

Automated Live Cell Imaging System

# Celloger<sup>®</sup> Mini Plus

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## Instruction manual



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# I. Package contents

**Celloger® Mini Plus** package includes the following items.

Item	Quantity
Celloger Mini Plus	1
Main Power Cable	1
POE	1
USB/LAN adapter	1
LAN Cable	2
USB memory	1
- Instruction Manual	1
- Celloger Mini Plus Scan App	1
- Celloger Mini Plus Analysis App	1
Vessel holder for well plate (Included)	1
Vessel holder for dish 35mm, dual (Optional)	1
Vessel holder for dish 60mm, dual (Optional)	1
Vessel holder for dish 90/100mm, single (Optional)	1
Vessel holder for T-flask 25cm <sup>2</sup> , single (Optional)	1
Vessel holder for T-flask 25cm <sup>2</sup> , dual (Optional)	1
Vessel holder for T-flask 75cm <sup>2</sup> , single (Optional)	1
Vessel holder for Slide, triple (Optional)	1

**When receiving the package,**

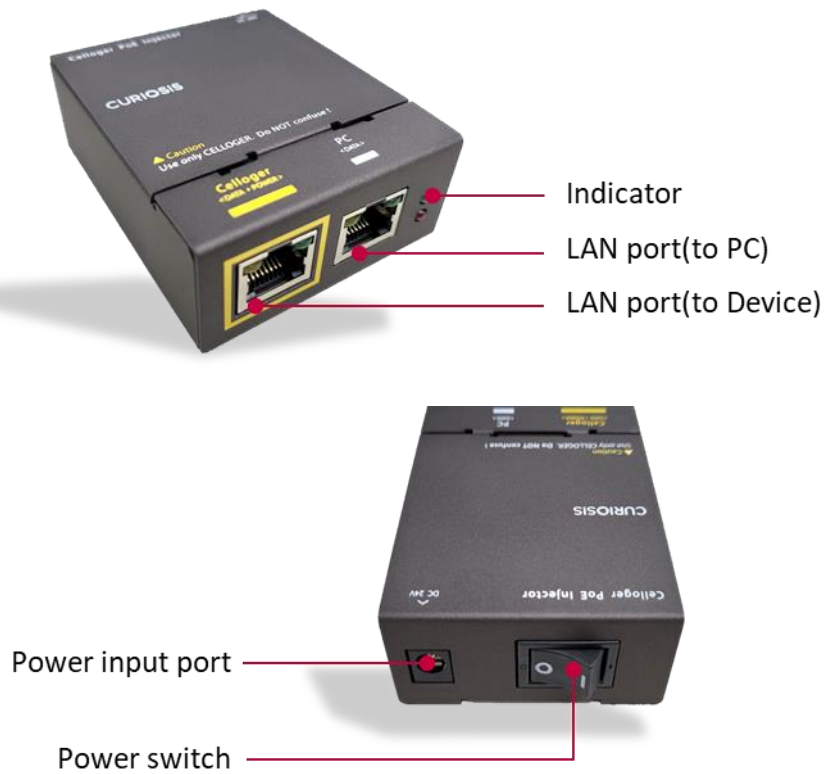
- Check that all items listed above are included in your package.
- Examine the device carefully for any damage during shipping.
- Contact your local distributor or [info@curiosis.com](mailto:info@curiosis.com) if any items are missing or damaged.
- Any loss or damage claims must be filed with the carrier.

## II. Device layout

### Front-left side



### POE



# III. Getting started

## 1. Pre-requirements

### Installation location

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

- Install **Celloger® Mini Plus** on a flat and level surface away from vibrating equipment.
- Place in dust- and other airborne particles-free area.
- Install away from direct light sources. Ambient room lighting can enter the imaging path and affect the image quality.
- During operation, place around 10 cm away from surroundings for proper air flow of the cooling fan.
- During operation inside the incubator, do not place the **Celloger® Mini Plus** close to the incubator door.
- Do not put any heavy materials on top of the **Celloger® Mini Plus**.
- If using multiple pieces of equipment, there should be a proper clearance among equipment.
- Keep the device at temperature between 10 ~ 40 °C.
- Relative humidity range is 20 ~ 95 %.

#### **Important!**

*It is not recommended to operate the device at low temperature (Below 10 °C). **PRIOR to putting it in the low temperature environment, warm up the device for over 10 minutes.***

### PC specifications (Refer to p.70)

- **Celloger® Mini Plus** software can be used on PCs with Window 10.
- A large capacity hard drive is necessary to save images from timelapse. If the capacity is not large enough, scanning will not function properly.
- For easy use, it is recommended to use a monitor with resolution of 1920 x 1080 mm.
- A LAN port is required in the PC to connect it to the equipment via LAN cable. If there is no LAN port, a LAN cable adapter should be prepared.
- A PC that supports USB 3.0 is recommended.

## 2. Installation

### 2.1 Unpacking

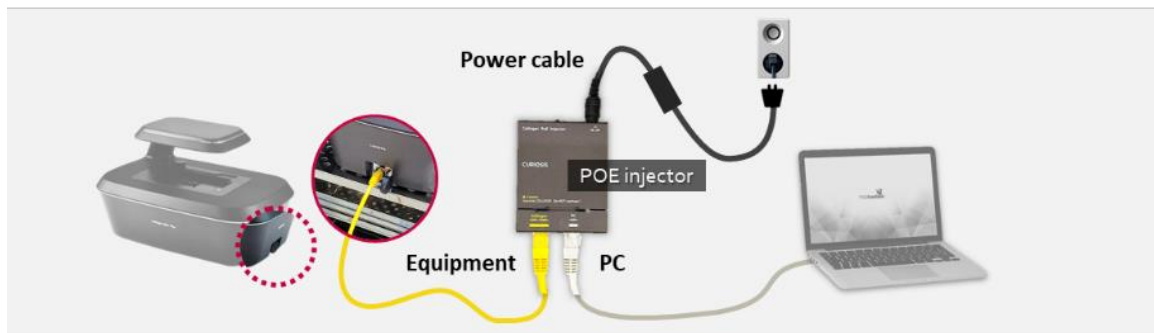
1. Lift the **Celloger® Mini Plus** from the box, remove all the covers, and place it on a clean and level surface.
2. Make sure all accessories are present by checking “**Package Contents**” (p.5). If any items are missing or damaged, please contact your distributor or Curiosis at [info@curiosis.com](mailto:info@curiosis.com).

### 2.2 Connect the device and computer

1. Prepare a computer that meets the specifications (refer to p.70 for PC specifications) and place it near the incubator.
2. Connect two LAN cables and a power cable to the POE. Connect one LAN cable to the device (colored) and another LAN cable to the PC (white).



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



### 2.3 Install the device inside an incubator

1. Install the vessel holder on the **Celloger® Mini Plus**. Then, place the device inside the incubator.

**Note.** Before using the device, make sure to remove all the covers including the lens cover (Black) and clean it with a soft, dampened wipe containing 70% ethanol.

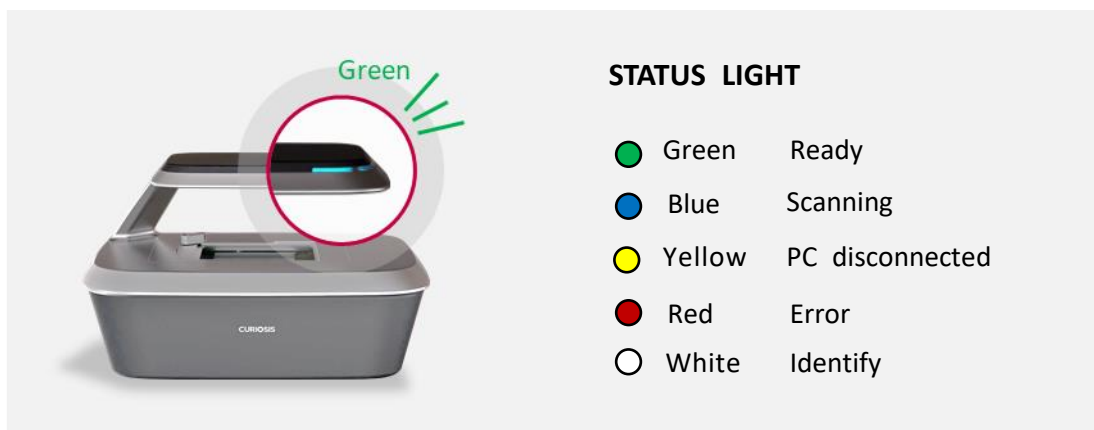




2. Place the sample on the stage.




3. Check the connection between the PC and the device. To connect the device and PC, refer to “**Connect the device**” (p.11).



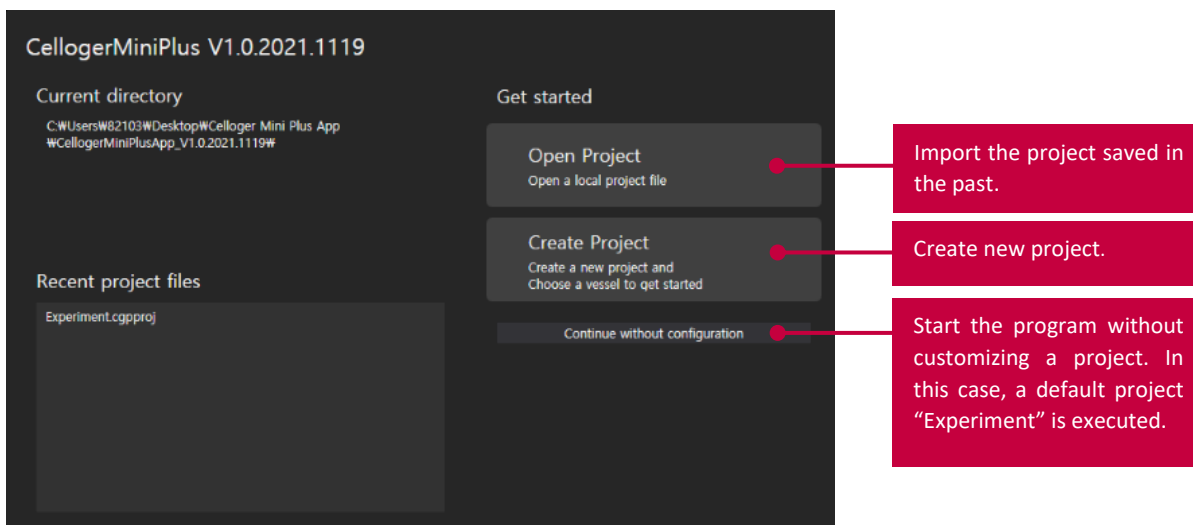
# Section 1. Celloger Mini Plus Scan App

## I. General Operation

### 1. Create or open a project

1. After installing **Celloger® Mini Plus**, execute the **Celloger Mini Plus Scan App** (  ).
2. A pop-up screen for project selection is displayed.

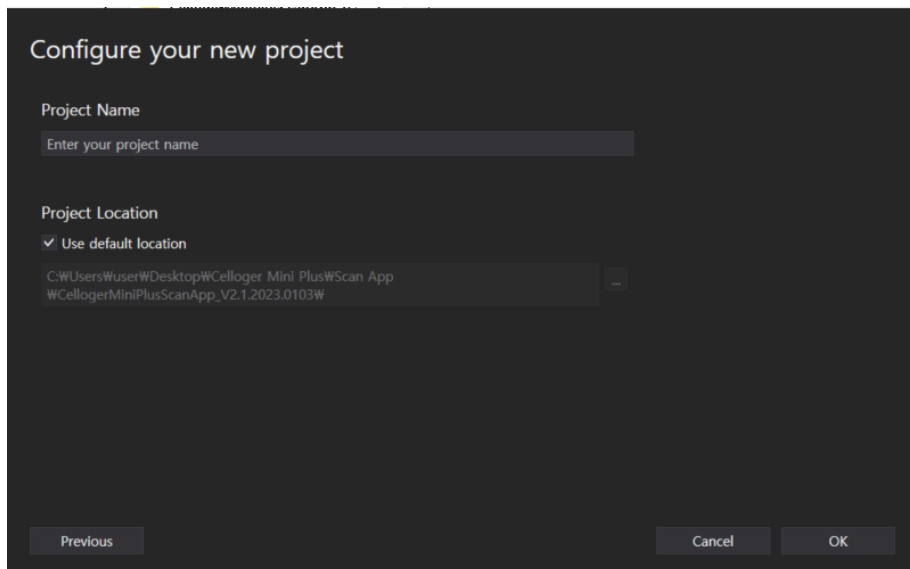
**Note.** The settings for **Celloger® Mini Plus** can be managed using the project file (".CGPPROJ" file format). Setting values (Light intensity, Focus, Position, Schedule, File storage location, etc.) are saved in the project.



<A pop-up screen for project selection>

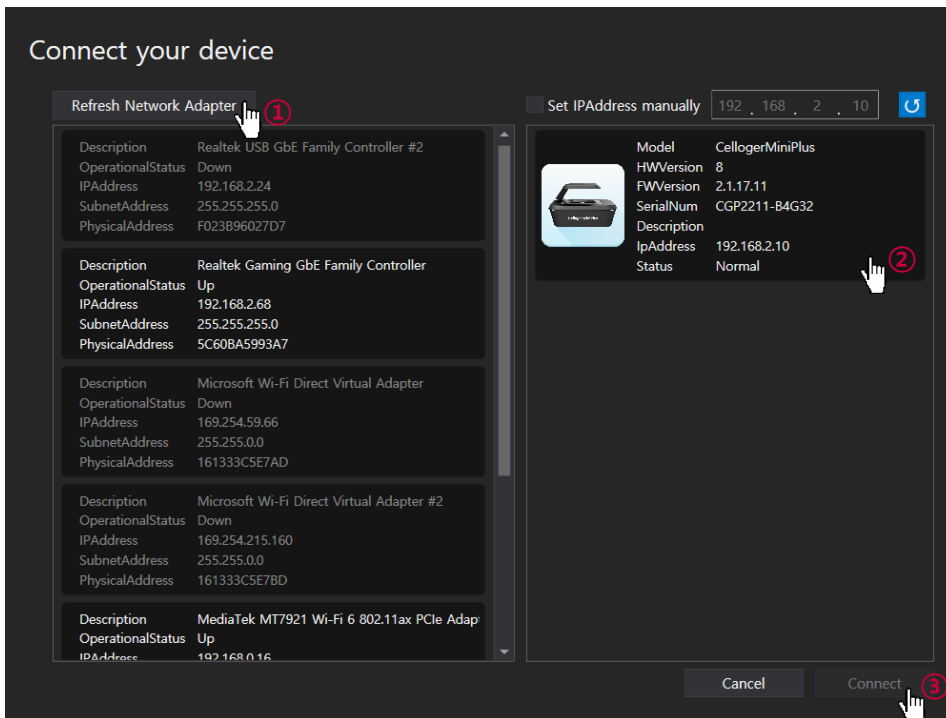
3. Create a new project or open an existing one. To start a program with default project settings, click **Continue without configuration**.


**Note.** Upon selecting **Create Project**, a pop-up window will appear, requiring you to choose a project name and storage location. You can start the program after entering the necessary information and press **OK**. The project will be stored in the default program file shown below. If you want to change it, uncheck the **"Use default location"** box and enter a new location.



<Create Project pop-up>

## 2. Connect the device



1. Open the **Celloger Mini Plus Scan App** (  ).
2. When the app is executed, the **Create or Open a project** popup appears. Select **Continue without configuration**.
3. The **Connect your device** popup will show.
4. Click **Refresh Network Adapter** to load the network adapters connected to the PC (step ① in the figure above).
5. Click the device icon (step ② in the figure above).

**Note.** If the icon is not shown, check the following cases and take actions if applicable.


### Case 1) The IP address setting of the PC network is not properly completed.

➔ Set the IP address of the PC network manually. If the PC, device, and router IP are the same, connection fails (Refer to Appendix B in p.75).

### Case 2) The LAN or power cable is not properly connected (LED status indicator is turned off).

➔ Check all cable connection status and POE injector power (refer to **Connect the device and computer** in p.8).

### Case 3) Separate network adapter is used.

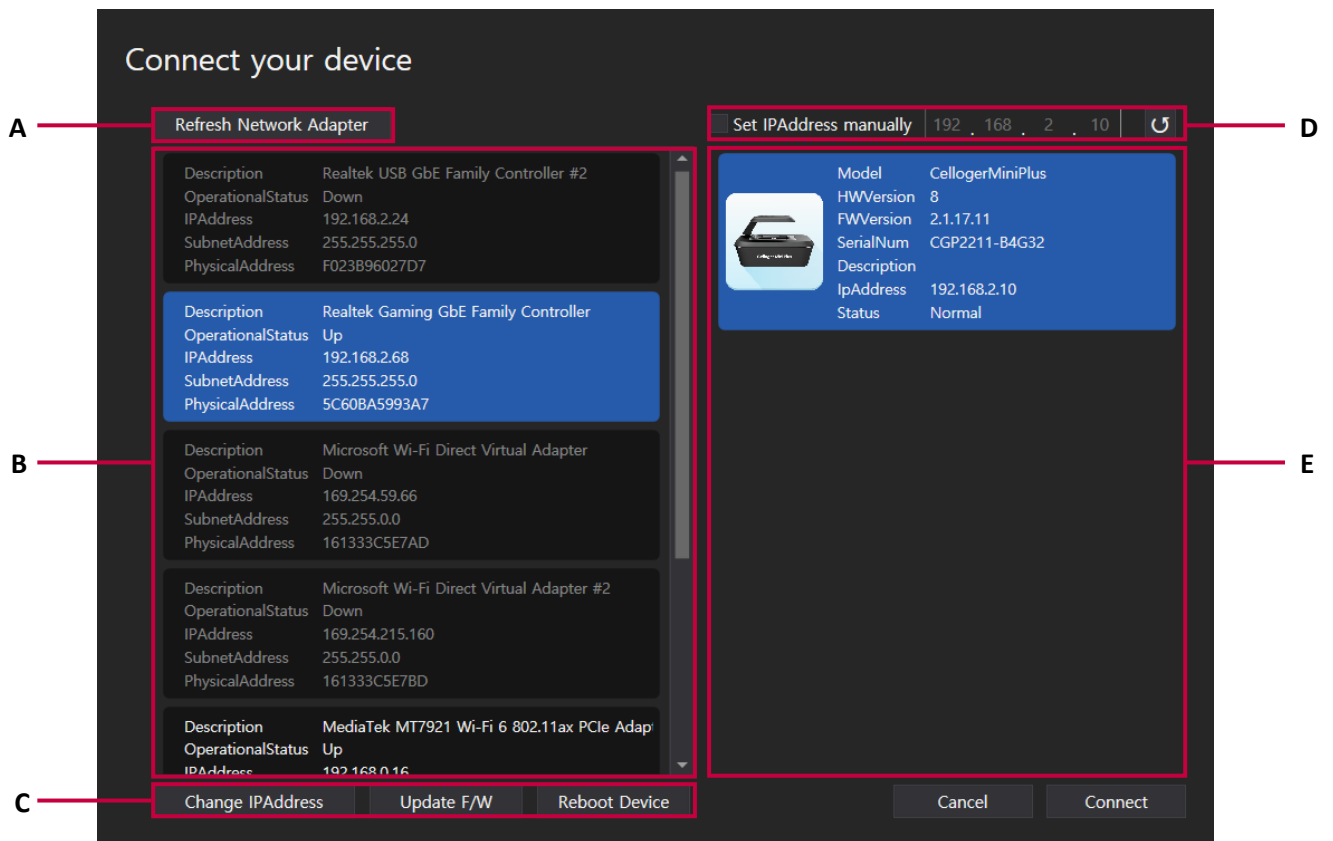
➔ Press the **Refresh Network Adapter** to select the adaptor to which the device is connected. If the device icon does not appear even when the network adaptor is selected, search for the device's IP address directly from  **Set IPAddress manually**  .

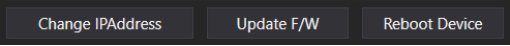
### Case 4) When using multiple devices in one PC, each device has the same IP.

➔ Initially, devices are released with the same IP. When using the device for the first time, it is necessary to reset the IP address (refer to p.72 for device IP setting).

6. Click **Connect** (step ③ in the figure above). When the connection has successfully completed, the LED status indicator changes color from yellow to green.

<Device connection pop-up description>



- A. **Refresh Network Adapter** reads the connected network adapter from the PC.
- B. **Network adapter list.** **Operational Status** shows the network connection status where **Up** means network is enabled, while **Down** means it is disabled.
- C. 
- ✓ **Change IPAddress:** Change the IP address of selected network and device.
  - ✓ **Update F/W:** Update the F/W of device (refer to **Firmware update guide**).
  - ✓ **Reboot Device:** Reboot the device.
- D. Manually search for the IP address of the device.
- E. The list of connected devices.

### 3. Software overview

**Celloger Mini Plus Scan App** is a program for the actual operation of the **Celloger® Mini Plus** device, which includes real-time cell monitoring and time-lapse imaging functions.

**Note.** Refer to p.29 for full software descriptions.



- ① Menu bar
- ② Toolbox
- ③ Display (Preview)
- ④ Light source control
- ⑤ Device status
- ⑥ Jog control
- ⑦ Vessel & Plate panel
- ⑧ Scan pattern
- ⑨ Position control
- ⑩ Time-lapse control
- ⑪ Connection status

## 4. Preparation (cell/device preparation)

- ① Install the vessel holder.
- ② Place the **Celloger® Mini Plus** in the incubator.

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

**Note.** The device should be pre-warmed for more than 1 hour prior to time-lapse imaging to prevent condensation.

- ③ Place the cell sample on the stage of the **Celloger® Mini Plus**.

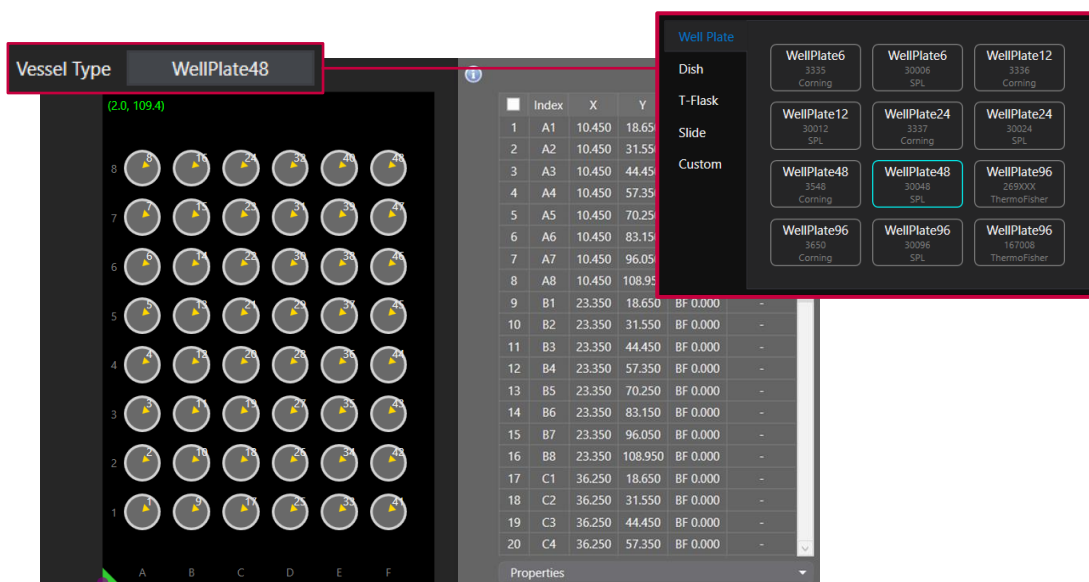
**Note.** Before covering the plate, warm the lid by slightly passing the lid over the fire of an alcohol lamp to prevent condensation. This step is not necessary, but it will prevent trapping the condensation in the lid.

## 5. Cell monitoring and image setting

### 5.1 Location adjustment

#### 1) Adjust X, Y position

- ① Select the vessel type and desired scan location on the **Vessel & Plate panel**.



- ② Press **Preview** for streaming while **BF** (means brightfield) 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 to be seen, run **5.2 Light intensity adjustment** in p.18 first.



**Note.** Double-clicking the mouse on the vessel image moves the lens to the center of the vessel. Additionally, double-clicking at the desired position on the vessel image while holding down the “Ctrl” key moves the lens to that position. Alternatively, you can use the arrow keys on the keyboard to move around. (refer to p.82).

## 2) Set BF Focus

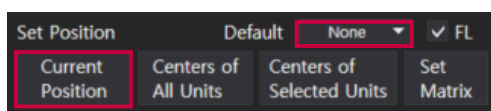
### ➤ Manual adjustment


- ① At the top right of the software screen, set the **Step** as “x1mm” and press the **Jog button** to move to the point where cells are visible. Conduct precise adjustments gradually by reducing the **Step**.

**Note.** Analysis can be precisely conducted when cells are clearly distinguishable from the background and the shape of cells are clearly visible. Therefore, selecting a clear focal position is one of the major factors that influence the accuracy of analysis. Ensure that the cells are focused clearly to enhance the quality of the analysis.

**Note.** The **Celloger® Mini Plus**’s Z-stage absolute coordinate error is 0.001 mm.

- ② Upon finding the best focal point for scanning, set the autofocusing frequency to **None** in **Default** and press **Current Position** in **Set Position** to specify the 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** button to apply the changes (refer to p.39 for **Properties**).

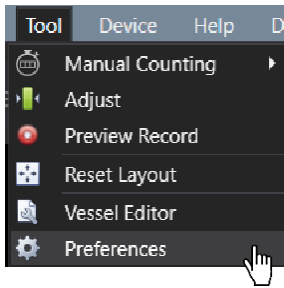
**Note.** When autofocusing frequency is set to a value other than **None**, autofocusing function is executed during scanning.

### ➤ Auto Focus

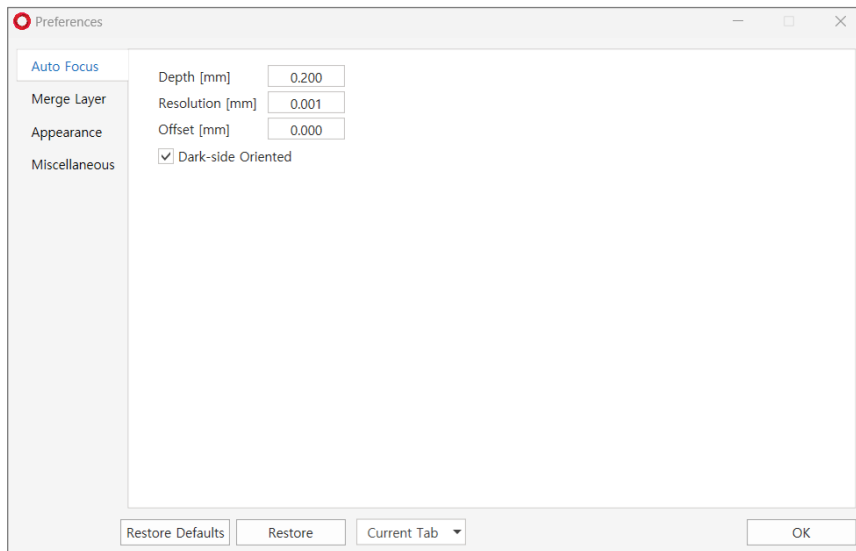
The autofocusing function of the **Celloger® Mini Plus** works by selecting a clear focal point after screening the top and bottom areas of the previously set focus point. For more successful autofocusing, set the focus location within the designated range of the autofocus parameter. Then use the autofocusing function after manually setting the Z-position.

- ① Move the focal plane using the **Jog** button.

- ② Open **Preferences** in the **Tool** menu.



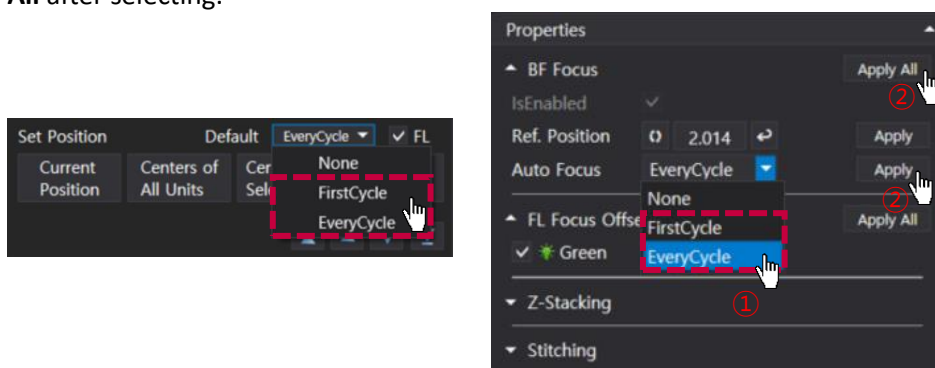
- ③ Designate parameters (**Depth, Resolution, Offset**) in the **Auto Focus** tab and press **OK**.



- ✓ **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 **Celloger® Mini Plus**, input the difference between the desired point and Z-position coordinates from autofocusing.
- ✓ **Dark-side Oriented:** Select whether to focus on the black side when performing autofocus. If checked, the focus will be on the black side; if not, the focus will be on the clearer side, either black or white.

**Note.** The larger **Depth** and the smaller **Resolution**, the higher autofocusing accuracy. Meanwhile, the longer the screening time and autofocusing time.

- ④ Set the value except for **None** in **Auto Focus** in **Default** or **Properties** to set the frequency of autofocusing function (refer to p.39 for autofocusing frequency). If selecting it from **Properties**, Click **Apply** or **Apply All** after selecting.





### 3) Set FL Focus

Since the focus of the **FL** (means fluorescence) channel may be different from that of the **BF**, it is recommended to set the focus position separately.

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

Index	X	Y	Focus	Util	
1	A1	13.780	17.460	BF 2.014	-
2	A2	13.780	36.760	BF 2.014	-
3	A3	13.780	56.060	BF 2.014	-
4	A4	13.780	75.360	BF 2.014	-
5	A5	13.780	94.660	BF 2.014	-
6	A6	13.780	113.960	BF 2.014	-
7	B6	33.080	113.960	BF 2.014	-
8	B5	33.080	94.660	BF 2.014	-
9	B4	33.080	75.360	BF 2.014	-
10	B3	33.080	56.060	BF 2.014	-
11	B2	33.080	36.760	BF 2.014	-
12	B1	33.080	17.460	BF 2.014	-
13	C1	52.380	17.460	BF 2.014	-
14	C2	52.380	36.760	BF 2.014	-
15	C3	52.380	56.060	BF 2.014	-
16	C4	52.380	75.360	BF 2.014	-
17	C5	52.380	94.660	BF 2.014	-
18	C6	52.380	113.960	BF 2.014	-
19	D6	71.680	113.960	BF 2.014	-
20	D5	71.680	94.660	BF 2.014	-

Properties

BF Focus Apply All

IsEnabled

Ref. Position  Apply

Auto Focus  Apply

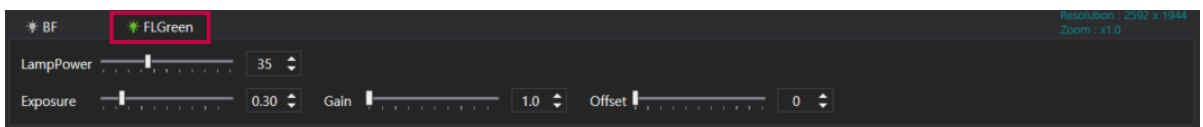
FL Focus Offset Apply All

Green  Apply

Z-Stacking

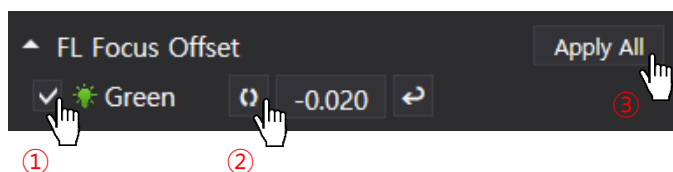
Stitching

- ② Select the **FL** channel in **Light Source Control** and adjust the focus using the **Jog** button by referring to the **Display**.



**Important!** When imaging fluorescent channels, avoid direct exposure to the light with your eyes.

- ③ Check that the **FL Focus Offset** checkbox under **Properties** and click on  to specify the focus and click **Apply All**. (If the position is specified after checking **FL** channel in the **Default** (refer to p.36 for **Default**), the **FL** channel checkbox is checked.)

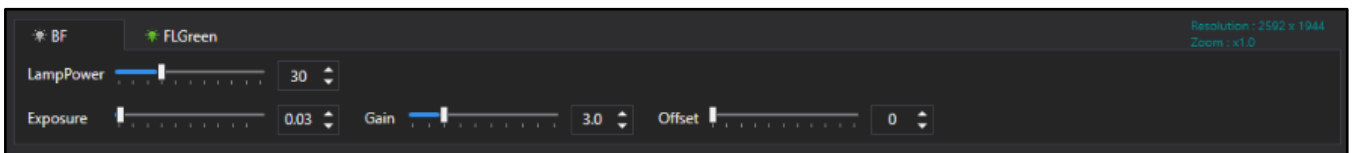


- ④ Check if the designated **FL** focus value is shown in **Scan table**.

Index	X	Y	Focus	Util	
1	A1	13.750	15.550	BF 2.014	-
2	A2	13.750	34.850	BF 2.014	-
3	A3	13.750	54.150	BF 2.014	-
4	A4	13.750	73.450	BF 2.014	-
5	A5	13.750	92.750	BF 2.014 FL -0.020	-
6	A6	13.750	112.050	BF 2.014	-
7	B1	33.050	15.550	BF 2.014	-
8	B2	33.050	34.850	BF 2.014	-
9	B3	33.050	54.150	BF 2.014	-
10	B4	33.050	73.450	BF 2.014	-
11	B5	33.050	92.750	BF 2.014	-
12	B6	33.050	112.050	BF 2.014	-
13	C1	52.350	15.550	BF 2.014	-
14	C2	52.350	34.850	BF 2.014	-
15	C3	52.350	54.150	BF 2.014	-
16	C4	52.350	73.450	BF 2.014	-
17	C5	52.350	92.750	BF 2.014	-
18	C6	52.350	112.050	BF 2.014	-
19	D1	71.650	15.550	BF 2.014	-
20	D2	71.650	34.850	BF 2.014	-

## 5.2 Light intensity adjustment

The light source options for the **Celloger® Mini Plus** is displayed on the lower tab. To adjust and customize the parameters, simply select the desired light source; the changes made are automatically saved. Given the significant impact of brightness on analysis accuracy, it is important to set the appropriate brightness level to ensure clear differentiation between cells and the background.



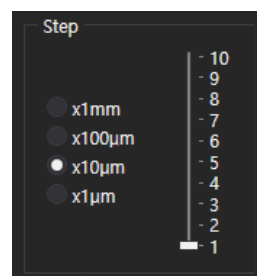
<Shows Light Source Control in the App connected to FL Green Celloger® Mini Plus>


- ① Select **BF** (at the bottom), press **Preview** (at the top) and check **Display** in the streaming on state.
- ② Adjust the brightness by changing **Lamp power**, **Exposure**, **Gain** and **Offset**.

## 5.3 Z-stacking

### 1) Manual Z-stacking

- ① By using the **Jog**, find the position to start stacking.
- ② Designate the stacking unit in the **Step** section.  
Ex) Step setting for stacking by 10  $\mu\text{m}$



- ③ Click **Current Position** for each position of the range you want to stack by using  in the **Jog**.

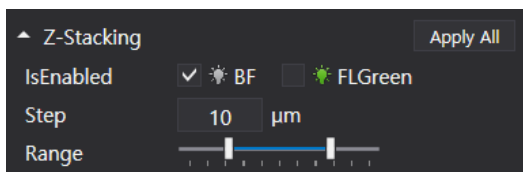
## 2) Automated Z-stacking

- ① Select the position in the **Scan table** where the Z-stacking function will be applied.

	Index	X	Y	Focus	Util
1	A1	11.200	14.300	BF 3.434	-
2	A2	11.200	23.300	BF 3.434	-
3	A3	11.200	32.300	BF 3.434	-
4	A4	11.200	41.300	BF 3.434	-
5	A5	11.200	50.300	BF 3.434	-
6	A6	11.200	59.300	BF 3.434	-
7	A7	11.200	68.300	BF 3.434	-
8	A8	11.200	77.300	BF 3.434	-
9	A9	11.200	86.300	BF 3.434	-
10	A10	11.200	95.300	BF 3.434	-

**Scan table**

- ② Select the channels to perform the Z-stacking function in the **Z-stacking** section under **Properties** (refer to p.40 for Z-stacking).
- ③ Specify the interval (= **Step**) and **Range** with which to execute the Z-stacking function.



**Z-stacking (under Properties)**

- ④ After clicking **Apply All**, check that the set Z-stacking value appears in **Util**.

	Index	X	Y	Focus	Util
1	A1	11.200	14.300	BF 3.434	-
2	A2	11.200	23.300	BF 3.434	-
3	A3	11.200	32.300	BF 3.434	ZS (-5,5)
4	A4	11.200	41.300	BF 3.434	-
5	A5	11.200	50.300	BF 3.434	-
6	A6	11.200	59.300	BF 3.434	-
7	A7	11.200	68.300	BF 3.434	-

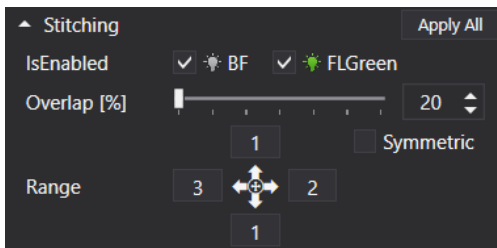
## 5.4 Stitching

- ① Select the position in the **Scan table** where the stitching function will be applied.

<input checked="" type="checkbox"/>	Index	X	Y	Focus	Util
4	A4	11.200	41.300	BF 3.434 FL 0.000	-
5	A5	11.200	50.300	BF 3.434 FL 0.000	-
6	A6	11.200	59.300	BF 3.434 FL 0.000	-
7	A7	11.200	68.300	BF 3.434 FL 0.000	-
8	A8	11.200	77.300	BF 3.434 FL 0.000	-
9	A9	11.200	86.300	BF 3.434 FL 0.000	-
10	A10	11.200	95.300	BF 3.434 FL 0.000	-

Scan table

- ② Select the channels to perform stitching function in the **Stitching** section under **Properties** (refer to p.40 for **Stitching**)
- ③ Set the overlapping area between the images using **Overlap** scroll bar and designate the range to be scanned in **Range**.



Stitching (under Properties)

- ④ After clicking **Apply All**, check that the set stitching value appears in **Util**.

<input checked="" type="checkbox"/>	Index	X	Y	Focus	Util
4	A4	11.200	41.300	BF 3.434 FL 0.000	-
5	A5	11.200	50.300	BF 3.434 FL 0.000	-
6	A6	11.200	59.300	BF 3.434 FL 0.000	-
7	A7	11.200	68.300	BF 3.434 FL 0.000	ST (3,1,2,1)
8	A8	11.200	77.300	BF 3.434 FL 0.000	-
9	A9	11.200	86.300	BF 3.434 FL 0.000	-
10	A10	11.200	95.300	BF 3.434 FL 0.000	-



**Note.** When performing stitching with autofocusing set to **None** and utilizing **FL** channel, please note that it takes approximately 2 minutes to scan 3 x 3 and 8 minutes for 7 x 7. The light conditions during scanning were as follows: **BF** (Lamp power: 30, Exposure: 0.06, Gain: 1), **FL** (Lamp power: 30, Exposure: 0.5, Gain: 1).

## 6. Image analysis

### 6.1 Capture

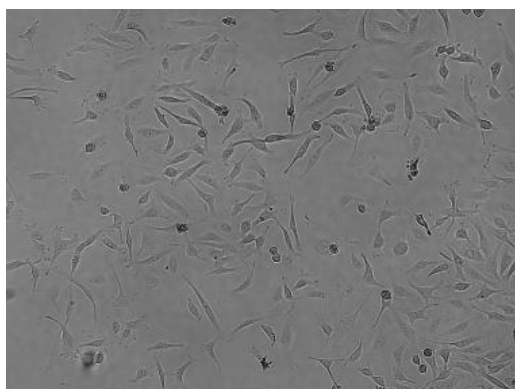
 icon in the **Toolbox** captures the image on **Display**. (Use “*Ctrl + I*” as a shortcut)

#### 1) An image with features

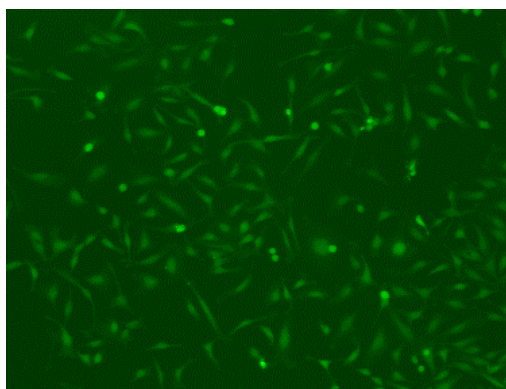
It is possible to generate and manipulate the image’s features, show it on **Display**, and save it as it is. To obtain the raw image without any manipulated features, press  (**Features layer**) for deactivation and then press  to save.

#### 2) BF or FL channel image

Press the desired tab of the channel in **Light Source Control** to show image on **Display** through **Preview** and press  to save.





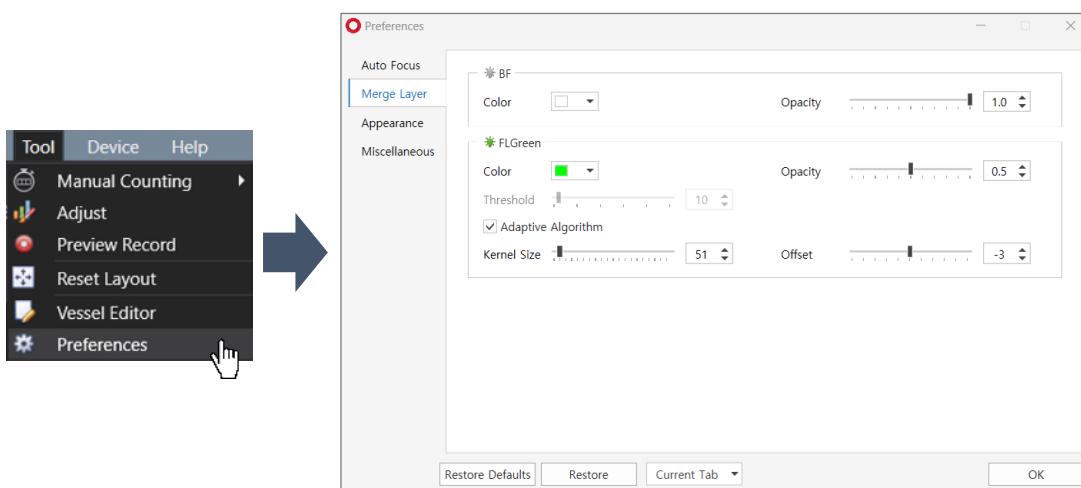
<BF channel image>



<FL (green) channel image>

### 6.2 Merge image

Press  (**Merge layer**) for activation, which is displayed as . This merged image will be shown on **Display**. To adjust the image, change the **Merge Layer** setting (**Opacity** and **Threshold**) in the **Preferences** tab of the **Tool** menu in the top left of your screen.



**Celloger® Mini Plus** conducts thresholding for **FL** channel images based on intensity and merges it with the **BF** image. There are two methods for intensity thresholding.

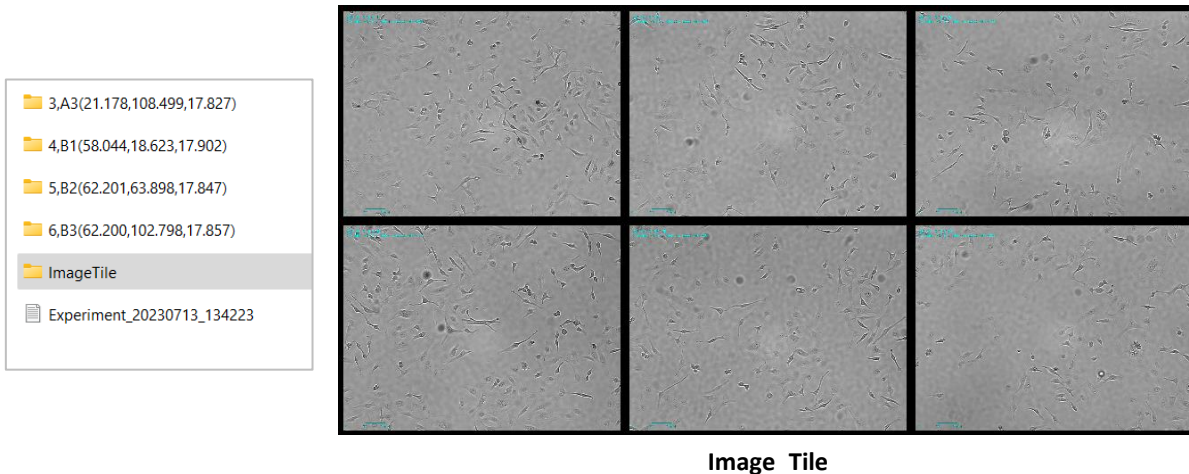
**Method 1) Global thresholding:** Thresholding is executed based on the overall intensity of the image. Adjust the parameter of **Threshold** while looking at the mask of an image, make sure to that **Adaptive Algorithm** box is unchecked.

**Method 2) Local adaptive thresholding:** Divide the image into local areas and thresholding is conducted by comparing relative intensity among these areas. Adjust parameters of the **Kernel Size** and **Offset** while looking at the mask of the image, with the **Adaptive Algorithm** box checked. (**Kernel Size** indicates size of local and **Offset** indicates the relative offset of the threshold within the local area.)

**Note.** When the fluorescence areas are distorted or clustered in one place, **Global thresholding** function is recommended; and when the fluorescence areas are evenly distributed, **Local adaptive thresholding** function is recommended.

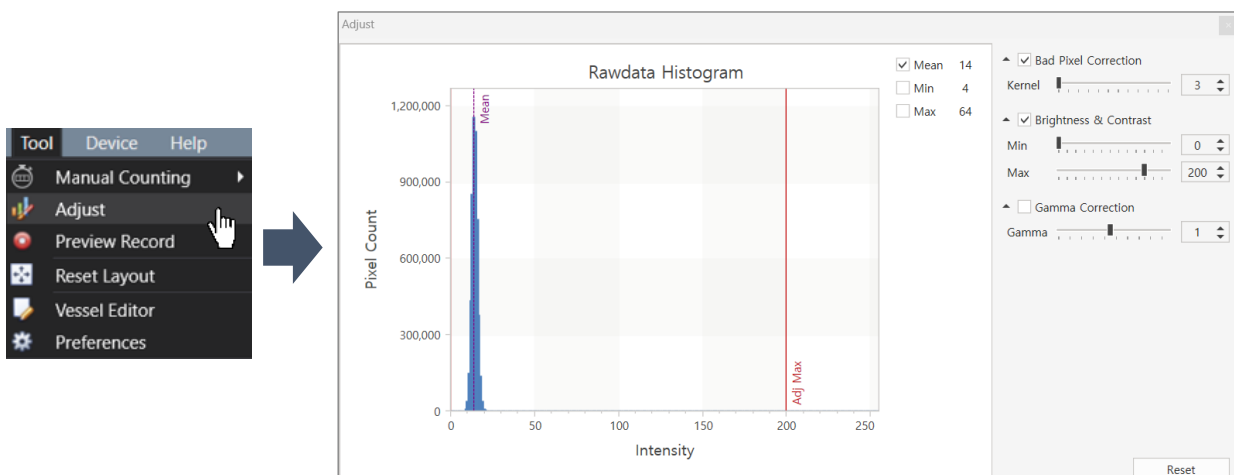
### 6.3 Image Tile

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

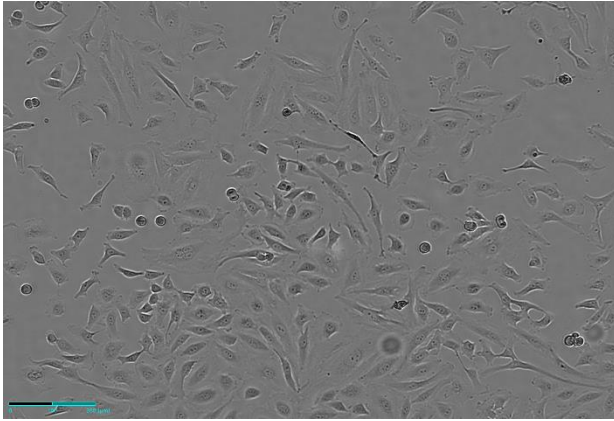


### 6.4 Image adjustment

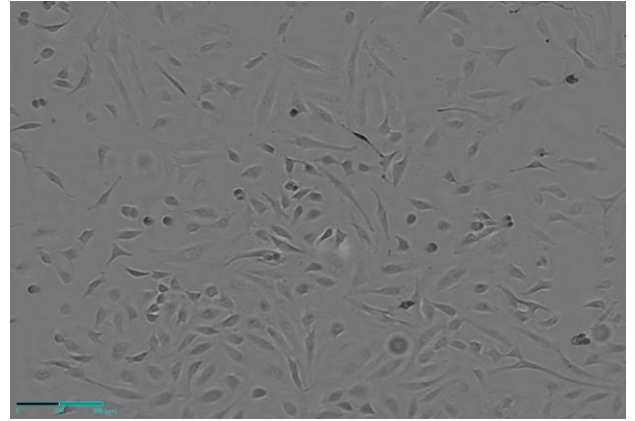
Adjust the image using parameters (Kernel size, brightness, contrast, gamma) in **Adjust** in the **Tool** menu, and save the modified image.



- ✓ **Bad Pixel Correction:** As a correction for defective pixels, the image is divided into pixels. Values that significantly stand out from a certain threshold between adjacent values are then smoothed or blurred.



<Before Bad Pixel Correction>

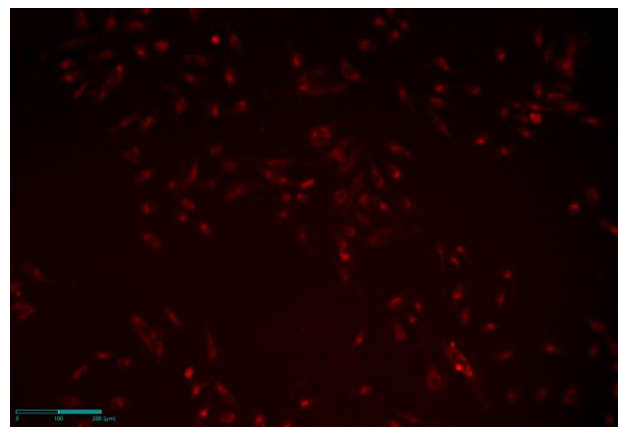


<After Bad Pixel Correction>

- ✓ **Brightness & Contrast:** It adjusts the overall brightness and contrast of the image.

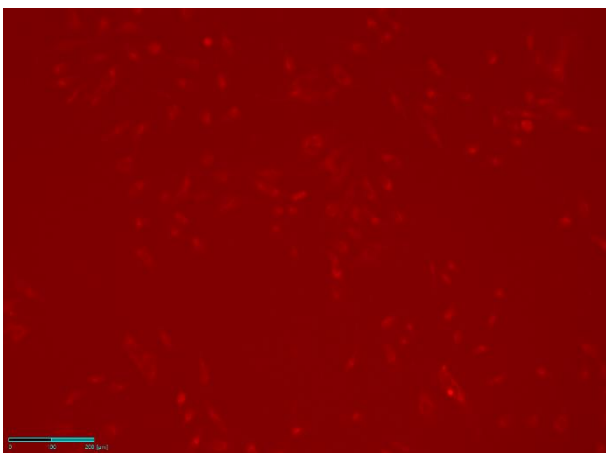


<Before Brightness & Contrast>

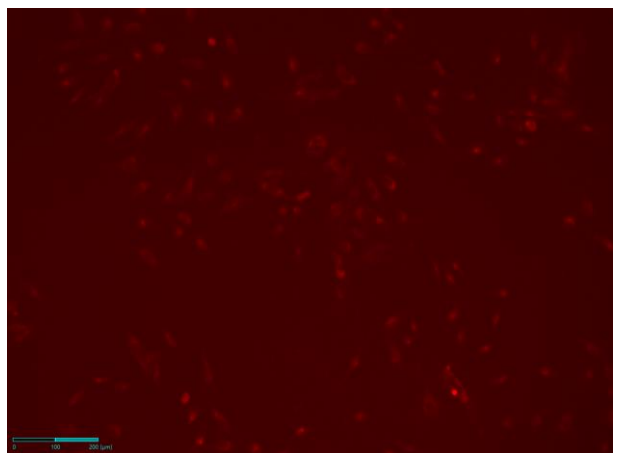


<After Brightness & Contrast>

- ✓ **Gamma Correction:** It is the process of adjusting the brightness of an image so that the colors appear correctly on the screen.



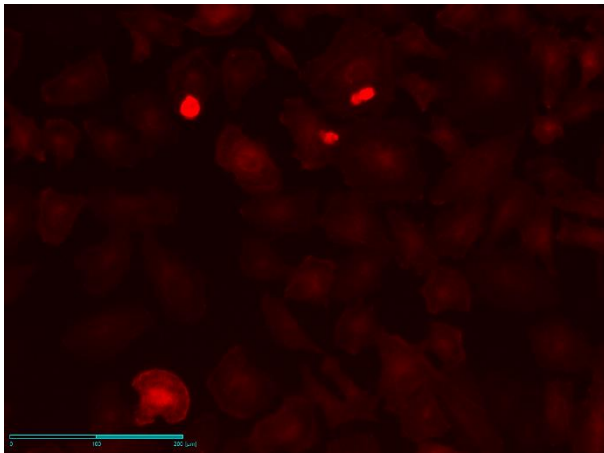
<Before Gamma Correction>



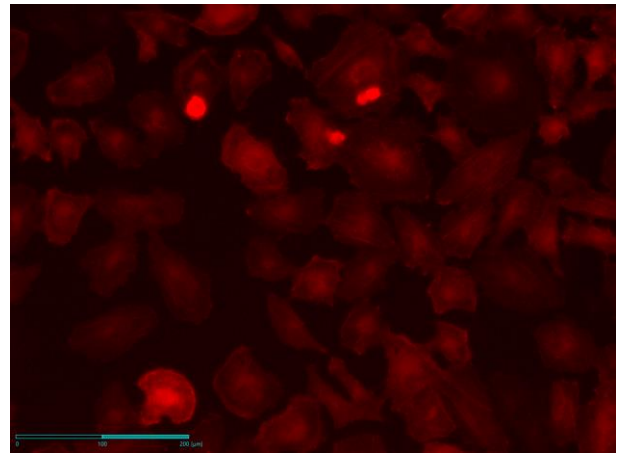
<After Gamma Correction>



- ✓ **Histogram Equalization:** It is a function that allows image where the histogram is concentrated in a specific area to have the histogram distributed evenly over the entire area.

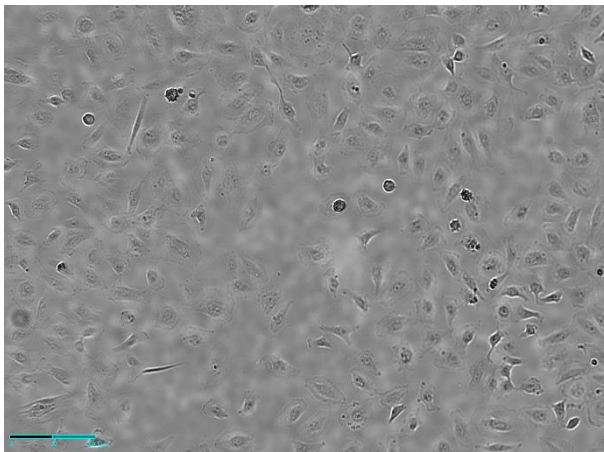


<Before Histogram Equalization>

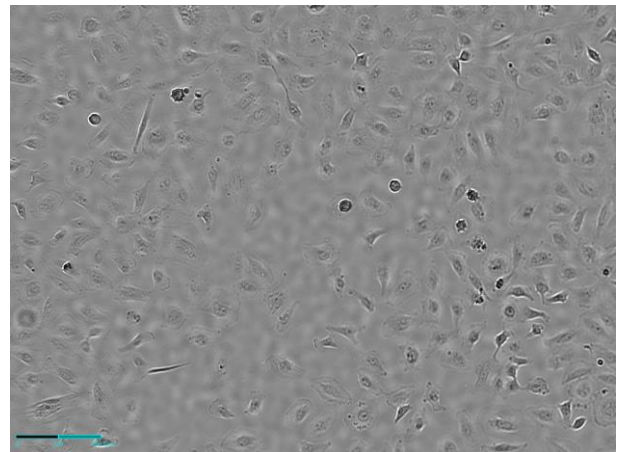


<After Histogram Equalization>

- ✓ **FFC (Flat Field Correction):** It is a function to uniformly correct the brightness value of the captured image.

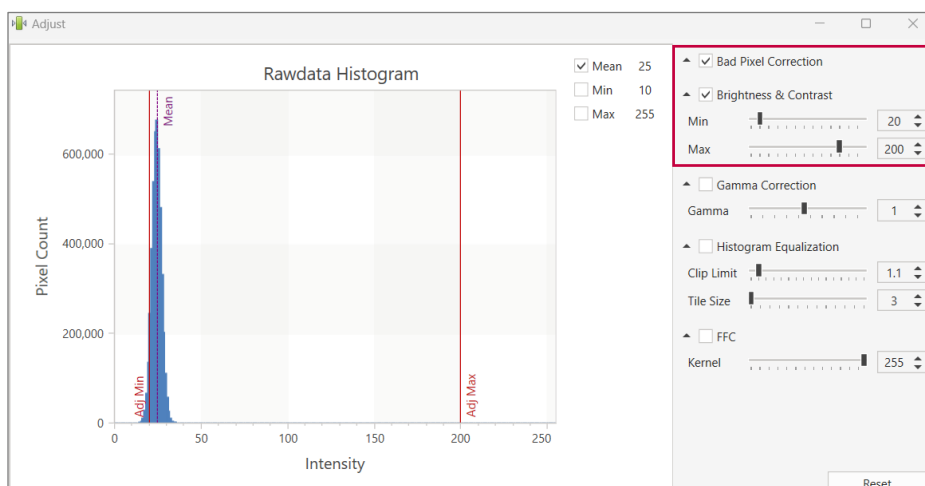


<Before FFC>



<After FFC>

**Note.** In case of fluorescence as shown in the image below, **Bad Pixel Correction** and **Brightness & Contrast** are set by default and may need adjustment.



**Note.** The adjustments are made with captured images, so cells are not affected during scanning.



## 6.5 Preview record

Upon activating **Preview Record** in the **Tool** menu, the image shown on the display is recorded and saved while streaming. The date and time of recording are displayed on the upper left corner of the recorded video.

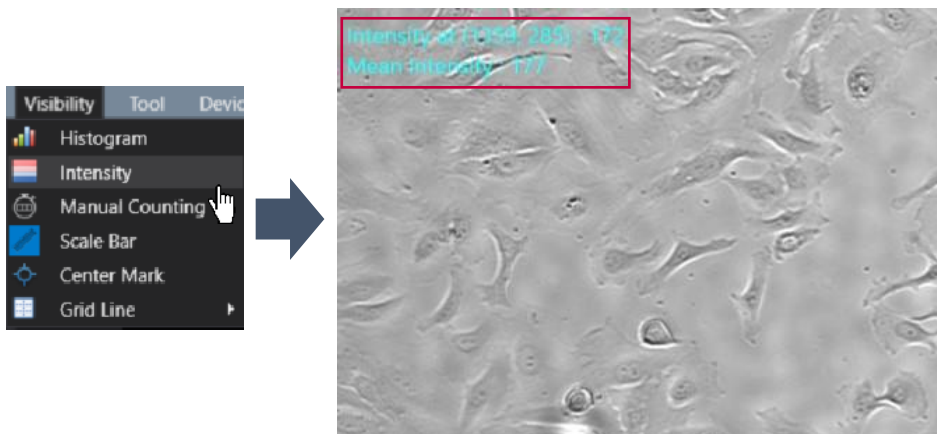


**Note.** When **Preview Record** is activated, the recording continues even when the **Preview** is turned off and on. To end the recording, you must deactivate it in the **Preview Record** tab.

## 6.6 Intensity

### 1) Point Intensity & Mean Intensity

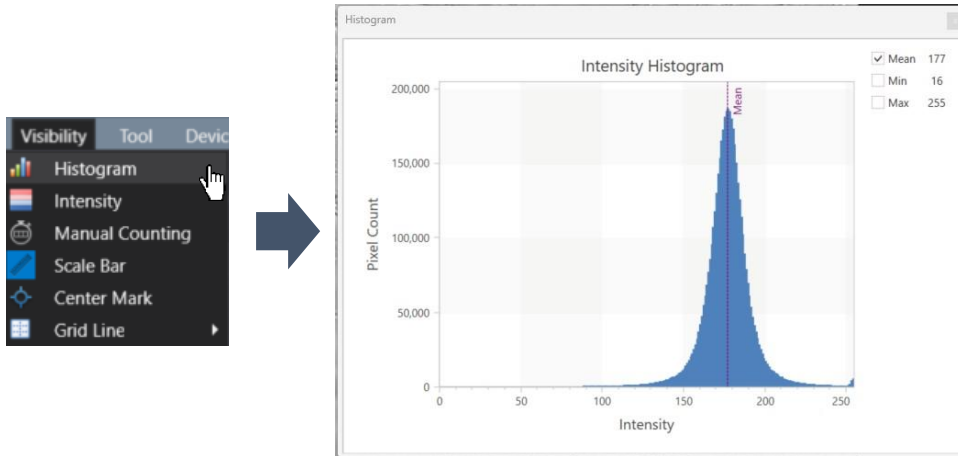
Upon activating **Intensity** in the **Visibility** menu, **Point Intensity**, and **Mean Intensity** information is displayed at the upper left corner of the **Display**.



- ✓ **Intensity at (X coordinate, Y coordinate):** Point intensity value of the X, Y coordinates indicated by the cursor. (If it is in the merge state, the intensities of **BF** and **FL** channel are displayed in order.)
- ✓ **Mean intensity:** Average intensity value of the image displayed on **Display**. (If it is in the merge state, the intensities of **BF** and **FL** channel are displayed in order.)

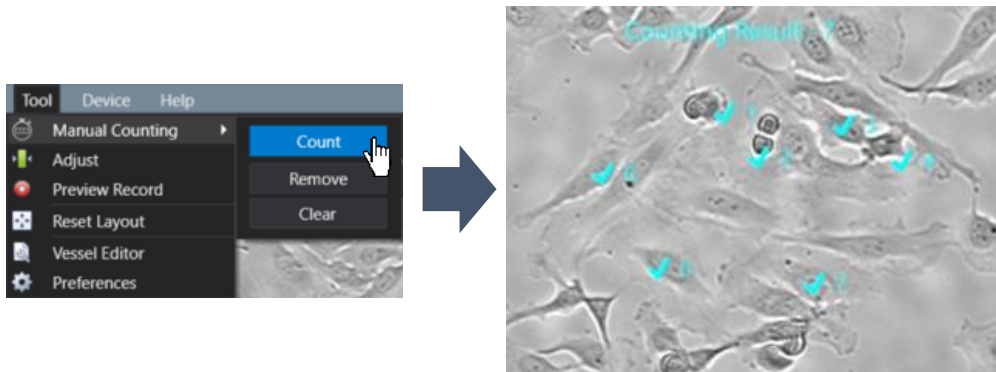
## 2) Histogram

Upon selecting **Histogram** in the **Visibility** menu, an **Intensity Histogram** is shown on **Display**.






## 6.7 Manual Counting

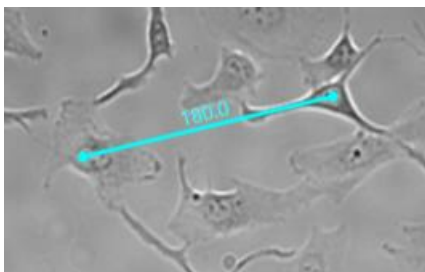
In the **Tool** menu, proceed to **Manual Counting** and select the **Count** option. To start counting, click on the desired area with the mouse. Each clicked area will show a check mark and a corresponding number. The total number of check marks/clicked area is shown at the top as the **Counting Result**.





To remove one selected area at a time, select the **Remove** option in the **Tool** menu's **Manual Counting** tab and click the items to be deleted. Select **Clear** in the same tab to delete all counting marks. To hide them without removing all data, go to the **Visibility** menu and select **Manual Counting**.

## 6.8 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 ( $\mu\text{m}$ ).

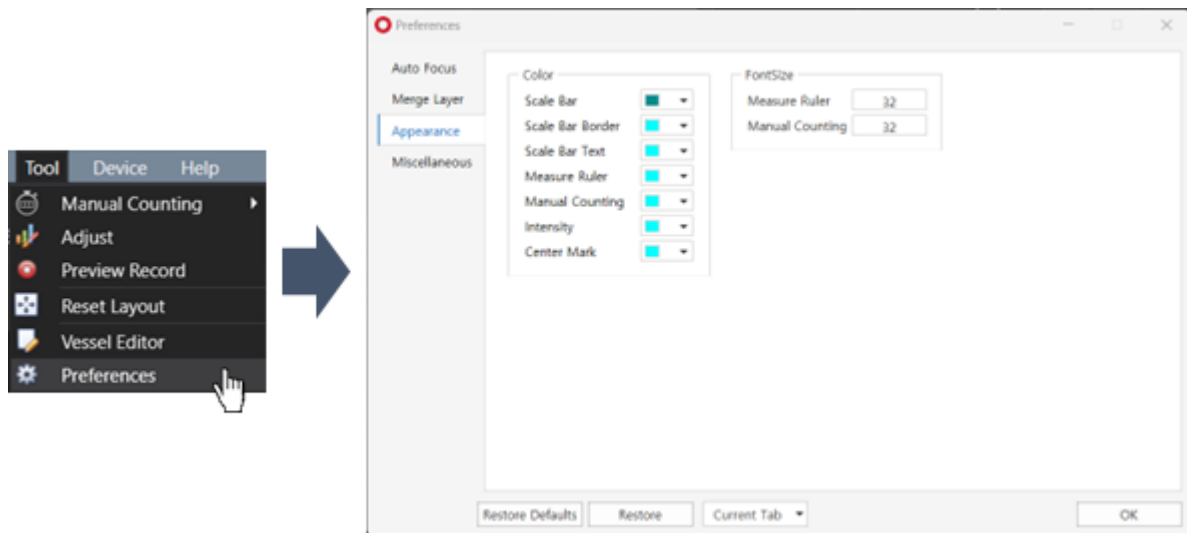


**Note.** To undo one line, select the  icon and click on the lines that are to be erased.

**Note.** To clear all lines created, click the  button.

**Note.** To undo the selected point (after selecting one point), press "Esc" on the keyboard.

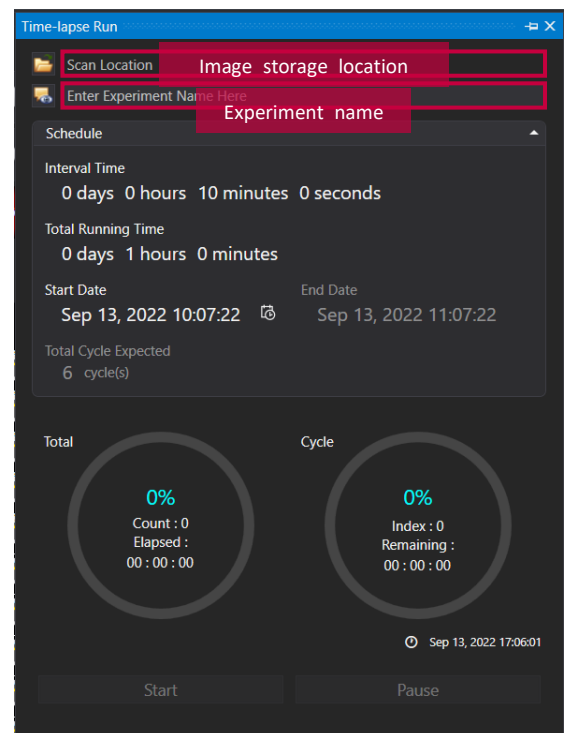
**Note.** The font size of the measurement can be changed in **Preferences** in the **Tool** menu.



## 7. Time-lapse imaging

- ① Select the vessel type (p.33).
- ② Click on **Preview** and adjust the light (p.18) and focus (p.15) by referring to the **Display**. If fluorescence will also be scanned, the quantity of light of fluorescence should also be adjusted.
- ③ Specify the position and focus to be scanned. At this time, select whether or not to execute the autofocusing function (p.39), Z-stacking function (p.40), and stitching function (p.40). If fluorescence is also scanned, the focus of fluorescence should also be adjusted.
- ④ Open **Time-lapse Control** and select a location where the images will be saved and enter the experiment name. If the image storage location is not specified, the file will be saved in the **Scan** folder within the **Celloger Mini Plus Scan App** folder. If the experiment name is not entered, a folder will be automatically created with the date you started scanning.

**Note.** The file name and location address should consist only of English letters, numbers, and some special symbols (avoid characters such as /, :, \*, ?, <, >, |). If the scan does not start, check whether the file name and location address is written correctly.



**Time-lapse Control**

- ⑤ Set the **Interval Time**, **Total Running Time**, and **Start Date** for the time-lapse imaging (p.42). If you want to start immediately, **Start Date** does not need to be adjusted.

**Note.** If **Start Date** is set as a past date, it is automatically updated to the present time.

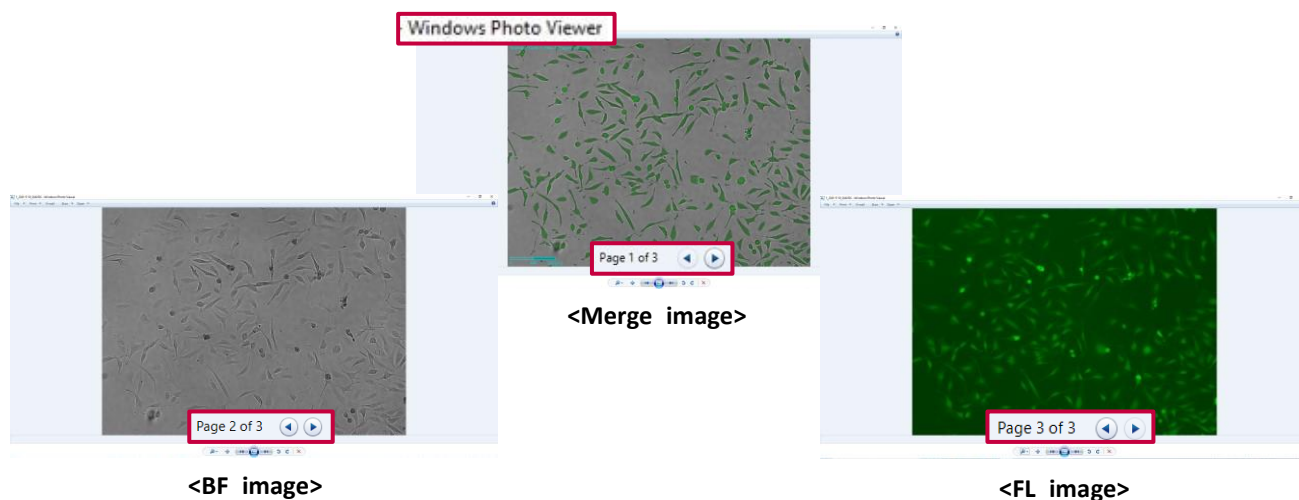
- ⑥ Click on **Start** to begin scanning.

**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 **Celloger Mini Plus Analysis App**.

**Note.** Images are saved as merge image and raw image. A merged image combines both Brightfield (**BF**) and Fluorescence (**FL**) images, displaying scanning information such as a scale bar and scanning time.

**Note.** Raw images are saved as “*Tiff files*” by default. If fluorescence was also captured, the saved images are stacked into one file, which will contain **BF** image and **FL** images. These stacked images can be viewed with the windows basic image viewer “*Windows Photo Viewer*”.

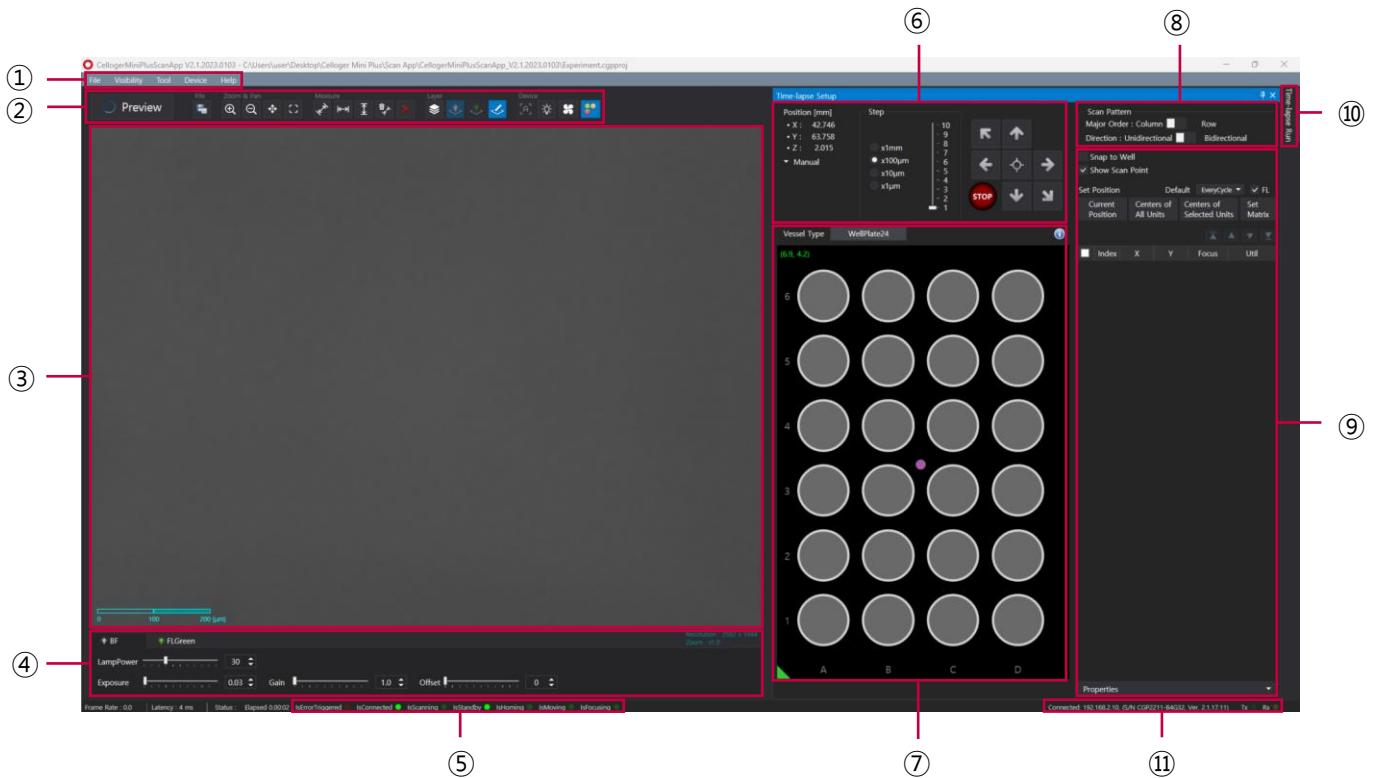


**[Z-stacking]** When an image is captured using only the **BF** channel, the first 2 images are the merged image (with measurements) and raw image of the reference position, followed by Z-stacked images (from low to high Z-axis). When an image is captured using both **BF** and **FL** channels, the first 3 images are the merged image and raw images of the reference position, followed by Z-stacked images of **BF** and **FL**, which are shown alternatively from the low to high Z-axis.

**[Stitching]** When an image is captured using only the **BF** channel, or both **BF** and **FL** channels, the first 2 or 3 images are merged image and raw images (same as Z-stacking). Followed by the images in scanning order (refer to p.41 for the stitching scanning order).

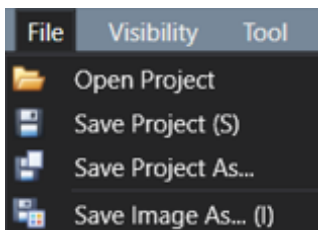
## II. Software description

Upon opening the **Celloger Mini Plus Scan App** and completing pre-setup (refer to p.10~12), the following main page is displayed.



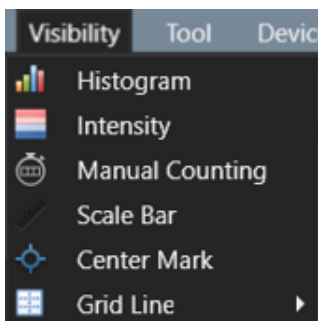
### ① Menu bar

#### [File]



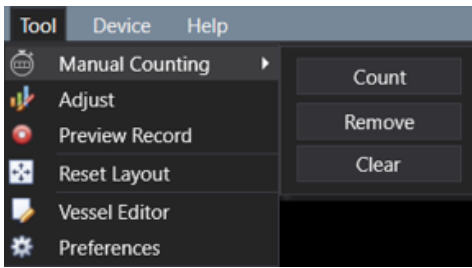
- ✓ **Open Project:** Import a previously saved project.
- ✓ **Save Project:** Save settings (Light intensity, Focus position, Schedule, etc.) on current project. Keyboard shortcut "*Ctrl + S*" can be used.
- ✓ **Save Project As...:** Create a new project with current settings.
- ✓ **Save Image As...:** Save the image shown on the **Display**. Keyboard shortcut "*Ctrl + I*" can be used.

#### [Visibility]



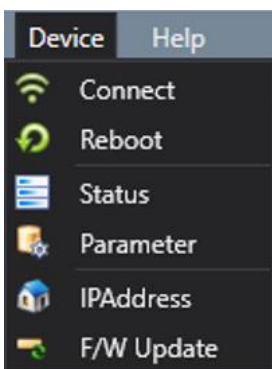
- ✓ **Histogram** (p.26): Show/hide intensity histogram of an image.
- ✓ **Intensity** (p.25): Show/hide point and mean intensity of an image.
- ✓ **Manual Counting:** Show/hide "Manual Counting" function.
- ✓ **Scale Bar:** Show/hide scale bar on the bottom left of **Display**.
- ✓ **Center Mark:** Show/hide a cross-shaped mark at the center of **Display** for reference.
- ✓ **Grid Line:** Show/hide grid line on **Display**. Number of grid lines can be changed.

## [Tool]



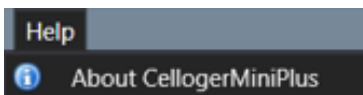
- ✓ **Manual Counting** (p.26): Count the points clicked by user.
  - ① **Count**: Add a point for counting.
  - ② **Remove**: Delete a clicked point.
  - ③ **Clear**: Delete all points.
- ✓ **Adjust** (p.22): Adjust an image.
- ✓ **Preview Record** (p.25): Record the streaming image.
- ✓ **Reset Layout** (refer to **Note** in p.43): Restore the software layout to its original state before any modifications.
- ✓ **Vessel Editor** (p.79): The specifications of the well plate are customizable.
- ✓ **Preferences**: Set **Auto Focus** (p.15~16), **Merge Layer** (p.21), **Appearance**, and **Miscellaneous**.

## [Device]



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

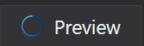
## [Help]



- ✓ **About Celloger Mini Plus**: Display app information.

## ② Toolbox

### [Preview]




 : Turns streaming on/off. The live image is shown based on the selected channel such as **BF**, **FL green**, and **FL red** the **Light Source Control** section of the software.

- ✓  : Streaming on
- ✓  : Streaming off

### [File]

 : Save the image of the **Display**.

### [Zoom & Pan]

- ✓  : Zoom-in (Digital zoom). Keyboard shortcut "*Ctrl + mouse scroll wheel*" can be used.
- ✓  : Zoom-out (Digital zoom). Keyboard shortcut "*Ctrl + mouse scroll wheel*" can be used.
- ✓  : Pan.

**Preview On:** When **Pan** is clicked and drag the screen using mouse, camera moves and shows the real-time image on the screen.

**Preview Off:** When **Pan** is clicked, it works only in Zoom-in status where the camera doesn't move and shows only the image within the display.






**Note.** It can be used by clicking and dragging the screen while pressing the "Shift" key.

**Note.** If you double-click a location within the display while pressing "Shift" key, the camera moves to center of selected position on the screen.






- ✓ : Return the zoomed image to its original state at once.

### [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.
- ✓ : Delete the selected measurement.
- ✓ : Delete all measurements.

### [Layer]

- ✓ : **Merge Layers**; It shows the merged image of **BF** and **FL** channels.
- ✓ : **BF** image layer
- ✓ , : **FL (FL green or 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 **Preferences** (refer to p.21).

### [Device control]

- ✓ : Execute autofocusing depending on the autofocusing settings in streaming status (refer to p.15~16 for autofocusing).
- ✓ : Turns on the white light in LED status indicator of the connected device.
- ✓ : Turns the fan on/off.
- ✓ : Turns the LED status indicator on/off.

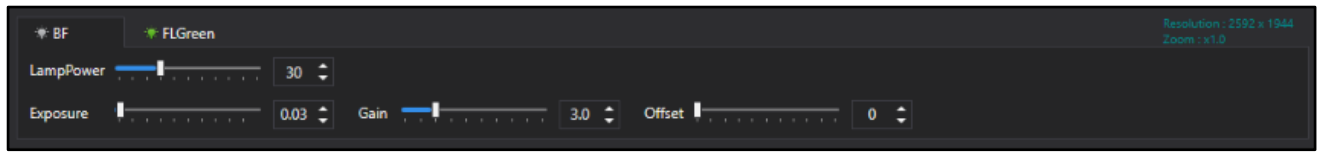
## ③ Display

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



#### ④ Light Source Control

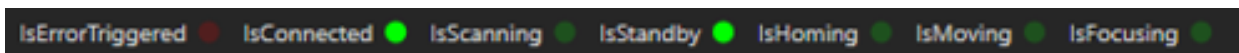
The tabs in **Light Source Control** are composed of **BF**, **FL green**, and **FL red**. The configuration of tabs is set depending on the light options of **Celloger® Mini Plus**.



- ✓ **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 30. This is a parameter associated with the contrast of the image. Be careful as noise becomes higher when gain value is high.
- ✓ **Offset (black level)**: Brightness of dark range of image. Be careful as contrast becomes low when offset value is high.

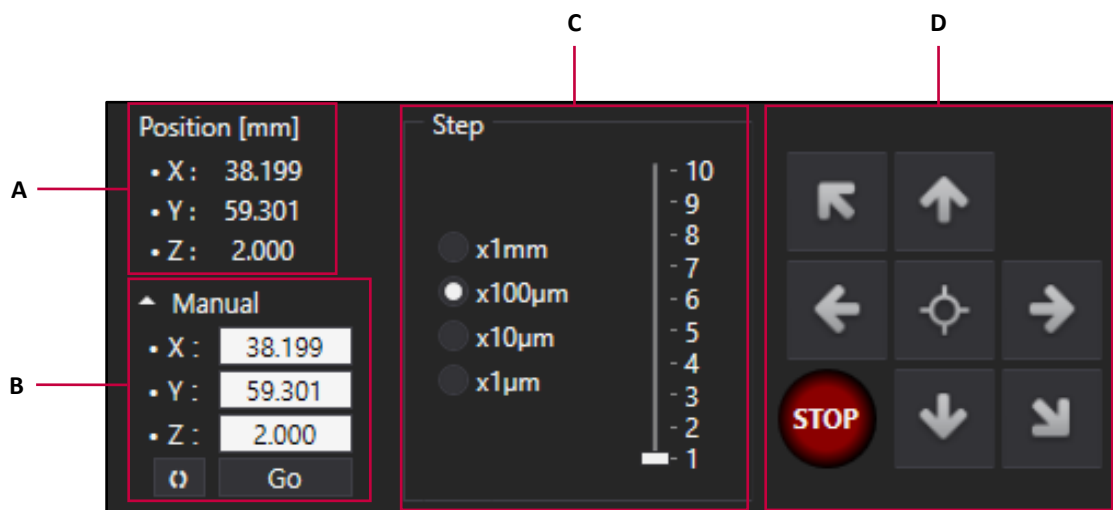
**Note.** For better results, it is recommended to adjust the parameters as follows. It is not recommended to increase the **LampPower** to high value as phototoxicity may occur. Moreover, it is not recommended to leave the **Preview** on state for a prolonged period as photobleaching may occur.


#### ⑤ Device status



It shows the status of connected equipment. When **IsErrorTriggered** lights in red, it means that an error has occurred. When others light in green, it means they are in progress. In the case of the above image, the green lights for **IsConnected** and **IsStandby** are turned on, which means the device is connected to the PC and is in standby state (not scanning).

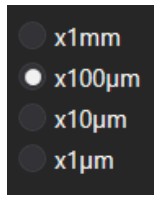
#### ⑥ Jog Control



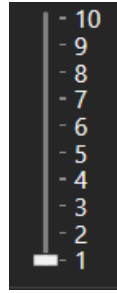
- A. Current X, Y, and Z-stage coordinate
- B. Upon pressing **Go** after entering the desired coordinates in the box, the stage is moved according to the set coordinates. Upon pressing , current coordinates are entered in the boxes.



- C. Distance moved upon pressing the **Jog button (D)**, also referred to as **Step distance**. Adjustments also be done using “\*”, “/” when “Num Lock” is off on the number pad of the keyboard.



: Step distance unit



: Step distance

**D. Jog button**

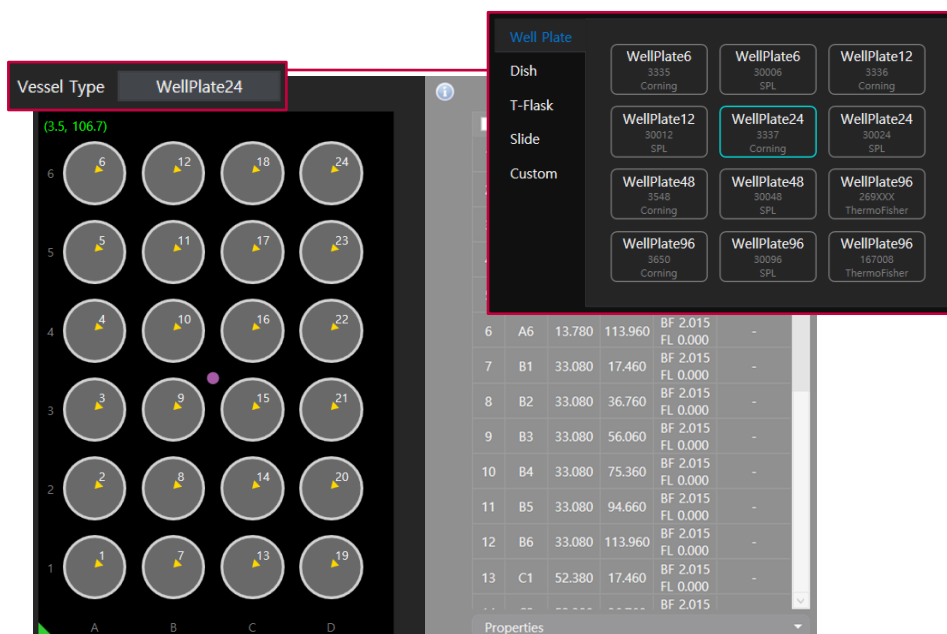
- ✓ : Move to the center of the stage.
- ✓ : Move the stage left and right (X-axis). Adjustments can also be done using keyboard arrows when “Num Lock” is off.
- ✓ : Move the stage up and down (Y-axis). Adjustments can also be done using keyboard arrows when “Num Lock” is off.
- ✓ : Move the stage up and down (Z-axis). Adjustments can also be done using “+”, “-” on the number pad of the keyboard when “Num Lock” is off.
- ✓ : Stop the stage.

⑦ **Vessel & Plate panel**

**[Vessel type]**

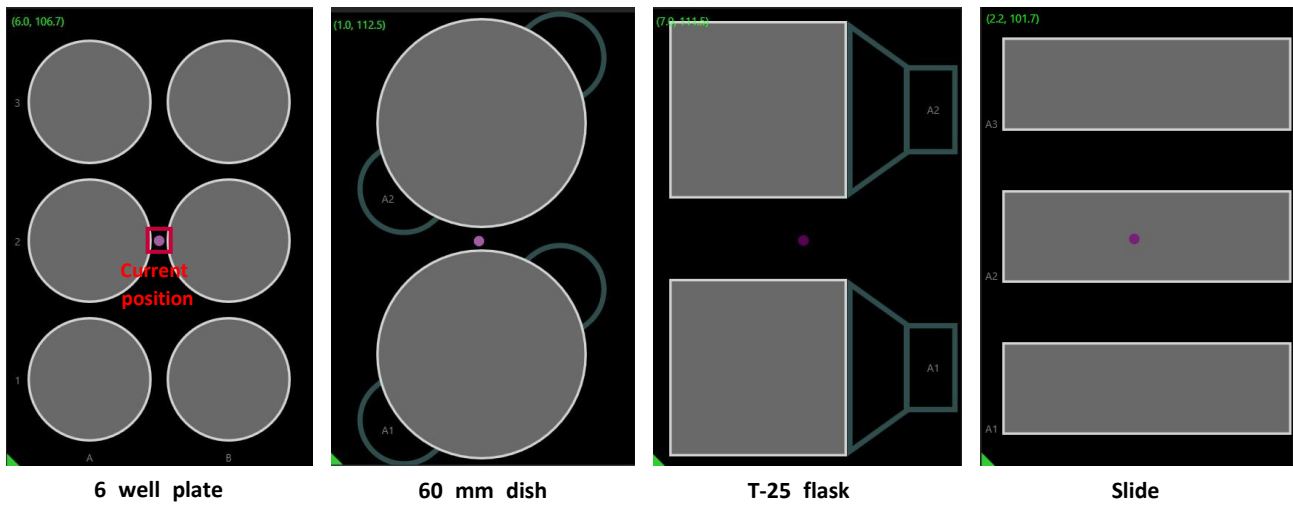
This is the tab where the vessel type is selected. Well plate (6, 12, 24, 48, 96 wells), Dish (35 mm, 60mm, 90 mm), slide, and T-flask (25 cm<sup>2</sup>, 75 cm<sup>2</sup>) can be selected.

**Note.** The specifications of the well plates are different for each brand. On the **Celloger Mini Plus 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 plate is used, it is recommended to change the vessel specifications in the **Vessel Editor** under the **Tool** menu (refer to p.79 for **Vessel Editor**).



## [Plate panel]

It shows the vessel selected under **Vessel type** as an image, and the current position of the camera on the stage (refer to p.35 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 the camera to the center of the vessel, double-click on the vessel image in the **Plate panel** (In the case of a well plate, it will move to the center of the selected well). To move to a position other than the center, double-click on the desired position while pressing "Ctrl" in the keyboard.

## ⑧ 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).

## ⑨ Position Control

The screenshot shows the Position Control software interface. Callout A points to the 'Snap to Well' and 'Show Scan Point' checkboxes. Callout B points to the 'Set Position' section, which includes buttons for 'Current Position', 'Centers of All Units', 'Centers of Selected Units', and 'Set Matrix'. Callout C points to the navigation arrows (up, down, left, right). Callout D points to the table of well positions. Callout E points to the 'Properties' section, which includes settings for BF Focus, FL Focus Offset, Z-Stacking, and Stitching.

Index	X	Y	Focus	Util	
1	A1	13.780	17.460	BF 2.015 FL 0.000	-
2	A2	13.780	36.760	BF 2.015 FL 0.000	-
3	A3	13.780	56.060	BF 2.015 FL 0.000	-
4	A4	13.780	75.360	BF 2.015 FL 0.000	-
5	A5	13.780	94.660	BF 2.015 FL -0.020	ZS (-2,3)
6	A6	13.780	113.960	BF 2.015 FL 0.000	-
7	B1	33.080	17.460	BF 2.015 FL 0.000	-

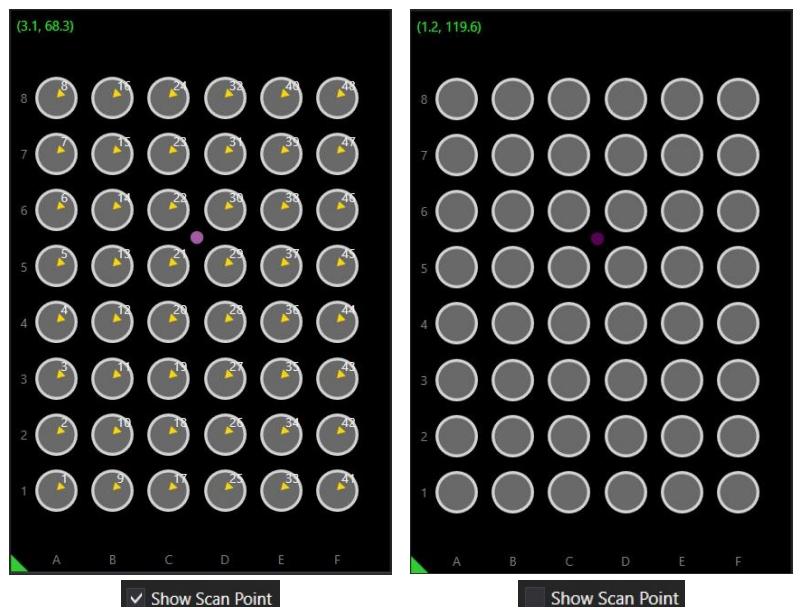
### A. Snap to Well & Show Scan Point

#### [Snap to Well]

If checked, it moves from well to well using the arrows on the keyboard when “Num Lock” is off. It can only be used when the vessel type selected is a well plate.

#### [Show Scan Point]

If checked, the position specified for scanning is indicated on the **Plate panel** as shown in the image below.



## B. Set Position

### [Default]

After setting the autofocusing frequency (p.39) and fluorescence channel in **Default**, the set **Default** value is automatically applied in every set position.

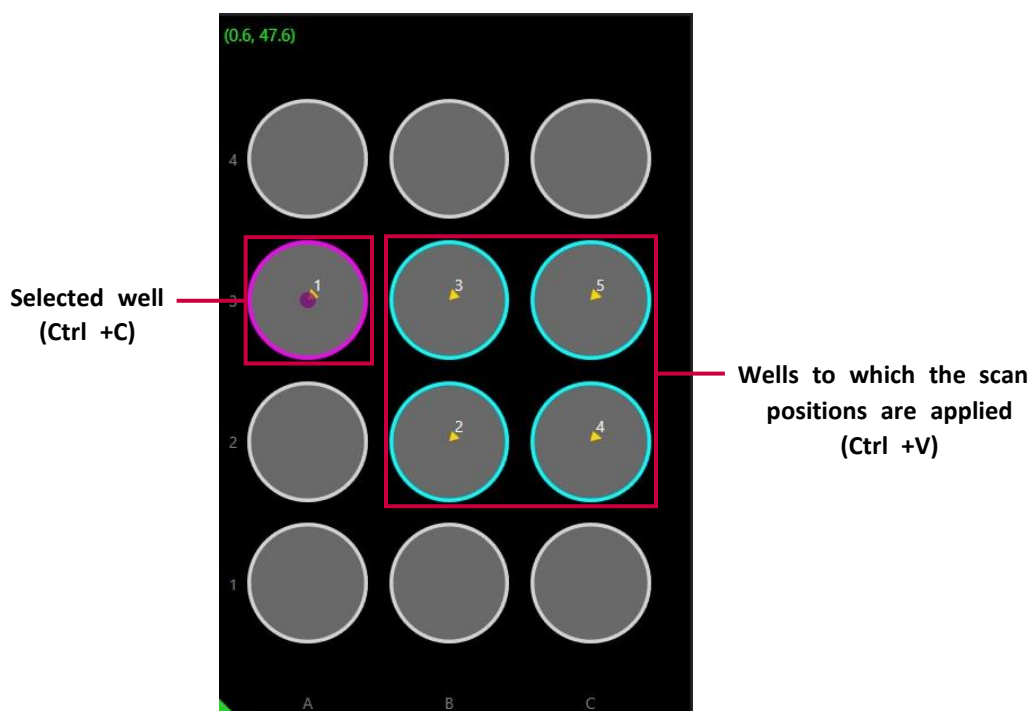
**Note.** If you want to execute the autofocusing function with a frequency other than the default at specific scan positions, you can select and change the desired position in the scan table (**D**). Fluorescence channel can also be changed in the same way.

### [Set Position]

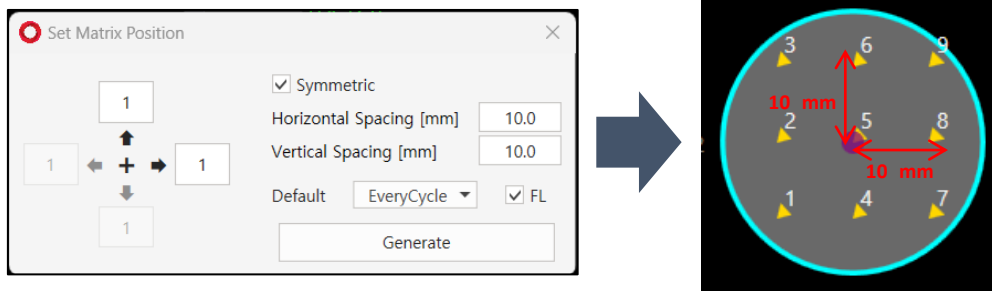
Set the position you want to scan.

- ✓ **Current Position:** Set the current position as the scan position.
- ✓ **Centers of All Units:** Set the centers of all vessels as the scan position.
- ✓ **Centers of Selected Units:** Set the centers of the selected vessels as the scan position. At this time, the wells are selected by dragging the mouse (It can be only used when the vessel type selected is a well plate).

**Note.** When the selected vessel type is a well plate, it is possible to select a specific well from the plate panel (*Ctrl + C*) and apply the scan positions to other wells (*Ctrl + V*). As shown in the image below, the selected well is highlighted in purple, and the other wells to which the scan positions are applied are highlighted in light blue. To deselect a well, click the selected well and press "*Ctrl + Q*".



- ✓ **Set Matrix:** Set scan positions at the top, bottom, left, and right based on the current position. For example, the image below shows that positions 10 mm above, below, left, and right are designated as scan positions based on the current location.



### C. Position reorder



It allows to change the scan order at the same **Index**.

- ✓ : Move to the first scan order.
- ✓ : Move to the previous scan order.
- ✓ : Move to the following scan order.
- ✓ : Move to the last scan order.

### D. Scan table

It shows the scanning order and their coordinates, as well as Z-stacking and stitching values.

Index	X	Y	Focus	Util	
1	A1	13.780	17.460	BF 2.015 FL 0.000	-
2	A2	13.780	36.760	BF 2.015 FL 0.000	-
3	A3	13.780	56.060	BF 2.015 FL 0.000	-
4	A4	13.780	75.360	BF 2.015 FL 0.000	-
5	A5	13.780	94.660	BF 2.015 FL -0.020	ZS (-2,3)
6	A6	13.780	113.960	BF 2.015 FL 0.000	-
7	B1	33.080	17.460	BF 2.015	-

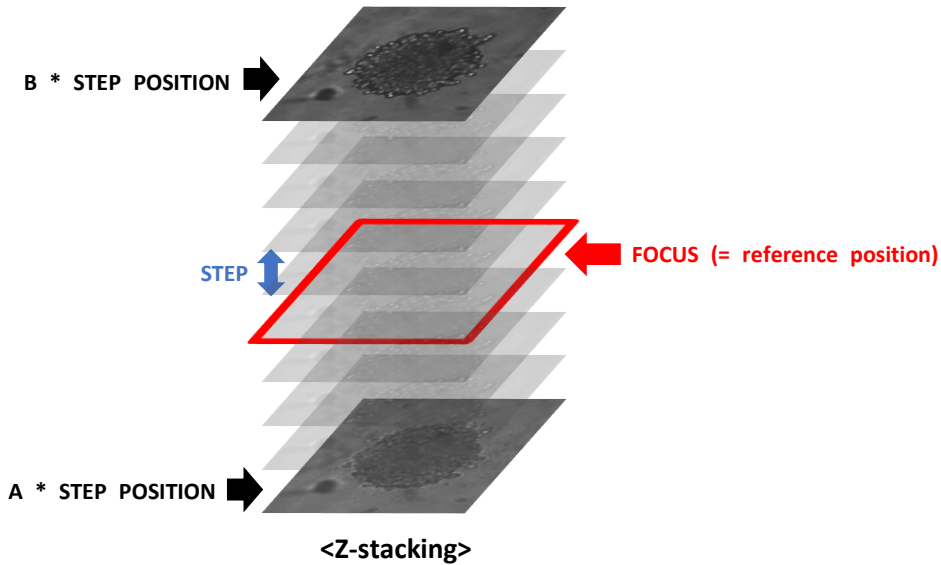
- ✓ **Index:** Well number of the well plate (If it is not a well plate, all indexes are indicated as A1).
- ✓ **X:** X-axis coordinate
- ✓ **Y:** Y-axis coordinate
- ✓ **Focus:** Z-axis coordinates of **BF** and **FL** channels

**BF** channel: Shows the focus of Z-axis coordinates when autofocusing function is off (=None) or it is the median coordinate when autofocusing function is on (=First cycle or Every cycle).

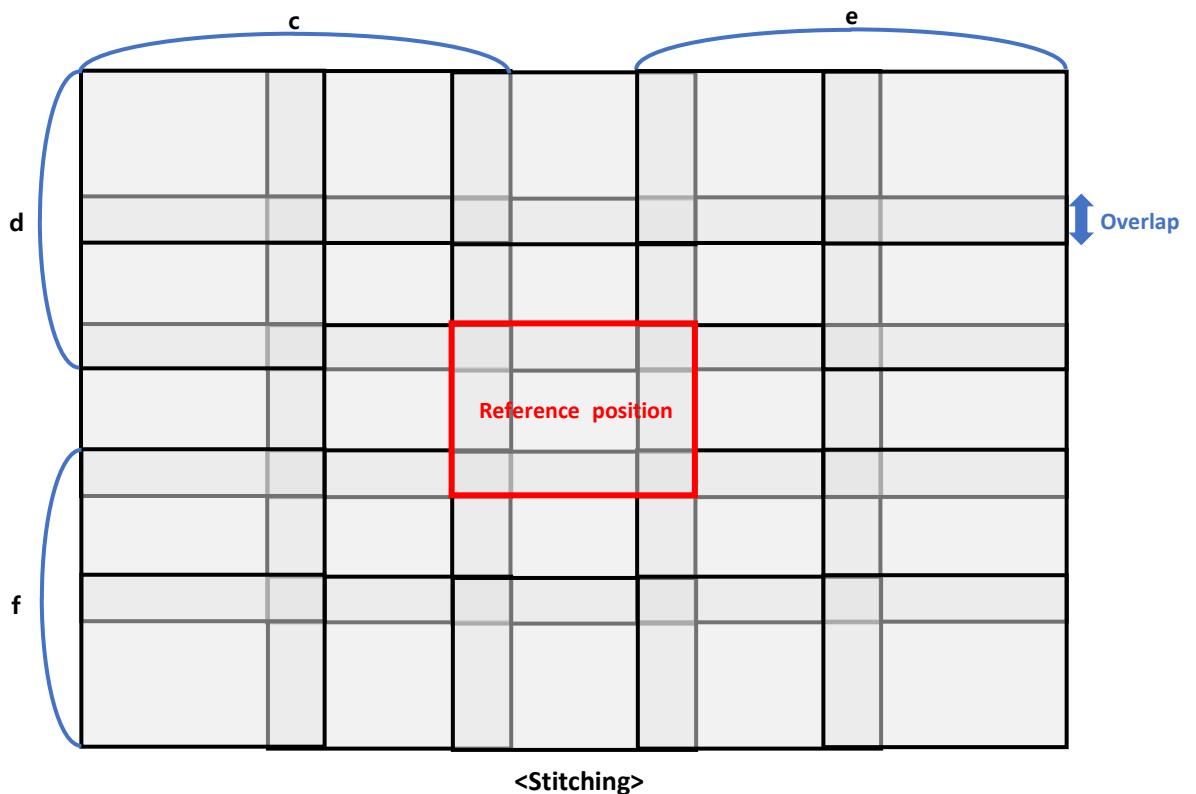
**FL** channel: Shows the focus difference from the **BF** channel. When **FL** channel has lower focus than **BF** channel, '-' is added, '0.000' appears when with the same focus, and only numbers are displayed when **FL** channel has higher value than that of the **BF** channel.

✓ **Util:** It is the Z-stacking or stitching value where **ZS** stands for Z-stacking while **ST** stands for stitching.

In case of **ZS(a, b)**, images are taken at intervals set in **Step** 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** (refer to p.40 for **Z-stacking**).



On the other hand, **ST(c, d, e, f)** stands for stitching. Based on the set position (= reference position), the image is taken with overlapping area set in **Overlap** while **c, d, e, f** stands for the range of stitched image left, up, right, and downwards respectively from the reference position that are set in **Range**.



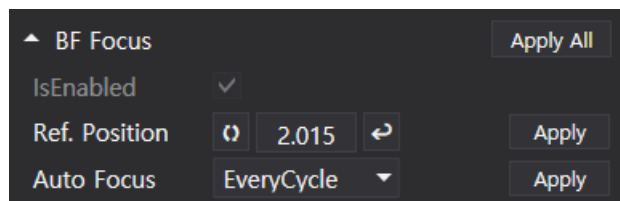
**Note.** For a detailed description of Z-stacking and stitching, refer to the example in Z-stacking and stitching on p.40~41.

## E. Properties

It shows the **Focus**, **Auto focus frequency**, and **FL** channel status of the selected position in the scan table (indicated in green). You can change to options other than the **Default** values and set the focus of fluorescence imaging. Furthermore, the Z-stacking and stitching settings can be adjusted after selecting whether or not to perform the Z-stacking and stitching.


### [BF Focus]

It shows the Z-axis coordinates of the **BF** channel to be captured while performing the time-lapse imaging. Moreover, the autofocusing frequency can be adjusted in the **BF Focus** section.

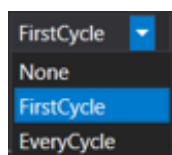


✓ **Ref. Position:** Position of the **BF** channel for time-lapse. **2.015** is the location of Z-axis coordinates of the **BF** channel.

✓ : Upon clicking, the current Z-axis coordinates is entered into the box next to it.

✓ : Upon clicking, the Z-axis position moves according to the coordinates shown on the left.

✓ **Auto Focus:** It shows the **autofocusing frequency** selected in **Default (p.)** and it can be changed.



1) **None:** Autofocusing is not performed during time-lapse imaging, and the images are taken based on the set coordinates.

2) **First Cycle:** Autofocusing is carried out in the initial cycle of time-lapse imaging, while the subsequent cycles are imaged based on the Z-axis coordinates designated in the first cycle.

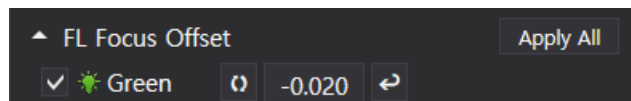
3) **Every Cycle:** Autofocusing is conducted in each cycle of time-lapse imaging.


✓ **Apply, Apply All:** Apply the Z-axis coordinates and autofocusing frequency settings.

**Note.** After changing the Z-axis coordinates and autofocusing frequency, the **Apply** button must be clicked on for each to apply the changes. Click the **Apply All** button to apply all functions at once.


### [FL Focus Offset]

It shows the Z-axis coordinates of the **FL** channel to be captured while performing the time-lapse imaging. In **FL** channel, the value shown is the difference between from the Z-axis of the **BF** channel. To capture the **FL** channel on the same coordinates or the **BF** channel, enter "0".



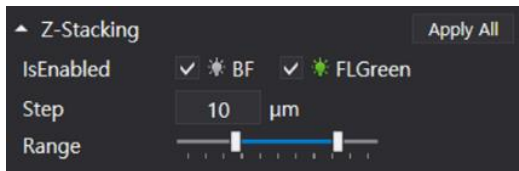
✓  **Green**: It shows the **FL** channel status. When checked, the fluorescence focus can be set. When unchecked, fluorescence imaging is not performed during the time-lapse.

✓ : Upon clicking, the current Z-axis coordinates is entered into the box next to it.

✓ : Upon clicking, it moves to the recalculated position by the value written on the left from the coordinates of the **BF** channel.

**Note.** After changing the Z-axis coordinates, the **Apply All** button must be clicked to apply the changes.

## [Z-stacking]

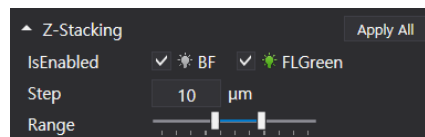


- ✓ **IsEnabled:** Select whether or not to run the Z-stacking function in **BF** and **FL** channels by clicking the boxes.
- ✓ **Step:** When executing the Z-stacking function, it sets the interval at which the Z-axis is to be imaged and it can be set up to 1,000 μm.
- ✓ **Range:** It sets the range for executing the Z-stacking function, and it can be adjusted from -10 to 10. The Z-stacking function can be executed from “-10 \* **Step** μm” to “10 \* **Step** μm” based on **Focus**.

## <Example>

Index	X	Y	Focus	Util	
1	A1	13.780	17.460	BF 2.015 FL 0.000	-
2	A2	13.780	36.760	BF 2.015 FL 0.000	-
3	A3	13.780	56.060	BF 2.015 FL 0.000	-
4	A4	13.780	75.360	BF 2.015 FL 0.000	-
5	A5	13.780	94.660	BF 2.015 FL 0.000	ZS (-2,3)
6	A6	13.780	113.960	BF 2.015 FL 0.000	-
7	B1	23.080	17.460	BF 2.015	-

Scan table



Properties (Z-stacking)

In the above example, the **Focus** of the position selected in the **Scan table** is 2.015 and the **Util** is ZS(-2,3). Along the Z-stacking channels set are **BF** and **FL green**, and the **Step** is 10 μm. This means taking images from 20 (= 2 x 10) μm below to 30 (= 3 x 10) μm above the **Focus** value of 2.015 at every 10 μm. As such, **BF** and **FL green** images are taken with the Z-axis of 1.995, 2.005, 2.015, 2.025, 2.035, and 2.045 at X (13.780) and Y (94.660) axis. The **Util** values of -2 and 3 correspond to the **Range** settings.

## [Stitching]



- ✓ **IsEnabled:** Click the boxes to select whether to run the stitching function in **BF** and **FL** channels.
- ✓ **Overlap:** Set the area of overlap between stitched images, ranging from 20% to 50%.
- ✓ **Range:** Set the range for the stitching function, with options from 1 to 3 (representing up, down, and sideways, respectively). When **Symmetric** is selected, the symmetrical range is set automatically.



<Example>

Index	X	Y	Focus	Util	
1	A1	13.780	17.460	BF 2.015 FL 0.000	-
2	A2	13.780	36.760	BF 2.015 FL 0.000	-
3	A3	13.780	56.060	BF 2.015 FL 0.000	ST (2,2,2,2)
4	A4	13.780	75.360	BF 2.015 FL 0.000	-
5	A5	13.780	94.660	BF 2.015 FL 0.000	-
6	A6	13.780	113.960	BF 2.015 FL 0.000	-
7	B1	33.080	17.460	BF 2.015	-

Scan table

Stitching Apply All

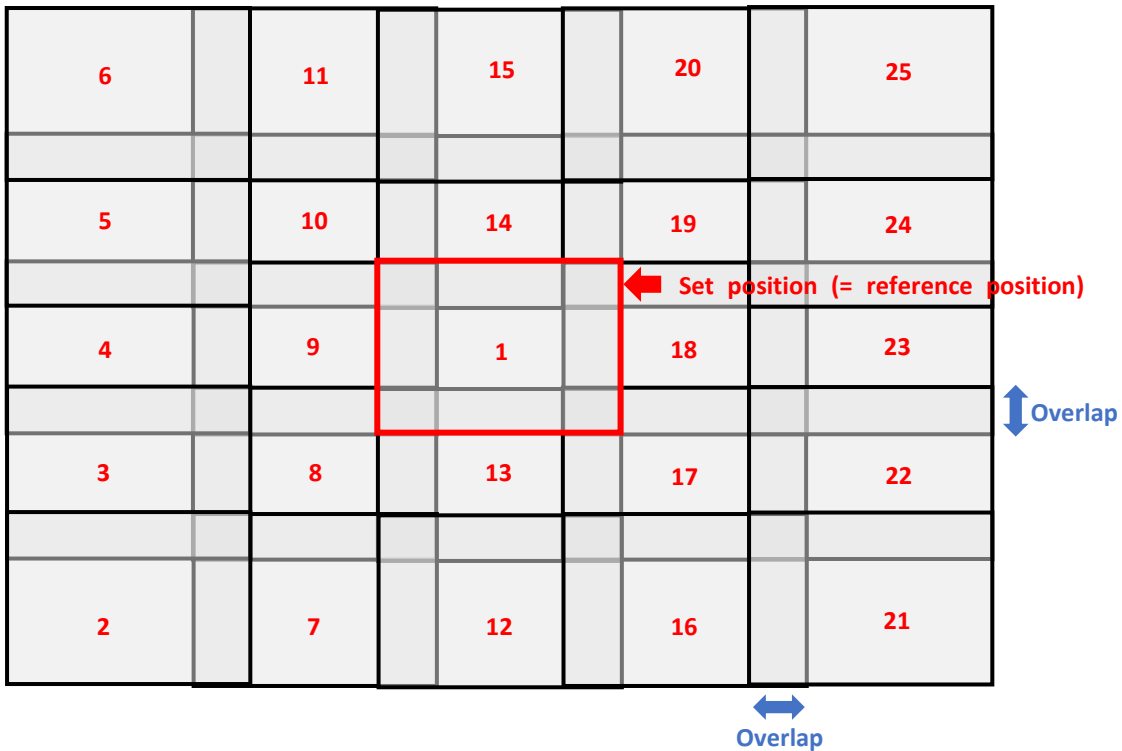
IsEnabled  BF  FLGreen

Overlap [%]

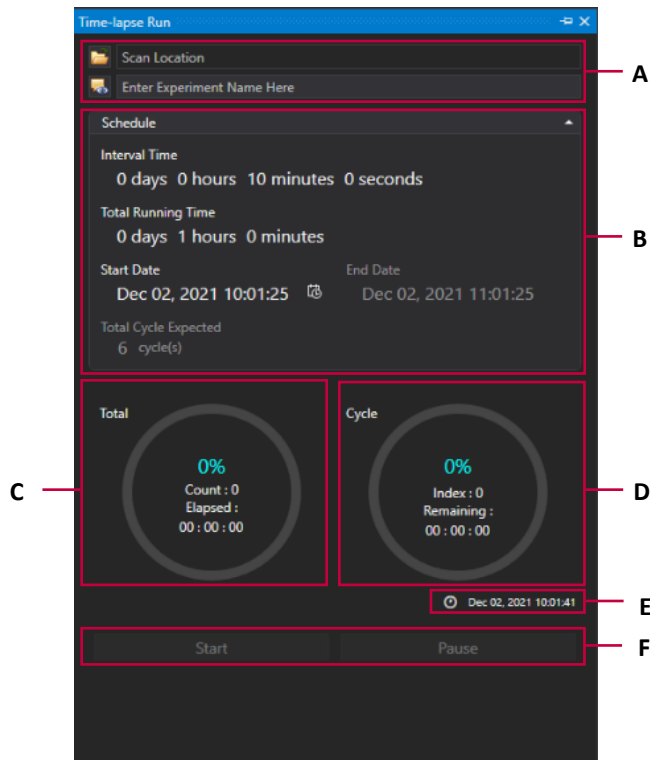
Range   Symmetric



Properties (Stitching)

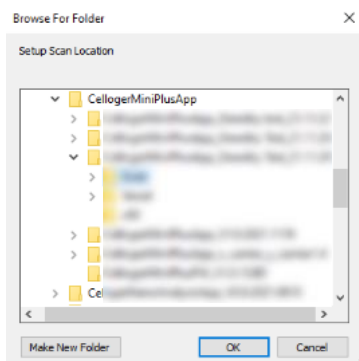
In the above example, the **Util** value of the selects position in the **Scan table** is ST(2,2,2,2) and the channel and **Overlap** settings of **Stitching** in **properties** are **BF** and **FL green**, and 20%. This means that based on the selected position (reference position of X: 13.780, Y: 56.060), two images are captured up, down, left, and right respectively to take the total images of 25 (= 5x5) with overlapping area of 20% as shown in the image below. The imaging sequence starts with the reference position (#1) and proceeds vertically from the bottom left (#2) to the top left (#6), continuing in this order until it reaches the top right (#25) image. The images are also stacked and stored in the same order as the imaging order.



## 10 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**. If a location is not designated, the file will be saved automatically in the **Scan** folder under the **Celloger Mini Plus Scan App** folder. The file name can be entered in the **Enter Experiment Name Here** 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 for **End Date** and **Total Cycle** cannot be selected. Click the mouse, turn the mouse wheel, or click the calendar to set the schedule.

- ✓ **Interval Time:** Interval between scanning cycles
- ✓ **Total Running Time:** Time-lapse execution period
- ✓ **Start Date:** Time-lapse start time (If the time is set to a past date, it changes automatically to the time when the **Start** button is pressed.)
- ✓ **End Date:** Time-lapse complete time (It is determined by **Total Running Time** and **Start Date**, and the user cannot directly change the value.)

- ✓ **Total Cycle Expected:** The number of scans during time-lapse period (It is determined by **Interval Time** and **Total Running Time**, and the user cannot directly change the value.)

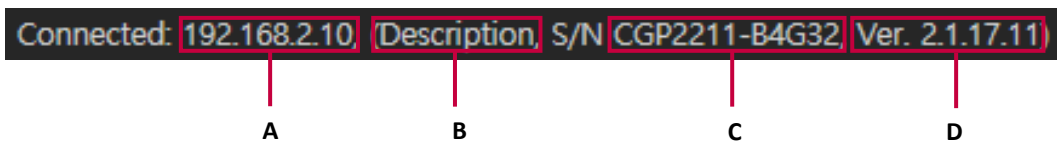
**Note.** The **Total Cycle Expected** is calculated by simply dividing **Total Running Time** by **Interval Time** and it does not consider the data processing time. Therefore, **Total Cycle Expected** could be different from the actual scanning cycles.

- C. “**Elapsed**” and “**%**” respectively denote the time elapsed and progress rate during the total time-lapse running time. **Count** means the number of times scanning occurred. Upon clicking **Start**, scanning begins, and this refers to count 1 (**Count** = cycle count).
- D. “**Remaining**” and “**%**” respectively denote the remaining time until the next cycle and progress rate within a cycle. **Index** shows the number of images taken within a cycle.
- E. Current date and time
- F. Upon clicking **Start**, scanning starts based on the set schedule.

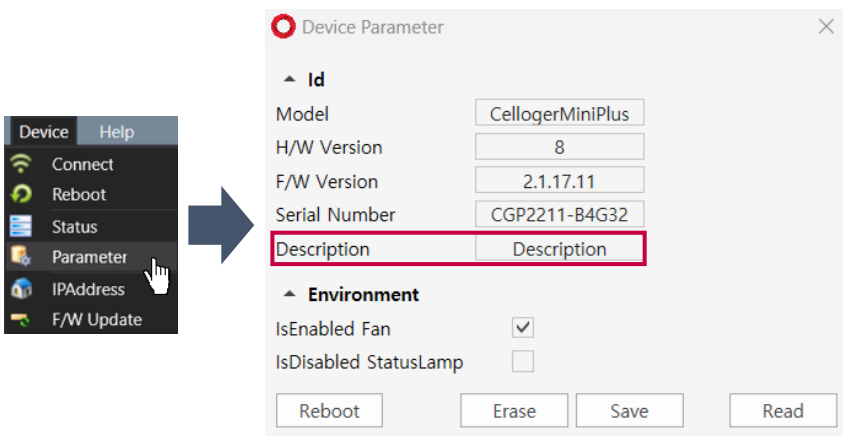
**Note.** Window can be separately floated so that it can fit on the monitor. It is possible to change the window by pressing   (**Autohide**) for floating or hiding. The layout is automatically saved upon terminating the program. To go back to the original state, press **Reset Layout** in the **Tool** menu before closing the **Celloger Mini Plus Scan App**. When the window disappears, delete “**LayoutCGP.XML**” in the **Celloger Mini Plus Scan App** folder to go back to default state after closing the app.

### ⑪ Connection status

It shows device connection status, and information on connected devices.



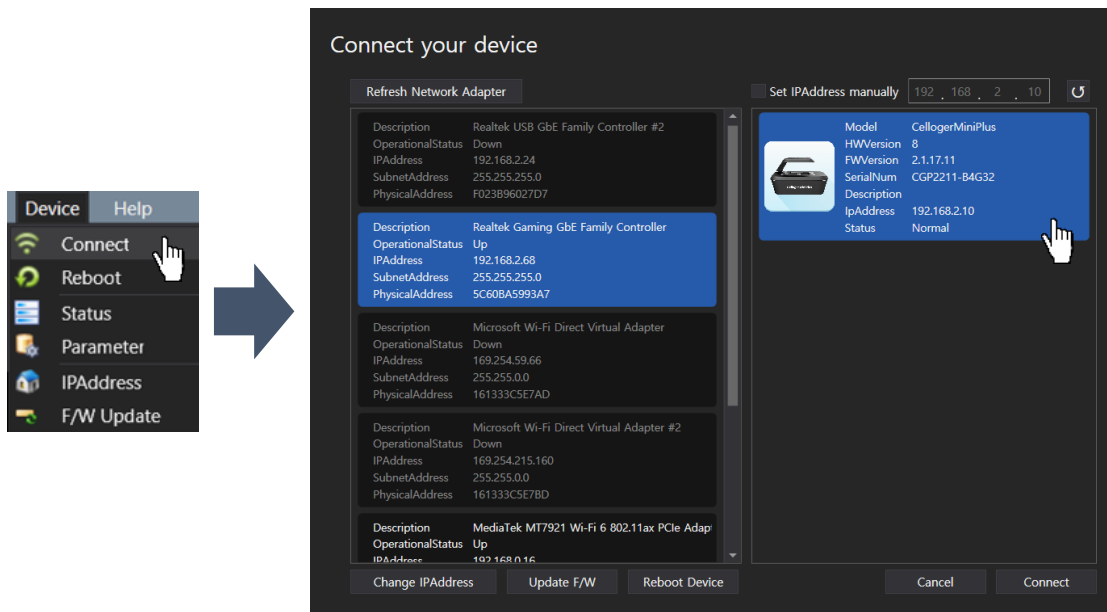
- A. IP address of connected device
- B. Description of connected device (It can be changed in **Parameter** in the **Device** menu.)



- C. Serial number of connected device

#### D. Firmware version of connected device

**Note.** If the device is not connected, **Disconnected: 0.0.0.0 Tx Rx** shows up. Click **Connect** in the **Device** menu for reconnection. If the connection window does not show the device icon, refer to **Note** in p.11.

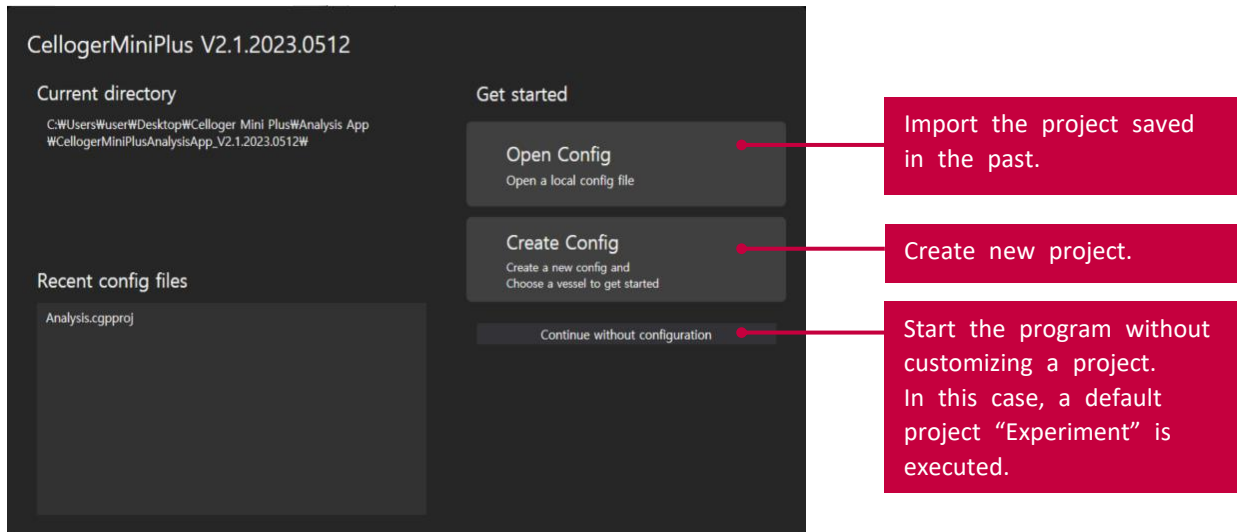


# Section 2. Celloger Mini Plus Analysis App

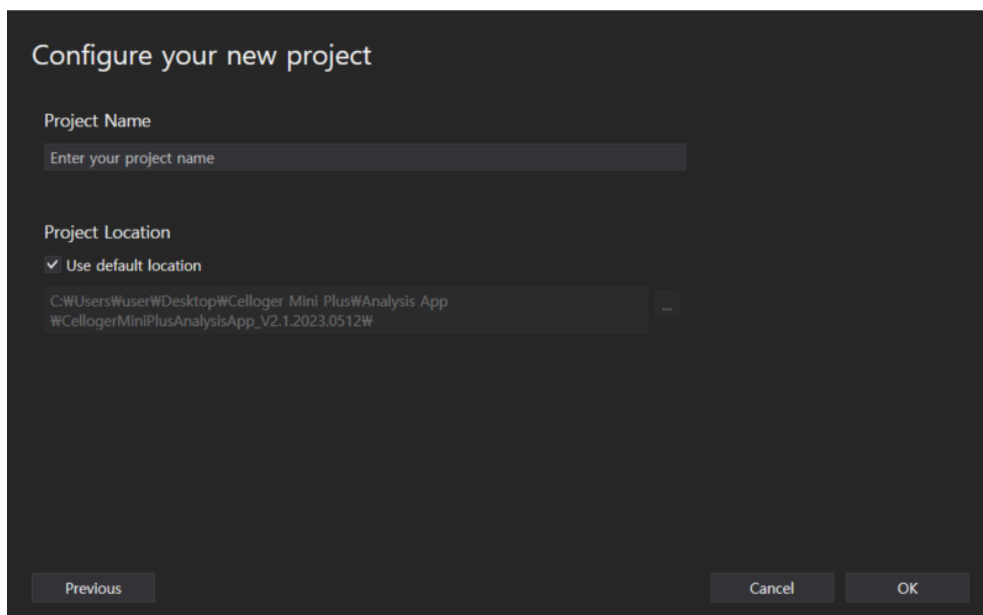
## I. General Operation

### 1. Create or open a config file

Upon executing the **Celloger Mini Plus Analysis App**, a pop-up screen for project selection appears. With **Celloger® Mini Plus**, the setting can be managed with the config file (“CGPPROJ” file format). Setting parameters, including Adjust, Coverage, Preferences, etc. are saved in this config file.



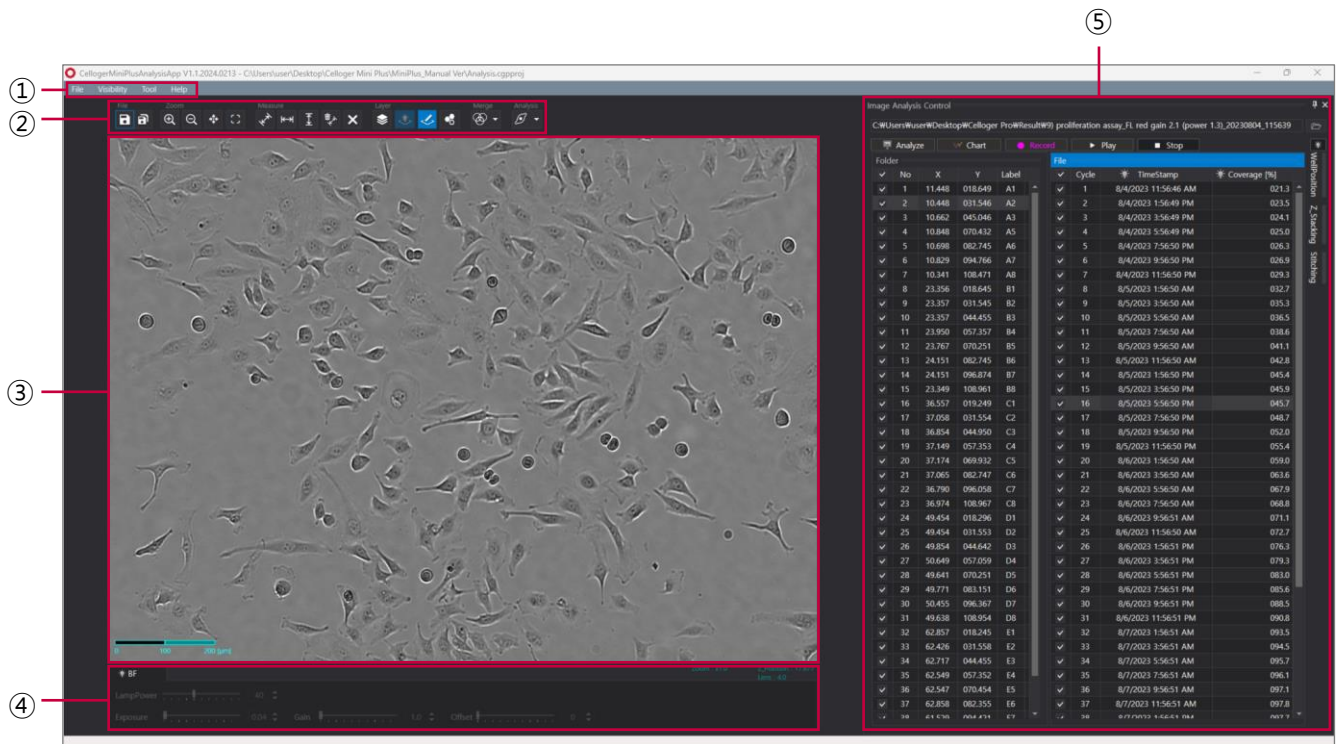
- Create a new config file or open an existing one. You can start a program with default project settings by clicking **Continue without configuration**.
- Upon selecting **Create Config**, a pop-up window appears, prompting the selection of a project name and storage location. You can start the program after entering the required information and press **OK**. The project will be stored in the default config file location shown below. If you want to change it, uncheck the **“Use default location”** box and enter a new location.



## 2. Software overview

**Celloger Mini Plus Analysis App** is a program dedicated to analyzing and post-processing images captured with **Celloger® Mini Plus**. This software can be operated without requiring a connection to the device.

**Note.** Refer to p.61 for full software descriptions.



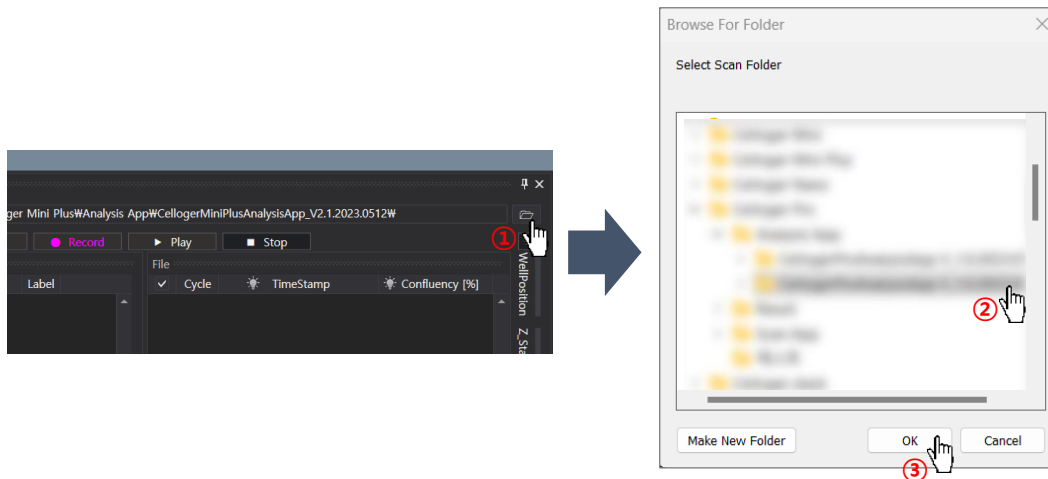
- ① Menu bar
- ② Toolbox
- ③ Display
- ④ Light source control & Z-axis coordinate
- ⑤ Image analysis control

### 3. Import the data

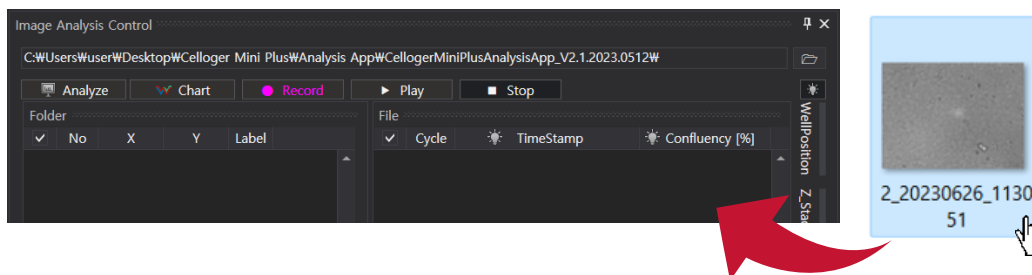
**Celloger Mini Plus Analysis App** can only import the original images taken with the **Celloger Mini Plus Scan App**.

① **Open the time-lapse folder or image file.**

Select the time-lapse folder taken by the **Celloger® Mini Plus**. Upon opening the selected folder, the images in the folder are listed following the order of the cycle. The higher-level folder shows scan position and lower-level folder shows the image files in the selected folder.







② **Drag the time-lapse folder or image file.**





**Note.** In order for the file to be correctly imported into the **Celloger Mini Plus Analysis App**, the file name and location address should consist only of English letters, numbers, and some special symbols (avoiding characters such as /, :, \*, ?, <, >, |). If the app fails to import the file, check whether the file name and location address is written correctly.

## II. Data analysis

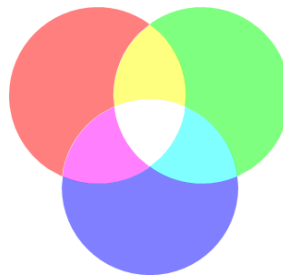
### 1. Merge the FL image

Press  (Merge layer) for activation, which is displayed as , and select the merge method (, ). The merged image will be shown **Display**.

- ①  (**Alpha Blending**): A method of combining images with a transparent effect. Individual color channels are presented in separate layers, and the merged image is displayed by combining the information from each layer with adjusted opacity. The opacity value determines the transparency, allowing for a smooth and blended final merged image.
- ②  (**Channel Merge**): A traditional method of combining multiple color images without a transparent effect. The intensity of each channel determines the brightness in the final merged image, where a higher intensity value results in a brighter or whiter appearance in the merged image.

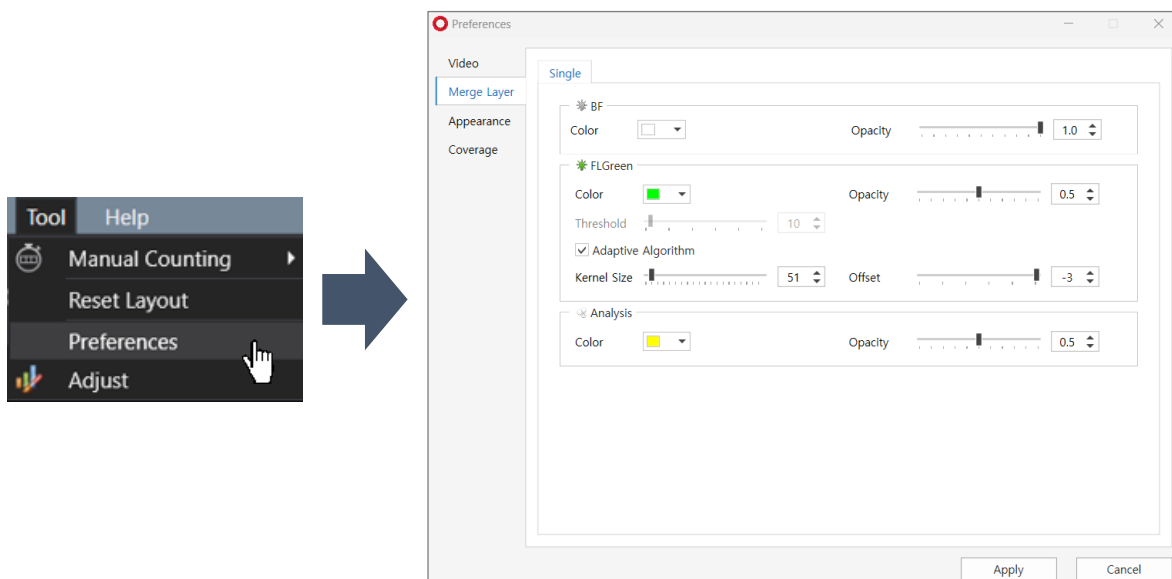


<Alpha Blending (opacity=50%)>



<Channel Merge>

Settings for the merged images can be changed by adjusting the **Merge layer** in **Preferences** of the **Tool** menu as shown below.



**Celloger® Mini Plus** conducts thresholding for **FL** channel image based on intensity and merge it with **BF** 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 **Threshold** while looking at the image mask after unchecking the **Adaptive Algorithm** box.


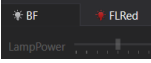
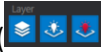
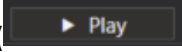
**Method 2) Local adaptive thresholding:** Divide the image into local areas, and thresholding is conducted by comparing relative intensity among these areas. Adjust parameters of the **Kernel Size** and **Offset** while looking at



the image mask after checking the **Adaptive Algorithm** box. (**Kernel Size** indicates the size of local area and **Offset** indicates the relative threshold offset of the local area.)

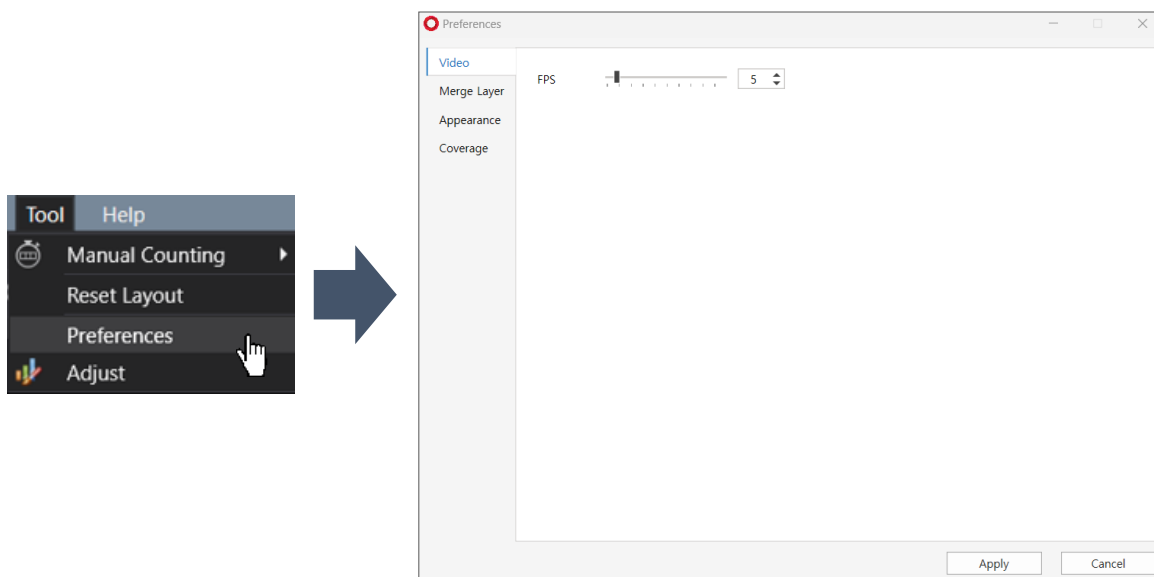
**Note.** When the fluorescence areas are distorted or clustered in one place, **Global thresholding** function is recommended; and when the fluorescence areas are evenly distributed, **Local adaptive thresholding** function is recommended.

## 2. Slide show

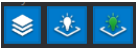
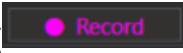
- ① Open the time-lapse folder.
- ② Select the light channel ( or ) or press **Merge Layers** ().
- ③ Press **Play** ().

**Note.** **FPS** (frame per second) can be changed in **Preferences** of **Tool** menu.

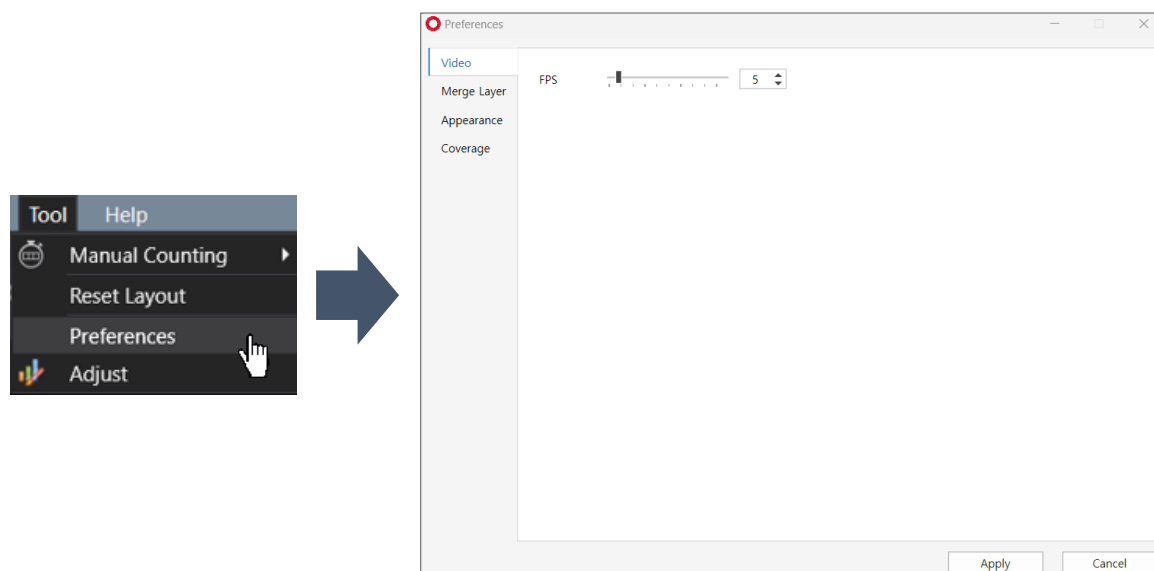
**Note.** The speed of the slide show and video can be different even with the same **FPS**.



## 3. Make video

- ① Open the time-lapse folder.
- ② Select light channel or press **Merge Layers** ().
- ③ Create a video by pressing **Record** ().
- ④ Open the video file for confirmation.

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



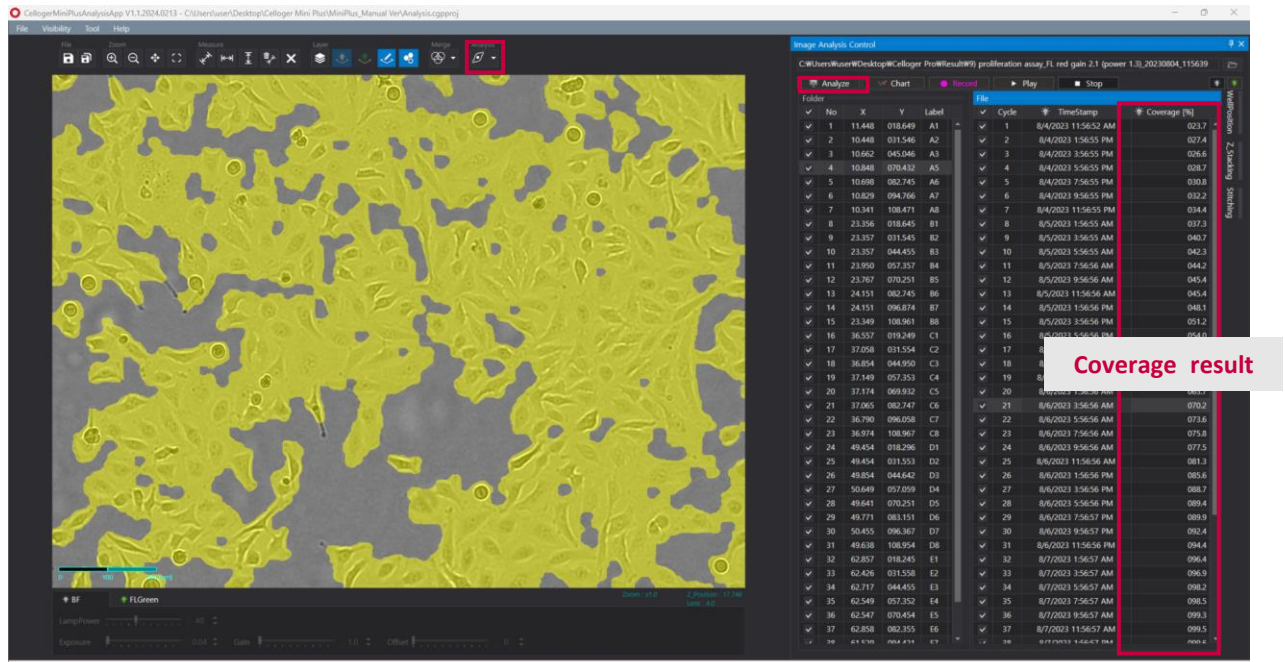
## 4. Estimate coverage

**Celloger® Mini Plus'** coverage analysis is divided into three modes: **Normal cell**, **Spheroid cell**, and **Wound area**. **Normal cell** and **Wound area** show coverage result in "%", and **Spheroid cell** shows it in "mm<sup>2</sup>".

### 4.1 Cell coverage

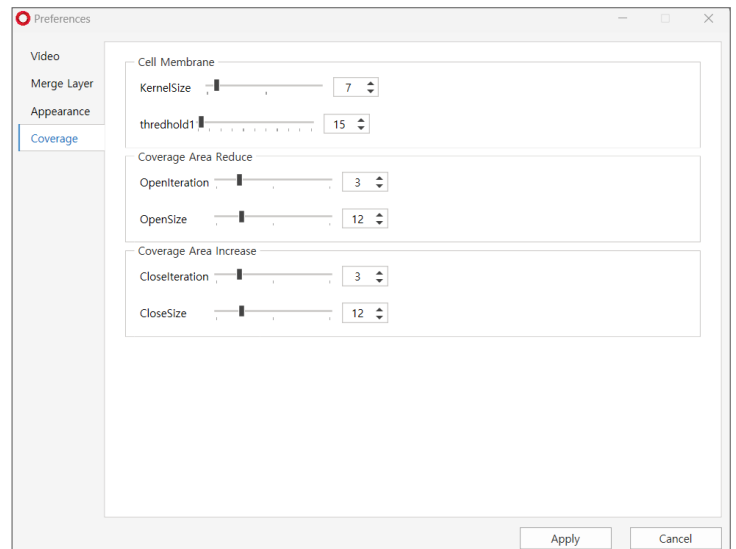
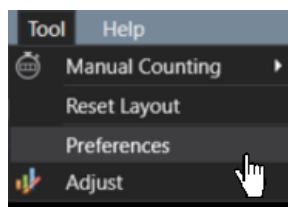
- ① Open the time-lapse folder or images captured with **Celloger® Mini Plus**.
- ② Select the analysis mode between **Normal cell** (👁️) and **Spheroid cell** (🎯).
- ③ Select the light channel in the **Light source control** or from the icon (🌞🌿) above the **Well Position**.
- ④ Press **Analyze**.

- ⑤ The coverage values and coverage mask (yellow) are indicated as shown below. Selecting a cycle from the list allows you to view the corresponding cycle's image and coverage mask.



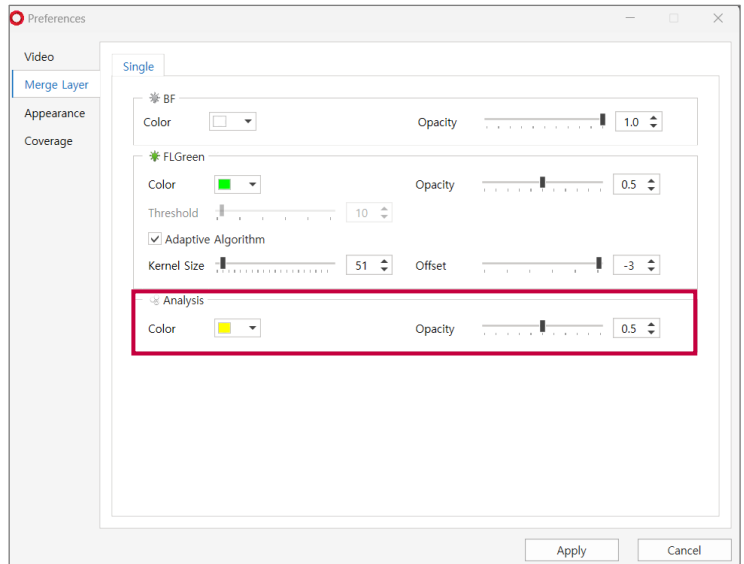
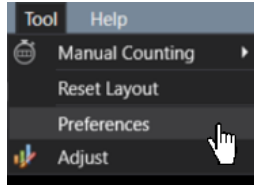
<Calculated coverage values and coverage mask>


**Note.** You can change the coverage parameters in **Preferences** of the **Tool** menu.





**Note.** The coverage of the fluorescence area is calculated after thresholding according to merge parameter. Therefore, to accurately calculate the coverage of the fluorescence area, adjust the appropriate thresholding parameters (refer to p.48) before executing the coverage analysis.

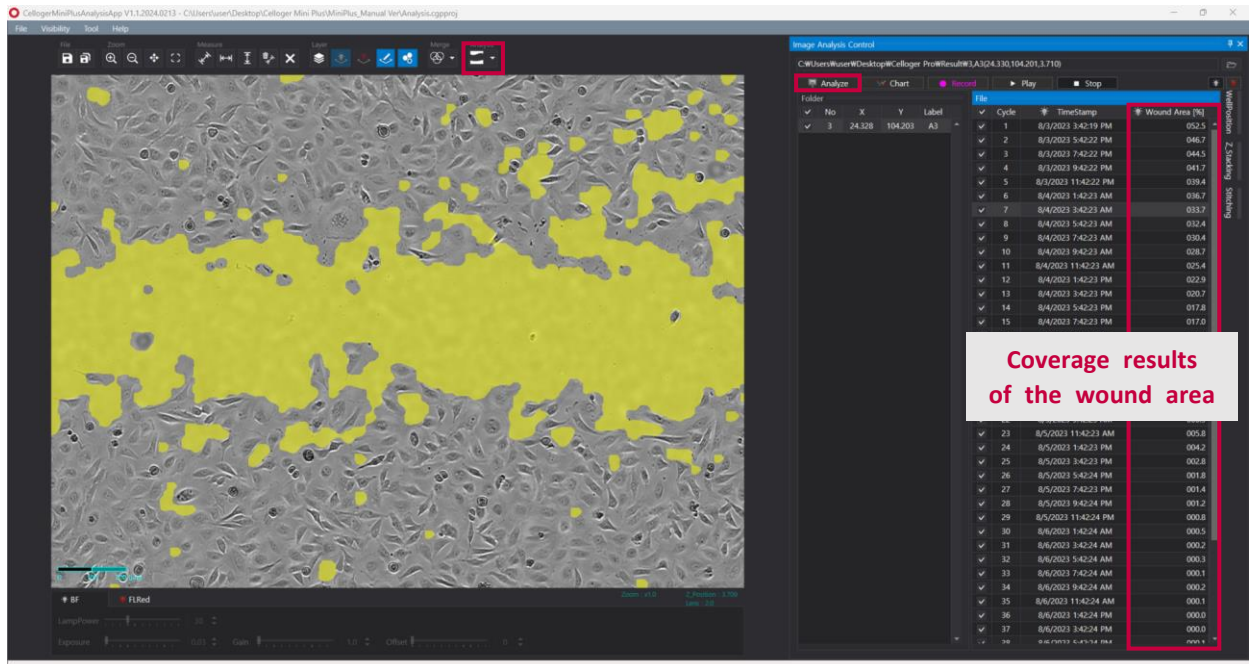
**Note.** If you want to adjust the coverage masking color or transparency, select **Preferences** under the **Tool** menu, change the parameters in **Analysis** under **Merge Layer** and click **Apply**.



**Note.** If you do not want to see the coverage mask, deactivate it by clicking on  under **Toolbox**.

## 4.2 Wound area

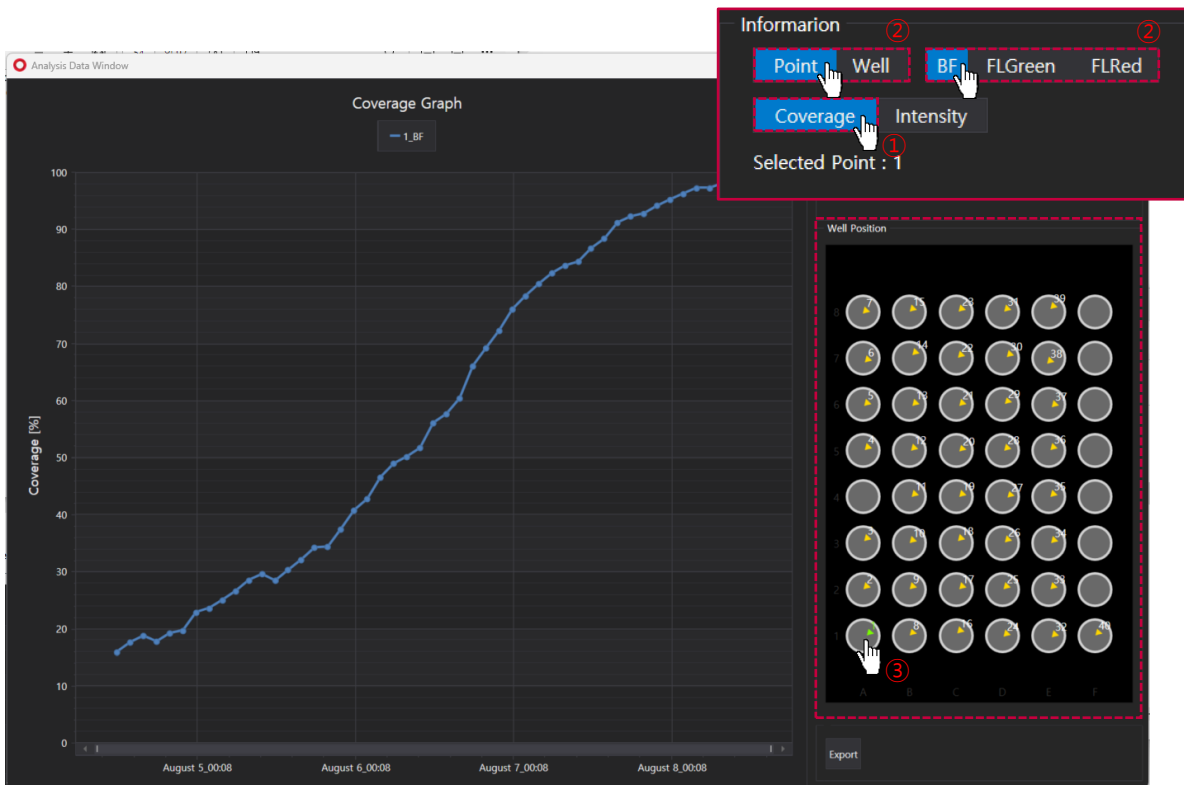
- ① Open the time-lapse folder or images captured with **Celloger® Mini Plus**.
- ② Select the **Wound area** () in **Analysis** mode.
- ③ Select the light channel in the **Light source control** or from the icon () above the **Well Position**.
- ④ Press **Analyze**.
- ⑤ The coverage value and coverage mask (yellow) of the wound area are indicated as shown below. Selecting a cycle from the list allows you to view the corresponding cycle's image and coverage mask.



<Calculated coverage values and coverage mask of the wound area>

## 5. Create coverage graph

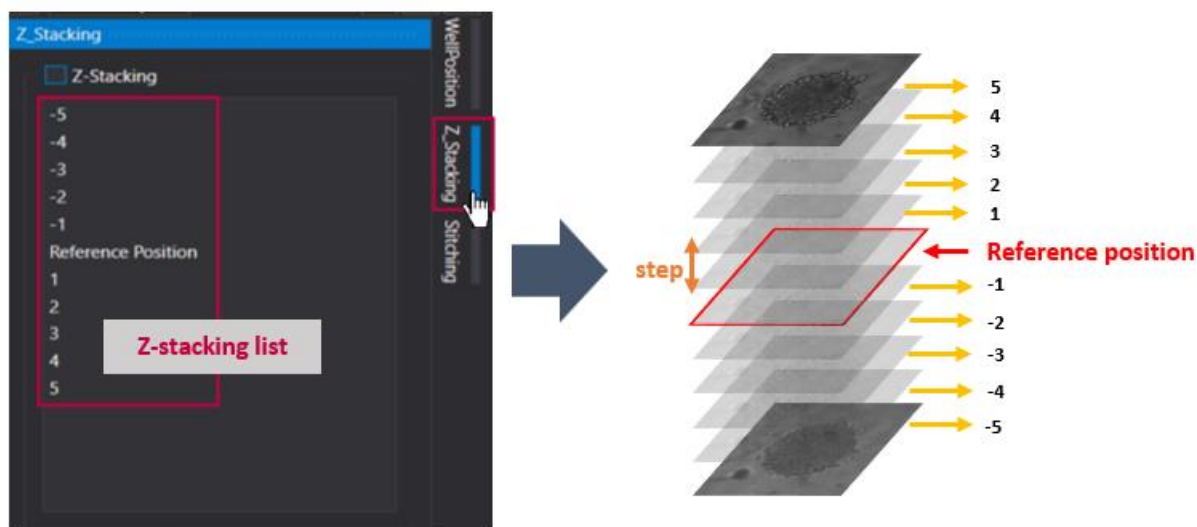
- ① Open the time-lapse folder or images captured with the **Celloger Mini Plus Scan App** and press **Analyze**.
- ② Press **Chart** to create the coverage graph.
- ③ Select **Coverage** in the **Information** column.
- ④ Select whether to create graph from scan positions (= **Point**) or well averages (= **Well**) and the light channel in the **Information** column.
- ⑤ In **Well Position**, select the scan position or well to create the graph.



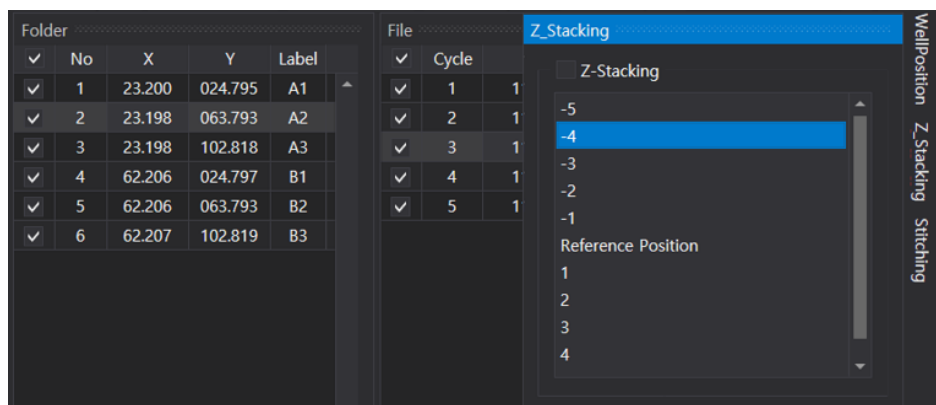
**Note.** Upon selecting the intensity in the **Information** column, intensity graph can also be seen in **Chart**.

## 6. Show Z-stacking image

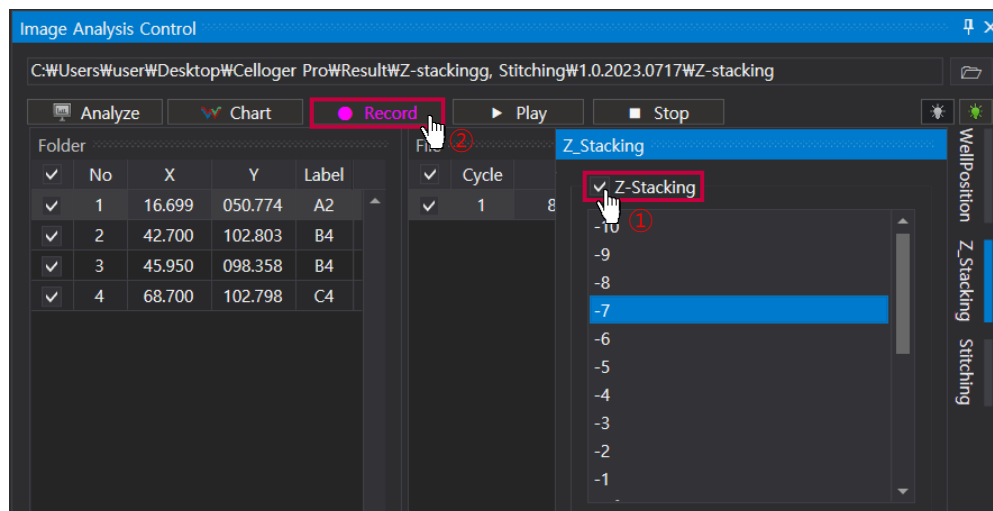
- ① Open the time-lapse folder or images captured with the Z-stacking function.
- ② After selecting the scan position with Z-stacked images, click the Z-stacking tab to see the **Z-stacking list**. The image below shows the **Z-stacking list** when the **Range** of Z-stacking function is set to ZS(-5,5).



- ③ Select one from the **Z-stacking list** and check the corresponding image on **Display**. In the example below, the image shows the selection of “-4” from the **Z-stacking list** for the third cycle at the second position (folder) of the **Scan Position**. In this case, the displayed image is captured four steps below the reference position (**Focus**) of the third cycle at the second position. For instance, if the **Step** is set to 10  $\mu\text{m}$ , selecting “-4” corresponds to an image taken 40  $\mu\text{m}$  below the reference position.



**Note.** If you want to make a video of the images in the **Z-stacking list**, ① Check the Z-stacking checkbox above the **Z-stacking list** and ② Click **Record**. The video appears in the order of the **Z-stacking list** (from low to high focus). The **FPS** of the video can be adjusted in **Properties** of the **Tool** menu (p.50).

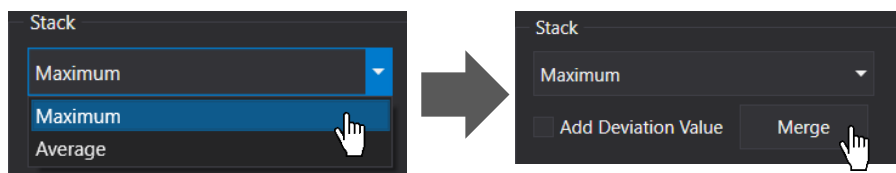


## 7. Merge Z-stacking images (Intensity Z-projection)

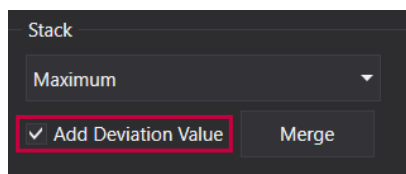
Intensity Z-projection is a method that compiles images into a 2D image by selecting pixels of the chosen intensity from each slice across the volume. Celloger®'s Z-projection parameters offer two options: **Maximum**, which merges pixels with the highest intensity value, and **Average**, which merges pixels with the average intensity value in each focal plane.

**Note.** This function can only be activated in the fluorescence image.

- ① Open the time-lapse folder or images captured with the Z-stacking function.
- ② After selecting the scan position with Z-stacked images, click the **Z-stacking** tab located on the right side of the analysis software.
- ③ Select the Z-projection parameters (**Maximum** or **Average**) and click **Merge**. The merged image appears on **Display**.



**Note.** **Add Deviation Value** is a function that increases the brightness of the merged image.

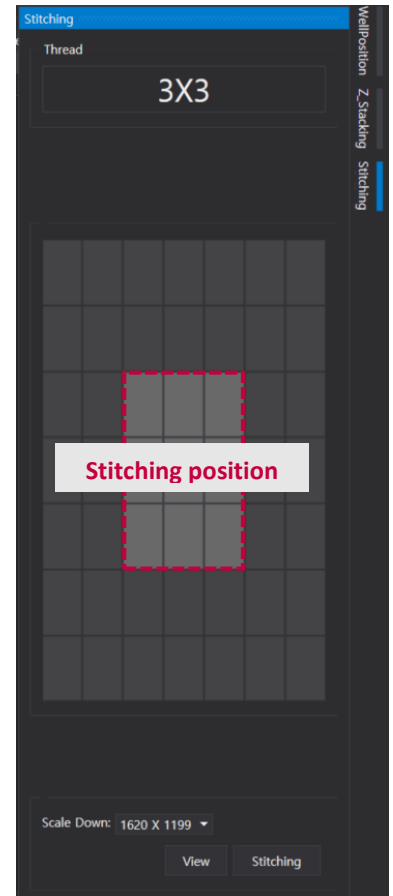


## 8. Stitch images

- ① Open the time-lapse folder or images captured with the stitching function.
- ② After selecting the scan position with stitching function, click the **Stitching** tab located on the right side of the analysis software to see the **Stitching position**. The image below shows the **Stitching position** when the **Range** of stitching is set to ST(1,1,1,1).

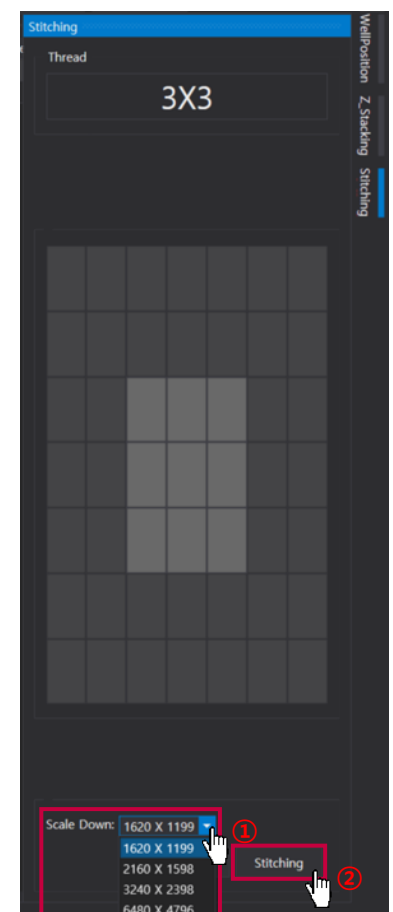
**Note.** **Thread** refers to the number of images taken to perform the stitching function (horizontal x vertical).

**Note.** If you select one of the **Stitching position**, you can view the image on **Display**.



- ③ Select the desired scale in **Scale Down** and click **Stitching** under **Stitching position** to merge the captured images.

**Note.** **Scale Down** is used to enhance the speed and reduce the file size. However, be aware that decreasing the scale also reduces the resolution.

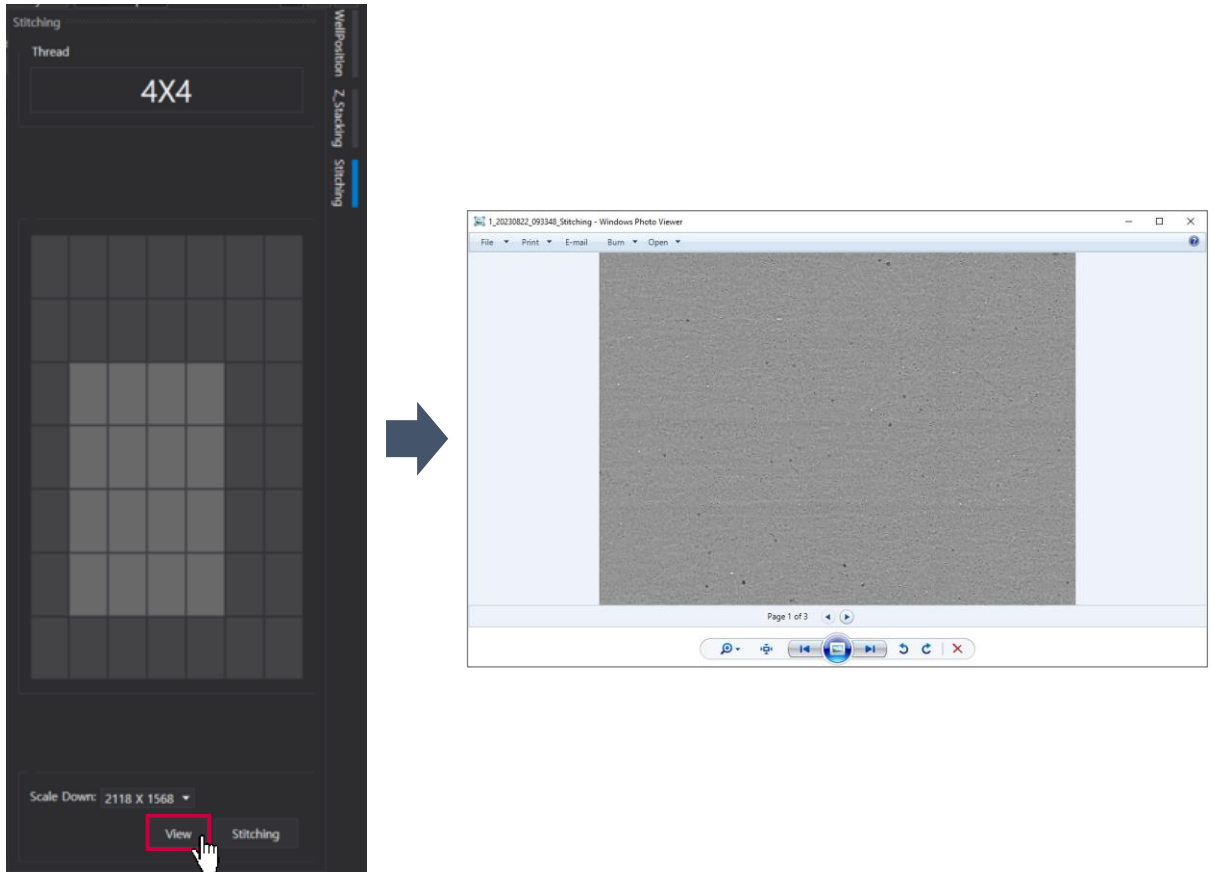




**Note.** When stitching is completed, images are saved in the stitching folder created in the raw image folder.

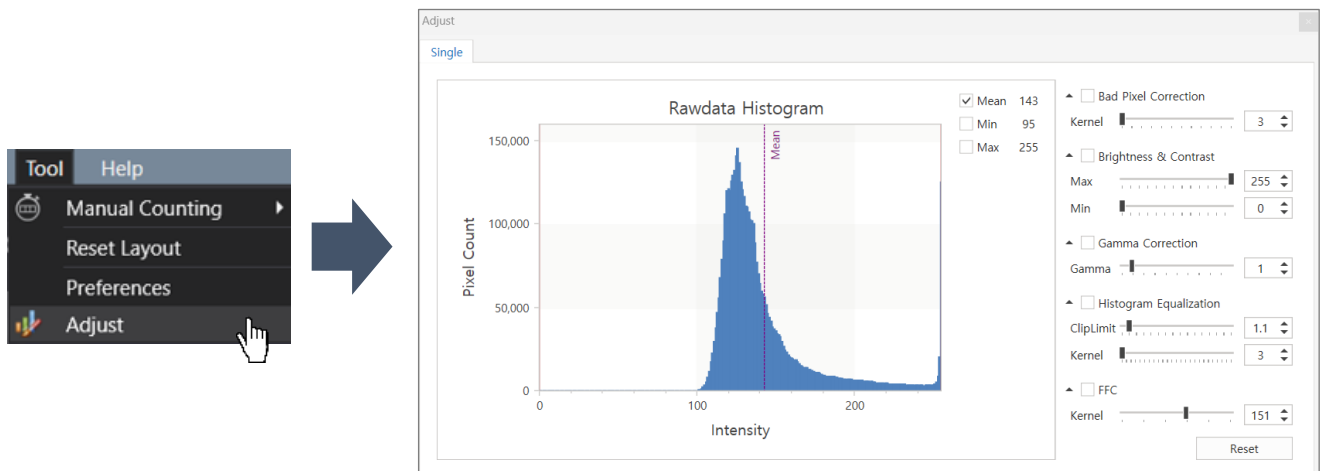


**Note.** Clicking **View** opens the stitched image file.

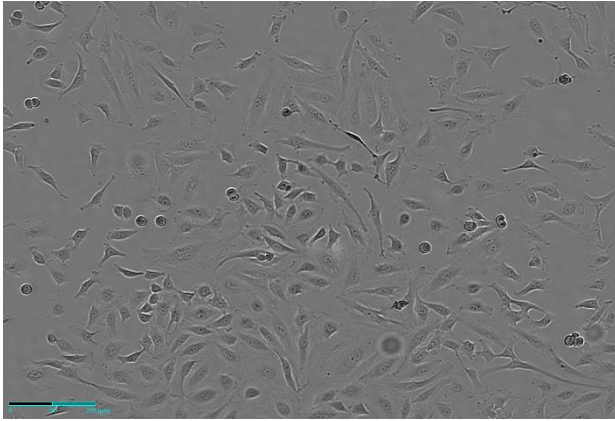


## 9. Image adjustment

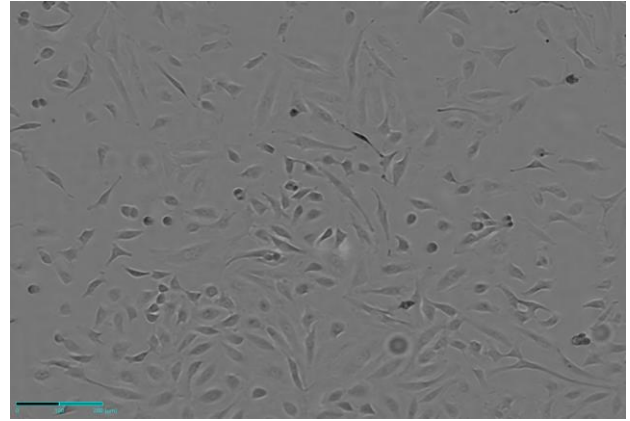
Adjust the image using parameters (Kernel size, brightness, contrast, gamma) in **Adjust** in the **Tool** menu, and save the modified image.



- ✓ **Bad Pixel Correction:** As a correction for defective pixels, the image is divided into pixels. Values that significantly stand out from a certain threshold between adjacent values are then smoothed or blurred.

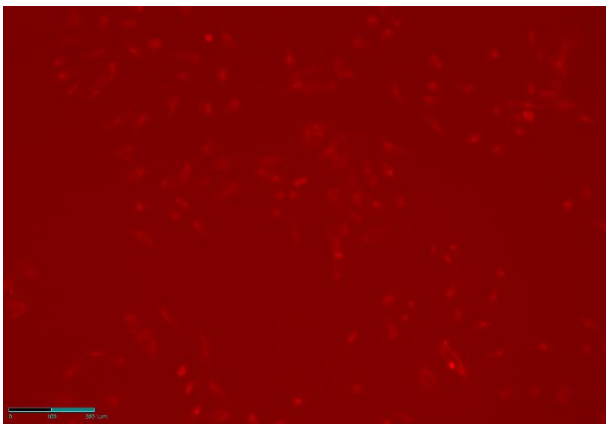


<Before Bad Pixel Correction>

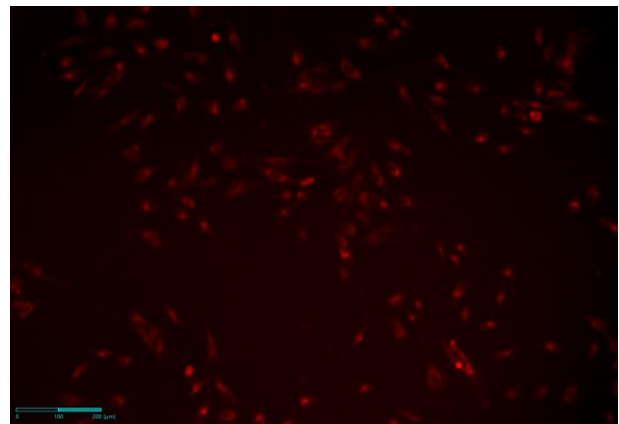


<After Bad Pixel Correction>

- ✓ **Brightness & Contrast:** It adjusts the overall brightness and contrast of the image.

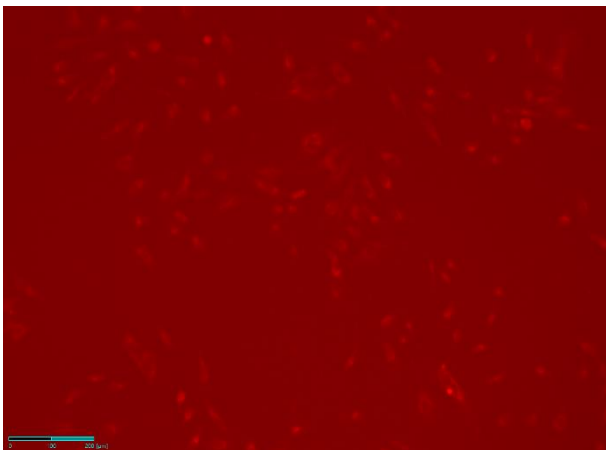


<Before Brightness & Contrast>

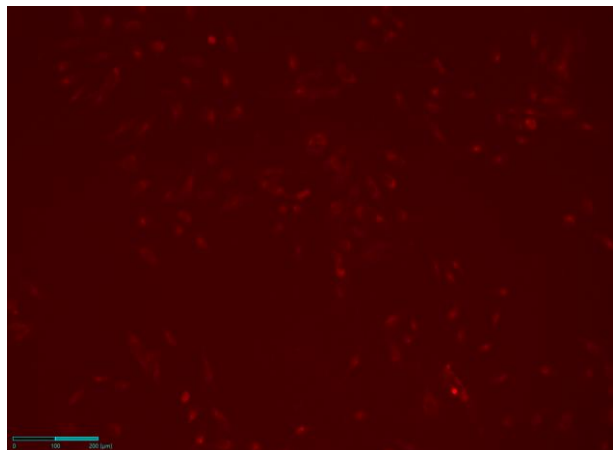


<After Brightness & Contrast>

- ✓ **Gamma Correction:** It is the process of adjusting the brightness of an image so that the colors appear correctly on the screen.

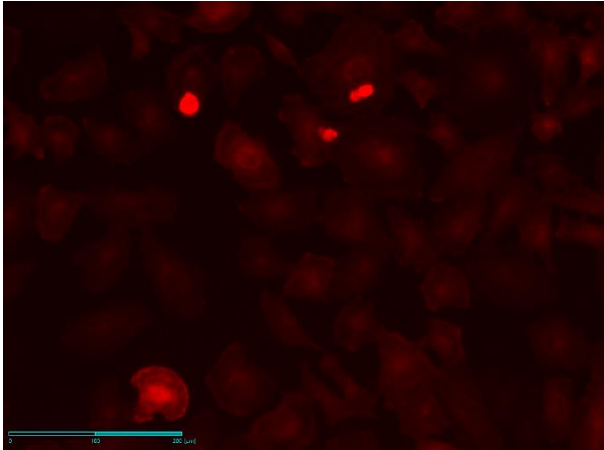


<Before Gamma Correction>

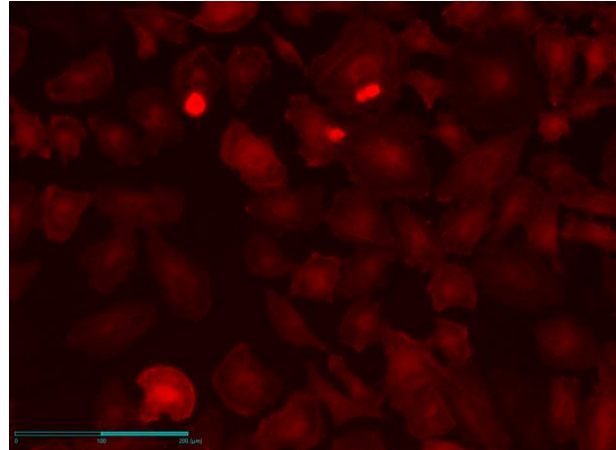


<After Gamma Correction>

- ✓ **Histogram Equalization:** It is a function that allows image where the histogram is concentrated in a specific area to have the histogram distributed evenly over the entire area.

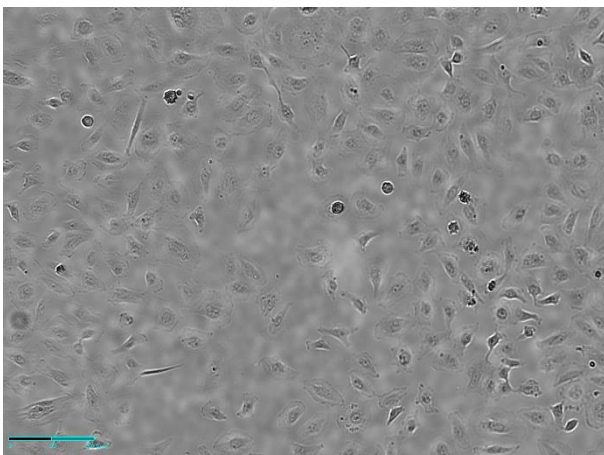


<Before Histogram Equalization>

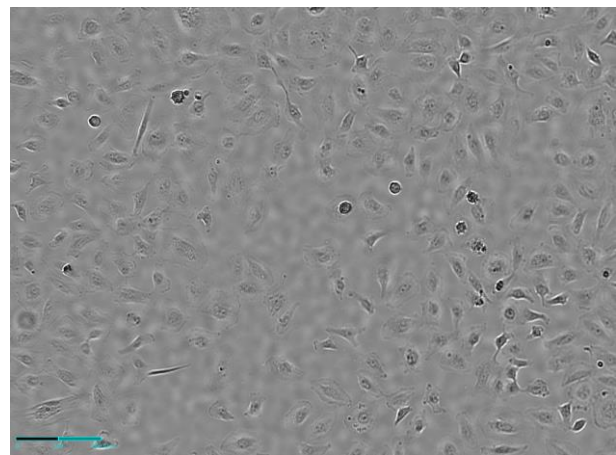


<After Histogram Equalization>

- ✓ **FFC (Flat Field Correction):** It is a function to uniformly correct the brightness value of the captured image.



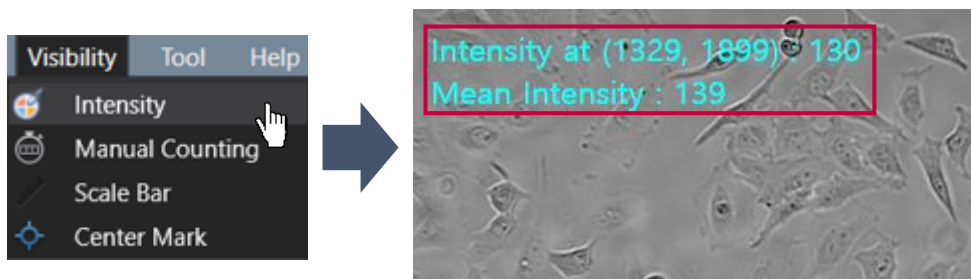
<Before FFC>



<After FFC>

## 10. Intensity

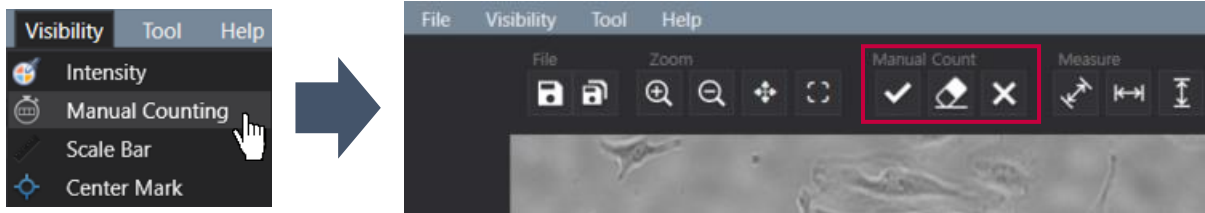
Upon activating **Intensity** in the **Visibility** menu, **Point intensity** and **Mean intensity** information is displayed at the upper left corner of the **Display**.



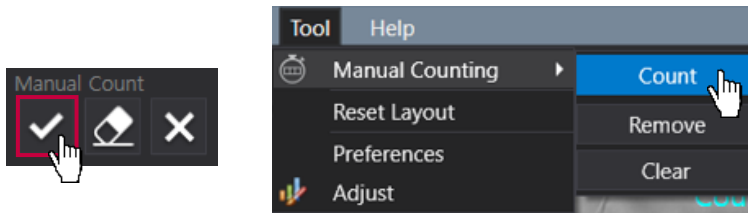
- ✓ **Intensity at (X coordinate, Y coordinate):** Point intensity value of the X, Y coordinates indicated by the cursor. (If it is in the merge state, the intensities of **BF** and **FL** channel are displayed in order.)
- ✓ **Mean intensity:** Average intensity value of the image displayed on **Display**. (If it is in the merge state, the intensities of **BF** and **FL** channel are displayed in order.)

## 11. Manual Counting

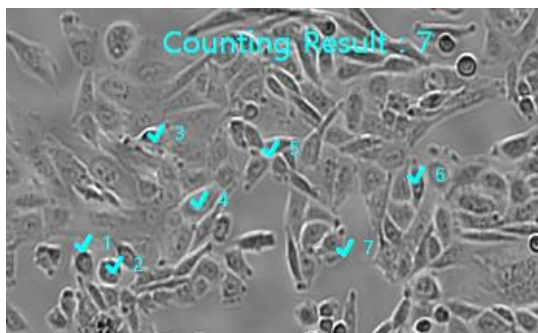
- ① Click **Manual Counting** in the **Visibility** menu. Then, this will generate the **Manual Count** section in the Toolbox.



- ② Click **Count** in the **Manual Count** section or proceed to **Manual Counting** and select **Count** option in the **Tool** menu.



- ③ Click on the desired area with the mouse to start counting. Each clicked area will display a check mark and a corresponding number. The total number of check marks/clicked areas is shown at the top as the **Counting Result**.



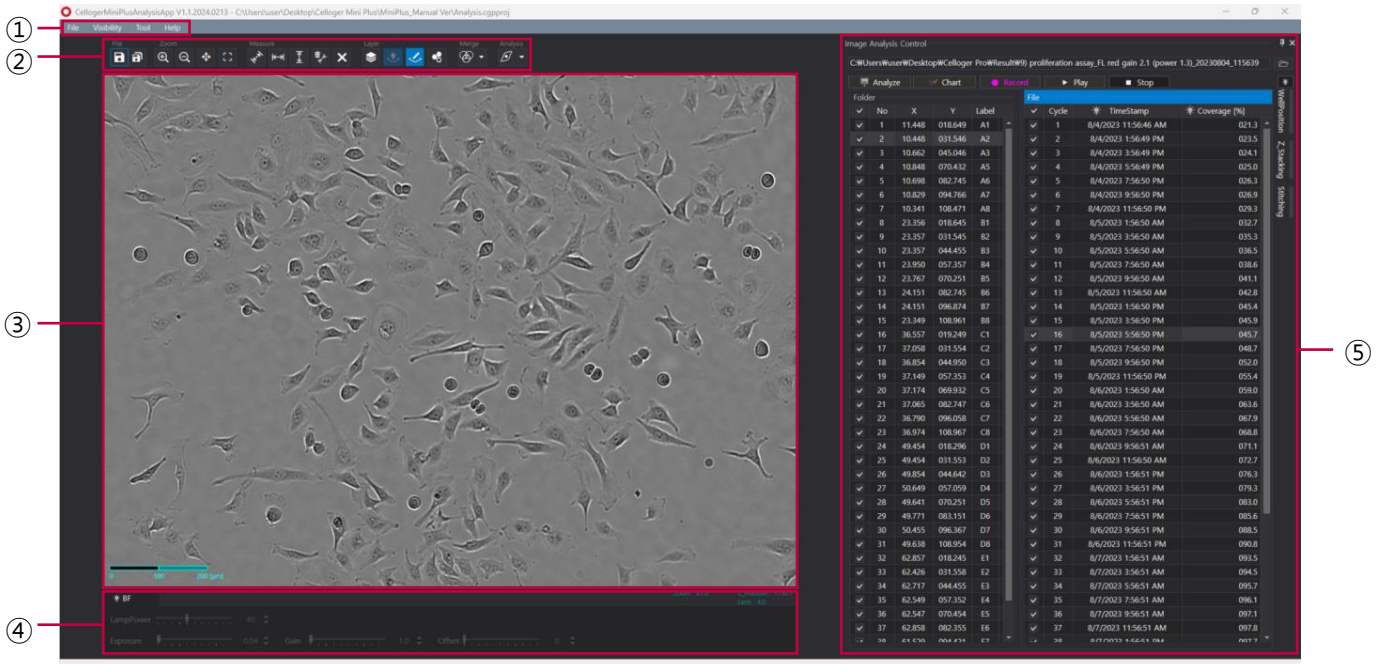
**Note.** To remove one selected area at a time, select the **Remove** option in the **Manual Counting** tab of the **Tool** menu or click **Remove** (🗑️) button in the **Manual Count** section.

**Note.** To delete all counting marks, select the **Clear** option in the **Manual Counting** tab of the **Tool** menu or click **Clear All** (✖️) button in the **Manual Count** section.

**Note.** To hide counting marks and **Counting Result** without removing all data, go to the **Visibility** menu and select **Manual Counting**.

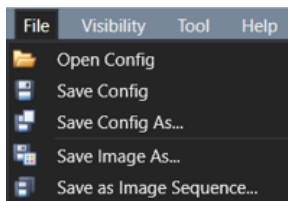
# III. Software description

Upon opening the **Celloger Mini Plus Analysis App** and completing pre-setup (refer to p.45), the following main page is displayed.



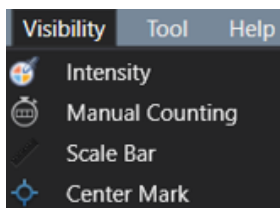
## ① Menu bar

### [File]



- ✓ **Open Config:** Import a previously saved config file.
- ✓ **Save Config:** Save settings (Coverage, Adjust, etc.) on current config file.
- ✓ **Save Config As...:** Create a new config file with current settings.
- ✓ **Save Image As...:** Save the image shown on the **Display**.
- ✓ **Save as Image Sequence...:** Save the selected images individually.

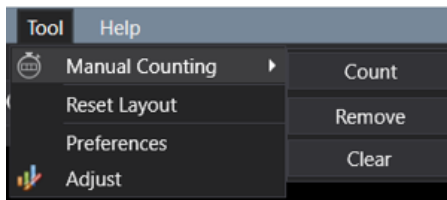
### [Visibility]



- ✓ **Intensity (p.59):** Show/hide point and mean intensity of an image.
- ✓ **Manual Counting:** Show/hide “Manual Counting” function.
- ✓ **Scale Bar:** Show/hide scale bar on the bottom left of **Display**.
- ✓ **Center Mark:** Show/hide a cross-shaped mark at the center of **Display** for reference.



## [Tool]



- ✓ **Manual Counting** (p.60): Count the points clicked by user.
  - ① **Count**: Add a point for counting.
  - ② **Remove**: Delete a clicked point.
  - ③ **Clear**: Delete all points.
- ✓ **Reset Layout** (refer to **Note** in p.63): Restore the software layout to its original state before any modifications.
- ✓ **Preferences**: Set **FPS** (p.50), **Merge Layer** (p.48), **Appearance**, and **Coverage** parameters.
- ✓ **Adjust** (p.57): Adjust the images.



## [Help]







- ✓ **About Celloger Analysis App**: Display app information.

## ② Toolbox




### [File]

- ✓  : Save the image of the **Display**.
- ✓  : Save the selected images individually.

### [Zoom & Pan]





- ✓  : Zoom-in (Digital zoom). Keyboard shortcut “*Ctrl + mouse scroll wheel*” can be used.
- ✓  : Zoom-out (Digital zoom). Keyboard shortcut “*Ctrl + mouse scroll wheel*” can be used.
- ✓  : Pan. It can be used by clicking and dragging the screen while pressing the “*Shift*” key in the Zoom-in state.
- ✓  : Return the zoomed image to its original state at once.


### [Manual Count]

- ✓  : Add a point for counting.
- ✓  : Delete a clicked point.
- ✓  : Delete all points.







### [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.
- ✓  : Delete the selected measurement.



- ✓  : Delete all measurements.

#### [Layer]

- ✓  : **Merge Layers**; It shows the merged image of **BF** and **FL** channels.
- ✓  : **BF** image layer
- ✓ ,  : **FL green** or **FL red** image layer
- ✓  : Features [Ruler (Measure), Scale bar, manual counting, etc.] layer
- ✓  : **Analysis Layer**; Mask the area recognized as coverage.




#### [Merge]

It is a way to combine images captured from different channels, **Celloger® Mini Plus** provides two methods. For more details about merge method, refer to p.48.

- ✓  (**Alpha Blending**): A method of combining images with a transparent effect.
- ✓  (**Channel Merge**): A method of combining images without a transparent effect.

#### [Analysis Mode]

Select the cell shape or wound area for coverage analysis.

- ✓  (**Normal Cell**): Ideal for analyzing cells that do not have a spheroid shape.
- ✓  (**Spheroid Cell**): It is suitable for spheroid cell analysis.
- ✓  (**Wound area**): It is suitable for wound area analysis.

### ③ Display



Display the image selected from the **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, coverage mask is shown and functions such as measure (= **Ruler**), **Intensity**, and **Manual Counting** can be performed on the screen.

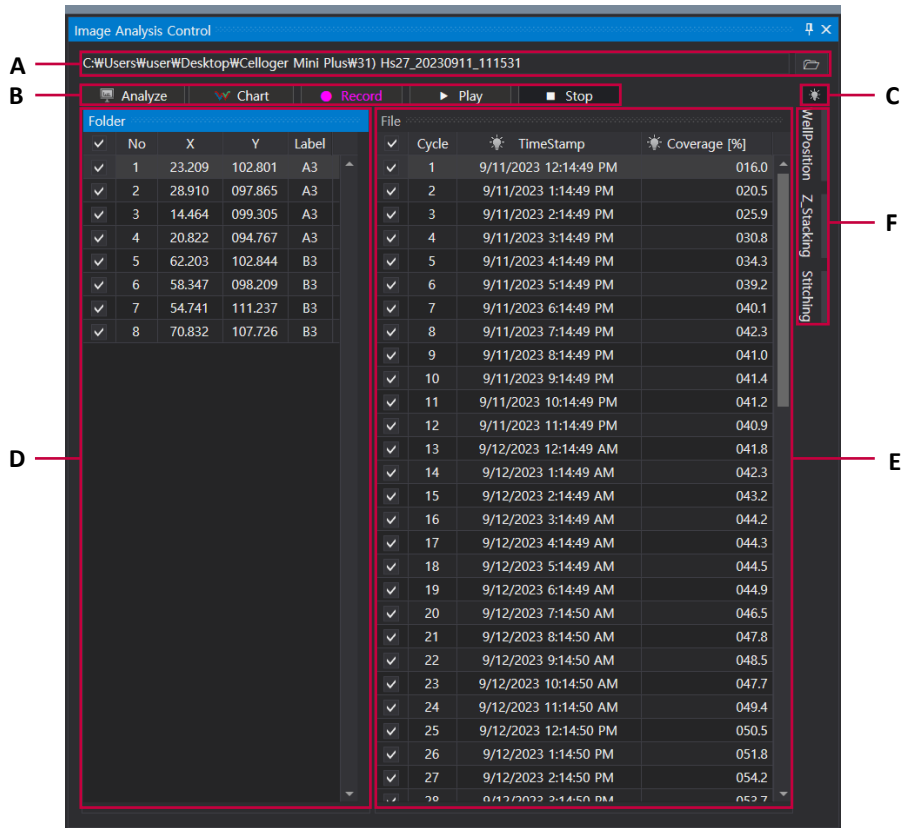
### ④ Light Source Control


Display the light source settings, Z-coordinates, and lens magnification of the **Celloger Mini Plus Scan App** and it cannot be changed.

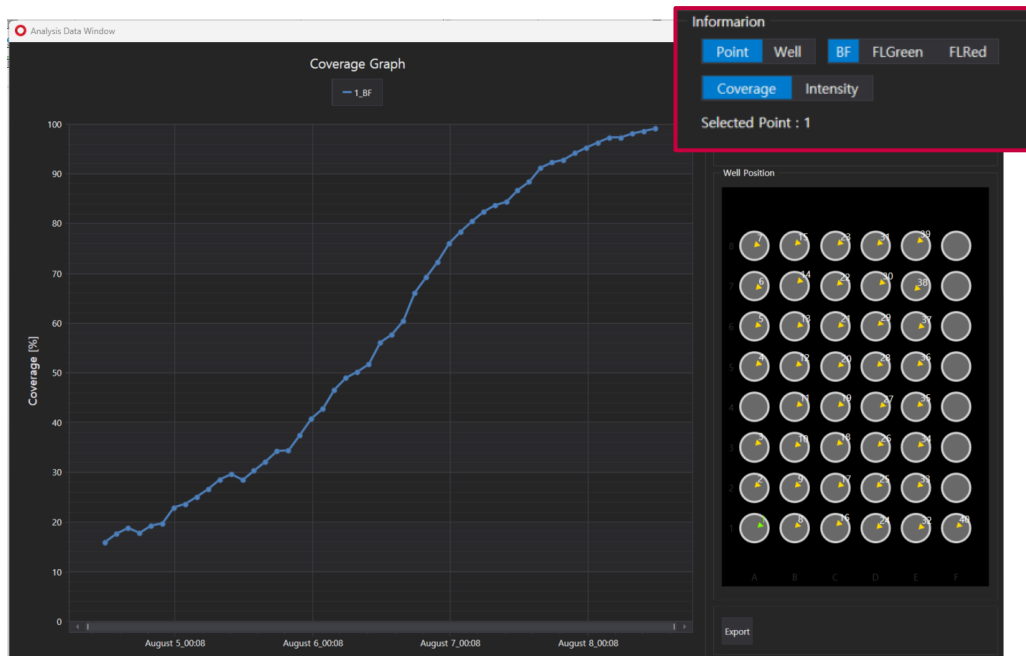
### ⑤ Image Analysis Control

Upon importing a folder, a file list is displayed. When a file is selected, the image is shown on **Display**.

**Note.** The window can be floated separately to fit on the monitor. You can adjust the window by pressing ,  (**Auto Hide**) for floating of hiding. The layout is automatically saved upon terminating the program. To go back to the original state, press **Reset Layout** in the **Tool** menu before closing the app. When the window disappears, delete the "**LayoutCGP.XML**" file in the **Celloger Mini Plus Analysis App** folder to go back to default state after closing the app.

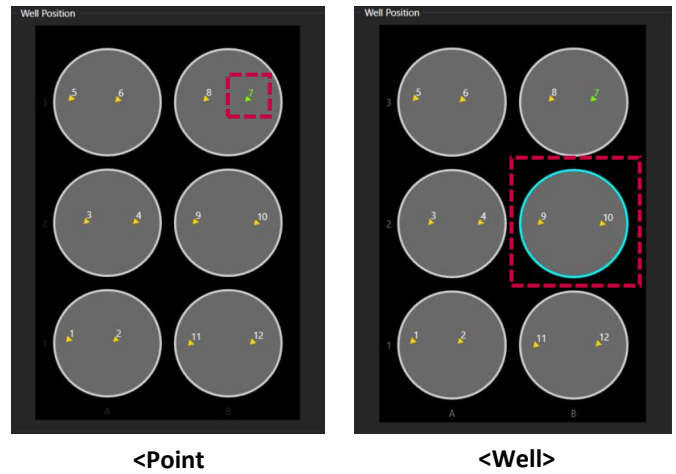


- A. Click  to load the image file taken with the **Celloger® Mini Plus** (refer to p.47).
- B. Analyze images or make time-lapse videos.
  - ✓ **Analyze:** Calculate the coverage of the files checked on the list.
  - ✓ **Chart:** Show the coverage graph and intensity graph (refer to p.53).

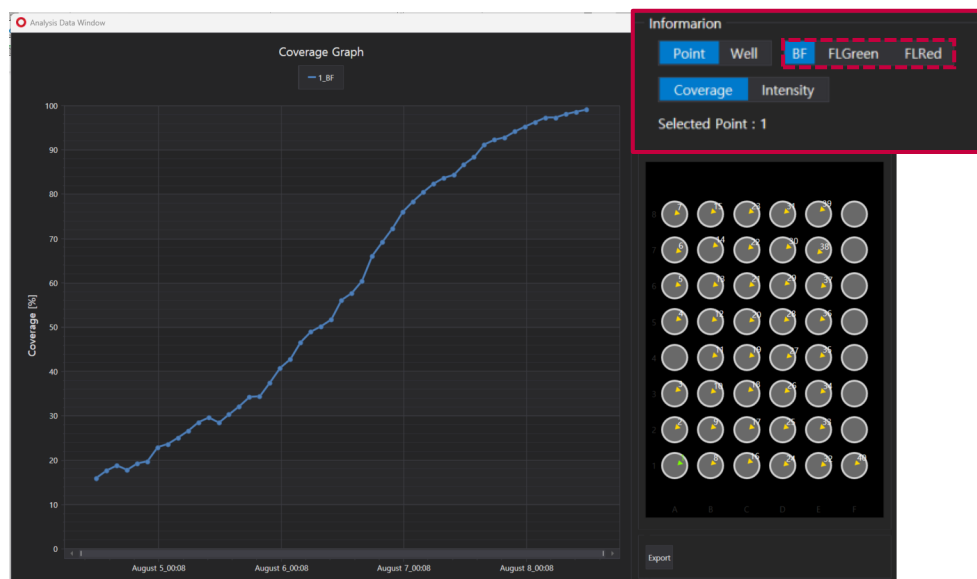




- **Point/Well:** Choose either a point or well to create the graph. Choosing a point shows the graph for each scan position, while selecting a well shows the graph of the average value for that specific well.



- **BF/FLGreen/FLRed:** Choose the light channel to create the graph.



- **Coverage/Intensity:** Choose whether to make a coverage graph or an intensity graph.
  - ✓ **Record:** Make a video (refer to p.49~50).
  - ✓ **Play:** Show the images in the list in order (refer to p.49 for slideshow).
  - ✓ **Stop:** Stop playing, analyzing, or stitching.
- C. The light source setting of the image taken. Once, clicked, the image of the selected channel appears on the display.
- D. **Scan positions:** The scan positions specified in the **Celloger Min Plus Scan App** are shown in order, along with the X and Y coordinates of those positions and the **Label**. The **Label** represents the well number in the well plate. (If it is not a well plate, all **Label** appears as A1.)
- E. **Image list:** Displays the cycles of the scan position. The image of the selected cycle is shown on the display. The time (**Time Stamp**) when the images were taken for each cycle is shown, and the result of coverage in percentage is shown after undergoing calculation using **Analyze**.
- F. It shows the well positions, a list of Z-stacking, and stitching.
- ✓ **Well Position:** Shows the well position at the time of imaging.
  - ✓ **Z-stacking:** Shows the list of Z-stacking images (refer to p.54) and merges stacked images (refer to p.55).
  - ✓ **Stitching:** Shows the location of captured images and stitches the images (refer to p.56).

# Safety Instruction

**\*\* Read all instructions before use.**





## General guidelines

- Install the device on a rigid and level place.
- Operate the device in conditions described in the operating condition section below.
- Use only the components provided and authorized by Curiosis Inc.
- 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.
- To avoid electrical shock, do not insert any metallic objects into the device through the bottom or side air vents; doing so can cause personal injury or device damage.
- Place the device around 10 cm away from the surroundings for proper air-cooling.
- Do not disassemble the device in any event. Contact your local distributor to arrange for service in case of malfunctioning. Malfunctions caused by disassembling of devices, external shock, or internal contamination will not be covered by the warranty even if the warranty period remains.
- Operate the device carefully as described in this manual.
- Do not bend the cable excessively.
- Use authorized accessories only.
- Do not move the lens with your hands.
- Do not look at the light directly with your eyes when imaging fluorescent channels.

## Operating condition

<b>Operating power</b>	100~240 VAC
<b>Frequency</b>	~50/60Hz
<b>Operating temperature</b>	10~40°C
<b>Relative humidity</b>	20~95%
<b>Installation site</b>	Indoor use only

## Safety standards

European standards		US standards	
UK Standards		Korean standards	
ROHS			

## Cleaning and Maintenance

- When cleaning optical elements, use only a damp cloth to avoid scratching soft lens coatings. Do not clean the lens with organic solvents.
- Lightly wipe the working surfaces of the **Celloger® Mini Plus** with a soft cloth dampened with 70% ethanol or hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Do not pour or spray liquids anywhere on the instrument.
- If liquid spills on the instrument, turn off the power and wipe dry immediately.
- Make sure that liquid or foreign matters do not enter fan holes or lens holes.
- In case of storage at 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 tools 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.
- Avoid leaving the equipment in the incubator without power for an extended period.
- When not using the equipment for a long period of time, turn on the fan and dry it before storing. Store it at room temperature on a flat place without humidity and vibration.

# Trouble Shooting

## Installation

### Device does not power up

- ✓ Check power source or contact your distributor.
- ✓ Replace power cable if it is in poor condition.

### Cannot connect with Celloger® Mini Plus device

- ✓ Make sure the device is connected to the computer.
- ✓ Unplug and re-connect all cables. Restart the **Celloger Mini Plus Scan App**.

## Focus

### Focus is irregular

- ✓ Make sure the sample thickness is uniform.
- ✓ Reload the vessel so that it lies flat on the stage. Be sure vessel the holder is mounted flat with respect to stage.

### Autofocusing does not work properly

- ✓ Before using **Auto Focus**, partially set the focus manually.
- ✓ Change the **Auto Focus** setting.

## Image monitoring

### Image display is dark

- ✓ Click the **Preview** button.
- ✓ Place sample on the center of objective lens.

### Image is not clear

- ✓ Carefully wipe off the objective lens with cotton swab.
- ✓ Eliminate any dust on culture dish and LED lamp.
- ✓ Remove any condensation on the lid of the culture dish.

### Problem in saving files

- ✓ Check the unused volume of the hard disk.

Check whether the file name and location address are written correctly. Only English, numbers, and some special symbols are allowed. Avoid characters such as /, :, \*, ?, <, >, |, etc.

### Time-lapse images become dark and bright

- ✓ To prevent any problem such as shaking of culture dish or inflowing light, pay special attention when you open or close incubator door during monitoring.

## Data

### Cannot play the video

- ✓ Video can be made only for time-lapse images obtained from the **Celloger Mini Plus Scan App**.

### Cannot analyze the coverage

- ✓ Check if the images are obtained from the **Celloger Mini Plus Scan App**.
- ✓ Before analyzing, check if the focus is clear.
- ✓ Conduct **FL** merge, set appropriate thresholding parameter and use coverage analysis to calculate the coverage of fluorescence.

## Others

### Water droplets from on the well plate, dish lid or top of the flask (Condensation occurs)

- ✓ Warm up the device 1 hour before starting monitoring.
- ✓ Check 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.).
- ✓ Make sure it is 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.** In case of using a 96-well plate, using sealing film would prevent condensation and drying of media.

## Product specifications

<b>Dimension</b>	226 x 358 x 215 mm
<b>Weight</b>	5.6kg / 12.3lb
<b>Objective Lens</b>	2X / 4X / 10X
<b>Imaging modes</b>	Brightfield, Fluorescence (Green/Red)
<b>Fluorescence</b>	Green : Excitation (470/40x) / Emission (510lp) Red: Excitation (510/84x) / Emission (570lp)
<b>Light source</b>	LED
<b>Camera</b>	5.0 MP CMOS
<b>Focus</b>	Auto / Manual
<b>Stage</b>	Motorized XYZ
<b>Imaging positions</b>	Multiple
<b>Field of view</b>	2.53 x 1.9 (2X), 1.185 x 0.895 (4X), 0.574 x 0.43 (10X) mm
<b>File export format</b>	TIFF, AVI, CSV
<b>Culture vessels</b>	Flask, dish, well plate, slide
<b>Operating environment</b>	10~40°C, 20~95% humidity
<b>Power requirements</b>	100-240V, ~50/60Hz
<b>Output ports</b>	Ethernet
<b>Accessories</b>	PoE adapter, ethernet cable, USB memory
<b>Warranty</b>	1 year
<b>Computer</b>	External PC
<b>O/S required</b>	Window 10 and above
<b>Processor (Recommended)</b>	CPU 3G
<b>Storage (Recommended)</b>	1TB
<b>Monitor (Recommended)</b>	1920*1080mm

## Ordering Information

Cat. No.	Description
<b>CRCLG-MPB02</b>	Celloger® Mini Plus, Live cell imaging system (Bright Field, 2X)
<b>CRCLG-MPB04</b>	Celloger® Mini Plus, Live cell imaging system (Bright Field, 4X)
<b>CRCLG-MPB10</b>	Celloger® Mini Plus, Live cell imaging system (Bright Field, 10X)
<b>CRCLG-MPBG04</b>	Celloger® Mini Plus, Live cell imaging system (Bright Field + Green Fluorescence, 4X)
<b>CRCLG-MPBG10</b>	Celloger® Mini Plus, Live cell imaging system (Bright Field + Green Fluorescence, 10X)
<b>CRCLG-MPBR04</b>	Celloger® Mini Plus, Live cell imaging system (Bright Field + Red Fluorescence, 4X)
<b>CRCLG-MPBR10</b>	Celloger® Mini Plus, Live cell imaging system (Bright Field + Red Fluorescence, 10X)

# Appendix A. Connecting multiple devices

With the **Celloger® Mini Plus**, multiple devices can be operated using one PC but this requires separate set up of a Device IP or purchase of a router. Because one program is used per device, the number of programs required is equal to the number of connected devices.

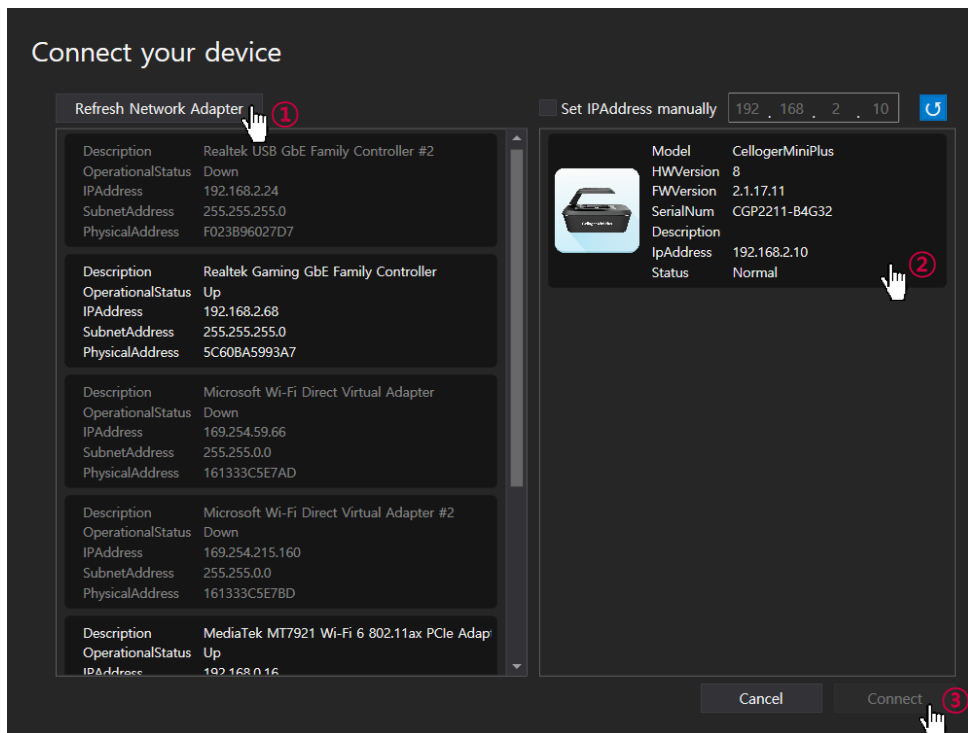
**Note.** Be careful as this system works abnormally if the same device is connected using two programs.

## 1. Installation

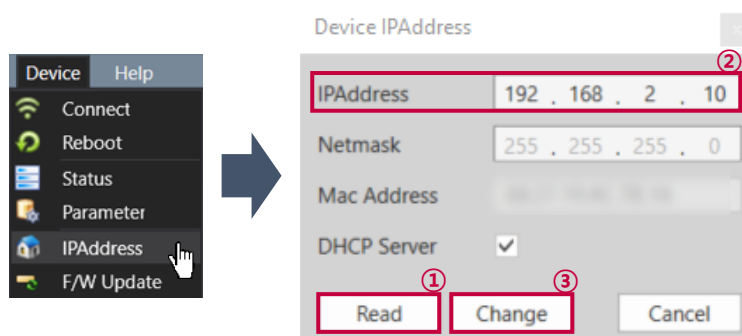
### 1.1 Device IP setup

**Celloger® Mini Plus** is operated based on the connection with a PC using a wired LAN cable (Ethernet connection type). Under the Ethernet connection method, devices with the same IP address cannot be properly connected. To connect multiple **Celloger® Mini Plus** units with the same IP address, it is necessary to set the IP address of the devices. The **Celloger Mini Plus Scan App** can be used to change the IP address of a device.

- ① After connecting one device to a PC, open the **Celloger Mini Plus Scan App**.
- ② Start the App after selecting the device (If the device does not show up, refer to p.11 for **Connect the Device**).



- ③ In the **Device** menu of the **Celloger Mini Plus Scan App**, open **IPAddress** and click **Read**.





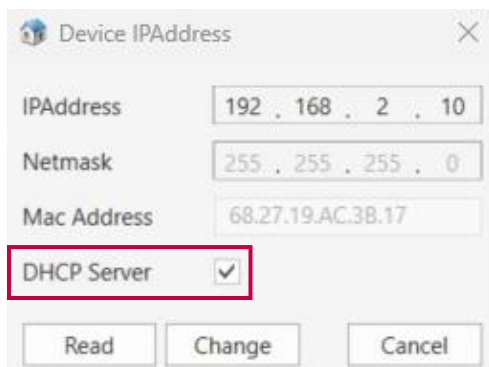
- ④ Change the IP address and click the **Change** button.

**Note.** Make sure that the device's IP address has a different final set of numbers than that of the PC or other devices. Choose a number between 2~254 to put in the last position.

**Ex) IP address setting for connecting 3 Celloger® Mini Plus devices and PC**

- PC IP address: 192.168.2.200
- Device 1. IP: 192.168.2.10
- Device 2. IP: 192.168.2.11
- Device 3. IP: 192.168.2.12

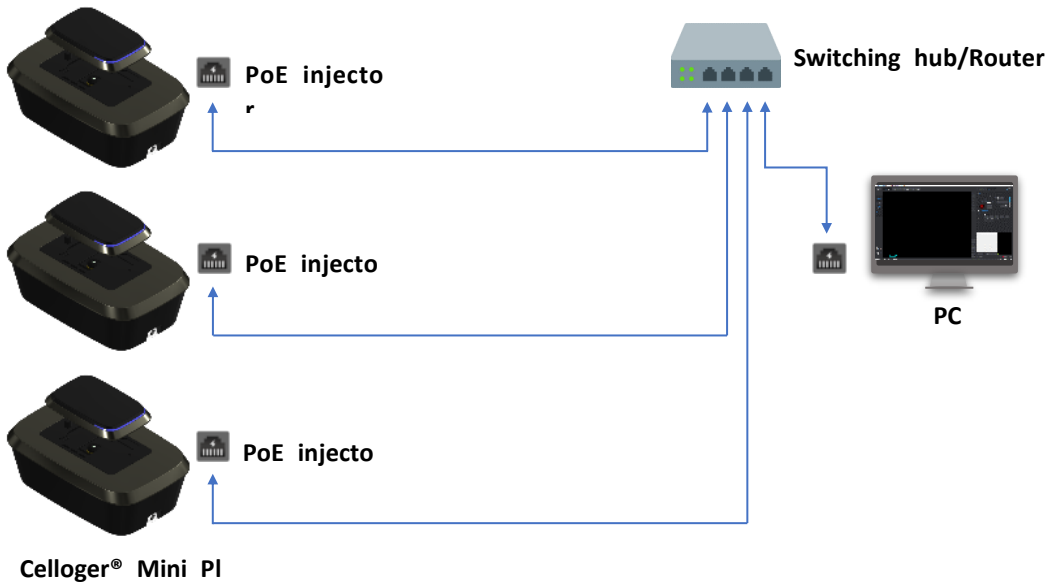
**Note.** When setting the IP address of the network adapter in the **Celloger Mini Plus Scan App**, only one of two or more devices must be checked in the **DHCP Server** and other devices must be unchecked. In case of setting the IP manually on a PC (p.75), connection can be done regardless of the **DHCP Server**.



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

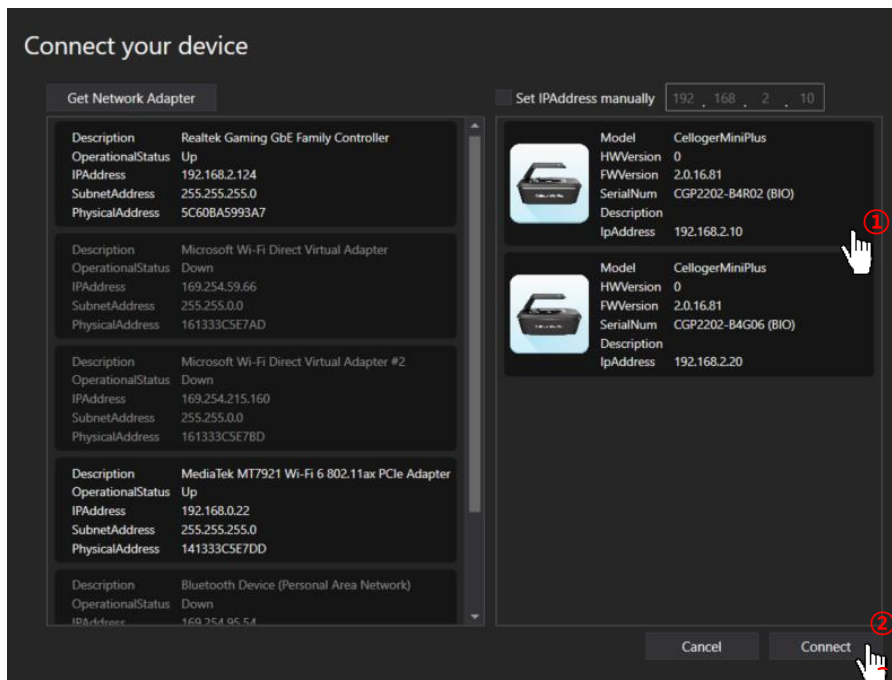
## 1.2 Using a router

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.



## 2. Operation

- ① Open the **Celloger Mini Plus 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:

- 1) Check the selected device description.
- 2) Check the IP address at the bottom of the program (refer to p.43 ①).
- 3) Click **Identify** (💡) and check if the white LED is blinking.

## Appendix B. Alternative PC network setup

Perform the PC network setting as follows to connect the device with PC. The steps below are based on “Windows 10”. For “Windows 11”, refer to the images below (p.77~78).

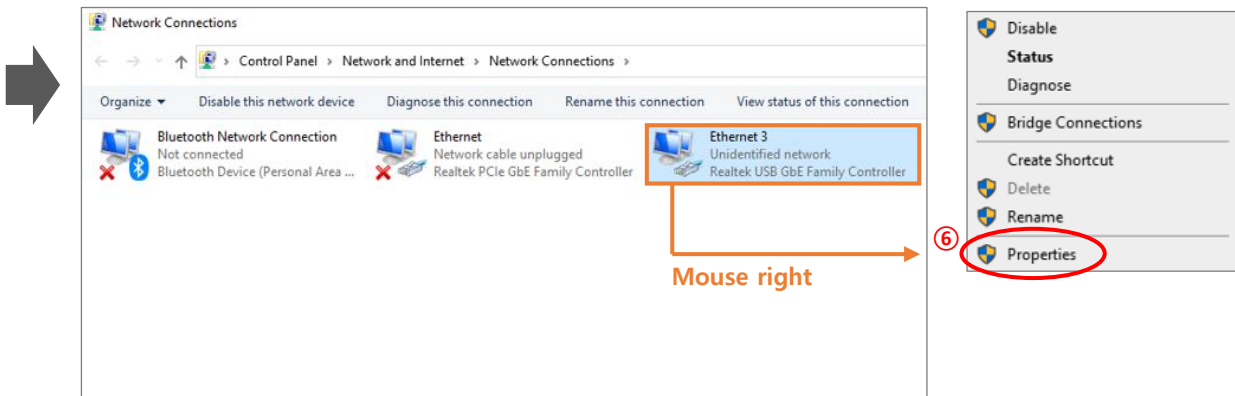
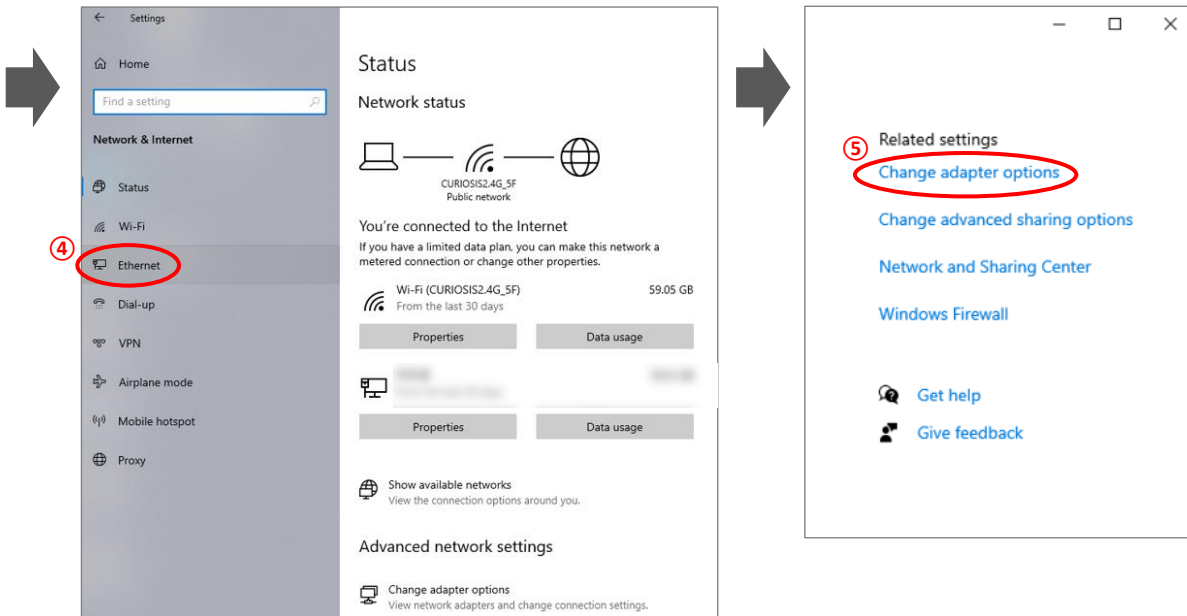
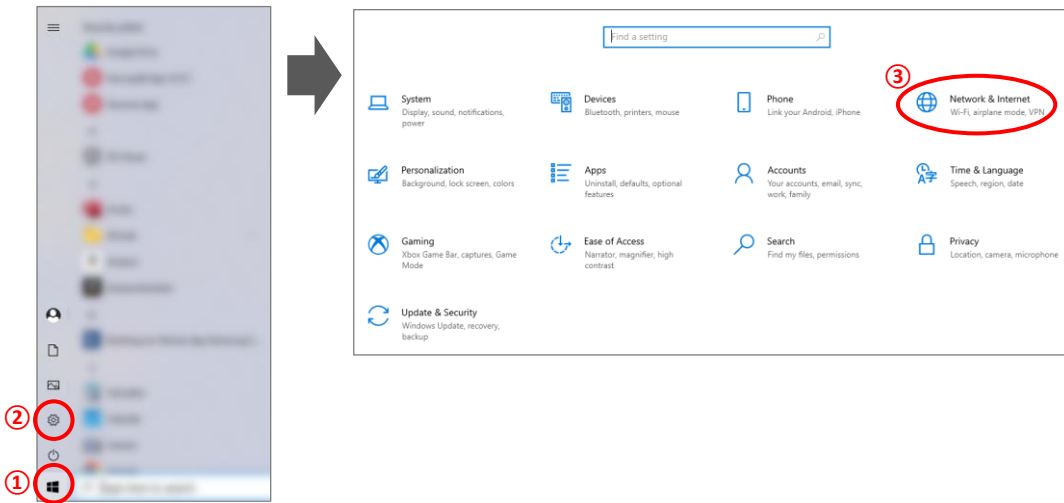
- ① Turn on the computer and click the “**Start**” or “**Window**” icon.
- ② Click “**Setting**”.
- ③ Click “**Network and Internet**” in the Control panel.
- ④ Click “**Ethernet**”.
- ⑤ Click “**Change adapter options**”.
- ⑥ Select “**Ethernet**” (this name can vary according to the PC.) and right-click the mouse and click “**Properties**” in the window that appears.
- ⑦ Select “**Internet Protocol Version 4(TCP/IPv4)**” and click “**Properties**”.
- ⑧ Select “**Use the following IP address**” and enter the **IP address (192.168.2.XX)** and **Subnet mask (255.255.255.0)** in the blank fields.

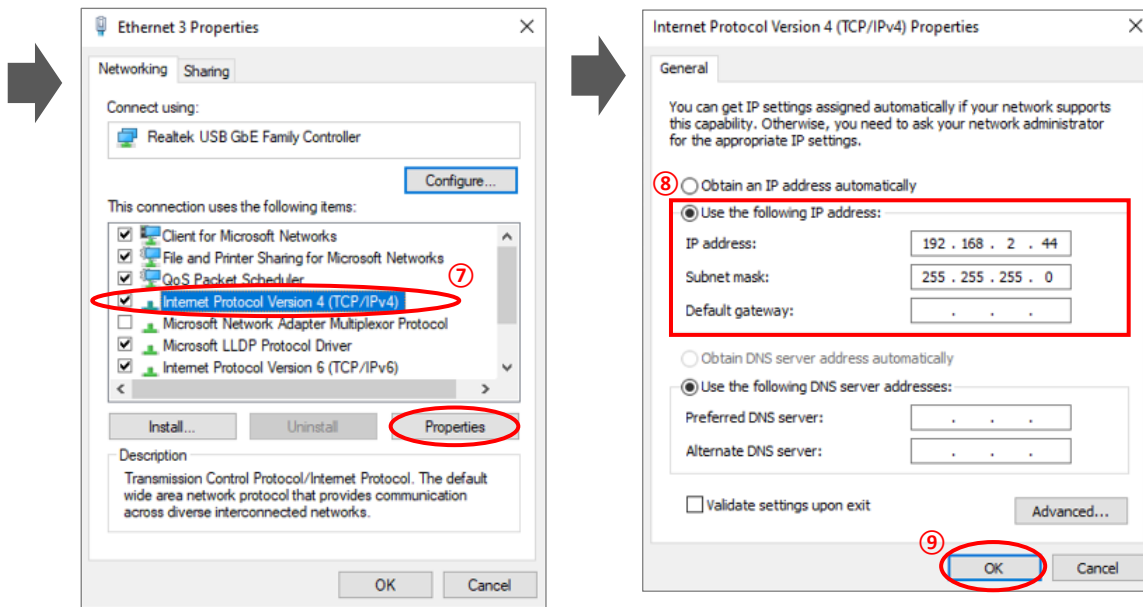
**Note.** Fill in any numbers from 2~254 except 10 in XX fields.

- The PC’s IP address should be set differently from the device’s 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 PCs depending on the PC network environment. In case of connection failure, contact us via [info@curiosis.com](mailto:info@curiosis.com) or your local distributor.
- ⑨ Click “**OK**”, then the network configuration is completed.
  - ⑩ To start the **Celloger Mini Plus Scan App**, first, connect the USB drive provided with device into the PC, then copy the app folder to PC and execute “**Celloger Mini Plus Scan App.exe**” in the app folder.

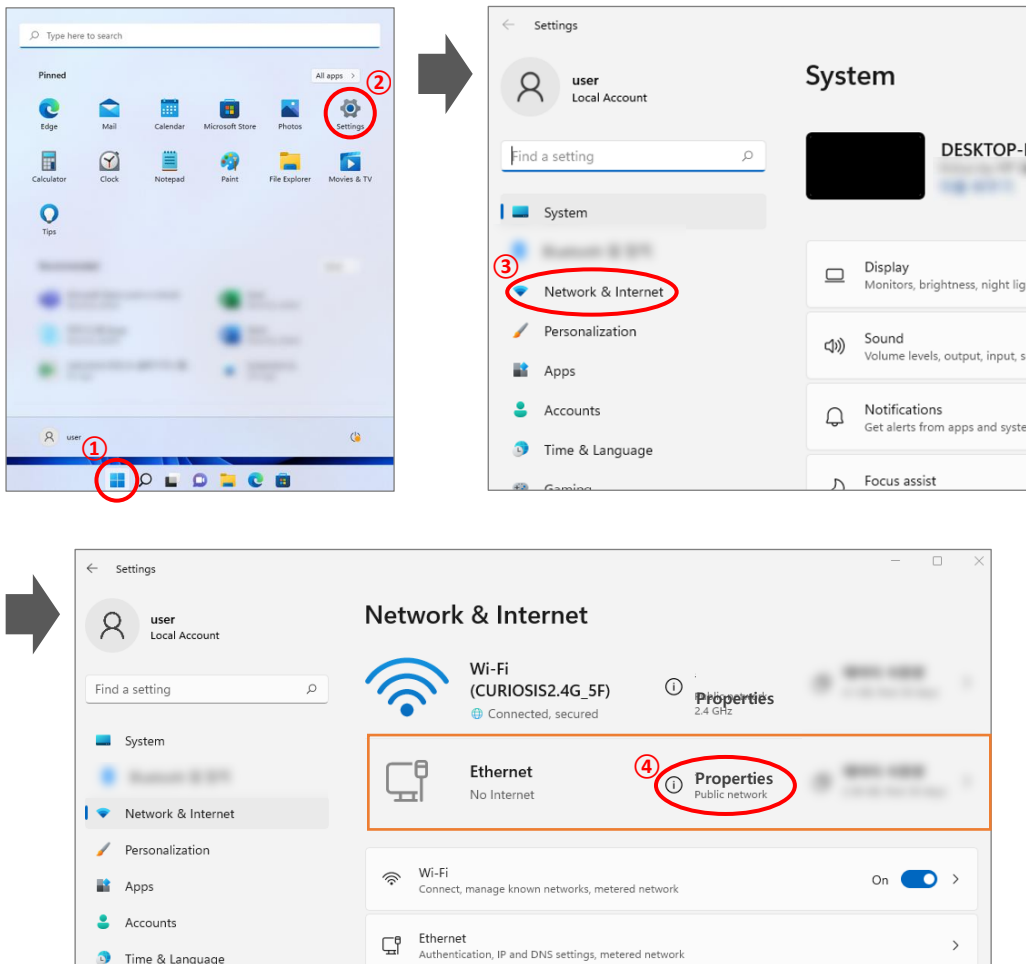
# < Image Descriptions of PC network setting >

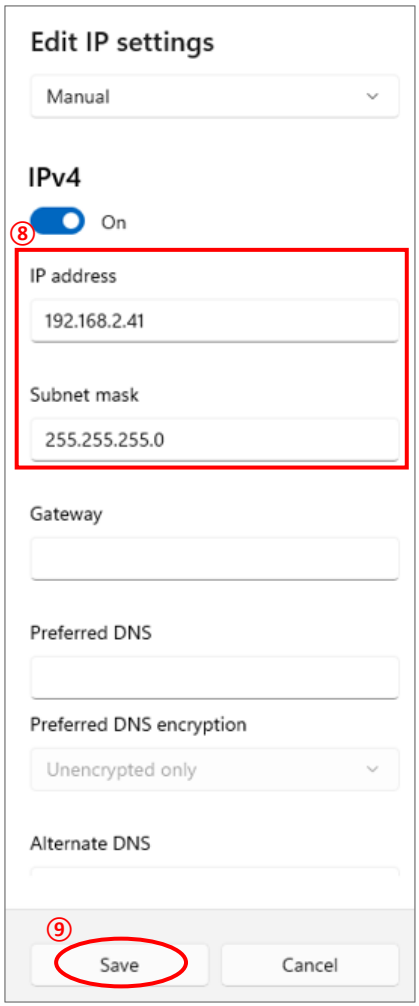
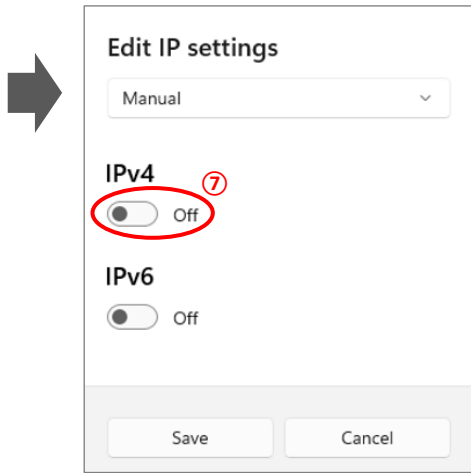
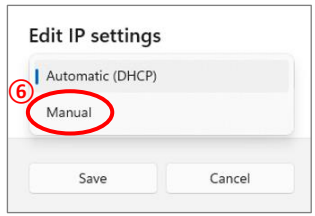
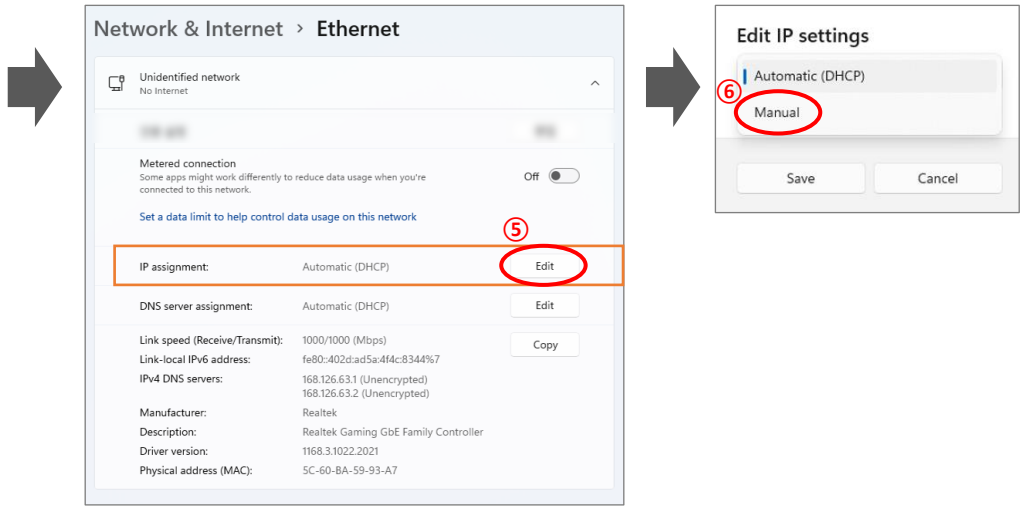
## 1) Window 10





## 2) Window 11

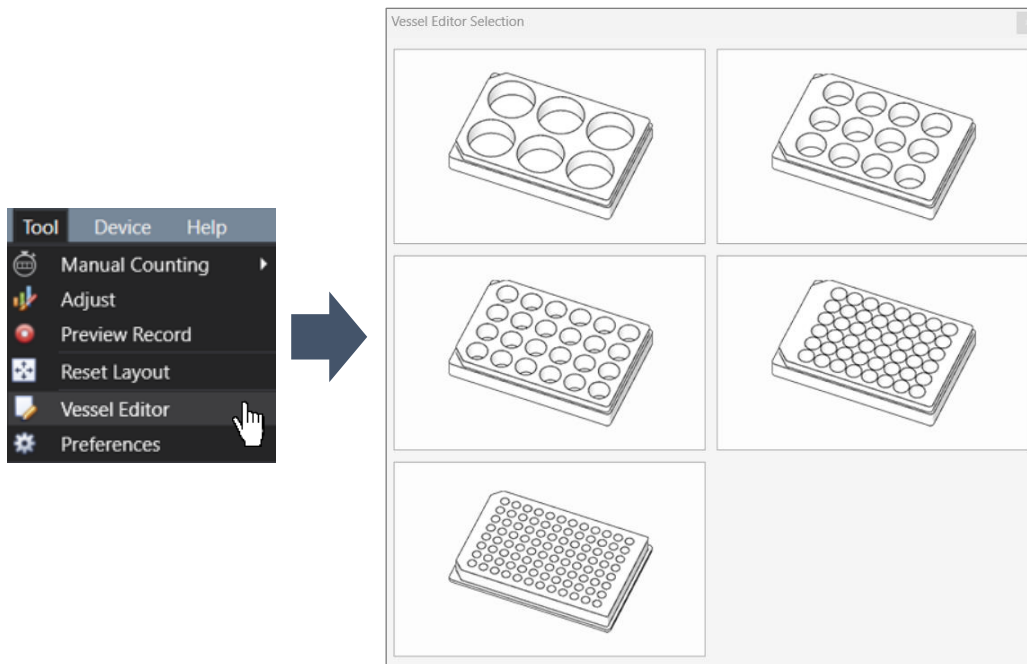




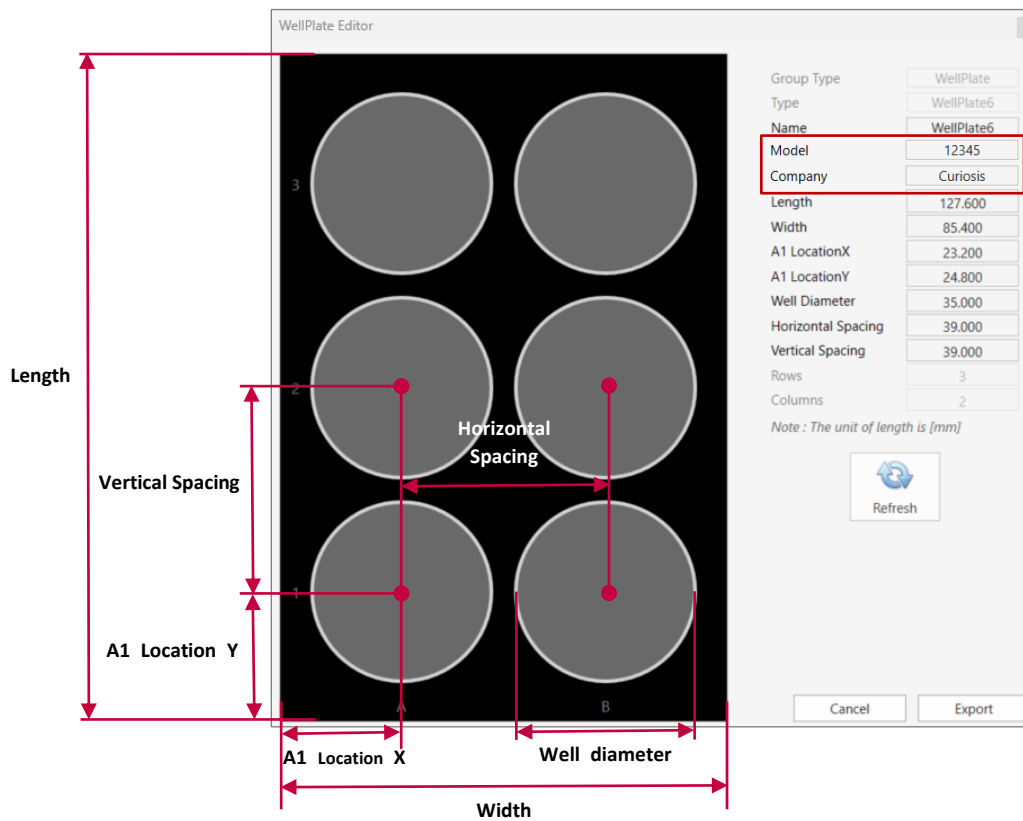
## Appendix C. How to use vessel editor

The **Celloger® Mini Plus**'s 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, it is recommended to set the well plate specifications of the brand used in **Vessel Editor**.

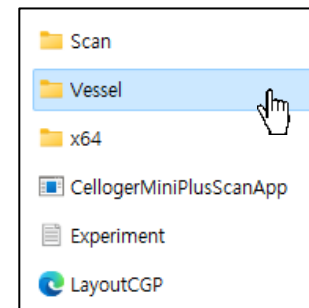
- ① Select **Vessel Editor** from the **Tool** menu.
- ② Select the type of well plate (6/12/24/48/96 well) to be used from the **Vessel Editor Selection** window.



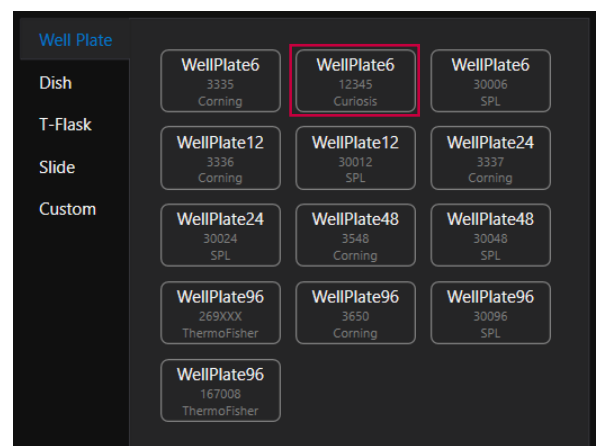
- ③ Change the specifications (**Length, Width, A1 location X, A1 location Y, Well Diameter, Horizontal Spacing, and Vertical Spacing**) of the well plate to be used after entering the **Model** and **Company** in the **Well plate Editor** window. Refer to the 6 well plate image below for your reference.



- ④ Click on **Refresh**.
- ⑤ Click on **Export** to save the setting values. The saved file can be found in the **“Vessel”** folder of the **Celloger Mini Plus Scan App**.



- ⑥ Re-run the **Celloger Mini Plus Scan App**.
- ⑦ After checking 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 holder to 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.



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

## Appendix E. The list of shortcut keys

Save the image	 
Save the project	 
Move to the double-clicked position	  Mouse double click
Zoom in & out	  Mouse wheel
When in Preview on state, camera moves according to drag.	  Mouse drag
The camera moves so that the double-clicked location becomes the center of the screen.	  Mouse double click

## Revision History

Date	Version	Description
2022/03/03	CR-IFU-008	Initial issue
2022/06/09	CR-IFU-008(Rev.1)	- Revised contents (Operating condition)
2022/09/15	CR-IFU-008(Rev.2)	<ul style="list-style-type: none"> <li>- Added contents (Grid line, Image adjustment, Preview record, Stitching, Chart)</li> <li>- Revised contents (Table of contents, Getting started, Z-stacking, Image analysis control, Autofocus, Cleaning and Maintenance, Troubleshooting, Appendix B)</li> <li>- Revised UI (Image analysis control)</li> </ul>
2023/08/15	CR-IFU-008(Rev.3)	- Added marks (Design award)
2023/12/12	CR-IFU-008(Rev.4)	- Added marks (Awards, revision version)
2024/04/04	CR-IFU-008(Rev.5)	<ul style="list-style-type: none"> <li>- Added contents [Create or open a config file, Estimate coverage, Intensity Z-projection, Image adjustment (Analysis app), Intensity (Analysis app), Manual counting (Analysis app), etc.]</li> <li>- Revised contents [Table of contents, Image adjustment (Scan app), Merge the FL image, Create coverage graph, Show Z-stacking image, Stitch images, etc.]</li> <li>- Revised UI (Analysis app)</li> </ul>



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