CELENA® X

HIGH CONTENT IMAGING SYSTEM

USER MANUAL

www.logosbio.com

LBSM-MD-ML-CLX-001
VC1812-01

DISCLAIMER
The contents of this document are subject to change without notice.
The CELENA® X High Content Imaging System is a set of electrical laboratory instruments for scientific research use only.
It is not a medical, therapeutic, or in vitro diagnostics device.
Do not disassemble the device on any occasion as this will invalidate your warranty.

TRADEMARKS
The trademarks used in this document are the property of Logos Biosystems, Inc. unless otherwise specified.
LED Filter Cubes are LTC Licensed Products provided under an intellectual property license from Life Technologies Corporation.
CellProfiler is provided under the BSD 3-Clause License.

©2018 Logos Biosystems, Inc. All rights reserved.

Table of Contents

1. Getting Started ... 4

1.1 Product contents 4
1.2 Product description 5
CELENA® X High Content Imaging System 5
CELENA® X Controller 5
Software 5
1.3 Setting up 6
Unpacking 6
Connections 6
Shipping guard/restraints 7
Turn on the CELENA® X 7
Install filter cubes and objectives 7
Shut down the CELENA® X 7

2. CELENA® X Explorer ... 8

2.1 User interface 8
Channels 9
Vessel 12
Z-stack 13
Time lapse 14
Project 15
Toolbar 16
Messages 16
Settings 17
2.2 Workflow 19
Create a project protocol 19
Save a project protocol 21
Load a project protocol 21
Run a project protocol 21
Pause/stop a project protocol 21
View project results 21

3. CELENA® X Cell Analyzer ... 22

3.1 Overview 22
Project 22
View 23
Image control 24
Analysis 25
Toolbar 25
Messages 26
3.2 Workflow 27
Set up project analysis 27
Load previously analyzed images 28
Annotate images and make simple measurements 28
Edit images 29
3.3 Pipeline module reference 30
Overview 30
Image processing 30
Object identification 33
Measurements 35

4. Maintenance 37

4.1 General care 37
4.2 Change filter cubes 37
4.3 Change objectives 38
4.4 Adjust objective correction collars 39

Appendix A: Troubleshooting 40

Image quality 40
Explorer 40
Mechanical 40

Appendix B: Specifications 41

CELENA® X High Content Imaging System 41

Appendix C: Safety Information 42

Instrument safety 42
Personal safety 43
Instrument symbols 43
Safety standards 43

Appendix D: Ordering Information 44

Instruments 44
Objectives 44
LED filter cubes 45
Accessories 45

Appendix E: Purchaser Notification 46

Limited use label license 46
LTC licensed products 46
BSD licensed products 46
Instrument warranty 47

1. Getting Started

1.1 Product contents

Your CELENA® X is shipped with the following components:

CELENA® X High Content Imaging System

- Camera module (installed as ordered)
- Condenser (installed as ordered)
- Laser autofocus module (installed as ordered)
- Filter cubes (installed as ordered)
- Objectives (installed as ordered)

CELENA® X Controller

PC

- CELENA® X Explorer (installed)
- CELENA® X Cell Analyzer (installed)

Accessories

- Universal Vessel Holder
- Microplate Holder
- Single Slide Holder

- Power Cord
- Cable PS-1
- Cable PS-2
- Cable SIG
- Cable USB 2.0
- Cable USB 3.0
- *(Optional)* Cable Laser AF (included with the Laser autofocus module)

- Flathead Screwdriver

- CELENA® X Cell Analyzer Verification Key

- USB drive, 64 GB (includes the user manual and installation guide)

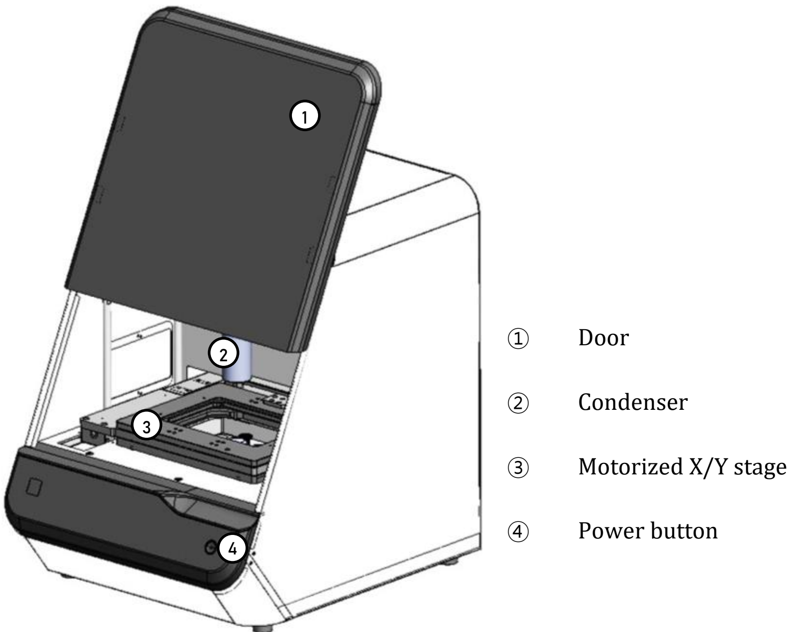
- Keyboard
- Mouse

Inspect the product package upon delivery to ensure that all components have been included. If anything is missing, contact your local sales representative. Damage that may occur during shipping and handling is not covered by warranty and must be filed with the carrier.

1.2 Product description

CELENA® X High Content Imaging System

Your CELENA® X High Content Imaging System is an integrated imaging system designed for rapid, high content image acquisition and analysis. Customizable imaging protocols, image-based and laser autofocusing modules, and a motorized XYZ stage simplify well plate imaging and slide scanning. The integrated CELENA® X Cell Analyzer software processes images and data for quantitative analysis. Analysis pipelines can be created and used to identify cellular or subcellular objects, process images for optimal data collection, and make various measurements.



CELENA® X High Content Imaging System

CAUTION! This instrument uses Class 3B ultraviolet LEDs that are in accordance with IEC/EN 60825-1. Make the CELENA® X door is closed when imaging to protect your eyes. Direct exposure to and diffuse reflections of the laser can be hazardous to the eye.

CELENA® X Controller

The CELENA® X Controller controls the power supply to and mechanical stages of the CELENA® X.

Software

CELENA® X Explorer

The CELENA® X is controlled by the integrated CELENA® X Explorer software. CELENA® X Explorer is pre-installed to the computer supplied with the instrument.

CELENA® X Explorer

CELENA® X Cell Analyzer

CELENA® X Cell Analyzer is used to process and analyze images to quantify numerous cellular phenotypes simultaneously. CELENA® X Cell Analyzer also provides tools to edit and annotate images as well as create videos.

CELENA® X Cell Analyzer

1.3 Setting up

Unpacking

Move the unpacked boxes to the site of operation.

CELENA® X

CAUTION! When moving the CELENA® X, do not attempt to lift or move the instrument without assistance. It is recommended that two or more people lift the instrument together while taking the proper safety measures to avoid injury.

IMPORTANT! Do not subject the CELENA® X to sudden impact or excessive vibration. Handle the instrument with care to prevent damage.

IMPORTANT! Wiping the computer supplied with the CELENA® X (i.e., erasing the hard drive to remove programs, etc.) voids the product warranty.

Open the CELENA® X box and remove the Styrofoam top and sides. Lift the CELENA® X out of its box by grasping its base firmly.

Place the CELENA® X on a flat, level surface that is free of vibration. Anti-vibration tables are recommended for optimal use. Leave sufficient space around the instrument for proper ventilation and to prevent overheating.

IMPORTANT! Do not expose the instrument to intense ultraviolet light.

CELENA® X Controller

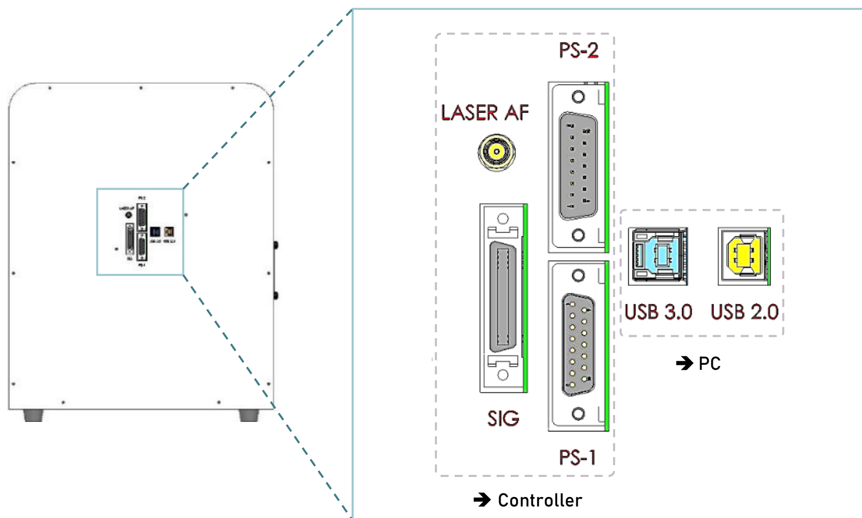
Place the Controller near the CELENA® X. A separate surface is recommended for optimal use but is not necessary.

Connections

Unpack the cables from the accessories box and attach as specified below:

CELENA® X & Controller	CELENA® X & PC
Cable PS-1 (I10331)	USB 2.0 (I10335)**
Cable PS-2 (I10332)	USB 3.0 (I10336)**
Cable SIG (I10333)	
Cable AF (I10334)*	

**Included if the laser AF module was purchased and installed*
***Make sure to plug into the blue USB 3.0 ports at the back of the PC, not the front*



Back of the CELENA® X

The CELENA® X is compatible with 4K Ultra HD (UHD) monitors. Use a DisplayPort (DP) cable to connect a 4K UHD monitor to the provided PC.

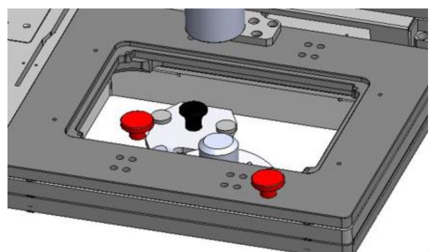
The CELENA® X Cell Analyzer Verification Key is a parallel or USB port hardware dongle that unlocks Cell Analyzer functionality. Attach to the provided PC.

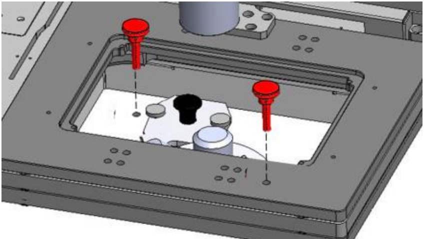
Shipping guard/restraints

Your CELENA® X is shipped with two shipping restraints installed (X/Y stage, LED filter cube stage) to prevent damage to the instrument from shock and vibration during transport.

IMPORTANT! The shipping restraints must be removed before the CELENA® X is turned on.

Remove shipping restraints





1. Unscrew screw A and pull it up to remove it from the LED filter cube stage cover.
2. Unscrew screw B and pull it up to remove it from the X/Y stage.

Note: Store the screws in the accessories box for future use. Make sure they are accessible in case you need to pack up for maintenance and servicing purposes.

Turn on the CELENA® X

IMPORTANT! The shipping restraints must be removed before the CELENA® X is turned on.

Turn on in this order:

- CELENA® X Controller
- CELENA® X
- Run CELENA® X Explorer

IMPORTANT! Using both Explorer & Cell Analyzer at the same time can affect both imaging and analysis time. Use just one program at a time.

Install filter cubes and objectives

IMPORTANT! CELENA® X Explorer must be on to install filter cubes and objectives.

For detailed instructions on how to install filter cubes, go to 4.2 Change filter cubes.

For detailed instructions on how to install objectives, go to 4.3 Change objectives.

Make sure the installed filter cubes and objectives match what is set in the CHANNELS panel.

Shut down the CELENA® X

IMPORTANT! Explorer must be shut down before the instrument to allow the stages to dock for safety.

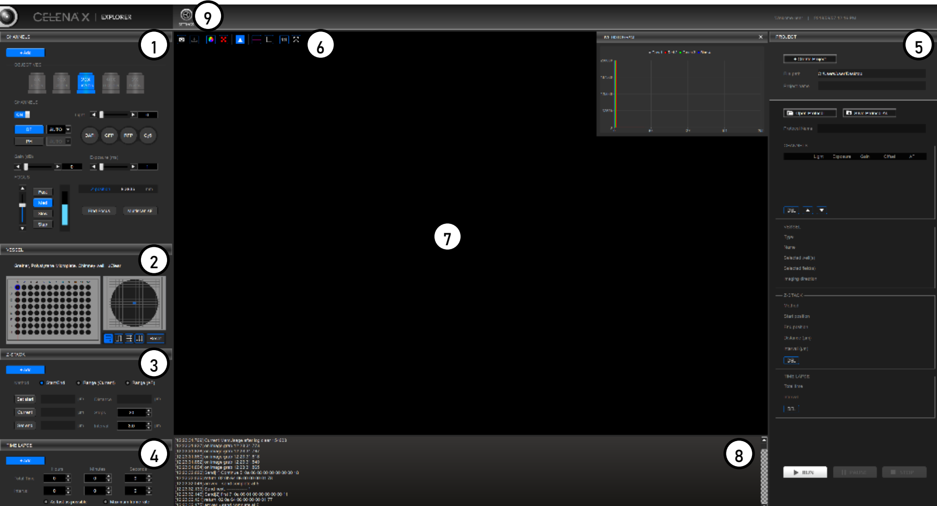
Turn off in this order:

- CELENA® X Explorer
- CELENA® X
- CELENA® X Controller

2. CELENA® X Explorer

2.1 User interface

The CELENA® X Explorer is the graphical user interface for the CELENA® X High Content Imaging System.

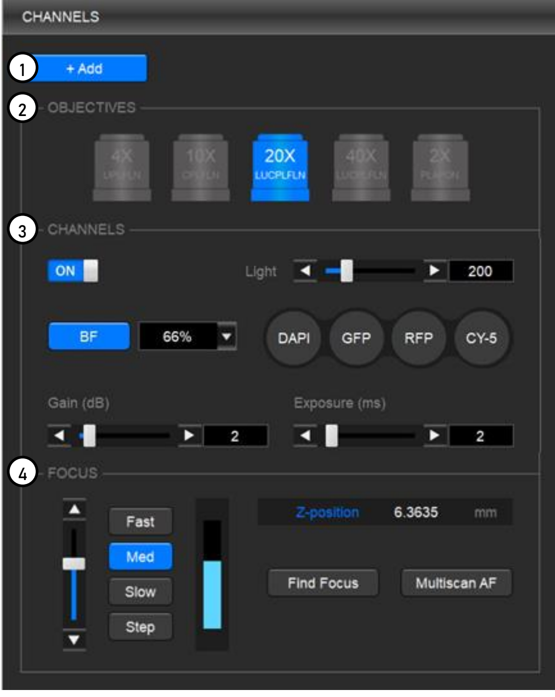


CELENA® X Explorer

① **CHANNELS:** Gives control over light, camera, and focus settings.
② **VESSEL**: Allows you to select the appropriate vessel, wells, and fields to capture.
③ **Z-STACK:** Allows you to capture multiple planes along the Z-axis.
④ **TIME LAPSE:** Allows you to set up time lapse sequences.
⑤ **PROJECT:** Allows you to run, load, save, and edit automated imaging projects.
⑥ **Toolbar:** Has tools for capturing and visualizing the current field of view.
⑦ **Viewing area:** Shows the current field of view.
⑧ **System messages:** Displays system messages.
⑨ **Settings:** Allows you to set system options and perform calibration procedures.

Channels

This panel is used to set light, camera, and focus parameters for a project.



① **Add:** Adds the CHANNELS settings to the project protocol.

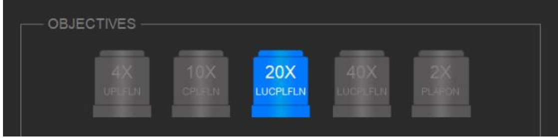
② **Objectives:** Allows you to select from the currently installed objectives.

③ **Channels:** Allows you to select from the currently installed filter cubes and adjust light and camera settings.

④ **Focus:** Allows you to find focus and set up autofocusing.

Objectives

This panel is used to select from the currently installed objectives.

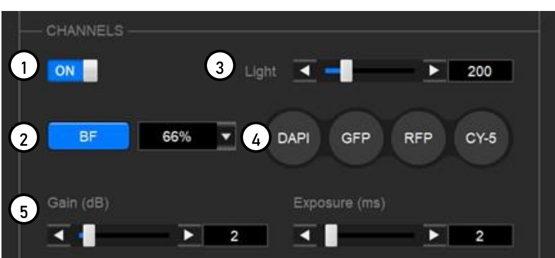


The magnification and label on each objective reflects its profile, which can be modified in **Settings > Objectives**.

Click the desired objective to select the corresponding magnification. You can select only one objective at a time. The selected objective is highlighted in blue.

Channels

This panel is used to set the light and camera settings.



① **ON/OFF:** Use to turn the light source on and off. When the light is on, the viewing area shows the sample illuminated with the selected light source.

CAUTION! This instrument uses Class 3B ultraviolet LEDs that are in accordance with IEC/EN 60825-1. Make the CELENA® X door is closed when imaging to protect your eyes. Direct exposure to and diffuse reflections of the laser can be hazardous to the eye.

IMPORTANT! Minimize the time that the sample is being exposed to light to prevent photobleaching and/or phototoxicity.

② **Condenser:** Is installed at the time of purchase (BF: brightfield, PH: phase condenser). Click the condenser (BF or PH) button for transmitted light. The dropdown menu allows you to control the condenser's iris diaphragm.

- AUTO Automatically adjusts to accommodate the selected objective
- 100% Used for objectives with high magnification
- 66% Used for objectives with medium magnification
- 33% Used for objectives with low magnification
- 0% Used for fluorescence imaging

③ **Light:** Controls the brightness of the selected channel. To adjust, move the slider in the desired direction or enter the desired value in the text box. Light intensity is controlled as a single parameter and expressed as a value between 1-1000.

④ **Filter cubes:** Represents the fluorescence channels available for imaging. Up to four interchangeable filter cubes may be installed at once for multichannel fluorescence imaging. Click the desired channel to select the corresponding light source. The selected filter cube is highlighted in blue. You can select only one channel at a time. Each filter cube can be renamed and its pseudocolor selected in **Settings ▸ Filter Cubes.**

⑤ **Gain and exposure:** Controls the camera capture settings. To adjust, move the slider in the desired direction or enter the desired value in the text box. Gain is the camera's amplification of the signal.

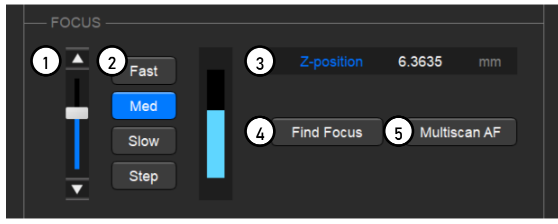
- Gain is the camera's amplification of the signal.

  $$\text{Gain(dB)} = 20 \times \log\left(\frac{V_{out}}{V_{in}}\right)$$

  8-bit: 0-36 dB
  12-bit: 0-24 dB
- Exposure is the amount of time that the camera shutter is open to allow light into the sensor.
  Exposure range: 0-10,000 ms

Focus

The focus panel is used to find focus and to set up autofocusing for batch processing in the currently selected channel.



① **Focus slider:** Used to adjust focus. The focus slider represents the full focal range. Adjust focus by moving the slider in the desired direction.

② **Z-stage speed:** Used to adjust the speed at which the Z-stage moves with each action. For fine focusing at high magnifications, set the focus speed to Slow. When Step is selected, the Z-stage moves the distance of the selected objective's depth of focus with each click.

③ **Z-position:** Shows the position of the Z-stage and used to adjust focus. The focus position is expressed in mm along the Z-axis. Adjust focus by entering the desired value in the text box.

④ **Find focus:** Used for instant autofocusing.

⑤ **Multiscan AF:** Used to set repeated autofocusing during an experiment.

The CELENA® X has two autofocus options: Find Focus for instant autofocusing and Multiscan AF for repeated autofocusing during an experiment.

Find Focus
Find Focus is used to have the CELENA® X find the optimal focal plane based on the image.

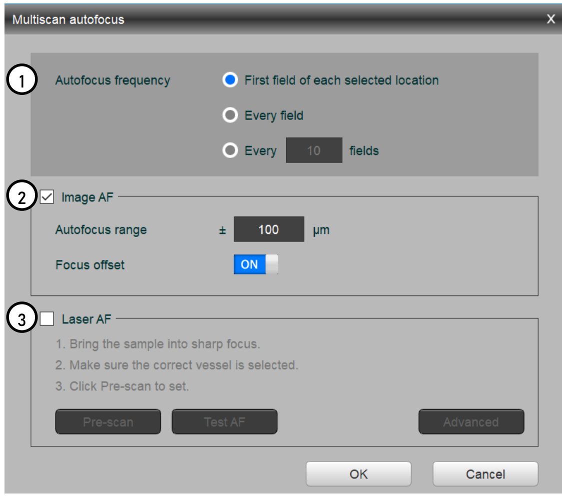
Set the range to scan from the current focal position.

- A long search range is useful when finding the focal plane of an unknown object.
- A short search range is useful for fine focusing.

Note: The speed of the image-based autofocus is entirely dependent on the set exposure. Reducing the exposure will increase focusing speed.

Multiscan AF
Multiscan AF is used to set up autofocusing for demanding batch image acquisitions such as multi-well plate imaging, slide scanning, and time-lapse imaging.

Prior to setting up Multiscan AF, make sure to bring the current field into sharp focus. The field must be focused sharply to setup subsequent autofocusing correctly.



IMPORTANT! If using this feature, Multiscan AF must be set up for each channel used.

① **Autofocus frequency:** Used to set the autofocus frequency to use during an automated scan.
- First field of each selected location
- Every field
- Every _ fields

Optimal AF frequency and range settings in Multiscan AF mode

	Optimal AF frequency	Optimal AF range
Multi-well plates	at least 1 field/well	±100 µm
Slides	every 10-20 fields	±10 µm

**Optimal AF frequency is also affected by objective magnification. Adjust accordingly.*
**Optimal AF range is also dependent on vessel bottom flatness. Adjust accordingly.*

When setting up Multiscan AF, you can select to use either the image-based or laser autofocus (optional; installed upon purchase).

A comparison of the CELENA® X autofocusing modes

	Image-based AF	Laser AF
Imaging speed	**Moderate**	**Fast**
	6 minutes 1 color, 10 ms exposure, 96-well plate	**2 minutes** 1 color, 10 ms exposure, 96-well plate
	9.5 minutes 3 colors, 10 ms exposure, 96-well plate	**3.5 minutes** 3 colors, 10 ms exposure, 96-well plate
Applicable magnifications	**All**	**10X-60X**
Photobleaching	**Yes**	**No**
Scratches, particles in sample	**Affected**	**Not affected**
Scratches, particles, and/or fingerprints on bottom surface	**Not affected**	**Affected**
Cell number, illumination conditions	**Affected**	**Not affected**

② **Image AF:** Select to set up image-based autofocusing.
Make sure the current field is focused sharply.

- **Autofocus range:** Use to set the range to scan from the current focal position.
- **Focus offset:** Choose to turn the focus offset on or off. The CELENA® X will calculate the difference between what the system defines as the optimal focal plane and what you define as the focal plane of interest and automatically calibrate the focus accordingly.

③ **Laser AF:** Select to set up laser-based autofocusing.
Make sure the current field is focused sharply and the correct vessel is selected.

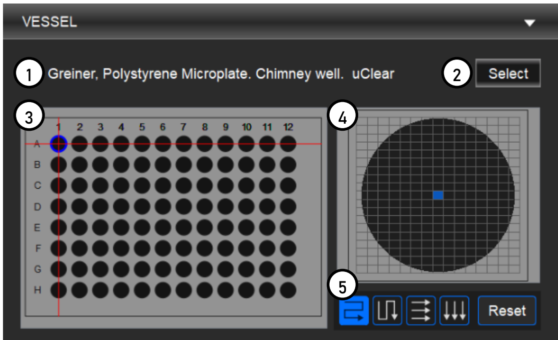
- **Pre-scan:** Use to have the CELENA configure the laser autofocus settings.
- **Test AF:** Use to test the accuracy of the configured laser autofocus.

IMPORTANT! Laser AF is not compatible with the following:

! Objectives with magnifications below 10X.
! PHC phase contrast objectives.
! LED filter cubes with an emission wavelength exceeding 750 nm.
! LED filter cubes with an excitation wavelength less than 350 nm.

Vessel

This section allows you to select the appropriate vessel, area, and fields to image.



① **Current vessel:** Shows the currently selected vessel.
② **Select vessel:** Allows you to select a vessel. Use the dropdown menus to select the vessel category and type. If the vessel you need is not available, go to **Settings > Vessels** to create a vessel.
③ **Vessel map:** Represents the currently selected vessel.
④ **Well map:** Represents the currently selected well. The field size within each well changes with the selected magnification.
⑤ **Acquisition order:** Allows you to specify the order in which selected areas are to be captured.

IMPORTANT! If using an objective with a correction collar, adjust the correction collar as necessary according to the bottom thickness of the selected vessel.

View a specific area/well: Double-click the desired area/well in the vessel map to move the stage to its respective location. The currently displayed area/well is rimmed in blue and indicated by red crosshairs.

Select a specific area/well for imaging: Click and drag to select multiple areas/wells in the vessel map. Otherwise, click each area/well. Selected areas/wells are filled in with yellow.

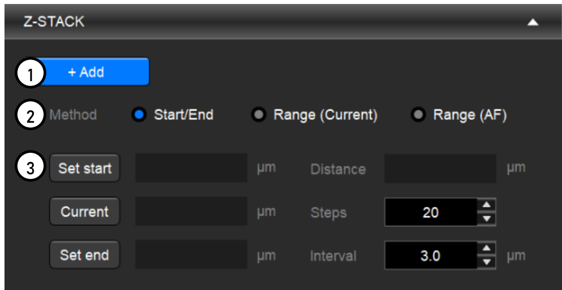
Select a specific field for imaging: Click and drag to select multiple fields in the well map. Otherwise, click each field. Selected fields are filled in with yellow.

Select the acquisition order: Click one of the following buttons to specify the order in which selected areas are to be captured.

	Horizontal, zigzag		Horizontal, rightward
	Vertical, zigzag		Vertical, downward

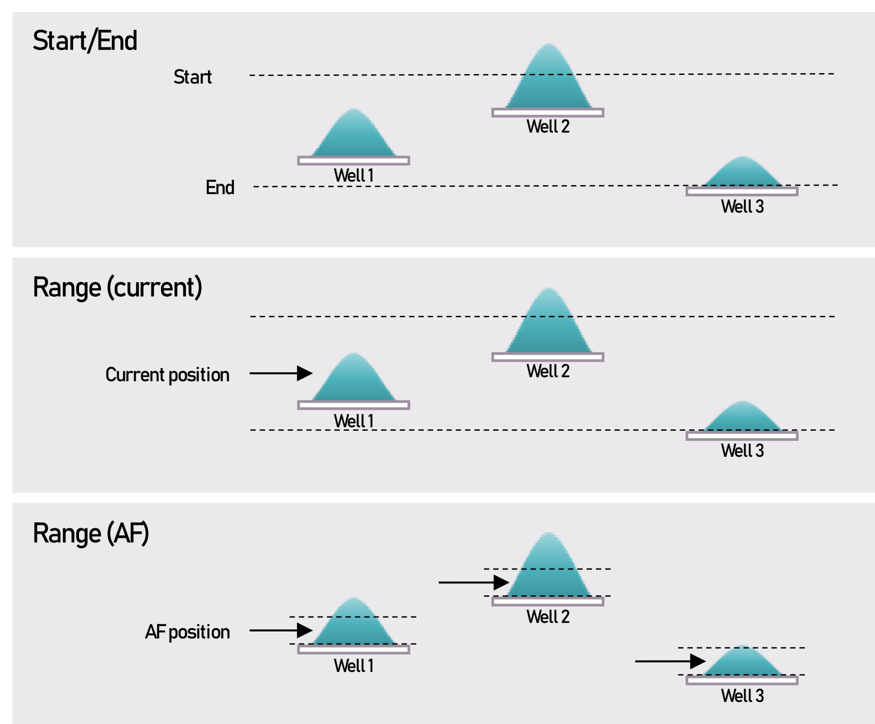
Z-stack

This panel allows you to set up Z-stack imaging. These settings apply to each added channel.



① **Add:** Adds the Z-STACK settings to the project protocol.
② **Method:** Allows you to select from three Z-stack imaging methods.
③ **Z-stack settings:** Allows you to set the Z-stack imaging parameters.

There are three methods of operation:



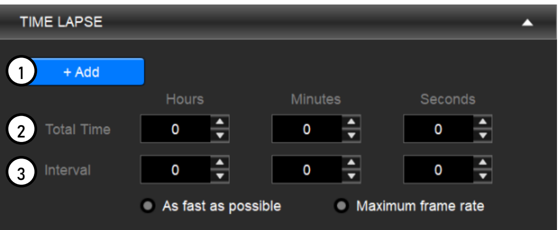
Z-stack methods

- **Start/End:** Set the start and end positions of the Z-stack.
  - Set start: Use to set the current focal plane as the start position.
  - Set end: Use to set the current focal plane as the end position.
  - Once the start and end positions have been set, the Z-stack distance is automatically calculated.
- **Range (Current):** Set the distance above and below the current Z-position.
  - Above (+): Use to set how far above the current focal plane to capture.
  - Below (-): Use to set how far below the current focal plane to capture.
- **Range (AF):** Set the distance above and below the autofocused position for each well. This method should be used when well-to-well focal variations are extreme. Multiscan AF must be set up first.
  - Above (+): Use to set how far above the autofocused position to capture.
  - Below (-): Use to set how far below the autofocused position to capture.

- **Distance:** The total distance between the start and end positions of the Z-stack. This is automatically calculated.
- **Steps:** The number of planes to capture along the Z-axis.
- **Interval:** The distance in µm between each focal plane captured.

Time lapse

This panel allows you to set up time lapse imaging for the project protocol. This applies to each channel. Selected fields are captured at set intervals over an allotted period of time.



① **Add:** Adds the TIME LAPSE settings to the project protocol.
② **Total time:** Allows you to set the total imaging time.
③ **Interval:** Allows you to set the time period that must elapse before a new set of images are captured.

The interval can be set manually or one of the two options below can be used to capture a new set of images immediately after capturing the previous set with no delay.

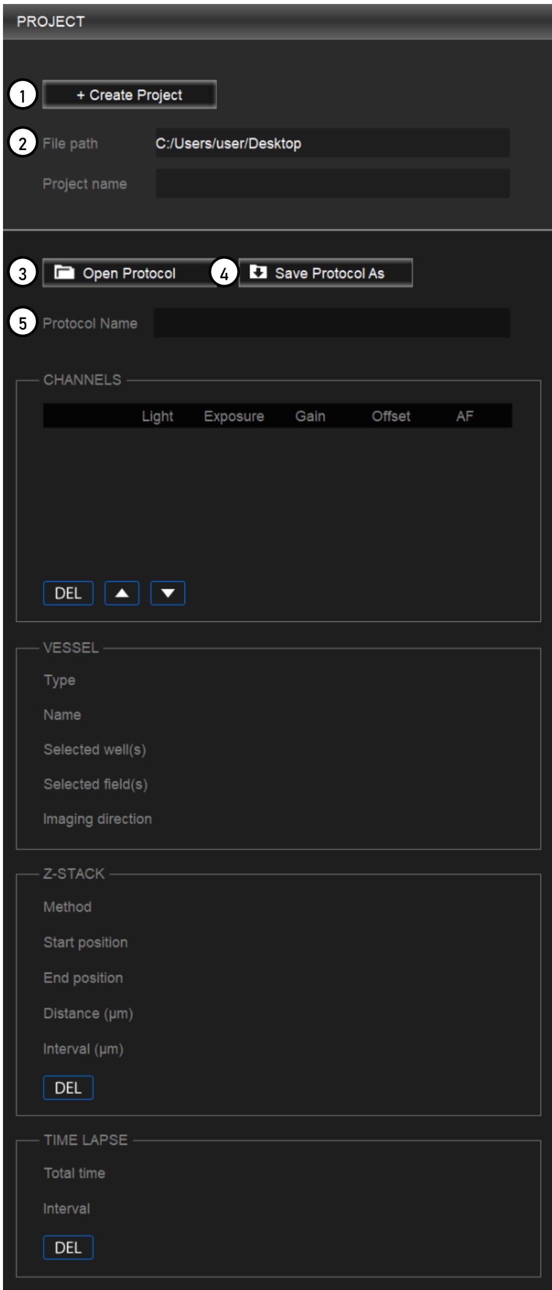
- **As fast as possible:** Can be used when imaging in multiple channels to capture the maximum number of images possible without stopping.
- **Maximum frame rate:** Can only be used when imaging a single field in a single focal plane with one channel to capture up to 30 frames per second. This option can be used for high-speed experiments such as calcium imaging.

Note: The interval will take into account other protocol settings such as the autofocus settings and exposure time.

Project

The project control panel is used to:

- Create and run a project
- Open or save a project protocol



① **Create project:** Allows you to start a project to image. This creates a project folder where all generated data will be stored.

- Project file (*.cxproj*): Stores project information, images, and associated metadata. This file can be opened in Cell Analyzer for analysis.
- Captured images
- Image thumbnails

Note: Save projects on the computer from which you are running Explorer. Do not save the project to an external hard drive or a USB drive as this can affect imaging time.

② **Project details:** Shows you the file path and name of a created project.

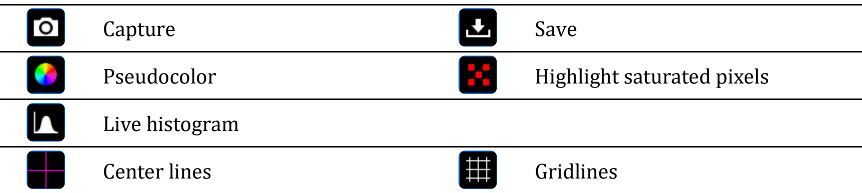
③ **Open protocol:** Allows you to open a previously saved protocol. This opens a previously saved protocol file (*.cxprotocol*). When you open a protocol, make the appropriate adjustments to each parameter as needed.

④ **Save protocol as:** Allows you to save a protocol. This saves a protocol file (*.cxprotocol*) for future use.

⑤ **Protocol details:** Shows you the protocol details.

Toolbar

The toolbar has tools for capturing and visualizing the current field of view.



	Capture		Save
	Pseudocolor		Highlight saturated pixels
	Live histogram		
	Center lines		Gridlines

① **Capture:** Click once to capture an image in the viewing area and turn off the light. Click again to clear the image from the viewing area and turn on the light.
② **Save:** Saves the captured image in the viewing area.
③ **Pseudocolor:** Shows the sample illuminated with the selected light source in pseudocolor. Go to **Settings > Filter cubes** to change the pseudocolor for each channel.
④ **Highlight saturated pixels:** Displays the pixels in saturated areas on an image. Go to **Settings > Camera** to change the color to label saturated pixels.
⑤ **Live histogram:** Shows a graphical representation of tonal values in real time.
⑥ **Center lines:** Shows center lines in the viewing area.
⑦ **Gridlines:** Shows gridlines in the viewing area.

Messages

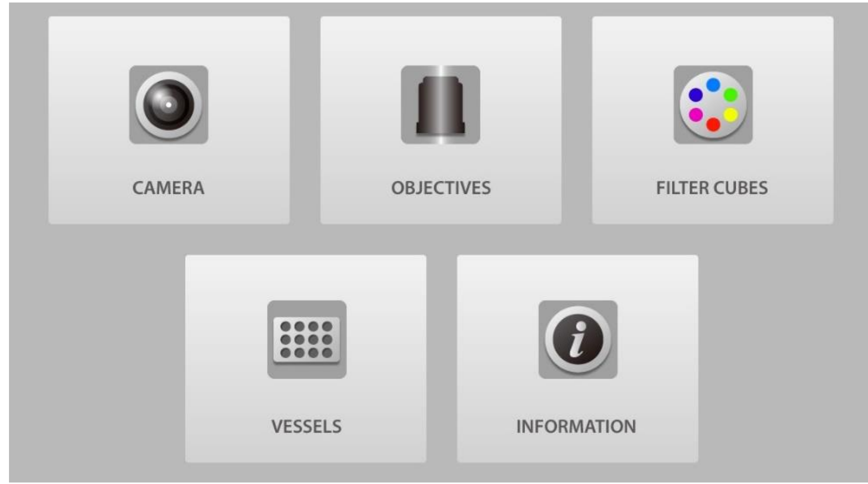
This panel is used to display system messages. You can resize the message panel by dragging the top border.
Not all system messages indicate problems with your system.

The message text can be copied for troubleshooting.

- To copy all the messages, right-click inside the message panel and click **Select All** from the context menu. Right-click the selection and select **Copy** from the context menu. The selection is copied and can be pasted as desired.
- To copy a specific message, select the desired message and right-click the selection. Select **Copy** from the context menu. The selection is copied and can be pasted as desired.

Settings

Settings allows you to set system options and perform calibration procedures. To access the Settings window, click the Settings wheel above the tool bar.



Camera

The camera settings allows you to select the camera, bit depth, saturated pixel color, as well as auto white balance the color camera.

① **Camera:**
   - Mono: Selects a monochrome camera.
   - Color: Selects a color camera,

② **Bit depth:** Can select to capture images in 8-bit or 16-bit with the monochrome camera (the actual bit depth of 16-bit images is 12-bit).

③ **Saturated pixel color:** Can select to color saturated pixels in red, green, or blue.

④ **Auto white balance:** Adjusts color intensities to render colors correctly when using the color camera.

IMPORTANT! Images captured with the color camera cannot be analyzed with CELENA® X Cell Analyzer.

Objectives

The objectives settings allows you to change objectives, adjust objective correction collars, and set the description for each installed objective. See 4.3 Change objectives to learn how to change objectives.

① **Change objectives:** Can be used to install and remove objectives.

② **Adjust correction collars:** Can be used to adjust the correction collar of objectives.

③ **Objective information**: Can be used to set the installed objectives and label them.

Filter cubes

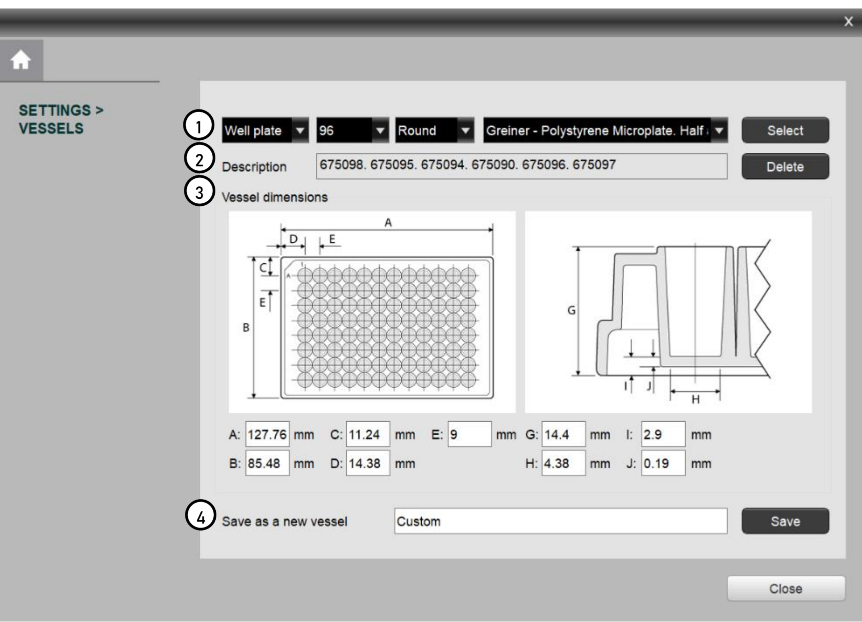
The filter cubes settings allows you to change filter cubes and set the pseudocolor and description for each installed filter cube. See 4.2 Change filter cubes to learn how to change filter cubes.

① **Change filter cubes:** Can be used to install and remove filter cubes.

② **Filter cube information:** Can be used to set the installed filter cubes, assign their associated pseudocolors, and label them.

Vessels

The vessels settings allows you to create and edit custom vessels.



① **Vessel details:** Can be used to select the vessel type, number of wells, well shape, and vessel name.
② **Vessel description:** Shows the associated catalog numbers of the selected vessel.
③ **Vessel dimensions:** Shows the dimensions of the selected vessel.
④ **Save as a new vessel:** Allows you to create a new vessel.

Create a vessel
Select the vessel type.

Select the number of wells.

Select the well shape.

Input the vessel dimensions: plate length (A), plate width (B), A1 row offset (C), A1 column offset (D), well spacing from center to center (E), plate height (G), well diameter bottom (H), flange/skirt height (I), well bottom thickness (J).

Name the vessel in Save as a new vessel.

Click **Save**.

Information

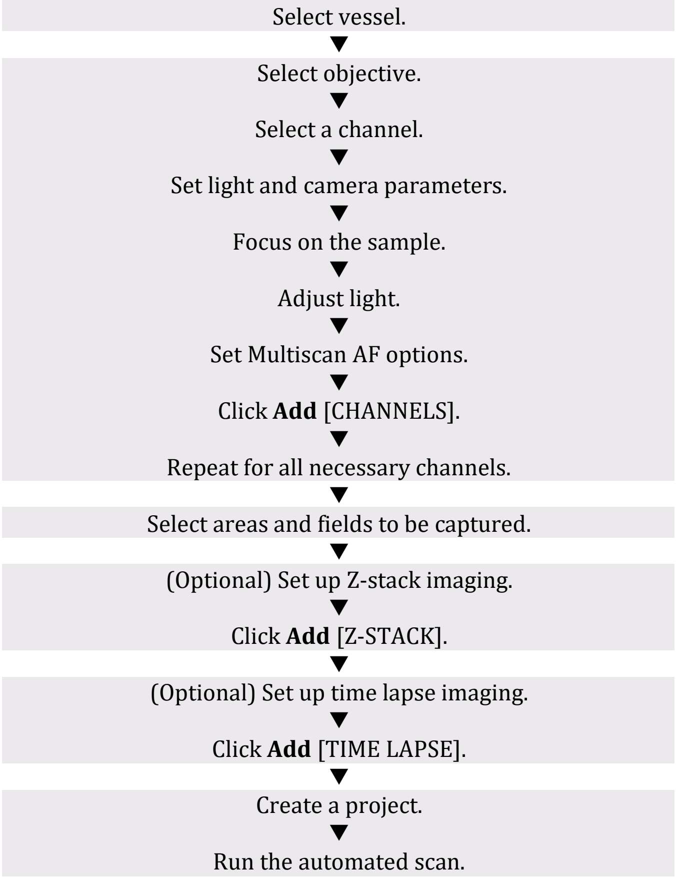
This section contains information about hardware, software, and the end user license agreement (EULA).

2.2 Workflow

Create a project protocol

Overview

Upon starting Explorer, you will create a new project protocol to capture images.



1. Select a vessel

In the VESSEL panel, click **Select** to bring up the vessel selection window.

Use the dropdown menus to select the vessel category and type.

Available vessel types are well plates, slides, dishes, and flasks.

It is crucial that you select the correct vessel to ensure proper focusing and vessel navigation. If the vessel you need is not available, go to **Settings > Vessels** to create a vessel.

IMPORTANT! Make sure the vessel doesn't fall into the CELENA® X.

2. Select an objective

In the CHANNELS panel, click the desired objective to select the corresponding magnification.

You can select only one objective for each project/protocol. If the objective you need is not available, go to **Settings > Objectives** to install a different objective.

3. Set up the channels

In the CHANNELS panel, set up channels as needed.

CAUTION! This instrument uses Class 3B ultraviolet LEDs that are in accordance with IEC/EN 60825-1. Make the CELENA® X door is closed when imaging to protect your eyes. Direct exposure to and diffuse reflections of the laser can be hazardous to the eye.

IMPORTANT! Minimize the time that the sample is being exposed to light to prevent photobleaching and/or phototoxicity.

Select a channel
Click the desired channel and adjust the condenser's iris diaphragm.

- For brightfield imaging, click BF or PH for transmitted light. Use the dropdown menu to control the condenser's iris diaphragm as desired.

- For fluorescence imaging, click the desired fluorescence channel to select the corresponding light source. If the channel you need is not available, go to **Settings > Filter cubes** to install a different filter cube.

Tips:

- When searching for a sample, increase gain and decrease exposure for a faster frame rate.
- Decrease gain to reduce background noise and increase exposure to improve signal intensity for imaging.

Adjust light intensity
Move the slider in the desired direction or enter the desired value in the text box.

Adjust camera gain and exposure
Move the slider in the desired direction or enter the desired value in the text box.

Focus sharply.
Move the focus slider in the desired direction or enter the desired value in the Z-position box. Alternatively, click **Find Focus** to have the CELENA® X find the optimal focal plane. Set the range to scan from the current focal position. A long search range is useful when finding the focal plane of an unknown object. A short search range is useful for fine focusing.

Note: The speed of the image-based autofocus is entirely dependent on the set exposure. Reducing the exposure (< 10 ms) will increase focusing speed.

Set up Multiscan AF.
Click **Multiscan AF** to set up autofocusing for demanding batch image acquisitions such as multi-well plate imaging, slide scanning, and time-lapse imaging. Select how often to autofocus during an automated scan. Select whether to use the image-based or laser autofocus.

- **Image-based:** Make sure the current field is focused sharply. Set the range to scan from the current focal position. You can choose to turn the user-defined focus offset on or off. The user-defined focus offset means that the system will calculate the difference between what the system defines as the optimal focal plane and what the user defines as the focal plane of interest and automatically calibrate the focus accordingly.
- **Laser AF:** Make sure the current field is focused sharply. The vessel information must be correct. Click Pre-Scan to have the CELENA® X configure the laser autofocus settings. Click Test AF to test the accuracy of the configured laser autofocus. Laser AF cannot be used with magnifications below 10X.

Note: When using this feature for imaging in multiple channels, Multiscan AF must be set for each channel. This is especially important when the fluorescent markers in different channels are in different focal planes.

Add to the project protocol.
Click **Add**.

Repeat for all necessary channels.

4. Select areas and fields to be captured

In the VESSEL panel, select the well(s) to image in the vessel map.

Click individual wells or drag and drop to select multiple wells. Wells selected for imaging will be filled with yellow.

Select the field(s) to image within each well in the well map.

Click individual fields or drag and drop to select multiple fields. Fields selected for imaging will be field with yellow.

5. (Optional) Set up Z-stack imaging

In the Z-STACK panel, select a Z-stack method and set appropriately.

- **Start/End:** Move the focal plane to the desired start Z-position and click **Set Start**. Move the focal plane to the desired end Z-position and click **Set End**. Select to capture images at specific intervals (µm) or to capture a specific amount of images (steps) and enter the desired value.

- **Range (current):** Move the to the desired start position. To set the imaging range, enter how far above (+) and below (-) the current position to set the imaging range. Select to capture images at specific intervals (µm) or to capture a specific amount of images (steps) and enter the desired value.
- **Range (AF):** Make sure Multiscan AF has been set. To set the imaging range, enter how far above (+) and below (-) the autofocused position. Select to capture images at specific intervals (µm) or to capture a specific amount of images (steps) and enter the desired value.

Click **Add.**

6. **(Optional)**
   Set up time lapse imaging

In the TIME LAPSE panel, set the total imaging time and imaging interval.

IMPORTANT! Set up time lapse imaging last so that the CELENA® X can account for the other imaging options you have set, which affect the time required to capture one image.

Click **Add.**

7. **Create a project**

In the PROJECT panel, click **Create Project**.

Name the project and designate where to save the project folder.

IMPORTANT! Save projects on the computer from which you are running Explorer. Do not save the project to an external hard drive or a USB drive as this can affect imaging time.

Save a project protocol

To save the set protocol for future use, click **Save Protocol As** in the PROJECT panel.

Name the protocol and designate the file path.

Load a project protocol

To load a previously saved protocol, click **Open Protocol** in the PROJECT panel.

Make the appropriate adjustments to each parameter as needed. This is especially important for the Multiscan AF feature and Z-stack imaging. Make sure to adjust Multiscan AF settings for each channel being imaged. To apply each change, click the **Add** button above each panel.

Run a project protocol

Once a protocol has been set and project has been created, click **RUN** at the bottom of the PROJECT panel.

IMPORTANT! Make sure the CELENA® X door is closed for fluorescence imaging applications to block ambient light and improve fluorescence image quality.

Pause/stop a project protocol

To pause a running project, click **PAUSE** at the bottom of the PROJECT panel.

To stop a running project, click **STOP** at the bottom of the PROJECT panel.

View project results

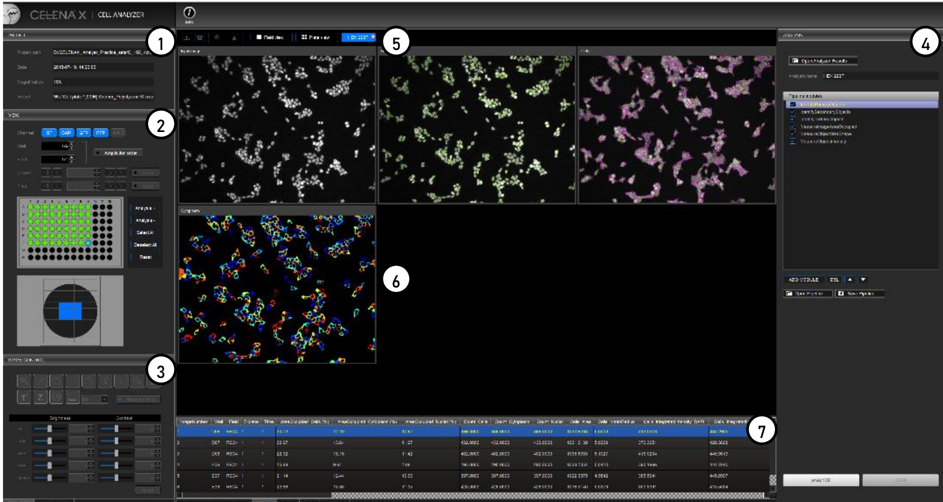
When a project is complete, you can scroll through the captured images using the vessel and well maps.

The project file (*.cxproj*) can be opened in CELENA® X Cell Analyzer for analysis.

3. CELENA® X Cell Analyzer

3.1 Overview

CELENA® X Cell Analyzer can be used to set up automated image analysis sequences to batch process images captured on the CELENA® X. Cell Analyzer also provides tools to edit and annotate images as well as create videos. The CELENA® X Cell Analyzer Verification Key must be plugged into use Cell Analyzer.



CELENA® X Cell Analyzer

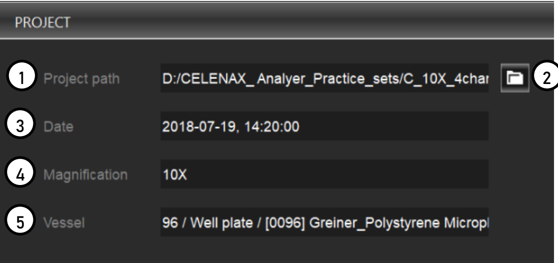
① **PROJECT:** Allows you to load a project for analysis and see project details.
② **VIEW**: Allows you view captured images and select wells for analysis.
③ **IMAGE CONTROL:** Allows you to edit images, add annotations, and make simple measurements.
④ **ANALYSIS:** Allows you to set up, edit, and run analysis pipelines.
⑤ **Toolbar:** Has tools to export images, create videos, and visualize images.
⑥ **Viewing area:** Shows captured and analyzed images.
⑦ **Messages:** Displays system messages, annotation measurement data, module details, and analysis results.

At the bottom of the window, there is a PROJECT tab and INFORMATION tab.

- **PROJECT:** Shows the PROJECT, VIEW, and IMAGE CONTROL panels.
- **INFORMATION:** Shows a detailed description of the project imaging details.

Project

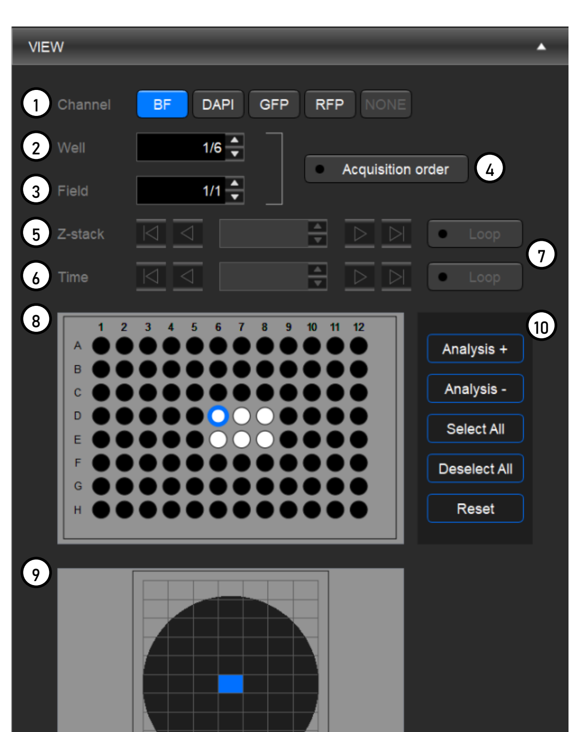
This panel is used to load a project for analysis and displays project details.



① **Project path:** Shows where the project file and images are located.
② **Folder icon:** Allows you to load a project for analysis.
③ **Date:** Displays the date and time the project was captured.
④ **Magnification:** Displays the objective magnification used for imaging.
⑤ **Vessel:** Displays the sample vessel used.

View

This panel allows you to view the captured images and select wells to analyze.



① **Channel:** Allows you to select which channels to display.
② **Well:** Allows you to select which well to view.
③ **Field:** Allows you to select the field in the selected well to view.
④ **Acquisition order:** Shows the images in the order they were captured.
⑤ **Z-stack:** Allows you to go through the captured Z-planes (if applicable).
⑥ **Time:** Allows you to go through the sequence of time lapse images (if applicable).
⑦ **Loop:** Sets the images in a loop so images can be cycled through continuously without stopping at the end of the sequence.
⑧ **Vessel map:** Represents the imaged vessel.
⑨ **Well map:** Shows the imaged fields within each well.
⑩ **Analysis buttons:** Allows you to select wells for analysis.

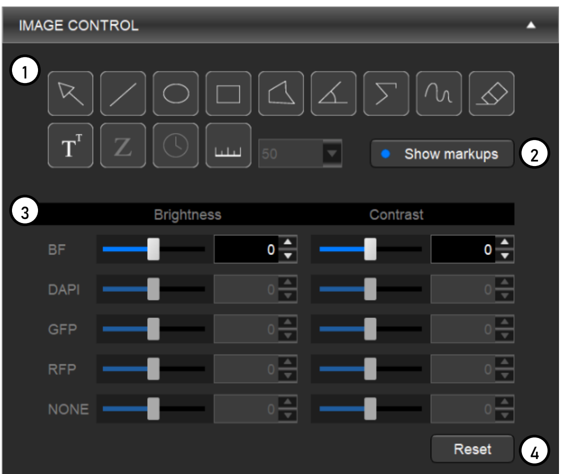
Click wells and fields to view their corresponding images. The currently displayed well is rimmed in blue and the displayed field is filled with blue.

Analysis buttons

- **Analysis +**: Adds wells to the list of wells to be analyzed.
  Wells to be analyzed will be filled with yellow.
- **Analysis –**: Removes wells from the list of wells to be analyzed.
  Imaged wells that are not set to be analyzed will be filled with white.
- **Select All**: Selects all imaged wells. Selected wells will be rimmed in blue.
  This only selects the wells. To add to the analysis list, you must click **Analysis +**.
- **Deselect All**: Deselects all wells. This only deselects wells. To remove from the analysis list, you must select the desired well(s) and click **Analysis -**.
- **Reset**: Clears the list of wells to be analyzed.

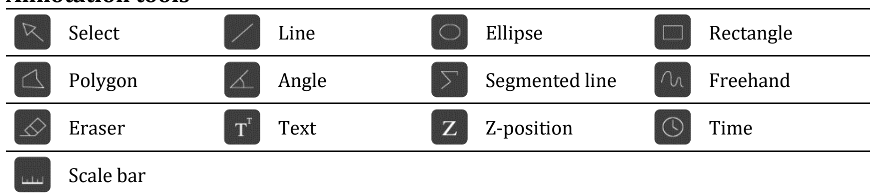
Image control

This panel allows you to edit images, add annotations, and make simple measurements.



① **Annotation tools:** Allows you to mark and measure specific areas of interest.
② **Show markups:** Shows or hides annotations.
③ **Editing tools:** Allows you to adjust the brightness and contrast of each channel
④ **Reset:** Resets all image adjustments.

Annotation tools



Select	Line	Ellipse	Rectangle
Polygon	Angle	Segmented line	Freehand
Eraser	Text	Z-position	Time
Scale bar			

Use the select tool to select and manipulate annotations.

Right-click on an annotation to change properties such as color and size as well as to copy, paste, and delete the annotation.

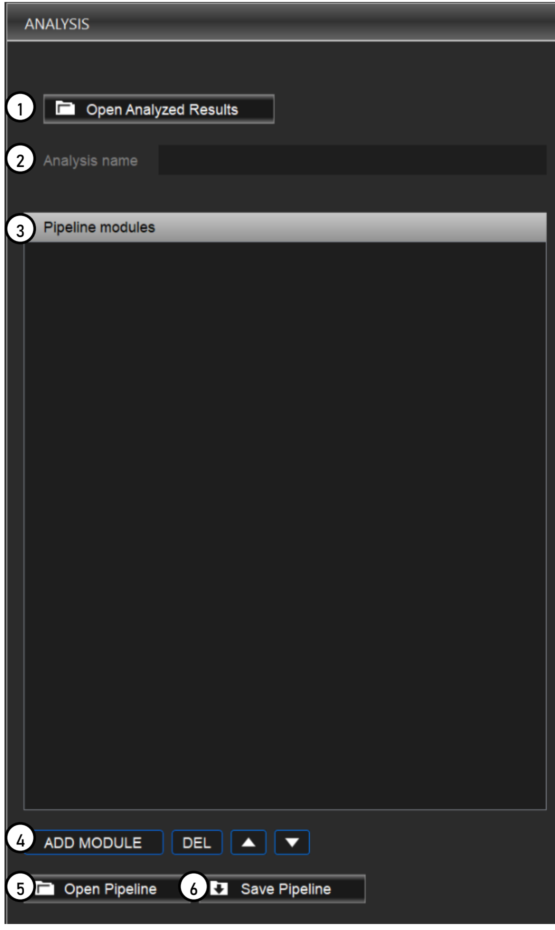
Double-click to deselect the annotation.

Editing tools
Adjust the brightness and contrast of each channel using the respective sliders or text boxes.

To select or deselect channels, use the channel buttons in the VIEW panel.

Analysis

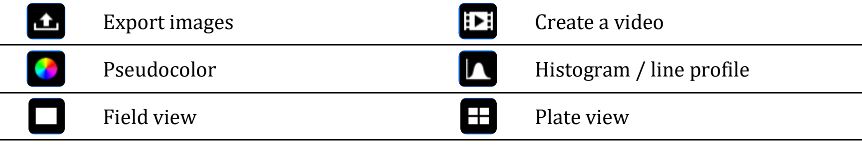
This panel allows you to set up, edit, and run analysis pipelines.



① **Open analyzed results:** Allows you open a previously analyzed project (*.cxasis*).
② **Analysis name:** Shows you the analysis name.
③ **Pipeline modules:** Shows you the modules in the pipeline.
④ **Module buttons:** Allows you to add, delete, or rearrange pipeline modules.
⑤ **Open pipeline:** Allows you to select a previously saved pipeline.
⑥ **Save pipeline:** Allows you to save a newly created or edited pipeline.

Toolbar

The toolbar has tools to export images, create videos, and visualize images



	Export images		Create a video
	Pseudocolor		Histogram / line profile
	Field view		Plate view

- **Export images:** Allows the export of annotated or edited images.
- **Create a video:** Allows the creation of a video of time lapse or Z-stack images.
- **Pseudocolor:** Shows each channel in its designated pseudocolor.
- **Histogram/line profile:** Displays tonal values of the whole image or a specific annotation.
- **Field view:** Shows a single field in the viewing area.
- **Plate view:** Shows the captured fields laid out according to their location in the vessel in the viewing area.

Messages

This panel is used to display system messages, annotation measurement data, module details, and analysis results. You can resize the message panel by dragging the top border.

There are four tabs: Messages, Data, Modules, and Results. Upon analysis, additional tabs will appear for each analyzed object.

Messages

This tab shows the analysis process.

The text can be copied for troubleshooting.

- To copy all the messages, right-click inside the message panel and click **Select All** from the context menu. Right-click the selection and select **Copy** from the context menu. The selection is copied and can be pasted as desired.
- To copy a specific message, select the desired message and right-click the selection. Select **Copy** from the context menu. The selection is copied and can be pasted as desired.

Data

This tab shows the values for measurements made with the annotation tools in the IMAGE CONTROL panel.

Data will appear in this tab as you mark specific areas of interest with the annotation tools.

- To see the location of an annotation, select the annotation from the list.
- To delete a specific measurement, select it and right-click to select **Clear**.
- To delete all data, right-click and select **Clear All**.
- To export measurement data as a CSV file, select the data to export and right-click to select **Export CSV**.

Modules

This tab allows you to adjust pipeline modules as needed.

Select a module in the pipeline module list in the ANALAYSIS panel to show module parameters.

For a complete list of available modules, go to 3.3 Pipeline module reference.

Results

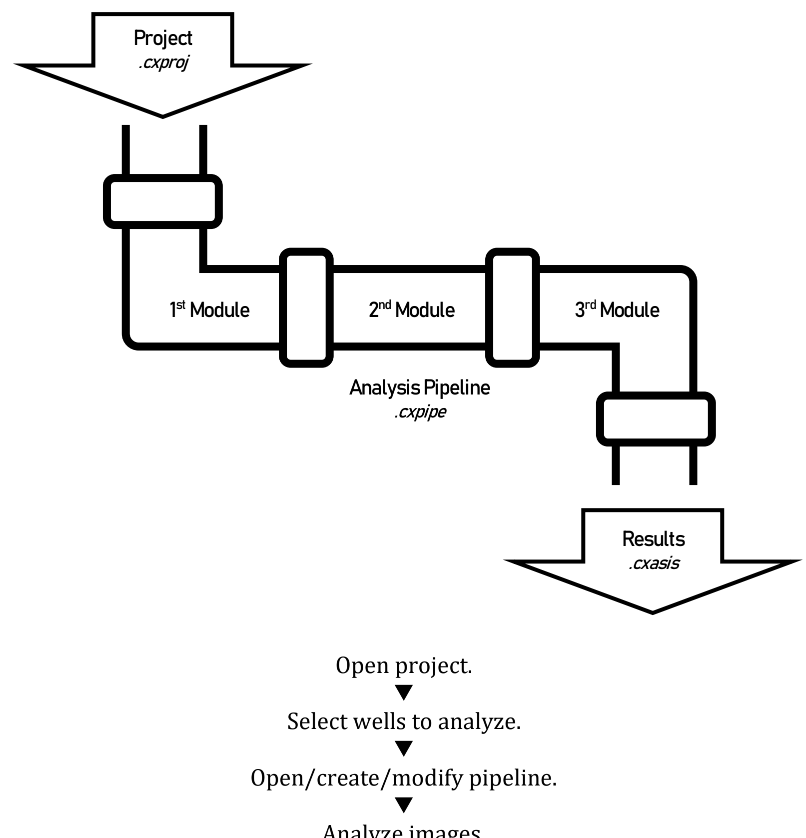
This tab allows you to examine analysis results.

3.2 Workflow

Set up project analysis

Overview

Using Cell Analyzer, users can create an image analysis pipeline, which is a sequence of modules that each perform a specific image processing task. This allows the quantitative analysis of multiple cellular features from images. Modules can be mixed, matched, and adjusted to measure phenotypes of interest quantitatively. Once a pipeline has been established, it can be used to analyze subsequent projects.



Open project.
▼
Select wells to analyze.
▼
Open/create/modify pipeline.
▼
Analyze images.

1. Open project

Make sure the project folder created by CELENA® Explorer is on your computer.

Click the folder icon next to Project path.

Select a *.cxproj* file. This loads the project file, which contains a list of the project image files, the file locations, and the associated metadata. The project images and metadata will be loaded.

Note: Make sure the images were captured with the monochrome camera. Pipelines require images to be in grayscale.

2. Select wells to analyze

Use the VIEW panel to go through images captured.

Select the wells to be analyzed and click **Analysis +**. Wells programmed for analysis will be filled in yellow.

3. Select a pipeline

Select a previously saved pipeline or create a new pipeline by using the modules located in the pipeline window.

Open a pipeline

- Click **Open Pipeline** in the ANALYSIS panel.
- Select a *.cxpipe* file. This loads the pipeline file and the pipeline modules with the saved settings will appear.
- Select a module in the pipeline to see its settings in the Modules tab. Adjust the settings for each module as needed.

Create a pipeline

- Click **ADD MODULE** in the ANALYSIS panel.
- Select the module(s) you want to use from the modules box and click **Add to Pipeline**. When finished, click **Close**.
- Modules are processed in the order specified. Adjust the sequence by dragging and dropping modules or by using the ▲ and ▼ buttons. Delete selected module(s) from the pipeline using the **DEL** button.
- Adjust the settings for each module as needed. Click a module in the pipeline to see its settings in the module pane.
- (Optional) Click **Save Pipeline** to save.

Note: Pipelines are automatically saved to the analysis folder once analysis is run.

For more detailed information on pipeline modules, see 3.3 Pipeline module reference.

4. Analyze images

Click **ANALYZE** at the bottom of the ANALYSIS panel.

Name the analysis to create a *.cxasis* file and begin image analysis.

The following files will be saved to the project folder:

- Analyzed images (*.tif*)
- Analysis results (*.csv*)
- Analysis file (*.cxasis*)
- Pipeline file (*.cxpipe*)

5. View data

Once analysis is complete, you can see a summary of the analysis results onscreen.

Click the **Results** tab in the messages panel to show the results pane. There will be a table that displays the results of all analyzed wells and fields.

Additional tabs will appear for each analyzed object. Click on these tabs to view object measurements.

Click wells and fields in the VIEW panel to view their corresponding images.

Load previously analyzed images

Previously analyzed projects can be reviewed in Cell Analyzer.

Click **Open Analyzed Results** in the ANALYSIS panel.

Select a *.cxasis* file. This will load the analyzed images, applied pipeline, measurement data, analysis results, and respective metadata.

Click the Results tab in the messages panel to show the results pane. There will be a table that displays the results of all analyzed wells and fields. Additional tabs will appear for each analyzed object. Click on these tabs to view object measurements.

Click wells and fields in the VIEW panel to view their corresponding images.

Annotate images and make simple measurements

1. Open project

Make sure the project folder created by CELENA® Explorer is on your computer.

Click the folder icon next to Project path.

Select a *.cxproj* file. This loads the project file, which contains a list of the project image files, the file locations, and the associated metadata.

2. Select image

Use the VIEW panel to go through images captured.

Select the desired image.

3. Add annotations

Use the annotation tools in the IMAGE CONTROL panel to add annotations and make simple measurements.

Click the Data tab in the messages panel to show the data pane. There will be a table that displays all the measurements related to each annotation.

4. Export (Optional) To export measurement data, select the desired measurement(s), right-click, and click **Export CSV**.

(Optional) To save the annotated image, click the export images icon in the toolbar.

Edit images

1. Open project Make sure the project folder created by CELENA® Explorer is on your computer.

Click the folder icon next to Project path.

Select a *.cxproj* file. This loads the project file, which contains a list of the project image files, the file locations, and the associated metadata. The project images and metadata will be loaded.

2. Select image Use the VIEW panel to go through images captured.

Select the desired image.

3. Edit image Use the IMAGE CONTROL panel to adjust the brightness and contrast of each channel.

In the VIEW panel, select the desired channels to display.

Adjust the brightness and contrast of each channel using the respective sliders or the text boxes.

To undo image adjustments, click **Reset**.

To save the edited images, click the export images icon in the toolbar.

4. Export (Optional) To save the annotated image, click the export images icon in the toolbar.

3.3 Pipeline module reference

Overview

Pipeline modules can be divided into the following categories:

1) **Image processing**
   a. ColorToGray
   b. EnhanceEdges
   c. EnhanceOrSuppressFeatures
   d. FilterObjects
   e. GrayToColor
   f. ImageMathOverlay
   g. Invert
   h. MaskImage
   i. OverlayOutlines
   j. Smooth
2) **Object identification**
   a. IdentifyPrimaryObject
   b. IdentifySecondaryObject
   c. IdentifyTertiaryObject
3) **Measurements**
   a. MeasureImageAreaOccupied
   b. MeasureObjectIntensity
   c. MeasureObjectSizeShape

Image processing

ColorToGray

The ColorToGray module converts RGB color images to grayscale images. Multiple channels can be merged into one grayscale image or converted into individual grayscale images.

Module settings:

1. Select the input image.
2. Select to:
   a. Combine multiple channels into one grayscale image or
   b. Split each channel to create individual grayscale images.
3. If 3a, name the output image.
   If 3b, select which channels to convert to gray and name the output image(s).
4. If 3a, the relative weights will adjust the contribution of the colors relative to each other. If necessary, adjust as needed.

EnhanceEdges

The EnhanceEdges module enhances or identifies edges in an image for downstream image processing and/or object identification. This can be used to enhance cell boundaries for effective determination of cell areas.

Module settings:

1. Select the input channel.
2. Name the output image.
3. Select an edge-finding method. Choose from the following:
   a. Sobel
   b. Prewitt
   c. Roberts
   d. LoG
   e. Canny
   f. Kirsch
4. If 3a or 3b, select edge direction to enhance.
   If 3d or 3e, select whether or not to calculate Gaussian's sigma automatically. If not, enter the Guassian's sigma value.
   If 3e, select whether or not to automatically calculate the threshold. If not, enter the absolute threshold value.
   If 3e, select whether or not to automatically calculate the value for low threshold. If not, enter the low threshold value.
   If 3e, enter the threshold adjustment factor.

Tips:

- All edge-finding methods besides Canny produce grayscale images on which Identify modules can be used downstream. The Canny method produces a black and white mask image of the edge pixels.

EnhanceOrSuppressFeatures

The EnhanceOrSuppressFeatures module enhances or suppress specific features in an image to improve downstream object identification.

Module settings:

1. Select the input channel.
2. Name the output image.
3. Select to:
    a. Enhance or
    b. Suppress features.
4. If 3a, select a feature type to enhance. Choose from the following:
    a. Speckles
    b. Neurites
    c. Dark holes
    d. Circles
    e. Texture
    f. DIC

   If 3b, select the feature size.
5. If 4a, select the speed and accuracy, and enter the feature size.
   If 4b, select the enhancement method and smoothing scale.
   If 4c, enter the range of hole sizes.
   If 4d, enter the feature size.
   If 4e, enter the smoothing scale.
   If 4f, enter the smoothing scale, shear angle, and decay.

FilterObjects

The FilterObjects module eliminates select identified objects based on certain measurements produced by another module. Objects can be also be filtered based on whether or not they touch image borders.

Module settings:

1. Select objects to filter.
2. Name the output objects.
3. Select the filtering mode. Choose from the following:
    a. *Measurements*: Specify a per-object measurement made by an upstream module in the pipeline.
    b. *Image or mask border*: Remove objects touching the border of the image and/or the edges of an image mask.
4. If 3a, select the filtering method. Choose from the following:
    a. *Minimal*: Keep the object with the minimum value for the measurement of interest. If multiple objects share a minimal value, retain one object selected arbitrarily per image.
    b. *Maximal*: Keep the object with the maximum value for the measurement of interest. If multiple objects share a maximal value, retain one object selected arbitrarily per image.
    c. *Minimal per object*: This option requires you to choose a parent object. The parent object might contain several child objects of choice. Only the child object whose measurements equal the minimal child-measurement value among that set of child objects will be kept.
    d. *Maximal per object*: Same as Maximal per object, except filtering is based on the maximum value.
    e. *Limits*: Keep an object if its measurement value falls within a range you specify.
5. If 4c or 4d, child object can overlap two parent objects and can have the maximal/minimal measurement of all child objects in both parents. Select to which parent to assign the overlapping child. Choose from the following:
    a. *Both parents*: The child will be assigned to both parents and all other children of both parents will be filtered.
    b. *Parent with most overlap*: The child will be assigned to the parent with the most overlap and a child with a less maximal/minimal measurement, if available, will be assigned to other parents.
6. If 5b, select the objects that contain the filtered objects.
7. Select whether or not to retain outlines of the identified objects.
    - Yes: Will retain the outlines of new objects for downstream modules.
    - No: Will not retain the outlines of new objects for downstream modules.

Tips:

- Any objects that are filtered are considered a new object, so the measurements associated with the original objects do not carry over to the new objects. For measurements on the new objects, make the measurements downstream.

Generated measurements:

- Count: The number of objects remaining after filtering.
- Parent: The identity of the input object associated with each filtered (remaining) object.
- Location_Center_X: The X coordinate of the center of mass of the filtered object.
- Location_Center_Y: The Y coordinate of the center of mass of the filtered object.

GrayToColor

The GrayToColor module converts grayscale images to color images.

Module settings:

1. Name the output image.
2. Select the images to convert.
3. Assign their respective colors.
4. Adjust the brightness of each color by using relative weights.

ImageMathOverlay

The ImageMathOverlay module multiplies image intensities.

Module settings:

1. Name the output image.
2. Select the image(s) to convert.
3. Enter how much to multiply each selected image by.

Invert

The Invert module inverts images.

Module settings:

1. Select the input channel.
2. Name the output image.

MaskImage

The MaskImage module hides specific areas in an image (based on objects identified upstream or a binary image) so they are ignored by downstream mask-respecting modules in the pipeline.

This module masks an image so you can use the mask downstream in the pipeline. The masked image is based on the original image and the masking object or image that is selected. If using a masking image, the mask is composed of the foreground (white portions); if using a masking object, the mask is composed of the area within the object. Note that the image created by this module for further processing downstream is grayscale. If a binary mask is desired in subsequent modules, use the Threshold module instead of MaskImage.

Module settings:

1. Select the input image.
2. Name the output image.
3. Select to:
    a. Use objects or
    b. An image as a mask.
4. If 3b, select the image.
5. Select whether or not to invert the mask.

OverlayOutlines

The OverlayOutlines module outlines objects in images.

Module settings:

1. Select the channel on which to display outlines.
2. Name the output image.
3. Enter the width of outlines.
4. Select objects to display.
5. Select outlines to display.

Smooth

The Smooth module smooths or blurs images to remove small artifacts.

Module settings:

1. Select the input channel.
2. Name the output image.

Object identification

Pipelines will depend on identifying the objects in the image. In Cell Analyzer, you will identify primary, secondary, or tertiary objects.

IdentifyPrimaryObject

The IdentifyPrimaryObject module identifies primary objects from grayscale images.

A primary object is an object that can be identified in an image without needing another object or image as a reference. Nuclei are good candidates for primary object identification as they are uniform in shape, have a high contrast relative to its background once stained, and are well-spaced apart from adjacent nuclei.

Module settings:
1. Select the input channel.
2. Name the primary objects to be identified.

Tips:
- Images must be grayscale.
- The regions of interest must be lighter than the background – if they are dark on a light background, invert the images using the **Invert** module upstream.
- If the images are phase or brightfield images, process the images using the **EnhanceOrSuppressFeatures** module upstream.

Generated measurements:
- Count: The number of primary objects identified.
- Location_Center_X: The X coordinate of the center of mass of the primary object.
- Location_Center_Y: The Y coordinate of the center of mass of the primary object.

IdentifySecondaryObject

The IdentifySecondaryObject module identifies secondary objects from grayscale images by using the primary object as a reference.

A secondary object is an object that can be identified in an image using another as a reference. Cells are challenging to identify without a reference as their borders are usually overlapping especially in the case of a confluent monolayer and are lower contrast due to diffuse staining. Cells are good candidates for secondary object identification as they need a previously identified primary object such as nuclei as a reference to detect cell borders.

Module settings:
1. Select the input channel.
2. Select the input objects. The input objects will be identified from a prior module. Although it is usually from the **IdentifyPrimaryObjects** module, it can be any an object identified by any other module.
3. Name the primary objects to be identified.

Tips
- Images must be grayscale. .
- Primary objects must be completely contained within a secondary object. Secondary objects must be larger than or equal in size to primary objects.

Generated measurements:
- Count: The number of secondary objects identified.
- Location_Center_X: The X coordinate of the center of mass of the secondary object.
- Location_Center_Y: The Y coordinate of the center of mass of the secondary object.

IdentifyTertiaryObject

The IdentifyTertiaryObject module identifies tertiary objects from grayscale images by using the primary and secondary object as a reference.

A tertiary object is an object that can be identified in an image by removing primary objects from the larger secondary objects. For example, cytoplasm is an object that is outside the nuclei but contained within the cell boundaries. This means that it can be identified by subtracting nuclei (smaller identified objects) from cells (larger identified objects).

Module settings:
1. Select the larger identified objects. This will be identified from a prior module. Although it is usually from the **IdentifySecondaryObjects** module, it can be any object identified by any other module.
2. Select the smaller identified objects. This will be identified from a prior module. Although it is usually from the **IdentifyPrimaryObjects** module, it can be any object identified by any other module.
3. Name the objects to be identified.

Tips:

- Images must be grayscale.
- The regions of interest must be lighter than the background – if they are dark on a light background, invert the images using the **Invert** module upstream.
- Primary objects must be completely contained within a secondary object. Secondary objects must be larger than or equal in size to primary objects.

Generated measurements:

- Count: The number of tertiary objects identified.
- Location_Center_X: The X coordinate of the center of mass of the tertiary object.
- Location_Center_Y: The Y coordinate of the center of mass of the tertiary object.

Measurements

MeasureImageAreaOccupied

The MeasureImageAreaOccupied module measures the total area occupied by identified objects within an image.

Module settings
1. Select objects to measure.

Generated measurements:
- AreaOccupied: The total area occupied by the input objects.

MeasureObjectIntensity

The MeasureObjectIntensity module measures the intensity of identified objects.

Module settings:
1. Select a channel.
2. Select objects to measure.
3. Select measurements to export.

Tips:
- Microscopes are not calibrated to an absolute scale, so when using intensity measurements in publications, the units of intensity can be called, "intensity units" or "arbitrary intensity units". Moreover, specify which intensity unit you are referring to (e.g. integrated intensity units, mean intensity units, etc.).

Generated measurements:
- IntegratedIntensity: The sum of the pixel intensities within an object.
- IntegratedIntensityEdge: The sum of the edge pixel intensities of an object.
- LowerQuartileIntensity: The intensity value of the pixel for which 25% of the pixels in the object have lower values.
- MADIntensity: The median absolute deviation (MAD) value of the intensities within the object. The MAD is defined as the median($|x_i$ - median(x)$|$).
- MassDisplacement: The distance between the centers of gravity in the gray-level representation of the object and the binary representation of the object.
- MaxIntensity: The maximal pixel intensity within an object.
- MaxIntensityEdge: The maximal edge pixel intensity of an object.
- MeanIntensity: The average pixel intensity within an object.
- MeanIntensityEdge: The average edge pixel intensity of an object.
- MedianIntensity: The median intensity value within the object.
- MinIntensity: The minimal pixel intensity within an object.
- MinIntensityEdge: The minimal edge pixel intensity of an object.
- StdIntensity: The standard deviation of the pixel intensities within an object.
- StdIntensityEdge: The standard deviation of the edge pixel intensities of an object.
- UpperQuartileIntensity: The intensity value of the pixel for which 75% of the pixels in the object have lower values.

MeasureObjectSizeShape

The MeasureObjectSizeShape module measures the area and shape of identified objects.

Module settings:
1. Select objects to measure.
2. Select measurements to export.

Tips:
- This module is only reliable for objects that are completely inside an image. If there are objects that touch the image borders, process images using the **IdentifyPrimaryObjects** module advanced settings upstream or the **FilterObjects** module downstream.

Generated measurements:
- Area: The number of pixels in the region.
- Center: The X, Y coordinates of the point farthest away from any object edge (the centroid). This is not the same as the Location-X and -Y measurements produced by the Identify modules.
- Compactness: The mean squared distance of the object's pixels from the centroid divided by the area. A filled circle will have a compactness of 1, with irregular objects or objects with holes having a value greater than 1.

- Eccentricity: The eccentricity of the ellipse that has the same second-moments as the region. The eccentricity is the ratio of the distance between the foci of the ellipse and its major axis length. The value is between 0 and 1. (0 and 1 are degenerate cases; an ellipse with an eccentricity of 0 is a circle, while an ellipse with an eccentricity of 1 is a line.)
- EulerNumber: The number of objects in the region minus the number of holes in those objects, assuming 8-connectivity.
- Extent: The proportion of the in the bounding box that are also in the region. Computed as the area/volume of the object divided by the area/volume of the bounding box.
- FormFactor: Calculated as 4*π*Area/Perimeter2. Equals 1 for a perfectly circular object.
- MajorAxisLength: The length (in pixels) of the major axis of the ellipse that has the same normalized second central moments as the region.
- MinFeretDiameter, MaxFeretDiameter: The Feret diameter is the distance between two parallel lines tangent on either side of the object (imagine taking a caliper and measuring the object at various angles). The minimum and maximum Feret diameters are the smallest and largest possible diameters, rotating the calipers along all possible angles.
- MaximumRadius: The maximum distance of any pixel in the object to the closest pixel outside of the object. For skinny objects, this is 1/2 of the maximum width of the object.
- MeanRadius: The mean distance of any pixel in the object to the closest pixel outside of the object.
- MedianRadius: The median distance of any pixel in the object to the closest pixel outside of the object.
- MinorAxisLength: The length (in pixels) of the minor axis of the ellipse that has the same normalized second central moments as the region.
- Orientation: The angle (in degrees ranging from -90° to 90°) between the x-axis and the major axis of the ellipse that has the same second-moments as the region.
- Perimeter: The total number of pixels around the boundary of each region in the image.
- Solidity: The proportion of the pixels in the convex hull that are also in the object.

4. Maintenance

4.1 General care

Clean surfaces with a soft cloth dampened with distilled water or 70% ethanol. Immediately wipe dry with a clean cloth.

Do not pour or spray liquids directly onto the instrument.

To avoid electrical shock or damage, do not wet electrical wires or connections.

If liquid is spilled on the instrument, turn off the power and wipe dry immediately.

Use only optical-grade cleaning materials to clean optical components.

Do not exchange components between instruments unless they have been provided or authorized by Logos Biosystems.

4.2 Change filter cubes

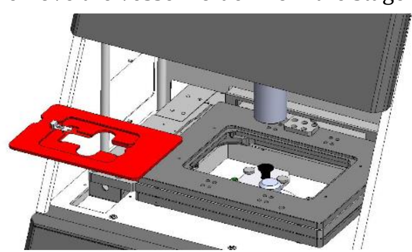
Procedure

Go to **Settings > Filter Cubes**.

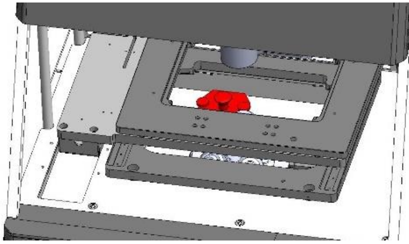
Click **Change filter cubes**.

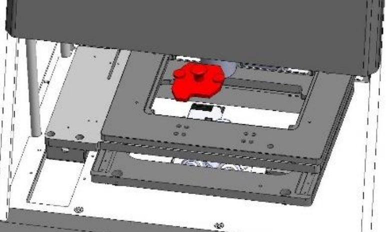
Click **Start**.

Remove the vessel holder from the stage.



Remove the filter cube stage cover.

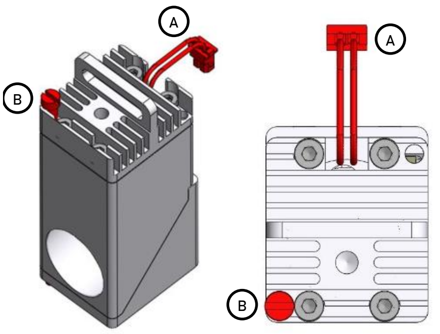




Click **Next**.

Click the filter cube you want to change. The filter cube stage will move to that position.

Unplug the connector (A) of the filter cube. Loosen the screw (B) in the cube with a flat-head screwdriver.



Gently pull out the filter cube.

Insert the desired LED filter cube, fasten the screw, and plug in its connector.

Repeat as necessary.

Click **Finish** when complete. This will return you to the original filter cubes settings window.

Select the installed filter cube from the registered filter cubes list. Select the post in which it was installed from the installed filter cubes list and click **>>**.

Double-click the label box to change how it shows up in the CHANNELS panel.

Use the Color drop-down menu to assign the filter cube a pseudocolor.

Use the **DEL**, ▲, and ▼ buttons to edit the list of installed filter cubes as needed.

Click **Apply**.

4.3 Change objectives

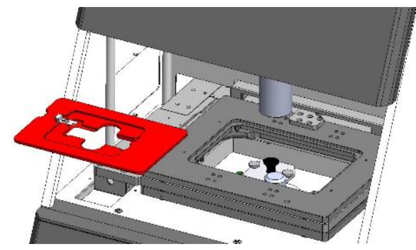
Procedure

Go to **Settings > Objectives**.

Select **Change objectives**.

Click **Start**.

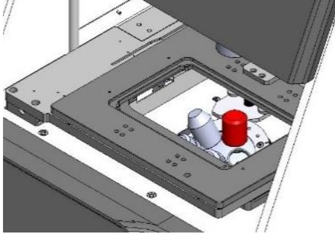
Remove the vessel holder from the stage.

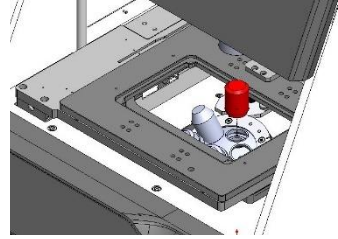


Click **Next**.

Click the objective you want to change. The turret will turn to that position.

Grasp the objective at its base and unscrew it from the turret.





Replace it with the desired objective and screw it in securely.

If applicable, set the correction collar (A) as needed.

Repeat as necessary.

Click **Finish** when complete. This will return you to the original objectives settings window.

Select the installed objective from the compatible objectives list. Select the post in which it was installed from the installed objectives list and click **>>**.

Double-click the label box to change how it shows up in the CHANNELS panel.

Use the **DEL**, ▲, and ▼ buttons to edit the list of installed objectives as needed.

Click **Apply**.

4.4 Adjust objective correction collars

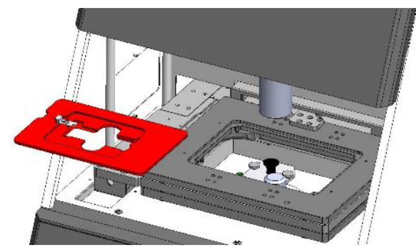
Procedure

Go to **Settings > Objectives**.

Click **Adjust correction collars**.

To adjust correction collars on applicable objectives, click **Start**.

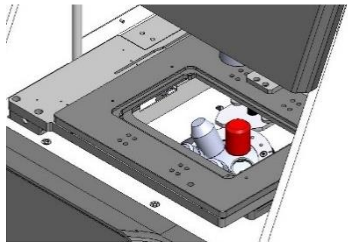
Remove the vessel holder from the stage.

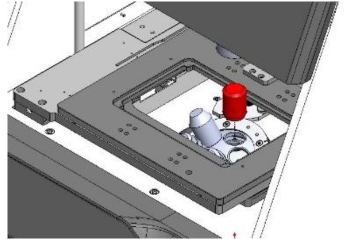


Click **Next**.

Click the desired objective. The turret will turn to that position.

Grasp the objective at its base and unscrew it from the turret.





Set the correction collar (A) as needed.

Reinstall the objective with care.

Repeat as necessary.

Click **Finish** to complete.

Appendix A: Troubleshooting

Image quality

Uneven focus	Make sure the vessel bottom is clean and free of fingerprints.
	Place the vessel in the appropriate vessel holder. Make sure it fits snugly and lies flat.
	Make sure you focus sharply on a sample before setting up the autofocus for Multiscan AF.
	Make sure you have selected the correct vessel.
	Make sure the objective correction collar (if available) is set to the correct vessel thickness.
Difficulty focusing on a coverslipped sample	Make sure the coverslip is facing up if using an objective corrected for 1.0 mm.
	Make sure the coverslip is facing down if using an objective corrected for 0.17 mm.
	If using an objective with a correction collar, make sure the objective correction is set to the desired vessel thickness and place the coverslipped sample accordingly.
Dim image	Set the iris diaphragm according to the objective and condenser used.
	Increase light intensity.
Spots or blurs on image	Clean the objective lens carefully and appropriately.
	Make sure the vessel bottom is clean and free of fingerprints.
Black viewing area	Turn on the light on in the CHANNELS panel.
	Center the sample over the objective.
Red viewing area, or red patches on image	Decrease light intensity until the red highlights disappear.
	Click to deactivate the Highlight Saturated Pixels button in the toolbar.

Explorer

Image irresponsive to changes in focus or stage position	Turn on the light in the CHANNELS panel.
Inactive buttons	Some of the buttons are contextual and only the controls relevant for the task at hand will be available.
Inactive save button	Click the Capture button in the toolbar first.
Inactive RUN button	Make sure channels have been added to the project protocol.

Mechanical

Stage does not move	Remove the shipping restraint.
Filter cube stage does not move	Remove the shipping restraint.
Vessel does not fit correctly	Use the appropriate vessel holder.

Appendix B: Specifications

CELENA® X High Content Imaging System

Supported labware	Slides, multi-well plates (6 to 1536 wells), petri dishes, culture flasks
Imaging modes	4-channel fluorescence, brightfield, phase contrast, color brightfield
Light source	High power LED filter cubes with adjustable intensity (>50,000 hours per filter cube)
Filter cube stage	Motorized; 4 interchangeable fluorescence filter cubes and 1 brightfield filter cube
Available filters	DAPI, EGFP, RFP, mCherry, ECFP, EYFP, DSRed, Cy5, Cy7, Cy3/TRITC Long Pass, GFP Long Pass, Cy5 Long Pass, custom filters
Objective turret	Motorized; 5 interchangeable objectives
Compatible objectives	1.25-100X; Olympus, Zeiss, and Logos Biosystems objectives
Condenser	Motorized; basic or phase contrast condenser Basic: 60 mm LWD condenser, 4-positions Phase contrast: 60 mm LWD condenser, 4-positions with 3 phase annuli
Camera	Single or dual camera module(s) Monochrome: CMOS, 1.92 MP *(optional)* Color: CMOS, 1.92 MP
Image outputs	Monochrome: 16-bit (12-bit dynamic range) TIF, PNG, or JPG Color: 24-bit color TIF, PNG, or JPG Movies: MP4
Autofocus method	Image-based autofocus *(optional)* Laser autofocus
Stage	Motorized X/Y-stage (120 mm x 80 mm); motorized Z-stage (10 mm)
Stage control	CELENA® X Explorer *(optional)* Joystick
Computer	External PC running Windows™ 10 Pro
Monitor	4K UHD monitor
Power	100-240 VAC, 250 W, 50/60 Hz
Dimensions	Main body: 39 x 46 x 50 cm (15.4 x 18.1 x 19.7 in) Controller: 17 x 30 x 23 cm (6.7 x 11.8 x 9.1 in)
Weight	Main body: 33 kg (72.8 lbs) Controller: 7 kg (15.4 lbs)

Appendix C: Safety Information

Instrument safety

General safety

Operate the instrument in the conditions described in the Operating Conditions.

Install the instrument on a level and sturdy surface. Avoid vibrations from other devices. The instrument can withstand light shock and vibration. However, excessive shock and/or vibration may damage the instrument. Leave sufficient space around the instrument for air circulation and cooling. Take care that the instrument does not overheat during long and continuous operation.

Do not touch the instrument or its components with wet hands.

Use components provided or authorized by Logos Biosystems. If the proper combination of components is not used, product safety cannot be guaranteed.

Use only the provided power cord and AC adapter. If the proper components are not used, electrical safety of the product cannot be guaranteed.

Ensure that the input voltage is compatible with the power supply voltage of the product.

Connect the grounding terminal of the instrument and electrical outlet properly. If the instrument is not grounded, electrical safety of the product cannot be guaranteed.

Turn on the instrument only after connecting the power cord and AC adapter to both the power source and the instrument. Turn off the instrument before disconnecting the power cord and/or moving the instrument.

Do not expose the instrument to intense ultraviolet light.

This instrument uses Class 3B ultraviolet LEDs that are in accordance with IEC/EN 60825-1. Always turn off the light before changing LED filter cubes or objectives.

Disconnect the power cord in the case of abnormalities.

Protect the computer from being infected with viruses and malware.

Operating conditions

Operating Power	100 - 240 VAC, 1.5 A
Electrical Input	12 VDC, 5.0 A
Frequency	50/60 Hz
Installation Site	Indoor use only
Operating Temperature	10 - 35°C
Maximum Relative Humidity	20 - 80%
Altitude	≤ 2,000 m
Pollution Degree	2

Instrument disassembly

Do not disassemble the instruments or wipe the supplied computer in any event as this will invalidate your warranty. If the instrument is damaged or malfunctioning, contact your local sales representative.

Personal safety

Safety guidelines

Read all user manuals thoroughly before using the instrument.

Keep all user manuals in a safe and accessible place for future reference.

Wear appropriate personal protective equipment (PPE) when handling reagents and samples to avoid exposure.

When using toxic agents, radioactive materials, or pathogenic microorganisms belonging to WHO Risk Groups 2-4, follow national laws and regulations for biosafety level requirements.

Instrument symbols

Electrical symbols

Symbol	Description
	Power symbol
	Protective earth (ground) terminal

Safety symbol

Symbol	Description
	WARNING! UV radiation hazard. Avoid looking directly at UV light.

Environmental symbol

Symbol	Description
	Waste Electrical and Electronic Equipment (WEEE). Do not dispose of this product as unsorted municipal waste. Follow local waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE.

Safety standards

European standards

Symbol	Description
CE	The CE mark indicates that this instrument conforms to all applicable European Community provisions for which this marking is required. Users must be aware of and follow the conditions described in this manual for operating the instrument. The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by Logos Biosystems.

Korean standards

Symbol	Description
	The KC certification mark indicates that this instrument conforms with Korea's product safety requirements for electrical and electronic equipment and components for which this marking is required.

United States standards

Type	Description
FCC Part 18	This device complies with Part 18 of the FCC Rules.

Appendix D: Ordering Information

Instruments

Cat #	Product		
CX30000	CELENA® X High Content Imaging System • CELENA® X Controller • HP Z240 Workstation • CELENA® X Cell Analyzer Verification Key • Universal Vessel Holder • Microplate Holder • Single Slide Holder		
	Options:		
	Camera	CX30200 CX30201	Monochrome Camera Module Dual Camera Module
	Condenser	CX30300 CX30301	Phase Condenser Brightfield Condenser
	AF module	CX30400 CX30401	Image-based AF Laser AF Module

Objectives

Olympus

High resolution fluorescence

Cat #	Objective	NA	WD (mm)	Correction (mm)
I10030	UPLFLN 4X	0.13	17	-
I10031	UPLFLN 10X2	0.3	10	-
I10034	LUCPLFLN 20X	0.45	6.6-7.8	0-2
I10035	LUCPLFLN 40X	0.6	2.7-4.0	0-2

Fluorescence and phase contrast

Cat #	Objective	NA	WD (mm)	Correction (mm)
I10038	UPLFLN 4XPH	0.13	17	-
I10039	UPLFLN 10X2PH	0.3	10	1
I10042	LUCPLFLN 20XPH	0.45	6.6-7.8	0-2
I10043	LUCPLFLN 40XPH	0.6	3.0-4.2	0-2

Low and high magnification

Cat #	Objective	NA	WD (mm)	Correction (mm)
I10046	PLAPON 1.25X	0.04	5	-
I10047	PLAPON 2X	0.08	6.2	-
I10050	UPLSAPO 60XO	1.35	0.15	0.17
I10051	UPLSAPO 100XO	1.4	0.13	0.17

Logos Biosystems

Plan fluorite

Cat #	Objective	NA	WD (mm)	Correction (mm)
I10005	TC PlanFluor 4X	0.13	17.5	1
I10006	TC PlanFluor 10X	0.3	7.5	1
I10007	TC PlanFluor 20X	0.4	7.5	1
I10008	TC PlanFluor 40X	0.6	2.9	1

Plan apochromatic

Cat #	Objective	NA	WD (mm)	Correction (mm)
I10013	Plan Apochromat Fluor 1.25X	0.04	3.7	-
I10014	Plan Apochromat Fluor 4X	0.13	17.2	-
I10009	Plan Apochromat Fluor 10X	0.3	8.6	0.17
I10010	Plan Apochromat Fluor 20X	0.65	0.7	0.17
I10011	Plan Apochromat Fluor 40X	0.8	0.2	0.17
I10015	Plan Apochromat Fluor Oil 40X	0.85	0.2	0.17
I10012	Plan Apochromat Fluor Oil 100X	1.25	0.19	0.17

LED filter cubes

Cat #	Filter cube	Excitation (nm)	Emission (nm)
I10130	DAPI	375/28	460/50
I10131	EGFP	470/30	530/50
I10132	RFP	530/40	605/55
I10133	mCherry	580/25	645/75
I10134	ECFP	436/20	480/40
I10135	EYFP	500/20	535/30
I10136	DSRed	530/40	620/60
I10137	Cy5	620/60	700/75
I10138	Cy7	710/75	810/90
I10139	Cy3/TRITC Long Pass	530/40	570lp
I10140	GFP Long Pass	470/40	500lp
I10141	Cy5 Long Pass	620/60	665lp
I10142	Custom	-	-

Accessories

Cat #	Product
CX31002	CELENA® X Cell Analyzer Verification Key
I10410	Joystick
I10411	Microscope Calibration Slide #1

Appendix E: Purchaser Notification

Limited use label license

Research use only

The purchaser of this product should use this product only for research for the sole benefit of the purchaser. By use of this product, the purchaser agrees to be bounded by the terms of this limited use statement whether the purchaser is a for-profit or a not-for-profit entity.

If the purchaser is not willing to accept the conditions of this limited use statement and this product is unused, the Company will accept return of the product with a full refund.

The purchaser cannot resell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party for Commercial Purposes.

Commercial Purposes mean any and all uses of this product and its components by a party for monetary or other consideration, including but not limited to, (a) product manufacture, (b) providing a service, information, or data, (c) therapeutic, diagnostic, or prophylactic purposes, or (d) resale of this product or its components whether or not such product and its components are resold for use in research.

Logos Biosystems, Inc. ("Company") will not claim any consideration against the purchaser of infringement of patents owned or controlled by the Company which cover the product based on the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine, or prophylactic product developed in research by the purchaser in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product.

For any use other than this limited use label license of research use only, please contact the Company or email info@logosbio.com for more information.

LTC licensed products

LED filter cubes

LED Filter Cubes are LTC Licensed Products provided under an intellectual property license from Life Technologies Corporation. The transfer of this product is conditioned on the buyer using the purchased product solely in research conducted by the buyer, and the buyer must not (1) use this product or its components for (a) diagnostic, therapeutic or prophylactic purposes; or (b) manufacturing, and/or (2) sell or transfer this product or its components for resale, whether or not resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@lifetech.com.

BSD licensed products

CellProfiler

Copyright © 2003 - 2018 Broad Institute, Inc. All rights reserved.

Redistribution and use in source and binary forms, with or without modification, are permitted provided that the following conditions are met:

1. Redistributions of source code must retain the above copyright notice, this list of conditions and the following disclaimer.
2. Redistributions in binary form must reproduce the above copyright notice, this list of conditions and the following disclaimer in the documentation and/or other materials provided with the distribution.
3. Neither the name of the Broad Institute, Inc. nor the names of its contributors may be used to endorse or promote products derived from this software without specific prior written permission

THIS SOFTWARE IS PROVIDED "AS IS." BROAD MAKES NO EXPRESS OR IMPLIED REPRESENTATIONS OR WARRANTIES OF ANY KIND REGARDING THE SOFTWARE AND COPYRIGHT, INCLUDING, BUT NOT LIMITED TO, WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, CONFORMITY WITH ANY DOCUMENTATION, NON-INFRINGEMENT, OR THE ABSENCE OF LATENT OR OTHER DEFECTS, WHETHER OR NOT DISCOVERABLE. IN NO EVENT SHALL BROAD, THE COPYRIGHT HOLDERS, OR CONTRIBUTORS BE LIABLE FOR ANY DIRECT, INDIRECT, INCIDENTAL, SPECIAL, EXEMPLARY, OR CONSEQUENTIAL DAMAGES (INCLUDING, BUT NOT LIMITED TO PROCUREMENT OF SUBSTITUTE GOODS OR SERVICES; LOSS OF USE, DATA, OR PROFITS; OR BUSINESS INTERRUPTION) HOWEVER CAUSED AND ON ANY THEORY OF LIABILITY, WHETHER IN CONTRACT, STRICT LIABILITY, OR TORT (INCLUDING NEGLIGENCE OR OTHERWISE) ARISING IN ANY WAY OUT OF THE USE OF THIS SOFTWARE, EVEN IF ADVISED OF, HAVE REASON TO KNOW, OR IN FACT SHALL KNOW OF THE POSSIBILITY OF SUCH DAMAGE.

If, by operation of law or otherwise, any of the aforementioned warranty disclaimers are determined inapplicable, your sole remedy, regardless of the form of action, including, but not limited to, negligence and strict liability, shall be replacement of the software with an updated version if one exists.

Development of CellProfiler has been funded in whole or in part with federal funds from the National Institutes of Health, the National Science Foundation, and the Human Frontier Science Program.

Instrument warranty

Warranty

Logos Biosystems, Inc. ("Company") warrants to the original purchaser ("Purchaser") that the instrument ("Instrument"), if properly used and installed, will be free from defects in materials and workmanship and will conform to the product specifications for a period of one (1) year ("Warranty Period") from the date of purchase. If the Instrument under this limited warranty fails during the Warranty Period, the Company, at its sole responsibility, will: within and up to 30 calendar days of purchase, refund the purchase price of the Instrument to the Purchaser if the Instrument is in original conditions; or, after 30 calendar days of purchase, only replace or repair the Instrument for up to the Warranty Period without issuing a credit.

In no event shall the Company accept any returned instrument (including its components) that might have been used or contaminated in some labs, including but not limited to, HIV or other infectious disease or blood-handling labs. This limited warranty does not cover refund, replacement, and repair incurred by accident, abuse, misuse, neglect, unauthorized repair, or modification of the Instrument. This limited warranty will be invalid if the Instrument is disassembled or repaired by the Purchaser.

In case that the Company decides to repair the Instrument, not to replace, this limited warranty includes replacement parts and labor for the Instrument. This limited warranty does not include shipment of the Instrument to and from service location or travel cost of service engineer, the costs of which shall be borne by the Purchaser. Every effort has been made to ensure that all the information contained in this document is correct at its publication. However, the Company makes no warranty of any kind regarding the contents of any publications or documentation as unintended or unexpected errors including occasional typographies or other kinds are inevitable. In addition, the Company reserves the right to make any changes necessary without notice as part of ongoing product development. If you discover an error in any of our publications, please report it to your local supplier or the Company. The Company shall have no responsibility or liability for any special, incidental, indirect or consequential loss or damage resulting from the use or malfunction of the Instrument.

This limited warranty is sole and exclusive. The Company makes no other representations or warranties of any kind, either express or implied, including for merchantability or fitness for a particular purpose with regards to this Instrument. To obtain service during the Warranty Period, contact your local supplier or the Company's Technical Support team.

Out of warranty service

Please contact your local supplier or the Company's technical support team in order to obtain out-of-warranty service. If necessary, repair service will be charged for replacement parts and labor hours incurred to repair the Instrument. In addition, the Purchaser is responsible for the cost of shipping the Instrument to and from the service facility and, if necessary, the travel cost of a service engineer after 30 calendar days of purchase, only replace or repair the Instrument for up to the Warranty Period without issuing a credit.

Logos Biosystems

HEADQUARTERS
FL 2 & 3
28 Simindaero 327beon-gil, Dongan-gu
Anyang-si, Gyeonggi-do 14055
SOUTH KOREA

Tel: +82 31 478 4185
Fax: +82 31 360 4277
Email: info@logosbio.com

USA
7700 Little River Turnpike STE 207
Annandale, VA 22003
USA

Tel: +1 703 622 4660
Tel: +1 703 942 8867
Fax: +1 571 266 3925
Email: info-usa@logosbio.com

EUROPE
11B avenue de l'Harmonie
59650 Villeneuve d'Ascq
FRANCE

Tel: +33 (0)3 74 09 44 35
Fax: +33 (0)3 59 35 01 98
Email: info-france@logosbio.com

www.logosbio.com