



WORLD
PRECISION
INSTRUMENTS

INSTRUCTION MANUAL

AutoLCI

Live Cell Imaging System

Serial No. _____

www.wpiinc.com

062122

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INTRODUCTION

Safety Instruction

* Read all instructions before use.

General Guidelines

- Install the device on a solid, flat and level platform.
- Operate the device in conditions described in the operating condition.
- Use only the components provided and authorized by WPI. Use authorized accessories only.
- Ensure the input voltage matches with the device's power supply voltage.
- Check if the power cable is properly grounded to avoid potential electric shock.
- Disconnect the power cable when abnormalities occur.
- Wait about 2-3 minutes for the device to boot.
- Do not insert any metallic objects into the device through bottom or side air vent to avoid electrical shock which could cause personal injury or damage the unit.
- Place the device around 10 cm away from the surroundings for proper air-cooling.
- NEVER disassemble the device. Contact your local distributor to arrange for service in the event of a malfunction. Malfunctions resulting from the disassembly of the unit, external shock or internal contamination void the warranty.
- Operate the device carefully as described in this manual.
- Do not bend the cable excessively.

Parts List

After unpacking, verify that there is no visible damage. Verify that all items are included:

- (1) AutoLCI™ Main Device
- (1) Main power cable
- (1) PoE
- (2) LAN Cable (5M, 4.8M)
- (1) USB/LAN Adapter
- (1) USB Memory Stick with Instruction Manual, Scan App, Analysis App
- (1) Vessel Holder for well plate (Included)

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 15 of this manual. Please contact WPI Customer Service if any parts are missing at (941) 371-1003 or customerservice@wpiinc.com.

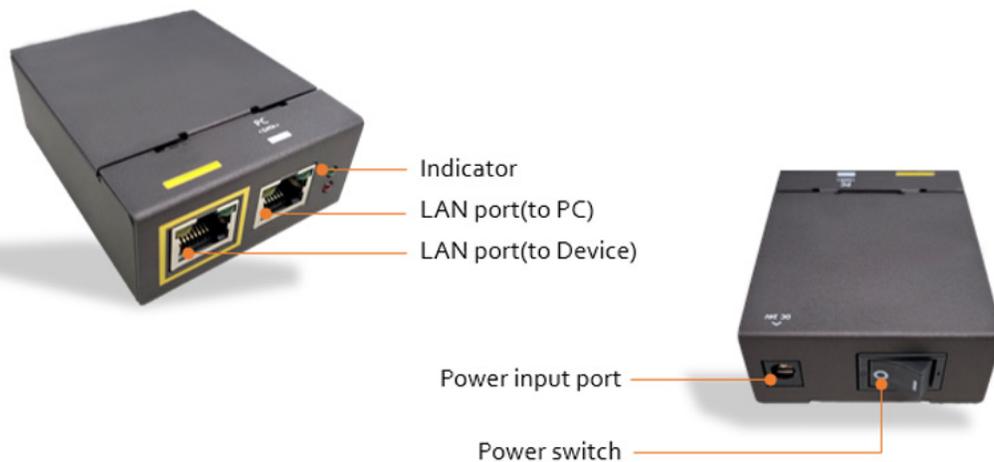
Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 15 of this manual.

INSTRUMENT DESCRIPTION

Front Left Side



PoE



GETTING STARTED

Pre-Requirements

Installation Location

For normal and stable operation of the device, the following environmental conditions should be satisfied.

- Install AutoLCI on a flat and level surface away from vibrating equipment.
- Place in an area free of dust or other airborne particles.
- During operation, place around 10 cm away from the surroundings for proper air flow of the cooling fan.
- During operation inside incubator, do not place close to the incubator door.
- Do not put any heavy materials on top of the AutoLCI .
- If using multiple devices, there should be a proper clearance between the equipment.
- Keep the device at temperature between 10 ~ 40°C with a relative humidity range of 20 ~ 95 %.

Important! Operation of the device at low temperature (Below 10°C) is not recommended. In those conditions, warm up the device for over 10 minutes.

PC Specifications (See p. 47)

- AutoLCI software can be used on PCs with Window 10.
- Large capacity hard drive is necessary to save images from Time Lapse. If the capacity is not enough, scanning will not function properly.
- For ease of use, we recommend using a monitor with resolution of 1920 x 1080.
- LAN port is required to connect the equipment to PC using LAN cable. If there is no LAN port, a LAN cable adapter should be prepared.

Installation

Unpacking

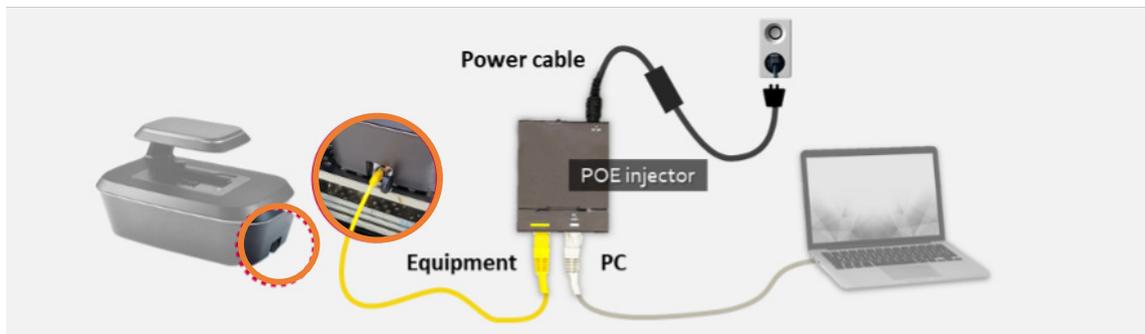
1. Lift the AutoLCI from the box, remove all the covers, and place it on a clean and level surface.
2. Make sure all accessories are present. If any items are missing or damaged, please WPI at wpi@wpiinc.com.

Connect the Device and Computer

1. Prepare a computer that satisfies the specifications (See p. 47 for PC specifications) and place it near the incubator.
2. Connect two LAN cables and the power cable to the POE injector. Connect one LAN cable to the device (yellow) and another LAN cable to the PC (white).



3. Connect power cable to an outlet.
4. Turn on the power by pressing the power switch located next to the power cable port and verify that the light indicator turns on.



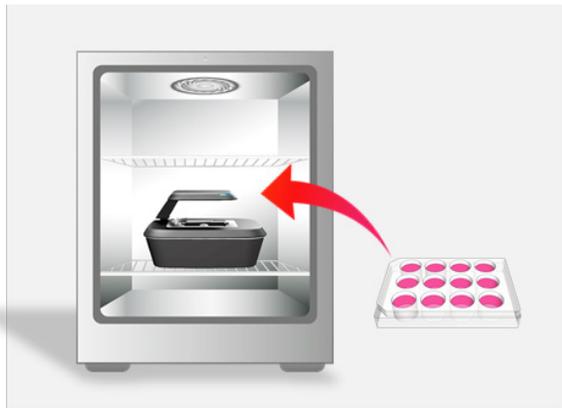
Install the Device Inside an Incubator

1. Install the vessel holder on the AutoLCI, then place the device inside the incubator.

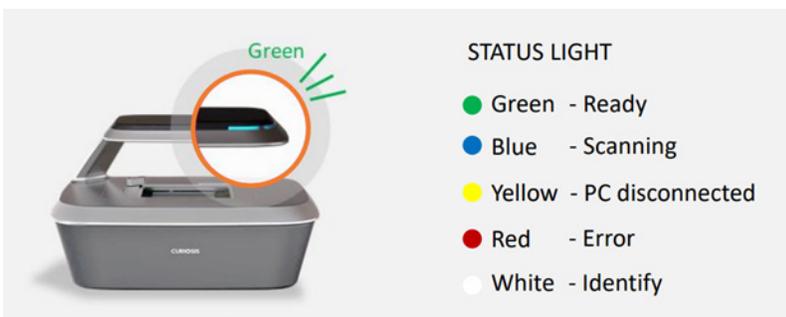
*Before using the device, make sure you remove all the covers, including the lens cover (Black).



2. Place the sample on the stage.



3. Check the connection between the PC and the device.



Set the PC Network

When the installation is completed, setup the PC network (changing PC IP address). Alternative method to set PC network within **AutoLCI Scan App** can be found in Appendix B (p. 52).

1. Turn on the computer and click “Start” icon or click the “Windows” button.
2. Click “Setting”.
3. Click “Network and Internet” in the Control Panel.

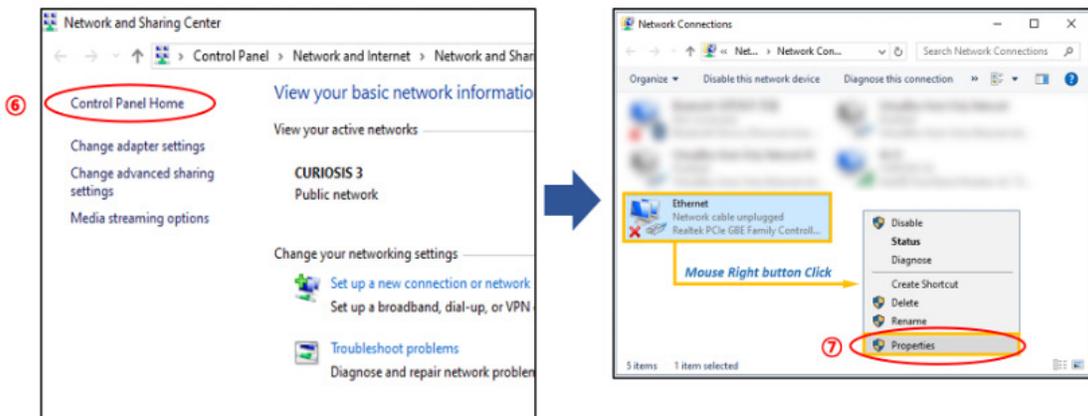
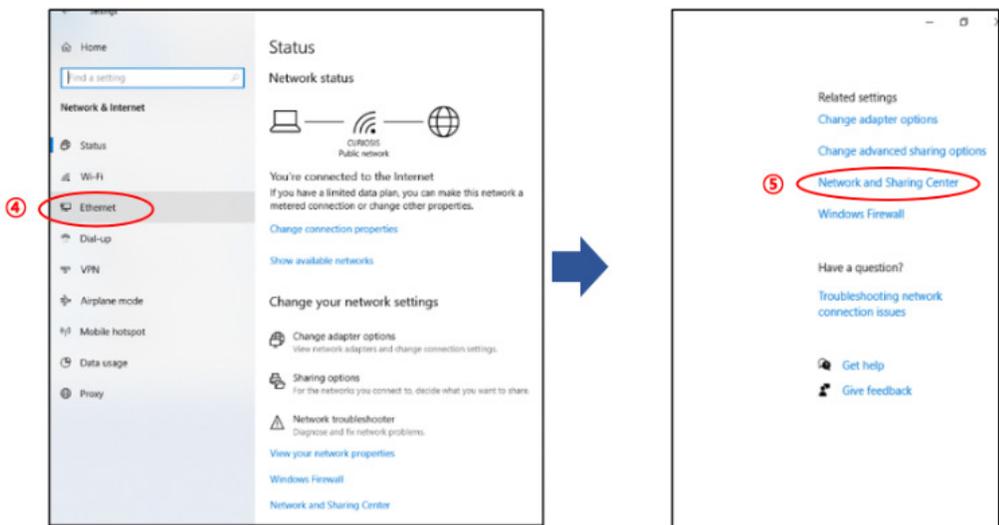
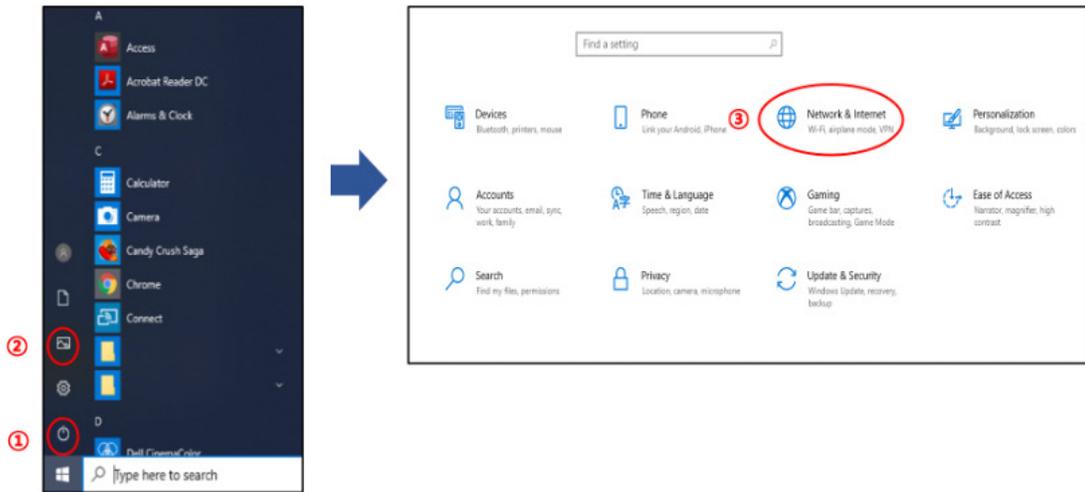
4. Click **"Ethernet"**.
5. Click **"Network and Sharing Center"**.
6. Click **"Change adapter settings"** on the left-side menu.
7. Select **"Ethernet."** (This name can vary according to PC.) Then, right-click the mouse and click **"Properties"** in the window that appears.
8. Select **"Internet Protocol Version 4 (TCP/IPv4)"** and click **"Properties"**.
9. Select **"Use the following IP address"** and enter **IP address (192.168.2.XX)** and **Subnet mask (255.255.255.0)** in the blank fields.

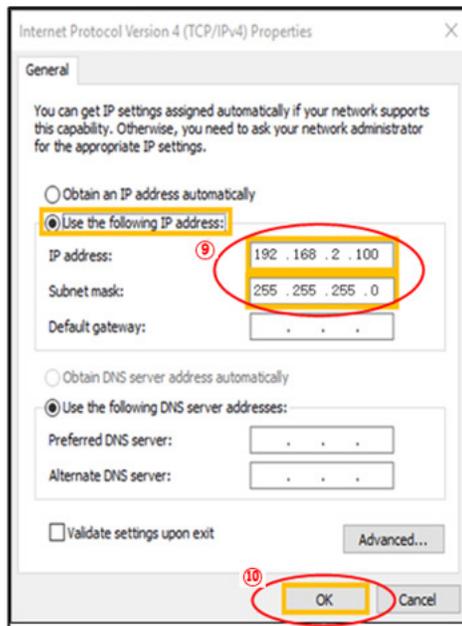
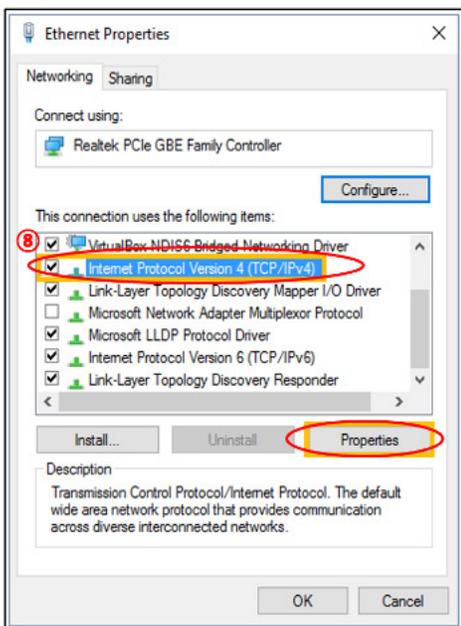
NOTE: Fill in any number from 2~254 except 10 in XX fields.

- The PC IP address should be set differently from the device IP address or the network adapter such as a router. The default device IP is 192.168.2.10, and to set up the PC IP address, enter 192.168.2.XX (in XX field, input 2~254 except 10).
- This IP setting guideline may not be applicable to some PC depending on the PC network environment. In case of connection failure, contact WPI at wpi@wpiinc.com.

10. Click "OK", then the network configuration is completed.

11. To start **AutoLCI Scan App**, first, connect the USB drive provided with device into the PC, then copy the APP folder to PC and execute "AutoLCI Scan App.exe" in the App folder.





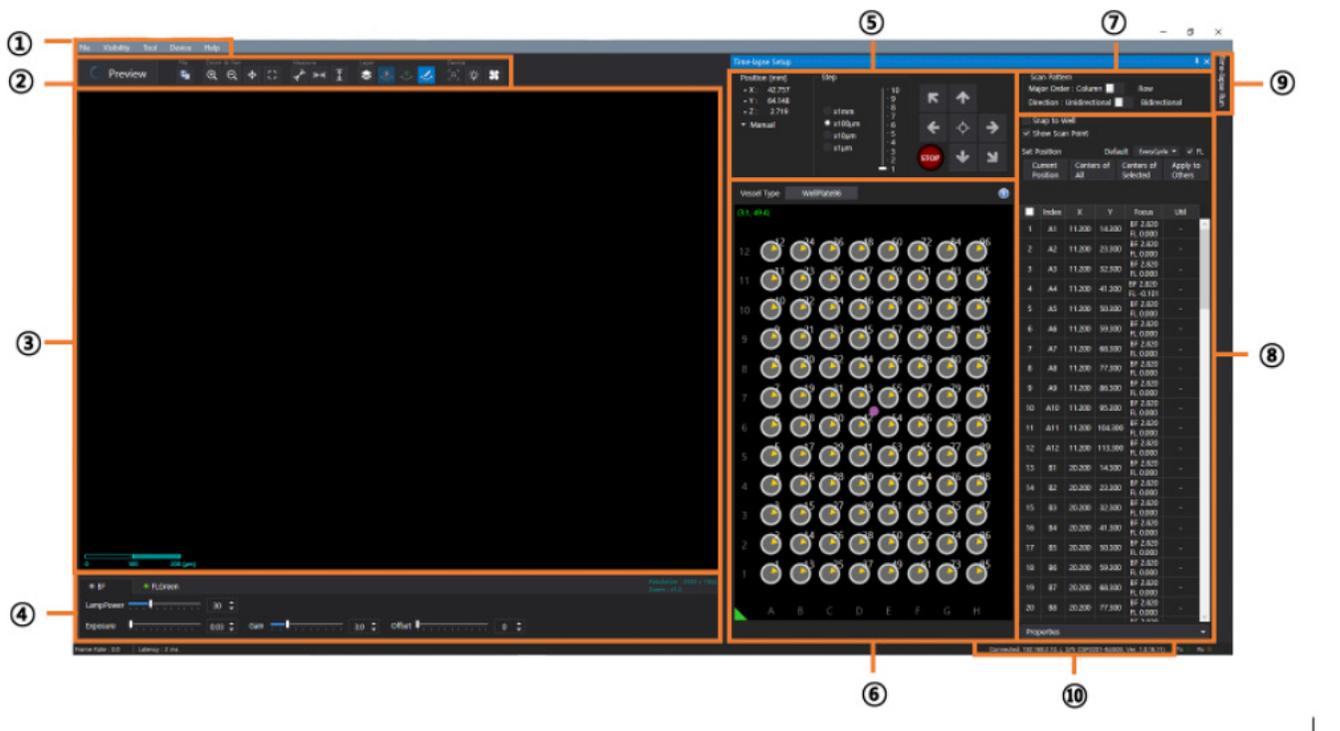
SOFTWARE DESCRIPTION

AutoLCI provides two software packages:

- **AutoLCI Scan App** is a program for the actual operation of AutoLCI device which includes real-time cell monitoring and time-lapse imaging function.
- **AutoLCI Analysis App** is a program for analyzing or post-processing the images obtained from AutoLCI. It can operate even without the device connected.

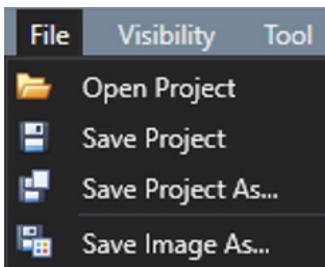
AutoLCI Scan App

When you open the **AutoLCI Scan App** and complete the pre-setup (See p. 26), the following main page displays.

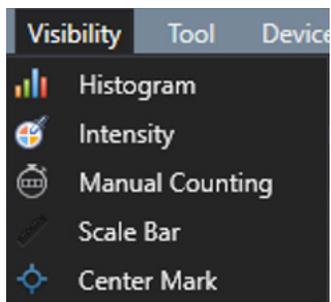


① Menu Bar

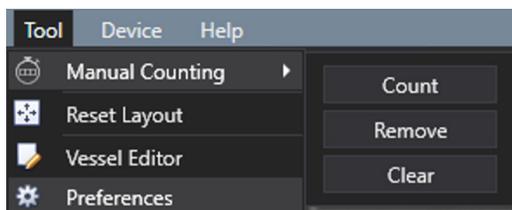
[File]



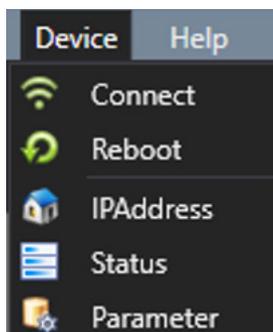
- ✓ **Open Project:** Import a previously saved project.
- ✓ **Save Project:** Save settings (Light intensity, Focus position, Schedule, etc.) on current project.
- ✓ **Save Project As...:** Create a new project with current settings
- ✓ **Save Image As...:** Save the image shown on the display.

[Visibility]

- ✓ **Histogram** (p. 35): Show/hide the intensity histogram of an image.
- ✓ **Intensity** (p. 34): Show/hide the point and mean intensity of an image.
- ✓ **Manual Counting**: Show/hide the “Manual Counting” function.
- ✓ **Scale Bar**: Show/hide the scale bar on the bottom left side of the display.
- ✓ **Center Mark**: Show/hide a cross-shaped mark at the center of the display for reference.

[Tool]

- ✓ **Manual Counting** (See p. 35): Count the points clicked by the user.
 - ① **Count**: Add a point for counting.
 - ② **Remove**: Delete a clicked point.
 - ③ **Clear**: Delete all points.
- ✓ **Reset Layout** (See note on p. 20): Return to “User Interface” before change.
- ✓ **Vessel Editor** (See p. 54): The specifications of the well plate are customizable.
- ✓ **Preferences**: Set Auto Focus (See p. 28), Merge Layer (See p. 33), and Appearance to change the color and font size of the feature.

[Device]

- ✓ **Connect**: Connect a new device or change the connection to another device.
- ✓ **Reboot**: Reboot the connected device.
- ✓ **IPAddress**: Change the IP address of the connected device.
- ✓ **Status**: Display the status of the connected device.
- ✓ **Parameter**: Display the values set for the connected device. Certain parameters can only be manipulated by an authorized person.

[Help]

- ✓ **About AutoLCI App**: Display App information.

② **Toolbox**

[Preview]



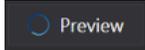
Preview

Turn the streaming on or off. The live image is shown based on the selected channel such as bright-field (Hereafter “BF”), green fluorescence (Hereafter “FL green”) and red fluorescence (Hereafter “FL red”) at the **Light Source Control**.



Preview

Streaming on



Preview

Streaming off

[File]



Save the image of the **Display**.

[Zoom & Pan]



Zoom in (Digital zoom)



Zoom out (Digital zoom)



Pan (Drag the display in Zoom-in status)



Return the zoomed image or relocated image to its original state immediately

[Measure]

Measure the length between two clicked points.



Measure the distance between two selected points in any direction.



Measure the distance between two selected points horizontally.



Measure the distance between two selected points vertically.

[Layer]



Merge layers. It shows the merged image of BF and FL channels.



BF image layer



FL (FL Green/FL Red) image layer



Features [Ruler (Measure), scale bar, manual count, etc.] layer

NOTE: Once activating **Merge Layers**, the displayed image is an image processed by setting **Opacity** and **Threshold** in the **Preferences** (See p. 33).

[Device control]



Execute autofocusing depending on the autofocusing settings in streaming status (See p. 28).



Turn on the white light in LED status indicator of the connected device.



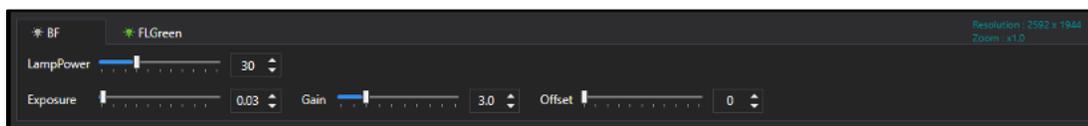
Turn fan on or off.

③ Display

Shows the real-time image upon activating **Preview** or the last captured image during the time-lapse.

④ Light Source Control

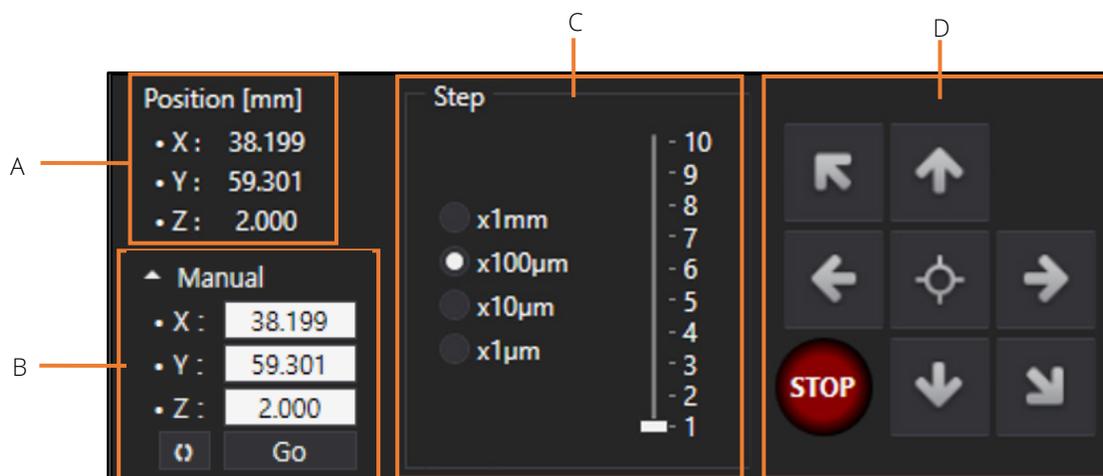
The tabs in **Light Source Control** are composed of BF, FL green and FL red. The configuration of tabs is set depending on the light options of AutoLCI .



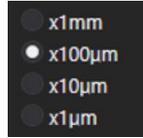
- **LampPower (%)**: Controls the lamp (illuminator) power that is adjustable from 0 to 100 %.
- **Exposure (second)**: The duration of exposure of cells to light, adjustable from 0.01 to 2 seconds. Be careful as photobleaching occurs when exposure value is high.
- **Gain**: The amount of signal amplification during the process of changing photon data to digital data, adjustable from 1 to 10. This is a parameter associated with the contrast of the image. Be careful as noise becomes higher when the gain value is high.
- **Offset**: (Black level) Brightness of dark range of image. Be careful as contrast becomes low when the offset value is high.

NOTE: For better results, we recommend adjusting the parameters as follows. To reduce the background noise, minimize the gain value while appropriately increasing the **LampPower** and **Exposure** as needed. We don't recommend increasing the **LampPower** to 70% or higher, because phototoxicity may occur. Do not leave the **Preview** on for a prolonged period as photobleaching may occur.

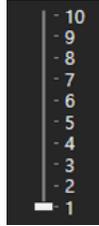
⑤ Jog Control



- Current X, Y, Z stage coordinate
- Enter the desired coordinates in the box and press **Go**. The stage moves according to the set coordinates. Press  to enter the current coordinates in the boxes.
- Define the distance to move when you press the **Jog button** (D). (Hereafter referred to as **Step distance**). Adjustments can also be made using "*", "/" on the number pad of the keyboard.



: Step distance unit



Step distance

D. Jog button



Moves to the center of the stage.



Moves the stage left and right (X axis). Adjustments can also be made using keyboard arrows.



Move the stage up and down (Y axis). Adjustments can also be made using keyboard arrows.



Move the stage up and down (Z axis). Adjustments can also be made using "+", "-" on the number pad of the keyboard.



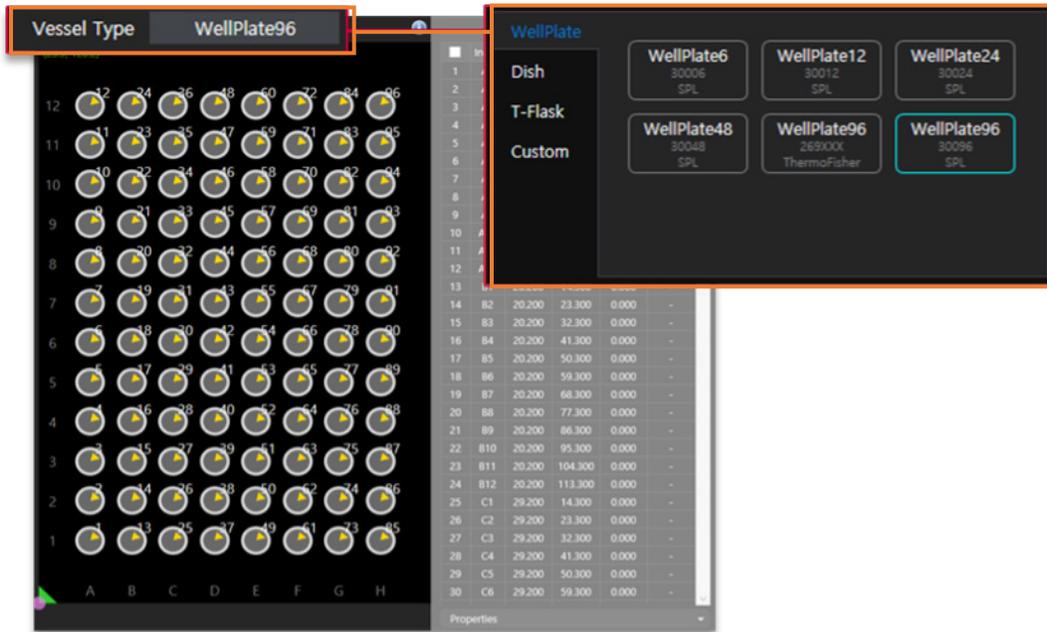
Stop the stage during a move.

⑥ Vessel & Plate Panel

[Vessel type]

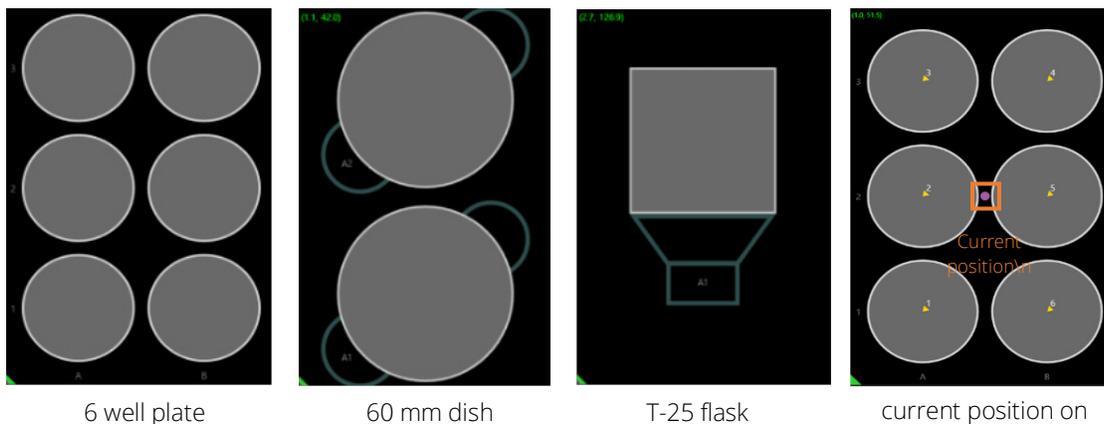
This is the tab where the vessel type is selected. Well plate (6, 12, 24, 48, 96 wells), Dish (35 mm, 60 mm, 90 mm), and T-flask (25 cm², 75cm²) may be selected.

NOTE: The specifications of the well plates are different for each brand. On the AutoLCI Scan App, the well plate specifications are set based on a certain brand's specifications. As such, if a well plate other than the set brands' well plates is used, we recommend changing the vessel specifications in the **Vessel Editor** under the **Tool** menu. (See p. 54 for **Vessel Editor**.)



[Plate Panel]

This menu item shows the vessel selected under Vessel type as an image, and the current position of the camera on the stage (See p. 17 for **Show Scan Point**). The image below is an example of vessels that appear on the **Plate Panel**, and the pink dot indicates the camera's current position.



To move to the center of the vessel, double click on the vessel image of the **Plate Panel**. (In the case of a well plate, it moves to the center of the selected well.) To move to a position other than the center, press "Ctrl" and double-click to move to the desired position.

⑦ Scan Pattern

- **Major Order:** When scanning multiple wells in a well plate, the scanning direction can be selected between the Column (↑↑↑) and Row (→→→).
- **Direction:** When scanning multiple wells in a well plate, the scanning direction can be selected between **Unidirectional** (scan in one direction) and **Bidirectional** (scan in zigzag).

⑧ Position Control

The screenshot shows the Position Control interface. Callout A points to the 'Snap to Well' and 'Show Scan Point' options. Callout B points to the 'Set Position' section, which includes 'Current Position', 'Centers of All', 'Centers of Selected', and 'Apply to Others' buttons. Callout C points to a table of well positions. Callout D points to the 'Properties' section, which includes settings for BF Focus, FL Focus Offset, and Z-Stacking.

Index	X	Y	Focus	Util	
1	A1	11.200	14.300	BF 2.453 FL 0.000	-
2	A2	11.200	23.300	BF 2.453 FL 0.000	-
3	A3	11.200	32.300	BF 2.453 FL 0.000	-
4	A4	11.200	41.300	BF 2.453 FL 0.000	-
5	A5	11.200	50.300	BF 2.453 FL 0.000	-
6	A6	11.200	59.300	BF 2.453 FL 0.000	-
7	A7	11.200	68.300	BF 2.453 FL -0.020	ZS (-2, 3)
8	A8	11.200	77.300	BF 2.453 FL 0.000	-
9	A9	11.200	86.300	BF 2.453 FL 0.000	-
10	A10	11.200	95.300	BF 2.453 FL 0.000	-
11	A11	11.200	104.300	BF 2.453 FL 0.000	-
12	A12	11.200	113.300	BF 2.453 FL 0.000	-

Properties

- BF Focus: BF Focus, IsEnabled, Ref. Position: 2.453, Auto Focus: EveryCycle
- FL Focus Offset: Green, FL Focus Offset, -0.020
- Z-Stacking: Z-Stacking, BF, FLGreen, Step: 10 μm , Range: [Slider]

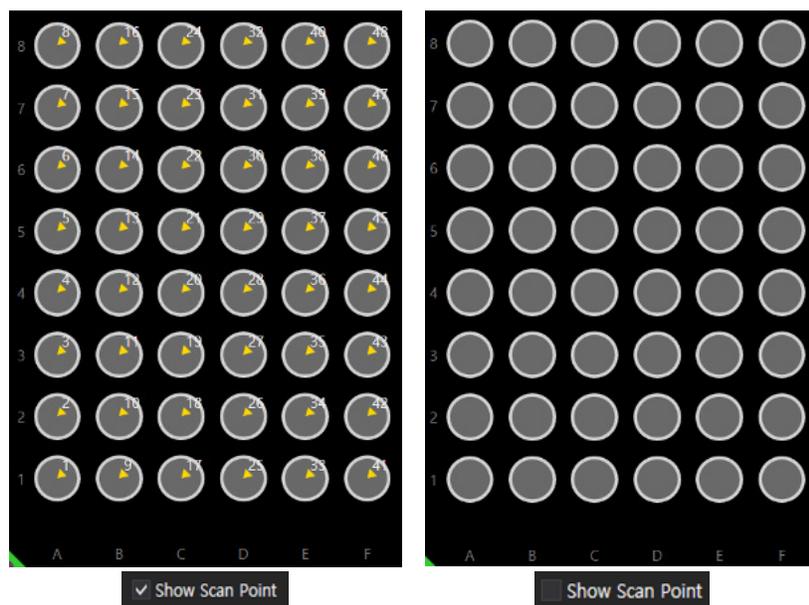
A. Snap to Well & Show Scan Point

[Snap to Well]

If selected, **Snap to Well** moves from well to well using the arrows on the keyboard. It can only be used when the vessel type selected is a well plate.

[Show Scan Point]

If **Show Scan Point** is selected, the position specified for scanning is indicated on the **Plate panel** as shown in the image below.

**B. Auto Focus Frequency & Set Position****[Auto Focus Frequency]**

Select the frequency of autofocusing to be executed during time-lapse imaging.



- ✓ **None:** During the time-lapse imaging, autofocusing function is not executed and the captures are based on the set coordinates.
- ✓ **FirstCycle:** During the time-lapse imaging, the autofocusing function is executed only for the first cycle, while the remaining cycles are imaged based on the z coordinates designated in the first cycle.
- ✓ **EveryCycle:** During the time-lapse imaging, the autofocusing function is executed every cycle.

[FL mode]

FL Select whether to run fluorescence imaging along with the time-lapse imaging.

[Set Position]

Set the position you want to scan.

- ✓ **Current Position:** Sets the current position as the scan position.
- ✓ **Centers of All:** Sets the center of all wells as the scan position. (This can only be used when the vessel type selected is a well plate).
- ✓ **Centers of Selected:** Sets the centers of the selected wells as the scan position. At this time, the wells are selected by dragging the mouse. (This may only be used when the vessel type selected is a well plate.)
- ✓ **Apply to Others:** Sets the same scan position specified at one well to other wells. (Select by dragging the mouse.) (This may only be used when the vessel type selected is a well plate).

NOTE: The selected **Auto Focus Frequency** and **FL mode** are set as default, and the set scan position is imaged based on the default settings. If you want to execute the autofocusing function at an **Auto Focus Frequency** other than the

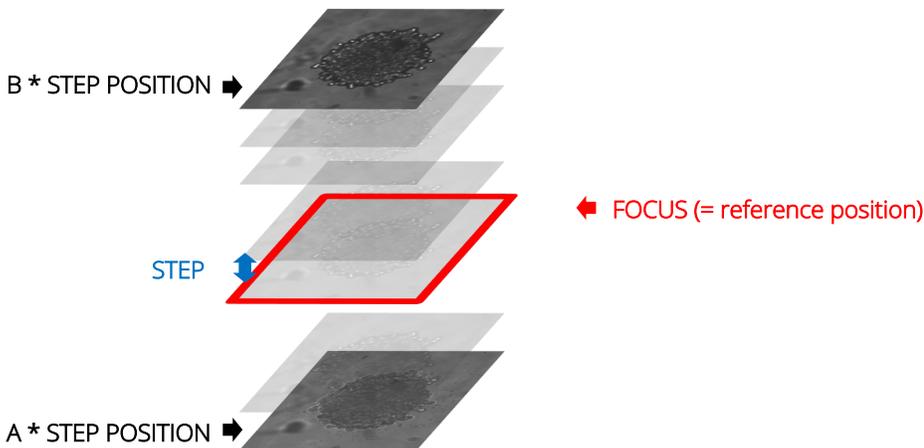
default value at some scan positions, the desired position can be selected and changed from the **Scan table (C)** (Refer to **D** on how to change the position). **FL mode** may also be changed in the same way.

C. Scan Table

The Scan Table shows the scanning order and the corresponding coordinates and Z-stacking values.

Index	X	Y	Focus	Util	
1	A1	11.200	14.300	BF 2.453 FL 0.000	-
2	A2	11.200	23.300	BF 2.453 FL 0.000	-
3	A3	11.200	32.300	BF 2.453 FL 0.000	-
4	A4	11.200	41.300	BF 2.453 FL 0.000	-
5	A5	11.200	50.300	BF 2.453 FL 0.000	-
6	A6	11.200	59.300	BF 2.453 FL 0.000	-
7	A7	11.200	68.300	BF 2.453 FL -0.020	ZS (-2, 3)
8	A8	11.200	77.300	BF 2.453 FL 0.000	-
9	A9	11.200	86.300	BF 2.453 FL 0.000	-
10	A10	11.200	95.300	BF 2.453 FL 0.000	-
11	A11	11.200	104.300	BF 2.453 FL 0.000	-
12	A12	11.200	113.300	BF 2.453 FL 0.000	-

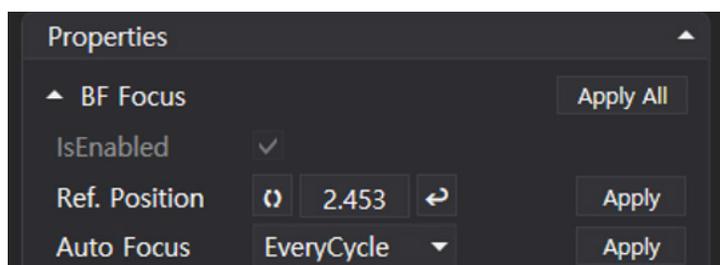
- ✓ **Index:** This is the well number of the well plate. (If it is not a well plate, all indices are indicated as A1).
- ✓ **X:** This is the X-axis coordinate.
- ✓ **Y:** This is the Y-axis coordinate.
- ✓ **Focus:** This is the Z-axis coordinate of BF channel when the **Auto Focus** function is off (= **None**) or it is the median coordinate when the **Auto Focus** function is on (= **FirstCycle** or **EveryCycle**).
- ✓ **Util:** This is the Z-stacking value, and images are taken at intervals set in **Step** (See **Z-Stacking** of p. 18) from top to bottom based on the Z-axis coordinates of the **Focus**. In **Util**, **ZS (a, b)** refers to taking images at intervals set in **Step** from **a * Step** bottom to **b * Step** top based on the reference position (= **Focus**). **a** and **b** are the values set in **Range** (See **Z-Stacking** of p. 20). (For a detailed description of Z-stacking, refer to the example in **Z-Stacking** on p. 2018)



D. Properties

The **Properties** shows the **Focus**, **Auto Focus Frequency**, and **FL mode** status of the selected position in the scan table (indicated in green). You can choose options other than the **Default** values and set the focus of fluorescence imaging (= if checked in the **FL mode**). The Z-stacking settings may be adjusted after selecting whether or not to perform the Z-stacking.

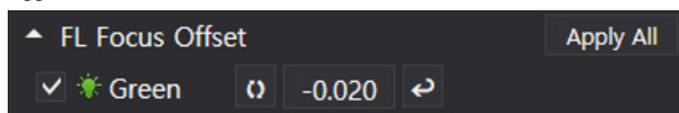
BF Focus



- ✓ **Ref. Position:** This is the position of BF channel for time-lapse. **2.453** is the location of Z-axis of BF channel when **Auto Focus** is **None** or the median coordinate when **FirstCycle** or **EveryCycle** is selected in **Auto Focus**.
- ✓  Click this button to enter the current coordinate into the box next to it.
- ✓  When you click this button, the Z position moves according to the coordinates shown on the left box. Click on the button in the state shown above to move to the 2.453 position.
- ✓ **Auto Focus:** This shows the **Auto Focus Frequency** selected in the **Position Control** (p. 17), and it can be changed.

NOTE: After changing the **Ref. Position** and the **Auto Focus**, click the **Apply** button for each to apply the changes. Click the **Apply All** button to apply both functions at once.

FL Focus Offset

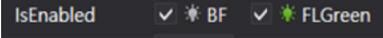


- ✓ **FL Focus Offset:** Position of FL channel for time-lapse. **-0.020** is the location of Z-axis of the FL channel captured while performing the time-lapse imaging. The value shown is the difference between the location of Z-axis captured in the BF channel. To capture the FL channel on the same coordinates of BF channel, enter 0.
- ✓  This shows the **FL mode** selected in the **Position Control** (p. 17), and if **FL mode** is selected (= if fluorescence imaging is performed at the same time), the fluorescence focus can be set. When unchecked, fluorescence imaging is not performed during the time-lapse.
- ✓  Click this button to enter the current coordinate into the box next to it.
- ✓  Click this button to move from the position of the BF channel to the position recalculated based on the value written in the left box. For example, referring to the image above (in the **Scan table** of p. 18), the Z-axis position of the BF channel is 2.453, and the Z-axis of the FL channel is 0.020 below 2.453. The fluorescence imaging is performed at 2.433 (= 2.453 – 0.020).

NOTE: Click on **Apply All** after adjusting **FL Focus Offset** to apply the changes.

Z-Stacking

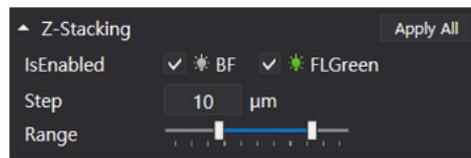


- ✓  Select whether or not to run the Z-stacking function in BF and FL by checking the boxes.
- ✓ **Step:** When executing the Z-stacking function, it sets the interval of the Z-axis to be imaged, and it can be set up to 1,000 μm.
- ✓ **Range:** This value sets the range for executing the Z-stacking function by moving the scroll bar, and it can be adjusted from -5 to 5. The Z-stacking function can be executed from $-5 * \text{Step } \mu\text{m}$ to $5 * \text{Step } \mu\text{m}$ based on the Focus.

<Example>

	Index	X	Y	Focus	Util
1	A1	11.200	14.300	2.453	-
2	A2	11.200	23.300	2.453	-
3	A3	11.200	32.300	2.453	ZS (-2, 3)
4	A4	11.200	41.300	2.453	-
5	A5	11.200	50.300	2.453	-
6	A6	11.200	59.300	2.453	-
7	A7	11.200	68.300	2.453	-
8	A8	11.200	77.300	2.453	-

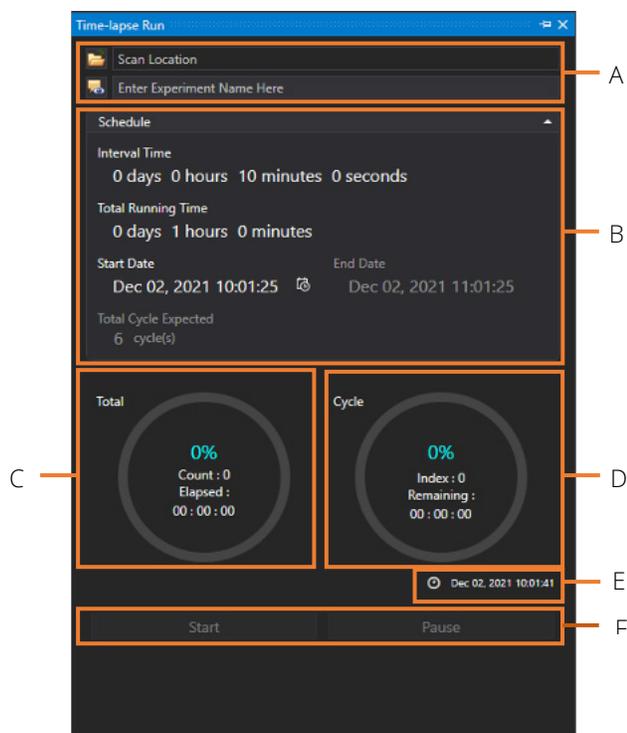
Scan table



Properties (Z-Stacking)

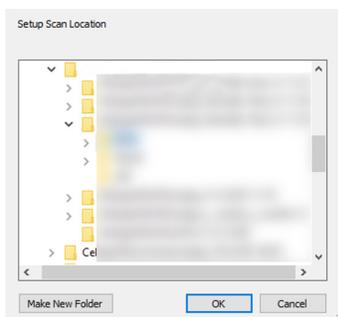
In the above figure, the **Focus** of the position selected in the **Scan table** is 2.453 and the **Util** is ZS (-2, 3), while the **Step** of **Z-stacking** set in **Properties** is 10 μm. This means that taking images from below 20 (= 2 x 10) μm to above 30 (= 3 x 10) μm every 10 μm based on the **Focus** of 2.453. As such, **BF** and **FL** images are taken with the Z-axis of 2.433, 2.443, 2.453, 2.463, 2.473, and 2.483 at x (11.200) and y (32.300) axis. At this time, -2 and 3 in **Util** are the values set in the **Range**.

⑨ Time Lapse Control



- A. The file name and location to save the file can be specified, and the saved file can be opened immediately.

Click on  to open the **Browse For Folder** window (refer to the image below). Select the location where you want to save the scan file and click **OK**. The selected folder location is shown on **Scan Location**, and if a location is not designated, the file is saved automatically in the **Scan folder** under the **AutoLCI Scan App** folder. The file name can be entered in the **Enter Experiment Name Here** field before saving. If a file name is not entered, the file will be automatically saved as the start time of the scan settings. Click on  to directly open the folder where the file is saved.



Browse For Folder

- B. Schedule

Set the schedule for time-lapse imaging. Time except for **End Date** and **Total Cycle** may be selected. Click the mouse, turn the mouse wheel or click the calendar to set the schedule.

- ✓ **Interval Time:** This is the Interval between scanning cycles.
- ✓ **Total Running Time:** This is the time-lapse execution period.
- ✓ **Start Date:** This is the time-lapse start time. (If the time is set to the past, it changes automatically to the time when the **Start** button is pressed.)

- ✓ **End Date:** This is the time-lapse complete time. (It is determined by the **Total Running Time** and the **Start Date**, and you cannot directly change the value.)
- ✓ **Total Cycle Expected:** This is the number of scans during the time-lapse period. (It is determined by the **Interval Time** and **Total Running Time**, and you cannot directly change the value.)

NOTE: The **Total Cycle Expected** is calculated by simply dividing the **Total Running Time** by the **Interval Time**, and it does not consider the data processing time. Therefore, **Total Cycle Expected** could be different from the actual scanning cycles.

- C. This is the time elapsed (**Elapsed**) and progress rate (%) during the total time-lapse running time. **Count** means the number of times scanning occurred. Click **Start** to begin scanning. This refers to count 1. (Count = Cycle count)
- D. This is the progress rate (%) within a cycle and the remaining time until the next cycle (**Remaining**). **Index** shows the number of images taken within a cycle.
- E. This shows the current date and time.
- F. Click **Start** to scan based on the set schedule.

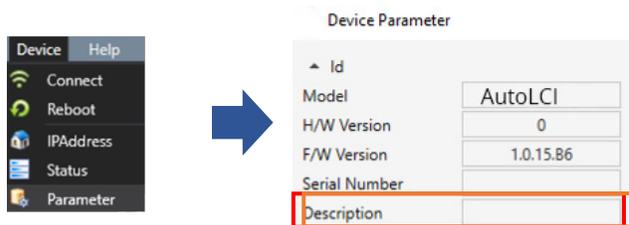
NOTE: The window can be separately floated so that it can fit on the monitor. It is possible to change the window by pressing  (Autohide) for floating or hiding. The layout is automatically saved when you terminate the program. To go back to the original state, press **Reset Layout** in the **Tool** menu before closing the **AutoLCI Scan App**. When the window disappears, delete "LayoutCGP.XML" in the **AutoLCI Scan App** folder to go back to default state after closing the App.

⑩ Connection Status

The Connection Status shows the device connection status and information on connected devices.

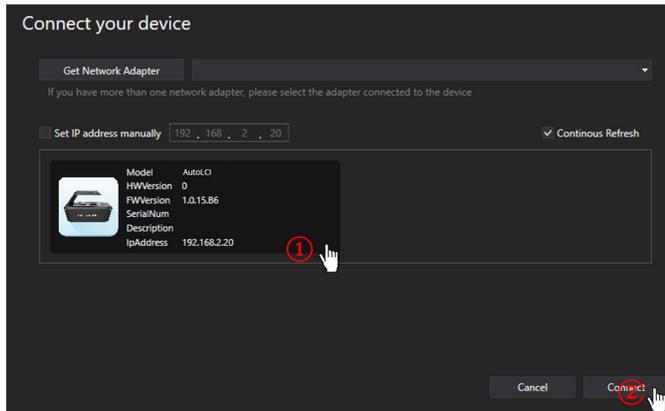
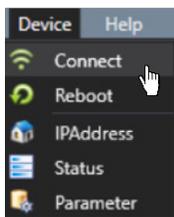


- A. This is the IP address of connected device.
- B. This is the description of connected device. (It may be changed in **Device -> Parameter**).



- C. This is the serial number of connected device.
- D. This is the firmware version of connected device.

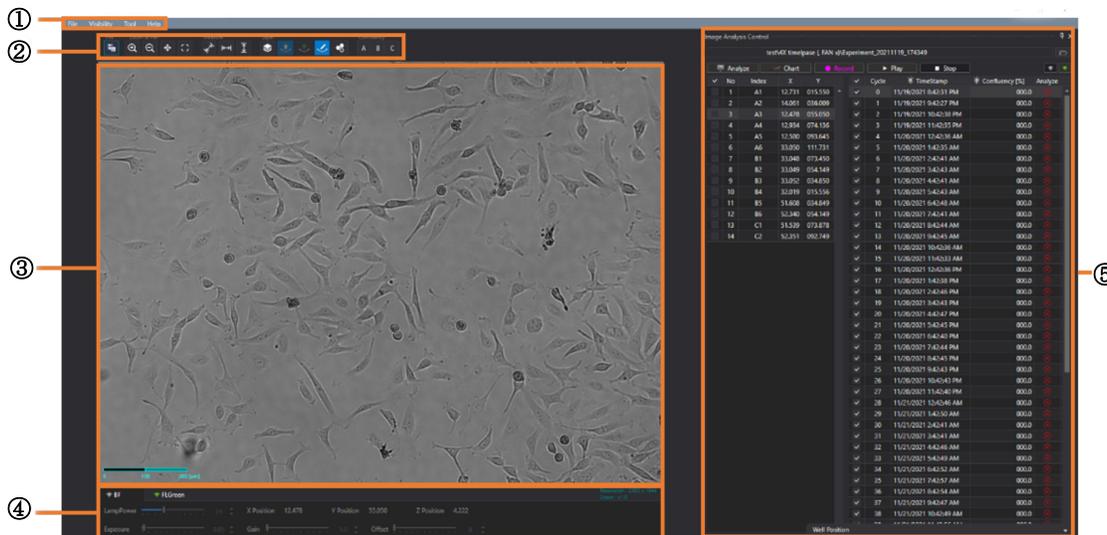
NOTE: If the device is not connected, **Disconnected: 0.0.0.0 Tx Rx** shows. Click **Device -> Connect** to reconnect. If the connection window does not show the device icon, see p. 27.



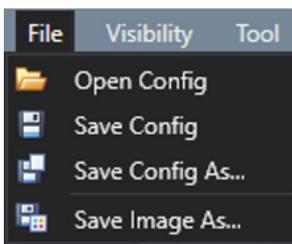
AutoLCI Analysis App

When you open the **AutoLCI Analysis App** and select the **Config file**, the following main page displays.

* **Config file**: It is a file that pops up when **AutoLCI Analysis App** starts. It is for saving and managing parameters set in the **Preferences** on the **Tool menu**.



① Menu Bar



The menu bar is managed by the “config file” instead of a project.

Other menus are the same as those of **AutoLCI Scan App**.

② Toolbox

[Layer]

 This button masks the area recognized as confluency. All other tools and functions are the same as those of **AutoLCI Scan App**.

③ Display

This item displays the image selected from **Image Analysis Control** (⑤) list. The image is displayed based on the activated channel that can be selected in **Light Source Control** (④). Upon completing analysis, a coverage mask is shown and functions like **measure (Ruler)**, **Intensity**, **Manual Counting** may be performed on the screen.

④ Light Source Control & Z Coordinates

This option displays the light source settings and Z coordinates of **AutoLCI Scan App**, and it cannot be changed.

⑤ Image Analysis Control

Upon importing a folder, this file list is displayed. When a file is selected, the image is shown on the **Display**. It is possible to analyze images or make a video. (See p. 39)

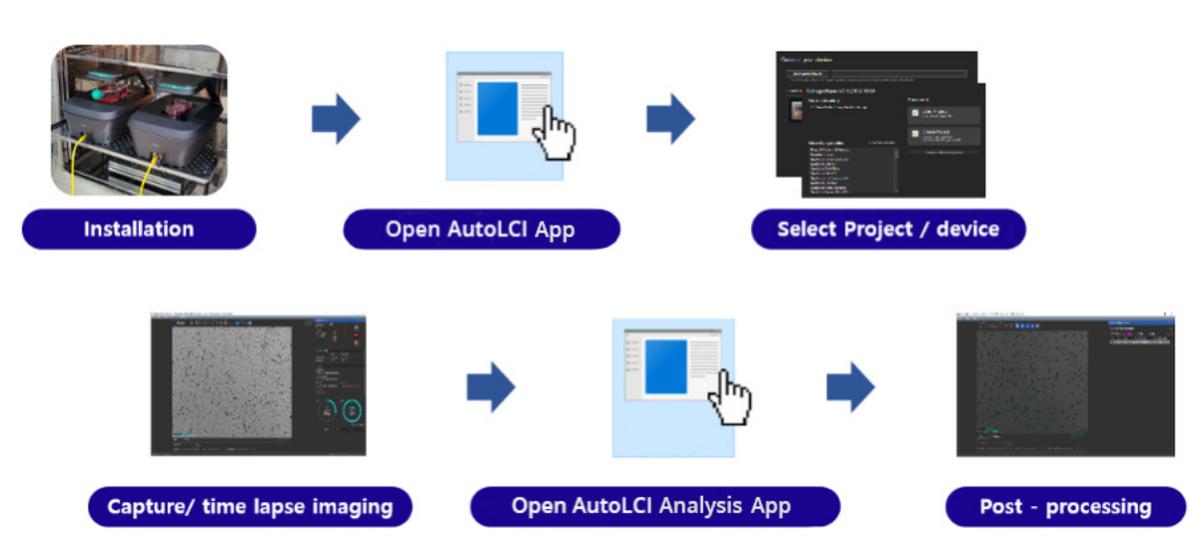
The screenshot shows the 'Image Analysis Control' window with a table of imaging data. The table has columns for No, Index, X, Y, Cycle, TimeStamp, and Confluency [%]. The data is as follows:

No	Index	X	Y	Cycle	TimeStamp	Confluency [%]
✓ 1	1	19.002	027.395	✓ 0	2022-01-15 오전 6:00:54	000.0
✓ 2	2	14.477	102.211	✓ 1	2022-01-15 오전 8:00:55	000.0
✓ 3	3	42.068	099.411	✓ 2	2022-01-15 오전 10:00:56	000.0
✓ 4	4	38.571	077.266	✓ 3	2022-01-15 오후 12:00:56	000.0
✓ 5	5	72.435	026.523	✓ 4	2022-01-15 오후 2:00:56	000.0
✓ 6	6	74.346	102.397	✓ 5	2022-01-15 오후 4:00:57	000.0
				✓ 6	2022-01-15 오후 6:00:58	000.0
				✓ 7	2022-01-15 오후 8:00:58	000.0
				✓ 8	2022-01-15 오후 10:00:58	000.0
				✓ 9	2022-01-16 오전 12:00:59	000.0
				✓ 10	2022-01-16 오전 2:00:58	000.0
				✓ 11	2022-01-16 오전 4:00:58	000.0
				✓ 12	2022-01-16 오전 6:01:03	000.0
				✓ 13	2022-01-16 오전 8:01:00	000.0
				✓ 14	2022-01-16 오전 10:01:00	000.0
				✓ 15	2022-01-16 오후 12:01:00	000.0
				✓ 16	2022-01-16 오후 2:01:00	000.0
				✓ 17	2022-01-16 오후 4:01:01	000.0
				✓ 18	2022-01-16 오후 6:01:04	000.0
				✓ 19	2022-01-16 오후 8:01:03	000.0
				✓ 20	2022-01-16 오후 10:01:03	000.0
				✓ 21	2022-01-17 오전 12:01:02	000.0
				✓ 22	2022-01-17 오전 2:01:03	000.0
				✓ 23	2022-01-17 오전 4:01:03	000.0
				✓ 24	2022-01-17 오전 6:01:04	000.0
				✓ 25	2022-01-17 오전 8:01:03	000.0
				✓ 26	2022-01-17 오전 10:01:04	000.0
				✓ 27	2022-01-17 오후 12:01:05	000.0
				✓ 28	2022-01-17 오후 2:01:06	000.0

At the bottom of the window, there are controls for 'Z-Stacking' and 'Well Position'.

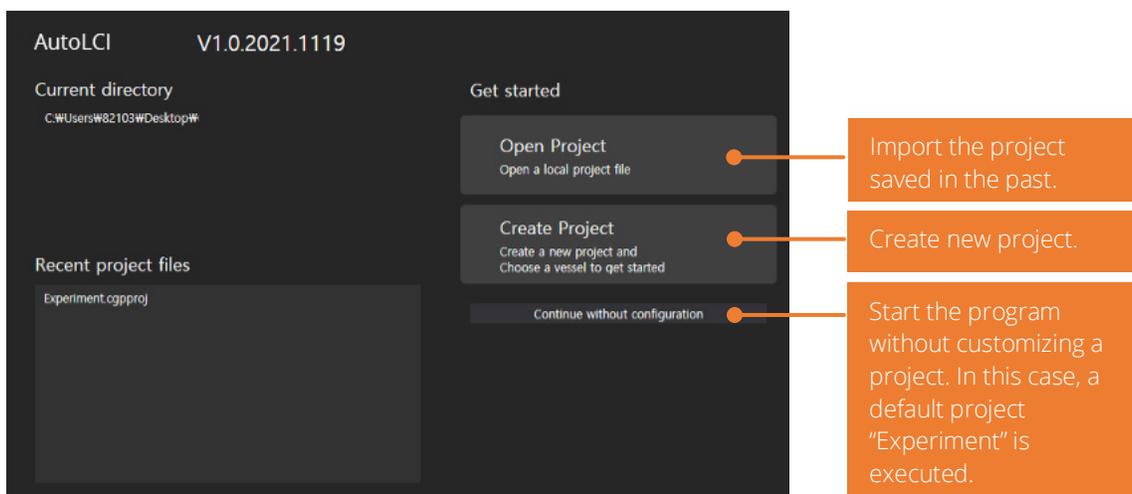
- ✓ **Analyze:** This calculates the confluency of the files checked on the list. Analyze the image of selected light channels on the right (See p. 41).
- ✓ **Chart:** This shows the confluency graph.
- ✓ **Record:** Press this to make a video (See p. 40).
- ✓ **Play:** This shows the images in the list in order (See p. 40 for slideshow).
- ✓ **Stop:** This button stops the play back.
- ✓ **Z-Stacking:** This option shows the list of Z-stacking (See p. 43)
- ✓ **Well Position:** This option shows the well position at the time of imaging.

OPERATIONS

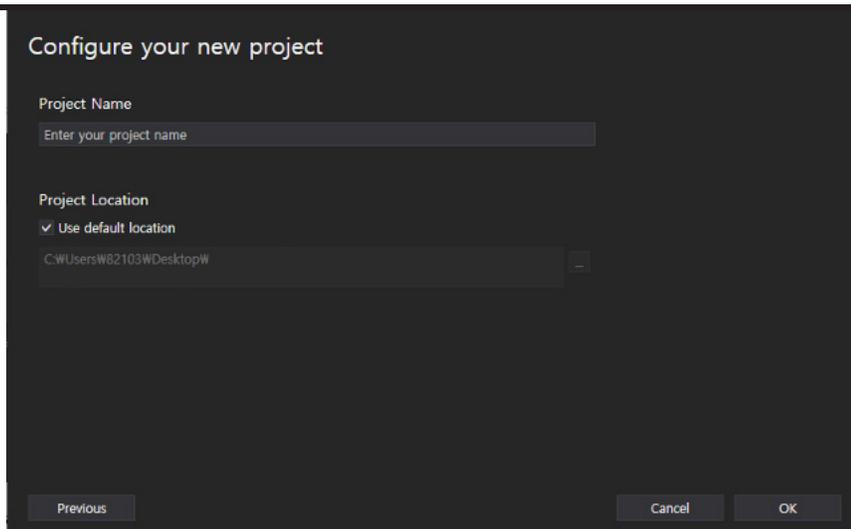


Create or Open a Project

- With AutoLCl , the setting can be managed by the project file (".cgnproj" file format). Setting values (Light intensity, Focus position, Schedule, file storage location, etc.) are saved in the project.
- When you start the App, the popup screen for project selection displays.

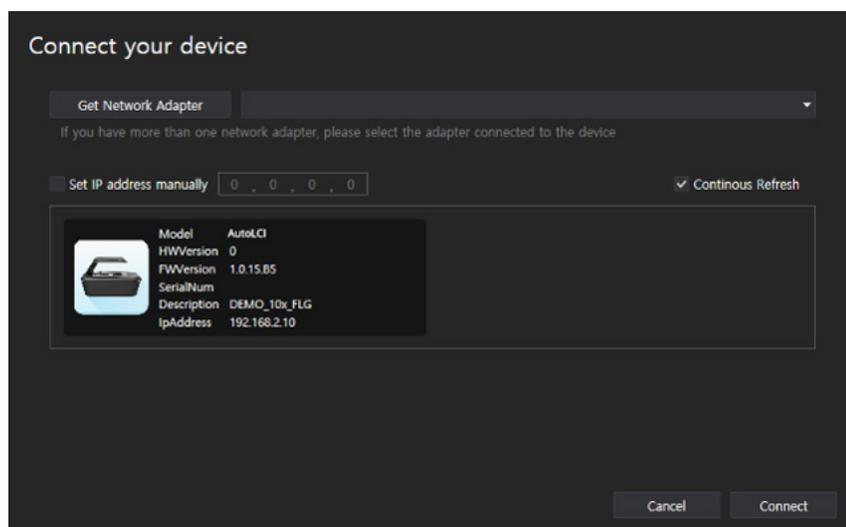


- Create a new project or open an existing one. You can start a program with default project by clicking **Continue without configuration**.
- Select **Create Project** and a popup window that requires the selection of project name and storage location appears. You can start the program after entering the required information and pressing **OK**. The storage location of the project is the address of default program file. If you want to change the storage location, cancel the **Use default location** and enter the correct address.



Device Connection

Icons for the devices connected by LAN cable are shown. To connect the device, select the icon and press **Connect**. When the connection is made, the color of LED status indicator changes from yellow to green.



NOTE: If the icon is not shown, check the following steps and take appropriate actions.

Case 1: The PC IP setting is not completed properly. Reset the PC IP. If the PC IP, device IP and router IP are the same, the connection will fail. (See **Set the PC network** in p. 6 for PC IP setting.)

Case 2: The LAN or power cable is not connected properly. (The LED status indicator is turned off.) Check all the cable connections and the POE injector power. (See **Connect the device and computer** in p. 5).

Case 3: A different LAN adaptor is used. Press the arrow on the right to select the adaptor to which the device is connected.



Case 4: When using multiple devices on one PC, each device has the same IP. Initially, devices are released with the same IP. When using the device for the first time, you **MUST** reset the IP address. (See p. 49 for device IP setting.)

GENERAL OPERATION

Preparation (Cell/Device Preparation)

The cell preparation method is different depending on the type of assay. The method below is a general process recommended for the monitoring of cells.

1. Before culturing cells, place the AutoLCI in the incubator for prewarming.

NOTE: Install the system as far as possible from the incubator door and place the device around 10 cm away from the surroundings for proper air flow of the rear cooling fan.

2. Prepare pre-warmed medium and cells on a plate.
3. Discard the medium on the vessel and wash the cells with PBS buffer.
4. Discard the PBS buffer and treat the trypsin-EDTA solution to detach the cell from the vessel.
5. Incubate for a few minutes and check the cell detachment using the AutoLCI or microscopy.
6. Move the cell and the Trypsin-EDTA to a conical tube and add fresh medium to neutralize the Trypsin reagent.
7. Centrifuge the suspension cells in the conical tube.
8. Discard the supernatant and add fresh medium to the cells.
9. Gently pipette the cell several times.
10. Transfer the appropriate amount of cells into a new vessel and dilute the cell concentration with fresh medium.
11. Cover the plate with a lid and gently shake the plate to spread the cells evenly.

NOTE: Before covering the plate, warm the lid by slightly passing the lid over the fire of an alcohol lamp to prevent condensation. This step is not necessary, but it will prevent trapping the condensation on a lid.

12. Place the plate on the AutoLCI .

NOTE: Both the plate and device should be pre-warmed for 30 minutes prior to time-lapse imaging to prevent condensation.

Cell Monitoring and Image Setting

Location Adjustment

Adjust X/Y position

1. Select the vessel type and desired scan location on the **Plate panel**.
2. Press the **Preview** button for streaming while **BF** is selected in the **Light Source Control**.



3. Move to the desired scan position using the **Jog button**. If the display is too dark or bright, preventing the cells from being seen, run the **Light intensity adjustment** in p. 31 first.)

Set BF Focus

Manual adjustment:

1. Select the **Step** as "X 1mm" and press the **Jog button** to move to the point where the cells are visible. Conduct precise adjustments gradually by reducing the **Step**.

NOTE: Selecting clear focal position is one of the major factors that influence the accuracy of analysis. Therefore, analysis can be precisely conducted when cells are clearly distinguishable from the background and the shape of cells are clearly visible.

NOTE: AutoLCI ' Z stage absolute coordinate error is 0.001 mm.

2. Upon finding the best focal point for scanning, designate the location by pressing **Current Position** in **Set Position**.

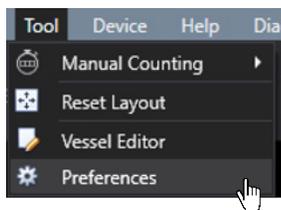
NOTE: If you want to change the focus, select the position you wish to change in the scan table, click  under **Properties** and click on **Apply** or **Apply All** to apply the change. (See p. 19 for **Properties**)

NOTE: When **Auto Focus Frequency** is set to a value other than **None**, autofocusing function is executed during scanning.

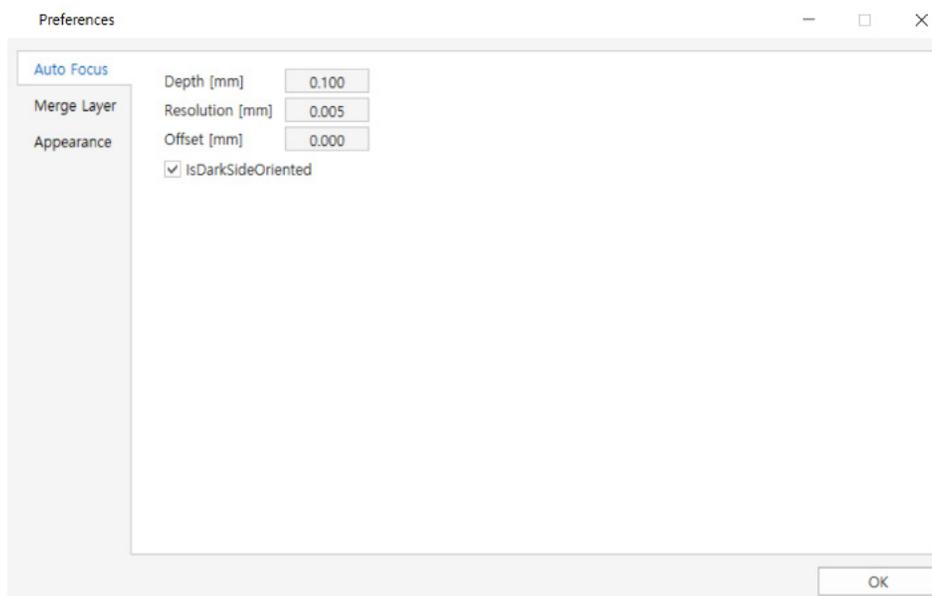
Auto Focus

Autofocusing function of **AutoLCI** works by selecting a clear focal point after screening the area up and down the location. Therefore, it is necessary to have the focus location within the designated range in **Auto Focus** parameter for a more successful autofocusing. Set the **Focus** roughly (manually) and use the autofocusing function.

1. Move to the focal plane using the **Jog button**.
2. Open the **Preferences** in the **Tool** menu.



3. Designate the parameter (**Depth, Resolution, Offset**) in the **Auto Focus** and press **OK**.



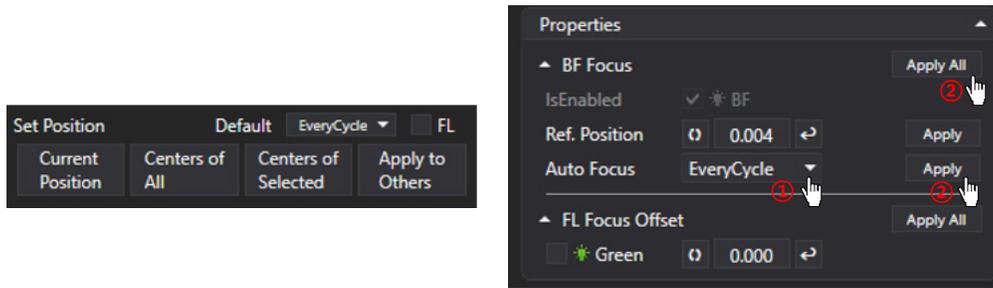
- **Depth:** Screening scope for autofocusing
- **Resolution:** The interval of autofocusing calculation within screening scope (0.010 mm or less for 4X optic)

and 0.005 mm or less for 10X optics)

- **Offset:** If you want to select the point different from focal plane recognized by AutoLCI, input the difference between the point and Z position coordinates from autofocusing.

NOTE: The recommended values are as follows. **Depth:** 0.100 mm or more, **Resolution:** 0.010 mm or less for 4X optic and 0.005 mm or less for 10X optic. The larger **Depth** and the smaller **Resolution**, the higher autofocusing accuracy. The longer the screening time, the longer the autofocusing time.

4. Set the value except for **None** in the **Auto Focus Frequency** or **Auto Focus** in **Properties** to set the frequency of autofocusing function. (See p. 17 for **Auto Focus Frequency**.) If you are selecting it from the **Properties**, click **Apply** or **Apply all** after making your selection.



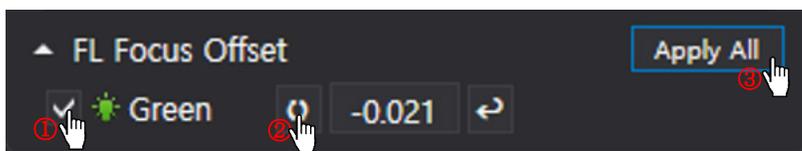
Set FL Focus Offset

Since the focus of the **FL** channel may be different from that of the **BF**, we recommend setting the focus position separately.

1. Select the desired scan position on the **Scan table**. It is highlighted in green once selected.

Index	X	Y	Focus	UH	
1	A1	11.200	14.300	0.004	-
2	A2	11.200	23.300	0.004	-
3	A3	11.200	32.300	0.004	-
4	A4	11.200	41.300	0.004	-
5	A5	11.200	50.300	0.004	-
6	A6	11.200	59.300	0.004	-
7	A7	11.200	68.300	0.004	-
8	A8	11.200	77.300	0.004	-
9	A9	11.200	86.300	0.004	-
10	A10	11.200	95.300	0.004	-
11	A11	11.200	104.300	0.004	-
12	A12	11.200	113.300	0.004	-
13	B1	20.200	14.300	0.004	-
14	B2	20.200	23.300	0.004	-
15	B3	20.200	32.300	0.004	-
16	B4	20.200	41.300	0.004	-
17	B5	20.200	50.300	0.004	-
18	B6	20.200	59.300	0.004	-
19	B7	20.200	68.300	0.004	-
20	B8	20.200	77.300	0.004	-

2. Select the **FL** channel in **Light Source Control** and adjust the focus using the **Jog** button by referring to the **Display**.
3. Check that the **FL Focus Offset** checkbox under **Properties** is checked, and click on  to specify the focus and click **Apply All**. (If the position is specified after checking in the **FL** mode, the **FL Focus Offset** checkbox is checked.)

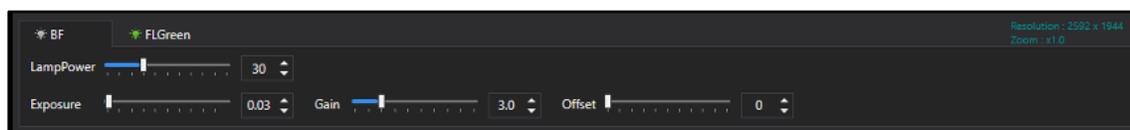


4. Check if the designated **FL Focus Offset** value is shown in **Scan table**.

Index	X	Y	Focus	Util	
1	A1	11.200	14.300	BF 2.890	-
2	A2	11.200	23.300	BF 2.890	-
3	A3	11.200	32.300	BF 2.890	-
4	A4	11.200	41.300	BF 2.890	-
5	A5	11.200	50.300	BF 2.890 FL -0.021	-
6	A6	11.200	59.300	BF 2.890	-
7	A7	11.200	68.300	BF 2.890	-
8	A8	11.200	77.300	BF 2.890	-
9	A9	11.200	86.300	BF 2.890	-
10	A10	11.200	95.300	BF 2.890	-
11	A11	11.200	104.300	BF 2.890	-
12	A12	11.200	113.300	BF 2.890	-
13	B1	20.200	14.300	BF 2.890	-
14	B2	20.200	23.300	BF 2.890	-
15	B3	20.200	32.300	BF 2.890	-
16	B4	20.200	41.300	BF 2.890	-
17	B5	20.200	50.300	BF 2.890	-
18	B6	20.200	59.300	BF 2.890	-

Light Intensity Adjustment

The light source option for **AutoLCI** is displayed on the lower tab. Select the desired light source to adjust and change the parameter. It is automatically saved. Brightness is one of the important factors that has an impact on analysis accuracy. It is important to set the appropriate brightness to distinguish cells from the background.



<Shows Light Source Control in the App connected to Green FL AutoLCI >

1. Select **BF**, press **Preview** and check **Display** in streaming on state.
2. Adjust the brightness by changing the **LampPower**, **Exposure**, **Gain** and **Offset**.

NOTE: High Gain value causes noise. Therefore, adjust **LampPower**, **Exposure** and **Offset** first. However, if brightness is not enough after the adjustment, change the **Gain** value. We recommend you run **LampPower** under 70 when performing time-lapse with FL.

NOTE: When analyzing the cell confluency, **Mean Intensity** of the image should be at least 120. (See p. 34 for **Mean Intensity**.)

Z Stacking

Manual Z Stacking

1. By using **Jog**, find the position to start stacking.
2. Designate the stacking unit in the **Step**.

Example: Step setting for stacking by 10 μm



3. In the **Jog** button, click **Current Position** for each position of the range you want to stack by using  .

Automated Z Stacking

1. Designate the position to scan. (p. 28)
2. Select the position where the Z-stacking function is to be executed in the scan table.
3. Select whether to execute the Z-stacking function in BF and FL in **Z-stacking** under **Properties**. (See p. 20 for Z-stacking)
4. After specifying the interval (= **Step**) to execute the Z-stacking function, adjust the scroll bar under **Range** to specify the range.
5. After clicking **Apply All**, check that the set Z-stacking value set shows in the **Util**.

<input type="checkbox"/>	Index	X	Y	Focus	Util	
<input type="checkbox"/>	1	A1	11.200	14.300	2.453	-
<input type="checkbox"/>	2	A2	11.200	23.300	2.453	-
<input checked="" type="checkbox"/>	3	A3	11.200	32.300	2.453	ZS (-2, 3)
<input type="checkbox"/>	4	A4	11.200	41.300	2.453	-
<input type="checkbox"/>	5	A5	11.200	50.300	2.453	-
<input type="checkbox"/>	6	A6	11.200	59.300	2.453	-
<input type="checkbox"/>	7	A7	11.200	68.300	2.453	-
<input type="checkbox"/>	8	A8	11.200	77.300	2.453	-

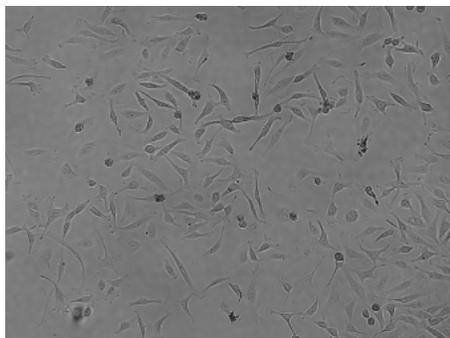
IMAGE ANALYSIS

Capture

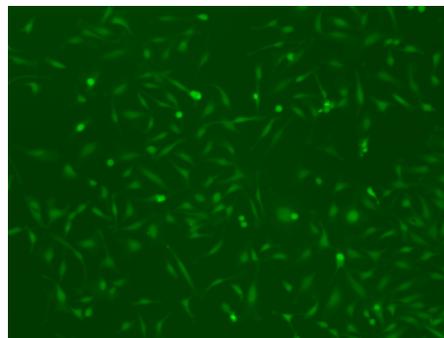


This icon in the **Toolbox** captures the image on the **display**.

- An image with features. It is possible to generate and manipulate the desired features, show it on the display and save it as it is. To obtain it without features, press (Features layer) to deactivate it and then press .
- BF or FL channel image. Press the desired tab of the channel in the Light Source Control to show the image on display through the preview and press .

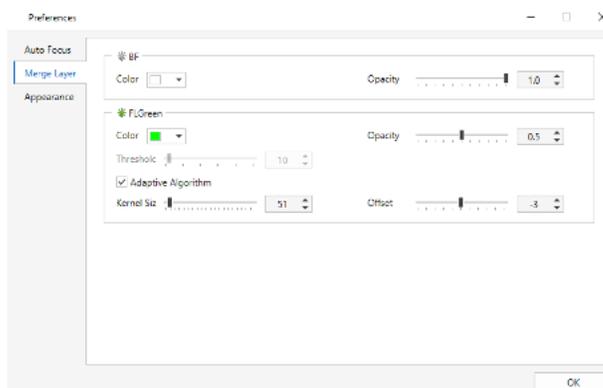
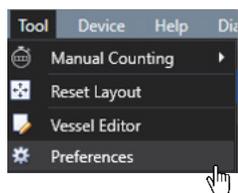


<BF channel image>



<FL channel image>

- Merge image. Press (Merge Layer) for activation . The merged image is shown on **Display**. The adjusted image as follows can be obtained by changing the **Merge Layer** setting (**Opacity** and **Threshold**) in the **Preferences** of Tool menu.



AutoLCI conducts thresholding for FL channel image based on the intensity and merges it with the **BR** image. There are two methods for intensity thresholding:

- **Method 1 Global thresholding:** Thresholding is executed based on the overall intensity of the image. Adjust a parameter of the **Threshold** while looking at the mask of an image after unchecking the **Adaptive Algorithm** box.
- **Method 2 Local adaptive thresholding:** Divide the image into local areas and thresholding is conducted by comparing relative intensity among areas. Adjust parameters of the **Kernel Size** and **Offset** while looking at the mask of the image after checking the **Adaptive Algorithm** box. (**Kernel Size** indicates size of local and **Offset** indicates the relative offset of the threshold of local area.)

NOTE: When the fluorescence areas are distorted or clustered in one place, the **Global thresholding** function is recommended. When the fluorescence areas are evenly distributed, **Local adaptive thresholding** function is recommended.

Image Tile

In the event of imaging using a well plate, each scan position is combined and displayed at once. An **ImageTile** folder is created automatically in the folder where the images are saved for each cycle.

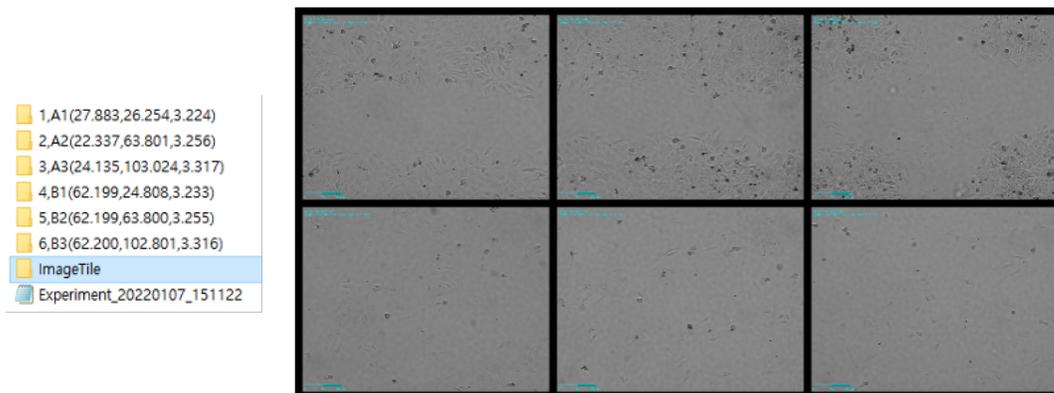
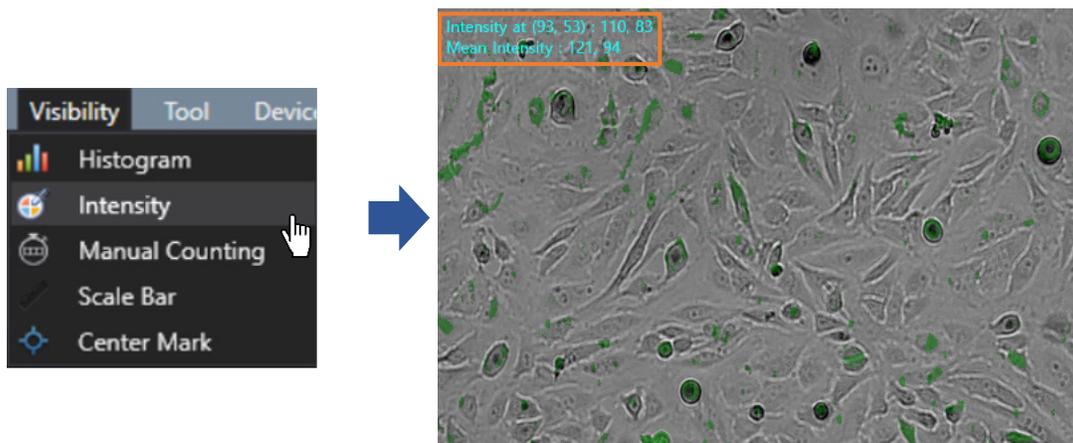


Image Tile

Intensity

Point Intensity & Mean Intensity

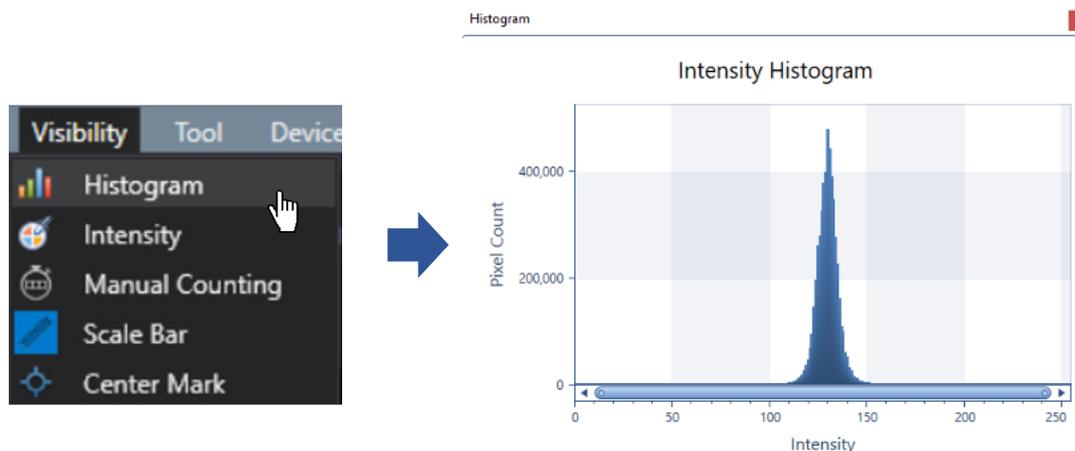
Upon activating **Intensity** in the **Visibility** menu, **Point Intensity** and **Mean Intensity** information is displayed at the top left side of the **Display**.



- **Intensity at (X coordinate, Y coordinate):** This is the point intensity value of the X, Y coordinates indicated by the cursor. (In the case of a merge, the intensity of the **BF** channel and the intensity of the **FL** channel are displayed in order.)
- **Mean Intensity:** This is the average intensity value of the image displayed on the **Display**. (If it is in the merge state, the intensity of the **BF** channel and the intensity of the **FL** channel are displayed in order.)

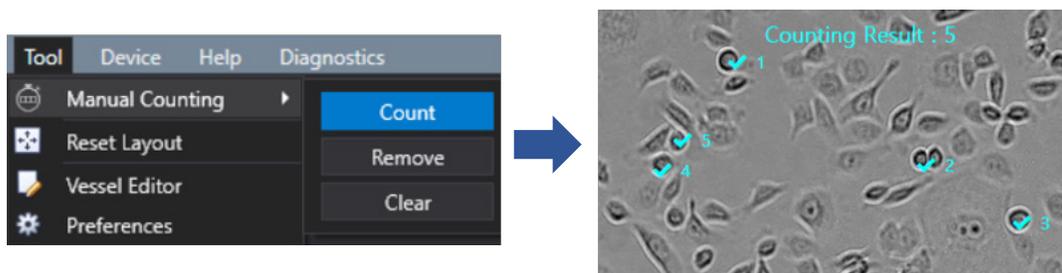
Histogram

Upon selecting **Histogram** in the **Visibility** menu, the **Intensity Histogram** is shown on the **Display**. As the **Histogram** does not provide information on **Merge Layers**, the **Intensity Histogram** of the channel selected in the **Light Source Control** is displayed in the case of the **Merge Layers**.



Manual Counting

Select **Count** in **Manual Counting** in the **Tool** menu and click the desired area to count with the cursor. The clicked area is checked and the counted number is shown in the **Counting Result**.

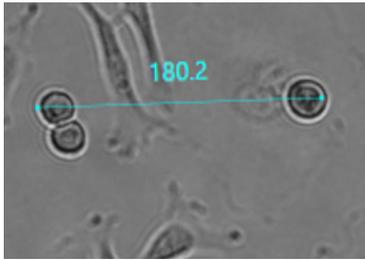


To delete part of the result, press **Remove** and click the items to be deleted. To delete all, press **Clear**.

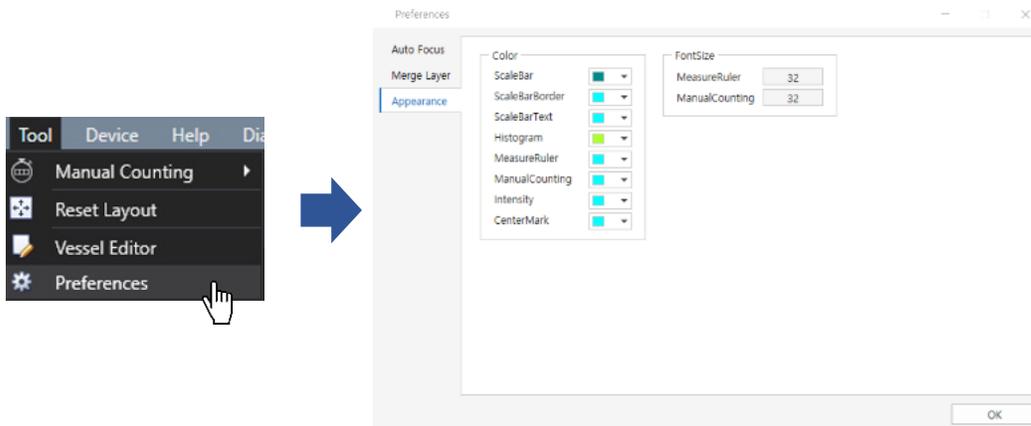
To hide them without removing all data, deactivate them by pressing **Manual Counting** activated in **Visibility** of the **Tool** menu.

Ruler

In **Measure**, select either  (Free),  (Horizontal) or  (Vertical) directions and select the desired area from point to point. The number on the line is the length measurement (μm).



NOTE: The font size of the number can be changed in **Preferences** in the **Tool** menu.



TIME-LAPSE IMAGING

1. Select the vessel type (p. 14).
2. Click on **Preview** and adjust the quantity of light (p. 31) and focus (p. 28) by referring to the **Display**. If fluorescence is also scanned at this time, the quantity of light of fluorescence should also be adjusted.
3. Specify the position and focus to be scanned. Then, select whether to execute the autofocusing function (p. 29) and Z-stacking function (p. 31). If fluorescence is also scanned at this time, the focus of fluorescence should also be adjusted.
4. Open **Time-Lapse Control** and select the location to save and enter the project name. If the image storage location is not specified, the file will be saved in the **Scan** folder under the **AutoLCI Scan App** folder, and if the project name is not entered, a folder will be automatically created under the scan start date.
5. Set the schedule (**Interval Time**, **Total Running Time** and **Start Date**) (p. 21). If you want to start immediately, **Start Date** does not need to be adjusted.

NOTE: If **Start Date** is set as past, it is updated automatically to the present time.

6. Click on **Start** to begin scanning.

NOTE: The LED indicator lights up blue while performing time-lapse.

7. Once the scanning is completed, check the saved images, make videos or perform additional analysis using the **AutoLCI Analysis App**.

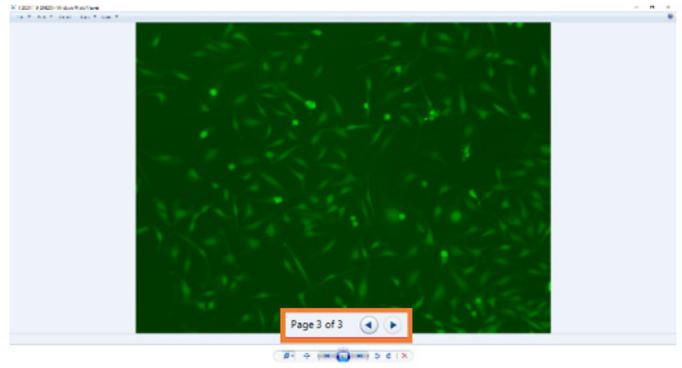
NOTE: Images are saved as "*Tiff files*" by default. If fluorescence was also imaged, the saved images are a stacked file of the merged image and raw images of each channel. A stack image may be viewed with the Windows basic image viewer "*Windows Photo Viewer*". When the Z-stacking function is executed, images are also stacked and saved. Of the stacked images, the first 2 or 3 images are the merged image and raw images of the reference position, and the following images are saved in a sequence from the low to high Z-axis. If the Z-stacking function was run for fluorescence as well, the BF images and FL images are shown alternately from the low Z-axis.



<Merge image>



<BF image>



<FL image>

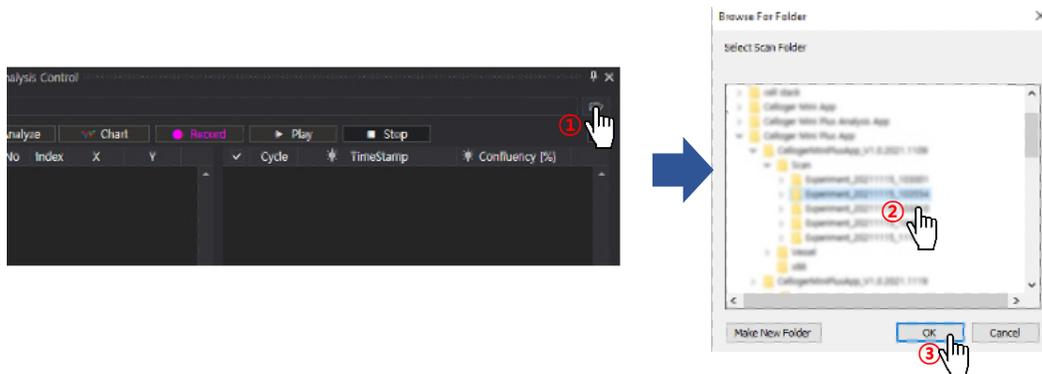
PROCESS SINGLE IMAGE OR TIME-LAPSE IMAGES

It is possible to conduct confluency analysis and make videos in **AutoLCI Analysis App** using time-lapse images taken by **AutoLCI**. In addition, intensity measurement, length measurement and manual counting are available in **AutoLCI Analysis App** and the methods are same as **AutoLCI Scan App**.

Import the Original Data Taken by AutoLCI

AutoLCI Analysis App can only import the original data taken by AutoLCI Scan App.

1. Open the time-lapse folder. Select the time-lapse folder taken by AutoLCI. Upon opening the selected folder, the images in the folder are listed following the order of the cycle on the right. The higher-level folder shows the scan position, and the lower-level folder shows the images in the selected folder.

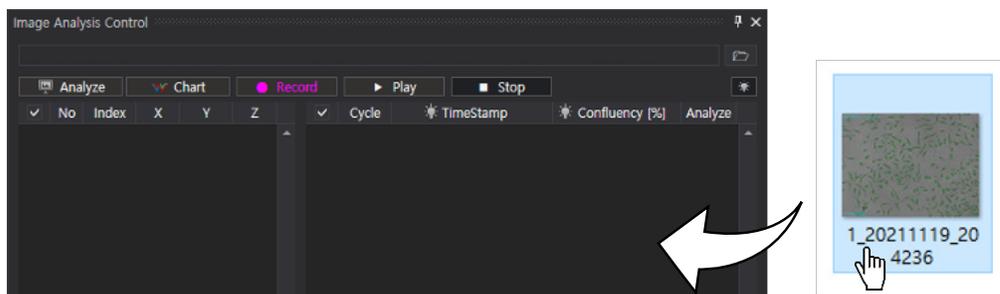


No	Index	X	Y	Cycle	TimeStamp	Confluency [%]
✓ 1	1	19.002	027.395	✓ 0	1/15/2022 6:00:54 AM	000.0
✓ 2	2	14.477	102.211	✓ 1	1/15/2022 8:00:55 AM	000.0
✓ 3	3	42.068	099.411	✓ 2	1/15/2022 10:00:56 AM	000.0
✓ 4	4	38.571	077.266	✓ 3	1/15/2022 12:00:56 PM	000.0
✓ 5	5	72.435	026.523	✓ 4	1/15/2022 2:00:56 PM	000.0
✓ 6	6	74.346	102.397	✓ 5	1/15/2022 4:00:57 PM	000.0
				✓ 6	1/15/2022 6:00:58 PM	000.0
				✓ 7	1/15/2022 8:00:58 PM	000.0
				✓ 8	1/15/2022 10:00:58 PM	000.0
				✓ 9	1/16/2022 12:00:59 AM	000.0
				✓ 10	1/16/2022 2:00:58 AM	000.0
				✓ 11	1/16/2022 4:00:58 AM	000.0
				✓ 12	1/16/2022 6:01:03 AM	000.0

Scan positions

Images

2. Open the image file. Drag the image file.



NOTE: Folder can be dragged.

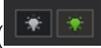
NOTE: The file location, address and file name should only be composed of English letters, number and some special symbols to import the file correctly in **AutoLCI Analysis App**. If it fails to import the file, check whether the file location address is written in languages other than English.

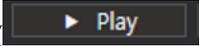
Merge FL Image

The same as **AutoLCI Scan App**. (See p. 33)

Slide Show

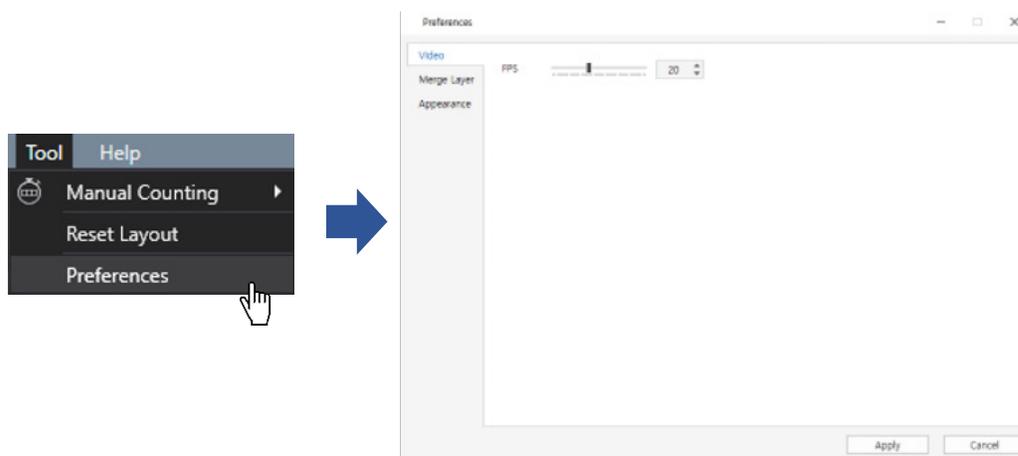
1. Open the time-lapse folder.

2. Select the light channel ( or ) or press **Merge Layers** ().

3. Press **Play** ().

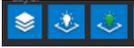
NOTE: In order to change the speed, change the **FPS** in **Preferences** of the **Tool** menu.

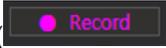
NOTE: **FPS** is a parameter indicating the speed of slide show and video. However, the speed of slide show and video could be different even with the same **FPS**.



Make a Video

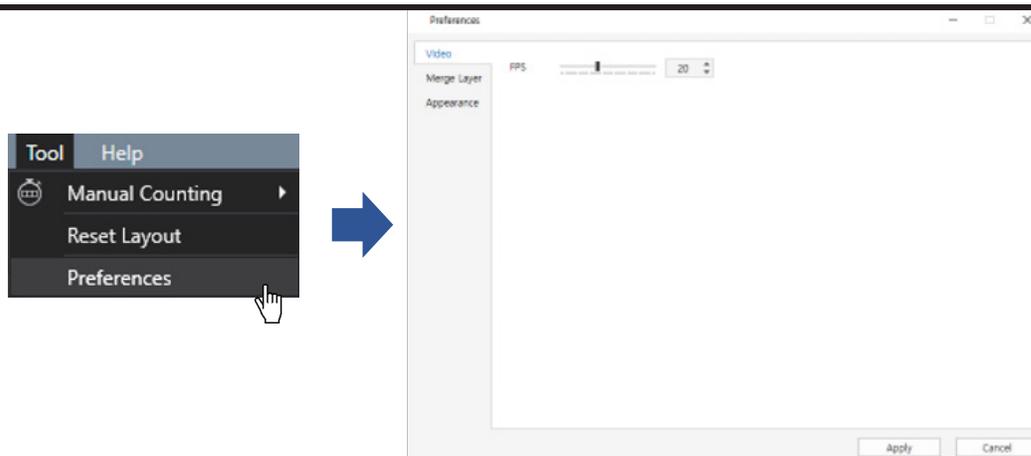
1. Open the time-lapse folder.

2. Select the light channel or merge (**Merge Layers** activation ).

3. Create a video by pressing **Record** ().

4. Open the file for confirmation.

NOTE: To change the speed, change the parameter in **Preferences** of the **Tool** menu. (Recommended value: 5~13)



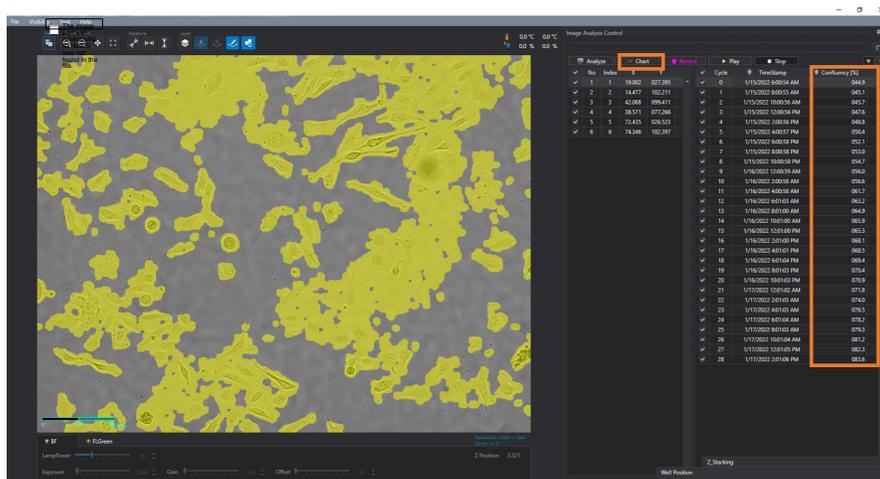
NOTE: FPS is a parameter indicating the speed of slide show and video. However, the speed of slide show and video could be different even with the same FPS.

Estimate Confluency (Coverage)

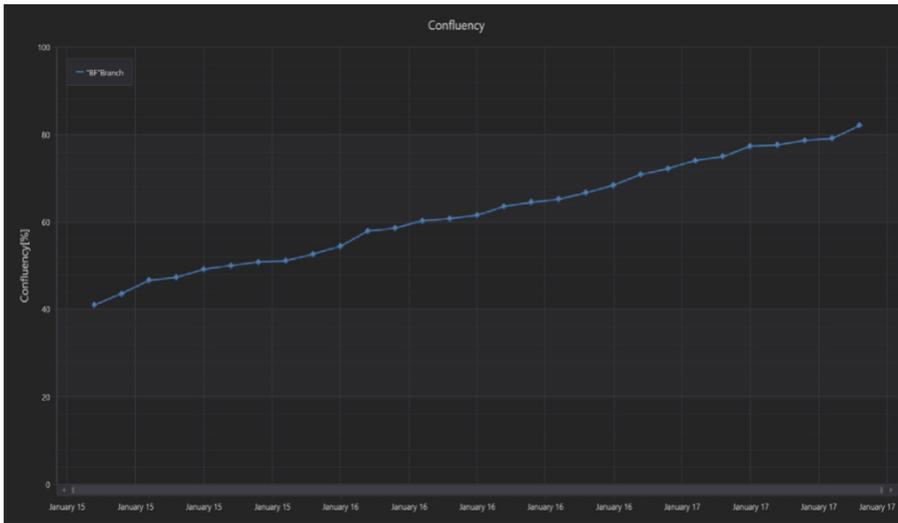
1. Open the file and images captured with **AutoLCI** .
2. Press **Analyze** (To analyze certain images from the retrieved file, use check function on the left.)

NOTE: Upon selecting the right icon  (Green FL device) or  (Red FL device), the coverage ratio of fluorescence area is calculated. At this point, coverage is calculated after thresholding according to the merge parameter. Therefore, to accurately calculate the coverage of a fluorescence area, adjust the appropriate thresholding parameters (See p. 33) before executing the coverage analysis.

3. Confluency values and confluency mask (yellow) are indicated. When a cycle is selected from the list, the corresponding cycle's image and confluency mask can be seen. The confluency graph appears when the **Chart** is clicked on.

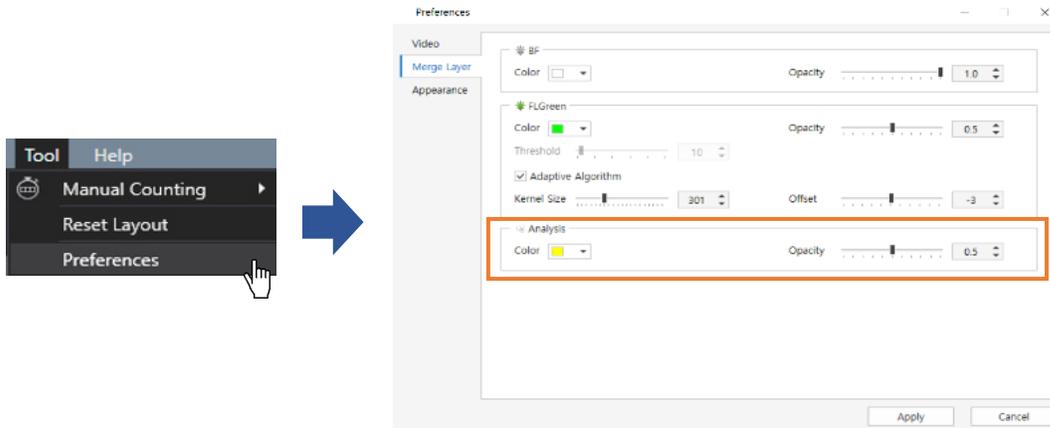


<Calculated confluency values and confluency



<Confluency graph>

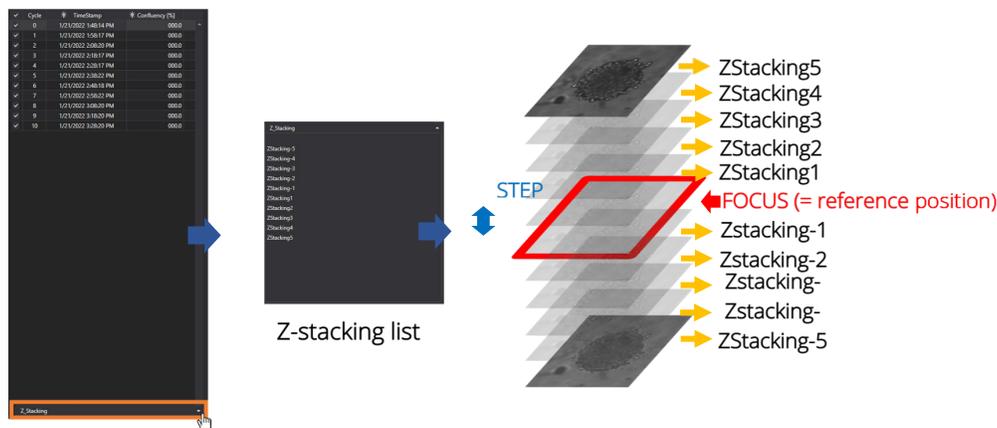
NOTE: If you wish to adjust the confluency masking color or transparency, select **Preferences** under the **Tool** menu, change the parameters in **Analysis** under **Merge Layer** and click **Apply**.



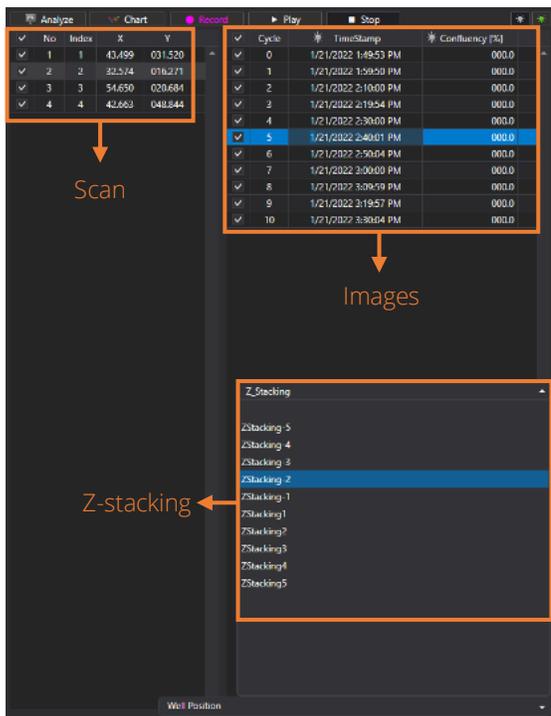
NOTE: If you do not want to see the confluency mask, deactivate it by clicking on  under **Toolbox**.

Show Z-Stacking Image

1. Open the time-lapse file or images captured with Z-stacking function.
2. When the arrow of Z-Stacking under **Image Analysis Control** is clicked, the **Z-stacking list** appears. The image below shows the **Z-stacking list** when the **Range** of the Z-stacking function is set to (-5,5).



3. Select one from the **Z-stacking list** and check the image on the **Display**. For example, the image below shows that the second image in the **Scan Positions**, the fifth cycle in **Images**, and "**ZStacking-2**" are selected in the **Z-stacking list**. In this case, you can see the "**ZStacking-2**" image of the fifth cycle captured at the second scan position on **Display**. "**ZStacking-2**" means the image was taken two **steps** below the reference position. (= **Focus**). For instance, if the **Step** is set to 10 μm , "**ZStacking-2**" is taken 20 μm below the reference position. (See p. 31 for more information on the Z-stacking setup.)



MAINTENANCE

Operating Conditions

Operating Power	100~240 VAC
Electrical Input	48 VDC, 1.04 A
Frequency	~50/60Hz
Operating Temperature	10~40°C
Relative Humidity	20~95%
Installation Site	Indoor Use Only

Cleaning and Maintenance

- When cleaning optical elements, use only a damp cloth to avoid scratching the soft lens coatings. Do not clean the lenses with organic solvents.
- Lightly wipe the working surfaces of the **AutoLCI** with a soft cloth dampened with 70% ethanol or hydrogen peroxide (H₂O₂). Do not pour or spray liquids anywhere on the instrument.
- If liquid spills on the instrument, turn off the power and wipe it dry immediately.
- Make sure that liquid or foreign matters do not enter fan holes or lens holes.
- In case of storage at a room temperature after taking it out of the incubator, store it in a dry and well-ventilated place.
- Never disassemble the instrument yourself. Do not remove any covers or parts that require use of tool to obtain access to moving parts.
- Operators must be trained before being allowed to perform the hazardous operation.
- Unauthorized repairs may damage the instrument or alter its functionality, which may void your warranty. Contact your local distributor to arrange for service.
- Do not place the POE injector inside in the incubator.
- Do not block the fan hole of the device.

TROUBLESHOOTING

Issue	Possible Cause	Solution
Device does not power up	Bad power supply.	Check the power source or contact your distributor.
	Bad power cable	Replace power cable if it is in poor condition
Cannot connect with AutoLCI device	Bad connection	Make sure the device is connected to the computer.
		Unplug and re-connect all cables. Restart the AutoLCI Scan App .
Focus is irregular	Vessel is improperly mounted.	Reload the vessel so that it lies flat on the stage. Be sure the vessel holder is mounted flat with respect to stage.
	Sample thickness varies	Make sure the sample thickness is uniform.
Autofocusing does not work properly	Auto Focus setting need to be adjusted.	Before using Auto Focus , partially set the focus manually.
		Change the Auto Focus setting.
Image display is dark	Not in preview mode.	Click Preview button.
	Sample is improperly positioned.	Place sample on the center of the objective lens.
Image is not clear	Lens is dirty.	Carefully wipe off the objective lens with cotton swab.
	Dish or lamp is dirty.	Eliminate any dust on culture dish and LED lamp.
	Condensation on the culture dish lid.	Remove any condensation on the lid of the culture dish.
Problem in saving files	No disk space.	Check the unused space of the hard disk.
Time-lapse images become dark and bright	Light changes when the incubator door opens.	To prevent any problem such as shaking of the culture dish or inflowing light, pay special attention when you open or close the incubator door during monitoring.
Cannot play the video	Not in time lapse mode.	The video can be made only for time-lapse images obtained from AutoLCI Scan App .
	Images are from unsupported source.	Check if the images are obtained from AutoLCI Scan App . Analysis can be performed only with the images obtained from AutoLCI Scan App .
	Focus is faulty.	Before analyzing, check if the focus is clear.
		Conduct FL merge, set the appropriate thresholding parameter and use the coverage analysis to calculate the coverage of fluorescence.
Water droplets	Condensation is forming.	Warm up the device 30 minutes before you start

Issue	Possible Cause	Solution
form on the well plate, dish lid or top of the flask		monitoring.
		Check for and remove any condensation on the lid of the culture dish. (In order to prevent condensation from occurring, it is better to warm up the plate or dish lid slightly when preparing the sample.)
		Check the recommended interval time.
		Do not install the device close to the front/door area of the incubator. Given that the incubator has high temperature and humidity, the area that comes in frequent contact with cool outside air may have condensation. It is recommended to install it inside the incubator as much as possible.
		Check that the fan is working. If it is turned off, condensation may occur. NOTE: If condensation continues to occur even after performing the above, it is recommended not to use the front wells (A1, B1, C1...) and rear wells as much as possible when using a well plate.

NOTE: If you have a problem/issue with that falls outside the definitions of this troubleshooting section, contact the WPITechnical Support team at (941) 371-1003 or technicalsupport@wpiinc.com.

SPECIFICATIONS

European standards 

US standards 

UK Standards 

Korean standards 

This unit conforms to the following specifications.

Dimension	226 x 358 x 215 mm
Weight	5.6kg / 12.3lb
Objective Lens	4X / 10X
Imaging Modes	Brightfield, Fluorescence (Green/Red)
Fluorescence	Green : Excitation (470/40x)/Emission (510lp) Red: Excitation (510/84x)/Emission (570lp)
Light Source	LED
Camera	5MP CMOS
Stage	Motorized XYZ
Imaging Positions	Multiple
File Export Format	TIFF, AVI (JPEG, PNG)
Culture Vessels	Flask, dish, well plate
Operating Environment	10~40°C, 20~95% humidity
Power Requirements	100-240V, ~50/60Hz
Output Ports	Ethernet
Computer	External PC
O/S Required	Window 10
Processor (Recommended)	CPU 3G
Storage (Recommended)	1TB
Monitor (Recommended)	1920*1080mm
Accessories	PoE adapter, ethernet cable, USB/LAN adapter, USB memory
Warranty	1 year

UNITS & ACCESSORIES

AUTO-LCI-BF4	AutoLCI, Brightfield, 4× Magnification
AUTO-LCI-BF10	AutoLCI, Brightfield, 10× Magnification
AUTO-LCI-BFGF4	AutoLCI Brightfield, 4× Magnification, with Green Fluorescence
AUTO-LCI-BFGF10	AutoLCI, Brightfield, 10× Magnification, with Green Fluorescence
AUTO-LCI-BFRF4	AutoLCI, Brightfield, 4× Magnification, with Red Fluorescence
AUTO-LCI-BFRF10	AutoLCI, Brightfield, 10× Magnification, with Red Fluorescence
505626	Attachment for T-Flask A25cm ² , SINGLE
505627	Attachment for T- Flask A75cm ² , SINGLE
505628	Attachment for 35 mm FluoroDish™
505629	Attachment for 60 mm Dish, Dual
505630	Attachment for 90 mm, Dish-Single

APPENDIX A: CONNECTING MULTIPLE DEVICES

With AutoLCI , multiple devices can be operated using one PC, but this requires a separate set up of the device IP or the purchase of a router. As one program is used for one device, it is necessary to open the same number of programs as the number of devices to be connected.

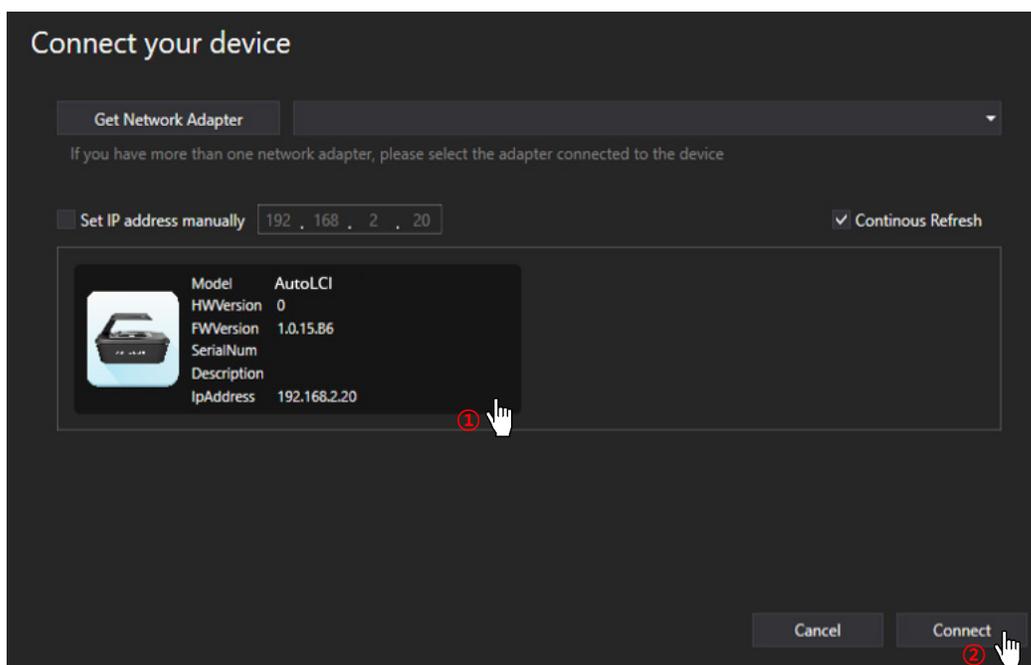
NOTE: Be careful as the system works abnormally if the same device is connected using two programs.

Installation

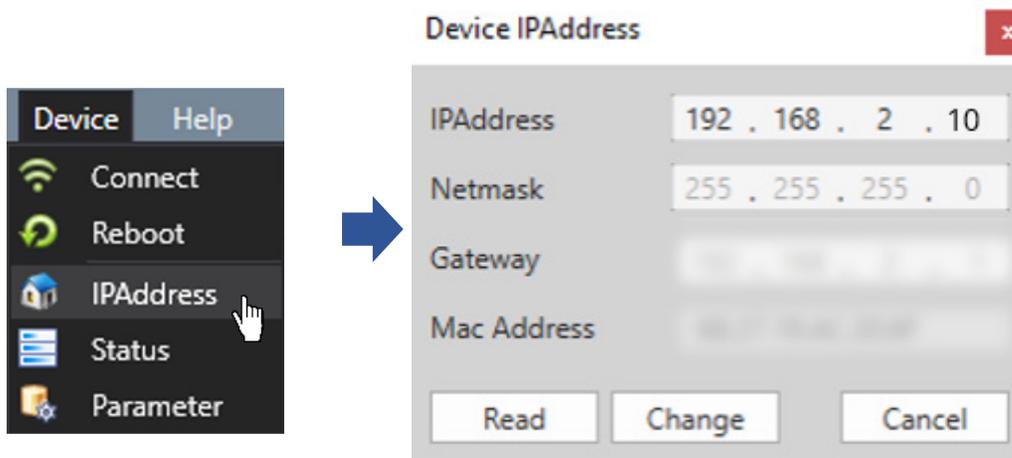
Device IP Setup

AutoLCI is operated based on the connection with PC using wired LAN cable (Ethernet connection type). Under the Ethernet connection method, devices with the same IP address cannot be properly connected. In order to connect multiple AutoLCI units with the same IP address, it is necessary to change the IP address of the devices. It is possible to change the IP address of a device in AutoLCI Scan App.

1. After connecting one device to the PC, open the AutoLCI Scan App.
2. Start the App after selecting the device. (If device does not show up, refer to p. 27 for Device Connection.)



3. In the menu of AutoLCI Scan App, open the IPAddress of the device and click Read.



4. Change the IP address and click the **Change** button.

NOTE: Make sure that the last digit of device IP address is different from the PC or other device's IP address. Put a numbers from 2 to 254 in last digit.

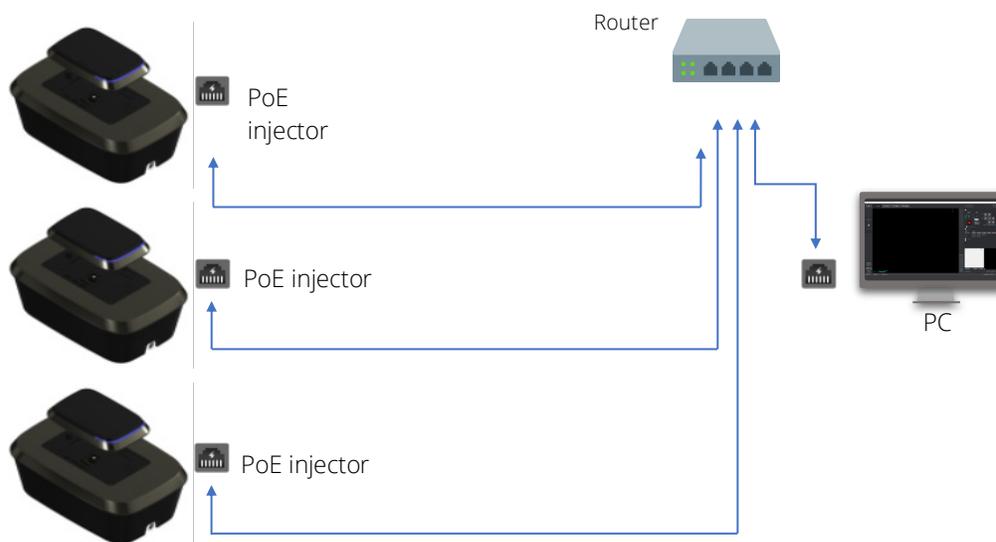
<Example. IP address setting for connecting 3 AutoLCI devices and PC>

- PC IP address: 192.168.2.200
- Device 1. IP address: 192.168.2.9
- Device 2. IP address: 192.168.2.11
- Device 3. IP address: 192.168.2.20

5. Click **Reboot** in the **Device** menu to complete the IP address setup.

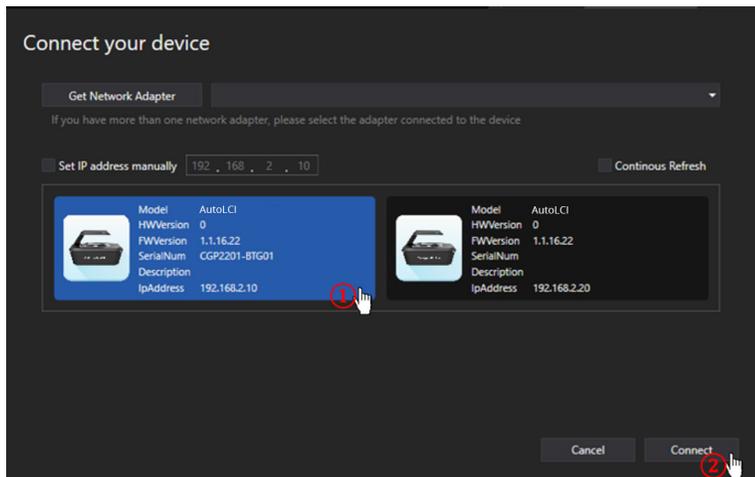
Using a Router

Having a router or switching hub enables the use of multiple devices in one PC. It is necessary to purchase a router or switching hub to use different devices at once.



Operation

1. Open **AutoLCI Scan App** after completing network setup.
2. Select the device and connect it.



NOTE: Devices connected to the App can be identified by choosing one of methods below:

- Check the selected device description.
- Check IP address at the bottom of the program (See p. 22).
- Click **Identify**  and check the white LED blinking.

APPENDIX B: ALTERNATIVE PC NETWORK SETUP

Perform the PC network setting as follows to connect the PC with the device using the **AutoLCI Scan App**.

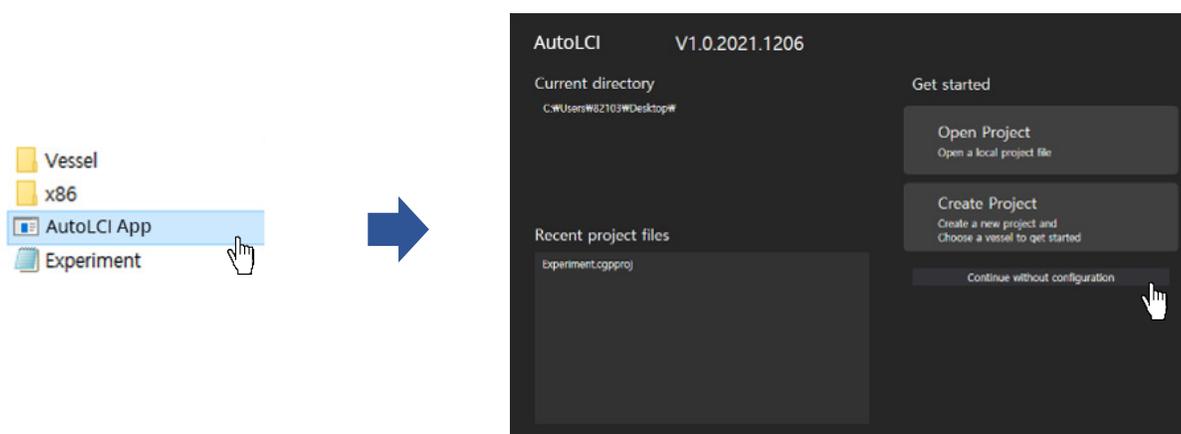
1. Open the AutoLCI Scan App.
2. The **Device Connection** popup displays after the **Create** or **Open a Project** popup (p. 26).
3. Click the arrow to the right of **Get Network Adapter** and select the network adapter connected with the AutoLCI in the **Device Connection** popup.
4. When you select the network adapter, the **Change PC IP Address** button displays at the bottom left. Click this button to open the **PC IP Setting** popup.
5. When you click the **Change PC IP Address** button, a confirmation message displays. Click **OK**. Changing the PC IP address needs administrator authorization. If the authorization setting is changed, the device connection restarts automatically and you need to perform the steps 2-5 again.
6. Change the **IP Address** and **Netmask** appropriately and click **Change**.

NOTE: The IP Address should be 192.168.2.XX and the netmask should be 255.255.255.0. Enter a number between 2 ~ 254 (except 10) in place of the XX number in the IP address.

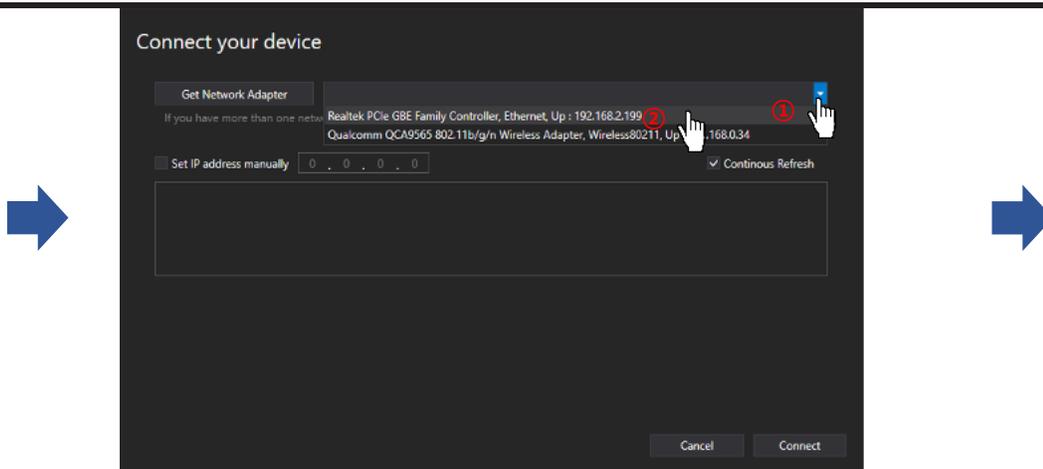
The PC IP address should be set differently from the device IP address or network adapter such as a router. The default device IP is 192.168.2.10 and to set up the PC IP address, enter 192.168.2.XX (in XX field, input 2 ~ 254 except 10).

This IP setting guideline may not be applicable to some PCs, depending on the PC network environment. In case of connection failure, contact us at wpi@wpiinc.com.

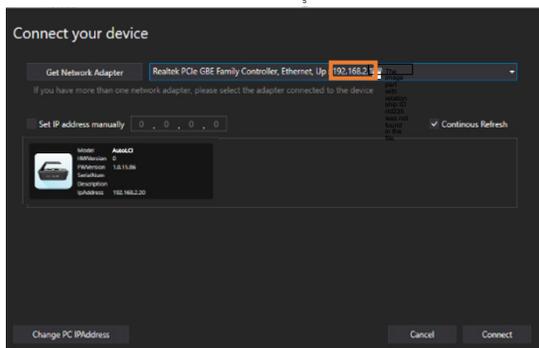
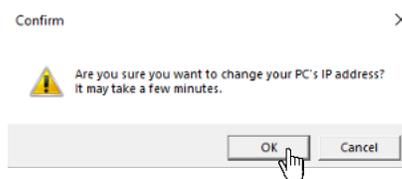
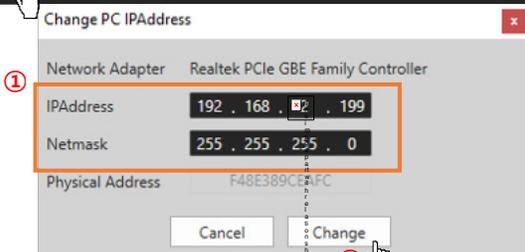
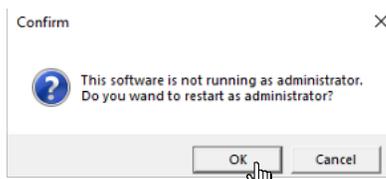
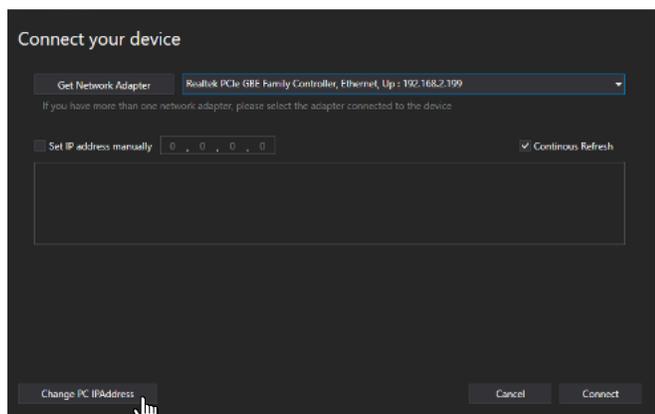
7. A confirmation message appears. Click **OK**. If the PC IP address setting is applied properly, the setting popup closes automatically.
8. Check if the IP address of the network adapter is applied correctly, then the PC network setting is completed.



<Create or open project popup>



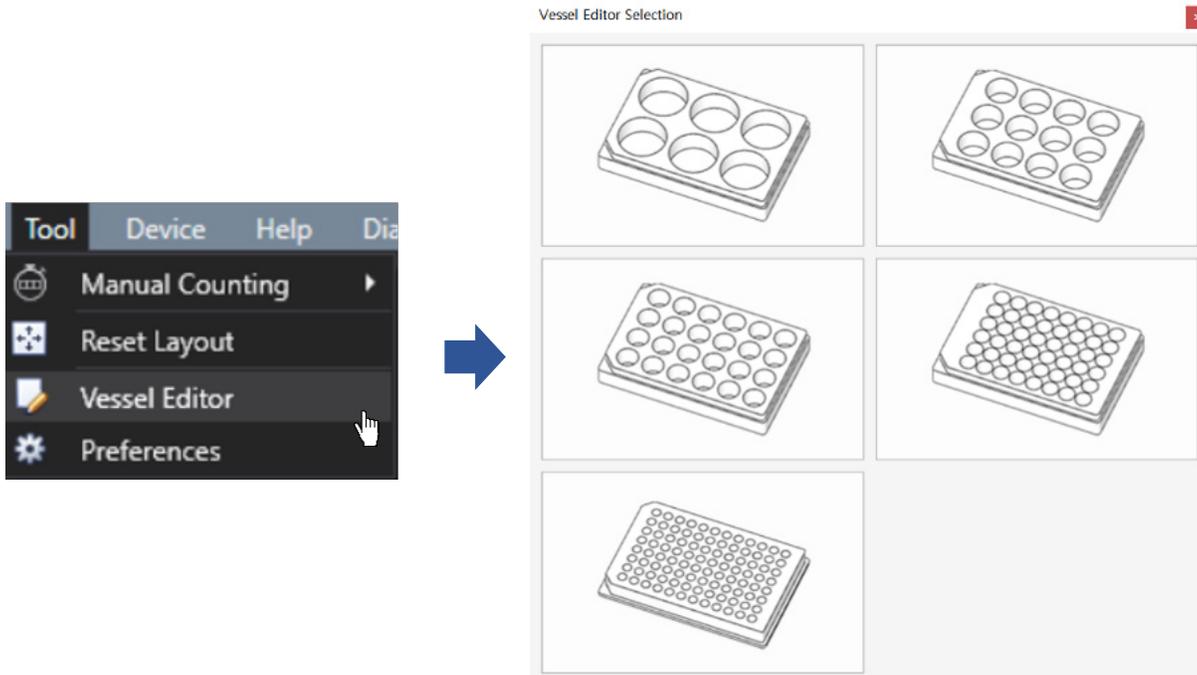
<Device connection popup>



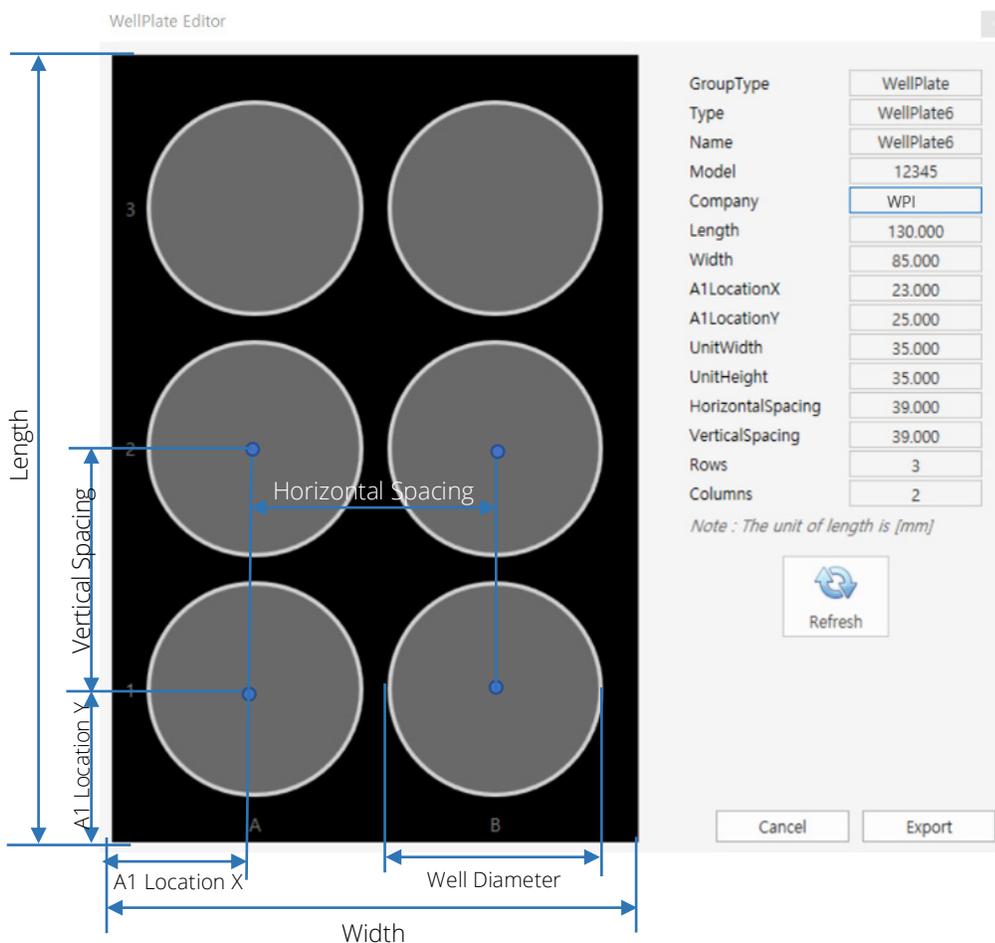
APPENDIX C: HOW TO USE VESSEL EDITOR

The AutoLCI well plate specifications are set based on a certain brand's specifications. As the specifications of the well plate differ for each brand, imaging may be difficult when the well plate you are using doesn't match the default well plate specification in **Vessel Type**. As such, we recommend setting the well plate specifications of the brand used in the **Vessel Editor**.

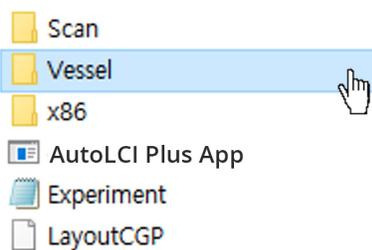
1. Select **Vessel Editor** from the **Tool** menu.
2. Select the type of well plate (6/12/24/48/96 well) to be used from the **Vessel Editor Selection** window.



- Change the specifications (**Length**, **Width**, **A1LocationX**, **A1LocationY**, **Well Diameter**, **Horizontal Spacing**, **Vertical Spacing**) of the well plate to be used after entering the **Model** and **Company** in the **Well Plate Editor** window. Refer to the 6 well plate image below for reference.

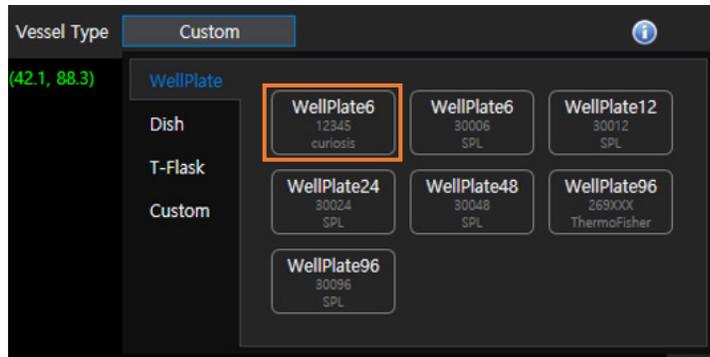


- Click the **Refresh** button.
- Click the **Export** button to save the setting values. The saved file can be found in the Vessel folder of the **AutoLCI Scan App**.



- Re-run the AutoLCI Scan App.

7. If there is a well plate of the brand saved in **Vessel Type**, click on it to use it.



APPENDIX D: HOW TO INSERT VESSEL HOLDER

Select the holder suitable for the vessel type. As shown in the figure below, insert the tip of the holder into the area where it faces the LAN port of the device first and mount it. Insert the screw into the hole and turn it clockwise to assemble it.



CAUTION! Please be careful when tightening the screw. It can fall into the device.

WARRANTY

WPI (World Precision Instruments) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

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