User's Manual

CQ1

Confocal Quantitative Image Cytometer

IM 80J01A01-01E

The translation of the original instructions

~However warning labels are not the same.~

This document corresponds to the following versions of software

Measurement software R1.04

Introduction

Thank you for purchasing the Confocal Quantitative Image Cytometer CQ1.

This manual describes the functions, installation and wiring procedures, operating procedures and handling precautions of the CQ1. Before you start to use the CQ1, please read this manual carefully to enable correct use of the system. After reading this manual, keep it in a handy place so that you can refer to it whenever necessary.

Notes

- The information contained in this manual is subject to change without prior notice due to improvements in performance and functionality or for other reasons.
- This manual has been prepared with the utmost care; however, if you have any questions, or note any errors, please contact our branch or sales office of the neighborhood.
- Unauthorized reproduction or reprinting of this manual in whole or in part is prohibited.
- Follow the operating instructions to keep stable system operation.

Trademarks

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- In this document, registered trademarks or trademarks are not indicated by ™ and ®.

History

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For Safe Use of This Equipment

To ensure safe and correct use of this equipment, be sure to follow the precautions below when using this equipment. If this equipment is used in a manner not specified in this manual, the protection provided by this equipment may be impaired. If you do not follow the precautions when using the equipment, we assume no responsibility nor provide any guarantee regarding its safety and function.

Following symbols are used in the manual in the following cases.



WARNING

This symbol describes precautions to avoid hazardous situations that could result in a bodily injury or death of the user upon mishandling and/or incorrect use of the equipment.



CAUTION

This symbol describes precautions to avoid hazardous situations that could result in a minor bodily injury of the user, or that could result in damage to the equipment upon mishandling and/or incorrect use of the equipment.

POWER ON

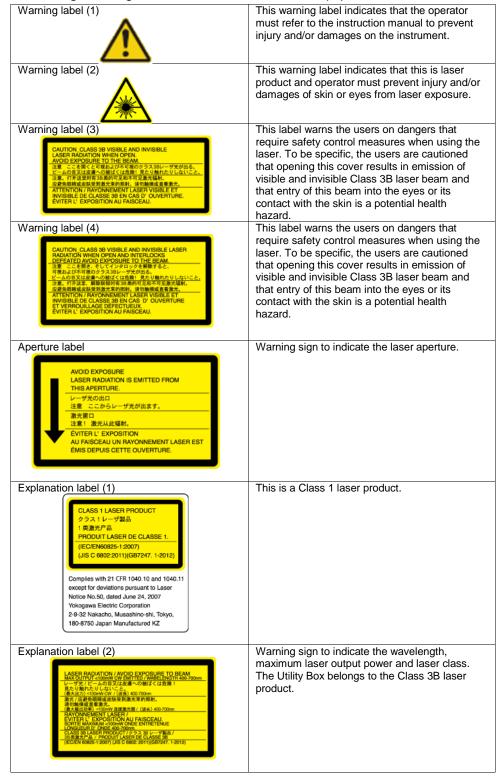
This symbol indicates POWER ON.



POWER OFF

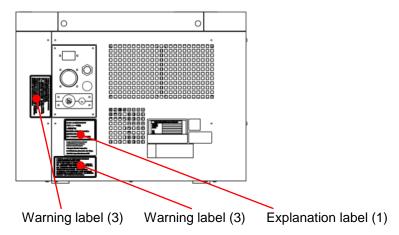
This symbol indicates POWER OFF.

Following warning labels are attached on this equipment.

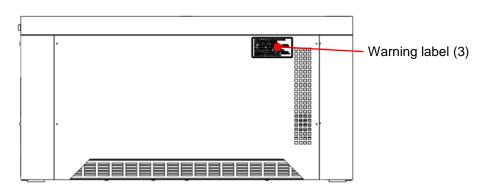


Location of Warning Labels

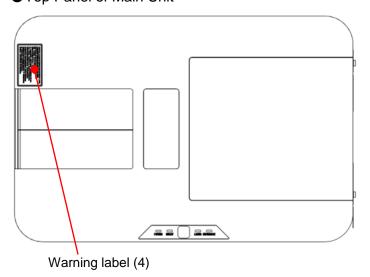
● Right Side of Main Unit



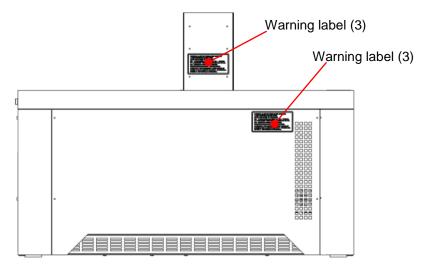
Rear Panel of Main Unit



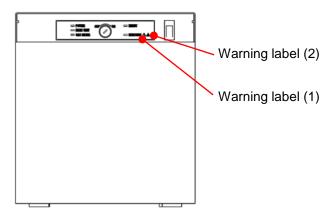
●Top Panel of Main Unit



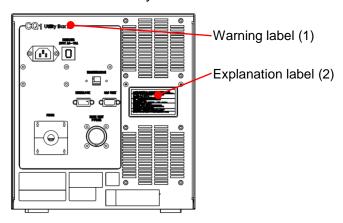
● Rear Panel of Main Unit (Transmission Illumination Option)



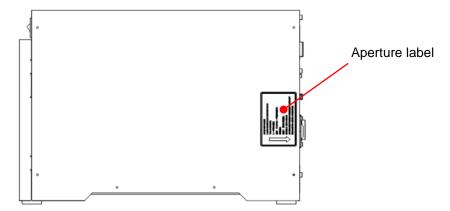
● Front Panel of Utility Box



Rear Panel of Utility Box



● Right Side of Utility Box



Observe the following precautions to prevent hazardous situations that could result in a bodily injury or death of the user, such as electric shock, or that could result in damage to the equipment.

/!

WARNING

- Be sure to turn on the power to the equipment after confirming that the power-supply voltage of the equipment matches the voltage of the supplied power. Always use the dedicated power cable supplied with your equipment.
- This is a Class 1 laser product. However, the equipment houses a Class 3B laser, which is protected by the enclosure and the interlocks provided at openings. When using this product, heed the precautions explained in "Laser Products Handling Precautions."
- Controls and adjustments other than according to the procedures specified herein will result in dangerous exposure to laser.
- If you use Gas Mixer, CQ1 emits max. 20 % CO₂ mixture gas at a rate of 200ml/min. If CO₂ concentration in the air exceeds 3%, it may be harmful to the human body. Be sure to provide ventilation, lead exhaust gas from CQ1 to outside of the room, or take other appropriate measures. It is also recommended that an alarm system be installed in case of emergency.
- If you use Gas Mixer, CQ1 emits min. 0.1% low O₂ gas at a rate of 200ml/min. If O₂ concentration in the air drops below 18%, it may be harmful to the human body. Be sure to provide ventilation, lead exhaust gas from CQ1 to outside of the room, or take other appropriate measures. It is also recommended that an alarm system be installed in case of emergency.
- Do not put a container with liquid or powder on the Main Unit,
 Utility Boxes or cables of this instrument. Also, do not spill liquid or powder on these materials. These materials may cause electric shock and suffer electrical or mechanical damages.
- Do not use the equipment by wet hand. It may cause electric shock and suffer electrical or mechanical damages.



✓! CAUTION

- Use the I/O terminals of the equipment within the ranges of the specifications to prevent damage to the equipment.
- This product uses precision optics. Therefore, do not use the
 equipment in locations where there are large vibrations, a lot of
 dust, high humidity and high temperature (in places near heating
 equipment or exposed to direct sunlight), rapid changes in the
 temperature (in places subject to dew condensation), or corrosive
 or combustible gases.
- Never touch any of the internal parts of this product. And never take apart or modify this product. The optical system housed in the product may become dirty, damaged or out of calibration and so on, leading to equipment failure.
- If the equipment has malfunctions or damages, please immediately cease use and contact us.
- This product is designed with the intention to use in industrial environment. If this product is used in residential environment, it may have influences to other equipments..

Laser Products Handling Precautions

- Laser products are classified according to their exposure emission limit determined by the wavelength and power characteristics of laser beam. A different set of common safety standards applies to each class of products. The product explained herein belongs to Class 1. However, the equipment houses a Class 3B laser, which is protected by the enclosure and the interlocks provided at openings.
- Only service personnel can remove the covers on which a warning label is attached. If these covers are removed, Class 3B laser beam will be emitted. Directly looking into Class 3B laser beam or beam reflected on a mirror may cause eye damage and is extremely dangerous. Exercise due caution when handling this product.

(Reference)

In the IEC standard, the laser classes are briefly explained as follows:

Class 1: Essentially safe. Continue to look into the Class 1 laser will not cause any danger.

Class 2: If the Class 2 laser is looked into for a period not more than 0.25 second, there is no risk of danger.

Class 3B:Looking into the laser beam from this class of equipment is dangerous. Even if the specification of the laser equipment used does not exceed this class, there is still a risk of eye injury.

Safety Standards

A class 1 laser is safe under all conditions of normal use. This
means the maximum permissible exposure can not be exceeded.
This equipment is designed in accordance with the IEC60825-1
Radiation safety standards for laser products and these lasers
must be labeled with the following label, but are exempt from the
requirements of the Laser Safety Program.

CLASS 1 LASER PRODUCT

Preventive Measures on Safety

Mirror reflection

Metals and other glossy reflective surfaces reflect the laser beam. Exercise caution to prevent unexpected mirror reflection.

Beam path

Never look directly into the laser beam or touch the laser beam.

WEEE(Waste Electrical and Electronic Equipment), **Directive**



(This directive is only valid in the EU.)

- This instrument complies with the WEEE Directive marking requirement. The marking above indicates that you must not discard this instrument in domestic household waste.
- Product Category
 With reference to the instrument type in the WEEE directive Annex
 1, this product is classified as a "Monitoring and Control instrument" product.
- When disposing this instrument in the EU, contact the distributer whom you bought it from. Do not dispose in domestic household waste.

Indemnity

- Yokogawa shall provide no warranty regarding this product, unless otherwise specified separately in the "Warranty Rule."
- Yokogawa shall assume no responsibility for any loss suffered by a customer or third party as a result of use of this product, or any loss or indirect loss suffered by a customer or third party due to a defect in this product or any other problem not predictable by Yokogawa.

Checking the Content of the Package

After opening the package box, check if the following items are included before beginning to use of this product. Should you find that a wrong product has been delivered or any of the items is missing or if you notice any abnormality on the exterior, please contact the dealer from which you purchased your product.

CQ1

Part	Quantity	Description
CQ1 Main Unit	1	Confocal fluorescent microscope system to observe cells cultured in vessels such as Microplate or slide glass
USB cable	1	Communication cable to connect CQ1 main unit and workstation
Main Unit⇔UTB cable	1	Power supply and communication cable to connect CQ1 main unit and utility box
Utility Box	1	Equipment for supply power and excitation laser light
Optical Fiber	1	Optical fiber to guide laser from utility box into main unit
AC Cord (CQ1)	1	AC cord for utility box (Supplied by dealer)

CQ1 (Continue from previous page)

Part	Quantity	Description
Key	1	Key switch on utility box
Tie-wrap	1	Tie-wrap for bundle USB cable and Main Unit⇔UTB cable
Remote Interlock	1	Remote interlock connector on Utility Box

Objective Lens

Part	Quantity	Description
9'9ZNJ/\to\m\ 9'9ZNJ/\to\m\ 00'0\xXII. 00dysueldii sndwxto Objective lens	1~6	To be installed on CQ1 main unit One to six lenses are installable depending on the specification. Please refer to Parts List: CMPL 80J01A01-01 for details.

Workstation

Part	Quantity	Description
Workstation	1	Attached with mouse, key board, back-up CD and instruction manual.
AC Cord (WS)	1	AC cord for workstation. (Supplied by dealer)

Option (Sold separately)

Option (Soid Sepa		
Part	Quantity	Description
Monitor	1	(Supplied by dealer)
AC Cord (Monitor)	1	(Supplied by dealer)
Monitor⇔WS cable	1	(Supplied by dealer)
Transmission Illumination	1	Transmitted light for phase contrast/ bright field observation There are following 2 types - Bright field/ Phase contrast switchable type (manual) - Phase contrast type
Gas mixer (TOKAI HIT, GM-8000)	1	Supply mixture gas that O ₂ and CO ₂ concentration is contolled to stage attachment
Gas mixer connecting tube	1	Tube to connect CQ1 main unit and Gas mixer Attached if stage heater option is selected
Stage attachment (All wells imaging type)	1	Stage attachment that all wells of microplate can be imaged

Option (Sold separately, continue from previous page)

Part	Quantity	Description
1 dit	Quantity	Description
Stage attachment (Chambered type)	1	Stage attachment which can keep the temperature, O ₂ CO ₂ concentration and humidity constant
Sample holder for	1	Sample holder for set three 35 mm dishes
triple 35 mm dishes		
Sample holder for single 60 mm dish	1	Sample holder for set one 60 mm dish
Sample holder for slide glass (ISO 8037/1)	1	Sample holder for set four slide glasses (ISO 8037/1)
Sample holder for cover glass chamber	1	Sample holder for set one cover glass chamber
60 mm dish ring	1	Hold silicone rubber when using chambered type stage attachment and 60 mm dish
Rubber for 60 mm dish	1	Set on 60 mm dish when using chambered type stage attachment
Holding plate for CGC	1	Hold silicone rubber when using chambered type stage attachment and cover glass chamber
Rubber for CGC	1 - 4	Set on cover glass chamber when using chambered type stage attachment Shape is different by type of cover glass chamber

Sample holder

Stage attachment

XY stage

Sample holders are set into the stage attachment as shown below.

How to use this manual

This user manual consists of Chapters 1 to 9, the details of which are explained below.

Chapter	Title and Content
1	Function Overview
	A functional overview of this equipment is explained.
2	Before Use
	Precautions for use and installation/wiring methods are explained.
3	Name of Each Part/Starting and Shutting Down the Equipment
	The name of each part and starting/shutdown of the equipment are explained.
4	Measurement procedures
	Procedures for measurement are explained
5	Functions of measurement software
	Screen of measurement software is explained
6	Maintenance
	Maintenance parts and consumable parts are explained.
7	Troubleshooting
	Error messages and troubleshooting procedures for this equipment are explained.
8	Specifications
	Specifications of this equipment are described.
9	Warranty Rule
	The rules of warranty applicable to this equipment are explained.

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1 Function Overview

1.1 About the CQ1

CQ1 is designed to quantitatively measure biological information from image data of each cell at high reproducibility. It enables acquisition of such information as cell functions, signal transduction, cell mobility (such as invasion) or morphological information from quantified image data after image processing, which is rather difficult to obtain by conventional flow cytometetric analysis. Different from flow cytometetric analysis in which cells are washed away, CQ1 measures the cells in their culture vessels such as microplate, thus it is possible to analyze the same cells repeatedly or follow their temporal changes.

Features of CQ1

High precision quantification of morphological information without detaching off the cells

It is possible to precisely quantify biological function or characteristics of each cell in a natural situation without breaking cell mass or detaching cell layers from culture dish. In addition to two-dimensional information, such as the area, various three-dimensional information, such as volume, surface area, cell number and location, granule location within each cell, fluorescent intensity, can be well visualized and displayed as graphs.

Live cell observation

Our proprietary confocal scanner unit, CSU series, is a confocal scanner which can be attached on an optical microscope to enable confocal observation. The best features of the CSU are the capabilities of high-speed confocal imaging with a minimal level of cellular photo-damage and photo-bleaching. Equipped with the CSU, the CQ1 enables three-dimensional and multi-color live cell observation. CQ1 is suitable for quality control, inspection and experiments of the studies in cell engineering field, since you don't have to spoil the cells after observation.

Highly reproducible data

Highly reproducible data can be acquired by stabilizing excitation laser power with the power monitor function, and also by periodical calibration to eliminate effects of any other variations.

Measurement values

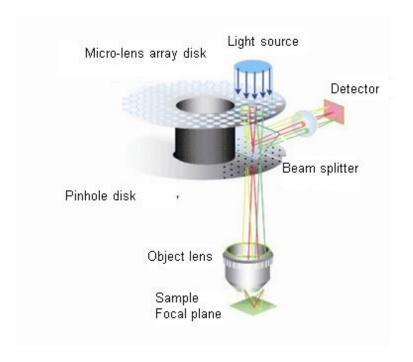
CQ1 outputs the following measurement values by analyzing confocal images of fluorescently labelled each cell.

	Measurement	Unit	Measurement Method	Description
Morphological features	Area	μm²	2D measurement	Measure the area of objects.
	Circumference	μm	2D measurement	Measure the circumference of object.
	Circularity	-	2D measurement	Compare the area of the circumscribed circle and the area of the object. Circularity is expressed by a positive number of less than 1.0, and perfect circle is 1.0
	Volume	µm ³	3D measurement	Measure total volume of objects.
	Surface area	μm²	3D measurement	Measure total surface area of objects.
	Sphericity	-	3D measurement	Compare the volume of an intracellular structure and its circumscribed globe. Sphericity is expressed by a positive number of less than 1.0, and perfect globe is 1.0.
	Diameter	μm	2D measurement 3D	Measure diameter of the circumscribed circle of an object. Measure diameter of the circumscribed
			measurement	globe of an object.
Intensity	Sum	-	2D/3D measurement	Measure the sum of pixel intensity of objects.
	Average	-	2D/3D measurement	Measure average pixel intensity of objects.
	Standard deviation	-	2D/3D measurement	Measure standard deviation of pixel intensity of objects.
	Minimum	-	2D/3D measurement	Measure minimum pixel intensity among objects.
	Maximum	-	2D/3D measurement	Measure maximum pixel intensity among objects.

1.2 Principle of the Nipkow Disk System with Micro-lens

Two disks, including the "pinhole array disk" having many pinholes arranged in a helical pattern, and the "Micro-lens array disk" that condenses excitation laser to individual pinholes, are rotated jointly at high speed to perform multiple scans over the observation area with approximately 1,000 laser beams.

Multiple beam scans are performed not only at high speed, but also with each beam exciting fluorochromes at high efficiency and very low laser intensity. These results in an optimal live cell observation system where phototoxicity and fluorescence photobleaching are suppressed notably compared to any conventional systems while allowing high-resolution real confocal imaging.



Operating Principle of the Micro-lens enhanced dual Nipkow Disk System

2 Precautions for Use/Installation

2.1 Precautions for Use

Handling Precautions for This Equipment

- This equipment uses plastic parts. When cleaning these plastic parts, wipe with a dry, soft cloth without using water or any solution.
 Never use benzene, thinner or any other chemical or cleaning solution. Doing so may result in discoloration, deformation or damage.
- Do not bring electrically charged objects near the signal terminals.
 Doing so may result in failure.
- Do not spill any volatile chemical over the display or keep the display in contact with any rubber or plastic product for an extended period of time.
- Do not install patches (Windows Update, service pack, security patch and so on) to update operating system. Also, do not change environment setting (display setting, power management and so on) of Workstation. Control board and Software may not work normally by these changes.
- Do not install the third-party software which YOKOGAWA doesn't specify to Workstation. The performance of CQ1 may reduce.In addition, 8.3 filename generation of Windows NTFS file system is invalid in Workstation for performance improvement. Therefore, the third-party software may not work normally.
- Do not install any third-party hardware to CQ1 Main Unit, Utility Box, and Workstation. The third-party hardware may fail.
- In case of connecting Workstation to Internet, please install an antivirus program which your affiliated facilitate designated and perform virus check periodically. Please don't perform virus check during CQ1 operation because performance may reduce.
- Do not give shocks to this equipment.
- Should you notice smoke coming out of the Main Unit, foul smell, abnormal noise or any other abnormality, immediately pull out the power cable from inlet of equipment.
- If the product has failed, contact us without attempting to disassemble any of the internal assemblies or take any other action to resolve the problem yourself.
- Do not put anything on CQ1 Main Unit.

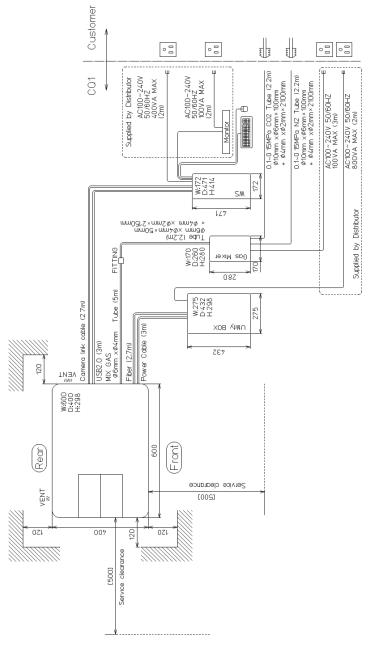
2.2 Installing the Equipment

Installation Conditions

Install this equipment in a location meeting the conditions specified below. Please call service personnel when you install or move the equipment.

Installation example 1)

In case of using CQ1 normal gas mixer.



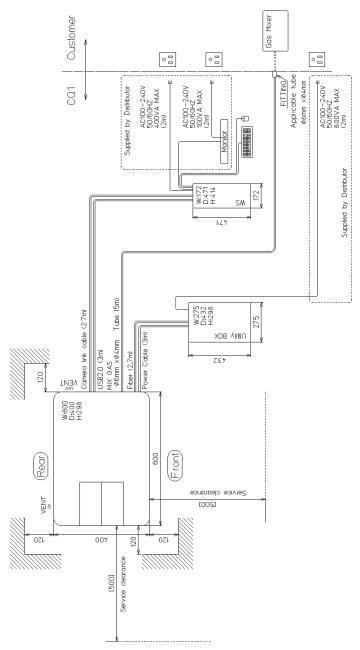
Note

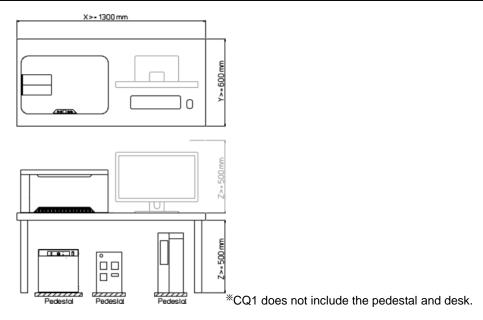
Please prepare following gas cylinder and regulator

Gas cylinder	Gas: N ₂ 100%, CO ₂ 100%
Regulator	Outlet pressure: Settable in 0.1 — 0.15MPa Piping: Silicone tube (inside diameter 6 mm, outside diameter 10 mm) is connectable

Installation example 2)

In case of using gas mixer that user prepares.





- Location where enough space is available
 If the ventilation openings are blocked, the equipment may become hot and eventually damaged. Provide enough space around CQ1 Main Unit and Utility Box.
 - -CQ1 Main Unit

Minimum Clearance

Front: 120 mm, Back: 120 mm, Above: 200 mm, Side: 120 mm

Recommended Table Size:

W 700 mm x D 500 mm x H 700 mm or larger

Withstand load: 100 kg or larger

Utility Box

Minimum Clearance

Back: 50 mm, Above: 50 mm, Side: 50 mm (Avoid placing on floor directly)

Gas Mixer

Minimum Clearance

Back: Provide enough space not to buckle tubes and cables Side: 50 mm (Avoid placing on floor directly)

- Avoid installing at highly humid and / or hot place such as close to heaters or under direct sunshine, or frequent temperature changes.**1
- Location of minimal mechanical vibration
 Never set any vibrating devices, such as centrifuge or mixer, on the same table with CQ1. Doing so may disrupt optical performance.

Level location

When installing this equipment, make sure the equipment does not tilt.

- Location of minimal soot, steam, dust, corrosive gases and so on.
- Location where electric power supply system of more than AC100V, 14A (1,400W) is available.

Installation Environment

Operating Temperature Range

CQ1: 15 - 35 °C

Gas Mixer: 20 − 35 °C

Operating Humidity Range

CQ1: 20 - 70 % RH, non-condensing

Gas Mixer: 10 - 85 % RH, non-condensing

^{*1} Subjecting the equipment to a sudden temperature shift may cause condensation.

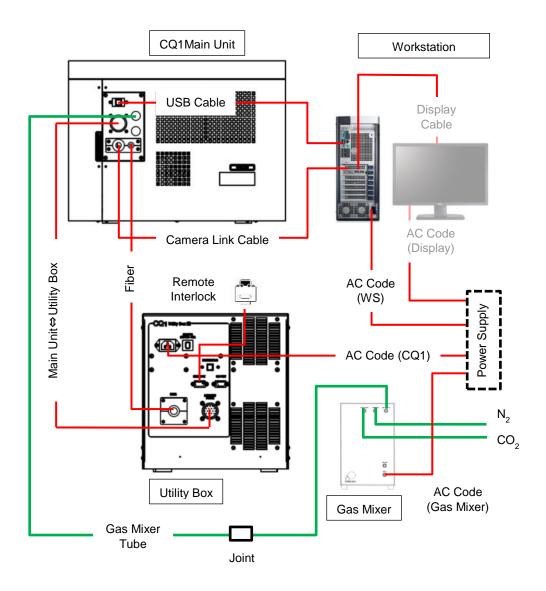
2.3 Wiring the Peripherals

Connect the cables after confirming that the power switch on the equipment is turned off.



CAUTION

Please don't connect any cables other than specified by YOKOGAWA.





WARNING

- Pulling a cable wired to this instrument with a strong force may cause damage to the cable or connected terminals on the instrument. Provide each cable with an ample allowance so that the input terminals on the instrument will not receive a direct pulling force.
- Securely connect all tubes from the gas cylinder (CO₂ 100%, N₂ 100%) through the gas regulator to the gas inlet tube to prevent gas leakage. It is recommended to install a gas leakage alarm system to avoid risk by gas leakage.



CAUTION

Please refrain from applying the following processing on the CQ1 workstation. If you do, there is a risk of malfunction of this instrument. In case, any problems happened as a result of such inappropriate processing, you are liable for the cost of repair.

- Install patches (Windows Update, service pack, security patch) to update operating system.
- Install any third-party software which YOKOGAWA doesn't specify.
- Install any third-party hardware.
- Use any third-party software and hardware.
- Connect to the internet.

2.4 Connecting the Power

Connect the plug on the other end of the power cable to a power outlet meeting the following conditions. For the power outlet, use a 3-pole socket with a protective grounding terminal.

Rated power-supply voltage: 100 to 240 VAC

Rated power frequency: 50 / 60 Hz

Allowable power-supply frequency fluctuation range: 48~63Hz Maximum power consumption: Main Unit, Utility Box 800VA

Gas Mixer 40VA

Workstation 400VA

Note

Subjecting the equipment to a sudden temperature shift may cause condensation.

Before connecting the power, take heed of the following warnings and cautions. Failure to do so may result in electric shock or equipment damage.



WARNING

Power supply

Be sure to turn on the power to the equipment after confirming that the power-supply voltage of the equipment matches the voltage of the power supply

Power cable and plug

To prevent electric shock and fire, use the power cable supplied by Yokogawa. For the main power plug, only use a power socket with a protective grounding terminal. Use of an extension cable without protective grounding wire will disable the protective operations

Protective grounding

To prevent electric shock, be sure to provide protective grounding before turning on the power to the equipment. The power cable supplied with this equipment is a 3-pin type with a grounding wire. Accordingly, use a 3-pin power socket with a protective grounding terminal. If a 3-pin to 2-pin conversion adapter (usable only in Japan) is used, securely connect the grounding wire of the conversion adapter to the protective grounding terminal.

Need for protective grounding

Do not cut the protective grounding wire running inside or outside this equipment or remove the connected wires from the protective grounding terminals. Doing so will put this equipment in a dangerous condition.

Defects in protective functions

If any of the protective functions such as protective grounding or any fuse is suspected as faulty, do not operate this equipment. Also check the protective functions to ensure absence of defects before operating this equipment

Use in gas

Do not operate this equipment in a location where it may come in contact with flammable or explosive gases or vapors. Using this equipment in such environment is very dangerous Removal of the case

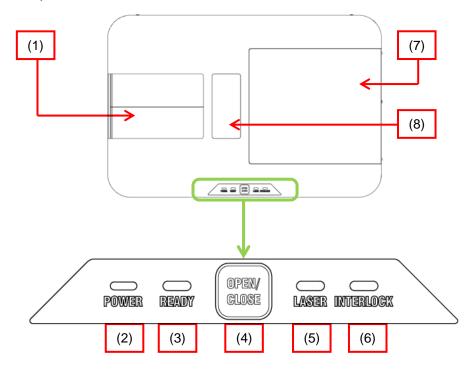
The case must not be removed except by Yokogawa's service personnel. Some parts in this equipment carry high voltage and a Class 3B laser is also housed inside. Accordingly, removing the case presents dangerous situations.

 External connections
 Connect each external device after providing proper protective grounding.

3 Name of Each Part/Starting and Shutting Down the Equipment

3.1 Name and Function of Each Part

CQ1 Main Unit



(1) Door

Door opens/closes either manually by the door button, or through the software; [CQ]button→[Sample]tab→[Sample Loader]→
[Open] button•[Close]button. Open door and load sample vessels, such as microplate, dish or slide glass, here.

(2) POWER Lamp

Lights when the power switch on the Utility Box is ON, and lights out when the power switch is OFF

(3) READY Lamp

Lights when the initialization of this equipment finished and get ready to use

(4) Door OPEN/CLOSE ButtonOPEN/CLOSE the door of the sample loading port

(5) LASER Lamp

Lights when the laser emits, and lights out when laser is OFF

(6) INTERLOCK Lamp

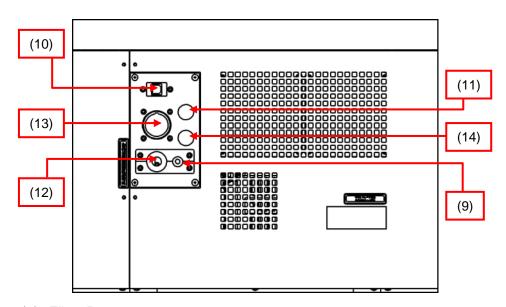
Lights when the door or cover is open. Laser emission stops when this lamp lights

(7) Sub Cover

Sub cover is opened when pouring distilled water into bath. Also, sub cover is opened when service maintenance.

(8) Illumination Setting Part

In case of transmission Illumination option, illumination unit is set on this part.



(9) Fiber Port

Port to connect the fiber to CQ1 Main Unit

(10) USB Connector

Connector for USB cable connection with the Workstation cable

(11) Gas Inlet Port

Port to connect the tube from Gas Mixer (option)

(12) Camera Cable Port

Inlet port to insert camera link cable to CQ1

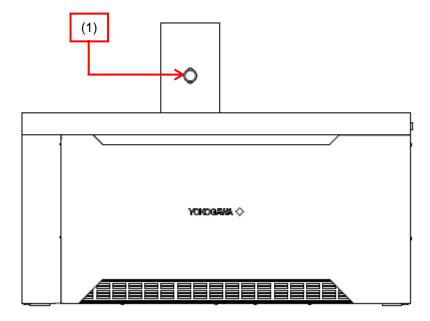
(13) Power Cable Connector

Port to connect the power cable from Utility Box

(14) Gas Outlet Port

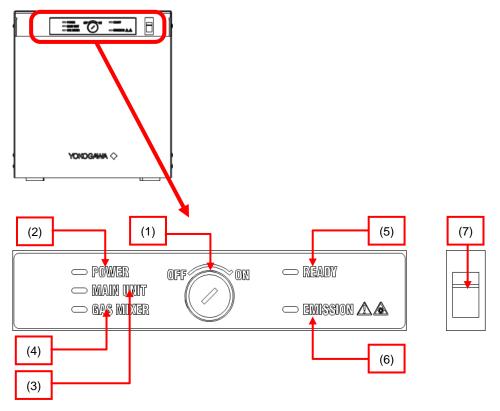
Port to exhaust the gas from chamber on XY stage when using Gas Mixer

Transmission Illumination



(1) Switching lever (bright field/ phase contrast) Switch the method of transmission illumination. Pull the lever toward to switch to bright field. Push the lever back to switch to phase contrast.

Utility Box



Key switch
 Laser power gets on and off by this key

(2) POWER Lamp

Lights when the power switch (7) of Utility Box is on, and lights out when the switch is off

- (3) MAIN UNIT Lamp
 Lights when connected with CQ1 Main Unit
- (4) GAS MIXER Lamp

Not in use

(5) READY Lamp

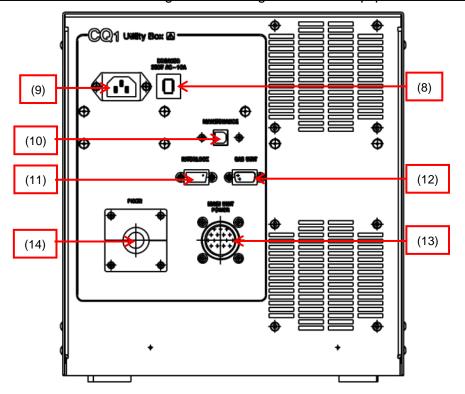
Lights when lasers are enable, and lights out when lasers are disable, and flicks when lasers are initializing

(6) EMISSION Lamp

Lights when laser emits, and lights out when laser is off

(7) Power Switch

Switch ON to power on CQ1 Main Unit Switch OFF to power off CQ1 Main Unit



(8) Breaker

For the Utility Box

(9) Power Cable ConnectorPort to connect power cable

(10) Maintenance Connector Not in use

(11) Remote Interlock Connector

Port to connect lase interlock connector

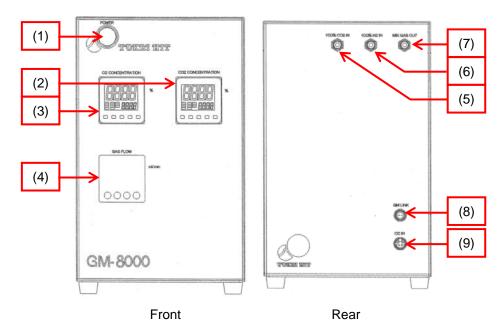
(12) Reserved Connector Not in use

(13) CQ1 Main Unit Power Cable Connector Port to connect power cable

(14) Fiber Port

Port to insert the fiber to Utility Box

Gas Mixer (Option)



(1) Power Switch

Switch ON to power on Gas Mixer Switch OFF to power off Gas Mixer

(2) CO₂ Concentration

Controller to set CO₂ Concentration of output mixture gas

(3) O₂ Concentration

Controller to set O₂ Concentration of output mixture gas

(4) Gas Flow

Controller to set flow rate of output mixture gas

(5) 100 % CO₂ IN

Port to connect the tube from CO₂ gas cylinder via the regulator

(6) 100 % N₂ IN

Port to connect the tube from N₂ gas cylinder via the regulator

(7) Mix Gas OUT

Port to connect the tube to CQ1 Main Unit

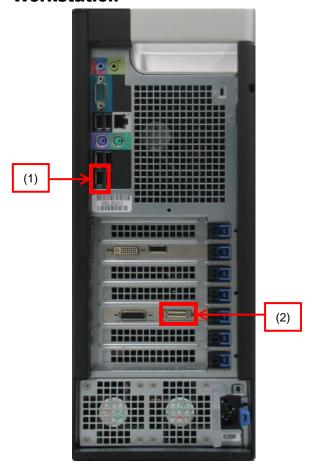
(8) GM LINK

Not in use

(9) DC IN

Port to connect cable of attached AC adapter

Workstation



(1) USB Connector

Connect to the USB connector (8) on the CQ1 Main Unit. Connect supplied cable as shown above.

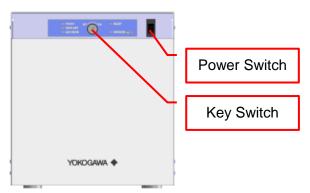
(2) Camera Link Cable Connector

Connect camera link cable out of CQ1 Main Unit as shown above.

3.2 Starting and Shutting Down of CQ1

3.2.1 Starting CQ1

(1) Turn the power switch ON, then turn the key switch ON..

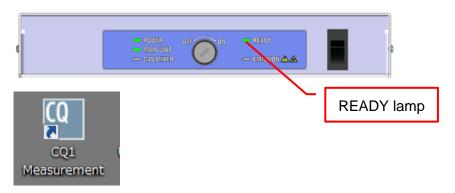




CAUTION

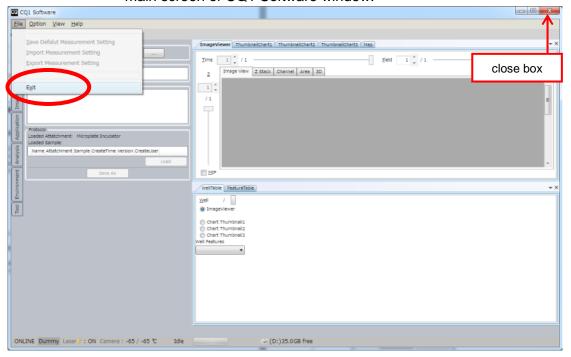
- Please confirm below before starting the equipment.
 - Is the equipment correctly installed?
 (Refer to 2.2 Installing the Equipment)
 - Are the cables correctly connected?
 (Refer to 2.3 Wiring the Peripherals)
- Immediately pull out the AC cord from inlet of equipment, when you notice smoke coming out of the main unit, foul smell, abnormal noise or any other abnormality.
- (2) Power ON the Workstation.
- (3) Confirm that there is enough free space in data (D:) disk drive. Data volume of one image that CQ1 outputs is about 11MB. Keep free space in data (D:) disk drive by considering total image quantity to acquire.
 - Also, free space of data (D:) disk drive is smaller than 1GB, measurement setting can't be carried out normally.

(4) Wait until Ready lamp on Utility Box turn ON after blinking. (It takes about 1 minute from turning the key switch on). Click the desktop icon of the Workstation to start CQ1 Software.



3.2.2 Shutting Down CQ1

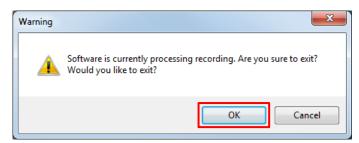
(1) Select [File] menu → [Exit] menu, or click the close box on the main screen of CQ1 Software window.



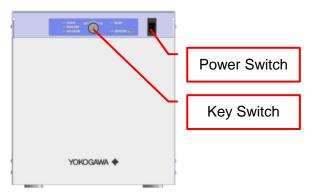
(2) Click [OK] button.



In case that measurement is running, following message is shown. Confirm that there are no problem to quit measurement then click [OK].



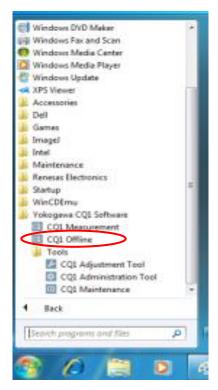
- (3) Shut down Workstation.
- (4) Turn the key switch on the Utility Box to OFF.
- (5) Turn the key switch OFF, then turn the power switch OFF..



3.2.3 Offline Mode

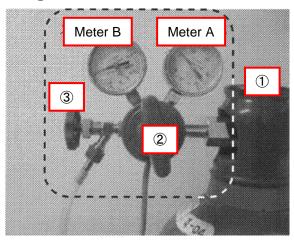
You can start CQ1 Software by "offline mode" without turning equipment on. It is convenient for viewing data or analyzing without measurement.

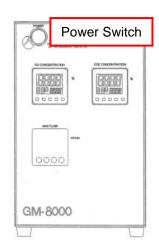
Starting procedure is different from above. Select"CQ1 Offline" from Windows start menu. (It is not icon on desktop).



3.3 Starting and Shutting Down of Gas Mixer (Option)

3.3.1 Starting Gas Mixer





- (1) Confirm that valve ③ of regulator is closed.
- (2) Confirm that valve ② is loosened by slight counterclockwise rotating.
- (3) Open valve ① of gas cylinder.
- (4) Open valve ③ of regulator.
- (5) By clockwise rotating valve ②, adjust outlet pressure (meter B) to be in the range of 0.1 MPa 0.15 MPa.
- (6) Turn the power switch of Gas Mixer.

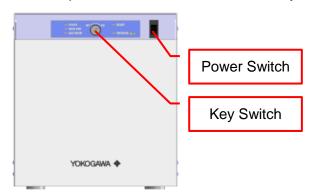
3.3.2 Shutting Down Gas Mixer

- (1) Close valve ③ of regulator to stop gas supply. (If the gas cylinder will not be used for long time, close valve ① of gas cylinder after closing valve ③.)
- (2) Turn the power switch of Gas Mixer.

4 Measurement Procedures

4.1 Measurement Procedures

Turn the power switch ON, then turn the key switch ON..



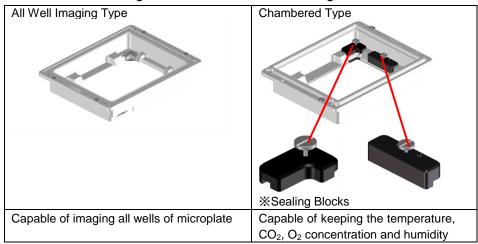
A CAUTION

Immediately pull out the AC cord from inlet of equipment, when you notice smoke coming out of the main unit, foul smell, abnormal noise or any other abnormality.

4.1.1 Exchanging Procedures of Stage Attachment

If you don't need to exchange stage attachment, go to procedure shown in 4.1.5 Setting Samples.

Features of each stage attachment are as following.

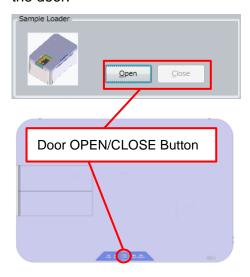


Note

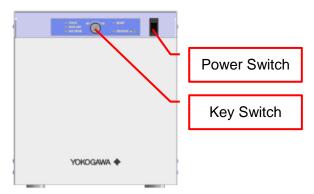
The range which can be imaged is different between types of stage attachment. For details, please refer to Technical Information TI 80J01A01-01E (Supported Sample Vessels).

<In case of exchanging All Well Imaging Type to Chambered Type>

Click "Sample" tab \rightarrow "OPEN" Button of CQ1 Software or do long press (3 seconds) of Door OPEN/CLOSE Button of CQ1 Main Unit to open the door.



Turn the key switch OFF, then turn the power switch OFF..



Loosen 2 screws.



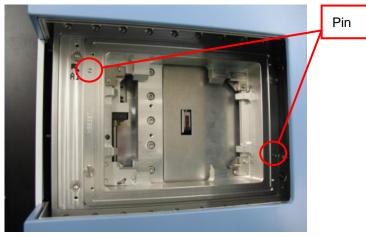
Push lever and lift up to remove Stage Attachment.





1 CAUTION

Lift up Stage Attachment vertically because it is positioned by pin. Be careful not to collide with cover.





Prepare Stage Attachment to set.

Direct clamp as following diagram and set Stage Attachment.

Be careful not to collide with cover.



Clamp

Put Stage Attachment on the stage and set it with pushing lever.



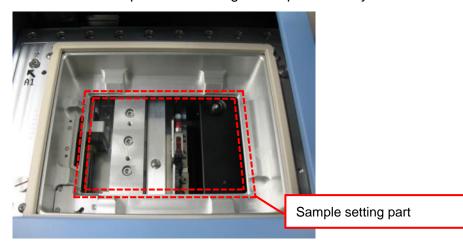
Lever



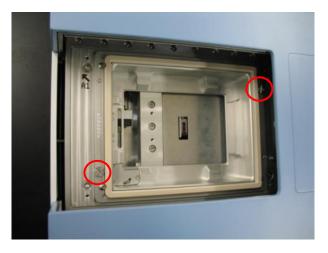


(CAUTION

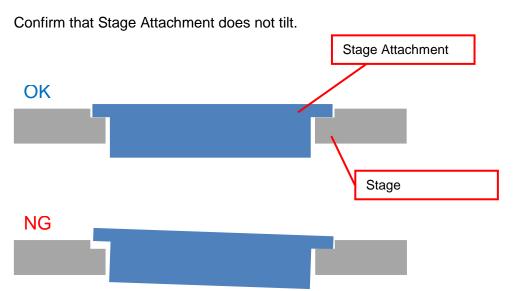
Be careful not to touch sample setting part of Chambered Type Stage Attachment when put it on the stage. This part is easily-broken.



Fasten 2 screws.







Set Sealing Block for Clamp on the clamp.



In case of using microplate, set Sealing Block for Microplate as following diagram.





Turn the power switch ON, then turn the key switch ON..



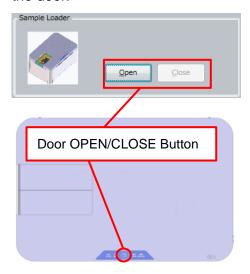
! CAUTION

After turning power switch ON, the door closes automatically. Please be careful not to pinch your fingers or others.

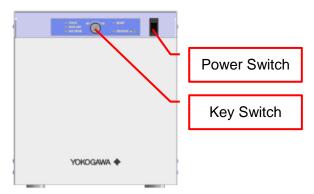
Immediately pull out the AC cord from inlet of equipment, when you notice smoke coming out of the main unit, foul smell, abnormal noise or any other abnormality.

<In case of exchanging Chambered Type to All Well Imaging Type>

Click "Sample" tab \rightarrow "OPEN" Button of CQ1 Software or do long press (3 seconds) of Door OPEN/CLOSE Button of CQ1 Main Unit to open the door.



Turn the key switch OFF, then turn the power switch OFF..



Remove Sealing Block for Microplate if it is set.



Remove Sealing Block for Clamp.

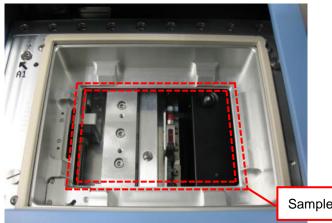


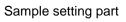
Exchange Stage Attachment referring to page 4-1.



CAUTION

Be careful not to touch sample setting part of Chambered Type Stage Attachment when remove it from the stage. This part is easily-broken.













Turn the power switch ON, then turn the key switch ON..



! CAUTION

After turning power switch ON, the door closes automatically. Please be careful not to pinch your fingers or others.

Immediately pull out the AC cord from inlet of equipment, when you notice smoke coming out of the main unit, foul smell, abnormal noise or any other abnormality.

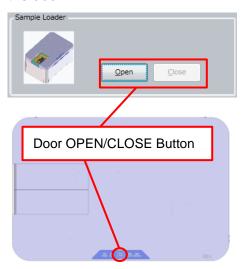
4.1.2 Procedures of Filling Water for Humidification

This procedure is only performed for controlling environment around sample. If you don't need environment control, go to procedure shown in 4.1.5 Setting Samples.

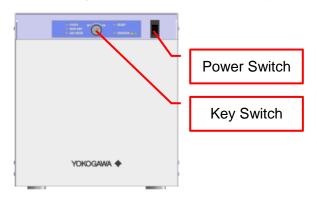
In case that measurement is running, click "Pause" button to pause.



Click "Sample" tab \rightarrow "OPEN" Button of CQ1 Software or do long press (3 seconds) of Door OPEN/CLOSE Button of CQ1 Main Unit to open the door.



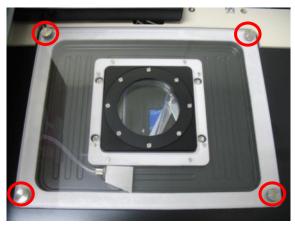
Turn the key switch OFF, then turn the power switch OFF..



Loosen 2 screws and remove sub cover.



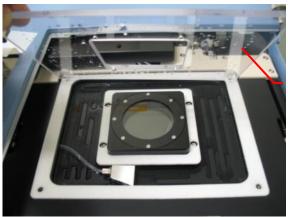
Loosen 4 screws and remove bath cover.





⚠ CAUTION

- Please don't remove screws from bath cover.
- During temperature controlling, water drops are formed on the downside of the bath cover. Please be careful not to drop water inside the equipment when removing bath cover. If water drops inside the equipment, wipe it completely before
 - turning CQ1 on.



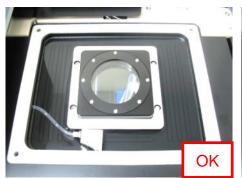
Water drop

If the waterbath is empty, pour 25 ml of distilled water.

(It is recommended to pour by syringe.)



Pour distilled water to cover whole area of the bath.



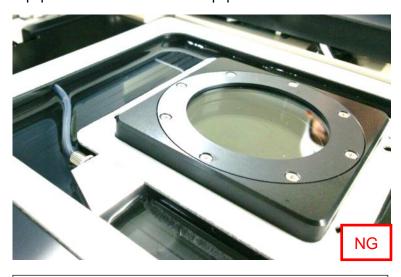




(CAUTION

It is acceptable that the bath is not filled with water.

If too much water is poured, it causes water immersion into the equipment and failure of the equipment.



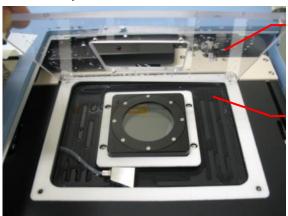
Note

Appropriate humidity level is maintained over 6 hours when 25 ml of water is poured.



extstyle ext

After measurement, wipe water in the bath and on the downside of bath cover everytime.

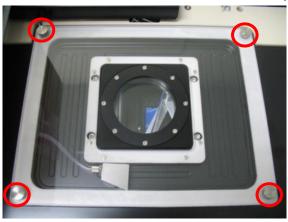


Water drop on the downside of bath cover

Water remains in the bath

Set bath cover and tighten 4 screws.

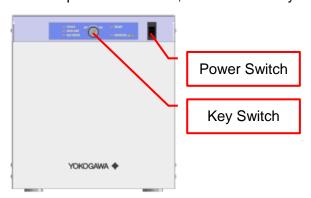
In case that the screws can't be fastened properly, adjust position of bath cover so that the screws are fastened properly.



Set sub cover and tighten 2 screws.



Turn the power switch ON, then turn the key switch ON..





After turning power switch ON, the door closes automatically. Please be careful not to pinch your fingers or others.

Immediately pull out the AC cord from inlet of equipment, when you notice smoke coming out of the main unit, foul smell, abnormal noise or any other abnormality.

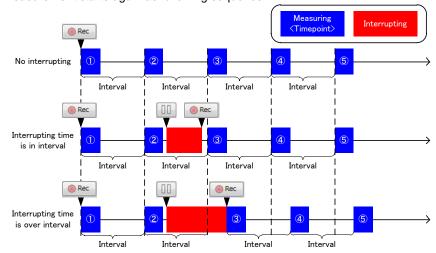
Click "Rec" button in case of restarting measurement.

(It takes about 2 minutes that "Rec" button becomes available after turning power switch ON.)



Note

In case that starting time of the next timepoint comes during the pausing time, measurement starts again as following sequence.



In case of the time-lapse measurement that pause for filling water and others is supporsed, it is recommended to set interval so that time span from finishing measurement of the timepoint to starting of next timepoint become longer than 10 minutes.

4.1.3 Procedures of Setting Gas Mixer

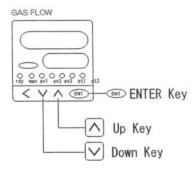
This procedure is only performed when controlling environment around sample. If you don't need environment control, go to procedure shown in 4.1.5 Setting Samples.

Start Gas Mixer by referring to <u>3.3 Starting and Shutting Down of Gas</u> Mixer (Option).

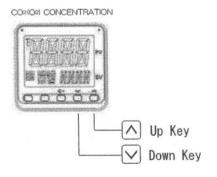
In this section, procedures are described for example by setting as following.

- Sample vessel: microplate
- CO₂ concentration in chamber: 5 %
- O₂ concentration in chamber: atomostic concentration (≠ 20 %) For other settings, please refer to Technical Information TI 80J01A05-01E (Setting Parameter of Gas Mixer).

By "Up/ Down" key of "GAS FLOW", set mixture gas flow rate to 150 (ml / min) and push "Enter".



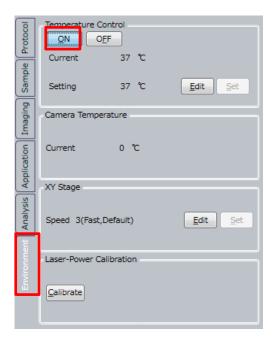
By "Up/ Down" key of " CO_2 CONCENTRATION", set CO_2 concentration to 6.0 (%). By "Up/ Down" key of " O_2 CONCENTRATION", set O_2 concentration to 20.0 (%).



4.1.4 Procedures of Setting Stage Heater

This procedure is only performed when controlling environment around sample. If you don't perform environment control, go to procedure shown in 4.1.5 Setting Samples.

Click "ON" button in "Environment" window -> "Temperature Control".



Click "Edit" button.

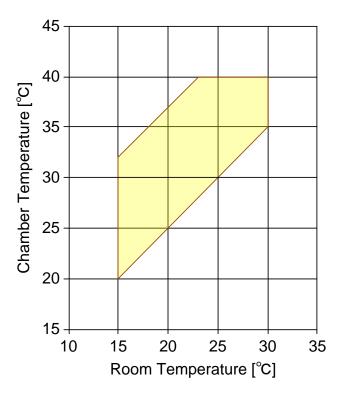


It changes to temperature setting window. Input control temperature to "Setting" and click "Set".



Guarranty range of chamber temperature depends on room temperature (refer to following chart).

For example, to set chamber temperature 37 $^{\circ}$ C, set room temperature 20 $^{\circ}$ C - 30 $^{\circ}$ C.



Note

If 35 mm dishes is used, input "control temperature – 0.8 $^{\circ}$ C" (example: If you want to set chamber temperature to 37 $^{\circ}$ C, input "36.2" to "Setting")

4.1.5 Setting Samples

<Caution when setting samples>

If the sample which is taken from cold environment, such as refrigerator, is set into CQ1 at once, condensation occurs on the bottom of sample vessel and autofocus may not operate normally.

Also, temperature of sample vessel changes rapidly after it is set into CQ1, strain of sample vessel occurs and autofocus may not operate normally.

In case of unusing Stage Heater, set sample into CQ1 after acclimating to room temperature. In case of the sample taken from refrigerator, acclimating time is about 30 minutes. In case that condensation occurs on the bottom of sample vessel, please wipe it away.

In case of using Stage Heater, set sample into CQ1 as soon as possible after taken it from incubator.

<In case of using 35 mm dish>Set dishes on sample holder.



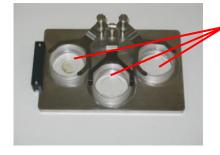
Lift up leaf spring, set a dish, and push down leaf spring to fix a dish.



Lift up leaf spring



Set a dish and push down leaf spring to fix a dish.



Set dishes on all 3 positions.

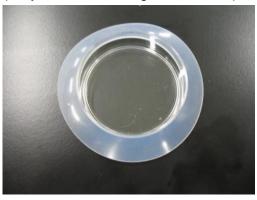


CALITION

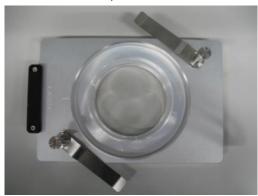
Same dishes must be set on all 3 positions.

<In case of using 60 mm dish>

(Only when controlling environment) Set rubber on the dish.



Set dish on sample holder.



(Only when controlling environment) Set a dish ring.



Dish ring

Fix a dish by leaf spring.



Lift up leaf spring



Set a dish and push down leaf spring to fix a dish



In case of performing environment control



In case of not performing environment control

<In case of using slide glass>

Note

Slide glass is not accommodate to measurement with environment contol

Set slides on sample holder.



Set a slide on sample holder with pressing to leaf spring. Please be careful not to tilt slides.



Set a slide with pressing to leaf spring.

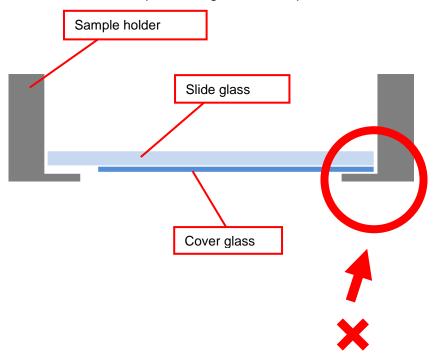


Please set slides such as label comes near side basically (depend on the slide glass setting in CQ1 Software; refer to page 5-19)

<u>^!\</u>

CAUTION

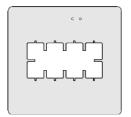
Set slides not to put cover glass on sample holder.



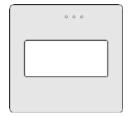
<In case of using cover glass chamber>

(Only when controlling environment) Remove rid from cover glass chamber, set rubber on the cover glass chamber fastly and set rid again.

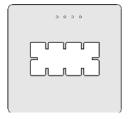
Rubber list



For IWAKI (2 holes)



For NUNC Lab-Tec I (3 holes)

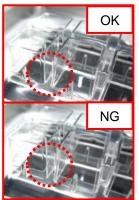


For NUNC Lab-Tec II (4 holes)



For MATSUNAMI (5 holes)





Set cover glass chamber pushing A1 well to corner indicated by arrow.

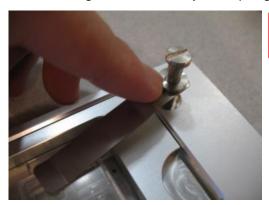


(Only when controlling environment) Set a holding plate.



Holding plate

Fix a cover glass chamber by leaf spring.



Set a cover glass chamber and push down leaf spring to fix



In case of performing environment control

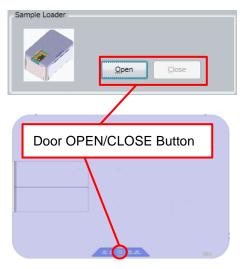


In case of not performing environment control

<Common procedures>

Click "Sample" tab → "Open" Button of CQ1 Software or do long press (3 seconds) of Door OPEN/CLOSE Button of CQ1 Main Unit, set microplate or sample holder on stage.

Click "Close" Button of CQ1 Software or do long press (3 seconds) of Door OPEN/CLOSE Button of CQ1 Main Unit to close the door.



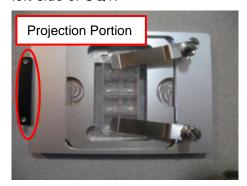




CAUTION

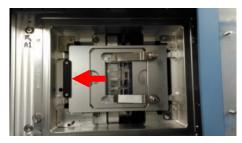
Be careful not to stuck fingers or others in door. If fingers or others are stuck, CQ1 will stop. In the case, please turn Power Switch of Utility Box off then turn it on again.

In case of using sample holder, set it in CQ1 as projection portion faces left-side of CQ1.



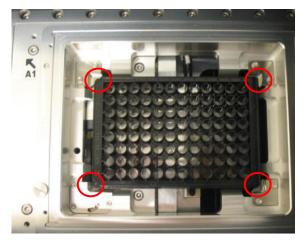






(L) CAUTION

If you don't set microplate or sample holder properly, it may result to equipment damage. Please confirm that 4 corners of the microplate or sample holder are set on stage and it is moved lightly (not to be stuck to edge of sample setting place).









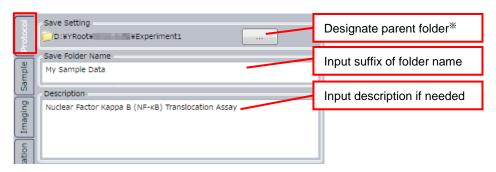
riangle caution

Immediately pull out the AC cord from inlet of equipment, when you notice smoke coming out of the main unit, foul smell, abnormal noise or any other abnormality.

If you perform measurement by protocol which has been made, go to procedure shown in <u>4.8 Sample Measurement</u>.

4.2 Basic Protocol Setting

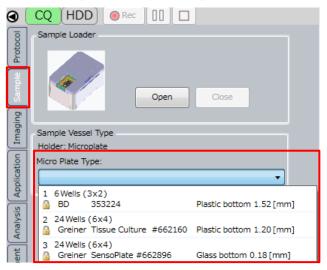
Designate the folder for saving the measurement data in "Protocol" window.



*Note

Please designate local drive (D:) as data storage folder. In case of designating other than local drive (D:), performance of HDD decreases, and it may cause errors during image acquisition.

Select sample vessel in "Sample" window.

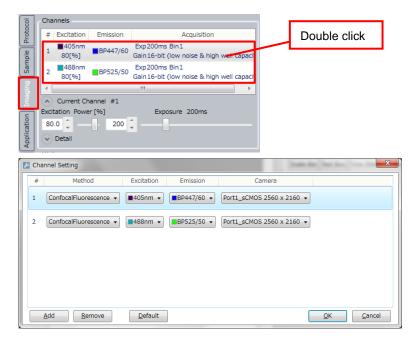


Switch to "Imaging" window.

Double click imaging channel setting part to open "Channel Setting".

Set the lasers and emission filters for each imaging channel.

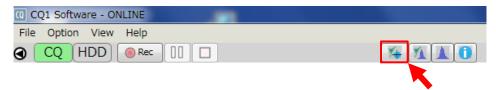
(For details, refer to 5.9 Channel Setting)



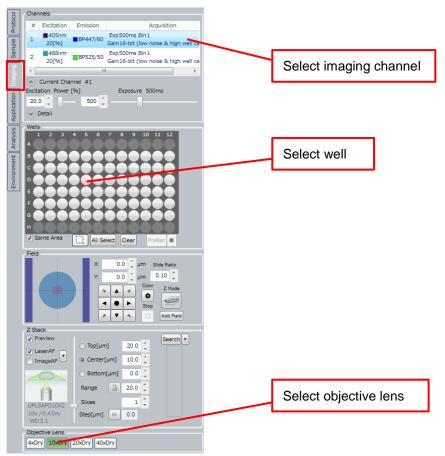
4.3 Sample Searching

Search the position that samples exist (suitable position to imaging) by low magnification objective lens.

Set the viewer layout to "Image & Map".

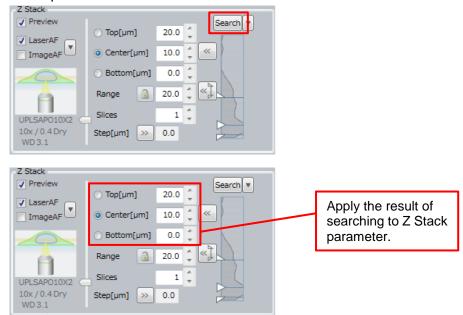


In "Imaging" window, select imaging channel, well and objective lens. For sample searching, use low magnification objective lens (2x - 10x).



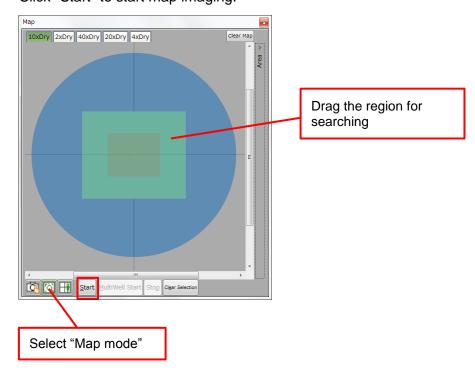
In "Z stack" of "Imaging" window (refer to page <u>5-28</u>), click "Search" to search Z position which samples exist.

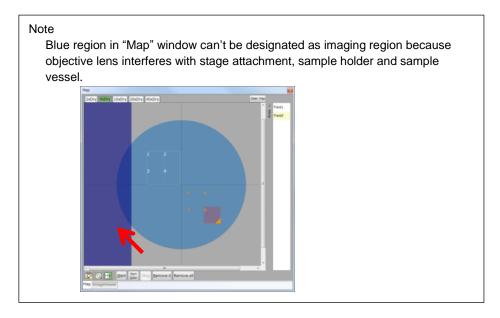
After Z position search, click "<<" to apply the result of searching to Z Stack parameter.



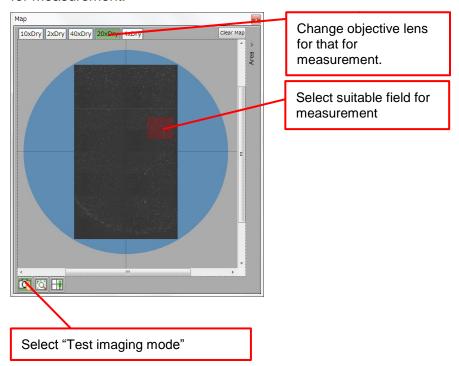
In "Map" window, select "Map Mode" (refer to page <u>5-95</u>) and select sample searching region by mouse drug.

Click "Start" to start map imaging.





After map imaging, select "Test imaging mode" (refer to page <u>5-94</u>), change objective lens to that for measurement, and select suitable field for measurement.

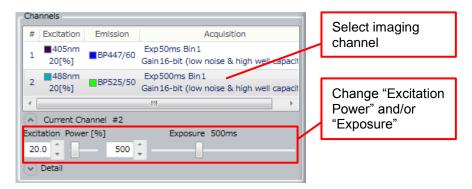


4.4 Imaging Setting

If needed, perform following (1) - (3) setting.

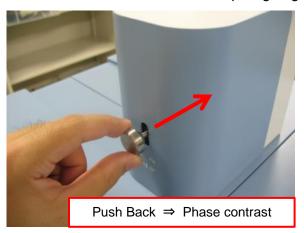
(1) Imaging channel setting

"Channels" window of "Imaging" window (refer to page <u>5-22</u>), change "Excitation Power" and/or "Exposure" of each imaging channel.

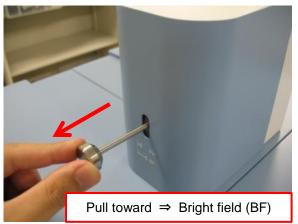


<In case of using Transmission Illumination with Bright Field/</p>
Phase Contrast>

Push the lever back in case of acquiring phase contrast image. Pull the lever toward in case of acquiring bright field image.







(2) Autofocus and 2D/ 3D imaging settingIn "Z Stack" window of "Imaging" window (refer to page <u>5-28</u>),

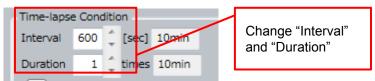
change autofocus setting.

Change "Range" and "Slices". As well, set 1 to "Slices" and 2D measurement is executed. Set 2 or more and 3D measurement is executed.



(3) Time-lapse setting

In "Time-lapse Condition" window of "Imaging" window (refer to page <u>5-32</u>), change "Interval" and "Duration". If time-lapse measurement is not performed, set "1" to "Duration".

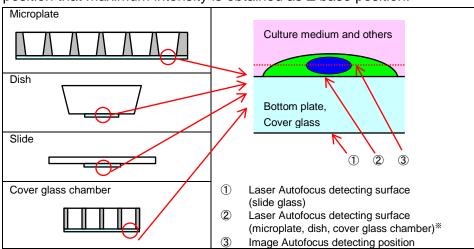


<About CQ1 autofocus>

CQ1 can perform 2 types of autofocus, which are laser based and image based.

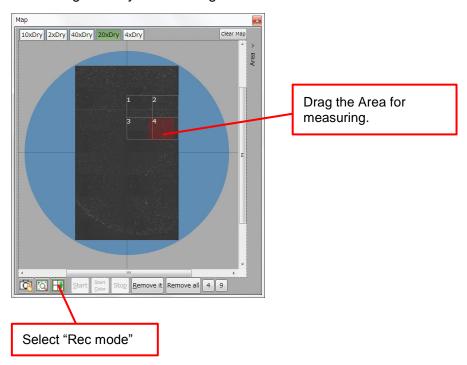
Laser Autofocus detects surface of bottom plate or cover glass by laser and set the surface as Z base position. Please note that surface to detect (Z base position) is different by the kind of sample vessel. (Refer to following table.)

Image Autofocus acquires image by designated channel and sets the Z position that maximum intensity is obtained as Z base position.



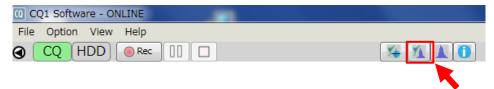
In case that laser autofocus doesn't operete normally when using microplate, dish or cover glass chamber, surface to detect would be changed to lower surface of bottom plate of sample vessel. For details, please refer to page <u>5-20</u>.

In "Map" window, select "Rec Mode" (refer to page <u>5-96</u>) and select measuring Area by mouse drug.



4.5 Analysis Protocol Setting

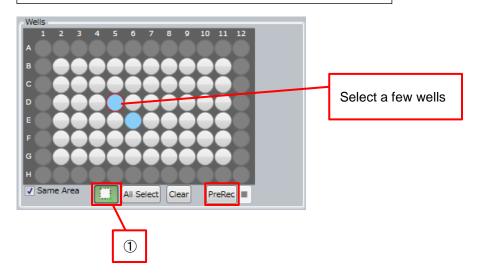
Set the viewer layout to "ImageViewer & Chart".



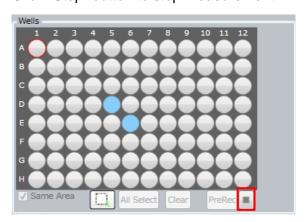
Pre-acquire the images for analysis setting.

In "Wells" window of "Imaging" window (refer to page <u>5-25</u>), make ① button to status ② (green), select a few wells from wells for measure ** (selected wells become light blue), and click "PreRec".

* Selection of negative control well(s) and positive control well(s) is effectual.



Click "Stop" button to stop measurement.



Select "Application" tab, check to "Execute Analysis", and click "Load".

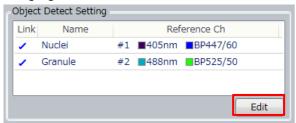
Select "User" tab if you use analysis protocol which is set and saved previously. Select "Application" tab if you use analysis protocol which is preloaded in CQ1 Software (Cell Cycle, FISH and so on). Select "Template" tab if you use basic protocol which is preloaded in CQ1 Software.

Edit

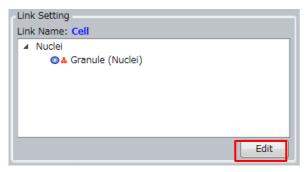
Select analysis protocol and click "Load" to load analysis protocol. (For details, refer to page 5-37)



If needed, in "Object Detect Setting" window of "Application" window (refer to page <u>5-44</u>), change setting of object recognizing of each imaging channel.



If needed, in "Link Setting" window of "Application" window (refer to page <u>5-46</u>), change link setting between each object.

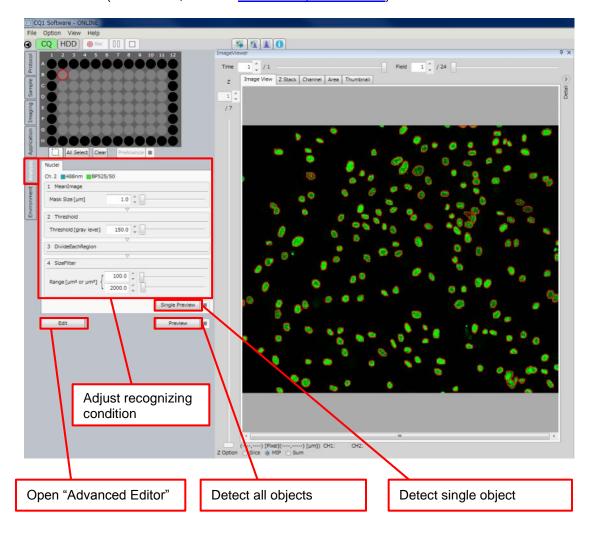


4.6 Object Recognizing Condition Setting

In "Analysis" window, click "Single Preview" (single object) or "Preview" (all objects) to confirm result of object recognizing. If it is necessary to improve the result, adjust recognizing condition as following.

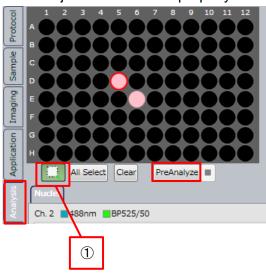
If it is necessary to improve more, click "Edit", open "Advanced Editor" and set detailed recognizing condition.

(For details, refer to 5.4.5 Analysis Window)



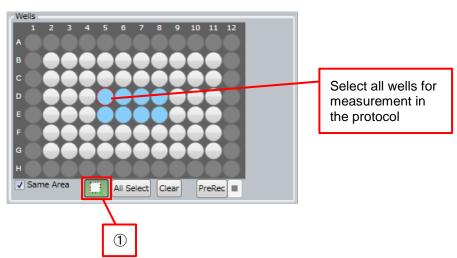
In "Wells" window of "Analysis" window (refer to page <u>5-51</u>), make ① button to status ② (green), select wells which were executed "PreRec" above procedure (selected wells become pink), and click "PreAnalyze".

Confirm that objects are detected properly in multiple wells. If it is necessary to improve the result, adjust detecting condition in the field which object isn't detected properly.

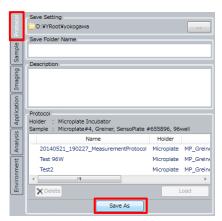


4.7 Protocol Saving

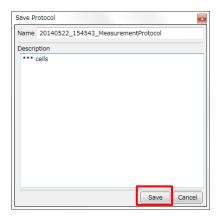
In "Wells" window of "Imaging" window (refer to page <u>5-25</u>), make ① button to status ② (green), select all wells for measurement in the protocol (selected wells become light blue).



Select "Protocol" tab and click "Save As".



Input name and descriptions of the protocol. Click "Save" to save the protocol.

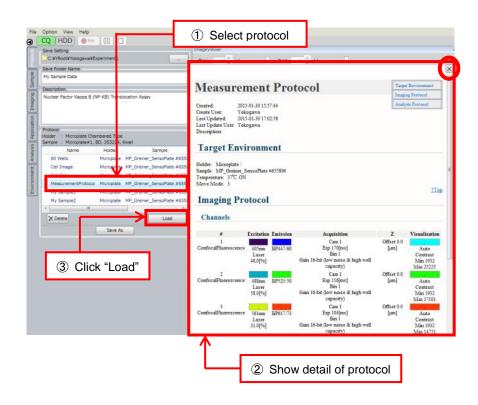


4.8 Sample Measurement

In case of measurement by saved protocol, select protocol from "Protocol" window.

Note

In case of measuring other sample in same protocol again, please load the protocol in each time. If the protocol is not loaded, autofocus may not operate normally.



Set the viewer layout to "ImageViewer & Chart".

Click "Rec" to start measurement. By clicking this button, imaging by the condition set in <u>4.4 Imaging Setting</u> and analyzing by the condition set in <u>4.5 Analysis Protocol Setting</u> and <u>4.6 Object Recognizing</u>

<u>Condition Setting</u> are executed.

Image dataset and analysis dataset are saved in the folder designated in <u>4.2 Basic Protocol Setting</u>.



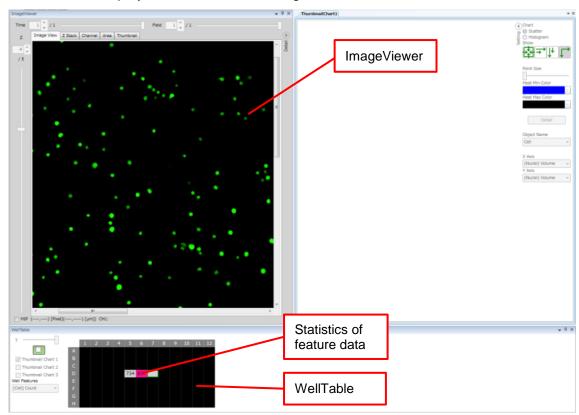
In case of pausing measurement, click "Pause" button.



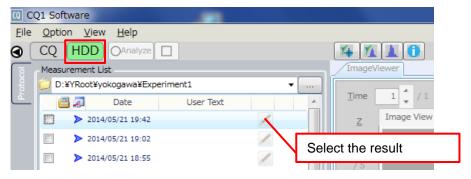
In case of re-starting measurement during pausing, click "Stop" button. In case of stopping, click "Stop" button.



Acquired images are displayed in "ImageViewer" and statistic of feature data (default is "Count") of wells which measurement is finished are displayed in "WellTable" during measurement.



After measurement, switch to "HDD" mode and select (double click) the result measured above.

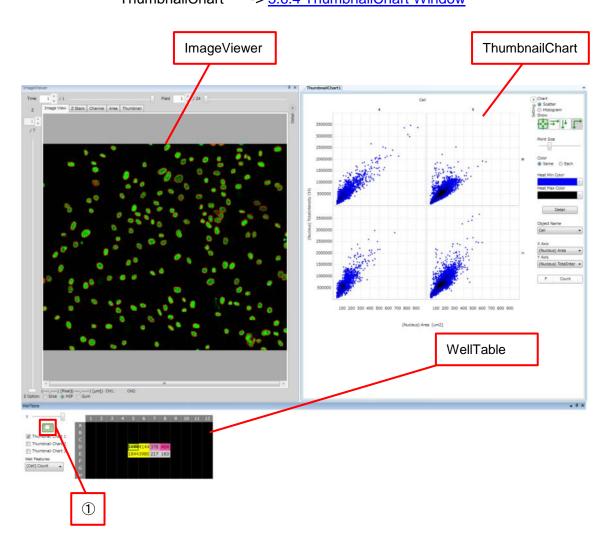


"ImageViewer".

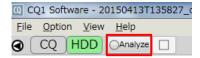
Make ① button to status ② (green), check number of Thumbnail Chart to display and select wells in "WellTable". Scattergrams or histograms are displayed in "ThumbnailChart".

For details of each window, refer to following

"WellTables" -> <u>5.6.5 WellTable Window</u>
 "ImageViewer" -> <u>5.6.2 ImageViewer Window</u>
 "ThumbnailChart" -> <u>5.6.4 ThumbnailChart Window</u>



In case of retrying analysis, reset analyzing condition by referring <u>4.5</u>
<u>Analysis Protocol Setting</u> and <u>4.6 Object Recognizing Condition Setting</u>, and click "Analyze"

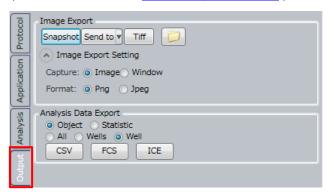


In case of stopping analysis process, click "Stop" button.



Output acquired images, window snapshot of CQ1 Software or CSV data and so on in "Output" window.

(For details, refer to 5.5.4 Output Window.)



5 Functions of CQ1 Software

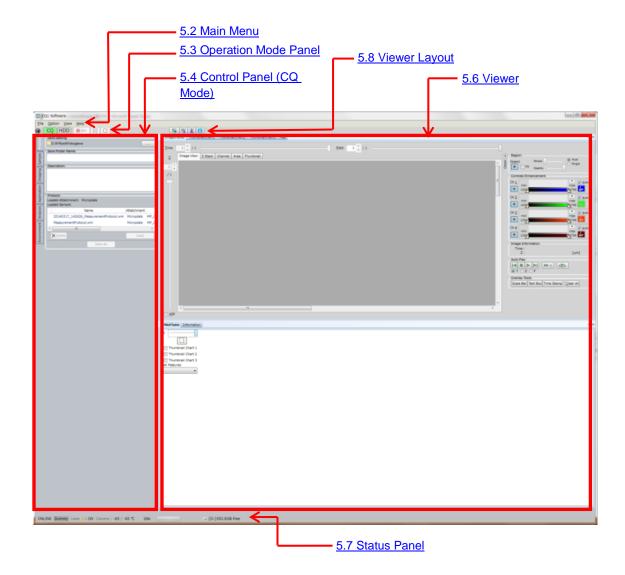
5.1 Outline

CQ1 Software is the application software to control both CQ1 Main Unit and Utility Box and runs sample measurement.

There are two operating modes; CQ mode and HDD mode, and the window display changes depending on the mode.

(1) CQ Mode

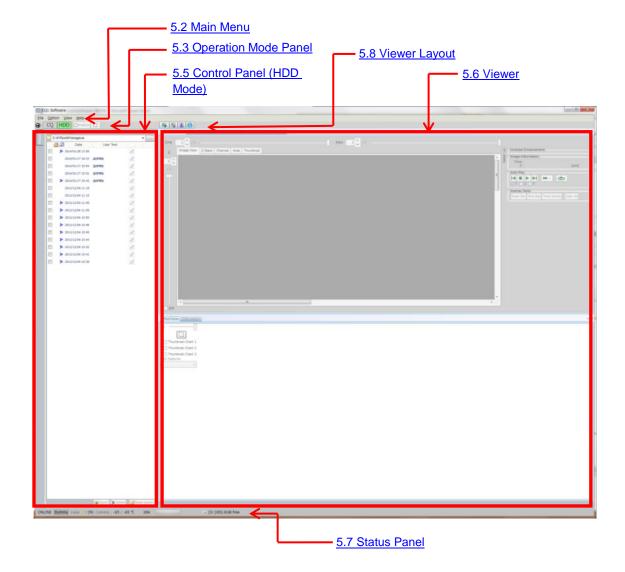
Controls both CQ1 Main Unit and Utility Box and runs sample measurement. Measurement data are saved in either the HDD of workstation or network drive.



(2) HDD Mode

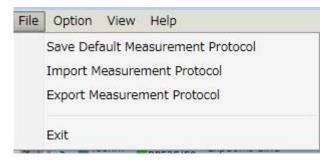
This mode is to view measurement data or to retry image analysis under modified analysis conditions.

In HDD mode, it is able to output data for report such as screen shot, movie, feature data and so on.

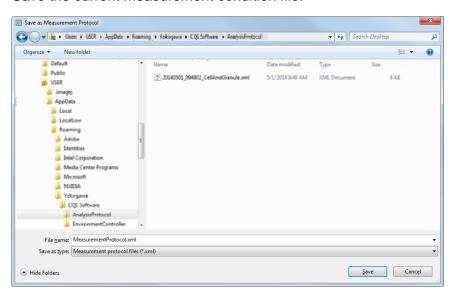


5.2 Main Menu

5.2.1 File Menu



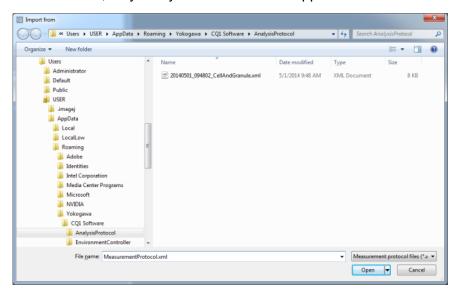
- (1) Save Measurement Protocol as Default Save the default measurement condition file. Its channel setting and analysis conditions shall be applied at the next start.
- (2) Save as Measurement Protocol Save the current measurement condition file.



(3) Load Measurement Protocol

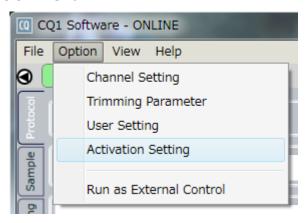
Load measurement condition fle designated by file read-in dialog. In CQ mode, conditions such as objective lens, sample vessel, Z stack, Fields to be imaged and analysis conditions are applied. Fields to be imaged aren't applied if stage attachment and sample holder isn't matched.

In HDD mode, only analysis conditions are applied.



- (4) Save Contrast and Region View Setting This function is valid only in HDD mode. Save "Contrast Enhancement" and "Region" setting of Tool function (Refer to 5.6.9 Tool Function). In case of not analyzed data, only "Contrast Enhancement" setting is saved.
- (5) Exit Exit from CQ1 Software.

5.2.2 Option Menu



(1) Channel Setting

Open Channel Setting window to set imaging channels. Please refer to "5.9 Channel Setting" for details.

- (2) Trimming Parameter
 Open Trimming Parameter window i to change stitch parameter.
 Please refer to "5.10 Trimming Parameter" for details.
- (3) User Setting Open User Setting window to change user setting. Please refer to "5.11 User Setting" for details.
- (4) Activation Setting

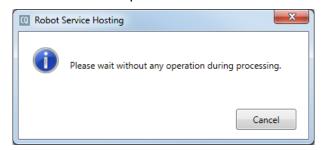
Open Activation Setting window to manage license of CQ1 Software. Please refer to "5.12 Activation Setting" for details.



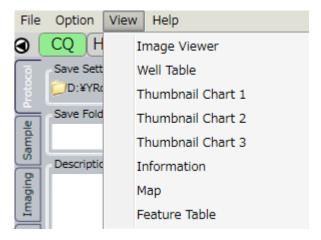
Please do not operate Activation Setting window except when necessary such as updating CQ1 Software. CQ1 Software might be inoperatable. For updating CQ1 Software, please contact the dealer.

(5) Run as External Control

Following "Robot Service Hosting" window opens and changes to the external control mode that high-order computer controls CQ1. Select this menu in case of operating CQ1 with external robot. Click "Cancel" to quit external control.



5.2.3 View Menu



Viewers corresponding to each menu item are shown. Please refer to "5.6 Viewer" for details.

5.2.4 Help Menu



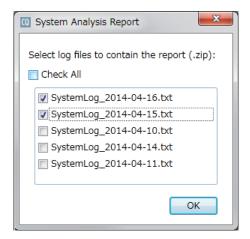
(1) About CQ1

Version information is shown.



(2) Make System Analysis Report

Collect system analysis information and output a compressed file of log and calibration file under CVSysRep folder on desktop. The output data will be used for system maintenance service when error occures.

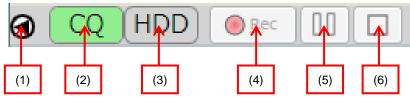


It takes a few minute to collect system information. It is recommended to check to select date when error is occurred because the all date log data is quite huge.

5.3 Operation Mode Panel

Operation mode panel screen changes depending on CQ mode and HDD mode.

5.3.1 Operation mode panel in CQ mode.



Control Panel Display SHOW/HIDE button
 Button to change SHOW/HIDE of control panel display.

Click to HIDE control panel display

D: Click to SHOW control panel display

(2) CQ Mode Button

Highlighted in the CQ mode but nothing happens by clicking this button. (Refer to <u>5.1 Outline</u> (1) CQ Mode)

(3) HDD Mode Button Click to shift CQ1 Software to HDD mode. Cannot click it during

measurement. (Refer to <u>5.1 Outline</u> (2) HDD Mode)

(4) Rec Button

Click to start measurement after setting measurement conditions on the control panel.

(5) Pause Button

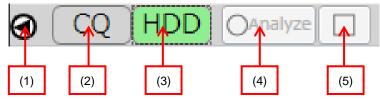
Click to pause measurement.

(6) Stop Button

Click to stop measurement.

This button becomes available when Pause button was clicked during measurement.

5.3.2 Operation Mode Panel in HDD Mode.



Control Panel Display SHOW/HIDE Button
 Button to change SHOW/HIDE of control panel display.

Click to HIDE control panel display

D: Click to SHOW control panel display

(2) CQ Mode Button

Click to shift CQ1 Software to CQ mode. Cannot click it during analyzing measurement data. (Refer to <u>5.1 Outline</u> (1) CQ Mode)

(3) HDD Mode Button Highlighted in the HDD mode but nothing happens by clicking this button. (Refer to <u>5.1 Outline</u> (2) HDD Mode)

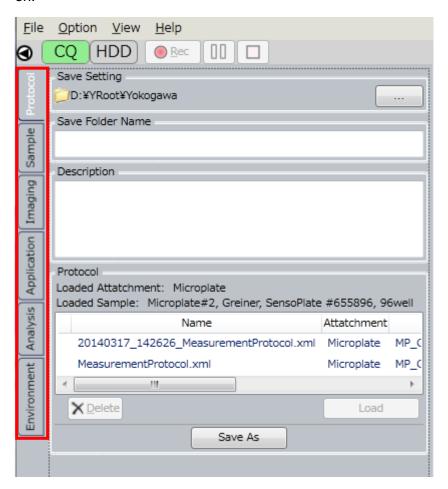
(4) Analyze Button

To start analysis of measurement data, select saved data on control panel, set analysis conditions at "Application window" and "Analysis window", and click this button.

(5) Stop ButtonClick to stop data analysis.

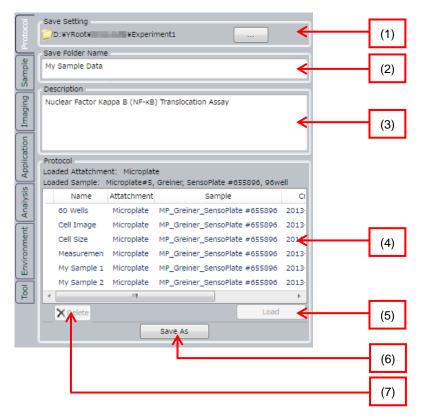
5.4 Control Panel (CQ Mode)

In CQ mode, click left-side tabs to set measurement conditions and so on.



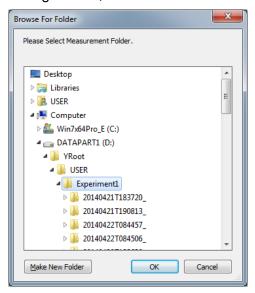
5.4.1 Protocol Window

Protocol means sample measurement conditions such as imaging conditions specified at "Imaging window", and analysis conditions specified at "Application window" and "Analysis window". "Protocol window" is to save or load Protocol, and specify the folder name to save measurement data obtained under the Protocol.



(1) Save Setting

Specify a storage folder to save measurement data. To change the storage folder, click button to show folder selection menu.



Note

Please designate local drive (D:) as data storage folder. In case of designating other than local drive (D:), performance of HDD decreases, and it may cause errors during image acquisition.

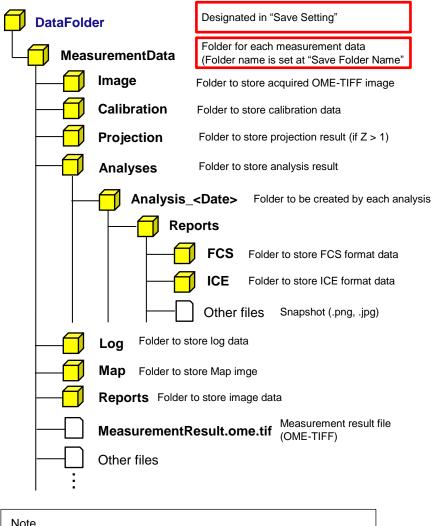
Absolute path (total folder name from "D:") of strage folder can't be over 90 letters.

(2) Save Folder Name

For each measurement, CQ1 Software prepares a measurement data folder within the data storage folder specified by "Save Setting", and save images and numerical data. "Save Folder Name" is used as a suffix of measurement data folder name.

Name format of MeasurementData folder is "yyyyMMddTHHmmss_<string input in "Save Folder name">". For example, in case of beginning measurement at April 1, 2014, 12° 34′ 56″, MeasuremenData folder name is "20140401T123456_Test".

Under MeasurementData folder, sub-folders are created as the following configuration. There are folders not to be created by mesurement setting.



Note

Over 39 letters can't be input for "Save Folder Name". Please utilize following "Description" as memo for measurement.

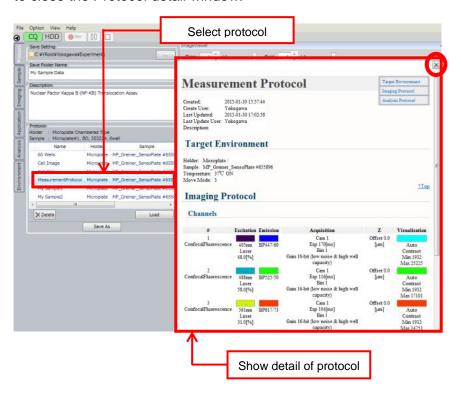
(3) Description

Fill in the outline of measurement data.

Input contents can be confirm in (9) Pen Button of 5.5.1 Protocol Window or <u>5.6.7 Information Window</u> (For details, please refer to each section).

(4) Protocol

List of Protocols saved in Workstation is shown. By selecting a Protocol, its details are shown. Click "x" button on the upper right to close the Protocol detail window.



(5) Load Button

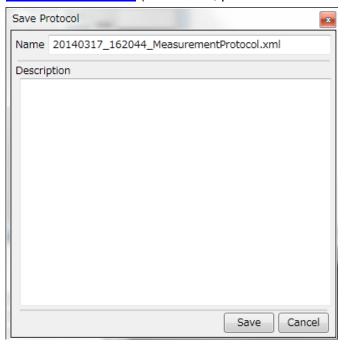
Click this button after selecting a Protocol from the list to load it in the control panel.

(6) Save As Button

File save dialog appears by clicking this button to save measurement conditions specified in the control panel. XML file name is shown in the "Name" column of Protocol list.

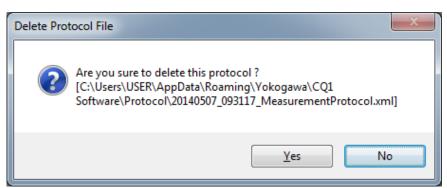
Fill in the outline of protocol.Contents can be confirm in 5.6.7

Information Window (For details, please refer to the section).



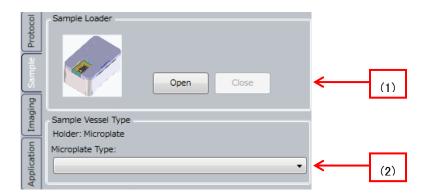
(7) Delete Button

Select a Protocol from the list, and click this button to show "Delete Protocol File" dialog. Click "YES" to delete Protocol.



5.4.2 Sample Window

Sample window is to select sample vessel type, such as microplate or slide glass, to load on CQ1.



(1) Sample Loader

Click Open button to open the door on CQ1 Main Unit when the XY-stage is located at the door.

Click Close button to close the door when XY-stage moves to the measuring position to get ready for imaging.

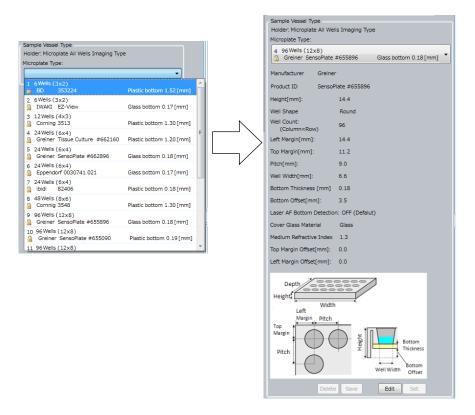
(2) Sample Vessel Type

Select sample vessel type loaded in CQ1 Main Unit.

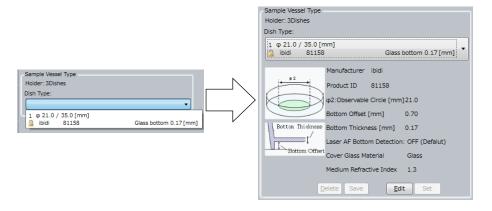
CQ1 Main Unit automatically recognizes the loaded sample holder type, and shows sample holder type name at the "Attachment" column of "Sample Vessel Type", to show selectable sample type in the menu depending on sample holder type.

Sample holder	Description
None	CQ1 Software recognizes as if microplate is loaded, and enables selection of "Microplate Type"
Triple 35 mm dishes	CQ1 Software recognizes a Triple 35 mm dishes sample holder is loaded in which up to three 35 mm dishes can be set, and enables selection of "Dish Type".
	$\angle!$ CAUTION
	Set dishes to all 3 positions.
Single 60 mm dish	CQ1 Software recognizes a Single 60 mm dish sample holder is loaded in which one 60 mm dish can be set, and enables selection of "Dish Type".
Slide glass	CQ1 Software recognizes a slideglass sample holder is
ISO 8037/1	loaded in which up to four slide glasses can be set, and enables selection of "Slide Type".
Cover glass chamber	CQ1 Software recognizes a cover glass chamber sample holder is loaded in which one cover glass chamber can be set, and enables selection of "Cover Glass Chamber Type".

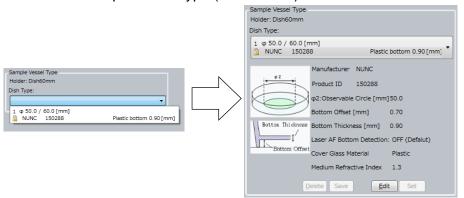
Selection of sample vessel type (Microplate)



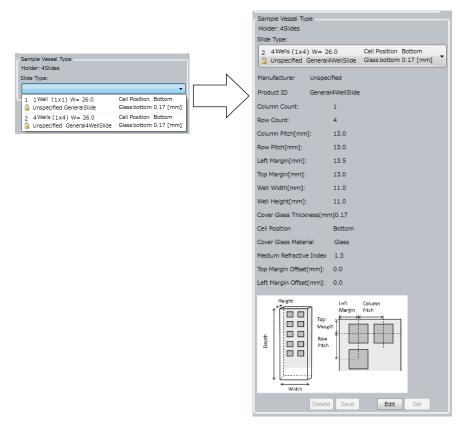
Selection of sample vessel type (35 mm dish)



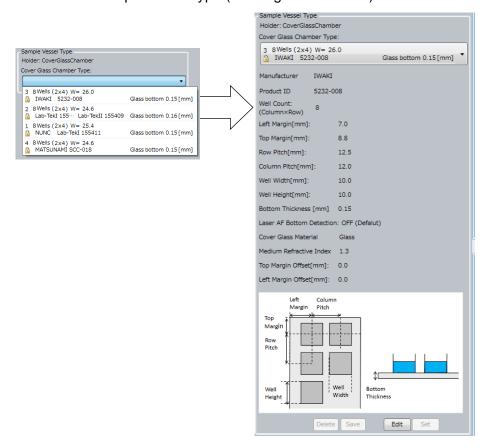
Selection of sample vessel type (60 mm dish)



Selection of sample vessel type (Slide glass)



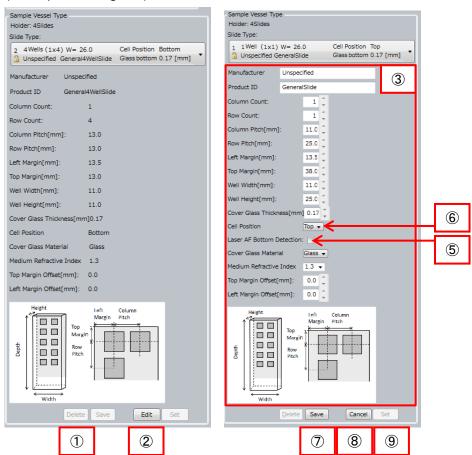
Selection of sample vessel type (Cover glass chamber)



By using "Edit" button at the bottom of "Sample Vessel Type", it is possible to modify sample shape and create customized sample type. (Example: 35 mm dish)

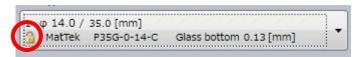


(Example: slide glass)



1 Delete Button

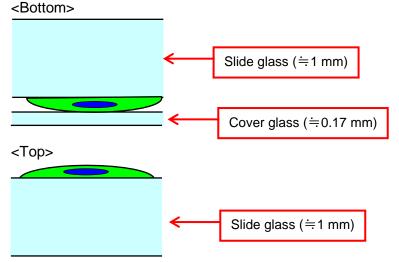
Delete the sample vessel type selected in "Sample Vessel Type" menu. Default sample vessel types are key-marked and cannot be removed by Delete Button.



2 Edit Button

Start editting parameters of selected sample vessel type in "Sample Vessel Type" menu in which name and size of vessel type can be modified. \rightarrow ③ Edit Window

- 3 Edit Window
 - Input parameters such as sample vessel name and dimensions. Refer to displayed diagram in the window for dimensions to be input.
- Measure Button (Invalid when using slide glass)
 Measure the bottom thickness of sample vessel. This function can be used when 10x dry objective lens (UPLSAPO10X2) is selected.
- (5) Laser AF Bottom Detection By checking the box, surface to detect by laser autofocus is changed to lower surface of bottom plate of sample vessel. This is checked when laser autofocus doesn't operete normally. For details of laser autofocus, please refer to page 5-28
- 6 Cell Position (Only valid when using slide glass) Select relative position between slide glass and sample.



To Save Button

Save modified parameters and finish editing.

8 Cancel Button

Cancel modified parameter setting and finish editing.

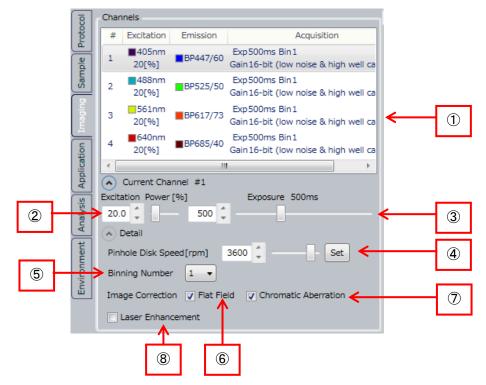
9 Set Button

Overwrite newly set parameters on selected sample vessel type and finish editing. You cannot click this button when a key-marked type is selected.

5.4.3 Imaging Window

Imaging window is to specify sample imaging conditions

(1) Channels



① Imaging channel list

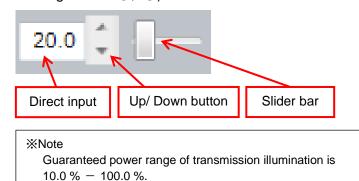
Show the list of imaging channels specified by; "Option" menu $\,\to\,$ "Channel Setting" menu. Also, by double-clicking each channel,

"Channel Setting" window (refer to 5.9 Channel Setting) opens.

Name	Description
#	Number of imaging channel
Excitation	Light source information (laser or transmission illumination)
Emission	Emission filter information
Acquisition	Image acquisition conditions of camera, such as exposure time or binning.

2 Excitation Power

Specify light source power (laser or lamp) in the range of $0.0\% \sim 100.0\%$ *. It is able to specify any way shown following. (The same goes for ③, ④)



3 Exposure

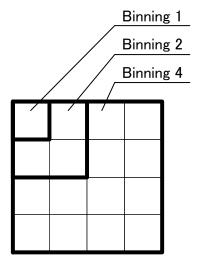
Specify camera exposure time in the range of 50ms~10,000ms (10 sec.)

④ Pinhole Disk Speed Specify rotation speed of the pinhole disk array of confocal unit in the range of 1,800rpm~4,000rpm. Default value is 3,600rpm.

⑤ Binning Number

Specify binning condition of the camera. Set binning number bigger and image resolution become lower, image brightness become higher, and data size become smaller

Binning Number	Description
1	Unuse binning function. One camera pixel is saved as one pixel of image data.
2	Combine adjacent 2X2 camera pixels and save as one pixel of image data.
4	Combine adjacent 4X4 camera pixels and save as one pixel of image data.
8	Combine adjacent 8X8camera pixels and save as one pixel of image data.



6 Flat Field Correction

Correct peripheral image darkness caused by shading of the microscope. (Default: checked)

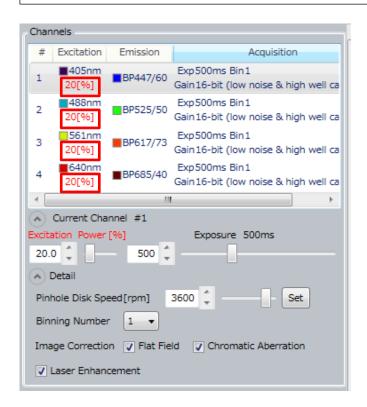
Chromatic Aberration
 Correct chromatic aberration caused by difference of wavelength.
 (Default: checked)

8 Laser Enhancement

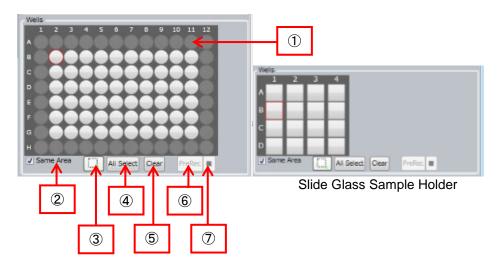
Check to enhance excitation power. If checked, value of excitation power turns red.

Note

Normally excitation power is calibrated by power irradiated on sample. If check this box, the calibration is disabled and excitation laser is output by non-calibrated laser power.



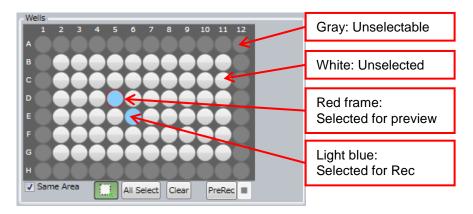
(2) Wells



Selected sample vessel

Diagram of sample vessel selected at "Sample window" is shown. Unselected wells are shown in white. Gray wells are unselectable due to interference of objective lens.

By clicking white well when button ③ is (white), the well is selected for preview (red frame). By left-clicking or left-dragging wells when button ③ is (green), the wells are selected for Rec (light blue). By right-clicking or right-dragging, selection for Rec is canceled.



2 Same Area

Check here to capture images of the same Area in all wells of microplate, slide. If unchecked, imaging Areas can be selected individually for each well.

- 3 Select measurement area Refer to above ①.
- 4 All Select

All wells are selected for Rec. This botton is displayed when "Same Area" is checked.

5 Clear

Selection for Rec of all wells are canceled. This botton is displayed when "Same Area" is checked.

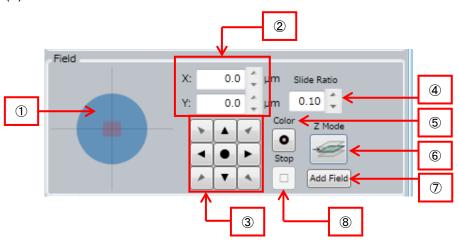
6 PreRec

Start preliminary imaging of wells selected for Rec. You need to select wells to be images by "Map" window beforehand.

Before performing object detects setting in "Analysis" window (refer to <u>5.4.5 Analysis Window</u>), preliminary imaging by this button must have done.

Stop PreRecStop preliminary imaging.

(3) Field



(1) Field selection

One well in the sample holder is shown here, within which location of current Field is shown. By clicking within the well, XY stage moves to the clicked point and capture an image, which will be shown as preview at the "ImageViewer".

2 XY coordinate

X-Y coordinate of current Field is shown for which the center of the well is the point of origin. Field location can be shifted by inputting values.

③ Imaging Field shift button

This button is to move imaging Field to the arrow direction. The center "• " button is to capture image at the current Field location by channel selected in (1) Channels .

4 Slide Ratio

Specify the travel distance when moving the imaging Field by imaging Field shift button. Field size is a travel distance unit. For example, by setting 0.10, the stage moves 1/10 of the Field size, and setting 1.00 moves by one Field size.

(5) Color

Click this button to capture images at the current Field position at all channels selected at "ImageViewer".

6 Z Mode

Select to run Z Stack imaging or not while preview imaging.

Z Mode	Description
-	Capture one image at the Center of Z Stack.
	Capture "Slices" images at "StepHight" interval between Top and Bottom of Z Stack.

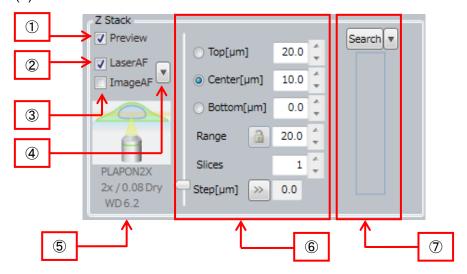
7 Add Field

Specify current imaging Field as the measurement location. The measurement filed is shown in "Map" window of "Viewer".

8 Stop

Click this button to stop imaging which was started by either mouse click of image Field or by clicking imaging Field shifting button.

(4) Z Stack

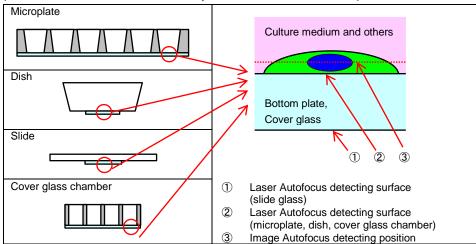


<About CQ1 autofocus>

CQ1 can perform 2 types of autofocus, which are laser based and image based.

Laser Autofocus detects surface of bottom plate or cover glass by laser and set the surface as Z base position. Please note that surface to detect (Z base position) is different by the kind of sample vessel. (Refer to following table.)

Image Autofocus acquires image by designated channel and sets the Z position that maximum intensity is obtained as Z base position.



In case that laser autofocus doesn't operete normally when using microplate, dish or cover glass chamber, surface to detect would be changed to lower surface of bottom plate of sample vessel. For details, please refer to page 5-20.

1) Preview

If checked, automatically capture images and show in "ImageViewer" whenever you change Z position.

2 Laser AF

Autofocus unit detects the interface of cover glass of the sample holder when capturing preview or time-lapse images, and save the Z-zero-position in the system. It is useful to avoid focus-out caused by tilted glass surface, when capturing images in wide field. Laser autofocus works at each measurement time when checked, but works only once for every Field if unchecked.

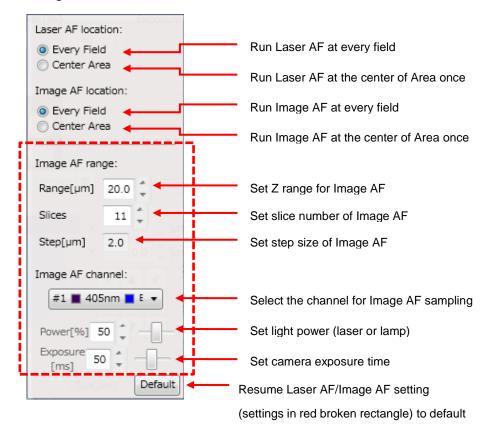
3 Image AF

Image Autofocus searches for objects by moving imaging position in Z direction, after Laser autofocus searched for the glass interface.

When checked, Image Autofocus runs when imaging started by "Start" button at "Map" window in "Viewer", or when measurement started by "PreRec" button in "Wells".

4 Laser AF-Image AF Setting

Click "▼" button to show Laser autofocus and Image autofocus setting window.



⑤ Objective lens information Information of selected objective lens such as model name, magnification and NA and working distance is shown.

6 Z Stack Parameter

Top is the upper limit of Z Stack, and Bottom is the lower limit of Z Stack. Center is the center position of Top and Bottom. When the Center value is changed, Top and Bottom values parallel shift by the same value. Input unit is 0.1um.

With the radio button at the left of "Top", "Center" and "Bottom", values can be changed by slider.

Range means the distance between Top and Bottom. To fix the Range value, click the key button at the left to click down. If either Top or Bottom value is changed when "Range" is fixed, the other value parallel shifts by the same amount.

"Slices" is to set the number of Z Stack images to capture.

"Step" is the imaging interval (step height) of Z Stack. Click left button to set suitable "Step" of selected objective lens*.

- * Action of clicking this button in each objective lens is as following.
 - Phase contrast lens -> This button is unavailable.
 - Dry lens: 2x, 4x -> "1" is set to "Slices"
 - Other than above, following step height is set to "Step". Dry lens: 10x -> 15 μ m, 20x -> 5 μ m, 40x -> 2 μ m Lens for thick bottom vessel: 20x -> 5 μ m Long working distance lens: 20x -> 9 μ m, 40x -> 4 μ m

Search

"Search" button is to search for sample position through Z Stack imaging.

- button is to set search conditions.
- button is to input search results on "Center" of Z Stack.
- button is to input search results on "Range" of Z stack.



(5) Objective Lens

Select objective lens here. Selected lens is highlighted.



(6) Time-lapse Setting

Switch the time-lapse acquisition mode (normal mode/ fast mode (option)).

If you didn't buy this option, this menu isn't shown and the time-lapse acquisition mode is always normal mode.



1 Normal

In this mode, sequence of imaging in each well, field, channel and Z slice has a priority. These sequences of imaging are performed at interval and duration set at "Time-lapse Condition" described below.

2 Fast (option)

In this mode, time-lapse imaging has a priority. Time-lapse imaging of speed and time length set at "Fast Time-lapse Condition" is performed in each well and field. Numbers of channel and Z slice are fixed to "1". When switching to this mode, the selected channel remains and the others are removed automatically. Also, "Chromatic Abberation" becomes invalid automatically.

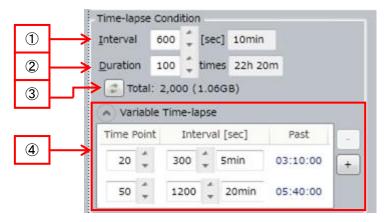
This mode is not correspond to on-the-fly analysis. To analyze image data acquired by this mode, it is necessary to acquire image data by CQ mode then analyze by HDD mode.

Note

Don't select "Fast" mode if setting of folder structure of saving data is "By Time Point" (Refer to <u>5.11 User Setting</u>). In case to use "Fast" mode, select Main Menu -> "Option" -> "User Setting" and select setting other than "By Time Point".

(7) Time-lapse Condition

Set time-lapse imaging setting in Normal mode



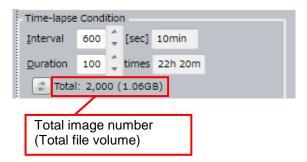
(1) Interval

Set the interval of time-lapse measurement in seconds. The specified time and acquisition time are shown on the right.

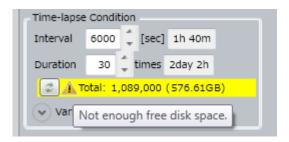
② Duration Specify the total number of repetitions. The total time is shown on the right.

3 Renewal Button

Display total image number and total file volume. By clicking this button, total file volume and total image number are renewed.



If HDD space is not enough, display warning sign.



4 Variable Time-lapse

An interval change event at a specific time point can be preset.

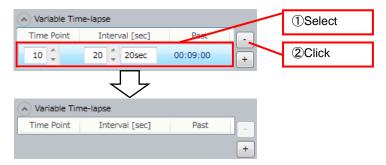
Time Point: Time point to change interval

Interval (sec): New interval

Past: Time for interval change

<In case of constant interval time-lapse>

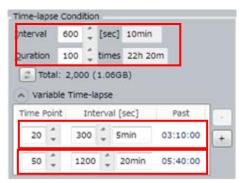
Set "Interval" and "Duration". Select row and click " - " to delete the "Variable Time-lapse" setting.



<In case of variable interval time-lapse>

Set "Interval", "Duration" and "Variable Time-lapse". In this case,

"Interval" is applied until first time point to change interval.



In case of setting as above

Time point	Interval (min)
11~19	10
220~49	5
③50 ~ 100	20

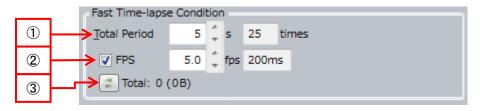
Note

If larger value than "Duration" is designated for "Time Point", measurement finishes at "Duration".

(8) Fast Time-lapse Condition

Set time-lapse imaging setting in Fast mode (option).

If you didn't buy this option, this menu isn't shown.



(1) Total Period

Set the term of time-lapse measurement by seconds. The calculated number of acquisition times is shown on the right.

2 Acquisition Speed

In case of setting acquisition speed and exposure individually, check "FPS". Upper limit of acquisition speed is determined by exposure, binning and trimming. If the value exceeding upper limit is input, value is changed to actual upper limit automatically. In case that "FPS" is unchecked, acquisition speed according to exposure is set automatically. Acquisition speed is limited by binning and trimming.

The calculated acquisition interval is shown on the right.

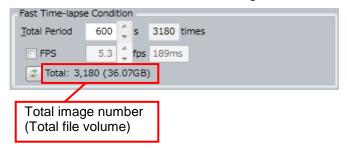
Note

Imaging speed in Fast mode is up to 20 fps. To perform 20 fps imaging, following setting is needed.

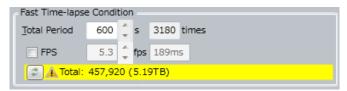
- Set exposure to 50 ms.
- Uncheck "FPS".
- Set binning 2 and over or narrow field of view to 1/4 and under in area by trimming (refer to 5.10 Trimming Parameter)

3 Renewal Button

Display total image number and total file volume. By clicking this button, total file volume and total image number are renewed.



If HDD space is not enough, display warning sign.



(9) Priority

In case of multiple Z slice, multiple channel imaging, set priority of imaging sequence.

(In Fast mode, numbers of channel and Z slice are fixed to "1". So setting of this item has not special meaning.)



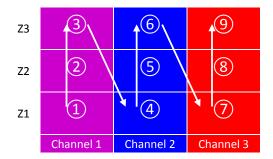
1 Z Stack

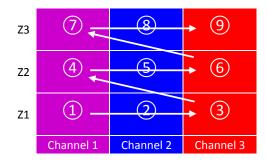
Multiple Z slice imaging by certain channel is performed in priority to multiple channel imaging.

2 Channel

Multiple channel imaging by certain Z slice is performed in priority to multiple Z slice imaging.

This setting becomes available in case that Z Offset of all channels are set to "0". (refer to 5.9 Channel Setting)



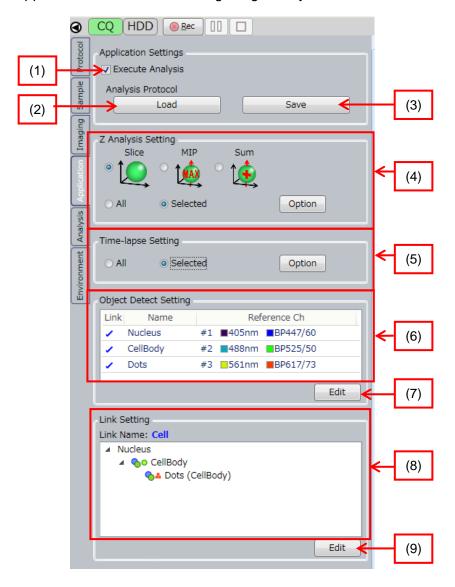


Z prior imaging

Channel prior imaging

5.4.4 Application Window

Application window is for setting image analysis conditions.



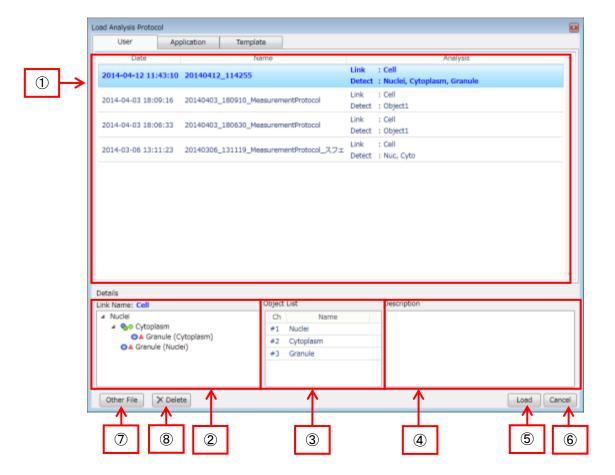
(1) Execute Analysis

When checked, runs measurement and analysis at the same time, and runs measurement only when unchecked.

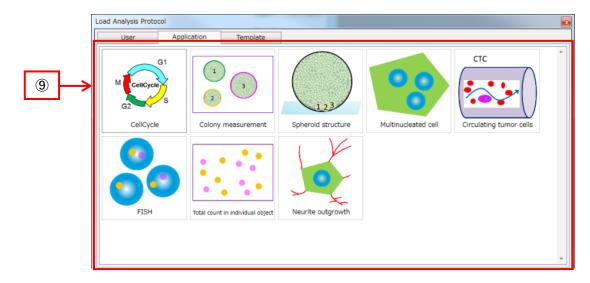
(2) Load

Load Analysis Protocol

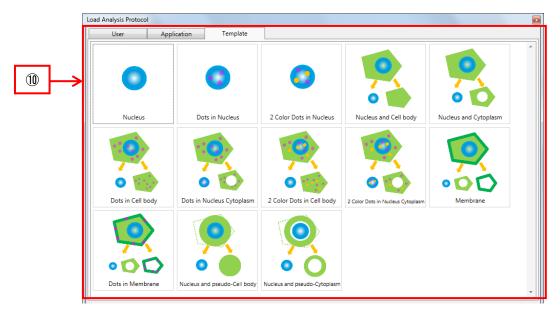
◆Load Analysis Protocol (User Window)



◆Load Analysis Protocol (Application Window)



◆Load Analysis Protocol (TemplateWindow)



1 List of Analysis Protocol (User Window)

Show the list of protocols saved by user.

Name	Description		
Date	Date of saving the analysis protocol		
Name	Name of analysis	protocol	
Analysis	Analysis target	Link	Name of Link Setting
		Detect	Show the name defined by Object Detect Setting

② Details of Link Setting

(In this document, "Link" means linkage among objects which is imaged by different channel.)

Show details of Link Setting of the Analysis Protocol selected at 1, 9 or 1.

3 List of Object Detection Setting

Show the Object Detect List of the Analysis Protocol selected at ①, ⑨ or ⑩.

Name	Description
Ch	Show the channel for detection
Name	Show the name of object detect

4 Description

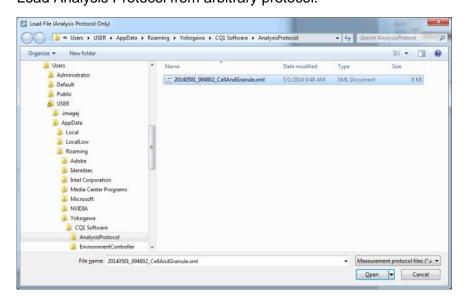
Description of the Analysis Protocol being selected at ①, ⑨ or ⑩.

⑤ Load

Load the Analysis Protocol being selected at ①, ⑨ or ⑩.

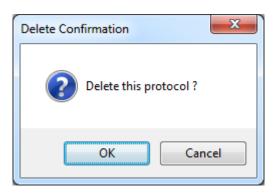
6 CancelClose the window

Other FileLoad Analysis Protocol from arbitrary protocol.



8 Delete

Delete Analysis Protocol selected in ①. Protocol selected in ② or ⑪ can't be deleted



Analysis Protocol List (Application Window)

Show Analysis Protocols preloaded in CQ1 Software.

Name	Description
CellCycle	Protocol for Cell Cycle
Colony	Protocol for analyzing colony
measurement	
Spheroid structure	Protocol for 3D Analysis
	(Spheroid, tissue slice and so on.)
Multinucleated cell	Protocol for analyzing multinucleated cell
Circulating tumor	Protocol for analyzing circulating tumor cell
cells	
FISH	Protocol for FISH Analysis
Total count in	Protocol for counting objects of multiple categories in
individual object	image
Neurite outgrowth	Protocol for analyzing neurite outgrowth

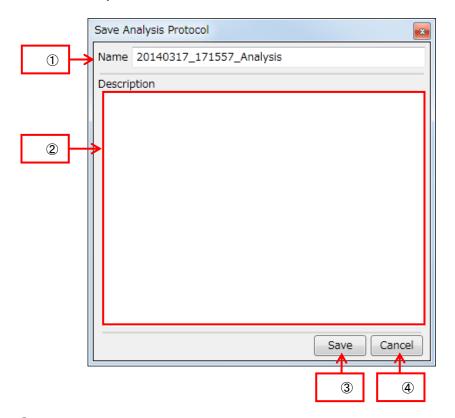
① Analysis Protocol List (Template Window)

Show basic Analysis Protocols preloaded in CQ1 Software

Name	Description
Nucleus	Protocol for analyze nucleus (one object)
Dots in Nucleus	Protocol for analyze nucleus and one category of dots
2 Color Dots in Nucleus	Protocol for analyze nucleus and two categories of dots
Nucleus and Cell body	Protocol for analyze nucleus and cell body
Nucleus and Cytoplasm	Protocol for analyze nucleus and cytoplasm
Dots in Cell body	Protocol for analyze nucleus, cell body and dots in cell body
Dots in Nucleus Cytoplasm	Protocol for analyze nucleus, cytoplasm and dots
2 Color Dots in Cell body	Protocol for analyze nucleus, cell body and two categories of dots in cell body
2 Color Dots in Nucleus Cytoplasm	Protocol for analyze nucleus, cytoplasm and two categories of dots
Membrane	Protocol for analyze nucleus, cell body and cell membrane
Dots in Membrane	Protocol for analyze nucleus, cell body, cell membrane and dots
Nucleus and pseudo-Cell body	Protocol for analyze nucleus and psudo cell body (uniformly expanded nucleus region)
Nucleus and pseudo-Cytoplasm	Protocol for analyze nucleus and psudo cytoplasm (uniformly expanded nucleus region)

(3) Save

Save the Analysis Protocol.



- 1) Name
 - Define the name of Protocol which is also used as file name.
- ② DescriptionInput description of the Protocol.
- 3 Save
 - Save the Protocol.
- 4 Cancel
 - Close the window without saving the Protocol.

(4) Z Analysis Setting

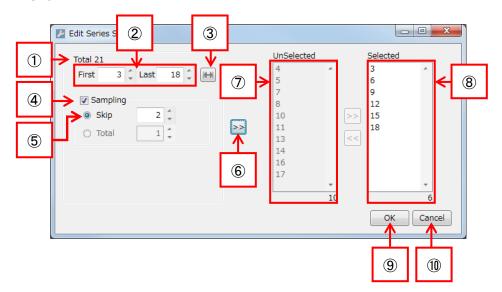
Set the analysis method for multi-Z images. Either one in following table must be selected.

Slice	3D image generated from Multi-Z images is analyzed
MIP	MIP image generated from Multi-Z images is analyzed MIP is a function to build a single image by putting together pixels associated with the largest signals (smallest signals in transmission illumination image) among identical pixels from multiple image data captured on the confocal plane in the Z-axis direction It is able to display and analyze 3D objects as one image
Sum	Sum image generated from Multi-Z images is analyzed Sum is a function to create a single image by putting together pixels associated with the summation signals among identical pixels from multiple image data captured on the confocal plane in the Z-axis direction It is able to display and analyze 3D objects as one image It is efficient if fluorescent intensity of sample is low

Select Z slice images to analyze

ALL	Select all Z slices for analysis target
Selected	Select Z slices for analysis target by following menu opened by
	"Option" button

Click "Option" button and select Z slices for analysis target by following menu.



- ① Total
 - Show all acquired Z slices number.
- 2 Range selection
 - Set range of Z slices to select for analysis target.
- 3 All range selection
 - Set whole range of Z slices to select for analysis target.
- 4 Sampling
 - Select Z slices for analysis target by interval designated by "Skip".
- Skip
 - Input interval of Z slices selection.

6 [>>]

Apply selection range of Z slices.

7 Unselected

Show unselected Z slices for analysis target. Total number of unselected Z slices is shown at the bottom right.

(8) Selected

Show selected Z slices for analysis target. Total number of selected Z slices is shown at the bottom right.

(9) OK

Fix Z slices range setting for analysis target.

① Cancel

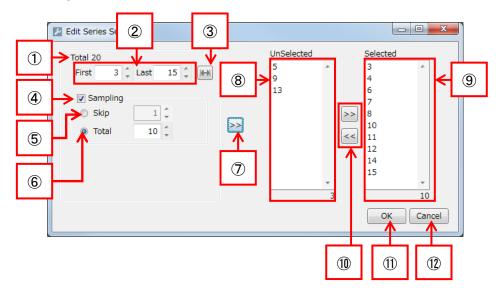
Cancel Z slices range setting and close window

(4) Time-lapse Setting

Select images of time point to analyze

ALL	Select all time point images for analysis target
Selected	Select images of time point for analysis target by following menu
	opened by "Option" button

Click "Option" button and select time point for analysis target by following menu.



1 Total

Show all acquired time points number.

2 Range selection

Set range of time points to select for analysis target.

3 All range selection

Set whole range of time points to select for analysis target.

4 Sampling

Select time points for analysis target by interval designated by "Skip" or total number of time points designated by "Total".

5 Skip

Input interval of time points selection.

6 Total

Input total number of time points to select.

7 >>

Apply selection range of time points.

8 Unselected

Show unselected time points for analysis target. Total number of unselected time points is shown at the bottom right.

9 Selected

Show selected time points for analysis target. Total number of selected time points is shown at the bottom right.

① [>>], [<<]

Move time points between "Unselected" <-> "Selected".

(1) OK

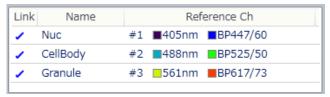
Fix time points range setting for analysis target.

(12) Cancel

Cancel time points range setting and close window

(6) Objective Detect Setting

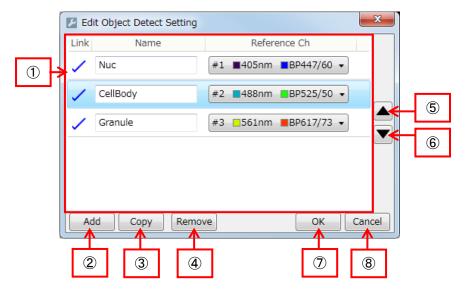
Show the list of current Object Detect Setting.



Name	Description		
Link	Show if the detected object is linked or not.		
	Checked	Linked	
	Unchecked	Unlinked	
Name	Show the object name to detect		
Reference Ch	Show the channel number, light source and BP filters of the object detect channel.		

(7) Edit Object Detect Setting

Edit object detect settings. Up to 10 objects can be added.



① Object list to detect

Show the list of object detect setting

Name	Description		
Link	Show if the detected object is being linked or not.		
	Checked	Linked	
	Unchecked	Unlinked	
Name	Edit name of the object to detect		
Reference Ch	Select object detect channel.		
	Only the confocal channel is selectable. However, Phase Contrast channel is selected if no confocal		
	channel is installed.		

2 Add

Add an Object Detect Setting to the end of the list.

(3) Copy

Copy selected Object Detect Setting and add to the end of the list. However, Link cannot be copied.

4 Remove

Remove selected Object Detect Setting.

(5)

Raise up analysis order of selected Object Detect Setting by one step.

⑥ ▼

Step down analysis order of the selected Object Detect Setting by one step.

(7) OK

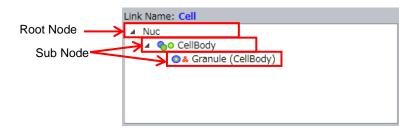
Close window after confirming the object Detect Setting. If a warning sign appears, reconfirm the Protocol at "Analysis" window.

8 Cancel

Cancel the modification and close window.

(8) Link Setting

Show the current Link Setting. The node one-step above the object is called "Root node", and the one below is called "Sub node".



◆Link Setting Items

Item	Description		
Link Name	Name of entire linked objects		
Link Tree	Tree to show correlations among objects		
	Root Node	Top node	
	Sub Node	Other nodes	

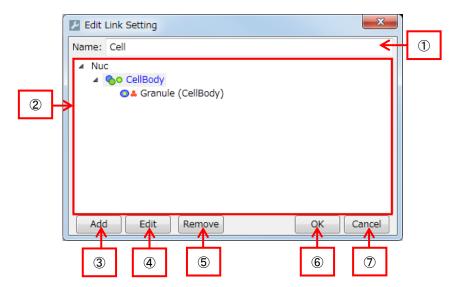
◆Conditions of Sub Nodes

Item	Description		
	Method for linking		
Method	P	Adjacent to Root Node	
	0	Included within Root Node	
	Relationship with Root Node		
Relationship	0	One-to-one relationship	
	&	One-to-many relationship	

(9) Edit Link Setting

Edit Link Setting. Please note the unlinkable conditions shown below.

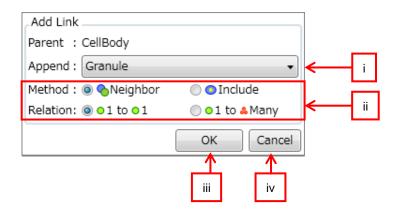
- Sub node cannot be added to an "one-to-many" link.
- Object Detect cannot be linked to "one-to-one" more than once.
- Object Detect cannot be linked to both "one-to-one" and "one-to-many".



- Name
 Set an entire name of all linked objects
- 2 Link Tree
 Show currently set Link Tree. Please refer to (7) Link Setting (page (8) Link Setting
- 3 <u>5-46</u>) for items in the tree.

4 Add

Add a Sub Node to the selected node.



i. Append

Select the Object Detect to be linked. The list of Object Detect Setting is shown.

- ii. Link Condition
 - Select link condition. Please refer to ◆Conditions of Sub Nodes in (7) Link Setting (page <u>5-46</u>).
- iii. OK

Close window after confirming changes.

iv. Cancel

Cancel changes and close window.

⑤ Edit

Edit selected node setting.



i. Append

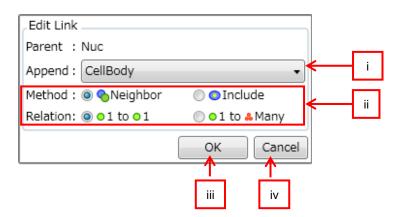
Select the reference Object Detect Setting from the list to be shown.

ii. OK

Close window after confirming changes.

iii. Cancel

Cancel changes and close window.



i. Append

Select a reference Object Detect Setting from the list to be shown.

ii. Link Condition

Select link condition. Please refer to ◆Conditions of Sub Nodes in (7) Link Setting (page <u>5-46</u>).

iii. OK

Close window after confirming changes.

iv. Cancel

Cancel changes and close window.

6 Remove

Remove selected nodes except for Root Node. Root Node cannot be removed. Sub nodes of removed node are also removed altogether.

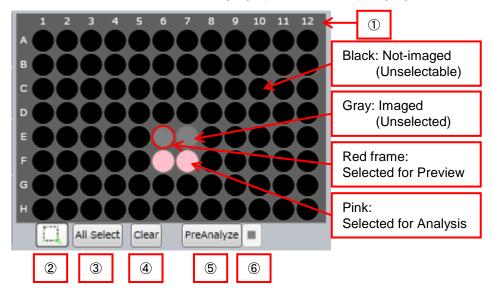
- OKClose window after confirming link setting.
- 8 Cancel Cancel changes and close window.

5.4.5 Analysis Window

Set each method and parameter of Object Detect.

(1) Wells

This function works after test imaging by "PreRec" at "Imaging" window.



Selected Sample Vessel

Diagram of sample vessel selected at "Sample window" is shown. Imaged wells are shown in gray. Not imaged wells are shown in black.

By clicking gray well when button ③ is ., the well is selected for preview (red frame). By left-clicking or left-dragging wells when button ③ is ., the wells are selected for Analysis (pink). By right-clicking or right-dragging, selection for Analysis is canceled.

- ② Selection of Analysis Area Refer to above ①.
- 3 All SelectAll imaged wells are selected for Analysis.
- 4 Clear Selection for Analysis of all wells is canceled.
- ⑤ PreAnalyze

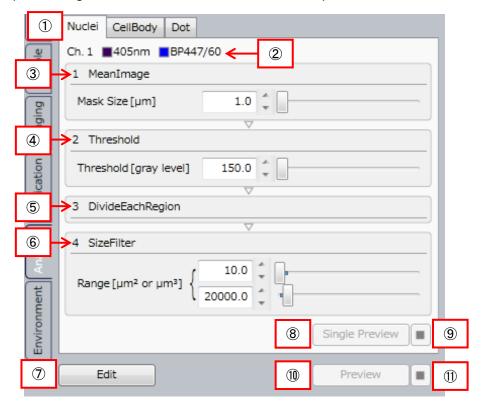
Start preliminary analysis of selected wells for Analysis.

Note
Results of preliminary analysis are not saved.

6 PreAnalyze stop Stop preliminary analysis.

(2) Object Detect Algorism

Set a series of image processing methods (Object Detect Algorism) and parameters to detect objects, such as nuclei or cytoplasm. Image processing methods will run in order from the top line.



- Object Window
 Exchange the Object Detect Algorism to be shown.
- ② Reference Channel Reference Channel is to show the imaging channel for object detect.
- ③ ImageFilter Method: MeanImage Apply averaging filter on images. Mask Size is to set the range of averaging.
- ④ Binarization Method: Threshold Apply binalyzation on images. Set the minimum threshold value in Threshold.
- S Labeling Method: DivideEachRegion Label each region of linked image pixels within the binarized image data.

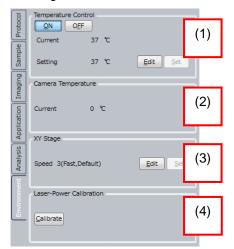
- 6 LabelTransform Method: SizeFilter Filter each labeled region based on its size (area for 2D analysis, and volume for 3D analysis).
- Edit
 "Advanced Editor" window opens. For detail, refer to <u>5.13</u>

 Advanced Editor.
- Single Preview Analysis
 Apply selected object detection to designated Field in well selected for preview.
- 9 Stop Single Preview Analysis
- ① Preview Analysis Apply all object detections to designated Field in well selected for preview.
- 1 Stop Preview Analysis

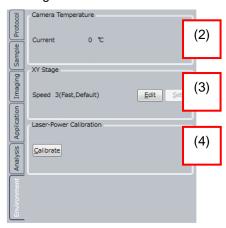
5.4.6 Environment Window

Environment window is to set the operation environment of CQ1 Main Unit.

<Stage Heater mounted model>



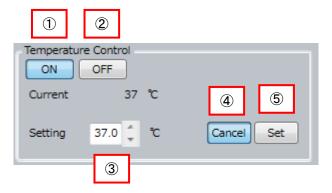
<Stage Heater unmounted model>



(1) Temperature Control

Activate/ inactivate of stage heater and its temperature.

Click "Edit" to set temperature.



(1) ON

Activate temperature control.

② OFF

Inactivate temperature control.

3 Setting

Set temperature. To apply, click ⑤Set.

4 Cancel

Cancel changes of temperature setting.

⑤ Set

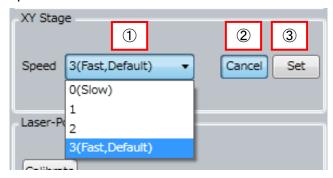
Apply changes of temperature setting.

(2) Camera Temperature

Show current camera temperature.

(3) XY Stage

Set moving speed of XY stage. [Edit] button is to select XY stage speed.



1 Speed

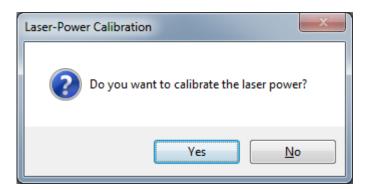
Select XY stage speed. Default setting is the fastest. If samples are easy to ablate from sample vessel, slow setting is recommended.

To apply, click 3Set

- 2 CancelCancel XY stage speed change
- ③ Set Set XY stage speed

(3) Laser-Power Calibration

Click "Calibrate" button to show "Laser-Power Calibration" dialog, and click "Yes" to proceed calibration, but no calibration by clicking "No".



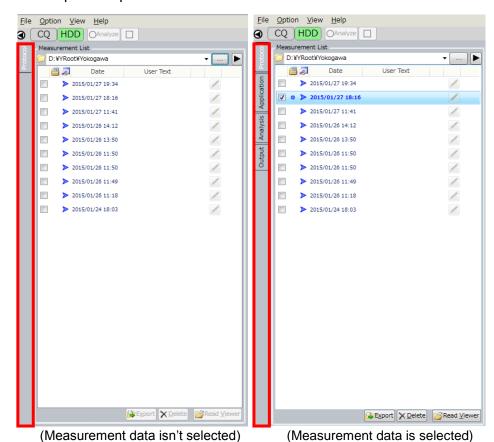
Laser power calibration is performed to maintain excitation laser power irradiated to samples against "Excitation Power" set in "Imaging" window (refer to <u>5.4.3 Imaging Window</u>). It takes a few minutes per 1 laser line to calibrate.

In case that error occurs in laser power calibration, optical adjustment is needed. Please contact the dealer.

5.5 Control Panel (HDD Mode)

In HDD mode, click left-side tabs to set analysis conditions and to output data such as screenshot, movie and so on.

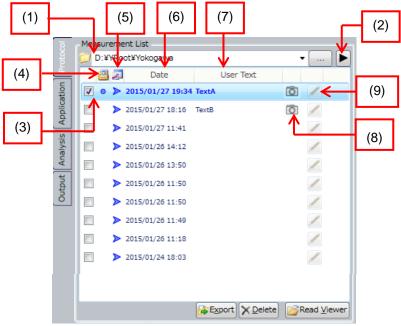
If measurement data isn't selected, only the "Protocol" tab is shown on control panel. Open measurement data and other tabs are shown.



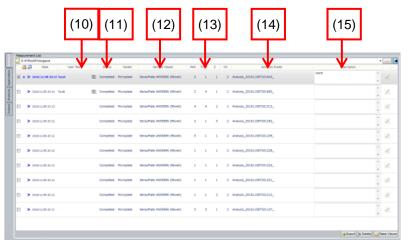
IM 80J01A01-01E

5.5.1 Protocol Window

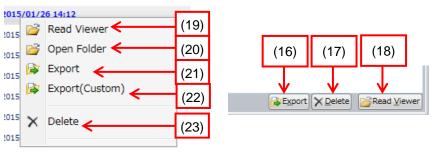
In HDD mode, measurement data list which is saved in the folder selected by "Measurement List" is shown at Protocol window. In detailed view, more information is shown than brief view.



Basical display mode



Advanced display mode

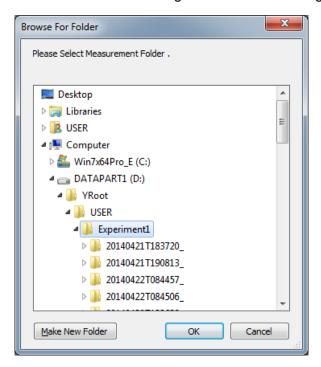


Right click menu

(1) Measurement Data Folder

Full-path-name of the storage folder of measurement data in the list is shown.

Click [...] button at the right end to show folder selection dialog where measurement data storage folder can be changed.



Click "▼" button at the right to show pull down list of previously selected folders. While pull down list is shown selected folder can be deleted from the list by right-click menu.

(2) Switch View Mode

Switch brief/ detailed view mode of measurement data.

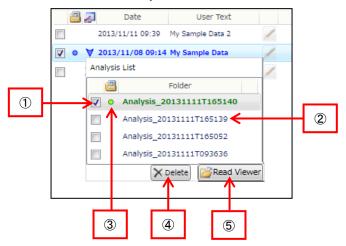
(3) Selection of Measurement Data

Check to select measurement data. Plural selection is available.

(4) Selection of Re-measurement Data

Re-measurement means either review data or repeat image processing after changing analysis conditions. This button is circle-marked when re-measurement data is selected.

(5) Existence of Feature Data



- Quantified data selection check box
 Check to select feature data. Plural selection is available.
- 2 Storage folder name of feature data.
- ③ Select re-quantify data Re-quantify data means to review or repeat image analysis on existing feature data. This button will be circle-marked when selected.
- Delete
 Delete selected feature data, available when more than 2 boxes are checked.
- Show the re-feature data (circled feature data) in "Viewer", available when re-feature data is selected.

(6) Date

Date and time of the measurement are shown.

(7) User Text

Show the text in "Save Folder Name" at "Protocol" window of CQ mode.

(8) Camera Icon

Show that report data such as snapshot exists. It is able to confirm snapshot and movie in report folder by putting pointer on this icon

(9) Pen Button

Show the text in "Description" of "Protocol" window of CQ mode. Click this button to show text, which can be overwritten. Click outside of this window to close.



Click pen button and input text

(10) Status

Show the result status of measurement data. It is able to confirm that measurement is finished or canceled and so on.

(11) Holder

Show sample holder used for measurement.

(12) Sample Vessel

Show sample vessel used for measurement.

(13) Measurement information

Show number of well, time point, Z-stack and channel.

(14) Analysis Folder

Show selected re-analyzed data.

(15) Description

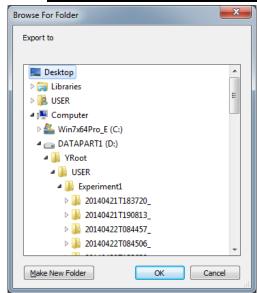
Show the text in "Description" at "Protocol" window of CQ mode.

(16) (21) Export

Export checked measurement data to other folder or drive.

Click this button after selecting measurement data to show folder selection dialog. Select either folder or drive to export data and click

"OK". Data format of saving folder must be NTFS.



After export is compleated, folder that measurement data is exported is opened. Name of measurement data folder is the same as that of original measurement data folder.

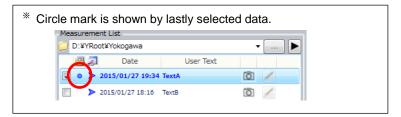
(17) (23) Delete

Delete checked measurement data. Click "Yes" for confirmation message to delete.

(18) (19) Read Viewer

Click to show lastly selected* data in the "Viewer".

Viewer display changes depending on the existence of quantified data as below.



1 No feature data:

Show images

(2) Feature data is selected:

Show selected feature data, which is the same with the case of clicking "Read Viewer" after selecting feature data.

③ Featured data is unselected: Show images

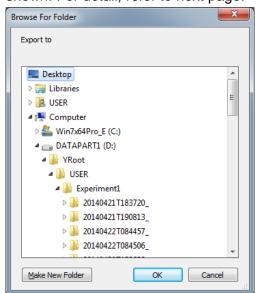
(20) Open Folder

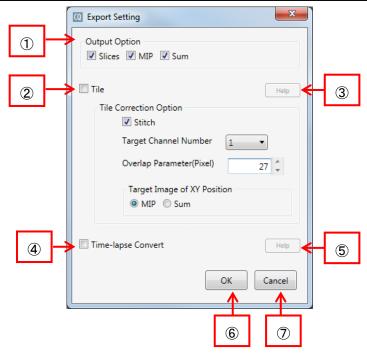
Open the folder that measurement data is saved by windows explorer.

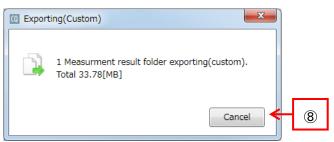
(22) Export (Custom)

Export measurement data with image processing.

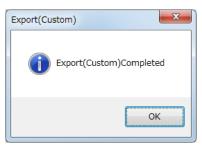
Click this button after selecting measurement data to show folder selection dialog. **Data format of saving folder must be** *NTFS***.** Select saving holder and click "OK". Then "Export Setting" window is shown. For detail, refer to next page.







Progress dialog



Dialog to notice completion

1 Output Option

Select the data to export. It is unselectable if there are no data corresponding to original data.

◆Items of Output Option

Item	Description	
Slices	Export slice images	
MIP	Export the MIP (refer to page 5-42) image	
Sum	Export the Sum (refer to page <u>5-42</u>) image	

2 Tile

Transform images acquired by Area imaging to single tiled image and output.

For using this function, it is necessary that images are acquired by same condition ("Same Area" in "Imaging" window is checked).

◆Items of Tile Correction Option

Item	Description	
Stitch	If checked, images are arranged by image processing and tiled. If unchecked, images are overlapped by trimming parameter and tiled. To use this function, it is necessary to enable overlap acquisition. Check Uncheck Trimming arameter X: 80 % Y: 80 % Save as trimmed image (OFF: as original image) QK Cancel	
	(For details, refer to 5.10 Trimming Parameter)	
Target Channel	Select channel to perform arrangement.	
Number	It is available if "Stitch" is checked.	
Overlap	Designate the image size to perform arrangemetn.	
Parameter (Pixel)	It is available if "Stitch" is checked.	
TargetImage of	Designate the image to perform arrangement. If	
XY Position	images are acquired as 3D, "MIP" and "Sum" is	
	selectable.	
It is available if "Stitch" is checked.		

3 Help (Tile)

Display help message in the case that this function is not available.

4 Time-lapse Convert

Convert plural measurement data as one time-lapse data and output. It is necessary to select plural measurement data acquired by same measurement condition.

5 Help (Timelaps Convert)

Display help message in the case that this function is not available.

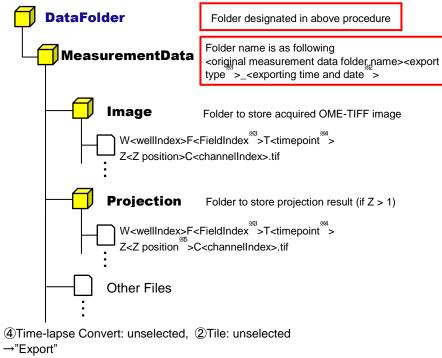
6 OK

Start data export by set parameter.

Progress dialog is displayed after export is started. Dialog to notice completion is displayed after export is completed.

Click "OK" and folder that measurement data is exported is opened.

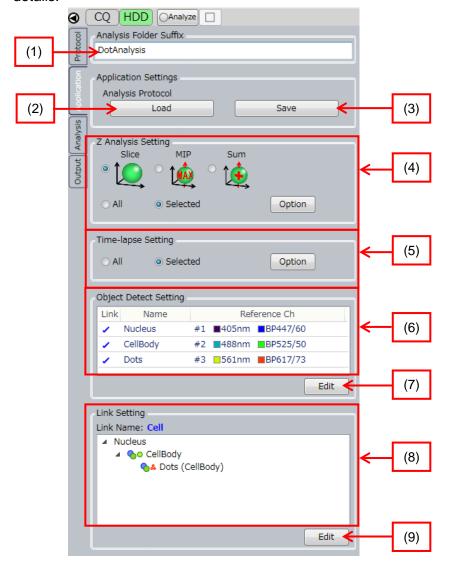
Folder configuration, folder name and file name of exported data are as following.



- - 4)Time-lapse Convert: unselected, 2)Tile: selected
 - →"Tile"
 - (4)Time-lapse Convert: selected
 - →"TimelapseConvert"
- *2 Name format is <yyyyMMdd>T<HHmmss>. In case of April 10, 2015, 12° 34′ 56"
 - →20150410T123456
- **3 In case that ②Tile is selected, FieldIndex is "0001"
- **4 In case that ④Time-lapse Convert is selected, continued number is assigned as
- *5 In case of MIP, <Z position> is "000". In case of Sum, <Z position> is "SUM".
- ⑦ Cancel (ExportSetting Window) Close "ExportSetting" window without export.
- 8 Cancel (Progress dialog) Cancel export.

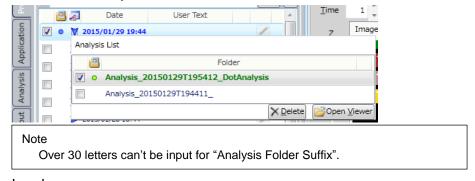
5.5.2 Application Window

Application window is to set image analysis conditions of existing measurement data. Please refer to <u>5.4.4 Application Window</u> for details.



(1) Analysis Folder Suffix

Set suffix of analysis folder name



(2) Load

Load Analysis Protocol

(3) Save

Save Analysis Protocol

(4) Z Analysis Setting Select Z slice images to analyze

(5) Time-lapse SettingSelect images of time point to analyze

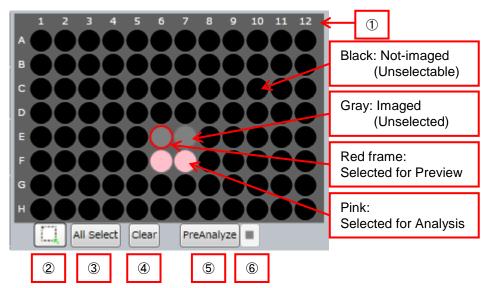
(6) Object Detect Setting Show existing Object Detect Setting list

- (7) Edit Object Detect Setting
- (8) Link Setting
 Show current Link Setting
- (9) Edit Link Setting

5.5.3 Analysis Window

Set methods and parameters for Object Detect

(1) Wells



Selected Sample Vessel

Diagram of sample vessel selected at "Sample window" is shown. Imaged wells are shown in gray. Not imaged wells are shown in black.

By clicking gray well when button ③ is (white), the well is selected for preview (red frame). By left-clicking or left-dragging wells when button ③ is (green), the wells are selected for Analysis (pink). By right-clicking or right-dragging, selection for Analysis is canceled.

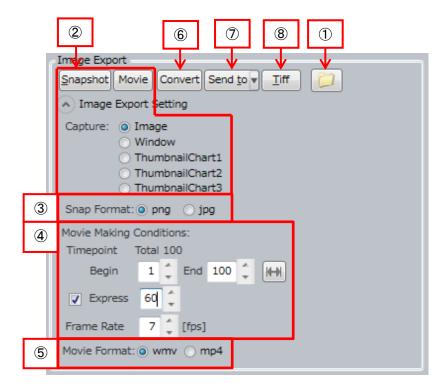
- ② Selection of Analysis Area Refer to above ①.
- 3 All SelectAll imaged wells are selected for Analysis.
- 4 Clear Selection for Analysis of all wells is canceled.
- ⑤ PreAnalyze
 Start preliminary analysis of selected wells for Analysis.
- PreAnalyze stop Stop preliminary analysis.
- (2) Object Detect Algorism

Please refer to (2) Object Detect Algorism of 5.4.5 Analysis Window (page <u>5-52</u>).

5.5.4 Output Window

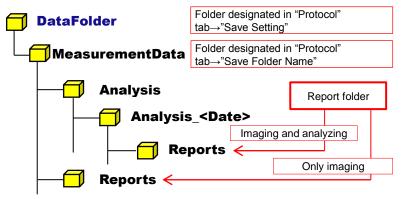
Output images and charts and others displayed in CQ1 software as images in designated folder (Report folder).

(1) Image Export



1 Output Folder

Click to open Report folder. Report folder is generated in following path.



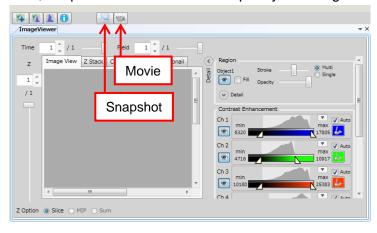
2 Snapshot/ Movie

Output snapshot or movie (Only time-lapse data) of displayed content of CQ1 Software. Content and file name to be output are as following.

Capture	Output Content	File Name
Image	Image shown in "ImageViewer"	Snapshot: Image_W <wellindex>F<fieldindex>T<timep oint="">Z<z position*1="">.png/.jpg Movie: Movie_W<wellindex>F<fieldindex>Z<z position*1="">T<start timepoint="">-T<end timepoint="">.wmv/.mp4</end></start></z></fieldindex></wellindex></z></timep></fieldindex></wellindex>
Window	Whole window of CQ1 Software	Snapshot: Window_ <time and="" date="" of="" outputting="" snapshot**2="">.png/.jpg Movie: Movie_W<wellindex>F<fieldindex>Z<z position**1="">T<start timepoint="">-T<end timepoint="">.wmv/.mp4</end></start></z></fieldindex></wellindex></time>
Thumbnail Chart*	Chart window	Snapshot: Chart*_ <time and="" date="" of="" outputting="" snapshot**^2="">.png/.jpg Movie: Movie_W<wellindex>F<fieldindex>Z<z position**^1="">T<start timepoint="">-T<end timepoint="">.wmv/.mp4</end></start></z></fieldindex></wellindex></time>

^{*1} In case of MIP, <Z position> is "MIP". In case of Sum, <Z position> is "SUM".

Also, snapshot or movie can be output by following button.



3 Snap Format

Set output format of snapshot.

"png" is for PNG format, and "jpg" is for JPEG format.

^{**2} Name format is <yyyyMMdd>_<HHmmss>. In case of April 10, 2015, 12° 34′ 56″

^{→20150410}_123456

4 Movie Making Conditions

Timepoint	Designate time point to save movie		
	Begin Time point of movie beginning		
	End Time point of movie ending		
	H-H	Set "Begin" and "End" to data existing range	
Express	Check to reduce images to convert to movie. If checked, designated number of image from "Begin" to "End" are picked up and converted to movie.		
Frame Rate	Designate frame rate of movie		

Note

In the case of time-lapse data which is acquired by "variable time-lapse" (refer to page <u>5-32</u>), movie is made by designated constant frame late without considering actual imaging "Interval.

In made move, the range of short "Interval" seems to be slow and the range of long "Interval" seems to be fast.

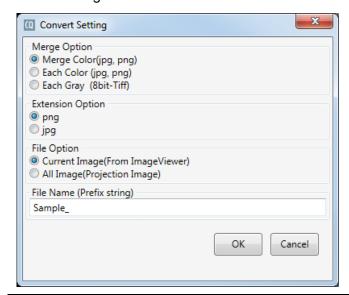
⑤ Movie Format

Set output format of movie.

"wmv" is for WMV format, and "mp4" is for MP4 format.

6 Convert

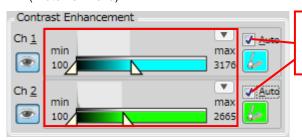
Click this button to open "Convert Setting" window. Click "OK" to convert images.



Merge Option	Select method for channel marging		
	Merge Color	Output with color marging. (Output format is PNG or JPG. Designate either by "Extension Option")	
	Each Color	Output each channel as color image. (Output format is PNG or JPG. Designate either by "Extension Option")	
	Each Gray	Output each channel as gray image (Output format is 8 bit-Tiff)	
Extension Option	Select output format		
	png	PNG format	
	jpg	JPG format	
File Option	ile Option Select output range		
	Current Image	Output images displayed in "ImageViewer"	
	All Image	Output projected images (MIP, Sum and so on) of all fields of all wells and all timepoints**	
File Name (Prefix String)	Designate letters to add to file name (Designated as "Sample_" in above diagram.)		

*Note

Contrast that set by "Contrast Enhancement" is applied to each output image. ("Auto" is invalid)



Contrast that set by left is applied to each output image even if "Auto" is checked

Sub folder is generated in Report folder and converted images are saved in the sub folder. Sub folder name and file name are as following.

Merge Option	Sub Folder Name	File Name
Merge Color	Convert_ <convert edtime and date*</convert 	<pre><string "file="" in="" input="" name"="">W<wellindex>F<fieldindex>T<timepoi nt="">Z<z position**2="">.png/.jpg</z></timepoi></fieldindex></wellindex></string></pre>
Each Color	Convert_ <convert edtime and date*</convert 	<pre><string "file="" in="" input="" name"="">W<wellindex>F<fieldindex>T<timepoi nt="">Z<z position**2="">C<channelindex>.png/.jpg</channelindex></z></timepoi></fieldindex></wellindex></string></pre>
Each Gray	Tiff_ <converted and="" date<sup="" time="">**1></converted>	<pre><string "file="" in="" input="" name"="">W<wellindex>F<fieldindex>T<timepoi nt="">Z<z position**2="">C<channelindex>.png/.jpg</channelindex></z></timepoi></fieldindex></wellindex></string></pre>

^{**1} Name format is <yyyyMMdd>T<HHmmss>. In case of April 10, 2015, 12° 34′ 56″ →20150410T123456

3D Viewer (ImageJ)

Transfer image data to ImageJ and display as 3D image. This function is available when "ImageViewer" setting is "Slice" (refer to page <u>5-87</u>).

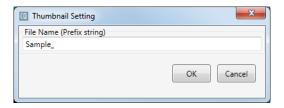
For detail, please refer to next page

Note

The purpose of this function is exporting CQ1 data to ImageJ, which is other software. If you don't use ImageJ whose version is different from that of installed in CQ1 Workstation (1.47v), this function is not guaranteed.

8 Image Output

Output images of measurement data selected at "Protocol" window in TIFF format. Click this button, designate letters to add to file name and click "OK".



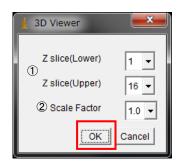
^{**2} In case of MIP, <Z position> is "000". In case of Sum, <Z position> is "SUM".

Procedure of 3D Viewing

①In "Image Export", select "Send to" \rightarrow "ImageJ 3D Viewer"



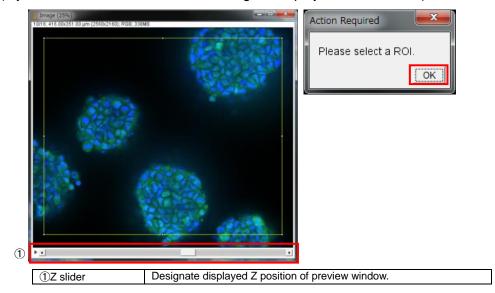
②Set the configuration of 3D Viewer. After setting, click "OK"



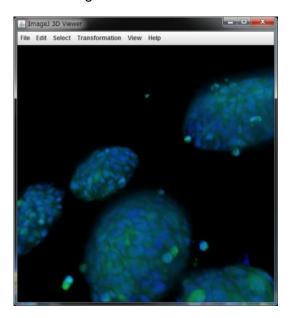
①Z slice	Designate the Z range for 3D projection
②Scale Factor	Condensation (1=100%, 0.5=50%)

③Set the ROI of 3D projection. After setting, click "OK".

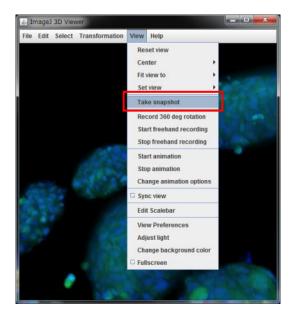
(If you don't set ROI, whole area of image is displayed in 3D Viewer.)



43D Viewer is displayed. Zoom by wheel of mouse. Rotate by left button drag of mouse.



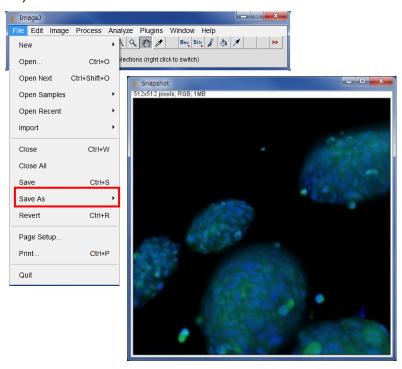
⑤Show the procedures to save snapshot of 3D Viewer. From menu of 3D Viewer, select "View" \rightarrow "Take snapshot".



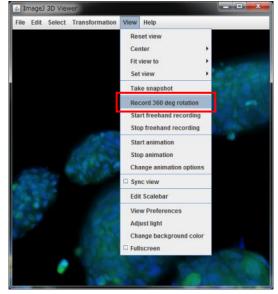
6Set image resolution of snapshot. After setting, click "OK".



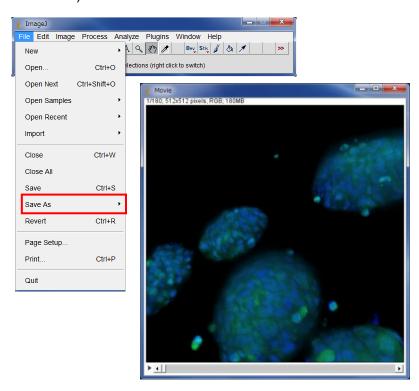
 \bigcirc Image of snapshot is displayed. From menu of ImageJ, select "File" \rightarrow "Save as" and save snapshot in arbitrary format (TIFF, PNG and so on).



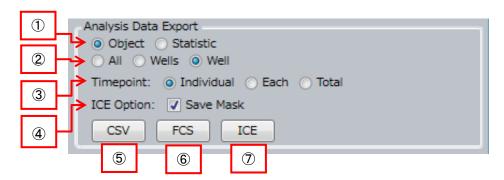
\$Show the procedures to save 3D rotation movie. From menu of 3D Viewer, select "View" \to "Record 360 deg rotation".



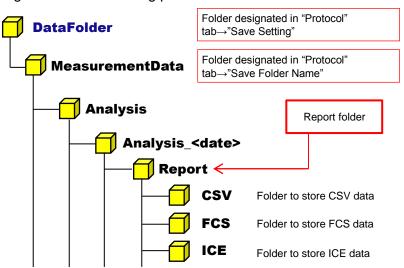
93D rotation movie is displayed. From menu of ImageJ, select "File" \rightarrow "Save as" and save 3D rotation movie in arbitrary format (TIFF, AVI and so on).



(2) Analysis Data Export



Click button in "Image Export" to open Report folder. Report folder is generated in following path.



Output Items

Select output items.

"Object" is to output feature data of each cell, and "Statistic" is to output statistic of feature data per each well

② Output Range (Well)

Select output data range. "All" is to cover all analyzed wells, "Wells" is to cover selected wells (yellow wells in WellTable) and "Well" is to cover currently selected wells (wells in green area in WellTable)

3 Output Range (Timepoint)

Select time point range to output as follows

- Individual: Time points of currently selected range
- Each: All analyzed time points
 (data file is devided corresponding to each time point)
- Total: All analyzed time points
 (all time points data is saved in one file)

4 ICE Option

Check to save mask file in ICE format.

(5) CSV Output

Output data in CSV format

Sub folder name and file name in CSU folder is as following

Output Item	Output Range (Timepoint)	Sub Folder Name	File Name
Object	Individual	<output range<br="">(Well)>_Individual_<o utputting time and date^{**1}></o </output>	[<name data="" folder="" measurement="" of="">]Feature_Cell.csv</name>
	Each	<output range<br="">(Well)>_Each_<output ting time and date^{*1}></output </output>	[<name data="" folder="" measurement="" of="">]Feature_T<time point="">_Cell.csv</time></name>
	Total	<output range<br="">(Well)>_Total_<output ting time and date^{**1}></output </output>	[<name data="" folder="" measurement="" of="">]Feature_Cell.csv</name>
Statistic	-	(No sub folder)	[<name data="" folder="" measurement="" of="">]Statistics.csv</name>

 $^{^{**1}}$ Name format is <yyyyMMdd>T<HHmmss>. In case of April 10, 2015, 12° 34′ 56″ →20150410T123456

Note

If row of CSV data exceeds 1,000,000, the data can't be opened by spreadsheet software such as Microsoft® Excel. In the case, reduce row of CSV file by outputting the data per well

Also, if file name (absolute path) of CSV data exceeds 256 letters, the data can't be opened.

6 FCS Output

Output data in FCS format. "Statistic" can't be selected as output item.

7 ICE Output

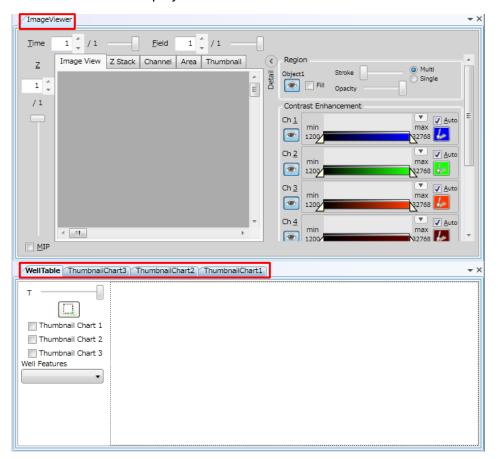
Output data in ICE format. "Statistic" can't be selected as output item.

5.6 Viewer

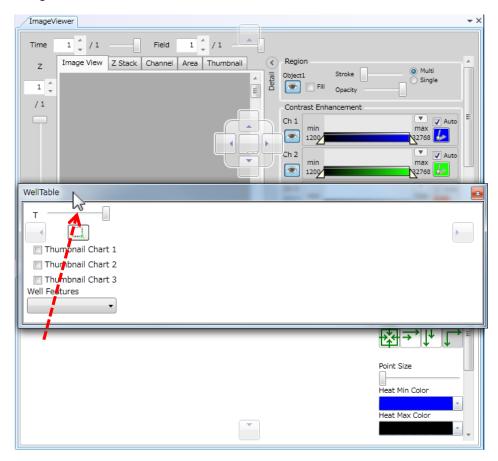
In "Viewer", captured images, image-detected regions, list of measurement and statistic of feature data, and scattergram of measurement data are shown.

5.6.1 Operation of Viewer

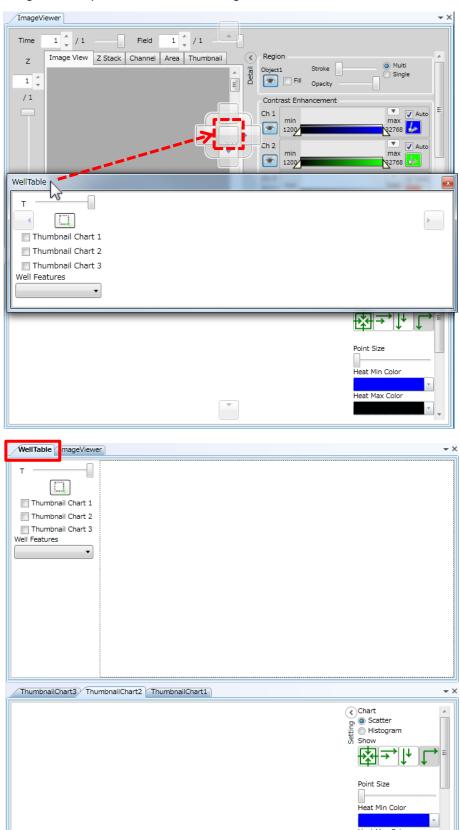
Click tab to switch display of each window.



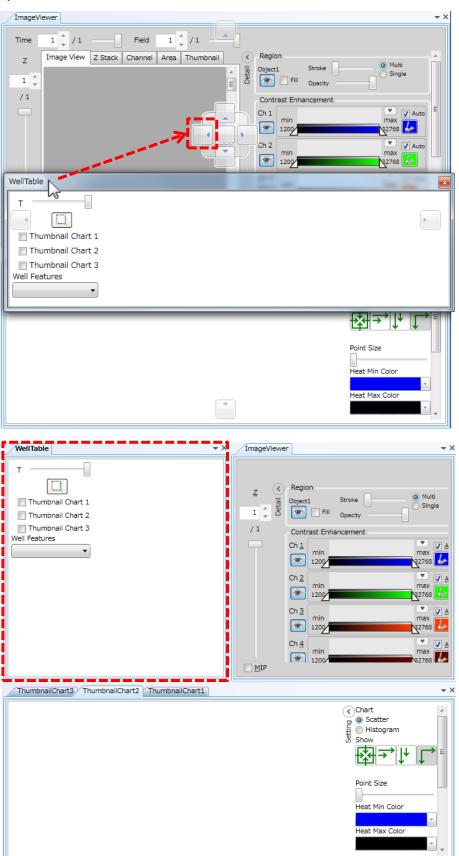
Drag tab to move the window.



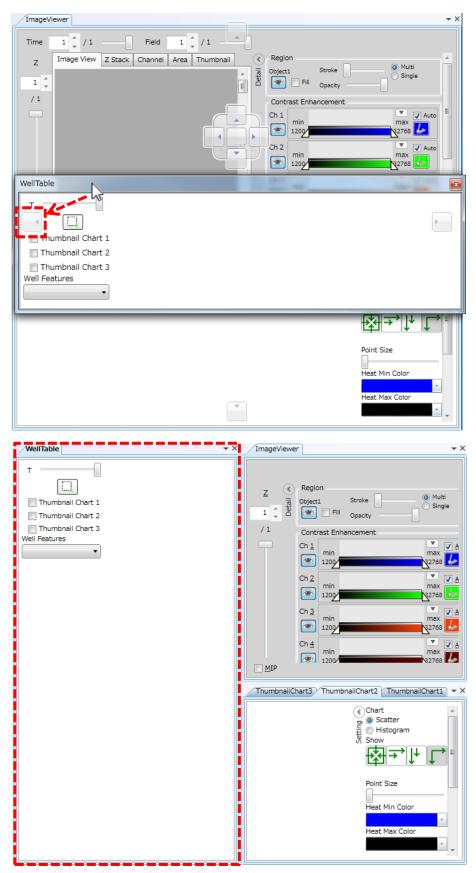
Drag tab to square mark as following to add the tab to the window.



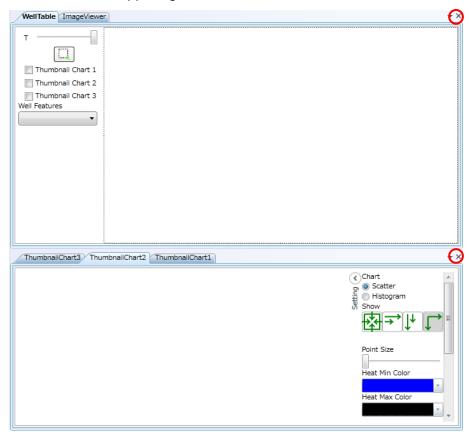
Drag tab to center arrow mark as following to allocate as new window by the window.



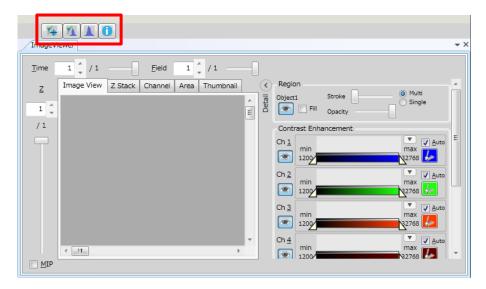
Drag tab to peripheral arrow mark as following to allocate as new window at end of "Viewer".



Click "x" mark in upper right of each window to close selected window.



Windows can be set to default position by "Viewer Layout" button. For detail, refer to <u>5.8 Viewer Layout</u>.



5.6.2 ImageViewer Window

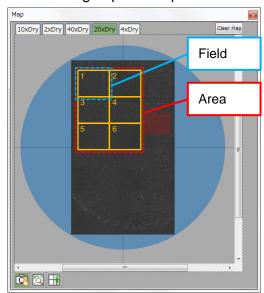
<Basic operation>

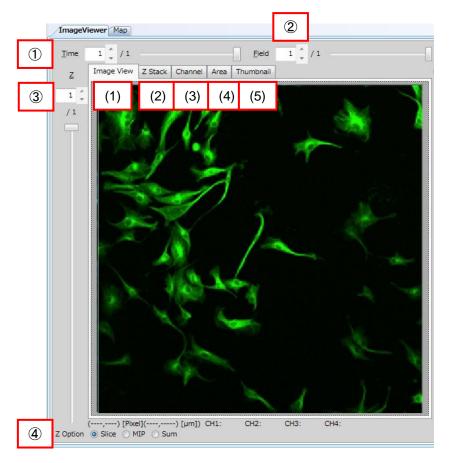
In "ImageViewer", it is able to move image by right-dragging. Also, it is able to zoom image by operation of mouse wheel.

This operation is same in <u>5.6.3 MAP Window</u>.

<Explanation of term: "Field" and "Area">

CQ1 can image not only one field but also multiple tiled fields. In this section and next section <u>5.6.3 MAP Window</u>, single field is described "Field" and group of multiple tiled field is described "Area".



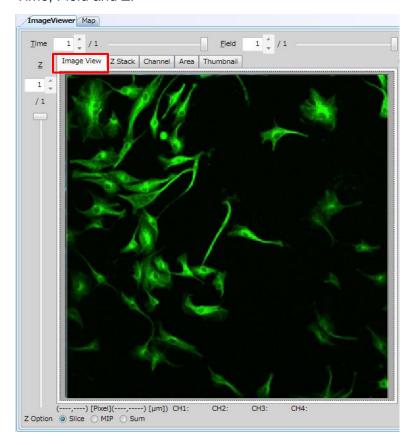


"ImageViewer" has five windows; (1) Image View, (2) Z Stack, (3) Channel, (4) Area, and (5) Thumbnail. Common functions of 5 windows are shown in 1~4, below.

- Time
 Set the time point to display.
- ② Field/Area "Image View", "Z Stack" and "Channel" windows are to set "Field" to display, and "Area" window is to set "Area" to display. During measurement, only the ongoing measurement is shown, thus display settings cannot be changed.
- ③ Z Set Z position to display. During measurement, only the ongoing measurement is shown, thus display settings cannot be changed.
- ② Z Option Check "MIP" to display MIP image. Check "Sum" to display Sum image.

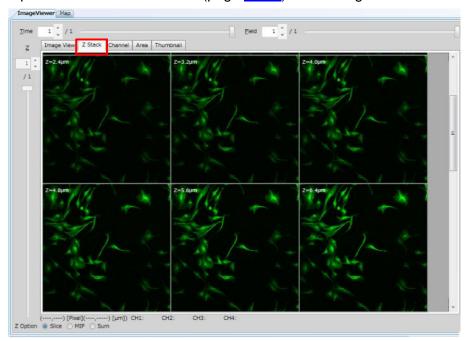
(1) Image View Window.

It is possible to display multi-channel images as one image by setting Time, Field and Z.



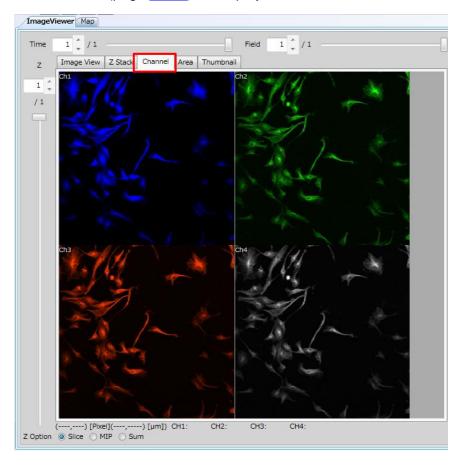
(2) Z Stack Window

Display tiled images from bottom to top in Z by setting Time and Field. MIP and Sum check box is not available here. Please refer to (6) View Option of 5.6.9 Tool Function (page <u>5-120</u>) about image size.



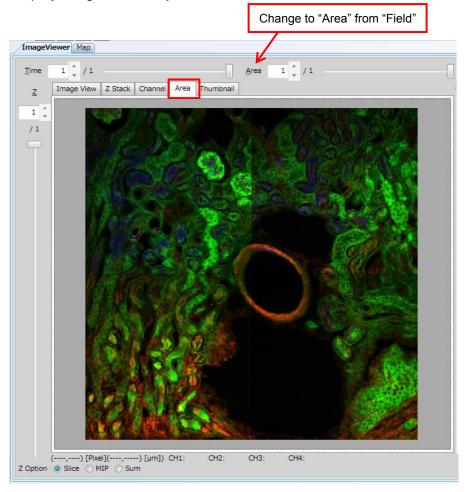
(3) Channel Window

Separately display images of each channel from a multi-channel image by setting Time, Field and Z. Please refer to (6) View option of 5.6.9 Tool Function (page <u>5-120</u>) for display modes.



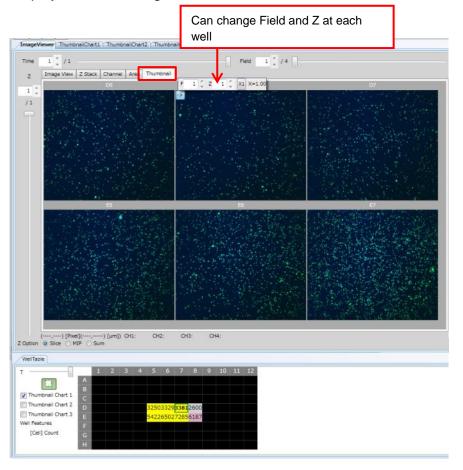
(4) Area Window

Display image of Area by tile.

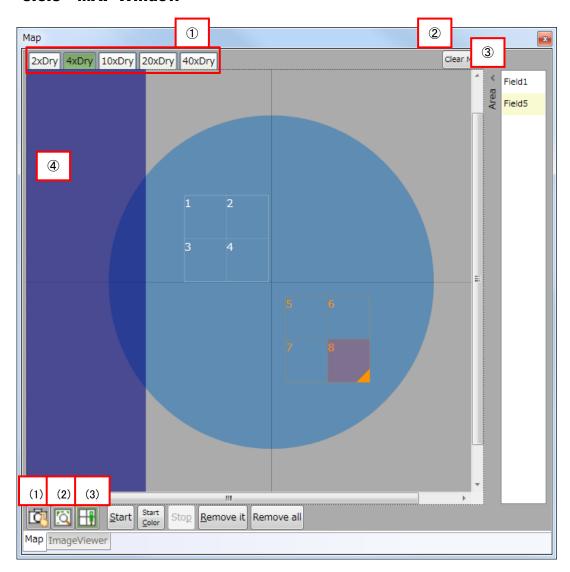


(5) Thumbnail Window

Display thumbnail images of wells selected at WellTable window. .



5.6.3 MAP Window

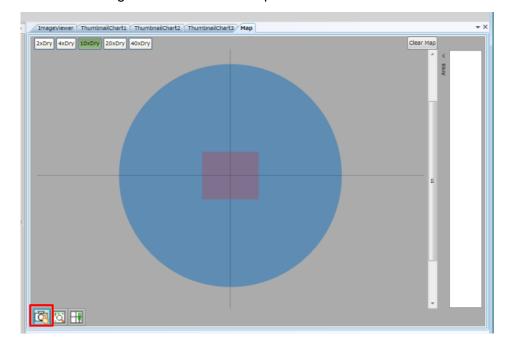


MAP viewer has (1) Test imaging mode, (2) Map mode, and (3) Rec mode. Their common functions are shown in ①~④ below.

- Objective lens selection
 Selected lens is highlighted.
- 2 Map clearClear the map in display.
- ③ Area List Display Area list. If there is more than one Area, the leading number of "Field" is shown.
- 4 Unimagable Region Blue region in "Map" window can't be designated as imaging region because objective lens interferes with stage attachment, sample holder and sample vessel.

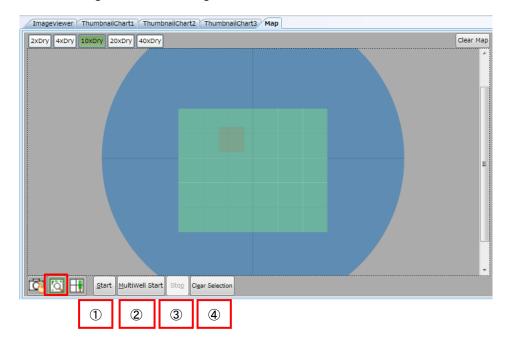
(1) Test Imaging Mode

Capture test image of clicked Field at a single channel by one-click. Field to be imaged is shown as red square.



(2) Map Mode

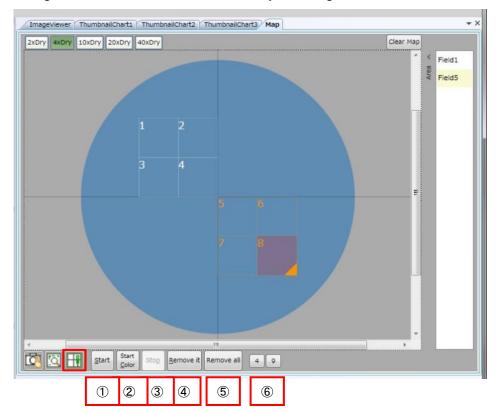
Capture map image by selecting a region by left-drug of mouse. Selected region is shown in green.



- Start
 Start map-imaging.
- ② MultiWell Start Start map-imaging of multiple wells.
- ③ StopStop map-imaging.
- Clear SelectionClear selection of map-imaging region.

(3) Rec Mode

Set Area(s) to be imaged. Clicked Area is selected and highlighted in orange. Selected Area can be moved by left-drag.



① Start

Capture test images of selected Area (highlighted by orage frame) at one channel, and display on the map.

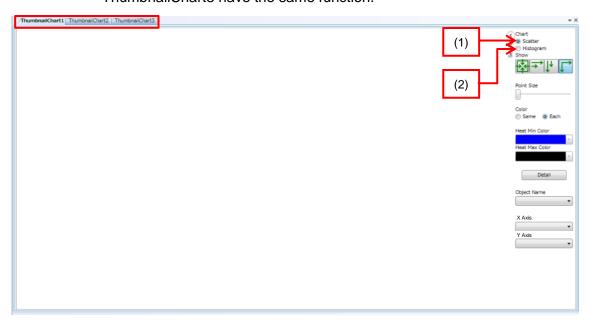
② Start Color

Capture test images of selected Area (highlighted by orage frame) by multiple channels under the same conditions for the measurement.

- 3 Stop
 Stop ① and ② above.
- Remove it
 Remove selected Area. Area numbers will be renewed.
- ⑤ Remove all Areas.
- 6 Define FOV Define the FOV (Field of View) at either 4 or 9, automatically.

5.6.4 ThumbnailChart Window

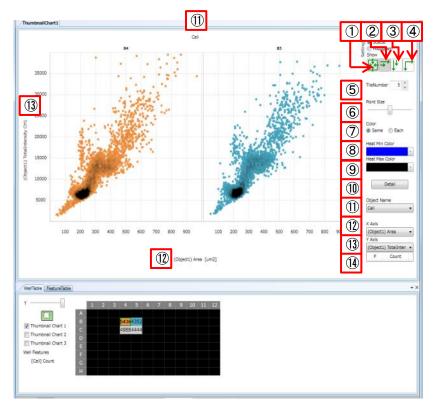
ThumbnailChart has two data display modes, (1) Scatter mode, and (2) Histogram mode. ThumbnailChart1, ThumbnailChart2 and ThumbnailChart3 have the same function.



(1) Scatter Mode

Display scattergram of measured data.

Measured data of wells selected at "WellTable" window are plotted.



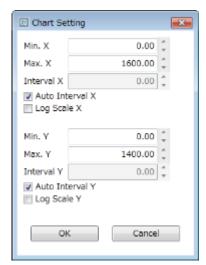
- Well Merge Display
 Display a measurement result of selected wells by one merged chart.
- Each Well Display (Horizontal Priority)
 Display charts of each well in line, or in horizontal priority.
- Each Well Display (Vertical Priority)
 Display charts of each well longitudinally, or in vertical priority.
- Each Well Display (Lattice)Display charts of each well aligned in lattice.
- Shown when either Vertical Priority Display or Horizontal Priority Display is selected. The number of charts to wrap lines can be specified. Charts above the specified number are in display at the next line.
- 6 Point Size Point size on a chart can be enlarged by moving the slider bar to the right.

7 Color

"Same" is to display all wells on a chart in the same color. "Each" is to color wells in different colors by each well.

- 8 Heat Min Color Select the color for low density area in a chart.
- (9) Heat Max ColorSelect the color for high density area in a chart.
- 10 Detail

Dialog to select display range of X-axis and Y-axis is shown, in which Min. and Max. display range and interval of X and Y axis can be set. Logarithmic scale is selectable for each axis. If enormously small value compared to axis size is set for Interval X or Interval Y, the value isn't applied.

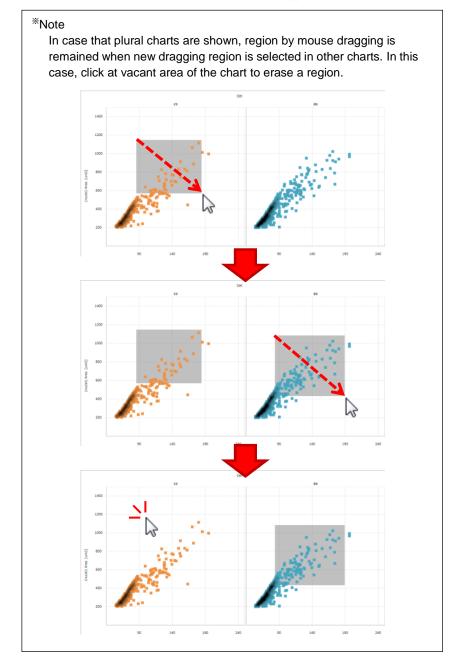


- Object Name
 Select object detect name to plot.
- X AxisSet the specified measurement value on X axis
- Y Axis
 Set the specified measurement value on Y axis

(14) Well, Field Count

"ImageViewer" shows clicked well or Field of the list.

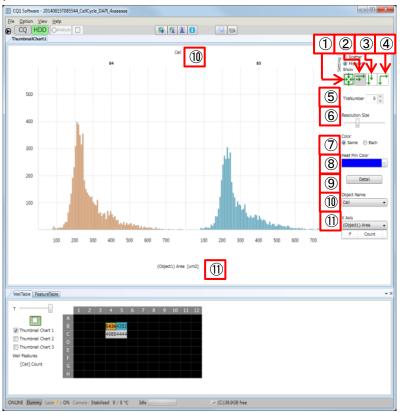




(2) Histogram Mode

Display histograms of measurement data.

Measurement data of yellow wells specified at "WellTable" window are plotted.



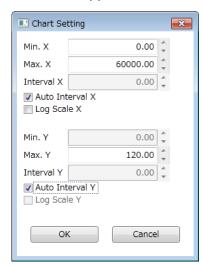
- Well Merge Display
 Display a measurement result of selected wells by one merged chart.
- Each Well Display (Horizontal Priority)
 Display charts of each well in line, or in horizontal priority.
- Each Well Display (Vertical Priority)
 Display charts of each well longitudinally, or in vertical priority.
- Each Well Display (Lattice)Display charts of each well aligned in lattice.
- ⑤ Tile Number Shown when either Vertical Priority Display or Horizontal Priority Display is selected. The number of charts to wrap lines can be specified. Charts above the specified number are shown in the next line.

6 Resolution Size

Change the width (resolution size) of the histogram. By moving slider bar to the right, width of the bar will be extended (to make the resolution size coarser.

- Heat Min Color
 Set the color of the bar on a chart.
- ® Detail

Dialog to select display of X-axis and Y-axis is shown. Min. and Max. display ranges, and interval of X and Y axis can be set. Logarithmic scale is selectable for each axis. If enormously small value compared to axis size is set for Interval X or Interval Y, the value isn't applied.



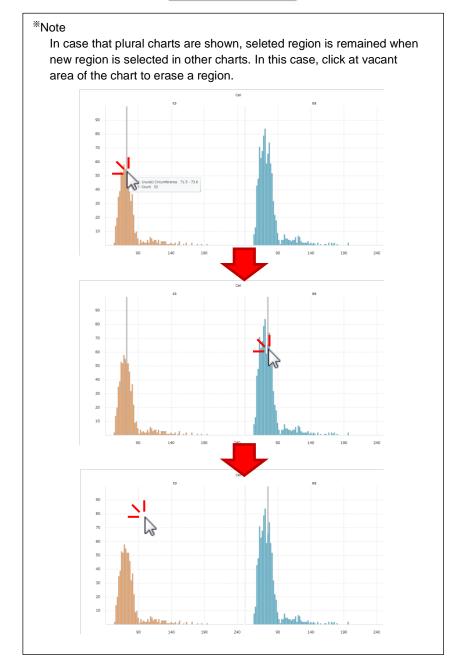
- 9 Object NameSelect object detect name to plot.
- X AxisSet specified measurement value on X axis.

11) Well, Field Count

When region is designated (by mouse dragging) in chart, show the number of points included in the region by the list. If chart display is Well Merge (above ①, 1), number of each well is shown. If chart display is Each Well (above ② - ④, 1, 1, 1), number of each Field is shown.

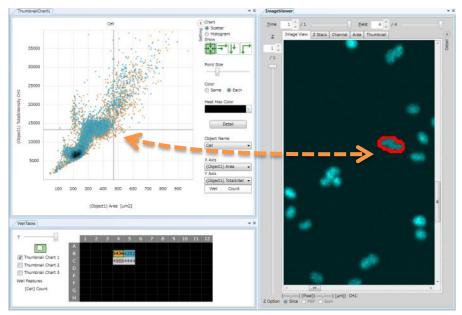
"ImageViewer" shows clicked well or Field of the list.

Well	Count	F	Count
B04	637	2	290
B05	554	4	268
		_ 1	245
		3	237

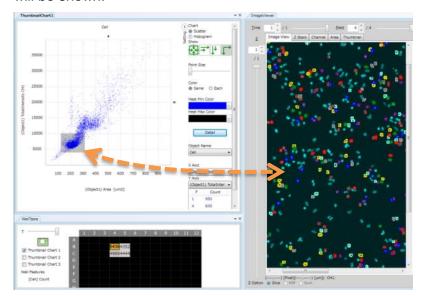


(3) Jump Function

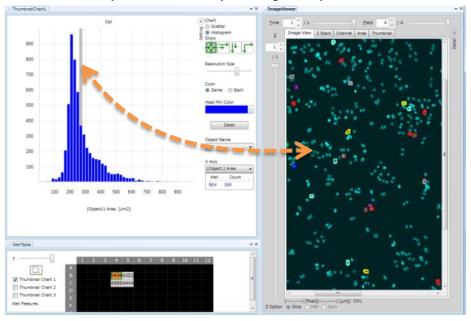
The detected object in corresponding image will be shown by clicking a spot plotted in scattergram. On the other hand, a corresponding spot in scattergram will be cross-marked by clicking an object.



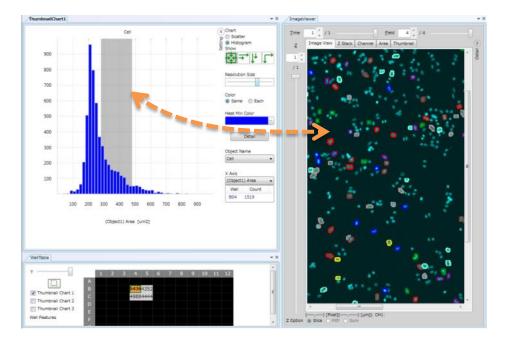
By designating rectangle region in scattergram by mouse dragging, the detected objects corresponding to spots in designated region in image will be shown.



The detected object in corresponding image will be shown by clicking a bin in histogram. On the other hand, a corresponding bin in histogram will be marked by a vertical line by clicking an object.

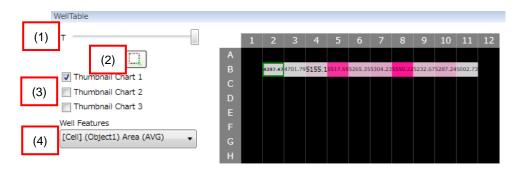


By designateing region in histogram by mouse dragging, the detected objects corresponding to bins in designated region in image will be shown.



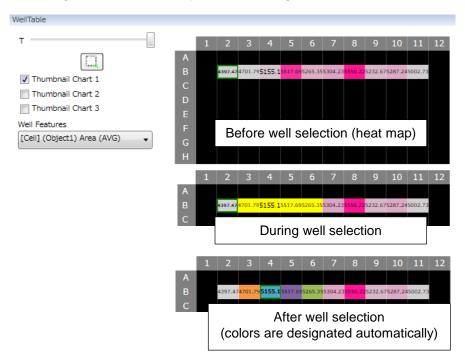
5.6.5 WellTable Window

In "WellTable" window, it is able to select wells to be shown at ThumbnailChart 1, ThumbnailChart 2 and ThumbnailChart 3 (2), and to select the statistic of feature data of each well (3).



- T
 Set time point to display.
- (2) Select Display Range

Wells become selectable by left button dragging when this button becomes (green) by clicking. By right button, wells are unselected. Wells become yellow during selecting. Color of wells are designated automatically after selecting.



(3) Thumbnail Chart Selection

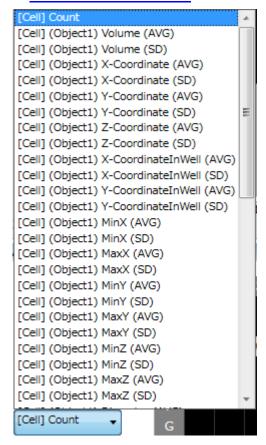
By checking [Thumbnail Chart 1] checkbox, the chart of selected wells will be shown at "ThumbnailChart1" window, and so are the same with [Thumbnail Chart 2] and [Thumbnail Chart 3].

(4) Measurement Data Selection

By selecting measurement data of each well, the data will be shown at WellTable. The red color becomes deeper proportionally to the relative values.

Selectable measurement data are cell number per well and average and standard deviation of data described in <u>5.6.8</u>

Measurement Data per well. Cell number is expressed as "Count", average of each data is expressed as "data name (AVG)" and standard deviation is expressed as "data name (SD)". Please refer to <u>5.6.8 Measurement Data</u> for details of measurement data.



5.6.6 FeatureTable Window

In "FeatureTable" window, it is able to confirm measurement value of detected object such as cell, granule and so on. Please refer to <u>5.6.8</u>
<u>Measurement Data</u> about shown items.

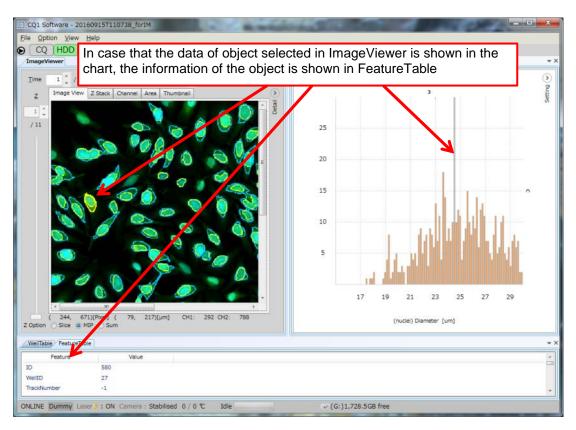
To confirm measurement value, click object in "ImageViewer" or data in either ThumbnailChart.

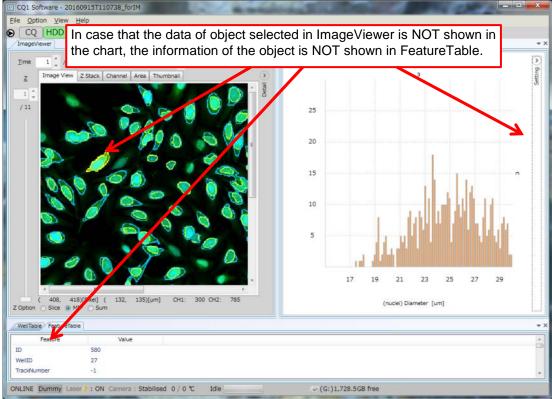


Note

Either case that there are no feature data or that cell or granule have never selected, nothing is shown in FeatureTable.

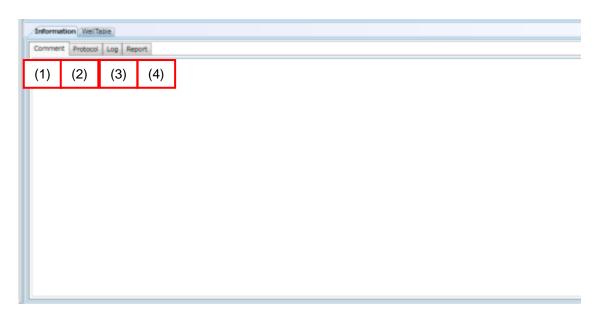
In case that range of either axis is designated, the data which is not shown in the chart is not shown at FeatureTable.





5.6.7 Information Window

"Information" window is, to confirm information of measurement data. This function is only for "HDD mode". In "CQ1 mode", display nothing.



- (1) Comment
 - Show Description of measurement data.
- (2) Protocol
 - Show Protocol of measurement data.
- (3) Log
 - Show log of measurement data.
- (4) Report
 - Show snap shot of measurement data.

5.6.8 Measurement Data

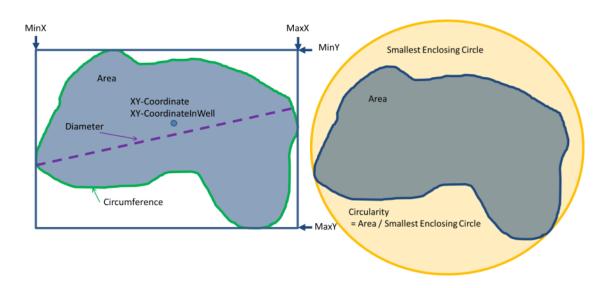
CQ1 Software outputs following measurement value.

(1) 2-Dimentional Measurement

Item	Description
Area	Area [µm²]
X-Coordinate	X coordinate of centroid [µm]
	(In reference to upper left of image)
Y-Coordinate	Y coordinate of centroid [µm]
	(In reference to upper left of image)
X-CoordinateInWell	X coordinate of centroid [µm]
	(In reference to center of well)
Y-CoordinateInWell	Y coordinate of centroid [µm]
	(In reference to center of well)
MinX	Min.X axis [μm]
	(In reference to upper left of image)
MaxX	Max.X axis [µm]
	(In reference to upper left of image)
MinY	Min.Y axis [µm]
	(In reference to upper left of image)
MaxY	Max.Y axis [µm]
	(In reference to upper left of image)
Diameter	Diameter [µm]
Circumference	Contour length [µm]
Circularity	Circularity
SkeletonLength	Length of line object [µm]
JunctionCount	Junction count of line object
BranchCount	Branch count of line object
TotalIntensityCHn	Total image intensity [*] in channel "n"
MeanIntensityCHn	Mean image intensity ^{**} in channel "n"
SdvIntensityCHn	Standard deviation of image intensity* in channel "n"
MinIntensityCHn	Min.image intensity ^{**} in channel "n"
MaxIntensityCHn	Max. image intensity ^{**} in channel "n"

^{*}Note

Value that background(about 100) is subtracted is output.

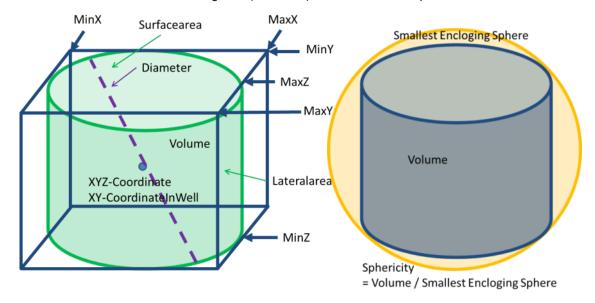


(2) 3-Dimentional Measurement

Item	Description
Volume	Volume [µm³]
X-Coodinate	X coordinate of centroid [μm]
	(In reference to upper left of Z=1-image)
Y-Coodinate	Y coordinate of centroid [μm]
	(In reference to upper left of Z=1-image)
Z-Coodinate	Z coordinate of centroid [µm]
	(In reference to upper left of Z=1-image)
X-CoordinateInWell	X coordinate of centroid [μm]
	(In reference to center of well)
Y-CoordinateInWell	Y coordinate of centroid [μm]
	(In reference to center of well)
MinX	Min.X axis [µm]
	(In reference to upper left of Z=1-image)
MaxX	Max.X axis [µm]
	(In reference to upper left of Z=1-image)
MinY	Min.Y axis [µm]
	(In reference to upper left of Z=1-image)
MaxY	Max.Y axis [µm]
	(In reference to upper left of Z=1-image)
MinZ	Min. Z axis [µm]
	(In reference to upper left of Z=1-image)
MaxZ	Max.Z axis [µm]
D	(In reference to upper left of Z=1-image)
Diameter	Diameter [µm]
Lateralarea	Lateral area [µm²]
Surfacearea	Surface area [µm²]
Sphericity	Sphericity
SkeletonLength	Length of line object [µm]
JunctionCount	Junction count of line object
BranchCount	Branch count of line object
TotalIntensityCHn	Total image intensity ^{**} in channel "n"
MeanIntensityCHn	Average image intensity* in channel "n"
SdvIntensityCHn	Standard deviation of image intensity [*] in channel "n"
MinIntensityCHn	Min. image intensity ^{**} in channel "n"
MaxIntensityCHn	Max. image intensity ^{**} in channel "n"

^{*}Note

Value that background(about 100) is substructed is output.



(3) Measurement Value with Link Setting

In case that link is set in "Application" window, object name is shown in front of the name of measurement value. Also, in case that one-to-many subnode is set in the link, linked number and average of each feature data is output.

For example, in case of following link, feature data described in following table is output.

■ Main

%o Sub1

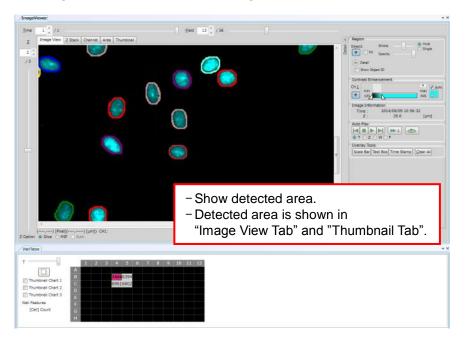
O& Sub2 (Main)

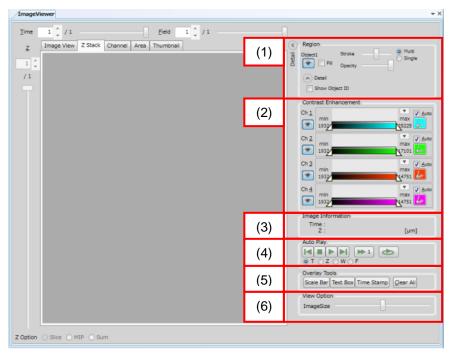
◆Output feature data (excerpts)

Item	Description
(Main) Volume	Volume [µm³] of Main object
(Main) X-Coordinate	X coordinate of centroid [µm] of Main object
(Main) Y-Coordinate	Y coordinate of centroid [µm] of Main object
(Main) Z-Coordinate	Z coordinate of centroid [µm] of Main object
•	
(Sub1) Volume	Volume [µm³] of Sub1 object
(Sub1) X-Coordinate	X coordinate of centroid [µm] of Sub1 object
(Sub1) Y-Coordinate	Y coordinate of centroid [µm] of Sub1 object
(Sub1) Z-Coordinate	Z coordinate of centroid [µm] of Sub1 object
•	
-	
(Sub2 (Main)) COUNT	Number of Sub2 object linked to Main object
(Sub2 (Main)) Volume AVG	Volume [µm³] of Sub2 object linked to Main object
(Sub2 (Main))	X coordinate of centroid [µm] of Sub2 object
X-Coordinate AVG	linked to Main object
(Sub2 (Main)) Y-Coordinate	Y coordinate of centroid [µm] of Sub2 object
AVG	linked to Main object
(Sub2 (Main)) Z-Coordinate	Z coordinate of centroid [µm] of Sub2 object
AVG	linked to Main object
•	
•	
•	

5.6.9 Tool Function

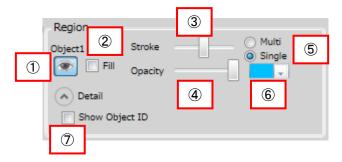
Tool Function is to change the display style of image-detected regions in "ImageViewer", or contrast of images.





(1) Region

Set display options for image-detected region. This setting is applied to only "Image View" window and "Thumbnail" window of "Viewer".



Display detected region

Eye-mark shuts or opens by each click.

When opened, the image-detected region is shown in "ImageViewer" and not shown when closed.

② Fill

All image-detected regions will be colored if checked, and only their outline will be colored if unchecked.

3 Stroke

Outline of region gets thicker by moving this slider to the right. The slider bar is not valid if "Fill" is checked.

4 Opacity

Opacity of the color of image-detected regions increase by sliding the bar to the left.

⑤ Multi/Single

[Multi] is to color detected regions with multiple colors. [Single] is to color detected regions with the one selected by "Color".

6 Color

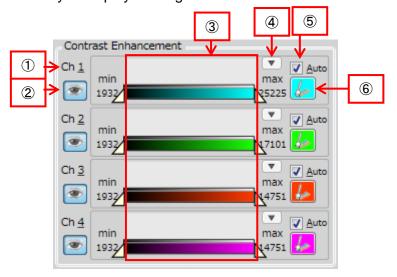
Select color of detected regions. Invalid if "Multi" is selected.



Show Object IDCheck to show ID of detected regions.

(2) Contrast Enhancement

Display intensity histograms of images of each channel, and Max. /Min, intensity of displayed images.



① Ch

Channel number

② Merge Setting Button

Eye-mark shuts or opens by each click of this button.

Images of current channel are shown in "ImageViewer" when the

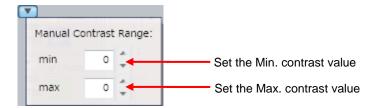
eye is open, and not shown if closed.

3 Intensity Display

Intensity information of displayed image is shown.

- Intensity Range (Max./Min,)
- Intensity distribution (Histogram)
- Intensity range of displayed image (Intensity range setting button)
- 4 Manual Contrast Range

Contrast range can be manually adjusted by unchecking [Auto].

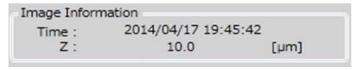


- ⑤ Automatic Contrast Adjustment By checking [Auto], contrast is automatically adjusted according to the intensity of images.
- Set the pseudo color of displayed image in the current channel.



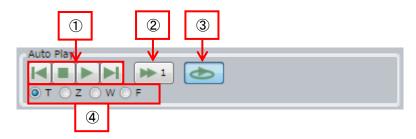
(3) Image Information

Show detail information of the image displayed in "ImageViewer".



(4) Auto Play

Operate each slider bar in "ImageViewer".



- Slider bar operation
 - Move to first imageStop auto playStart auto playMove to last image
- Auto play speed
 Designate speed of auto play from 1 (slow) 4 (fast).
- 3 Looping auto play
- Slider bar selectionSelect slider to operate.
 - T: Time Z: Z position W: Well F: Field

(5) Overlay Tools

Overlay information such as text or size on the displayed images in "ImageViewer".



1) Scale Bar

Add "scale bar" on images.

By dragging in "ImageViewer", position and length can be changed. Also, by right-click menu, color, with/ without of caption, with/ without of edge step and pitch of length changing can be changed and delete can be performed.

2 Text Box

Add any text on images.

Edit text in "ImageViewer". By dragging, position can be changed. Also, by right-click menu, color and font can be changed and delete can be performed.

3 Time Stamp

Add time stamp on images.

By dragging in "ImageViewer", position can be changed. Also, by right-click menu, color and font can be changed and delete can be performed.

(4) Clear All

Clear all added information from images.

(6) View Option

Slider to change size of images displayed in "ImageViewer", when "Z Stack" window at "ImageViewer" is open.

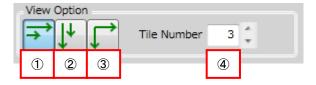


Items to change display mode in "ImageViewer" are shown when "Channel window" at "ImageViewer" is open.



- Each Channel
 Display all channels independently
- ② Laser and Lamp Marge confocal channels, but display transmission illumination channel separately.
- 3 Show Caption
 Show captions such as channel number on each image.

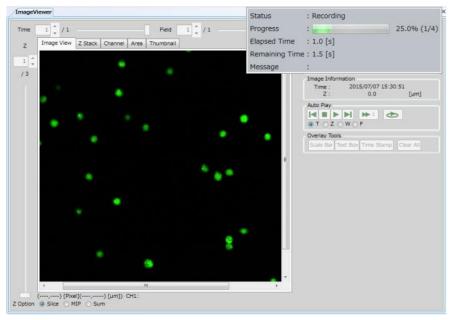
Items to change display mode in "ImageViewer" when "Thumbnail window" at "Imageviewer" is open.



- Each Well Display (Horizontal Priority)
 Display image of each well in line or in horizontal priority.
- Each Well Display (Vertical Priority)
 Display image of each well longitudinally or in vertical priority.
- ③ Each Well Display (Lattice)
 Display image of each well in lattice.
- Tile Number Shown when either Vertical Priority Display or Horizontal Priority Display is selected. The number of images to wrap lines can be specified.

5.6.10 Progress Bar

During measurement, progress information is displayed in upper right of viewer.





- 1) Status
 - Executing process is shown.
- 2 Progress
 - Progress of measurement is shown
- 3 Elapsed Time
 - Elapsed time of measurement is shown.
- 4 Remaining Time
 - Remaining time of measurement is shown
- ⑤ Message

5.7 Status Panel



(1) Communication Status with CQ1 Main Unit

ONLINE: Connected with CQ1 Main Unit

OFFLINE: Unconnected with CQ1 Main Unit

(2) Laser Status

ON: Lasers are ready to use

LOCKED: Interlock is working

(3) Chamber Status (Only in Stage Heater mounted model)

Show chamber temperature (Current/ Setting).

Back ground become red if Current > Setting + 1°C.

Back ground become light blue if Current < Setting -1°C

Display of setting temperature become "OFF" if temperature

control is off.

(4) Camera Status

Camera Temperature (Current value/Set value) is shown

(5) Software Status

Idle: Idling

Recording: Under measurement

Pause Waiting: Status in between "Pause" and "Stop" buttons on

Status Mode Panel under CQ Mode.

Preview: Preview imaging is ongoing

Analysis: Analysis is ongoing

Connecting: Connection with CQ1 Main Unit is ongoing

(6) Progress Status

Progress status of measurement is shown by well number (completed wells/total wells) and by the progress bar.

(7) HDD Disk Space

Show free disk space of HDD

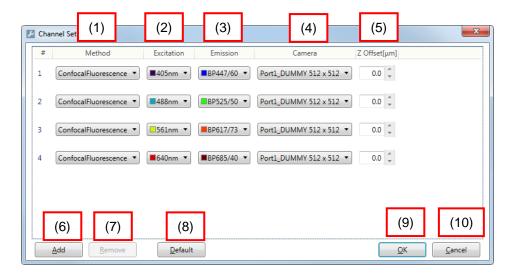
5.8 Viewer Layout

4 layouts are selectable by "Viewer Layout".



- ImageViewer & Map
 Change viewer layout for image capturing
- (2) ImageViewer & Chart Change viewer layout suitable for the analysis of each single cell by using charts
- (3) Chart
 Change viewer layout for chart analysis.
- (4) InformationChange viewer layout for viewing information of the CQ1

5.9 Channel Setting



(1) Method

Select imaging method

Note <In case of Transmission Illumination (Phase Contrast) is mounted> If "PhaseContrast" is selected, phase contrast lens must be selected. "BrightField" is unselectable. <In case of Transmission Illumination (Bright Field/ Phase Contast) is mounted> If "PhaseContrast" is selected, push the lever of transmission illumination back and select phase contrast lens. If "BrightField" is selected, pull the lever of transimission illumination forward. <In case of Transmission Illumination is unmounted> "PhaseContrast" and "BrightField" are unselectable.

(2) Excitation

Select light source

(3) Emission

Select emission filter

(4) Camera

Select camera (unselectable in previous version)

(5) Z Offset [µm]

Set Z offset of each channel (default: 0.0 (µm))

(6) Add

Add imaging channel. Up to 10 channels can be set.

This button is invalid if 10 channels are already selected.

(7) Remove

Remove selected channels. This button is invalid if only one channel is selected.

Note

If channel which is used in analysis setting, the other channel is applied to the setting automatically.

(8) Default

Restore channel setting to the default.

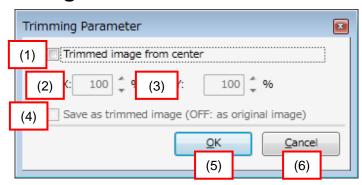
(9) OK

Close "Channel Setting" window with applying changes. Analysis conditions are reset if channel setting is changed.

(10) Cancel

Close "Channel Setting" window without applying changes.

5.10 Trimming Parameter



(1) Trimmed image from center

Check to trim acquired images by following paremeter.

Trimmed area isn't displayed.

In case of tiling images with stitching (refer to page $\underline{5-64}$), check the box.

(2) X

Set the crosswise trimming ratio of images

(3) Y

Set the vertical trimming ratio of images

(4) Save as trimmed image

If checked, trimmed images are saved. If unchecked, images without trimming is kept are saved.

In case of tiling images with stitching (refer to page <u>5-64</u>), uncheck the box.

Note

In case that correction of "Chromatic Aberration" is available in "Imaging" tab (refer to <u>5.4.3 Imaging Window</u>) and the box is unchecked, blank space may occure in saved images.

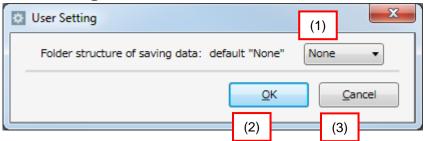
(5) OK

Close "Trimming Parameter" window with applying changes.

(6) Cancel

Close "Trimming Parameter" window without applying changes.

5.11 User Setting



(1) Folder structure of saving data

Set folder structure for saving image as following.

None:

Save all images in 1 folder

By Field:

Create folders for each Field to save images by Field.

By Well:

Create folders for each well to save images by well.

By Time Point:

Create folders for each time point to save images.

(2) OK

Close "User Setting" window with applying changes.

(3) Cancel

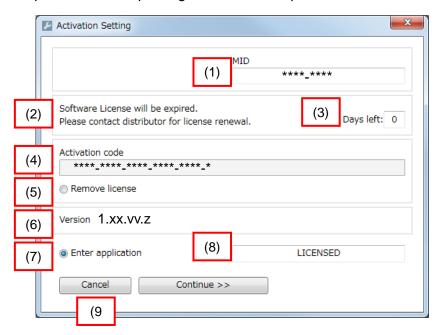
Close "User Setting" window without applying changes.

5.12 Activation Setting



CAUTION

Please do not operate Activation Setting window except when necessary such as updating CQ1 Software. CQ1 Software might be inoperatable. For updating CQ1 Software, please contact the dealer.



(1) MID

MID is shown.

(2) Message

Message to explain status of license is shown.

(3) Days left

Days until license expiration is shown.

(4) Activation code

Activation code is shown.

(5) Remove license

Select this radio button and click "Continue >>" to remove license.

(6) Version

Version of CQ1 Software is shown.

(7) Enter Application

Select this radio button and click "Continue >>" to return to Main window.

(8) Status

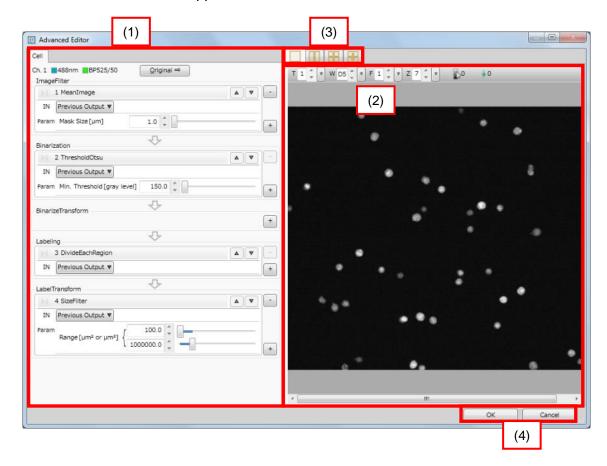
Status of license is shown.

(9) Cancel

Close "Activation Setting" window without applying changes.

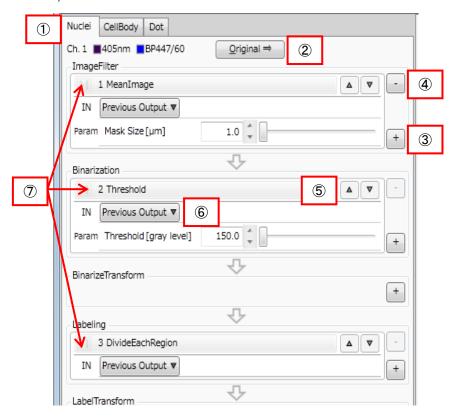
5.13 Advanced Editor

In the "Advanced Editor" window, "object detecting algorism" which was defined at "Application" window, can be edited.



(1) Edit object detecting algorism

Object detecting algorism which was defined at "Application" window, can be edited.



- Selection of Object
 Select the object detecting algorism.
- ② Display of Image Click this button to show the image of reference channel used as input image for object detection in "Analysis Viewer" (refer to following (2)).

3 Addition of Image Processing Method Click "+" button to show the list of image processing methods which are usable within the processing group. The selected method will be added at the end of the group.

Group	Method Name	Description
ImageFilter	MeanImage	Average images by mean value
	Sobel	Acquire Sobel image as the primary differential value,
	Laplace	Acquire Laplace image as the secondary differential value,
	Gaussian	Process linear convolution by Gaussian mask
	DilationImage	Dilate images
	ErosionImage	Erode images
	MedianImage	Average images by median value
	ThresholdTruncation	Change value over threshold to threshold value
	ThresholdToZero	Change value under threshold to "0"
	AddImage	Add images
	AddSImage	Add defined value to images
	SubImage	Subtract images
	SubSImage	Subtract defined value from images
	Mullmage	Multiply images
	MulSImage	Multiply images by defined value
	Divlmage	Divide images
	DivSImage	Divide images by defined value
Binarization	Threshold	Binarize all images with the same threshold
	ThresholdOtsu	Binarize all images with a fixed threshold calculated by Otu's method.
	AdaptiveThreshold	Binarize images with variable threshold according to image location
	ThresholdSigma	Binarize images with fixed threshold calculated from the standard deviation of background image
	AdaptiveSigmaThresh old	Binarize with dynamic thresholds calculated from the standard deviation of background image
BinarizeTransform	DilationCircle	Dilate binarized region in a circular shape
	DilationRectangle	Dilate binarized reign in a rectangular shape
	ErosionCircle	Erode binarized region in a circular shape
	ErosionRectangle	Erode binarized region in a rectangular shape
	ClosingCircle	Close circularly dilated binarized region
	ClosingRectangle	Close rectangular dilated binarized region
	OpeningCircle	Open circularly eroded binarized region
	OpeningRectangle	Open rectangular eroded binarized region
	FindMaximumDistance	Obtain maximum metric value of from background
	FindMaximumImage	Obtain maxmal value in binalized region
	Orlmage	Obtain union of two binarized regions
	Skeleton3D	Extract center line of binarized region
	Pruning3D	Remove branches of extracted center line
	AndImage	Obtain cross union of two binarized regions
	Xorlmage	Obtain symmetric difference set of two binarized regions

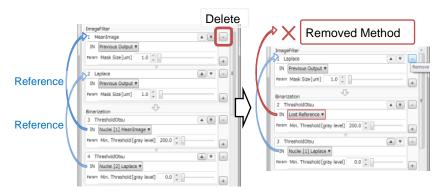
Group	Method Name	Description
Labeling	DivideEachRegion	Attach the same label on adjacent axis
	EndPointLabeling	Attach label on endpoint of center line
	JuncPointLabeling	Attach label on junction point of center line
	LineLabeling	Attach label on center line
LabelTransform	DilateRegion	Dilate labeled region
	ErodeRegion	Erode labeled region
	SubRegion	Subtract labeled region
	AndRegion	Obtain cross union of labeled region
	ExpandRegion3D	Expand labeled region within the specified
		range
	OrExpandRegion3D	Expand after obtaining the union of
		labeled region and specified range
	ExpandImage3D	Expand labeled region within the specified
		range gradually
	OrExpandImage3D	Expand after obtaining the union of
		labeled region and specified range
		gradually
	FillUp	Fill up holes of region
	ExcludeEdge	Exclude edge of region
	SizeFilter	Filter by size of region
	DiameterFilter	Filter by diameter of region
	RoundnessFilter	Filter by roundness of region
	MeanIntensityFilter	Filter by mean intensity* of region
	ROIFilter	Filter by location of region

^{*}Note

4 Removal of Image Processing Method

Click "-" button to remove methods. Within "Binarization" and "Labeling" groups, at least one method has to be selected, thus the last one method cannot be removed.

If a method specified as "Input" for succeeding method is removed, the "Input" of its referenced method will become "Lost Reference" and cause error. To correct such error, it is necessary to re-specify "Input".



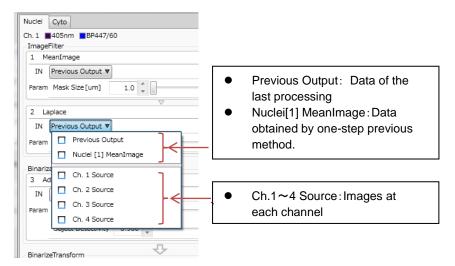
⑤ Moving Image Processing Method

Image Processing Method can be moved up and down by clicking "▼" or "▲" buttons at the right side. However, moving a method above "Input" specified method also results in "Lost Reference ".

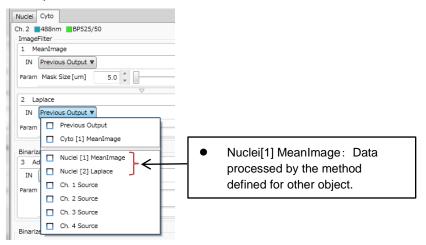
[&]quot;MeanIntensity" in this procedure contains background (about 100). It is different from "MeanIntensity" output as measurement value.

6 Input Setting

"Custom Object Detection Methods" setting window can be selected from drop-down menu.



In the case of the second or later Object Detect, it is possible to use the output data by the methods of the previous object detect, as "Input".



Performing Image Processing Method

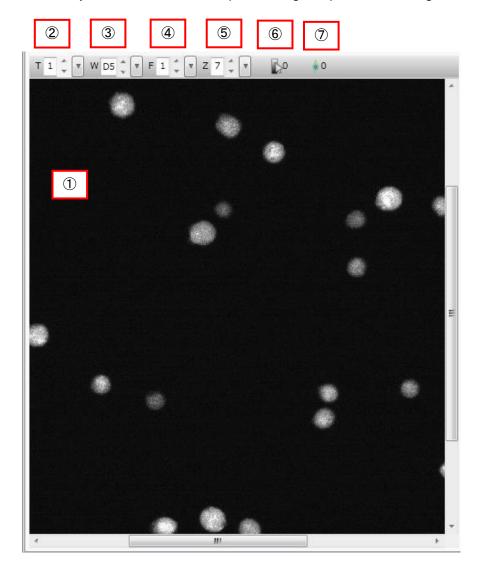
Click each method name to perform image processing until clicked method and to display the result at "Analysis Viewer".

In "CQ1 mode", image processing is performable after test imaging (by clicking "PreRec" of "Imaging" window). In "HDD mode", performable consistently.

If "Lost Reference" exist at "Input" setting, image processing is unperformable because algorism is not proper.

(2) Analysis Viewer

"Analysis Viewer" shows acquired image or processed image.

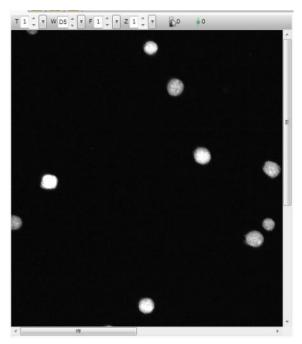


① Displaying Image

Show following 4 types of image. Call ii, iii and iv processed image.

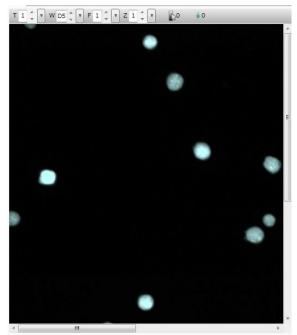
i. Acquired Image

Acquired image of reference channel to use as input image for object detection. This image is displayed by contrast of black \rightarrow white.



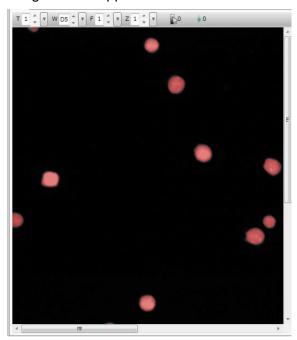
ii. Filtered Image

Processed image after performing methods of "ImageFilter" group. This image is displayed by contrast of black \rightarrow light blue.



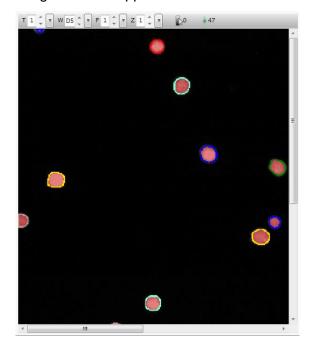
iii. Binarized Image

Processed image after performing methods of "Binarization" or "Binarize Transform" group. This image is displayed by binary image (objects are red and background is black). Finally filtered image is overlapped.



iv. Region Image

Processed image after performing methods of "Labeling" or "LabelTransform" group. Detected regions are displayed by individually colored outline. Finally filtered image and binarized image are overlapped.



② Time

Set time point of image to display.

3 Well

Set well to display.

4 Field

Set Field to display.

Note

⑤ Z

Set Z position of image to display.

6 Brightness Value

Show the brightness value of pointed pixel.

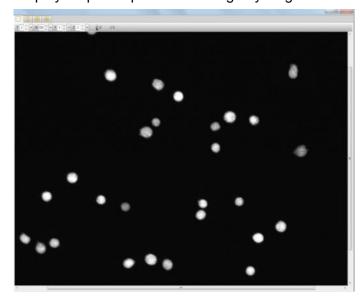
Number of Detected Object

Show number of detected object when region image is displayed.

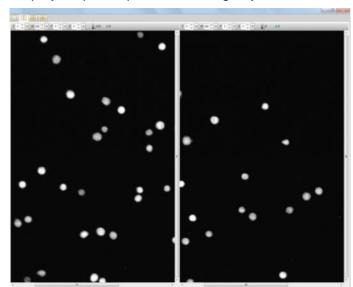
(3) Analysis Viewer Setting
Change the setting of "Analysis Viewer"



Single View Mode
 Display acquired/ processed image by single view.

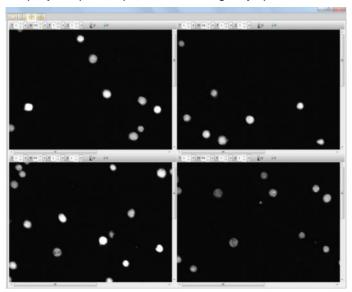


② Dual View Mode Display acquired/ processed image by dual view.



3 Quad View Mode

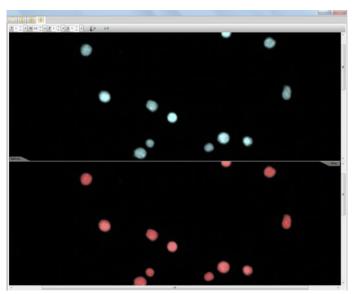
Display acquired/ processed image by quad view.



4 Before/ After View Mode

Display pre-processed image above of window and post-processed image below.

In this view, it is convenient to confirm how image processing of the method works.



(4) OK

Determine the editing and close the window. If "Lost Reference" exists, this button is unavailable.

(5) Cancel

Cancel the editing and close the window.

Then run [Defragmentation of disk].

6 Maintenance

6.1 Maintenance of Workstation

- System requirement
 CQ1 system requires system (C:) disk drive whose volume is larger than 200GB.
- Keep free space of hard disk drive
 For measurement, please keep free space of system (C:) disk
 drive larger than 30GB, and data (D:) disk drive larger than 1GB.
- Defragmentation of hard disk drive
 Fragmentation becomes advanced by repeating write and deletes data. Please do defragmentation of hard disk drive (both data (D:) and system (C:)) once in a half year, at least.
 Procedure: Open the defragmentation window from property of disk. Run [Analyze disk] and check the progress of fragmentation.

7 Trouble Shooting

7.1 Trouble Shooting during Measurement Preparation

- Shooting during Meast	-
Problem	Probable Cause / Corrective Action
The power cannot be turned ON.	It might be that the power plug is not
	connected to the power outlet.
	⇒ Check the connection between
	them.
	It might be that CQ1 Main Unit and
	Utility Box are not conneted by the
	power calbe.
	⇒ Check the connection between
	them.
	It might be that the main power switch
	on the front panel of Utility Box is not
	turned ON.
	⇒ Turn the Power switch ON.
The door of CQ1 Main Unit can not	It might be that the initilalization does
open.	not end.
	⇒ Wait until the initialization ends.
	It might be that the measurement is
	being processed.
	⇒ Wait until the measurement ends.
	⇒ It may take time for door opening
	in case that stage speed is set to
	low.
The stage does not run.	It might be that the door of CQ1 Main
	Unit is open.
	⇒ Close it.
	It might be that sub cover of CQ1
	Main Unit is open.
-	⇒ Close it.
The micorplate can not be set on the	It might be that the stage attachment
stage.	for microplates is not set on the
	stage.
	⇒ Set the stage attachment on the
	stage.
	⇒ The microplate can not be set
	depending on its shape. For detail,
	please contact the dealer ^{*1} .
The slideglass(es) can not be set on	It might be that the stage attachment
the stage.	is not set on the stage.
	⇒ Set the stage attachment on the
	stage.
	It might be that the sample holder for
	slideglass is not set on the stage
	attachment.
	⇒ Set the slideglass(es) on the
	sample holder for slideglass and
	set sample holder on the stage
	attachment.

T. Hodelo erreett	
Problem	Probable Cause / Corrective Action
The dish(es) can not be set on the	It might be that the stage attachment
stage.	is not set on the stage.
otago.	listication and stage.
	→ Cat the atama attachment on the
	⇒ Set the stage attachment on the
	stage.
	It might be that the sample holder for
	dish is not set on the stage
	attachment.
	⇒ Set the dish(es) on the sample
	holder for dish and set sample
	holder on the stage attachment.
The cover glass chamber can not be	It might be that the stage attachment
set on the stage.	is not set on the stage.
	⇒ Set the stage attachment on the
	stage.
	Juago.
	10. 11.11.11.11.11.11.11.11.11.11.11.11.11
	It might be that the sample holder for
	cover glass chamber is not set on the
	stage attachment.
	9
	→ Cat the account place about an en
	⇒ Set the cover glass chamber on
	the sample holder for cover glass
	chamber and set sample holder
	on the stage attachment.
	on the stage attachment.
The COA Coffee and do not make	It winds by the table 004 Coffee and in
The CQ1 Software does not run.	It might be that the CQ1 Software is
	run before Windows startup process
	ends.
	⇒ Select "CQ1 Software" on Start
	Menu of Windows.
	iviend of vviridows.
	It might be that the connection
	between CQ1 Main Unit and
	Workstation is not built.
	⇒ Connect them by the USB Cable.
	⇒ Confirm that door is not open.
	·
Sample vessel cannot be	It might be that the sample holder on
recognaized.	the stage is not in proper condition.
	⇒ Set sample holder on the stage
	properly.
	1 -1 - 7
	It maintain the theory are also believed
	It might be that the sample holder is
	not set in proper direction.
	⇒ Set sample holder on the stage as
	projection portion faces left-side of
	CQ1.
	⇒ Recognition of microplate may
	difficult depending on its shape.
	For detail, please contact the
	dealer ^{※1} .
	1

Problem	Probable Cause / Corrective Action
The laser calibrating function does	Output power of lasers does not
not operate properly.	stabilize.
not operate property.	⇒ After while, re-calibrate them.
	⇒ In case of failure in re-calibration,
	please contact the dealer ^{*1} .
	⇒ Confirm that CQ1 Main Unit is
	turned on.
CQ1 Software freezes. / Change	Is the sample loading port open?
between "CQ" and "HDD" modes can	⇒ Close it.
not be done.	It might be that "OFFLINE" and
	"Laser: LOCKED" are displayed in
	status panel (refer to <u>5.7 Status</u>
	Panel)
	⇒ Please do following procedure.
	① Close CQ1 Software.
	② Confirm that power switch and key
	switch of Utility Box are ON.
	3 Confirm ready lamp on Utility Box
	is turned on. If it is blinking, wait
	until stop blinking (about 1
	minute).
	Start CQ1 Software.
Measurement protocol can not be	It might be that different Stage
read.	Attachment is set.
	⇒ Set Stage Attachment designated in measurement protocol.
Chamber temperature doesn't get to	Setting temperature might be low.
setting temperature.	⇒ Confirm setting temperature and
county temperature.	set proper temperature.
	Is chamber temperature set over than
	room temperature + 5°C?
	⇒ Confirm room temperature and set
	proper chamber temperature
CO ₂ , O ₂ concentrations of Gas Mixer	Remaining amount of each gas
don't get to setting concentration.	cylinder might not be enough
	⇒ Confirm remaining amount of
	each gas cylinder. Tubes might be buckled or collapsed.
	⇒ Confirm tubes and if there are
	abnormalities, please fix it.
	Is outlet pressure of regulator proper?
	⇒ Set it in the range of 0.1 – 0.15
	MPa
Measurement data cannot be found	Confirm that the folder designated to
by HDD mode.	"Measurement List" is proper.
	⇒ Designate the upper folder of
	measurement data folder, whose
	name is as <date_****>.</date_****>
Sometimes temperature display isn't	⇒ Temperature display is renewed
renewed.	at the time point that more than 5
	minute elapse from previous
	display renewal.

^{**1} In the case of inquiring the dealer about trouble, it may need to send system log file. For outputting of log file, please refer to page 5-7.

7.2 Trouble Shooting during Imaging

Problem	Probable Cause / Corrective Action
The image cannot be acquired. / The	It might be that the exposure time is
acquired image is dark.	short.
	⇒ Set it longer.
	It might be that the laser intensity is
	low.
	⇒ Set it higher.
	It might be that the imaging camera is
	not recognized.
	⇒ Restart the whole system
	properly. Please refer to 3.2
	Starting and Shutting Down.
	It might be that the microplate is not
	set in precisely.
	⇒ Set it so the "A1" well locates on
	the left rear position from the front of CQ1 Main Unit.
	⇒ Set it so that 4 corners set on
	stage precisely.
	It might be that the slideglass(es) are
	(is) not set in proper direction.
	⇒ Set it(them) in proper direction(s).
	⇒ Set slide glass to move over to left
	side of sample holder.
	It might be that the door of CQ Main
	Unit opens.
	⇒ Close it.
	It might be that the key switch of
	Utility Box is OFF.
	⇒ Turn the key switch ON.
	⇒ Confirm ONLINE and Laser: ON in
	status panel (refer to <u>5.7 Status</u>
	Panel)
	⇒ Confirm that contrast setting is
	proper. (Refer to page <u>5-117</u>) It might be that viewer status is
	turned to undisplayed ().
	⇒ Turn it to displayed status ().
	(Refer to page <u>5-117</u>)
Image become bright suddenly	Confirm whether the check box
anage accome angin cadaciny	"Laser Enhancement" of "Imaging"
	window is checked or not.
	⇒ If checked, laser power can
	increase suddenly.
	⇒ Confirm that contrast setting is
	proper. (Refer to page <u>5-117</u>)
The laser autofocus does not operate	It might be that there are scratches /
properly.	dusts on the bottom surface of
	sample.
	⇒ Clean the surface and remove
	dusts and water on it.
	⇒ Use the sample without scratches on the bottom surface.
	Sample vessel might tilt
	⇒ Remove sample vessel once and
	set it again carefully.
	set it again carefully.

Problem	Probable Cause / Corrective Action
The laser autofocus does not operate	Kind of sample vessel might be
properly.	wrong.
	⇒ Confirm that sample vessel
	corresponds to that of designated
	in measurement protocol.
	It might be that sample vessel which
	is not registered is used.
	⇒ Register the sample vessel (refer
	to page <u>5-19</u>)
	⇒ Use registered sample vessel
	Is gel used for cell culture?
	⇒ In "Sample Vessel Type" of
	Sample window, check "Laser AF
	Bottom Detector". (Refer to page
	5-19)
	Is sample vessel whose cell culture surface is processed especially?
	⇒ In "Sample Vessel Type" of
	Sample window, check "Laser AF
	Bottom Detector". (Refer to page
	5-19)
	⇒ In case of measuring other sample
	in same protocol again, please
	load the protocol in each time.
The Image autofocus does not	It might be that sample is too dark or
operate properly.	bright dusts exist in sample vessel.
	⇒ Uncheck "ImageAF"
Quality of map image is bad	⇒ Zoom map image, and map image
	is shown in original resolution
Despite focusing by "Search"	In case that "Rec" position is far from
function, images acquired by "Rec"	"Search" position, Z position that
are defocused.	sample exist may different.
	⇒ Perform focusing by "Search" at
Waiting occurs during the	"Rec" position.
measurement.	It might occur in case when image acquisition and heavy duty analysis
measurement.	are processed concurrently.
	⇒ It is recommended that image
	acquisition and analysis are
	processed separately.
"Rec" is unavailable	It might be that imaging Area is not
	set.
	⇒ Set imaging Area.
	It might be that well for "Rec" isn't
	selected.
	⇒ Select well for "Rec".
The error occurs just after "Rec"	It might be that the parameter(s) set
button being clicked.	in analysis is (are) not appropriate.
	⇒ Reset the parameters.
	It might occur that memories of workstation run out because of heavy
	duty analysis.
	⇒ Reset protocol.
	It might occur that memories of
	workstation run out because of too
	many number of Z stack.
	⇒ Reduce the number of Z stack.
	. todado trio ridiribor di Z stadit.

Problem	Probable Cause / Corrective Action
The laser calibrating function does	Output power of lasers does not
not operate properly.	stabilize.
	⇒ After while, re-calibrate them.
	⇒ In case of failure in re-calibration,
	please contact the dealer*1.
The brightness on the corners of	Confirm that the check box "Flat Field
image being acquired is dark.	Correction" of "Imaging" window is
	checked.
	⇒ In case that the corners of image
	are still dark despite the check box
	is checked, please adjust
	Trimming Parameter (refer to <u>5.10</u> <u>Trimming Parameter</u>) to cut the
	edge of images for temporary
	measure.
	The whole system needs
	maintenance for primal measure.
	Please contact the dealer ^{*1} .
Objects in image look like they shift to	Sample vessel might tilt
XY direction by Z movement	⇒ Remove sample vessel once and
	set it again carefully.
	Sample vessel might be distorted.
	⇒ Acquire images of 4 corners of
	sample vessel and compare their
	tilt. Additionally, acquire images of
	plural Fields in the well and compare their tilt.
	If direction of object shift is
	different, sample vessel might be
	distorted.
	If direction of object shift is same,
	CQ1 might have an abnormality of
	stage. In this case, please contact
	the dealer ^{*1} .
	Sample set part of Stage Attachment
	may be distorted.
	⇒ If sample set part of Stage
	Attachment is distorted, replacement is needed. In this
	case, please contact the dealer ^{*1} .
Sample position is different among	Confirm that the check box
channels.	"Chromatic Aberration" of "Imaging"
	window is checked.
	⇒ If it is checked and sample
	position is different, maintenance
	is needed. Please contact the
	dealer ^{ж1} .
It is impossible to acquire whole well	⇒ It is impossible to acquire outer
	area of well by combination of
"Ol"	objective lens and sample vessel.
"Chromatic Aberration" correction	⇒ In case of using low magnification
doesn't work properly at image edge	lens, there are affect of aberration
in measurement using low magnification lens	which cannot correct by CQ1
magnineation lens	function. If there are problems in analysis, cut the edge of image
	(about 10 %) by trimming (refer to
	5.10 Trimming Parameter).
	o. to mining raidifictory.

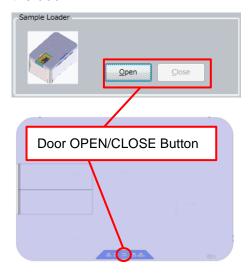
I rouble Snooting> 1 -		
Problem	Probable Cause / Corrective Action	
Z position of bottom of sample vessel can not be found.	 ⇒ Reset the range of "Search" function wider by using "▼" button beside "Search" button. ⇒ Confirm that sample vessel is set properly. 	
The sample can not be found.	It might be that there are not any cells in F.O.V.	
	⇒ First, run "Preview map image acquisition" function in "Map" window, and, after that, reset the Field position to region containing cells according to the result of the function described above.	
Well(s) can not be selected by "Imaging" / "Analysis" / "Well Table" windows.	It might be that "Select measurement area" mode is ineffective.	
	⇒ Click button to set the mode effective.	
Measurement and analysis can not be run simultaneously.	It might be that "CQ" mode is not selected.	
	⇒ Select "CQ" mode.	
	It might be that the check box of "Execute Analysis" in "Application" window is not checked.	
	⇒ Check the check box described above.	
Lasers do not operate.	It might be that the key switch switch is not turned ON.	
	⇒ Turn it ON.	
	It might be that the remote interlock	
	connector is not connected to the paired port.	
	⇒ Connect the connector to the paired port.	
	It might be that the door is open.	
	⇒ Close it.	
	It might be that sub cover is open.	
	⇒ Close it.	
	⇒ Confirm ONLINE and Laser: ON in status panel (refer to <u>5.7 Status</u> <u>Panel</u>)	
There is(are) gap(s) in resultant image of "Map" window.	⇒ There is occasionally happened that the gap appears according to the magnification of resultant image of "Map" window.	
There are dark fields sometimes in	⇒ Uncheck auto contrast. (refer to	
map imaging.	page <u>5-117</u>)	

Problem	Probable Cause / Corrective Action
It is impossible to acquire time-lapse by set interval	It might be that time for acquiring sequence per one time point exceeds time-lapse interval. ⇒ Set interval longer or reduce number of well, field and Z slice so that acquiring sequence per one time point doesn't exceed interval time
Condensation occurs on the rid of sample vessel when using Stage Heater	It might be caused by dust or water on the condensation reducer. ⇒ Clean the condensation reducer by referring 7.2.1 Cleaning Procedure of the Condensation Reducer Condensation occurs on room temperature sample when using Stage Heater. ⇒ Please warm the sample up to 30 °C and over.

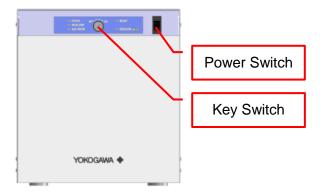
^{*1} In the case of inquiring the dealer about trouble, it may need to send system log file. For outputting of log file, please refer to page 5-7.

7.2.1 Cleaning Procedure of the Condensation Reducer

Click "Sample" tab \rightarrow "OPEN" Button of CQ1 Software or do long press (3 seconds) of Door OPEN/CLOSE Button of CQ1 Main Unit to open the door.



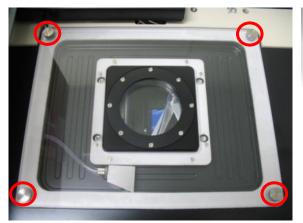
Turn the key switch OFF, then turn the power switch OFF..



Loosen 2 screws and remove sub cover.



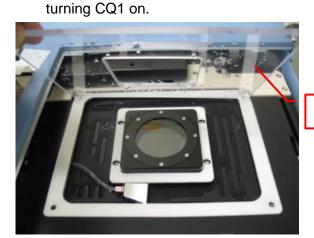
Loosen 4 screws and remove bath cover.





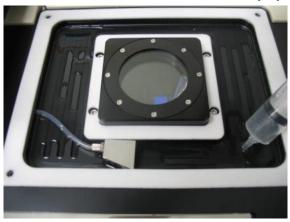
extstyle ext

- Please don't remove screws from bath cover.
- During temperature controlling, water drops are formed on the downside of the bath cover. Please be careful not to drop water inside the equipment when removing bath cover. If water drops inside the equipment, wipe it completely before

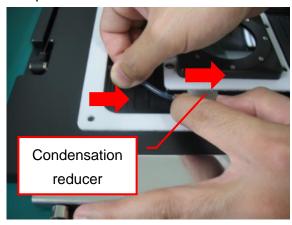


Water drop

If there is water in the bath, remove it by syringe and others.



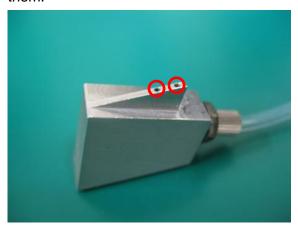
Hold the tube and the block of condensation reducer, slide them right and pull off the tube from the bath.



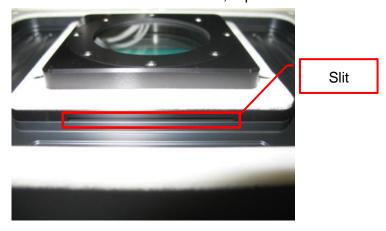
Rotate the block counterclockwise then pull off the block.



If there is dust or water on the 2 holes on the edge of the block, wipe them.



If there is dust or water in the slit, wipe them.



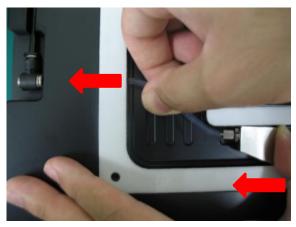
After cleaning, insert the edge of the block into the slit of the bath and rotate it clickwise.



Slide the tube and the block left and insert the tube to the bath hole.

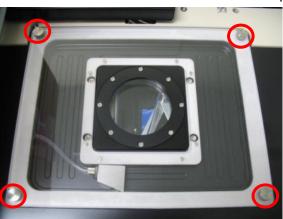


Hold the tube and the block then slide them left to insert the tube to the hole until the end.



Set bath cover and tighten 4 screws.

In case that the screws can't be fastened properly, adjust position of bath cover so that the screws are fastened properly.



Set sub cover and tighten 2 screws.



7.3 Trouble Shooting during Analyzing

Darkton	
Problem	Probable Cause / Corrective Action
"Pre Analysis" function does not run	"Pre Analysis" function does not run
after the measurement.	in "CQ"mode.
	⇒ Change the oprerating mode from
	"CQ" mode to "HDD" mode, and
	then run "Pre Analysis".
Nothing is shown in Thumbnail Chart	⇒ Select well to show chart in Well
window.	Table window
Heat map display in Well Table	Heat map isn't shown if wells are
window disappeares.	selected in Well Table window.
инизан энемере энем	⇒ Unselect wells
Cells can not be recognized.	It might be that the threshold of
Cond dan not be recognized.	binarization is too high.
	⇒ First, reconfirm the intensity range
	of image by using "Image Viewer
	function image acquisition"
	function, and, after that, reset the
	threshold properly (and, set
	"Object Detectivity" higher).
	It might be that the range of "Size
	Filter" is not porper.
	⇒ Reset the range of "Size Filter"
	properly with visualization of
	"Chart" function.
	It might be that viewer status for is
	turned to undisplayed (-).
	⇒ Turn it to displayed status (🖭).
	(Refer to page <u>5-115</u>)
	It might be that Area window is
	selected in ImageViewer
	⇒ Recognized result is not shown in
	Area window. Select Image View
	window. (refer to page <u>5-88</u>)
	It might be that Z position that sample
	doesn't exist is shown by viewer.
	⇒ Adjust Z position.
The defined line of boundary on cells	It might be that the intensity on the
is not smooth.	boundary fluctuates or is too dark.
	⇒ Increase the intensity of image by
	making the exposure time longer
	or increasing laser output power.
	⇒ To define outer envelope of the
	boundary as the resultant
	boundary, add "Closing Circle"
	command to "Binarize Transform"
	block by using "Edit" of "Analysis"
	window.
	⇒ To define inner envelope of the
	boundary as the resultant
	boundary, add "Opening Circle"
	command to "Binarize Transform"
	block by using "Edit" of "Analysis"
	window.
How to clear recognition showing?	⇒ Turn viewer status to undisplayed
11011 to oldar robogilition showing:	status (). (Refer to page <u>5-115</u>)
	siaius (<u>)</u> . (Neiei io page <u>5-115)</u>

Problem	Probable Cause / Corrective Action
Well(s) can not be selected by	It might be that "Select measurement
"Imaging" / "Analysis" / "Well Table"	area" mode is ineffective.
windows.	
	⇒ Click button to set the mode effective.
Chart(s) can not be displayed in	It might be that the check box whose
"Thumbnail Chart" window.	title is the same as that of the chart
	window which should display the
	chart is not checked.
	⇒ Check the check box described
	above.
The measurement data can not be	Is proper folder selected?
read.	⇒ Select "MeasurementData" folder
	described in diagram at page
	<u>5-13</u> .
"Send to" can not be selected	Isn't "MIP" or "Sum" in "Z Option" at
	ImageViewer (refer to <u>5-88</u>)
	selected?
	⇒ Select "Slice" in "Z Option" to
	show 3D image by "Send to"
Analysis result, by stored analysis	Analysis is not performed normally if
protocol, is strange	measurement condition (channel,
	exposure and so on) is changed.
	⇒ Edit analysis protocol to match
	measurement condition.
Where are plural analysis results of	⇒ Analysis result list is shown by
one measurement data by different	clicking ">". (refer to page 5-59)
analysis protocols?	, , , , , ,
"3D Viewer" of "Image J" freezes.	It might be that the size of image is
	too large.
	⇒ Set the value of "Scaling" smaller.
	It might be that the display driver of
	WS or PC is unproper. It might
	occure when OS is upgraded.
	⇒ Please inquire the WS or PC
	vender.
Analysis does not operate properly	⇒ Set the size of cell(s) to detect
because it recognizes wall of well(s)	properly by using "Size Filter"
as the menbrance of cell(s).	command.
	⇒ Set the value of "ROI Size Raito"
	of "ROI Filter" function smaller so
	that ROI becomes inner area of
	each well.
Analysis is run repeatedly on the	It might be that adjecet images are
same cell(s).	overlayed partially.
	⇒ Acquire images not so that
	adjacent images are overlayed.
	⇒ Set the value of "ROI Size Raito"
	of "ROI Filter" function smaller not
	so that adjacent images are
Only partial range of image is system.	overlayed.
Only partial range of image is output	By "Snapshot", range of image shown
by "Snapshot"	in ImageViewer is output.
	⇒ Show whole image at
	ImageViewer and click
	"Snapshot".

Problem	Probable Cause / Corrective Action
Measurement data cannot be found	Confirm that the folder designated to
by HDD mode.	"Measurement List" is proper.
	⇒ Designate the upper folder of
	measurement data folder, whose
	name is as <date ****="">.</date>

^{*1} In the case of inquiring the dealer about trouble, it may need to send system log file. For outputting of log file, please refer to page 5-7.

7.4 Other Trouble Shootings

Problem	Probable Cause / Corrective Action
Image Viewer window and/or	⇒ These windows can be shown by
Thumbnail Chart window disappear	Menu -> "View" menu.
Access to hard disk drive becomes	It might be that the free space on the
slower.	HDD is small.
	⇒ Keep the free space by deleting unused files.
	(It is recommended that Windows command "Defrag" is run after deleting unused files.)
While image acquisition and analysis	⇒ There are no influences to
are performed simultaneously, sound	measurement.
of moter movement become noisy.	

^{**1} In the case of inquiring the dealer about trouble, it may need to send system log file. For outputting of log file, please refer to page <u>5-7</u>.

8 Specifications

8.1 Details of MS codes

8.1.1 CQ1 Main Unit

Model	Suff	ix Cc	ode		Option	Description	
Code					-		
CQ1						Confocal Quantitative Image Cytometer	
Laser	-L11					Wavelength: 488 nm	
	-L21					Wavelength: 488, 561 nm	
	-L22	2				Wavelength: 405, 488 nm	
	-L23	3				Wavelength: 488, 640 nm	
	-L31					Wavelength: 405, 488, 561 nm	
	-L32	2				Wavelength: 405, 488, 640 nm	
	-L33	3				Wavelength: 488, 561, 640 nm	
	-L41					Wavelength: 405, 488, 561, 640 nm	
Dichroic		-M1				DM: 405, 488, 561, 640 nm	
mirror							
Transmi	ssion		·P			Phase Contrast	
Illuminat	ion		·T			Bright Field/ Phase Contrast	
		-	·N			-	
Stage He	eater		-H			With Stage Heater	
			-N			Without Stage Heater	
Stage At	ttachn	nent	-A1			All Wells Imaging Type	
			-A2			Chambered Type	
Worksta	tion		-V	/ 1		Standard Workstation	
Languag	je,		•	-J		Japanese, with Monitor, with AC Cord	
Shipping		n		-N		Japanese, without Monitor, with AC Cord	
AC Cord	ĺ			-E		English, without Monitor, without AC Cord	
Dummy	Code			-N		Dummy Code	
Subcode				-0	00	Always "-00"	
Software	Opti	on		•	/FTL	Fast Time-lapse	
Custom					/Z	Custom Order	

8.1.2 Objective Lens

Model	Suffix	Code	Option	Description	
Code					
CQ1TBL				CQ1 Objective Lens	
Holder	-P0			Objective Lens Holder Position	
Position	-NN			No Objective Lens Holder	
Objective I	Lens	-L002N		2x Dry (NA=0.08) PLAPON2X	
		-L004N		4x Dry (NA=0.16) UPLSAPO4X	
		-L010N		10x Dry (NA=0.40) UPLSAPO10X2	
		-L020N		20x Dry (NA=0.75) UPLSAPO20X	
		-L020M		20x for thick bottom vessel	
			(NA=0.7) UCPLFLN20X		
		-L040N		40x Dry (NA=0.95) UPLSAPO40X2	
		-L020L		20x Long Working Distance	
				(NA=0.45) LUCPLFLN20X	
		-L040L		40x Long Working Distance	
			(NA=0.60) LUCPLFLN40X		
		-L010P		10x Phase Contrast	
		-L020P		(NA=0.30) UPLFLN10X2PH	
				20x Phase Contrast	
				(NA=0.45) LUCPLFLN20XPH	
	-N0000			Holder (Without Objective Lens)	
Custom O	rder		/Z	Custom Order	

8.1.3 Emission Filter

Model	Suffix Code	Option	Description
Code			
CQ1FLT			
	-D		Dummy code
	specification code	/△01	EM B525/50 for 488nm Excitation
EM (emiss	•	/△02	EM B617/73 for 561nm Excitation
△: Position 1 – 9 Selling separately 0		/△03	EM B447/60 for 405nm Excitation
		/△06	EM B685/40 for 640nm Excitation
		/△08	EM B520/35 for 488nm Excitation
		/△09	EM BR488/568 for 488 / 561nm
			Excitation
Custom O	rder	/Z	Custom Order

8.1.4 Options

Model Code	Suffix Code	Option	Description	
CQ1PRT				
Language	-J		Japanese	
	-E		English	
Gas Mixer		/MX2-D	Gas Mixer	
Stage Attach	nment	/SAT1	All Wells Imaging Type	
		/SAT2	Chambered Type	
Sample Hold	der	/HDA01	For Triple 35 mm Dishes	
		/HDA02	For Single 60 mm Dish	
		/HDA03	For Slide Glass (ISO 8037/1)	
		/HDA04	For Cover Glass Chamber	
Sealing Parts for Sample Holder		/CB601	60 mm Dish Ring	
		/CB602	Rubber for 60 mm Dish	
		/CBT01	Holding Plate for CGC	
		/CBT02	Rubber for CGC (IWAKI)	
		/CBT03	Rubber for CGC	
			(NUNC Lab-Tek I)	
		/CBT04	Rubber for CGC	
		/CB104	(NUNC Lab-Tek II)	
		/CBT05	Rubber for CGC	
		,00100	(MATSUNAMI)	

8.2 General Specifications

Main Unit	Туре	Standard Model	Transmission Illumination Option		
	Confocal	Microlens enhanced dual Nipkow disk system			
	Scanning				
	Method				
	Scanning speed	4000 rpm			
	Excitation laser	Select 1 – 4 lasers from 405 nm. 4	88 nm. 561 nm. and 640 nm		
	wavelength	,	, ,		
	Phase contrast	-	Option: LED transmission		
	illumination		illumination		
	Camera	sCMOS, 2560 x 2160 pixel, 16.6 n	nm x 14.0 mm		
	Objective lens	Up to 6 lenses from below list are in	nstallable		
		Dry:2x, 4x, 10x, 20x, 40x			
		Long working distance: 20x, 40x			
		Phase contrast: 10x, 20x			
	External	W 600 mm x D 400 mm	W 600 mm x D 400 mm		
	dimensions	x H 298 mm	x H 437 mm		
	Mass	Without Stage Heater: 38 kg	Without Stage Heater: 40 kg		
	IVIASS	With Stage Heater: 41.3 kg	With Stage Heater: 43.3 kg		
	Noise level	46 dB max			
	NOISC ICVCI	A-weighted sound pressure level is	less than 70dB		
Utility Box	External	W 275 mm x D 432 mm x H 298 m			
,	dimensions				
	Mass	18 kg			
Gas Mixer	External	W 170 mm x D 260 mm x H 280 m	m		
	dimensions				
	Mass	5.2 kg			
Workstation for	External	W 172 mm x D 417 mm x H 298 m	m		
measurement	dimensions				
	Mass	14 kg			
Measurement softwar	е	Set measurement condition; Displa	y image data and charts, Map		
		imaging; Quantified data analysis; Reporting; 3D Imaging; Multi-color			
		imaging; Multi-position imaging			
		Data format: 16 bit OME-TIFF file			
Operating	CQ1	15 − 35 °C			
temperature range	Gas Mixer	20 – 30 °C			
Operating humidity	CQ1	20 – 70 % RH No condensing			
range	Gas Mixer	10 – 85 % RH No condensing			
Power consumption		100 – 240 VAC 50 / 60 Hz			
		Main Unit, Utility box 800 VA			
		Gas Mixer 40 VA			
		Workstation 400 VA			

Note

Specifications are subject to change without notice.

8.3 Measurement sample vessels

(1) Microplate

Following microplate types are applicable

- 6 well
- 12 well
- 24 well
- 48 well
- 96 well
- 384 well^{**1}
- 1536 well^{**1}

(2) Dish

Following dish types are applicable

- 35 mm Dish (By using 35 mm dish sample holder, up to 3 dishes are measurable in one experiment)
- 60 mm Dish (By using 60 mm dish sample holder, one dish is measureable in one experiment)
- (3) Slide glass

By using slideglass sample holder, up to 4 slide glasses are applicable in one experiment

(4) Cover Glass Chamber

By using cover glass chamber holder, one cover glass chambdr is applicable in one experiment

If 1536 well microplate is measured by 2x/ 4x objective lens, multiple wells are measured by one FOV. Therefore in this case, measurement is inhibited. (Warning is displayed.)

For details of sample vessels usable in CQ1, please refer to Technical Information (TI 80J01A01-01E).

^{**1} If 384 well microplate is measured by 2x objective lens, multiple wells are measured by one FOV. Therefore in this case, measurement is inhibited. (Warning is displayed.)

8.4 Applicable Standards

- **◆**CE Marking
- EMC Directives:

This product belongs to Class A which is designed to be used under industrial environment. If used under household environment, risk of radio interference could arise, and the user may have to take appropriate measures.

This product satisfies the requisite minimal immunity level. If being used under other environment than controlled area, this product may suffer external influence, and the user may have to take appropriate measures

EN/IEC 61326-1 Class A, Table 1 (Basic immunity requirements)
Electrical equipment for measurement, control and laboratory use
- EMC requirements - Part 1: General requirements

Performance Criteria

	Performance Criteria*
ESD IEC 61000-4-2	В
Radiated electromagnetic field IEC 61000-4-3	А
EFT/Burst IEC 61000-4-4	В
Surge IEC 61000-4-5	В
Conducted disturbance IEC 61000-4-6	А
Power freq. magnetic field IEC 61000-4-8	А
Voltage dips IEC 61000-4-11	B (0.5,1cycle 0%) C (25 cycles 70%)
Short interruptions IEC 61000-4-11	С

^{*}Performance Criteria

- A: The equipment continues to operate to specification.
- B: When the noise is applied, the equipment will go to be fluctuated. However, if the noise is removed, it continues to operate to specification.
- C: When the noise is applied, temporary degradation or less of function is observed and to recover the normal condition, an operator intervention or system reset is required.

EN/IEC 55011 Group 1, Class A

Industrial, scientific and medical equipment.

Radio-frequency disturbance characteristics. Limits and methods of measurement

EN/IEC 61000-3-2 Class A

Electromagnetic compatibility (EMC)

- Part 3-2: Limits - Limits for harmonic current emissions (equipment input current <= 16 A per phase)

EN/IEC 61000-3-3

Electromagnetic compatibility (EMC)

- Part 3-3: Limits - Limitation of voltage changes, voltage fluctuations and flicker in public low-voltage supply systems, for equipment with rated current ≤16 A per phase and not subject to conditional connection

- **◆CE Marking**
- Machinery Directive:

ISO12100

Safety of machinery

- -- General principles for design
- -- Risk assessment and risk reduction

EN/IEC 13849-1

Safety of machinery

- -- Safety-related parts of control systems
- -- Part 1: General principles for design

EN/IEC 61010-1

Safety requirements for electrical equipment for measurement, control, and laboratory use

- Part 1: General requirements

EN/IEC 60825-1

Safety of laser products

- Part 1: Equipment classification and requirements
- Environmental Standard:

EN 50581

◆Laser Safety Standard

EN/IEC 60825-1

Safety of laser products

- Part 1: Equipment classification and requirements

FDA Laser Safety Regulation

Information of Inner laser parts

405 nm laser for excitation

- AEL laser safty class: 3B

- Maximum output power: 100 mW- Wavelength: 405 nm

- Pulse duration & repeating frequency: Continuous mode

- Angular subtense: 0.1 rad

488 nm laser for excitation

- AEL laser safty class: 3B

- Maximum output power: 60 mW- Wavelength: 488 nm

- Pulse duration & repeating frequency: Continuous mode

- Angular subtense: 0.1 rad

● 561 nm laser for excitation

- AEL laser safty class: 3B- Maximum output power: 50 mW- Wavelength: 561 nm

- Pulse duration & repeating frequency: Continuous mode

- Angular subtense: 0.1 rad

640 nm laser for excitation

- AEL laser safty class: 3B

- Maximum output power: 100 mW- Wavelength: 640 nm

- Pulse duration & repeating frequency: Continuous mode

- Angular subtense: 0.1 rad

Laser for autofocus

- AEL laser safty class: 3R

- Maximum output power: 2.5 mW- Wavelength: 785 nm

- Pulse duration & repeating frequency: Continuous mode

- Angular subtense: 0.1 rad

9 Warranty Rule

The warranty period is one year from the date of purchase. Failures that occur within the warranty period will be repaired free of charge. The warranty only covers this product.

If any of the following conditions applies, repair will be charged even within the warranty period:

- Failures or damage caused by inappropriate handling or use
- Failures or damage caused by handling, use, or storage that exceed the tolerance of the design and specifications
- Failures or damage caused by repairs and modifications made by the user
- Failures or damage caused by transportation, movement, dropping, or the like occurring after purchase
- ◆Failures or damage caused by a fire, natural disaster (earthquake, storm, flood, lightning and so on), salt damage, gas damage, and abnormal voltage.
- Failures caused by the following processing on the workstation.
 - ■Install patches (Windows Update, service pack, security patch) to update operating system.
 - ■Install the third-party software which YOKOGAWA doesn't specify.
- Failures caused by water leakage due to following processing.
 - ■Over filling water into Stage Attachment (Chambered type).

Any other damage not attributable to Yokogawa is outside the scope of this warranty.

Should you require any repair, please consult Yokogawa.

Manufacturer

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Please contact the dealer inquiries about this product.