example, to make a very short test pulse duration, you should start by setting an even shorter online cursor duration to be allowed to set the short test pulse.

5.2.6.9 How to use the automatic V_{xx} feature

After the V_{xx} block has been set up, the subsequent blocks in the experiment protocol can make use of the V_{xx} setting for voltage clamp segments as described in section 5.2.6.7. Figure 54 shows an example of an experiment protocol where a block for measuring V_{xx} is followed by a block which can utilize the V_{xx} values. The block for measuring V_{xx} can be recognized by the S Curve symbol.

All experiment blocks added after the V_{xx} block can make use of the V_{xx} values determined in the V_{xx} block. If updated values of V_{xx} are desired, simply add a new V_{xx} block where needed. The subsequent experiment blocks will always use the latest determined V_{xx} values for each site.

Tip! If subsequent V_{xx} blocks are inserted as copies of the first V_{xx} block, the parameters for the V_{xx} protocol will automatically be linked. It is not possible to link V_{xx} blocks that have been added individually from the 'Add block' menu.

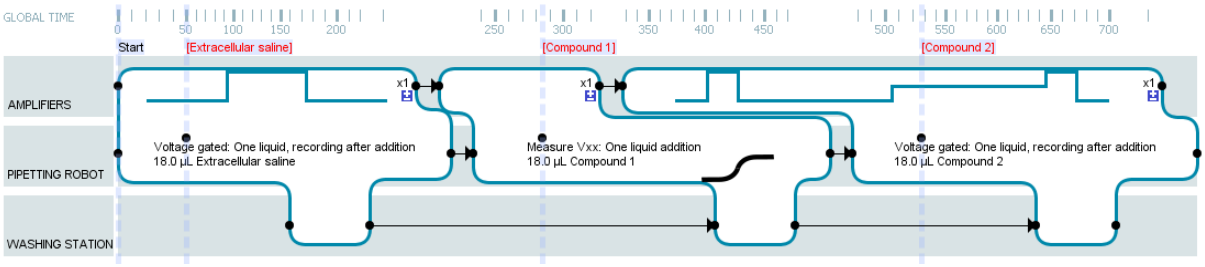


Figure 54 Example of an experiment protocol where the second block is for measures V_{xx}, which is then utilized for V_{xx} segments in the third block

5.2.6.10 Use V_{xx} as holding potential (V_{hold}) between sweeps

After a V_{xx} block is set up, subsequent blocks in the experiment protocol can make use of the V_{xx} setting as a holding potential between the sweeps of a voltage protocol. Figure 55 shows an example of a block that sets the 'Between sweeps (V_{hold})' holding potential to the latest V_{xx} value if applicable, i.e. V_{xx} is measured in a previous block and is also within the desired range controlled by ['Min', 'Max']. Otherwise, the 'Fallback' value will be used.

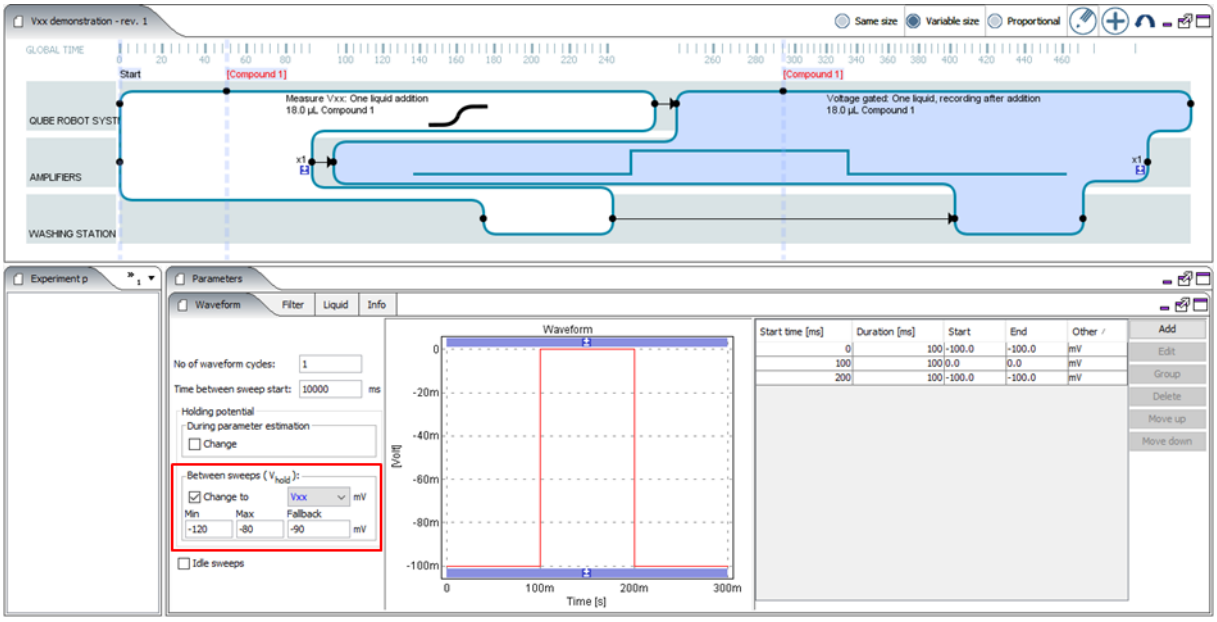


Figure 55 An example of a block where the holding potential between sweeps is set to V_{xx}

Tip! The 'Holding potential Between sweeps (V_{hold})' can also be set to a specific, fixed value via its drop-down menu.

Figure 56 illustrates how the holding potential can be fine-tuned along an experiment.

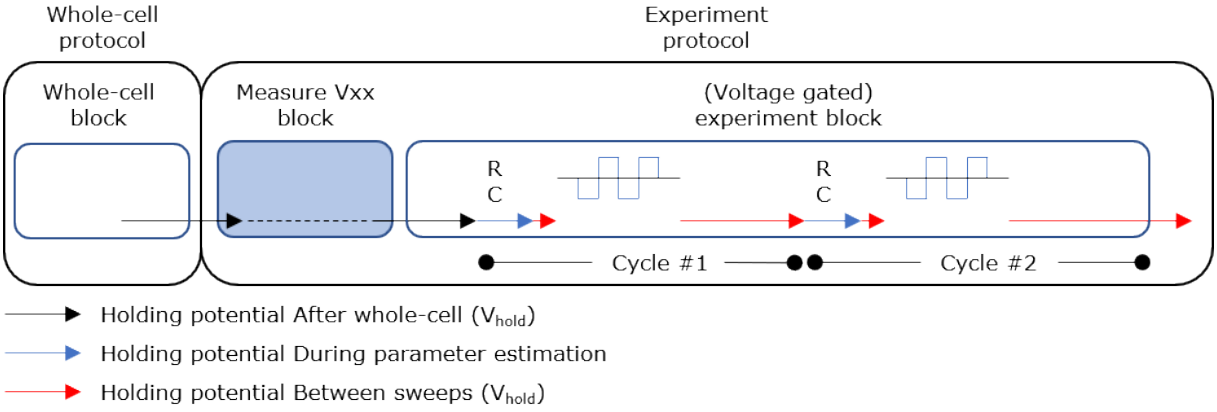


Figure 56 An example of possible holding potential changes during a protocol run. The 'Holding potential Between sweeps (V_{hold})' may be V_{xx} or a specific, fixed value. If holding potentials are not actively changed during the experiment protocol, they will instead use the value from the whole-cell protocol throughout the experiment protocol run

5.2.6.11 Cell rescue feature in Current Clamp mode

Amplifiers in the Qube have a unique feature that monitor the voltage when doing Current Clamp experiments. The feature disables Current Clamp circuitry on the affected site(s) during an experiment if the voltage exceeds approximately +/-250 mV. The amplifier returns to Voltage Clamp mode with the holding potential set by V_{hold}. This feature ensures that the cell survives in case voltage increases above this level. ViewPoint reports this as a warning with the following text 'Current Clamp was disabled. Position: xx'.

5.2.6.12 Cell rescue feature when using R_s compensation

The amplifiers in Qube also have a feature that monitors the voltage when doing R_s compensation. It is well known that when compensating for access resistance in Voltage Clamp, there is a risk that the clamp voltage will start to oscillate. This can kill the cell and destroy the experiment. If the R_s compensation circuitry detects an oscillation, R_s compensation is disabled on the affected site(s) and the voltage protocol is executed without it. ViewPoint reports this as an Error with the following text 'Rs compensation was disabled. Positions: xx…'. R_s compensation will automatically be re-enabled before the next voltage protocol is executed.

5.2.7 Cleanup protocols

The 'Cleanup' protocol cleans the CTP and removes the used QChip 384 from the BCI either in preparation for the next QChip 384, or as clean-up of the last run.

For Qubes equipped with a stacker, the cleanup protocol can discard MTPs and QChips automatically and rearrange pipette tips depending on the chosen block.

Create a new 'Cleanup' protocol and add the appropriate block. Cleanup blocks adapt the cleanup process automatically and therefore do not require any parameter changes.

5.2.8 Summary panel

In the 'Summary' panel (Figure 57), all the relevant protocols are selected. Usually all five protocols are selected, but they are independent and hence can be executed in different combinations and also used in an assay development mode.

In the 'Summary' panel, the 'Repetitions' field will specify the number of QChip 384 plates to run. If the number of repetitions exceeds the number of QChip 384 plates available on the worktable, Qube will stop after the last available QChip 384 and output an error. If an optional stacker has been mounted on your Qube, execution will continue until there are no more QChip 384 plates available from that source.

It is possible to export all protocols by clicking on the 'Wrench' icon button and choose 'Export all protocols…'. This can be valuable if you need to share them with your Application Scientist or use them on another Qube in your company.

The 'Wrench' icon button can also be used to add additional processing to protocol runs after they have completed:

- 'Reduce data': Will reduce data as described in section 4.7.4.2 on page 44.
- 'Automatic project': Will create a new project with the protocol run or add the protocol run to an existing project.

  If you combine this with automatic export of reports or result tables (see the Sophion Analyzer manual), then the project will automatically be calculated as soon as the protocol run has completed, and you will not have to wait for calculations to finish when you open the project.

  You can also use the automatic export of reports to send you an email when the protocol run has completed. This way you will know immediately that the Qube is ready to start the next protocol run.

When clicking on the 'Load protocols' button, plans are loaded into Qube and the screen changes to the 'Run' tab (see page 83).

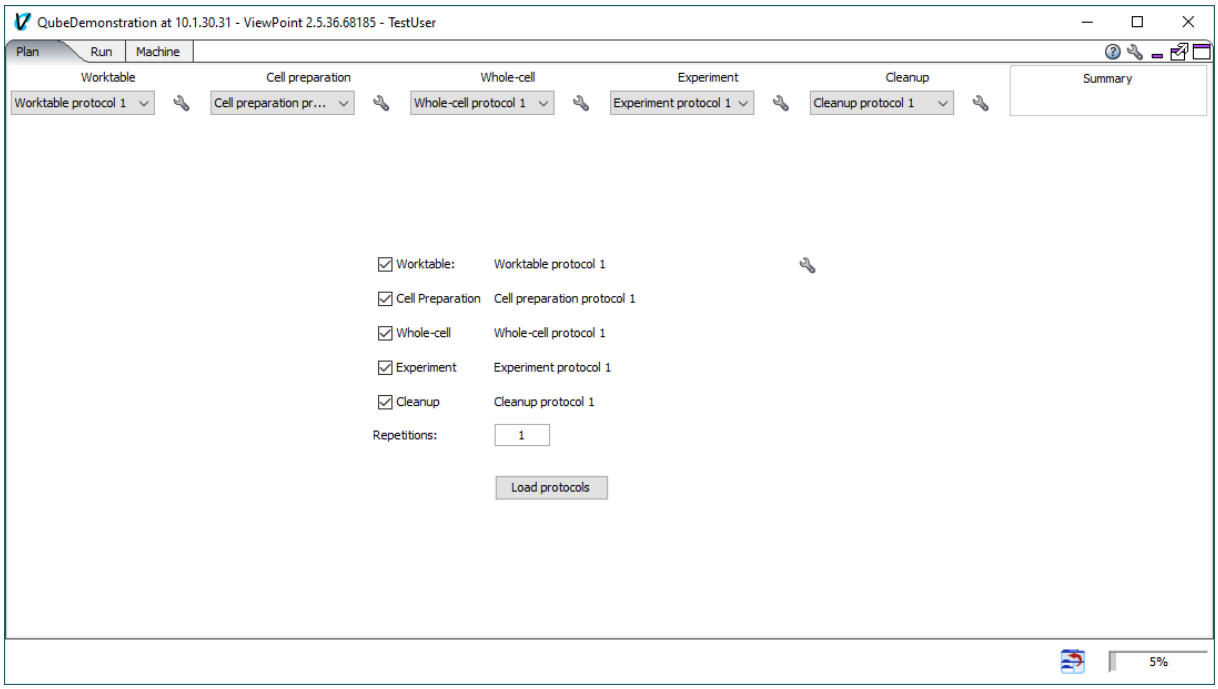


Figure 57 Summary panel, the last step in preparing the plan

Tip! In the 'Summary' panel, if 'Repetitions' are set to 1 and 'Cleanup' protocol deselected, the QChip 384 will stay in the BCI with the cells in a 'Whole-cell' configuration. Utilize this to run multiple experiment protocols on the same cells in a QChip in a truly exploratory assay development scenario. Experiment protocols can be changed on the fly and reloaded to run them on the QChip.

To do this, all subsequent 'Experiment' protocol runs on the same QChip 384 should only be run with 'Experiment' checked in the 'Summary' panel (see Figure 57).

Cleanup and finalize exploratory assay development by loading and running the 'Cleanup' protocol by itself.

Such exploratory assay development should not be combined with automatic projects.

5.3 Run tab

The plans, which have just been created and loaded, can be started by clicking the start button in the green 'Instrument' panel.

The 'Run' tab in ViewPoint shows the status of running experiments or the status of previous experiments. The Run tab gives access to all data, including sweeps, resistance measurements at various points, cell parameters, and so forth.

Figure 58 is a schematic of the basic navigation tools in the 'Run' tab. It is also in the 'Run' panel that the compound plates are assigned to an experiment to be used in the analysis.

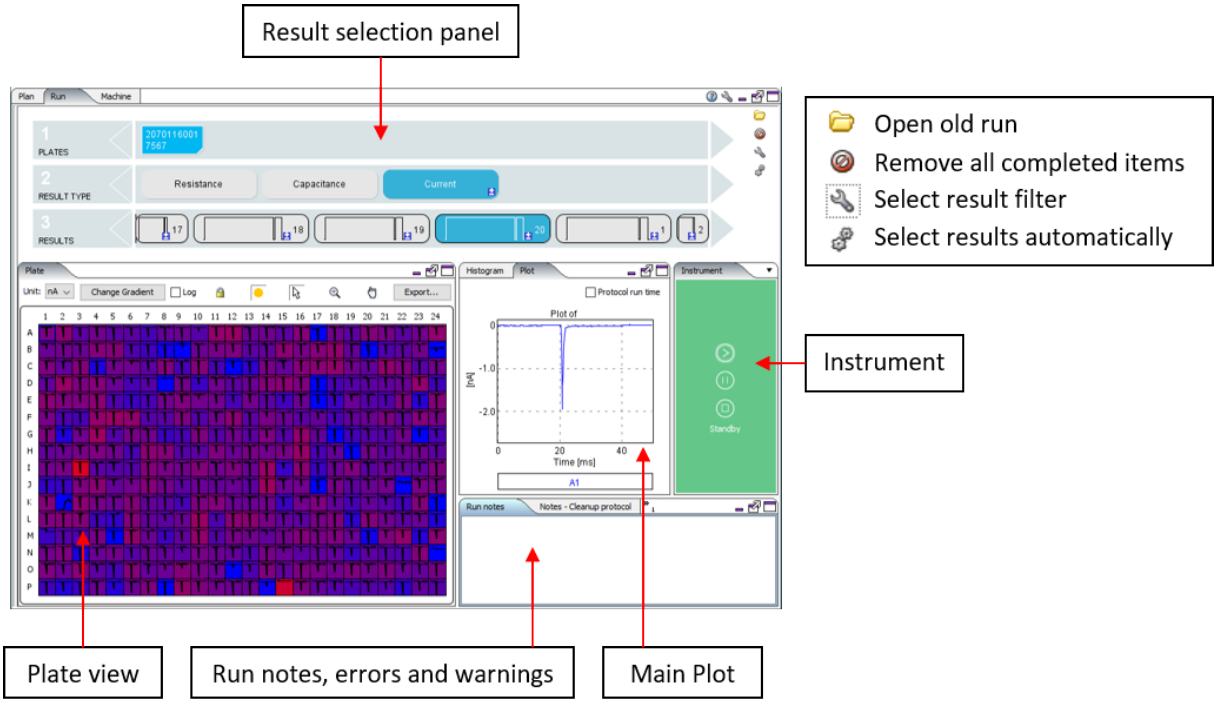


Figure 58 Overview of the 'Run' tab with main controls highlighted

For detailed experiment analysis, use the Analyzer software.

The 'Run' view is separated into five sub-panels, namely:

- The 'Result selection' panel (top): Track based view with 2-4 tracks depending on the setting of the filters
- The 'Plate view' (left): Plate view with 384-well representation of the plate (QChip 384 or Compound plate) selected in the 'PLATES' track. Displayed values are selected in the 'RESULTS' track
- The 'Main Plot' (center right): Main plot with data from the site(s) selected on the 'Plate view' and histogram showing distribution of all data
- The 'Instrument' (right): Qube control buttons (with start, pause, and stop) together with the instrument status
- The 'Run notes' and 'Errors and warnings' (bottom right): Text field for adding various notes relevant to the experiment run and list of warnings and errors encountered during the run

Tip! Hovering the mouse over each plate in the 'Plates' track displays additional plate details.

5.3.1 Result Selection

Results are shown in real time and can be revisited at any time. The 'Result selection' panel changes depending on filter settings and what is selected. Selecting a plate from the 'Plates'

track changes the available choice of results for the next track, and so forth. Both the type of results and the number of tracks changes depending on the filter setting.

Tip! It is possible to view results from previously executed plate runs as well as the currently running plate run. Qube prioritizes execution and data recording over result display so there is no risk of compromising an ongoing assay. Multiple plate runs can be opened at the same time in ViewPoint even though Qube is actively running.

The 'Result selection' panel is controlled with the highlighted buttons in the top right corner in Figure 58.

- 'Load protocol runs' brings up a search function to find previously run protocols
- 'Remove all completed items' removes completed protocol runs from the result selection panel
- 'Select result filter' has several options to alter the way different results are presented. As Qube records and logs a lot of data during an experiment, it is often useful to filter the results. See Table 14 for an overview of the default filters
- Enable 'Select results automatically' to automatically select and display the latest results as they are generated

Table 14 Results filters and options

Filters and options	Description
Debug	Displays a moderate degree of data
Exp results	Filter for the most relevant experiment data
Plates	Used for assigning compound plates and shows data based on the plate (How to assign compounds is described in section 5.3.5)
No filter	Displays everything
Show building blocks	Option that enables results display to be filtered at block level in combination with results filters

Tip! It is possible to set up your own filters with the 'setup filters' menu. Use this to make personalized result filters. The filters will be visible for all users from any PC connected to the Qube.

5.3.1.1 Protocol run export

It is possible to export plate runs in the 'Run' panel. To export the runs, right click on the appropriate plate in the 'Plates' track and choose the 'Export…' option. An export dialog identical to the one described in section 4.7.6 on page 48 will appear. The export function will automatically export all protocol runs coupled to the selected plate.

Note! There is **no anonymization** of data or other information in exported protocol runs.

Note! Plate runs, i.e. protocol runs belonging to the same QChip 384, will always be exported as bundled runs with all protocols in a single file even if not all of them are selected for export. It is not possible to export individual protocol runs if they belong to a plate run.

Note! Very large runs will be split into zip files of around 4 GB each. The first file has the extension .zip, the second file has the extension .z01, etc. Make sure that all the files are available in the same folder for subsequent import.

5.3.1.2 Protocol re-run

It is possible to access and re-run previously executed plate runs. To do so, follow these instructions

1. Find and open the old plate run via the 'Open old run' button
2. Right-click the plate with the QChip number in the 'Plates' track
3. Choose 'Run again' (see Figure 59)
    a. 'Run again' is only available when Qube is not in a running state – check the green panel under 'Instrument'
4. If one or more of the protocols have been edited since the chosen plate run was executed, a dialog will appear giving the option of using either the original or newest version (see Figure 60)
5. Choose 'Original' version if you want to re-run the protocols with the same settings as used in the chosen plate run. Choose 'Newest' if you want to re-run with the newest settings
6. The protocols will now appear in the 'Plan' tab and can be loaded via the 'Summary' panel

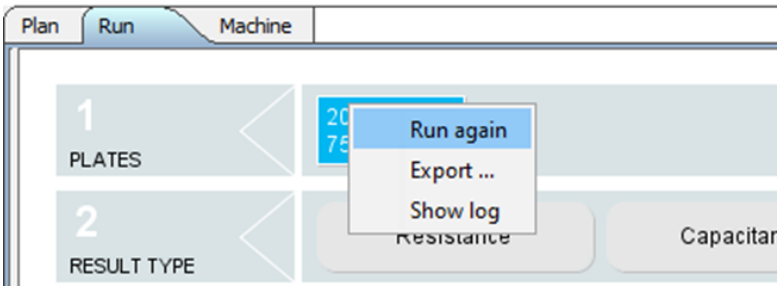


Figure 59 Right-click a plate run in the 'Run' tab to access the 'Run again' option for viewing and optionally re-running old protocols

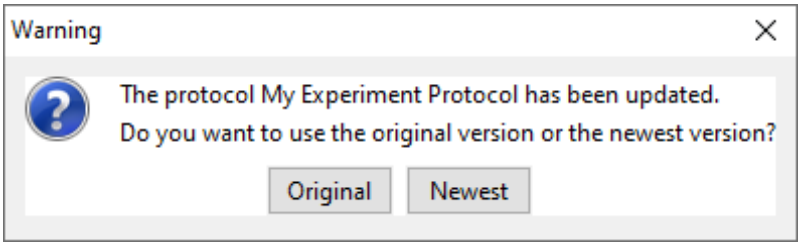


Figure 60 If 'Run again' is used for an old version of a protocol, it is possible to choose between re-running the old, original version or the newest version

Note! If an old version of a protocol has been chosen via the re-run function, the protocol will appear in read-only mode in the Plan tab. It is not possible to edit the protocol or save it as a new version or new protocol.

Old versions of a protocol can only be saved as new, separate protocols via the 'Run' panel, see tip below.

Tip! An old version of a protocol can be saved as a new protocol via the 'Run' panel. To do so, choose the 'No filter' setting in the result filter, right-click the requested protocol in the 'Protocols' track, and choose 'Save as new protocol…'. It is not possible to save the old protocol as a new version of the same protocol, it is only possible to save it as a new, unique protocol.

Tip! The re-run function can also be used to access to old versions of a protocol without executing an actual re-run.

5.3.2 Plate view

The 'Plate view' displays the results of the 'Result selection' panel. In Figure 58, for example:

1. A plate is selected (blue highlight) in the 'PLATES' track
2. 'Current' is selected in the 'RESULT TYPE' track
3. The trace # 17 is selected in the 'RESULTS' track
4. The plate view then displays this specific trace in all the 384 sites

Selections can also be made in 'Plate view', and the specific result will be highlighted in the detailed view called the 'Main plot'. Depending on the type of result and the number of selections, the 'Main plot' will display the results in either the 'Plot' or 'Histogram' tab.

Tip! Using Shift-key and Ctrl-key allows several selections to be made at once, both in the tracks and in the 'Plate view'.

At the top of the 'Plate view' panel, there are several buttons related to the display of data:

Figure 61 Top of the 'Plate view' panel

- 'Unit' dropdown will change the prefix of the values displayed in the 'Plate view'
- 'Change Gradient' button enables data to be colored with different color schemes for a visual interpretation of it, which is particularly useful for histograms
- 'Log' checkbox toggles logarithmic y-values of the data where it is relevant
- 'Padlock' icon selects the data scaling
- 'Yellow circle' icon toggles to display error indicators on the 'Plate view'
- 'Arrow' icon is used for selecting a subset of the data in the 'Plate view' to be shown in the main plot. To select multiple data:
  - Drag over the data
  - Use Shift-key and left-click for continuous selections
  - Use Ctrl-key and left-click for discontinuous selections
- 'Magnifying glass' icon enables zooming in the 'Plate view':
  - Drag over the area to zoom in
  - Double click to zoom out
- 'Hand' icon enables panning if a zoom factor higher than full plate view is used
- 'Export…' button is used to export data to clipboard or a separate file. Note that results analyzed in Analyzer are typically more interesting to export than raw data from ViewPoint

Tip! The scroll wheel on the mouse can be used as an alternative to the 'Magnifying glass' icon to zoom in to fewer sites in the 'Plate view' panel.

5.3.3 Main plot

The 'Main plot' panel has two main tabs whose relevance depends on the data being examined:

- The 'Plot' tab is best when looking at currents or anything related to sweep data
- The 'Histogram' tab is useful for data with single numerical values, for instance, resistance measurements

The output in the 'Main plot' depends on the selection in the 'Plate view'. Right clicking in both the 'Histogram' and 'Plot' view brings up a menu to alter the axis and export the view.

5.3.3.1 'Plot' tab

In the 'Plot' tab, it is possible to zoom in and toggle the 'Protocol run time', which either:

- Sorts out the displayed data according to the data generation timestamp
- Or overlays it for better comparison and with a time axis that reflects the voltage or current clamp protocol for the selected type of sweep

5.3.3.1 'Histogram' tab

In the 'Histogram' tab (Figure 62), numerical data is displayed in a histogram. In contrast to the plot view, the histogram view always uses values from all 384 sites from the 'Plate view', i.e. from a whole QChip 384. Zoom on the plot by dragging the mouse or by right click on the relevant axis.

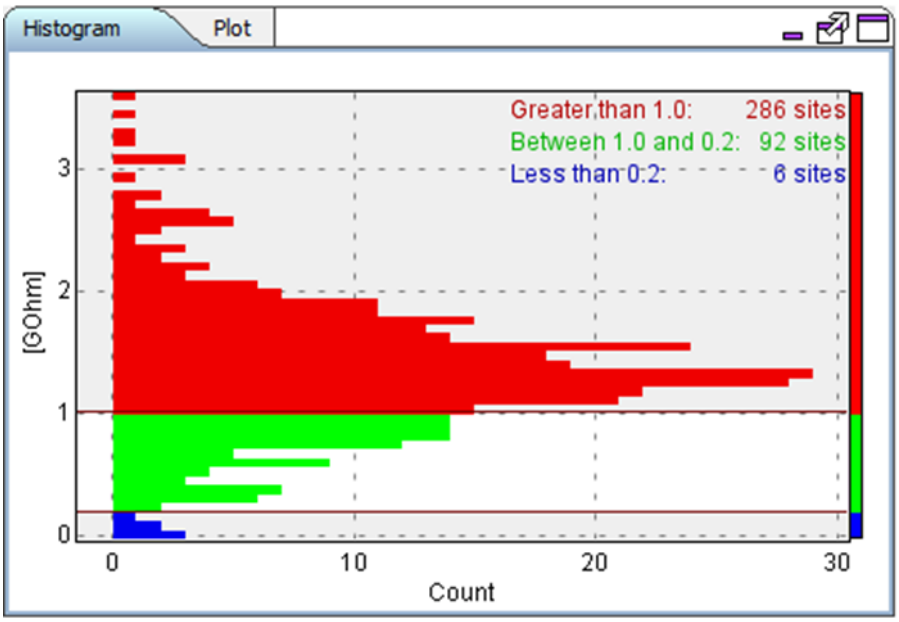


Figure 62 Histogram in main plot. The example shows total resistance during experiment execution on a single hole QChip 384

The color gradient is adjusted by dragging the top (respectively the bottom) boundary of the main plot down (up) to the desired effect. The color bins are thus created and entries in each bin are given in the upper right corner of the histogram view. The colors follow the setting made under the 'Change gradient' button in the 'Plate view'. The 'Plate view' will follow the same color coding as the histogram, which means that dragging the boundaries up and down is very useful in identifying specific sites on the QChip 384 with a certain result.

5.3.4 Add Run notes

The panel under the 'Plot' and 'Histogram' tabs contains a 'Run notes' tab if a plate run with an experiment protocol is selected. This field can be used to write notes about the specific plate run and is coupled to the experiment protocol run. These notes will also be available as 'Run notes' in the 'Analyzer' software.

The run notes can only be edited after run execution has finished, i.e. it is not possible to edit the field while experiments are ongoing.

The 'Run notes' tab is visible for all result filters, except for the 'Plates' filter. Since there is a 'Run notes' tab for each experiment protocol run, it is possible to have multiple 'Run notes' tabs shown simultaneously. This can occur, if multiple plate runs are selected in the 'Plates' track, or if one plate run contains multiple experiment runs, cf. Tip! in Section 5.2.8.

It is recommended to only having a single 'Run notes' tab visible at a time to ensure that notes are added to the correct run.

Tip! Use the 'No filter' setting to select an individual experiment protocol run from plate runs containing multiple experiment runs, cf. Section 5.2.8.

Note! It is not possible to lock the 'Run notes' field for editing, i.e. the run notes can always be edited or even deleted by any user. The only restriction is a lock against simultaneous editing from multiple applications.

5.3.5 Assign compounds

When the 'Result filter' (5.3.1 page 83) is set to 'Plates', the 'Main plot' panel gets a new tab labeled 'Compounds' (Figure 63).

This calls up a menu where a compound list can be assigned to a specific plate that contains any of the liquid types mentioned in section 4.7.7:

- If the barcode of the physical compound plate matches an entry in the compound list directory, ViewPoint will suggest this match. The assignment is done when the user approves it
- If there is no obvious match, a new compound list must be imported, or created by using the 'Assign range of compound' button, or an existing list assigned to the plate you want despite a name-mismatch
- Any mis-assignment can always be corrected by unassigning the list by selecting the plate and clicking on the cross in *italics* in 'Plate view'

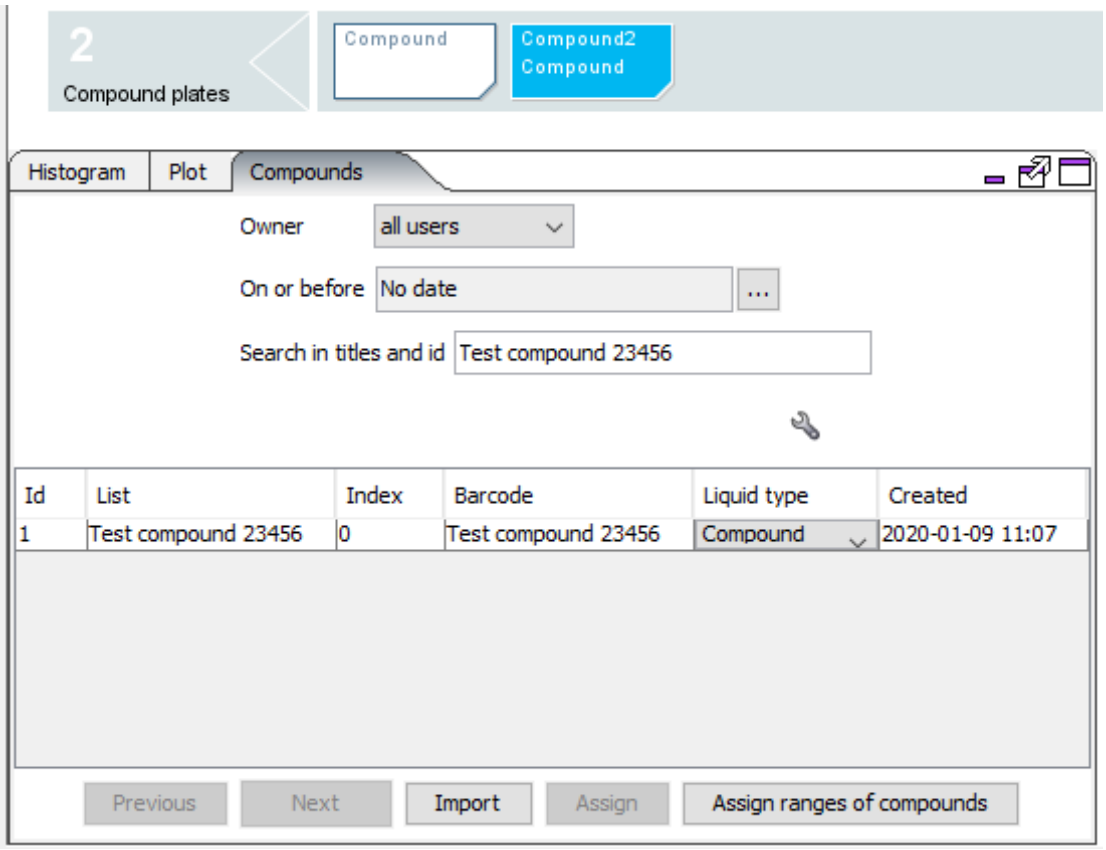


Figure 63 Adding compound lists to plates. If the plate barcode has been used before suggestions are made automatically

New compound lists can be imported to the database using either .xlsx, .xls or .csv formats. Note that:

- For the file to be recognized, the data is best stored as seen in Figure 64
- The unit for concentration is important for correct analysis of e.g. IC_{50} values
- ViewPoint will try to determine if the unit is M or µM based on units in the compound list

	A	B	C	D
1	Pos.	Compoun	Concentration [µM]	
2	A1	Test 1	1	
3	A2	Test 2	1	
4	A3	Test 3	1	
5	A4	Test 4	1	
6	A5	Test 5	1	
7	A6	Test 6	1	

Figure 64 The optimum layout for compound lists

After importation:

1. A dialog box will appear that informs of import success
2. As soon as compounds have been assigned, the compound names and concentrations will be available in Analyzer for detailed analysis
3. Compound information will also be written in 'Plate view' for the selected plate and it is possible to select different prefixes for the concentration using the drop down in the upper left corner

5.3.6 Instrument tab

The green 'Instrument' tab displays the following 3 buttons:

- Start: After protocols have been loaded from the 'Plan' tab, experiments are started with Start
- Pause: Pause Qube at the next possible time. It is possible to resume the experiment from where it was paused
- Stop: Stops Qube and cancels the rest of the planned runs. When clicking stop, a confirm request appears that must be acknowledged. Thereafter, some time may pass to complete the active action. Meanwhile, the word 'Stopping' flashes below the button

Caution! Opening the cabinet door during a pause will engage the safety circuit and terminate the run. The pause functionality can therefore not be used to interact with the instrument in any way that requires opening of the cabinet door. Make sure that Qube is not executing any protocols before opening the door to avoid unintended interruption.

Note! Stopping the run execution prematurely is likely to require use of recovery utility protocols before Qube is ready to start a new plate run, see Table 15.

5.3.7 Errors and warnings tab

The 'Errors and warnings' tab displays warnings or errors that happen during the run related to a site or system in general. Examples of warnings could be: Clipped trace data, disabled R_s compensation, and other warnings relevant for the experiment. Furthermore, errors related to interruption of execution are also displayed in this window.

5.4 Machine tab

The 'Machine' tab has two sub-tabs: 'Info' and 'Tools'. The 'Tools' tab is only available on the User PC configured to control Qube, as this tab is where Qube is directly controlled.

5.4.1 'Tools' tab

The 'Tools' tab is where Qube is initialized, checked, and prepared using various utility protocols, e.g. loading tips, preparing the washing station, etc. All utility protocols have descriptions and warnings that are available when you hover the mouse over the icon. The 'Tools' tab also contains information about the input pressure levels and state of the safety circuits. Figure 65 shows the tool panel in four different conditions to demonstrate available warnings and status changes when the cabinet door is open, or the emergency stop is engaged. Please note that warnings about safety relay status and BCI pressure levels are automatically cleared when the cabinet door is closed, and the emergency stop is disengaged.

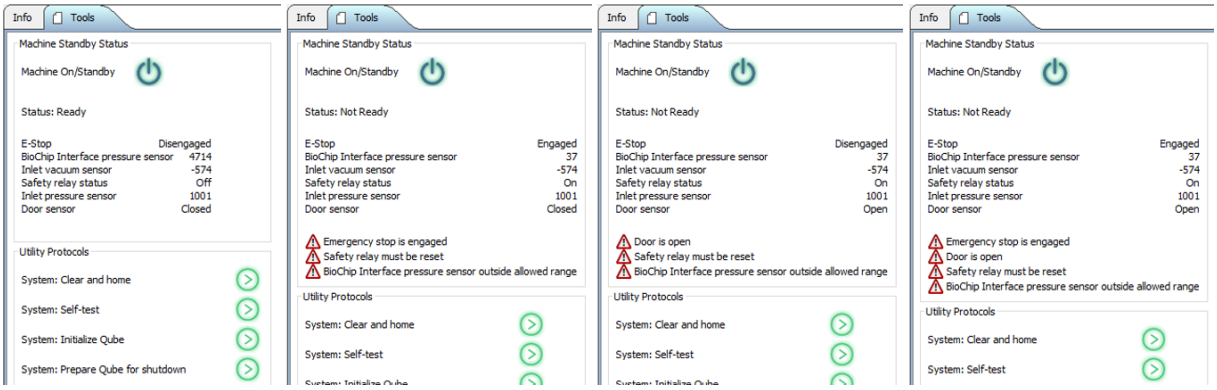


Figure 65 Snippet of the 'Tools' panel in four different conditions. From left to right: Instrument is ready to use, Instrument is not ready due to engaged emergency stop, instrument is not ready due to open cabinet door, and instrument is not ready due to engaged emergency stop and open cabinet door. Only a sample of utility protocols are shown here

5.4.1.1 Temperature control

Qubes equipped with a temperature control module have a 'Temperature control' section in the 'Tools' panel. This section is described in more detail in Section 6.4.2.

5.4.1.2 Utility protocols

'Utility Protocols' are predefined plans found in the 'Tools' tab (Figure 65) that are loaded and run with a single click. There are some for normal operations and some for recovery operations if an error has occurred. The available utility protocols depend on the specific configuration of your Qube. Table 15 gives the name and explanation for all utility protocols:

Table 15 List of 'Utility' protocols

Utility protocol name	Action	Caution/note
System: Clear and home	Resets and homes the ACP and the 384-robot, stops the centrifuge, and closes the manifold in the BCI. This utility protocol or 'System: Initialize Qube' must be run after waking Qube from standby	Must not be run if the 384-robot has stalled with pipette tips loaded – instead run 'Recovery: Eject 384 pipette tips at e6'
System: Self-test	Test moving the 384-robot, barcode reader, BCI manifold open/close, amplifier, and pressure system	Run at the beginning of the day to check that Qube works as intended

Utility protocol name	Action	Caution/note
System: Initialize Qube	Similar to 'System: Clear and home' with added functionality that primes the LMS and loads an ACP pipette tip	Before running the utility protocol, an ACP pipette tip should be placed in the ACP tip ejector and the LMS should be prepared with filled inlet containers and empty waste containers Cannot be run if the ACP pipettor already has an ACP pipette tip mounted
System: Prepare Qube for shutdown	Ejects the ACP tip and flushes and empties the LMS	LMS supply containers should be empty and connected before running the utility protocol, otherwise the LMS tubing will not be emptied Cannot be run if the ACP pipette tip has already been ejected Do not run if a tip is already sitting in the ejector
Robot: Transfer 384 new tips from e4 to a4	Transfers 384 pipette tips from a source pipette tip rack at physical position e4 to the tip loading tool at physical position a4	Ensure that the tip loading tool at a4 is properly mounted and that the tool is empty
Robot: Transfer 384 new tips from e5 to a5	Transfers 384 pipette tips from a source pipette tip rack at physical position e5 to the tip loading tool at physical position a5	Ensure that the tip loading tool at a5 is properly mounted and that the tool is empty
Robot: Transfer 384 new cell tips from e6 to a4	Transfers 384 pipette tips from a source pipette tip rack at physical position e6 to the tip loading tool at physical position a4	Ensure that the tip loading tool at a4 is properly mounted and that the tool is empty
Robot: Transfer 384 new compound tips from e6 to a5	Transfers 384 pipette tips from a source pipette tip rack at physical position e6 to the tip loading tool at physical position a5	Ensure that the tip loading tool at a5 is properly mounted and that the tool is empty
Robot: Discard and shift cell pipette tips	Used to get clean cell pipette tips. The robot loads the 4 left-most columns of tips from the tip loading tool at physical position a4 and discards them at physical position e6. Then it moves the remaining pipette tips in e4 four columns to the left.	Place an empty plastic pipette tip rack (SB2200) or an empty 300 mL reservoir (SB2252) at physical position e6 to receive the discarded pipette tips
Washing station: Flush	Runs water through the LMS and washing station	Use for weekly cleaning Is also included as part of the utility protocol 'System: Initialize Qube'
Washing station: Empty	Empties the LMS tubing and washing station	Use for weekly cleaning Is also included as part of the utility protocol 'System: Prepare Qube for shutdown' LMS supply containers should be empty and connected before running the utility protocol, otherwise the LMS tubing will not be fully emptied

Utility protocol name	Action	Caution/note
ACP: Load ACP pipette tip	Loads the ACP tip from the ACP tip ejector	Cannot be run if a pipette tip is already mounted at the ACP pipettor Is also included as part of the utility protocol 'System: Initialize Qube'
ACP: Eject ACP pipette tip	Ejects the ACP tip and leaves it in the ACP tip ejector	Do not run if a tip is already sitting in the ejector Is also included as part of the utility protocol 'System: Prepare Qube for shutdown'
ACP: Pre-wet CTP	Uses the ACP tip to aspirate saline from the EC reservoir on the ACP to wet the surface of the trough of the CTP	It is important to do this before running Qube to ensure even distribution of cells The CTP can also be manually pre-wetted
Recovery: Stop centrifuge	Stops the centrifuge	May be necessary if Qube was interrupted while the centrifuge was spinning
Recovery: Open gripper arms	Releases the QChip 384 or any other plate by homing the gripper arms	May be necessary if Qube was stopped while the gripper was holding a plate **Warning!** This utility protocol only opens the gripper arms and drops the plate from where the 384-robot is positioned when the protocol is executed. If necessary, the 384-robot should manually be moved, and the gripper manually lowered via the toothed belt to a position where the plate can be dropped safely. Refer to Section 8.4.2 for instructions on how to lower the gripper manually
Recovery: Remove QChip 384 from BCI	Opens the BCI and lets the gripper remove the QChip 384 and release it on the worktable in front of the BCI	May be necessary if Qube was stopped while the QChip 384 was inside the BCI The gripper will drop the QChip 384 from a small distance above the plane – this is intentional
Recovery: Eject 384 pipette tips at e6	Relevant robot axes are homed. Hereafter any pipette tip contents are emptied into the washing station and the tips are ejected at physical position e6	Must be used if Qube was stopped while the tips were loaded. Do not run normal 'System: Clear and home' Place an **empty** plastic pipette tip rack (SB2200) or an empty 300 mL reservoir (SB2252) at physical position e6 to receive the discarded pipette tips
Manual override: Reset pipette tip state	Qube will remember whether pipette tips are loaded or not, both on the 384-robot and the ACP. If Qube has been put in standby with the tips loaded on the 384-robot it is not possible to home or initialize the system This utility protocol resets the pipette tip state to "Not loaded" for both 384-robot and ACP	**Warning!** This utility protocol must **only** be used if all pipette tips have been removed from the 384-robot. Refer to Section 8.4.1 for instructions to manually remove 384 tips before running this utility protocol. If the ACP tip is loaded, it is of utmost importance that the correct actions are taken after running this utility protocol. Refer to Section 8.4.3 for how to handle a loaded ACP pipette tip before running this utility protocol

Utility protocol name	Action	Caution/note
Manual override: Reset BCI state	Qube has a state to inform whether gripper arms are inside or outside of the BCI, i.e. placed under the manifold. If the gripper arms are inside the BCI, you will be prevented from starting most robot moves. The state is remembered even if Qube is set to standby This utility protocol resets the state to assume that the gripper arms are outside of the BCI	**Warning!** This utility protocol must **only** be used if the gripper arms are outside of the BCI. Refer to Section 8.4.5 for instructions to manually move the robot out of the BCI if it has accidently stopped with the gripper under the manifold. **Warning!** Do **not** set Qube to standby or power off the robot if the gripper arms are inside the BCI. The manifold will close if doing so.

5.4.2 'Info' tab

The 'Info' tab displays the same information as the general 'Machine' tab in ViewPoint Maintenance. Refer to section 4.9 for a detailed description.

5.5 Customizing the user interface layout

The graphical user interface of both ViewPoint and ViewPoint Maintenance can be customized by rearranging panels. Panels can be rearranged by dragging the panel name tab and resized by dragging the borders between the different panels.

Panel layout buttons can be used to maximize, restore, disconnect and reconnect each panel. Maximizing and restoring panel size can also be done by double-clicking the white space next to the panel name tab, which is a quick way to, for instance, temporarily maximize the plate view panel.

Panels are disconnected to allow them to float outside the main window. In this case, use the panel borders to move and resize the floating panels. Panels are reconnected using the reconnect button.

Figure 66 Tools for customizing panel layouts. From left to right: Panel name tab, layout buttons in default condition, layout buttons in a maximized size condition, and layout buttons in a disconnected condition.

The interface layout is saved for each user on the specific PC when ViewPoint or ViewPoint Maintenance is shut down. This means that different Qube users can have each their personalized layouts on the same PC, and that the same user can have different layouts on different PCs.

If the layout needs to be reset, use the 'Restore panel positions' button at the bottom of the application window to restore to the default layout.

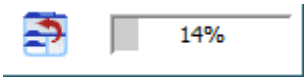


Figure 67 'Restore panel positions' button is placed next to the memory indicator at the bottom of the application window

6. Running Qube

Before starting the experiments, Qube needs to be loaded with cells, pipette tips, QChip 384 plates, and compound plates.

The position of these items depends on the worktable layout (Figure 6 on page 18 and Figure 37 on page 59). The worktable layout and standard positions can vary depending on the use of external stackers, non-standard setups, or layouts.

Generally, for an experiment to run, it requires:

- Two tip loading tools containing pipette tips, one tool containing pipette tips for cell handling and one containing pipette tips for compound handling
- Additionally, at least:
  - One QChip 384
  - One reservoir for IC and one for EC – check the position in the Viewpoint worktable protocol
    - One or more reservoirs may be autofill versions depending on the Qube model
  - One reservoir for pre-aspiration liquid
- Depending on the experiment, compound plates and additional QChip 384 plates must be placed at specific worktable positions or in the stacker as needed according to the worktable layout and Qube model

Warning! For stacker-based assays with automated tip exchange, the tip loading tool for compound tips should be empty before starting the run. Refer to the 'Info' section in the appropriate worktable block.

On the ACP module, there must be:

- An ACP pipette tip – load the tip in the tool on the ACP module
- A centrifuge tube – place it in the QFuge and ensure the holder swings out freely
- A CTP – place it according to the worktable in Viewpoint protocol
- A cell waste bottle mounted under the waste funnel – place the funnel + bottle in the square hole at the back of the ACP module
- A reservoir with EC solution – place it according to the worktable in Viewpoint protocol
- A QStirrer cup with cell suspension – remember to place a magnetic stirring bead in the suspension

Check the waste and supply level in the LMS, empty and fill as needed.

Before starting the actual experiment, Qube should be initialized. Depending on the current state, this can be done in one step by using the utility protocol 'System: Initialize Qube' or in separate steps. In all, the following requirements must be checked before starting an experiment:

- ACP tip loaded – Qube will keep track of the load status
- Cells added to the appropriate cup (small/large) with a magnetic stirrer bead and placed on the stirrer, optionally with the ACP-compatible black lid
- Washing station flushed
- CTP must be wetted either manually with a pipette or automatically by a utility protocol
- Tips transferred to the tip loading tools in physical positions a4, and respectively a5
- Adequate level of water in LMS system (approx. 0.75 L per QChip 384 plate run)

When complete, the protocols are loaded via the 'Summary', and the start button is pressed in the 'Run' tab. The plate run will now start.

Warning! The ACP must **not** have a tip loaded for cell clone testing. Refer to section 6.6 on page 106 for details.

6.1 Correct placement of consumables

Besides placing MTPs and QChips correctly oriented with site or well A1 in the rear left corner and barcodes to the left, it is also important to pay attention to correct mechanical placement of the consumables.

It is possible to mount plates and ACP pipette tips incorrectly. Doing so may result in mechanical damage to the Qube instrument. The following figures show examples of how to place consumables correctly.

6.1.1 MTPs and reservoirs in standard slot plates

Due to the hollow perimeter of most MTPs and reservoirs, it is possible to position the plates on top of the slot plate alignment pins. Please pay attention to position consumables between the alignment pins and the leaf springs in standard slot plates as shown in Figure 68.

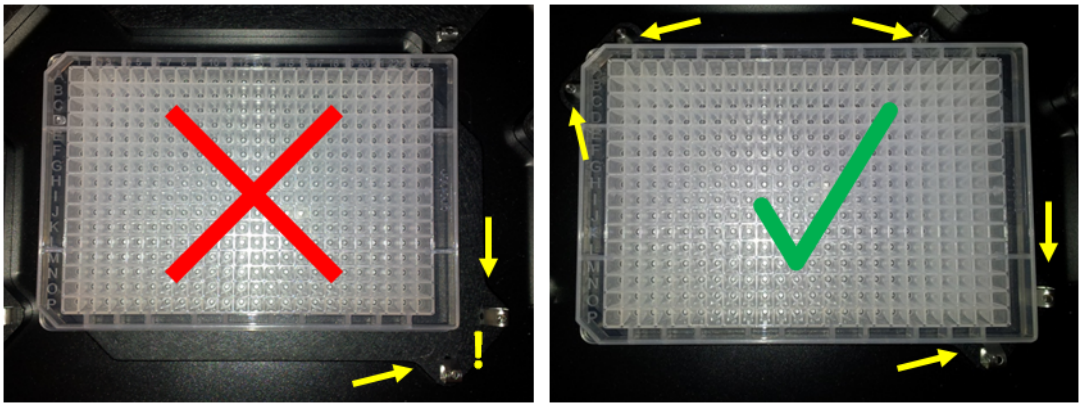


Figure 68 Placement of MTPs in standard slot plates. Left: Incorrect placement with MTP placed on top of alignment pins and with a gap to the leaf springs. Right: Correct placement of MTP between alignment pins and leaf springs

6.1.2 MTPs and pipette tip racks in active tip rack slots

The slot plates equipped with locking retainers, typically mounted at physical positions e4 to e6, are primarily designed to lock plastic pipette tip racks in place while transferring pipette tips to the tip loading tools.

However, in some cases the slot plates can also accept MTPs and reservoirs that comply with short flange height standards.

When mounting plates or pipette tip racks, it is important that the flanges are properly pushed in place and locked under the retainers. See Figure 69 and Figure 70 for examples of incompatible and compatible MTPs, respectively.

Figure 69 Example of an MTP with medium flange height in an active tip rack slot. The flange is too high to fit under the retainers and the MTP is therefore not compatible with the slot plate

Figure 70 Example of and MTP with short flange height correctly placed in an active tip rack slot. The flange can fit under the retainers as shown by the yellow arrows. This MTP is therefore compatible with the slot plate

6.1.3 ACP pipette tip in ACP tip ejector

The ACP pipette tip must be placed in the steel-based ACP tip ejector for automated pipette tip loading and ejection. When placing the pipette tip in the ejector, it is important to place the collar of the tip inside the notch and to ensure it does not rest on top of the tool. The pipette tip must be pushed all the way to the backside of the ejector cavity to be held in place by the integrated ball springs, and the collar must rest on the lower surface of the notch. See Figure 71 for correct placement.

Figure 71 Placement of the ACP pipette tip in the tip ejector. Left: Incorrect position with the pipette tip resting on top of the ejector. Centre: Correct placement with the pipette tip collar inside the notch. Right: Close-up showing the collar pushed all the way to the back and is resting on the lower notch surface. The collar must not be squeezed into the lower part of the tool

6.2 Correct placement of barcodes on compound plates

The Qube instrument is equipped with a dual-mode barcode reader. The barcode reader scans in **both** High Density (HD) and Wide Field (WF) mode when reading barcodes. The High Density mode reads barcodes with a higher resolution, and thereby finer pitched barcodes, than the Wide Field mode, but at the expense of a smaller field of view.

Figure 72 shows a schematic representation of a typical compound plate seen at the edge where barcodes are placed. The two hatched areas indicate where barcodes must be placed in order to be read in Wide Field mode (red hatching) and High Density mode (blue hatching).

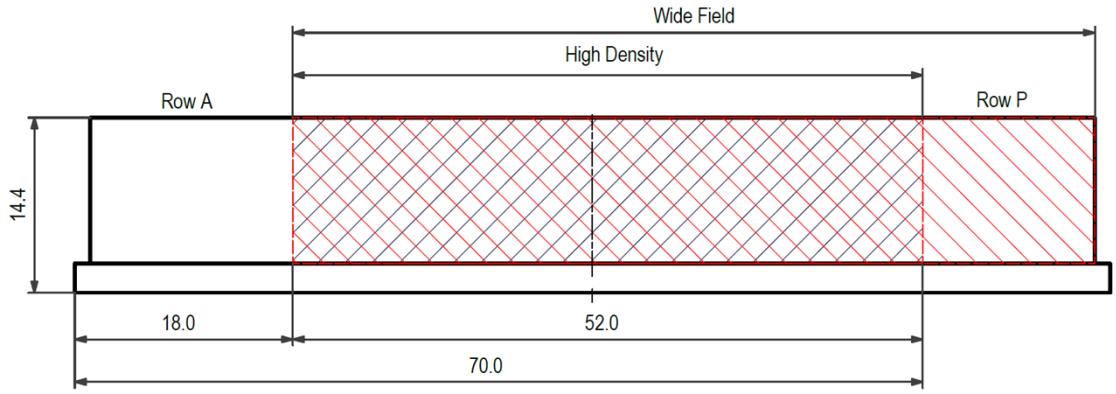


Figure 72 Compound plate seen at the short edge, i.e. towards column 1. The hatched areas indicate where barcodes must be placed within for High Density and Wide Field mode, respectively. Barcodes (CODE128 standard) with a minimum line width down to 0.25 mm have been tested compatible in Wide Field mode

Barcodes (CODE128 standard) with a minimum line width down to 0.25 mm have been tested compatible in Wide Field mode. The supported barcode formats can be found in System specifications, page 128.

6.3 Using the optional stacker and autofill module

Qube stacker (SB3310) can be fitted on a Qube (SB1110). The stacker delivers 384 format MTPs and QChip 384 plates to the 384-robot inside Qube. The stacker module includes an autofill reservoir functionality that supplies up to three autofill reservoirs from bottles placed in the stacker module. The capacity of the stacker module is 4 hours unattended

operation. Other containers such as the ACP waste bottle and LMS containers do not need modification to sustain 4 hours of operation.

6.3.1 Description of the stacker

The stacker has two stacks each with a capacity of 43 cm stacking height. One stack is dedicated to QChip 384 plates and the other stack is dedicated to 384 format MTPs. The position of each is clearly labeled on the stacker and must be followed.

All connections in terms of power, pressure, and communication go via the main Qube instrument, and the stacker is operated via the ViewPoint software as a device seamlessly integrated in the available block templates. No separate initialization or handling is needed besides loading the stacker with plates before starting the experiment.

6.3.2 Placing plates in the stacker

384 format MTPs and QChip 384 plates can be loaded into the stacks in two ways, either by detaching the stacks and lowering them over the pre-arranged plate stack placed on a small elevation as shown in Figure 73. Or they can be added from the top of the stacks while the stacks are either mounted on the stacker unit or standing on a separate table. All plates should be mounted with the barcodes facing left and at A1 in the top left corner.

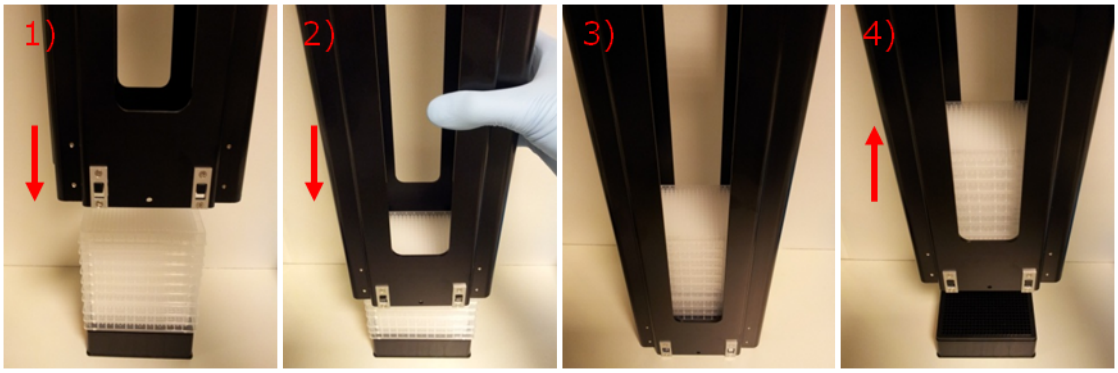


Figure 73 Sequence for plate loading by lowering the stack over a plate stack. The example shows a plate stack of 384 format MTPs placed on top of an empty 384 pipette tip rack. The stack is lowered over the plates in steps 1) and 2) until it is standing on the surface in step 3). Hereafter, the MTP containing stack can be lifted as shown in step 4)

The system will use the plates in the order that they are supplied, taking plates from the bottom of the stacks. For experiments that need multiple compound plates, it is very important that the user places the compound plates in the stacker in the correct order and in the correct numbers. The system does not have any knowledge of which plates belong to which experiments and consequently cannot check that the plates have been placed in the stack in the correct order before executing the experiment.

Similarly, Qube cannot check if the two stacks for MTPs and QChip 384 plates have been placed correctly or have been interchanged. It is therefore of vital importance that the stack for QChip 384 plates and the stack for MTPs are mounted in the correct positions on the stacker. Stacks and stacker are clearly labeled.

It is possible to reload the stacker with plates while Qube is running, either by adding plates to the mounted stacks or temporarily detaching the stacks. However, if the plate reloading coincides with the stacker being actively handling plates, Qube will fail and terminate further execution.

Caution! Ensure all plates in the stacks can be easily separated to ensure stable operation. Occasionally two plates can be mechanically jammed to each other if they are not placed correctly in the stacks.

Caution! Only 384 format MTPs and QChip 384 plates are supported by the stacker. The module is not compatible with plates such as 1 x 1 reservoirs, 16 x 1 reservoirs, and 1 x 24 reservoirs. Make sure to use the correct plates only.

6.3.3 Operating the stacker

Operation of Qube with a stacker is very similar to operating a Qube without a stacker. When a stacker is mounted, Qube will be configured with block templates that are tailored for use with the stacker. Each of the five protocol types used to set up a complete assay are created as described in chapter 5. However, since the stacker is both adding and removing plates to and from the Qube worktable, it is important to combine the 'Worktable protocol' with a matching 'Cleanup protocol' to ensure that Qube is always properly prepared for the next run. The integrated 'Info' section of the blocks contains specific guidance about this.

A Qube equipped with a stacker can be used both in an assay development mode, where compound plates are manually placed on the worktable by the user, and in a screening mode, where both compound plates and QChips are handled fully automated by the stacker. Please refer to the built-in documentation in the block templates for more details on the two modes.

The stacker will not restack either QChips or MTPs, but discard and dump them directly into the waste bin during the 'Cleanup protocol' execution. Therefore, in addition to preparation described in the first section of chapter 6 on page 94, you should prepare the following:

- Place a waste bin for receiving MTPs and QChip 384 plates under the transparent waste bin pipe at the end of the stacker support
    - Use a waste bin for biohazardous and toxic waste in accordance with local regulations

Warning! Do not mount a closed bag on the waste bin pipe. The pipe is only constructed to guide the discarded plates into a floor-based waste bin and the stacker and autofill module is not constructed to support the load of a waste bag.

6.3.4 Description of the autofill module

The autofill module consists of three main parts

- Source bottle(s)
- Control and pump module
- Autofill reservoir(s) with sensors

The source bottles are 1 L standard laboratory blue cap bottles. The bottles are equipped with a customized cap containing a quick coupling, suction tube, and venting hole. Both parts of the quick-connect coupling contain automatic shut-off valves to avoid spill when decoupling the bottles. Due to these valves, the bottles should not be decoupled while the autofill module is in 'Full/standby' or 'Autofill' mode.

The control and pump module ensure that the autofill reservoir(s) are always filled to a pre-defined liquid level as long as liquid is available from the source bottle. The autofill module operates completely independently of the remaining of the Qube system, with the exception that the module responds to opening and closing of the cabinet door and to the emergency stop.

Caution! Due to the autonomous nature of the autofill module, Qube will continue to run for as many repetitions as specified in ViewPoint even if the autofill reservoir(s) run out of liquid and the autofill module goes into an error state. It is therefore important to ensure that the autofill module is in the correct state and has sufficient source liquid before commencing plate runs.

The autofill reservoir(s) consist(s) of a slot plate with two sensors and a removable reservoir containing a quick-connect coupling for connecting the source tube. Two sensors detect if the reservoir is mounted and if it is filled to the pre-defined level, respectively. The reservoirs and tubes contain automatic shut-off valves to avoid spill when decoupling the reservoirs.

Warning! The autofill reservoirs must always be connected to the tubes while the autofill module is in 'Full/standby' or 'Autofill' mode. Otherwise, pressure can build up in the tubes, among other things resulting in leaks in the autofill system or uncontrolled spray of liquid when reconnecting the reservoirs.

Warning! The sensors are pre-calibrated for optimum and safe performance and the sensor calibration must not be altered.

6.3.5 States and LEDs on the autofill module

The pump and control module can control up to three autofill reservoirs that all operate independently. Each autofill reservoir is controlled via a single push button which has an associated status LED. An overview of the functionality is summarized in the following table.

Table 16 Description of the various states and concurrent LEDs on the autofill module

State	LED	Description
Idle	Yellow constant on	Module is powered but autofill functionality is not active
Full/standby	Yellow flashing	Module is powered and the autofill functionality is active with the reservoir currently detected full. The pump will automatically start when the reservoir liquid level drops below the threshold
Autofill normal mode	Green constant	The pump is actively running and refilling the reservoir to the pre-defined level. The pump has a short timeout limit for safe operation
Autofill priming mode	Green flashing	The pump is actively running and priming the tubes and reservoir until the pre-defined level is reached. The pump has an extended timeout to enable system priming
Error	Red flashing	The module is in error state. The error must be resolved before the system can be re-engaged
Unpowered pumps	No light	The pumps are unpowered if the cabinet door is open, the emergency stop is engaged, or the entire Qube is unpowered
Unpowered module	No light	The entire module is powered off. The module is only powered off when the entire Qube is powered off

6.3.6 Operating the autofill module

Since the autofill module is an autonomous unit, it will not respond to any operations performed via the ViewPoint software including going to and from standby. All operations of the autofill module must be done via physical push buttons mounted on the control and pump module.

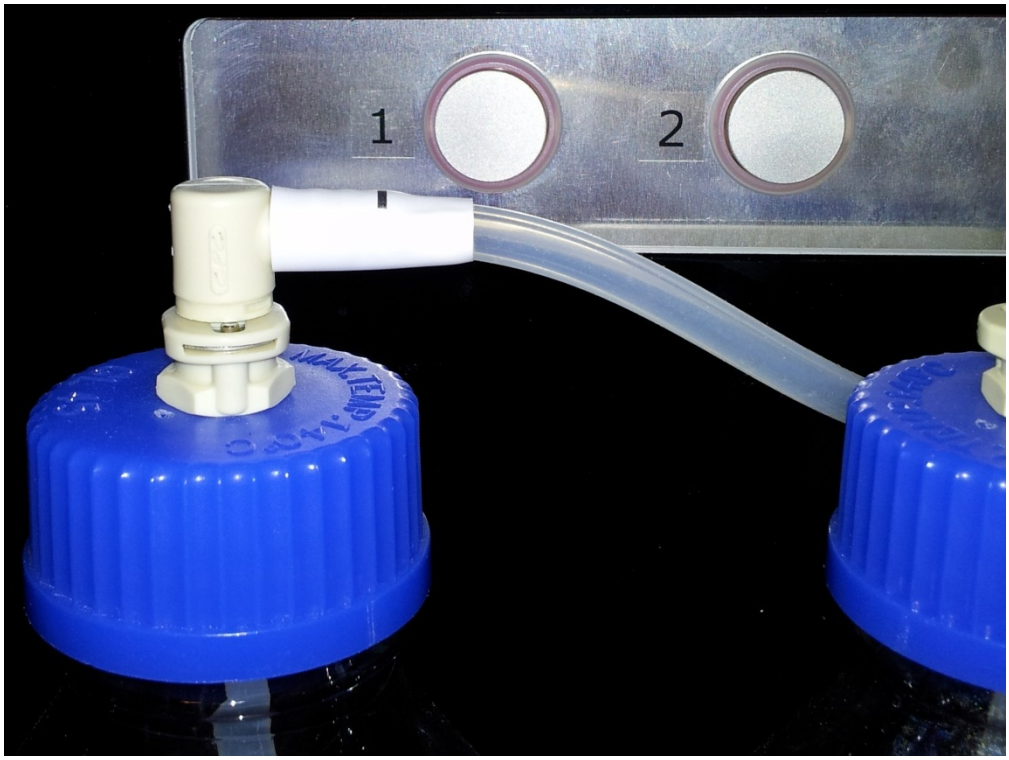


Figure 74 Photo showing two of the push buttons on the control and pump module placed behind the source bottles. The status LEDs light up the circumference of the buttons

When powering up Qube using the main switch, the default state of the autofill module is 'Idle'.

Any change of the state of the autofill module goes via the 'Idle' state when using the push buttons. Pushing a button for less than 3 s will switch to and from 'Idle' mode. Pushing a button for more than 3 s when the system is in 'Idle' state will engage the 'Autofill priming mode'. Pushing a button for less than 3 s when the system is in 'Idle' state will engage the 'Autofill standard mode'.

To prime the autofill reservoir, follow these steps:

1. Mount and connect the autofill reservoir
2. Mount and connect a filled source bottle
3. Put the autofill module into 'Idle' state by pushing the button less than 3 s
    a. If the system is in 'Error' mode, the cause of the error should be resolved first in order to enable the module
4. Put the module into 'Autofill priming mode' by pushing the button for at least 3 s. The button light starts flashing green when it is ready to be released
5. The pump will now fill the tubing and reservoir. Please monitor the system while priming takes place; due to the extended timeout in priming mode the reservoir can overflow in case of sensor failures. If there is a risk of overflow, stop the pump by pushing the button less than 3 s or by pressing the emergency stop
6. After successful priming, please verify that the module has automatically changed to 'Full/standby', i.e. the LED is flashing yellow

To engage the 'Full/standby' state, follow these steps:

1. If the module is not already in an 'Idle' state, push the button less than 3 s to enter 'Idle' state
2. If the module is in 'Idle' state, push the button for less than 3 s to engage 'Full/standby' state
    a. If the autofill reservoir is not full, the module will automatically go into 'Autofill normal mode' until the reservoir is full, thereafter 'Full/standby' state engages

The autofill module will go into the 'Error' state as a safety precaution when one of the following conditions is met:

- The reservoir is missing
- Timeout has been reached during pumping
- The emergency stop is or has been engaged

To recover from an 'Error' state, the cause should be resolved, and the push button pressed for less than 3 s. The system then goes into 'Idle'. From here, one of the two autofill modes can then be re-engaged as described above.

Opening the cabinet door will cut the power to the pumps while the door is open, but not engage an error state. The autofill module will go back into the previously used state as soon as the door is closed again.

6.3.7 Care of the stacker and autofill module

The stacker and the entire support structure are maintenance-free and only require being kept clean and free of dust and contaminants.

The autofill module must be flushed with clean water and emptied after use to prevent salt residues in pumps and tubes.

See the Quick Guide on page 12 for more details about cleaning the modules.

6.4 Using the optional temperature control module

Qube temperature control module (SB3360, SB3361, or SB3362) is an add-on that can be fitted on Qube 384 both with and without stacker. The temperature control module controls the temperature at the BCI and can both cool and heat to enable experiment execution at temperatures ranging from below room temperature to above physiological temperature.

The temperature control module consists of a refrigerated, heating circulator, a specially designed BCI with an integrated temperature sensor, a heat exchange system, as well as numerous components for seamless module integration with Qube hardware and software.

During experiment execution, temperature is automatically recorded and is available as a sweep property in Analyzer. Temperature is recorded regardless of whether the module is actively regulating or not.

6.4.1 Connecting the temperature module

The temperature control module will be installed and verified by a service engineer authorized by Sophion. All liquid, sensor, and communication connections are directly integrated in Qube. The circulator is powered by its own wall outlet.

Warning! The circulator has a current draw of up to 12 A (230 V version) or 13 A (100 V and 115 V version). Ensure that installation site mains power is capable of handling both the circulator and Qube. Use separate groups for powering the circulator and Qube if required. The wall outlet must have a proper ground connection.

6.4.2 Operating the temperature control module

The temperature control module is fully integrated with the Qube software and is controlled via ViewPoint. The temperature control module is controlled via the 'Tools' panel in ViewPoint (see Figure 75).

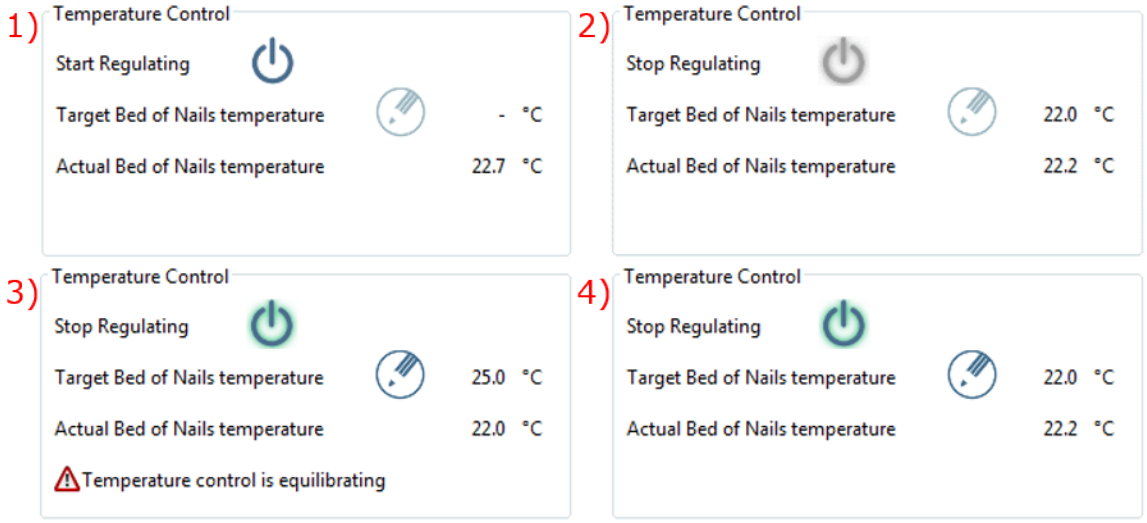


Figure 75 The 'Temperature Control' section on the 'Tools' panel under different conditions. 1) The module is not active. 2) The module is actively regulating the temperature and Qube is executing a protocol. It is not possible to stop or change the regulation. 3) The module is regulating, and the actual temperature has not yet reached the target temperature. A warning is shown if the difference between actual and target temperature is more than 1 °C. 4) The module is regulating and the difference between actual and target temperature is less than 1 °C

The module does not start automatically when Qube is turned on, but it will automatically go into standby when the entire Qube system is set to standby. This allows Qube to run with or without the temperature control module activated.

To use the temperature module, follow these steps:

1. Wake Qube from standby
2. Start the temperature control module on the 'Start Regulating' button
    a. The temperature control module will, by default, start regulation at 22 °C
3. Press the 'Pencil' icon and choose a 'Target Bed of Nails temperature'
    a. Use the instrument specific calibration curve to choose a proper value
4. Allow Qube to stabilize thermally. Recommended stabilization time is **two hours** after having reached the target value
    a. Keep the Qube front door and liquid drawer closed for maximum thermal stability

The target temperature can only be changed when Qube is in 'Ready' state, i.e. it is not possible to change target temperature during protocol execution. Qube cannot automatically change target temperature during execution either.

Similarly, regulation can only be started and stopped when Qube is in 'Ready' state to avoid accidentally changing conditions during protocol execution.

Warning! Qube 384 must only be operated under non-condensing conditions. Depending on the laboratory conditions and target temperature, the temperature control module may lead to condensation inside the Qube 384 and at the bed of nails. The temperature control module must always be operated at a target temperature at least 2 °C above the dew point of the laboratory to avoid condensation. Failing to comply with this may short circuit the measurement sites.

Note! When using the temperature control module, keep the Qube front door and liquid drawer closed as much as possible, also when Qube is not running, to ensure maximum thermal stability. Opening the door and drawer is, of course, inevitable for exchange of liquid and consumables, but opening time should be minimized.

Note! It is also recommended to handle all consumables in a consistent way, so their temperature is the same for all experiments. It is recommended to let the MTPs equilibrate for 15 minutes on the Qube worktable before starting the experiment.

Note! The temperature is set on the Bed of Nails. Use the calibration curve to calculate the proper Target Bed of Nails temperature based on a desired QChip temperature.

Warning! The circulator has several built-in safety and regulation settings. These have been pre-configured for optimum safety and performance with the Qube temperature control module. Altering any other settings than the temperature set point will void the warranty of the Qube instrument. The only exception is during the bi-annual safety check. Contact your Sophion Service Engineer for detailed instructions in case you need to perform the safety check yourself.

Tip! The temperature can be displayed in either °C or °F. Click on the unit in the 'Tools' panel to switch between the two scales.

6.4.3 Care of the temperature control module

The temperature control module must be regularly cleaned to ensure optimum performance.

Likewise, the liquid level should be checked regularly, and the circulator should be refilled if needed. See section 7.4 on page 110 for more details on how to clean the temperature control module, exchange the liquid, and maintain safe operation.

Warning! The circulator must always be unplugged from the wall outlet when cleaning, emptying, and filling the system.

6.5 Using ARQ plates

Qube is delivered with an Artificial Reference QChip (ARQ). This is a measurement plate with integrated electronics that emulate passive channels and can be used for system tests.

The ARQ is run in the same way as a standard QChip 384, however, with the important difference that the ARQ is not made for liquid handling and is therefore not mechanically compatible with the 384-robot pipette tips.

When using the ARQ ensure the following:

1. Remove the top part of both tip loading tools to ensure that the 384-robot does not have access to pipette tips
2. Place the ARQ at the worktable position normally used for a QChip 384
    a. Ensure that the ARQ is oriented correctly with the barcode facing to the left and the small A1 indicator at the upper left corner
3. Run a single repetition in the summary panel only

4. If using a standalone Qube with stacker, take precautions not to discard the ARQ directly into the waste bucket

Compounds, cells, etc. are not needed when using the ARQ. However, since all hardware is engaged, the LMS must be equipped with sufficient system water for the ARQ run as well as the ACP must be equipped with a waste bottle in order to allow Qube to run at all.

6.6 Using a ccCTP for cell clone test

Qube can also be used for testing different cell clones or different cell lines on the same QChip. To do this, the optional Cell Cloning Cell Transfer Plates (ccCTP) with either 4 (SB3304), 8 (SB3308) or 16 (SB3300, Figure 76) chambers should be used.

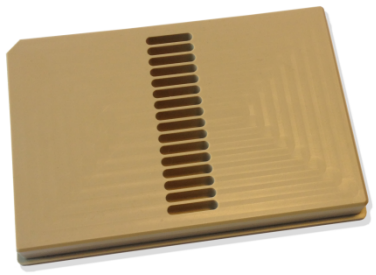


Figure 76 SB3300 ccCTP with 16 chambers

The volume requirements for the different versions of the ccCTPs are shown in Table 17.

Table 17 Volume requirements for different ccCTPs

Version	Max volume per compartment	Min volume per compartment
ccCTP4 (SB3304)	1.8 mL	680 µL
ccCTP8 (SB3308)	900 µL	340 µL
ccCTP16 (SB3300)	450 µL	170 µL

Cells should be harvested the same way as when preparing a standard Qube experiment with a cell density of app. 3.0 mio/mL and kept in suspension. Lower cell density down to 0.25 mio/mL can be used at some risk of lowered success rates. Make sure Qube is ready to be used.

Prepare Qube for running the cell clone assay in the same way as if a standard screening assay was to be run, however, with the following differences:

- The ACP must **not** have a pipette tip mounted
- The CTP is exchanged with a pre-filled ccCTP, see below
- The run is set up not to use a 'Cell preparation' protocol

Warning! Make sure that the ACP pipette tip is not mounted. If the ACP has a pipette tip mounted when running cell clone testing with a ccCTP the ACP pipettor may break during the cleanup protocol execution.

Cells should be prepared manually for the cell clone testing on Qube:

- Spin down 2 mL of each cell line/clone at 120 rpm for 120 s
- Remove supernatant and re-suspend pellet in 1 mL of extracellular saline
- Spin down again at 120 rpm for 120 s
- Remove supernatant and bring tubes with the cell pellet to your Qube platform

Procedure for Qube:

- Open the cabinet door
- Re-suspend cells in 1 mL extracellular saline using one auto pipette
- Aliquot a volume that obeys the requirements dependent of ccCTP type (see Table 17) of cell suspension into a compartment in the ccCTP with another auto pipette to save time
- Continue with the appropriate number of cell clones keeping track of where which cell clone goes
- Place the ccCTP on the worktable in the standard CTP slot (see Figure 77), and close the cabinet door
- Load protocols and execute the plan run
    - A 'Cell preparation' protocol should not be used

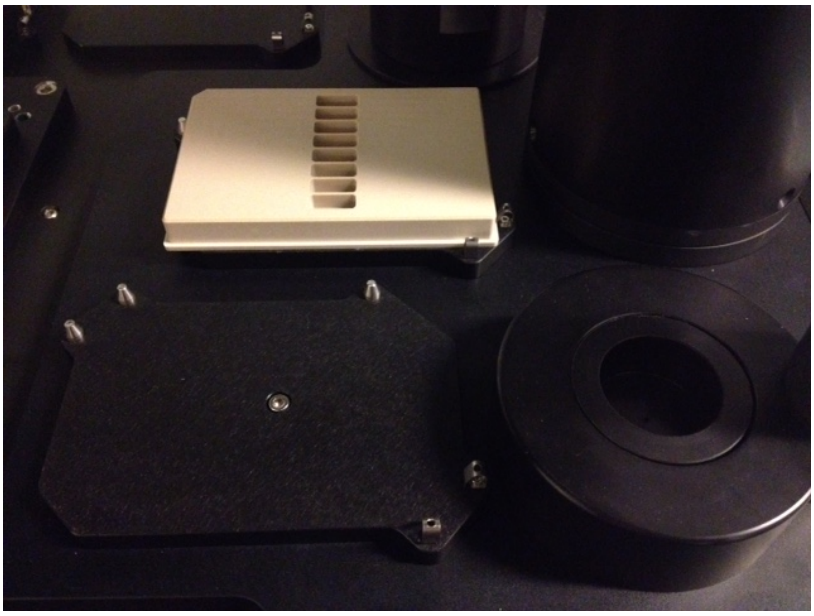


Figure 77 Placing the ccCTP

Procedure for the analysis:

- Prepare a 384 compound list in excel with identifiers/names for the cell clones according to Table 18
- Upload the compound list
- In Viewpoint, select 'plates' on the wrench on the 'Run' tab and assign the list to the 'EC' plate

Now the clones can be identified in Sophion Analyzer.

Table 18 Key for clone names in compound list for use of ccCTPs starting from the top

ccCTP version	Clone placed in…	Clone name in compound list entry…
ccCTP4	Top compartment Second compartment Third compartment etc.	Clone name in row A-D Clone name in row E-H Clone name in row I-L etc.
ccCTP8	Top compartment Second compartment Third compartment etc.	Clone name in row A-B Clone name in row C-D Clone name in row E-F etc.
ccCTP16	Top compartment Second compartment Third compartment etc.	Clone name in row A Clone name in row B Clone name in row C etc.

7. Taking care of Qube

7.1 Record Keeping

To verify that Qube has been properly maintained, you must keep a record of the daily, weekly, monthly, and quarterly care tasks performed. Appendix E at the end of this manual contains a logbook for record keeping.

7.2 Daily care

At the end of each day:

1. Follow the Quick Guide on page 12

7.3 Weekly care

At the end of each week:

1. Perform all daily care tasks, see the Quick Guide on page 12
2. Perform all weekly care tasks, see the Quick Guide on page 13
3. If your Qube system is equipped with a temperature control module:
    a. Check the liquid level in the temperature control module and refill if needed
    b. If you are using an antigrowth agent with indicator dye for the temperature control circulator, check the indicator dye. Exchange all liquid if needed. Exchange all liquid at minimum once every 3 months regardless of indicator dye
    c. Check the ribbed condenser for dust and clean if needed – clean at minimum once a month
4. If your Qube is equipped with a stacker and autofill module:
    a. If the waste bin pipe has become contaminated, use a proper decontaminant which is compatible with acrylic (PMMA) and polycarbonate (PC). Wear protective equipment and observe the general safety precautions as described in 2 and Appendix C
    b. Ensure that the stacker base is clean without any debris or dust in the area where the stacks (aluminum towers for QChip and compound plates) rest on the base unit. Remove the stacks and use a cotton swab once a week to clean the base area below the stacks (see Figure 78). Take care to prevent debris and dust entering into moving, mechanical parts

Warning! The temperature control module works by circulating liquid in an open circuit. The circulator must always be unplugged from the wall outlet when cleaning, emptying, and filling the system. See section 7.4.6 for refill instructions.

Warning! Make sure that the stacker is unpowered when performing the weekly cleaning procedure.

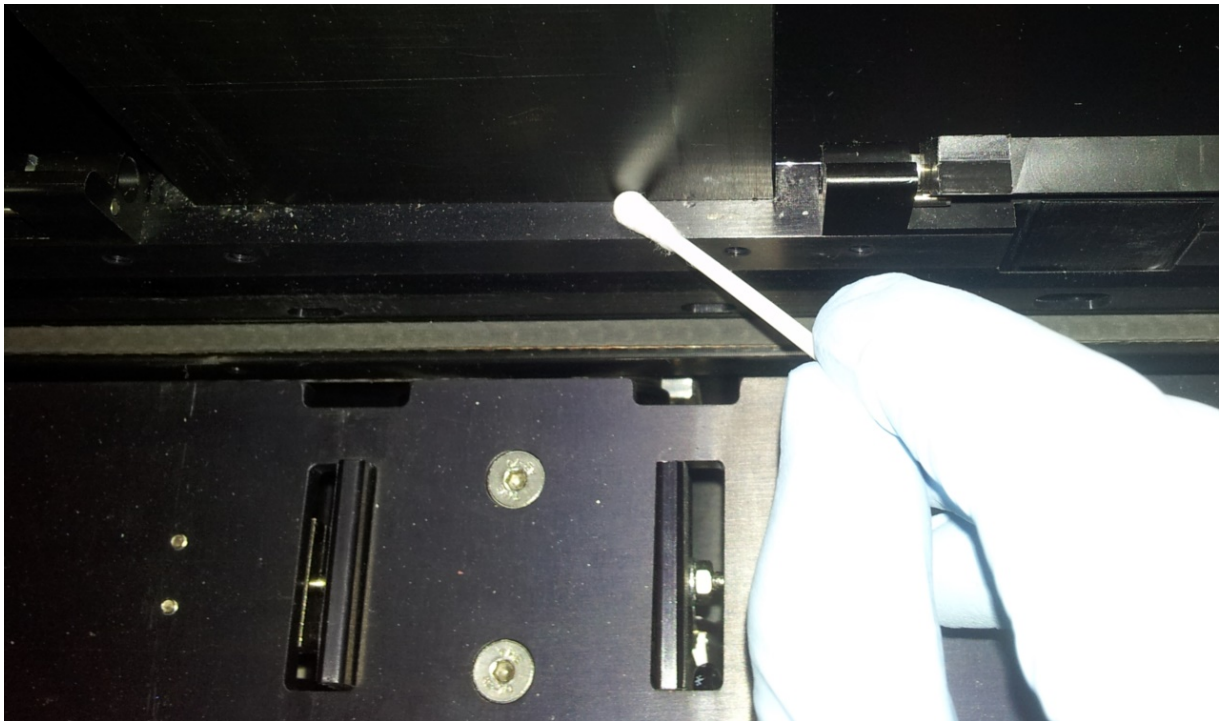


Figure 78 Clean the area where the aluminum stacks rest on the stacker area with a cotton swab

7.4 Additional care of the temperature control module

If your Qube is equipped with a circulator based temperature control module, please follow these guidelines for taking care of the module.

If the temperature module is regularly used, the care tasks listed for various intervals should be followed.

If the temperature control module will not be used for a prolonged period, it should be emptied to avoid contamination, and the device should be disabled in the software. Instructions for how to empty and disable the system are found in the following sections.

Warning! The temperature control module works by circulating liquid in an open circuit. The circulator must always be unplugged from the wall outlet when cleaning, emptying, and filling the system.

7.4.1 Liquids and refilling of the temperature control module

The liquid level in the internal bath should be checked once a week and refilled to counteract evaporation loss, if needed. See section 7.4.6 for refill instructions. Refill with soft, decalcified water only, in general the antigrowth agent does not need to be refilled since overall evaporation is typically very limited. Do not use DI or Milli-Q filtered water.

7.4.2 Monthly care of the temperature control module

The refrigeration unit condenser should be cleaned every month.

1. Make sure that Qube is in standby
2. Switch off the circulator, both at the refrigeration unit and the pump unit
3. Unplug the circulator's mains cable from the wall outlet
4. Remove the front venting grid
5. Clean the ribbed condenser, preferably with a vacuum cleaner
    - Take care not to damage the condenser ribs
    - Do not clean the condenser with compressed air
6. Reattach the vent grid
7. Reconnect the mains cable

7.4.3 Quarterly care of the temperature control module

The circulation liquid should be exchanged when the protective antigrowth agent needs exchange. The maximum time span between liquid exchange is 3 months, but it may be shorter depending on utilization of the circulator and on the specific agent. Please refer to the data sheet for the antigrowth agent. See the following sections for instructions to empty and fill the system.

7.4.4 Bi-annual care of the temperature control module

The circulator is equipped with low liquid level and over temperature safety features. The functionality of these features must be verified bi-annually. The safety check is performed as part of the preventive maintenance included in the Sophion service contracts.

Warning! Please contact your Sophion Service Engineer for detailed instructions on how to perform the safety check if you do not have a service contract.

7.4.5 Emptying the temperature control module

There are two variants of temperature control module, one with the 'FEED' tube attached to the lid and one with the 'FEED' tube attached to a rear side fitting. Follow the instruction suitable for your installation.

7.4.5.1 Emptying models with 'FEED' tubing through the lid

1. Wake Qube from standby
2. Start temperature regulation and set the regulation to room temperature
3. Lift the lid with the feed tubing so the system is filled with air
    - Hold the lid above the reservoir to avoid liquid spills
4. Keep the pump running until the tubing is emptied. After a few seconds of pumping, the noise level of the system increases as air is pumped through it.
5. Stop the circulator by pressing the 'Stop Regulating' button in ViewPoint
6. Put the lid back in place
7. Set Qube to standby
8. Switch off the circulator and unplug the mains cable from the wall outlet
9. Remove the front venting grid
10. Mount a drain tube on the barbed drain outlet
11. Place a suitable container under the drain tube
    - The total volume in the circulation system is approximately 5 L
12. Open the drain valve by turning it counterclockwise
13. Close the drain valve again by turning it clockwise and tighten it after having emptied the system
14. Remove the drain tube and remount the front venting grid on the circulator

7.4.5.2 Emptying models with 'FEED' tubing through the rear side fitting

1. Wake Qube from standby
2. Start temperature regulation and set the regulation to room temperature
3. Empty the system by gently disconnecting the 'FEED' tube, labelled  at the circulator output  while the circulator is running
    - After a few seconds of pumping, the noise level of the system increases as air is pumped through it
4. Stop the circulator by pressing the 'Stop Regulating' button in ViewPoint
5. Reconnect the 'FEED' tube
6. Set Qube to standby
7. Switch off the circulator and unplug the mains cable from the wall outlet
8. Remove the front venting grid
9. Mount a drain tube on the barbed drain outlet
10. Place a suitable container under the drain tube

- The total volume in the circulation system is approximately 5 L

11. Open the drain valve by turning it counterclockwise
12. Close the drain valve again by turning it clockwise and tighten it after having emptied the system
13. Remove the drain tube and remount the front venting grid on the circulator

7.4.6 Filling the temperature control module

1. Make sure that the Qube is in standby
2. Switch off the circulator, both at the refrigeration unit and the pump unit
3. Unplug the circulator's mains cable from the wall outlet
4. Ensure the bottom drain valve is closed
5. Remove the lid
6. Fill the circulator with soft, decalcified water until the liquid level is about 5 mm above the indentation in the bath side walls
    - Water quality must conform to the specifications in the circulator operating manual. Do not use DI or Milli-Q filtered water
7. Mount the lid
8. Connect the mains cable and switch on the refrigeration unit and pump unit
9. Wake Qube from standby
10. Start the pump by pressing 'Start Regulating' in ViewPoint
11. Let the pump run for at least one minute to prime the entire temperature control module
12. Stop the pump by pressing 'Stop Regulating' in ViewPoint
13. Set Qube to standby
14. Switch off the circulator, both at the refrigeration unit and the pump unit
15. Unplug the mains cable from the wall outlet
16. Check the liquid level in the bath
17. Refill until the liquid level is a few mm above the indentation in the bath
18. Add a suitable antifungal and antibacterial water protective agent approved for circulators and water baths. Refer to the documentation of the specific agent for a proper concentration
19. Mount the lid
    - Secure the lid if it is a variant with 'FEED' tubing through the lid
20. Reconnect the mains cable and switch on the refrigeration unit and pump unit

7.4.7 Disabling and enabling the temperature control module

By default, the Qube system requires the temperature control circulator to be powered, filled, and connected to run. If Qube needs to be used with an unpowered circulator, e.g. if it has been emptied, the temperature control can be disabled.

1. Make sure that Qube is in standby mode
2. Go to the 'Features' tab in 'ViewPoint Maintenance'
3. Uncheck the 'Temperature control' checkbox

The Qube system will now ignore the temperature control hardware and can be used with the circulator unpowered. The module can be enabled again using the same checkbox.

7.5 Cleaning the BioChip Interface

The BioChip Interface (BCI) should be touched as little as possible but should be kept free from dust and other particles. Never touch the gold pins with your fingers, since grease from the skin can affect amplifier connections. Furthermore, the amplifiers can be damaged by electrostatic discharge.

Before handling the BCI in any way, ensure that Qube instrument is in standby state and the safety circuit engaged. The best way to remove dust is by blowing clean, pressurized air or nitrogen on the Bed of Nails (BON). Larger particles can be removed using tweezers. Ensure that you touch other metal parts on the Qube worktable first (e.g. the system

frame), to ground yourself, and avoid electrostatic discharge onto sensitive parts of the system.

8. Troubleshooting

Qube has various safety features which hinder incorrect use of the system. Examples could be preventing pipette tips from being loaded if they are already loaded on either 384-robot or ACP, hindering execution in general if the proper ACP waste container is not mounted, or if pressure or vacuum are outside a safe operating range. In case Qube does not respond as expected from normal operating conditions, either due to engaged safety features or unexpected errors, ViewPoint and ViewPoint Maintenance software will typically report error messages or warnings which can be used to identify the cause.

Depending on the type of error, their messages will be found different places in the software as described in the following section.

8.1 Basic troubleshooting steps

The following steps can be used as a quick guide to identify and resolve issues in general in a step-by-step manner.

8.1.1 Problems encountered during run execution

1. Check for run related error messages in the ViewPoint 'Run' panel
    a. Hover the mouse over the affected run in the 'Plates' track
    b. Check for messages in the 'Errors' panel for the affected run
2. Check for machine related errors in the ViewPoint 'Tools' panel
3. Check for device related errors in the ViewPoint 'Info' panel
4. Use the troubleshooting matrix in the following section to identify a possible solution
5. Contact your Application Scientist or service engineer authorized by Sophion if the above steps have not resolved the issue

8.1.2 Problems with starting up Qube

1. Check for machine related errors in the ViewPoint 'Tools' panel
2. Check for device related errors in the ViewPoint 'Info' panel
3. Use the troubleshooting matrix in the following section to identify a possible solution
4. Contact your Application Scientist or service engineer authorized by Sophion if the above steps have not resolved the issue

8.2 Exporting debug information

There are numerous possibilities for exporting debug information if a problem cannot easily be resolved with the help of the troubleshooting matrix. With this information, your Application Scientist or service engineer authorized by Sophion can assist in resolving the problem.

Debug information can consist of log files, machine configuration files, or the exported run with or without log and measurement data.

Depending on the situation, the relevant type of debug information differs. Follow these overall guidelines for creating debug information.

8.2.1 Debug run related errors

For issues which have occurred during the execution of a protocol, use one of the following options:

- Option 1: Export the run without data, see section 4.7.6
    - This will include the protocols, protocol run, and log files covering the time span of the selected protocol(s). Raw current and voltage trace data is omitted
- Option 2: Export the run with the log only, see section 4.7.6
    - This will only contain the log file covering the time span of the selected protocol(s)

- Option 3: Save a debug file with a log time span covering the time of the incident, see section 4.2.4 page 37
    - This will contain machine configuration and metrics files, screenshot, and log files

8.2.2 Debug data interpretation

For issues which are related to data interpretation, use one of the following options:

- Option 1: Export the run without data, see section 4.7.6
    - This will include the protocols, protocol run, all recorded data, and log files covering the time span of the selected protocol(s)
- Option 2: Make screenshots of selected data manually

8.2.3 Debug instrument or software related errors

- Option 1: Save a debug file with a log time span covering the time of the incident, see section 4.2.4 page 37
    - This will contain machine configuration files, screenshot, and log files
- Option 2: Save a debug file from error message dialogs (see Figure 79)
    - This will contain machine configuration and metrics files, screenshot, and log files covering the time span near the occurred error

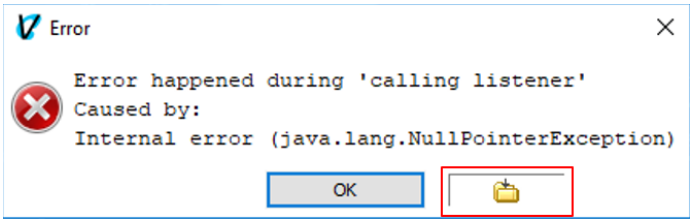


Figure 79 If an unexpected error occurs, the yellow folder icon in the error message dialog can be used to save a debug file

Note! There is **no anonymization** of data or other information in exported protocol runs, log files, or debug files.

Note! Debug files and machine info files contain a screenshot of the whole display. Make sure that there is no confidential content in the screenshot before uploading the file. Delete the screenshot or make a new debug file if needed.

Tip! Debug files can also be exported directly with the keyboard shortcut 'Ctrl+Shift+D'. This works in both ViewPoint, ViewPoint Maintenance, and Sophion Analyzer.

8.3 Troubleshooting matrix

The following table lists a number of symptoms, possible causes, and solutions. The list is not exhaustive and there may be other causes for the symptoms described.

If the scenarios listed in the following are insufficient in resolving your problem, please do not hesitate to contact your Application Scientist or service engineer authorized by Sophion.

Symptom	Possible cause	Possible solution
Homing is not possible	Qube is in standby	Wake Qube from standby
	384-robot has pipette tips loaded	Run recovery utility protocol 'Recovery: Eject 384 pipette tips at e6'
	System is not ready	Check the 'Tools' panel for possible causes Check the state of the individual devices in the machine 'Info' panel
	One or more devices are not powered	Check the 'Power Supplies' section of the 'Tools' panel in ViewPoint Maintenance. If a power supply is off put Qube to standby and wake from standby again afterwards **Warning!** If the 384-robot has pipette tips loaded, these must be removed manually before waking Qube from standby. See the following sections for manual recovery.
	ACP waste bottle is full or missing	Mount an empty ACP waste bottle
Utility protocols cannot execute	The system is not homed	Home the system
	System is not ready	Check the 'Tools' panel for possible causes Check the state of individual devices in the machine 'Info' panel
Protocols cannot be loaded from the 'Summary' section	System is not ready	Check the 'Tools' panel for possible causes Check the state of individual devices in the machine 'Info' panel
It is not possible to set Qube to standby	System is running	Check system state and wait for execution to finish
	ViewPoint has lost connection to Qube	Restart ViewPoint
Gripper failed to remove QChip from the BCI	QChip mechanically stuck	Run utility protocol 'Recovery: Remove QChip 384 from BCI'. If above failed, remove QChip 384 manually. See separate section for manual recovery
Robot stopped with gripper inside BCI	Gripper has failed to handle a QChip mechanically correct	See section 8.4.5 for how to manually move the gripper out of the BCI Subsequently, run the utility protocol 'Manual override: Reset BCI state' to re-enable robot movement, see Table 15 at page 90.

Symptom	Possible cause	Possible solution
		 **Warning!** Do **not** run the abovementioned utility protocol if the gripper is still inside the BCI. Do **not** set Qube to standby or power off the robot if the gripper arms are inside the BCI. The manifold will close if doing so.
Centrifuge tube swings out abruptly	Centrifuge tube holder or pins are dirty	Clean pins and centrifuge holder
Amplifier board(s) is not responding	Multiple possible causes	Set Qube to standby and subsequently wake from standby  Warning! Ensure the appropriate utility protocols are run to remove pipette tips and QChips if needed before placing Qube into standby.
A single site consistently shows high or infinite resistance	Dirt in connector pin in BON	Contact service engineer authorized by Sophion for assistance
	A pipette is malfunctioning	Run an ARQ job (without pipette tips) to determine if the error is related to the 384-robot or the BCI Call service engineer authorized by Sophion for assistance
A single site consistently shows low resistance	Short circuited BON pins	Call service engineer authorized by Sophion for assistance
Run stopped with the message 'Error: The QChip is not placed correctly in the BCI or the QChip has a pressure leak.'	QChip 384 has a leaking site or has not been placed correctly	Run utility protocols 'Recovery: Eject 384 pipette tips at e6' if tips are loaded followed by 'Recovery: Remove QChip 384 from BCI'
	Leak in manifold or pressure system	Run an ARQ job (without pipette tips) to verify leak cause Call service engineer authorized by Sophion for assistance
Run stopped with the message 'The pressure unit has detected at least one blocked channel. Please check if the BCI is empty. Refer to the troubleshooting chapter in the user manual for further instructions.'	A QChip has been left in the BCI from a previous run	Run utility protocol 'Recovery: Remove QChip 384 from BCI'
	Blockage in manifold or pressure system	Contact service engineer authorized by Sophion for assistance
Cannot log in to Qube via ViewPoint	User is not registered in the Qube database	Register user via ViewPoint Maintenance

Symptom	Possible cause	Possible solution
	Controller software is not running	Start Controller Software from the 'Software' panel in ViewPoint Maintenance
	Controller PC is not running	Check controller PC status from the 'Software' panel in ViewPoint Maintenance. If the Controller PC is not running, shut down the Data PC and power cycle Qube Wait at least 30 s before powering on Qube again
Cannot log in to Qube via ViewPoint Maintenance	External network error	Check if the User PC has network connection in general Check if Qube is physically connected to the network **Note!** Qube must be connected to a network before being powered on. If Qube has been powered on while disconnected, it must be powered off and connected before being powered on again.
	Internal Qube PCs have been powered off	Power cycle Qube Wait at least 30 s before powering on Qube again
	Qube is powered off	Power on Qube
ViewPoint or ViewPoint Maintenance do not seem to have connection to Qube	External network error	Check network connections and resolve issues
	Temporary loss of network connection	Restart ViewPoint and ViewPoint Maintenance
The 'Tools' panel is not available in ViewPoint	The PC is not configured to control Qube	Only the Qube User PC is configured to show the 'Tools' panel
Execution stopped during experiment with the error message 'No plate in [position name]'	The experiment protocol has been set up to use a position that is not enabled, or where the plate type has not been defined in the worktable protocol	Use matching worktable and experiment protocols
384-robot stopped with the gripper arms closed over an empty slot plate	Assay has been set up to grab a plate that is not physically placed on the worktable	Run appropriate utility protocol(s) Possibly cleanup ACP and centrifuge Make sure to match number of repetitions with the number of placed plates
Execution stopped with the error message 'Worktable is	The protocols have been loaded with more repetitions than available in	Possibly cleanup ACP and centrifuge Make sure to match number of repetitions with the available array in the worktable protocol

Symptom	Possible cause	Possible solution
missing logical position(s) with name(s) [...], index [...]'	the chosen worktable array	
Pipette tips on 384-robot are bent	Mismatch between plate types	Ensure that the plate type chosen in the worktable protocol matches the actual plate used in the instrument
	Misplaced consumable	Ensure to place consumables correctly between the guide pins and springs in the slot plates
Not all protocol types were executed	Not all protocol types were selected in the 'Summary' section	Select all required protocol types
Execution stopped with the error message 'Waste pump pressure switch activated'	LMS waste container connector was released while the waste container was in use	Run appropriate recovery utility protocol(s) Run utility protocol 'Washing station: Flush' to check if the problem is resolved Avoid disconnecting containers in use
Execution stopped with the error message 'Supply pump pressure switch activated'	LMS tubing at the backside of the liquid drawer has been kinked or blocked	Run appropriate recovery utility protocol(s) Check tubing for blockages
'Tools' panel states 'BioChip Interface pressure sensor outside allowed range'	The cabinet door is open	Close the cabinet door
	The emergency stop is engaged	Disengage the emergency stop
	There is an error in the external pressure supply	Check the external pressure supply
'Tools' panel states 'Safety relay must be reset'	The cabinet door is open	Close the cabinet door
	The emergency stop is engaged	Disengage the emergency stop
Power supply buttons are lit although Qube machine state is 'Standby'	Controller software or internal PCs have been restarted while device power supplies were on	Turn off the individual power supplies in ViewPoint Maintenance and wait 30 s before waking Qube from standby
Amplifiers and Pipetting robot devices do not connect after waking Qube from Standby	Device power was on before waking Qube from standby	Set Qube to standby and verify that power supply indicators in ViewPoint Maintenance go to off state Wait 30 s to ensure power supplies are off and wake Qube from standby again
	An internal fuse has been blown	Contact a service engineer authorized by Sophion for assistance

Symptom	Possible cause	Possible solution
Autofill module is in 'Error' state	Emergency stop has been pressed	Use the autofill push button as required to re-engage the desired state
	The reservoir is not mounted or not mounted correctly	Mount the reservoir correctly and use the autofill push button as required to engage the desired state
	Pump timeout has been reached because priming has been attempted with 'Autofill normal mode'	Use the 'Autofill priming mode' to prime the autofill reservoir
	Pump timeout has been reached because the source bottle has run dry	Refill the source bottle and use the autofill push button to re-engage the desired state
	Pump timeout has been reached because one or more of the quick-connect couplings has not been properly mounted	Reconnect the quick-connect coupling and use the autofill push button to re-engage the desired state **Warning!** If the reservoir quick-connect coupling has not been properly mounted when the pump was running, the system may be pressurized. Reconnecting the quick-connect coupling will release the pressure. Wear protective equipment and cover the reservoir temporarily to avoid spaying liquid onto yourself or the instrument.
	Pump timeout has been reached because of a leakage or blockage	Clean up the spill and let the autofill module stay in 'Error, state. Contact a service engineer authorized by Sophion for assistance

8.4 Manual recovery

Qube is designed to use software controlled hardware as the primary mean for execution, including recovery after unintended interruptions. However, there may be situations where a manual recovery is the only way to get Qube back into a safe operational state.

Manual recovery should always be the last resort. In general, software based recovery functions should be employed whenever possible.

The following sections describe the situations where a manual recovery is necessary and guides how to do the recovery.

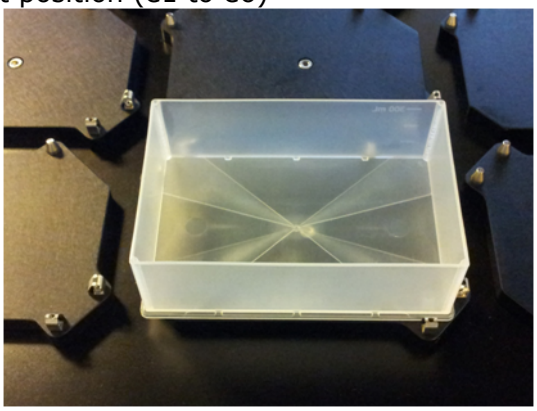
Warning! For maximum safety, the emergency stop must always be engaged when performing any of the following manual recovery operations.
The user should wear proper protective equipment such as lab coat, gloves, and safety goggles during the recovery.

8.4.1 Manual removal of pipette tips from 384-robot

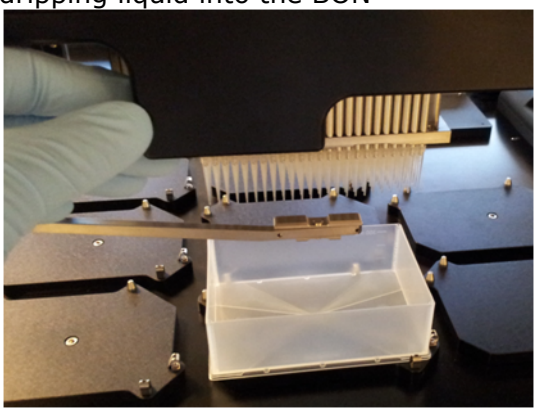
If the power for the 384-robot has been cut while the pipette tips were loaded, the mounted pipette tips must be removed manually before Qube can be used again. A power cut to the robot can, for instance, have been caused by setting the Qube to standby in an improper manner, or by a general power cut to the entire instrument. In the latter case, the Controller Software will allow the homing to take place due to loss of tip load state

knowledge. However, the pipette tips should still be removed manually before homing in order not to risk contamination of the pipetting head.

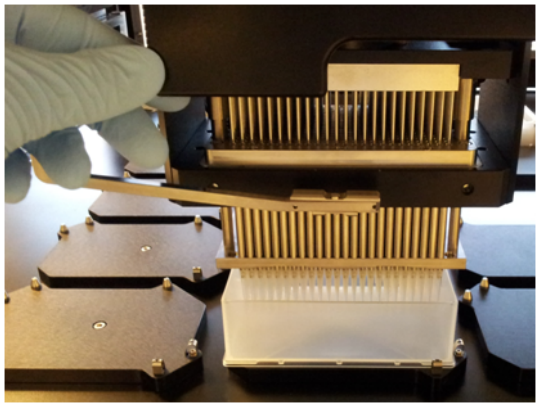
1. Place a suitable container to receive the pipette tips and possible liquid in a front row slot position (e1 to e6)



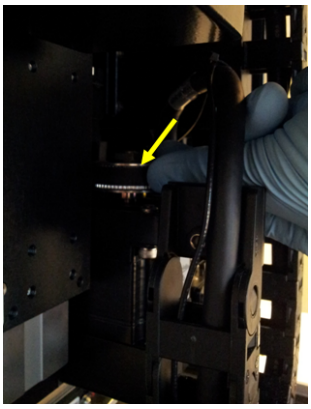
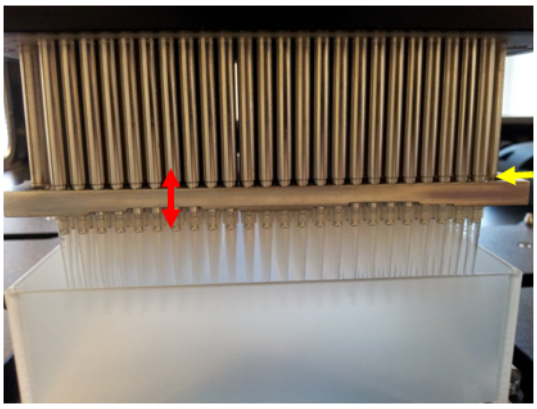
2. Manually move the 384-robot head to the selected slot position
    a. Be careful not to traverse the robot across the BCI to eliminate the risk of dripping liquid into the BON



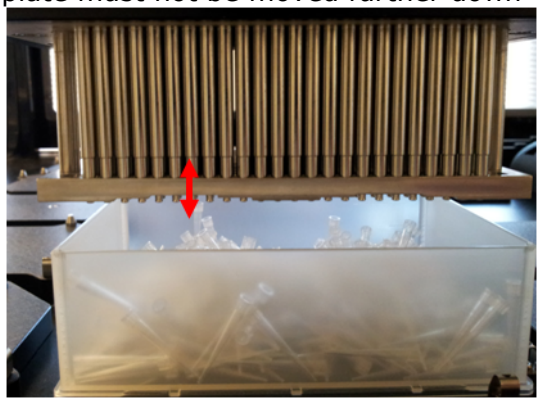
3. Manually move the pipetting head down towards the container by turning the Z axis motor pulley with the toothed belt at the top rear side of the Z axis assembly
    a. Position the 384-robot head 10 mm to 20 mm above the container



4. Eject the pipette tips by turning the pipette motor pulley with the toothed belt at the rear side of the pipetting head. This will move the pipette pistons and the ejector plate
    a. Ensure that the pulley is turned the correct direction by looking at the pipette pistons which should go downwards

b. When the bottom of ejector plate has cleared the pipette tip cones the ejector plate must not be moved further down



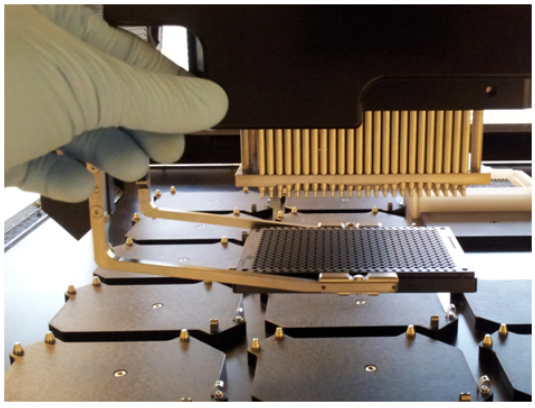
5. Retract the ejector plate using the pipette motor pulley until the ejector plate has reached its upper end stop
6. Move the 384-robot to a safe position
7. After recovery, run the utility protocol 'Manual override: Reset pipette tip state' followed by 'System: Clear and home'
    a. If the ACP pipette tip is mounted, make sure to eject the ACP pipette tip immediately after homing, see section 8.4.3

8.4.2 Manual removal of plates from the gripper

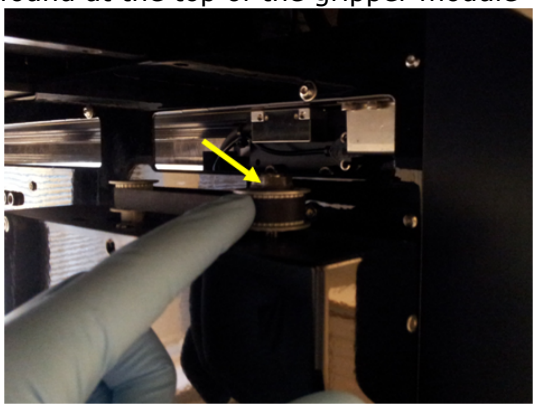
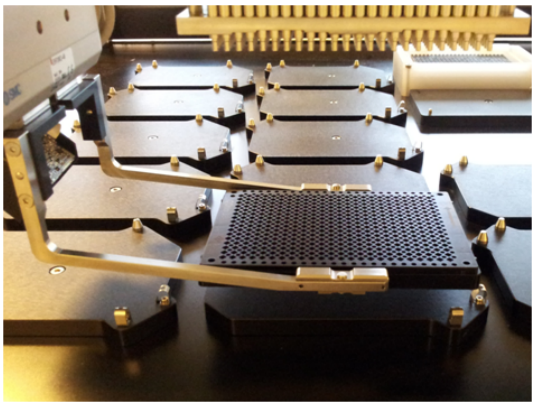
If the robot has been powered off while a plate was kept in the gripper, the plate must be released manually before Qube can be used again.

Warning! The following operation can and must only be done when the 384-robot is powered off.

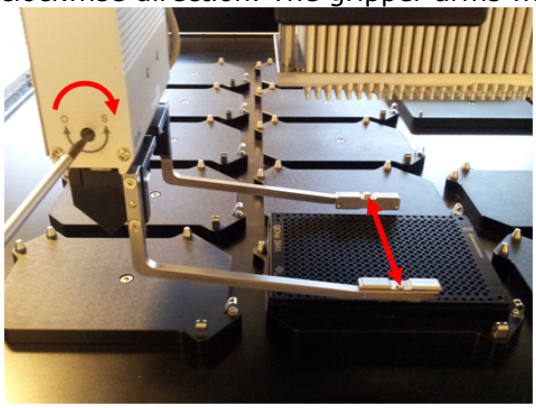
1. Manually move the 384-robot to a safe position where the gripper arms are free to open



2. Lower the gripper towards the worktable by turning the gripper z axis motor pulley found at the top of the gripper module

3. Use a flat tip screwdriver to turn the screw at the side of the gripper module in the clockwise direction. The gripper arms will now open and release the plate



4. Run the utility protocol 'System: Clear and home' after waking Qube from standby. Make sure that the gripper is free to retract to homing height at the position where it was left after the recovery

8.4.3 Automated or manual removal of pipette tip from the ACP

In contrast to the 384-robot, the ACP can perform a full homing with a pipette tip loaded. There is, therefore, no problems in executing a standby cycle with an ACP pipette tip loaded.

8.4.3.1 Automated removal sequence

If the entire Qube system, the Controller PC, or the Controller Software have been restarted, or the utility protocol 'Manual override: Reset pipette tip state' has been run, the software based tip load state has been reset and the ACP pipette tip must be removed using the following sequence:

1. Wake Qube from standby
2. Run the utility protocol 'System: Clear and home'
3. Run the utility protocol 'ACP: Eject ACP pipette tip'

The ACP is now ready to operate safely again.

8.4.3.2 Manual removal sequence

The ACP pipettor is delicate, and manual removal of the ACP pipette tip should therefore only be performed if the automated sequence is not possible. This situation could, for instance, occur if the loaded pipette tip has been damaged or misplaced such that it does not fit the ACP tip ejector or if it for different reasons is not possible to home the instrument.

Follow the instructions below to remove the pipette tip:

1. If the pipette tip contains liquid, make sure to perform the removal over a suitable reservoir to prevent liquid spill in the instrument
2. Grab the pipette tip around the collar and unscrew the metal tip adapter, including the pipette tip (see Figure 80 a))
3. Ensure that the small O-ring for sealing between the tip adapter and the pipettor is placed correctly as shown in Figure 80 b)
4. A spare tip adapter is delivered with your Qube. Mount the spare metal tip adaptor onto the pipettor. The adapter should only be mounted finger tight onto the pipettor without any use of tools (see Figure 80 c))

Warning! The ACP pipettor is delicate. Be careful not to exert any sideways forces or to excessive force to the pipette tip, tip adapter and pipettor while mounting the tip adapter. The threaded part of the pipettor can break off.

A pipette tip that is stuck on a removed tip adapter can be very difficult to remove. Do not use tools that can scratch the tip adapter if you attempt to remove the tip. Contact your Sophion Service Engineer for assistance if needed.

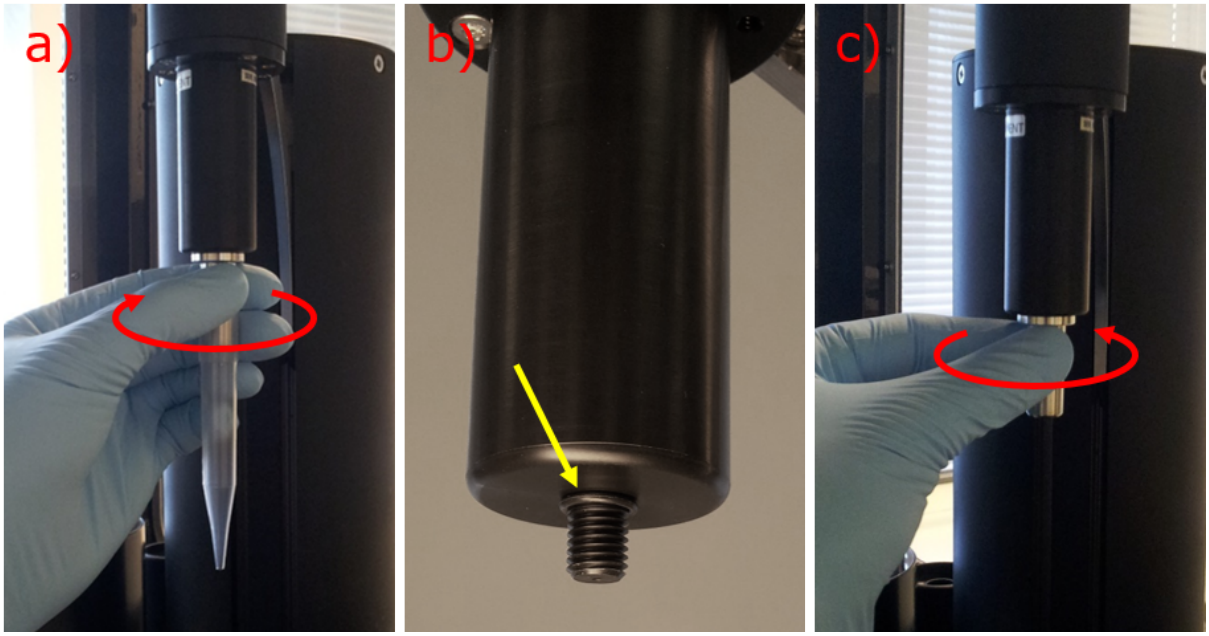
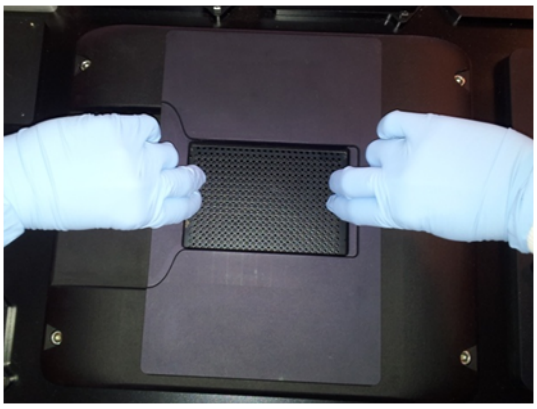


Figure 80 Sequence for manual removal of ACP pipette tip. a) Unscrew the metal tip adaptor from the pipettor. b) Check that the sealing O-ring is positioned correctly. c) Mount the spare tip adaptor on the pipettor.

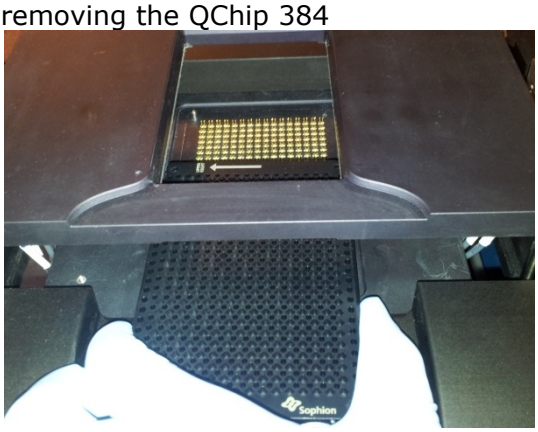
8.4.4 Manual removal of a QChip 384 from the BCI

Whenever possible, the QChip 384 should always be removed by the 384-robot using the utility protocol 'Recovery: Remove QChip 384 from BCI'. If it is not possible to have the robot remove the QChip 384, follow these steps:

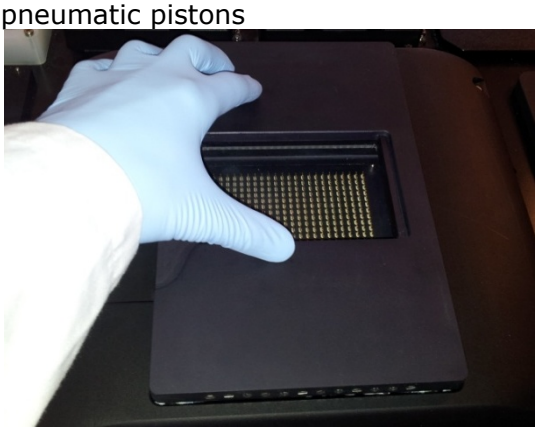
1. Lift the unpressurized manifold by pulling at the left and right sides of the manifold opening
    a. Be careful not to touch the gaskets under the front and rear sides of the manifold opening
    b. Be careful not to touch the gold pins in the BON



2. It is advised to place a mechanical obstruction under the manifold to mechanically blocking it from movement
    a. Be careful not to touch the gaskets or the tubing
3. Grab the QChip 384 by its edges and lift it out of the BCI
    a. Be careful not to tilt the QChip 384
    b. Be careful not to drag the QChip 384 along the gold pins in the BON when removing the QChip 384



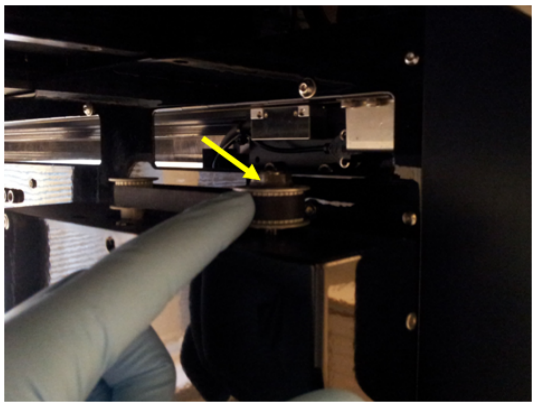
4. Remove the mechanical obstruction
5. Close the manifold by gently pushing it down
    a. Make sure to keep the manifold level when closing it to avoid damaging the pneumatic pistons



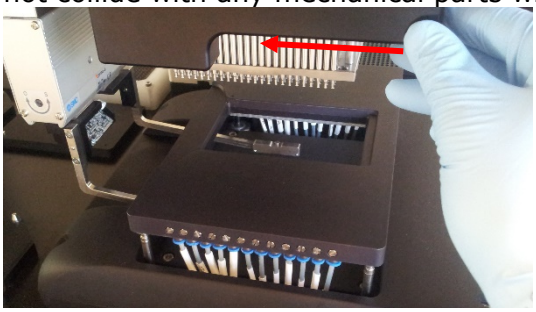
8.4.5 Manually moving the gripper out of the BCI

If the 384-robot has stopped with the gripper inside the BCI, i.e. with the gripper arms placed under the manifold, the robot must be manually moved out of the BCI before proceeding.

1. Adjust the height of the gripper to ensure that it is free to be moved laterally. Turn the pulley on the top of the gripper module to adjust the height



2. Move the 384-robot and thereby gripper arms fully out of the BCI by gently moving it by hand. Be careful to ensure that the gripper arms and potentially QChip 384 do not collide with any mechanical parts when moving the robot



3. Re-enable robot movements by running the utility protocol 'Manual override: Reset BCI state', see Table 15 at page 90.

Warning! Do **not** set Qube to standby or power off the robot if the gripper arms are inside the BCI. The manifold will close and severely damage the gripper if this is done.

9. System Requirements and Specifications

9.1 System requirements

Main supply	100–240 V AC 50–60 Hz 3.5–6 A		
Additional main supply for temperature control module (add-on)	SB3360 Version	SB3361 Version	SB3362 version
	230 V AC 50 Hz 12 A	100 V AC 50–60 Hz 13 A	115 V AC 60 Hz 13 A
Vacuum (ISO 8573-1:2010)	Minimum –620 mbar Maximum –900 mbar at a peak consumption of 1 m^3/hour (4.4 gallons/minute)		
Pressure range (ISO 8573-1:2010)	6–8 bar (85–115 psi) at a peak consumption of 1.8 m^3/hour (7.9 gallons/minute)		
Compressed air quality	ISO 8573-1:2010 [1:4:1]		
Network connection	1000 BASE-T Fast Ethernet (1000 Mbit/s) connection		
IP classification	IP20		

9.2 Environment

The Qube instrument is designed for indoor use only, primarily for operation in commercial laboratories.

The hardware of the instrument will operate safely in the ambient temperature range of +5°C to +40°C (40°F to 105°F). Nevertheless, the system has only been tested at room temperature. Ambient temperatures deviating from ordinary room temperature (18°C to 26°C, 64°F to 79°F) are expected to seriously affect the lifetime of the cells in the QStirrer cup and their ability to form gigaseals.

The maximum safe altitude is 2000 m. The maximum safe relative humidity is 80% for temperatures up to 31°C, decreasing linearly to 50% relative humidity at 40°C.

Note! If the relative humidity is below 40%, it can affect the automatic handling of tips due to build-up of static charge.

If condensed water is present in the Qube instrument, it must be acclimatized before being switched on. In some cases, such as if it is extremely cold (below 0°C), 24 hours should elapse before operating the instrument. We do not recommend that it is placed in an environment with large temperature fluctuations, because this increases the likelihood of condensed water repeatedly appearing in the instrument.

The instrument should be kept in a clean, dry room and must not be operated in explosive, corrosive, dusty, or damp environments.

9.3 ViewPoint Software

ViewPoint Software is a Java application that theoretically can run on any computer and operating system that supports a Java Runtime Environment version 1.8 or later; for example, a standard PC running Windows XP or later, Mac OS X, or UNIX systems. The ViewPoint Software has currently only been extensively tested on standard PCs with

Microsoft Windows 7 (64 bit) and Microsoft Windows 10 (64 bit), so these are the recommended platforms.

The hardware requirements for the computer running the ViewPoint Software are:

- Graphics resolution: minimum 1024 ⨉ 768 pixels, 16 million colors; recommended 1920 ⨉ 1080 pixels
- RAM: 1 GB minimum, 2 GB or more recommended for optimal performance

ViewPoint Software includes software modules for which licenses apply (see Appendix A).

9.4 System specifications

Dimensions	Width: 128 cm (195 cm with stacker) Depth: 85 cm Height: 187 cm (206 cm with front door open)
Weight	Approximately 600 kg
Maximum power consumption	750 W
Compound plate formats	MTP-384 (ANSI SLAS standard)
Pipetting volume	4 µL to 20 µL (but not changeable by the user)
Parallel recordings	384
Automated operation	≥4 hours fully automated operation
Supported barcode standards	CODE39, CODE128, EAN/UPC, and Codabar
Barcode reader ambient light immunity	Sunlight: Up to 97000 lux
Centrifuge	Speed controlled by the software in the range of 1 g to 300 g
Declarations of Conformity	CE
Fuses (Mains)	T6.3AH/250Vac

9.5 QChip 384 plate specifications

Waste reservoir capacity	25 µL (waste is continuously removed by the 384-robot)
Electrodes	Ag/AgCl (individual for all 384 sites)

9.6 Data acquisition system

The Qube data acquisition system consists of a custom QAmp amplifier system comprising 12 amplifier boards that each contain 32 individual patch clamp amplifiers operating in parallel. The technical specifications of each QAmp amplifier can be found in Table 19.

Table 19 QAmp Patch Clamp Amplifier technical specifications

Sampling rate and resolution	50 kHz, 16 bit
RMS noise	<40 pA at full bandwidth
C_{fast}, R_{series}	C_{fast}, and R_{series} compensation in single-hole mode (optional)
Bandwidth	20 kHz
Input current range	–300 nA to +300 nA
Control output voltage range	–400 mV to +600 mV
Voltage range in Current Clamp	–200 mV to +200 mV
Set current precision in Current Clamp	<2 pA

The raw data sampled at 50 kHz may optionally be filtered and down sampled to save data storage and improve the performance of the data analysis software.

Appendix A Licenses for software modules

Third-party software

The following open source third-party software is distributed with the Qube system and software:

Amazon AWS SDK 1.11.256 from https://aws.amazon.com/tools/

Apache Ant 1.8.4 from http://ant.apache.org/

Apache Commons Codec 1.9 from https://commons.apache.org/proper/commons-codec/

Apache Commons Exec 1.1 from http://commons.apache.org/proper/commonsexec/

Apache Commons IO 2.4 from http://commons.apache.org/proper/commonsio/

Apache Commons Lang 3.3 from http://commons.apache.org/proper/commonslang/

Apache Commons Logging 1.1.3 from https://commons.apache.org/proper/commons-logging/

Apache Commons Math 3.3 from http://commons.apache.org/proper/commonsmath/

Apache HTTPComponents 4.5.2 from https://hc.apache.org/

Apache OpenOffice 4.1.3 from https://www.openoffice.org/

Apache POI 3.9 from http://poi.apache.org/

Apache XMLBeans 2.3.0 from http://xmlbeans.apache.org/

Batik 1.7 from http://xmlgraphics.apache.org/

Bridj 0.7.0 from https://code.google.com/p/bridj/

Boost 1.58.0 from http://www.boost.org/

Docking Frames 1.1.2 from http://dock.javaforge.com/

Dom4j 1.6.1 from http://dom4j.sourceforge.net/

Guava 14.0.1 from https://code.google.com/p/guavalibraries/

IPMItool 1.8.18 from https://sourceforge.net/projects/ipmitool/

IText 2.0.8 from http://itextpdf.com/

Jackson FasterXML from https://github.com/FasterXML

Jasper Reports 1.1.1 from http://sourceforge.net/projects/jasperreports/

Java 1.8.0 from http://www.java.com

JavaMail 1.4.1 from http://www.oracle.com/technetwork/java/javamail

Java Native Access 4.0.0 from https://github.com/twall/jna

JCIFS 1.3.18 from https://jcifs.samba.org/

JCommon 1.0.0 from http://www.jfree.org/jcommon/

JDatePicker 1.3.2 from http://sourceforge.net/projects/jdatepicker/

JFreeChart 1.0.0 from http://www.jfree.org/jfreechart/

JGoodies Looks 2.6.0 from http://www.jgoodies.com/

Joda-Time 2.8.1 from https://www.joda.org/joda-time/

JOpt Simple 4.5 from http://pholser.github.io/jopt-simple/

jSSC 2.6.0 from https://code.google.com/p/javasimpleserialconnector/

JUnit 4.10 from http://junit.org/

MariaDB Java Client 2.6.2 from https://mariadb.org/

MigLayout 4.0 from http://www.miglayout.com/

Netty 4.1.17 from https://netty.io/

Phymem revision 5 from https://www.codeproject.com/script/Articles/ListVersions.aspx?aid=35378

PuTTY 0.62 from http://www.chiark.greenend.org.uk/~sgtatham/putty/

RXTX 2.2 from https://github.com/grafjo/rxtx/

Silk icon set 1.3 from http://www.famfamfam.com/lab/icons/silk/

Simple Logging Façade for Java 1.7.2 from http://www.slf4j.org/

Swinglayout 1.0.3 from https://java.net/projects/swinglayout/

UltraVNC 2.0.5 from http://www.uvnc.com/

Webcam Capture 0.3.10 from http://webcam-capture.sarxos.pl/

WinSCP 5.9.6 from https://winscp.net/

You can find the license conditions for the third-party software in the installation folders of the software.

You can also find the license conditions and the source code for the third-party software at http://opensource.sophion.com/opensource.

Disclaimer: The above list reflects the versions and modules used at the writing of the current manual revision. Earlier and later versions of the Qube software and system may deviate from the above list.

Appendix B Consumables, accessories, and spare parts

The consumables, accessories, and spare parts used in or addable in a Qube system can all be purchased from Sophion.

Description	Order number
QChip 384 (1 patch clamp hole per site)	SB2110*
QChip 384X (10 patch clamp holes per site)	SB2115*
QChip 384D (1, 2, 6, 10, 16 & 36 holes per site)	SB2171*
QChip hole size 2 (range 1-9)	SB2192**
QChip hole size 7 (range 1-9)	SB2197**
QChip custom hole size or custom hole numbers	SB21xx***
Pipette tips for 384-robot (30 µL), 10 racks of 384	SB2200
Pipette tips for the ACP (5 mL), 5 x 24 tips	SB2210
QClean for daily and weekly care, 500 mL	SB2058
QStirrer cup small (60 mL), 70 pcs	SB2050
QStirrer cup large (125 mL), 30 pcs	SB2250
Magnets for QStirrer, 10pcs	SB3070
QFuge tubes (5 mL), 200 pcs	SB2251
ACP reservoir for extracellular saline (300 mL), 4 pcs	SB2252
ACP reservoir for extracellular saline (500 mL), 1 pcs	SB2255#
ACP waste bottle (500 mL), 50 pcs	SB2253
1 x 1 MTP, low profile, 25 pcs	SB2260
1 x 16 MTP, low profile, 25 pcs	SB2261
24 x 1 MTP, low profile, 25 pcs	SB2262
QVac for securing steady and sufficient vacuum, region specific	SB301x
Wheels for Qube for laboratory use	SB3302§
Cell Transfer Plate (CTP), 1 pcs	SB3301
ccCTP, CTP with 16 troughs for cell cloning, 1 pcs	SB3300
ccCTP8, CTP with 8 troughs for cell cloning, 1 pcs	SB3308
ccCTP4, CTP with 4 troughs for cell cloning, 1 pcs	SB3304
Stacker module, automation package	SB3310
Autofill reservoir	SB3311
Series resistance compensation capability	SB3320
LMS supply container	SB3331
LMS waste container	SB3332
External data server, software solution	SB3340
Current Clamp capability	SB3350
Temperature control, region specific	SB336x
Training module on-site	SB4060¤
Training module at Sophion in your region	SB4080¤¤
Service and support, Silver level	SB4410
Service and support, Gold level	SB4430
Service and support, Platinum level	SB4450
Periodic Maintenance a la carte	SB4445
Customer specific block templates – contact your app. scientist	SB66xx

*These items must be ordered in multiples of five due to packing. **minimum order size is 100. *** possible hole numbers are 2, 6, 16 & 36, minimum order size 100. #Necessary for stacker operation. §Does NOT mean that Qube can be wheeled around without contacting Sophion, rather it is intended for earthquake region requirements. ¤Service contracts have a varying number of days. ¤¤Included in Gold and Platinum contracts and reduced price for Silver contract.

Appendix C Safety guides

This appendix describes examples of potential accidents or malfunctions in using the Qube instrument and suggestions for what to do if they occur. The procedures listed in the following tables are only meant as suggestions, and the list does not cover all possible events. It is important that this list is considered together with regional and national safety regulation and that both integrated into local safety procedures of the laboratory.

Hardware safety guide

If this happens	We suggest doing this
Cabinet glass or other parts of the cabinet are broken	The Qube instrument is unsafe to use if the cabinet is not intact 1. Discontinue use 2. Disconnect the power supply cable 3. Call a service engineer authorized by Sophion
The 384-robot pipetting head, the robot gripper arm or other moving parts jam, crash, or act strangely	1. Press the emergency stop button 2. If liquids are spilled into the Bed of Nails, set Qube to standby, shut down the internal PCs and turn off power immediately 3. Contact a service engineer authorized by Sophion
If the LMS tubes or LMS containers are leaking	 **Warning!** The LMS tubes or LMS containers may contain cells and hazardous or toxic materials. Wear protective clothing. 1. 'Stop' the run 2. Disconnect and empty all supply containers 3. Reconnect the supply containers 4. Run utility protocol 'Washing station: Empty' 5. Empty the LMS waste containers 6. Turn off the internal PCs and shut down Qube on the main switch 7. Call Service engineer authorized by Sophion  **Note!** If the leak has drained into the bottom of the LMS drawer, the LMS safety feature will automatically stop Qube operation. Call a Service engineer authorized by Sophion for further assistance.

Cell handling safety guide

If this happens	We suggest doing this
If the QStirrer cup for cells is dropped and cells are spilled on the worktable	1. Wear protective clothing 2. Remove the QStirrer cup 3. Wipe up the spill with paper towels 4. Clean the affected area with decontaminant 5. Clean up using 70% ethanol 6. Dispose of the waste in containers appropriate for biohazardous waste 7. If large amounts of liquid were spilled and there is a risk that liquid has penetrated to the interior of the Qube instrument, call a service engineer authorized by Sophion. The service engineer should be informed about the nature of the spilled liquids
If the user drops the centrifuge test tube and cells are spilled on the worktable	1. Wear protective clothing 2. Remove the centrifuge test tube 3. Wipe up the spill with paper towels 4. Decontaminate the affected area with decontaminant 5. Clean up using 70% ethanol 6. Dispose of the waste in containers appropriate for biohazardous waste
If the centrifuge splashes droplets out onto the worktable	1. Wear protective clothing 2. Wipe up the spill with paper towels 3. Decontaminate the affected area with decontaminant 4. Clean up using 70% ethanol 5. Dispose of the waste in containers appropriate for biohazardous waste
If the centrifuge tube with its contents is thrown out of the centrifuge	1. Press the emergency stop button 2. Wait until the centrifuge has stopped spinning 3. Remove the centrifuge tube 4. Wipe up the spill with paper towels 5. Decontaminate the affected area with decontaminant 6. Clean up using 70% ethanol 7. Dispose of the waste in containers appropriate for biohazardous waste 8. Examine the Qube worktable for damage and call a service engineer authorized by Sophion if damage has occurred
If a used QChip 384 is dropped and droplets are splashed onto the worktable	1. If the robot gripper arm dropped the QChip 384, press the emergency stop button. See 8 for further information 2. Wear protective clothing 3. Dispose of the used QChip 384. Wipe up the spill with paper towels 4. Decontaminate the affected area with decontaminant 5. Clean up using 70% ethanol 6. Dispose of the waste in containers appropriate for biohazardous waste
If the user touches a well on a used QChip 384, potentially containing cells and drugs	If gloves were not used, wash hands thoroughly in tap water If gloves were used, dispose of the gloves in an appropriate container

Compound application safety guide

If this happens	We suggest doing this
If a reservoir or MTP is dropped on the worktable and the content is spilled	1. If the robot gripper arm dropped a compound plate, press the emergency stop button. See chapter 8 for further information 2. Wear protective clothing 3. Dispose of the MTP or reservoir 4. Wipe up the spill with paper towels 5. Decontaminate the affected area with decontaminant 6. Clean up using 70% ethanol – except the manifold 7. Dispose of the waste in containers appropriate for biohazardous waste 8. If large amounts of liquid were spilled and there is a risk that liquid has penetrated to the interior of the Qube instrument, call a service engineer authorized by Sophion. The service engineer should be informed about the nature of the spilled liquids
If a user gets any liquid on his or her hands	If gloves were not used, wash the hands thoroughly using soap and running water If gloves were used, dispose of the gloves

Appendix D Terms and abbreviations

Term (Abbreviation)	Definition
Amplifier (AMP)	This device makes it possible to record current or voltage depending on clamping mode In voltage clamp mode, it can record currents in the pA to nA range. The maximum current is +/- 300 nA In current clamp mode, it can record voltages in the µV to mV range. The maximum voltage is above +/- 200 mV
Artificial Reference QChip (ARQ)	Measurement plate with integrated electronics for testing the Qube system
Automated Cell Preparation Module (ACP)	The functions of the ACP: • Keeps the cells (suspended in cell media and added to the cell reservoir by the user) from sedimenting in the reservoir using a magnetic stirrer • Transfers the amount of suspension necessary for a single measurement plate from the container to a vial in the centrifuge • Spins the cell suspension, removing the supernatant, and adds extracellular fluid (EC) • Dispenses the cell suspension into the Cell Transfer Plate (CTP) from where the cells are pipetted onto the measurement plate four columns at a time
Auxiliary sweeps	The sweeps used for estimating parameters such as R_{total} and C_{total}, etc. All auxiliary sweeps are executed with negative voltage steps from the holding potential during parameter estimation. The step size is either -10 mV or -30 mV depending on the parameter to be estimated
BioChip Interface (BCI)	The interface between the Qube and the QChip 384. The BCI is the complete measurement station including amplifiers, BON, and the pneumatically operated manifold connected to the pressure system
Bed of Nails (BON)	Electrical connections between the QChip 384 and the Qube. Part of the BioChip Interface (BCI)
Block template	Templates that can be used to create protocols in the ViewPoint software
Block	A copy of a block template that has been inserted into a protocol
Compound list	List of compounds used for mapping the compounds in the plate. The list designates the site, name, and concentration of the compounds on the compound plate
Compound plate	A 384-well Microtiter Plate (MTP) on which some or all of the wells contain compounds
Cell Cloning Cell Transfer Plate (ccCTP)	A plate with 4, 8 or 16 troughs where different cell suspension can be added manually and picked up by the 384-robot. Used for cell cloning assays

Term (Abbreviation)	Definition
Cell Transfer Plate (CTP)	The plate into which the ACP pipettes the cell suspension and from which the 384-robot picks up the cells
Device	A software component that controls a specific instrument component, e.g. Pipetting Robot device, Pressure Unit device, or Amplifiers device
Dose-response study	A study of the relationship between the concentrations of a compound applied and the recorded current responses from cells
Extracellular Saline Solution (EC)	The Ringer's solution in the front side (FS) channels
Flow channel	Liquid flow channels in the QChip 384
Giga seal	A high-electrical-resistance seal between the chip orifice and the cell membrane above 1 GΩ
Hit	A compound has the desired effect or any other effect on the ion channels in the screening
Intracellular Saline Solution (IC)	The Ringer's solution in the rear side (RS) channel
Liquid Management System (LMS)	The liquid exchange system for pipette tip wash in the Qube
Measurement site	An individual, physical measurement site on a QChip 384. This comprises the chip, the electrodes, the flow channels and the waste reservoir. A QChip 384 consists of an array of measurement sites
Microtiter plate (MTP)	A format primarily used to store compounds in an array of wells
Plan	An overall term for a group of device actions that can be executed in Qube software. Protocols and utility protocols are examples of plans
Plan run	Executing a plan leads to the creation of a Plan run which contains the results generated during the plan execution. Plan runs that are based on execution of protocols are also denoted Protocol Runs
Plate run	Collection of plan runs associated to a specific QChip 384
Priming	The process by which intracellular and extracellular saline solutions are brought to fill the space on either side of the patch clamp hole(s) in the QChip 384
Protocol	A user defined plan that consists of blocks. There are five different protocol types: • Worktable protocol • Cell preparation protocol • Whole-cell protocol • Experiment protocol • Cleanup protocol

Term (Abbreviation)		Definition
Protocol run		A plan run based on a protocol execution
QChip 384		Measurement plate for Qube
QClean		Special cleaning liquid for the LMS
QFuge		A robot-accessible micro-centrifuge. The QFuge is part of the ACP
QStirrer		A continuously running magnetic stirrer for cell suspension stored in a QStirrer cup. The QStirrer is part of the ACP
Qube Instrument		The Qube instrument that performs the screening, including robotics and hardware components
Qube System		Automated patch clamping system using the QChip 384 measurement plate
Screening		Electrophysiological experiments performed to determine whether compounds affect the current from ion channels expressed in the cells
Sophion ViewPoint		Sophion proprietary software to control the Qube instrument
Sophion Analyzer		Software for analysis of Qube instrument data
Sophion ViewPoint Maintenance		Sophion proprietary software for administering the Qube instrument
V_{xx}	Inactivation	A potential where $_{xx}$% of the channels are in the inactivated state (when using that specific protocol)
	Activation	A potential where $_{xx}$% of the channels are in the activated state (when using that specific protocol)
Whole-cell configuration		A measurement configuration in which it is possible to record the current response from ion channels expressed in the entire cell membrane.

Appendix E Logbook for daily and weekly care

Date	Initials	Flush	Deconta-minate washing station	Empty system water and waste	Care		Comments/errors
					Daily	Weekly	

Appendix F Declaration of Conformity

Declaration of Conformity

Manufacturer:

Sophion Bioscience
Baltorpvej 154
2750 Ballerup
Denmark

Product description:

Qube 384 Basic
Qube 384 w Stacker

We declare under our sole responsibility that the products, to which this declaration relates, are in conformity with the Council Directives on the approximation of the laws of the EEC Member States relating to the following:

Directives

Electromagnetic compatibility 2004/108/EC
Low Voltage Directive 2006/95/EC
Machinery Directive 2006/42/EC

and

Standards

EN 61326: 2013	Product family standard, Measurement, control and laboratory equipment
EN 61010-1: 2010	Safety requirements for electrical equipment for measurement, control and laboratory use
EN 61010-2-081: 2015	Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes
EN 12100: 2010	Risk assessment and risk reduction

Year of applying CE marking: 2013

Location: Ballerup

Name : Thais Johansen
CEO
Sophion Bioscience a-s

16-1-2017
Date / Signature