

Celloger[®] Mini Plus

Automated live cell imaging system

Instruction Manual

Developed and manufactured by CURIOSIS Inc.

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I. Package contents

Celloger® Mini Plus Live cell imaging device package includes the following items.

Item	Quantity
Celloger® Mini Plus main device	1
Main power cable	1
PoE	1
LAN Cable (5M, 4.8M)	2
USB/LAN adapter	1
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 ² , single (Optional)	1
Vessel holder for T-flask 25cm ² , dual (Optional)	1
Vessel holder for T-flask 75cm ² , single (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 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 at dust- or other airborne particles-free area.
- During operation, place around 10 cm away from the surroundings for proper air flow of the cooling fan.
- During operation inside incubator, do not place close to the incubator door.
- Do not put any heavy materials on top of the Celloger® Mini Plus.
- If using multiple equipment, there should be a proper clearance among equipment.
- Keep the device at temperature between 10 ~ 40 °C.
- Relative humidity range is 20 ~ 95 %.

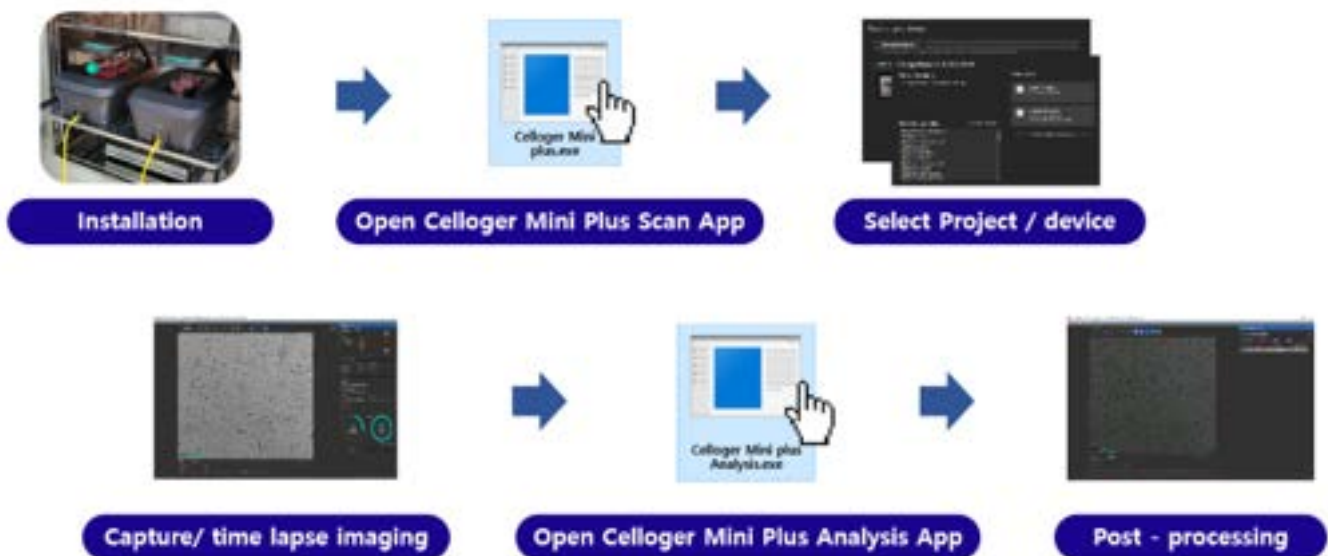
Important!

It is not recommended to operate the device at low temperature (Below 10 °C). In those conditions, warm up the device for over 10 minutes.

PC specifications (p.55)

- Celloger® Mini Plus software can be used on PCs with Window 10.
- Large capacity hard drive is necessary to save images from Timelapse. If the capacity is not enough, scanning will not function properly.
- For the ease of use, it is recommended to use a monitor with resolution of 1920 x 1080.
- LAN port is required to connect the equipment to PC using LAN cable.

2. Operation overview



3. Installation

Unpacking

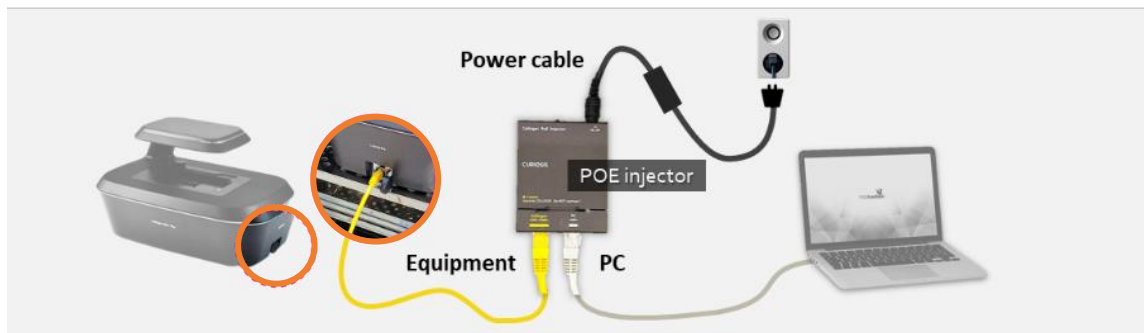
1. Lift the Celloger® Mini Plus from the box, remove all the covers, and place it on a clean and level surface.
2. Make sure all accessories are present by checking “Package Contents” (p.4). If any items are missing or damaged, please contact your distributor or Curiosis at info@curiosis.com.

Connect the device and computer

1. Prepare a computer that satisfies the specifications (refer to p.55 for PC specifications) and place it near the incubator.
2. Connect two LAN cables and power cable to the POE injector. Connect one LAN cable to the device (yellow) and another LAN cable to the PC (white).



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

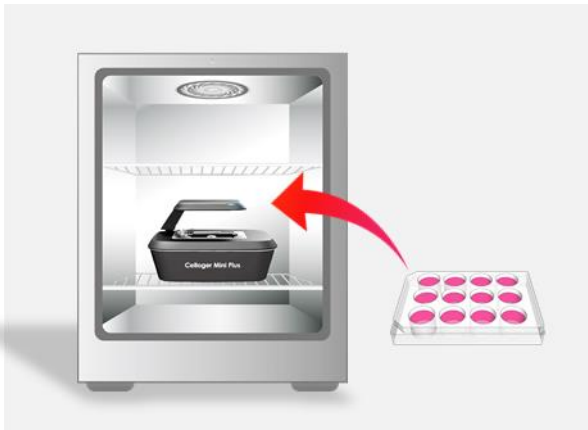


Install the device inside an incubator

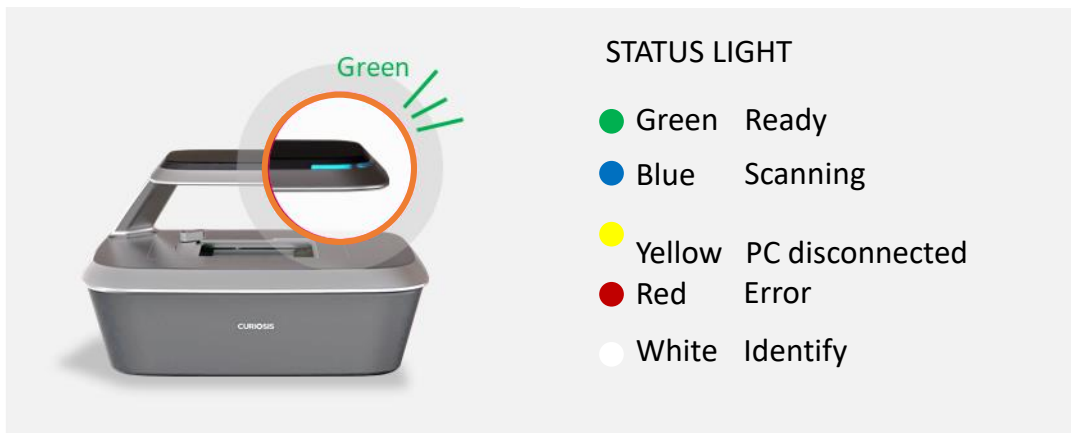
1. Install the vessel holder on Celloger® Mini Plus, then place the device inside the incubator.
*Before using the device, make sure to remove all the covers including the lens cover(Black) and clean the device with soft dampened with 70% ethanol.



- Place the sample on the stage.



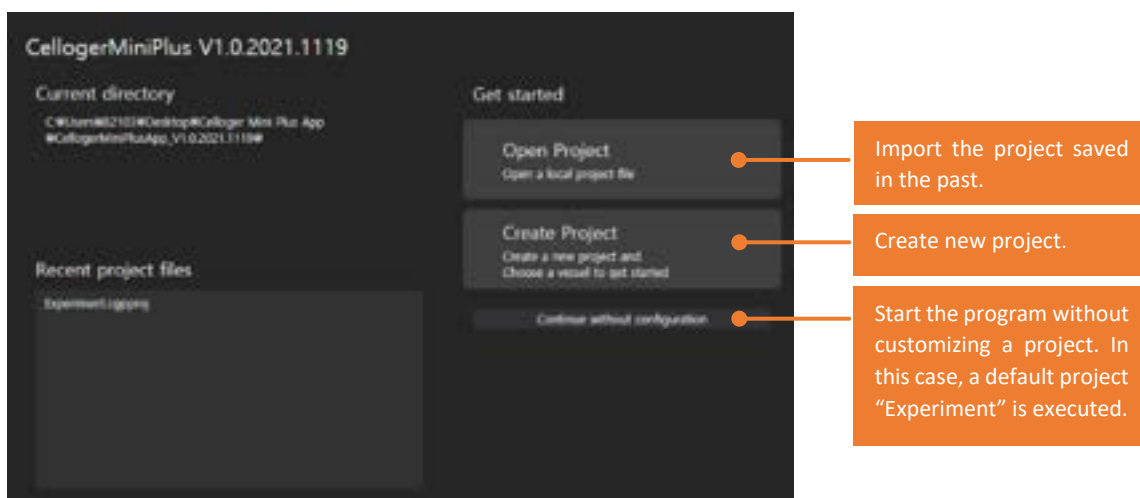
- Check the connection between the PC and the device. To connect the device and PC, refer to “**Connect the device**” (p.9)



4. Create or open a project

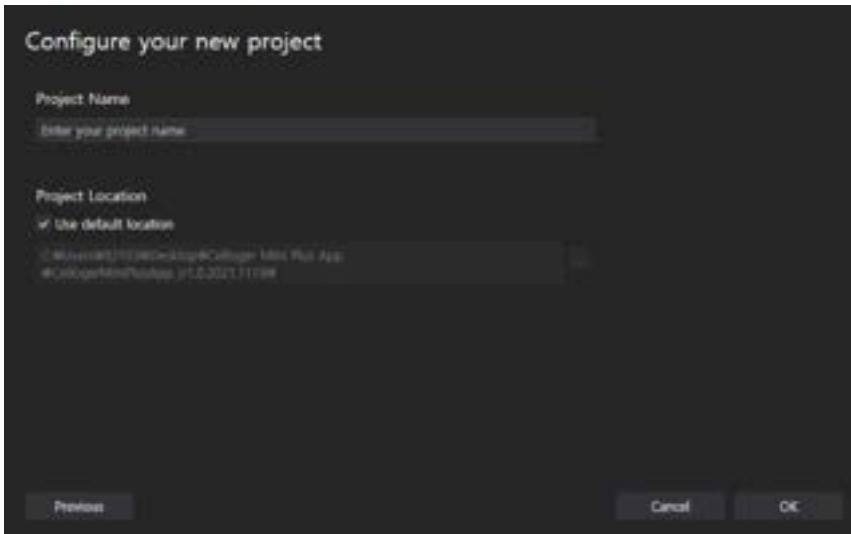
Upon executing the **Celloger Mini Plus Scan App**, popup screen for project selection is displayed. With Celloger Mini Plus, the setting can be managed by the project file (“.cgnproj” file format). Setting values(Light intensity, Focus position, Schedule, File storage location, etc.) are saved in the project.

Note. In **Celloger Mini Plus Analysis App**, the setting is managed by config file instead of project file.

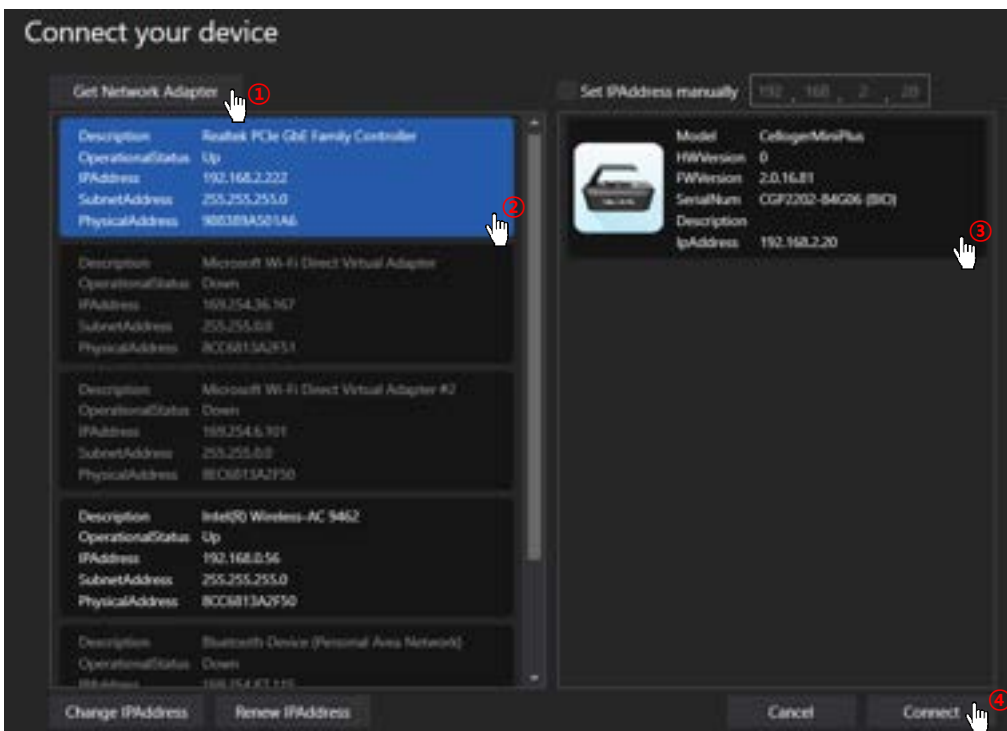


- Create a new project or open an existing one. You can start a program with default project by clicking **Continue without configuration**.

- Upon selecting **Create Project**, popup window that requires the selection of project name and storage location shows up. You can start the program after entering required information and press **OK**. The storage location of the project is the address of default program file and if you want to change the storage location, cancel **Use default location** and enter address.



5. Connect the device



1. Open **Celloger Mini Plus Scan App**.
2. When the app is executed, **Create or Open a project** popup appears. Select **Continue without configuration**.
3. **Connect your device** popup will show.
4. Click **Get Network Adapter** to load the network adapters connected to the PC.
5. Select PC network from **Network adapter list** then the device icon will appear on the right.
6. Click the device icon.

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

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

Set the IP address of PC network manually. If the PC IP, device IP and router IP are the same, connection fails (refer to Appendix B).

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

Check all cable connection status and POE injector power (refer to **Connect the device and computer** in p.7).

Case 3. Separate network adaptor is used.

Press the **Get Network Adapter** to select the adaptor to which the device is connected. If the device icon does not appear even when the network adapter is selected, search for device IP address directly from.

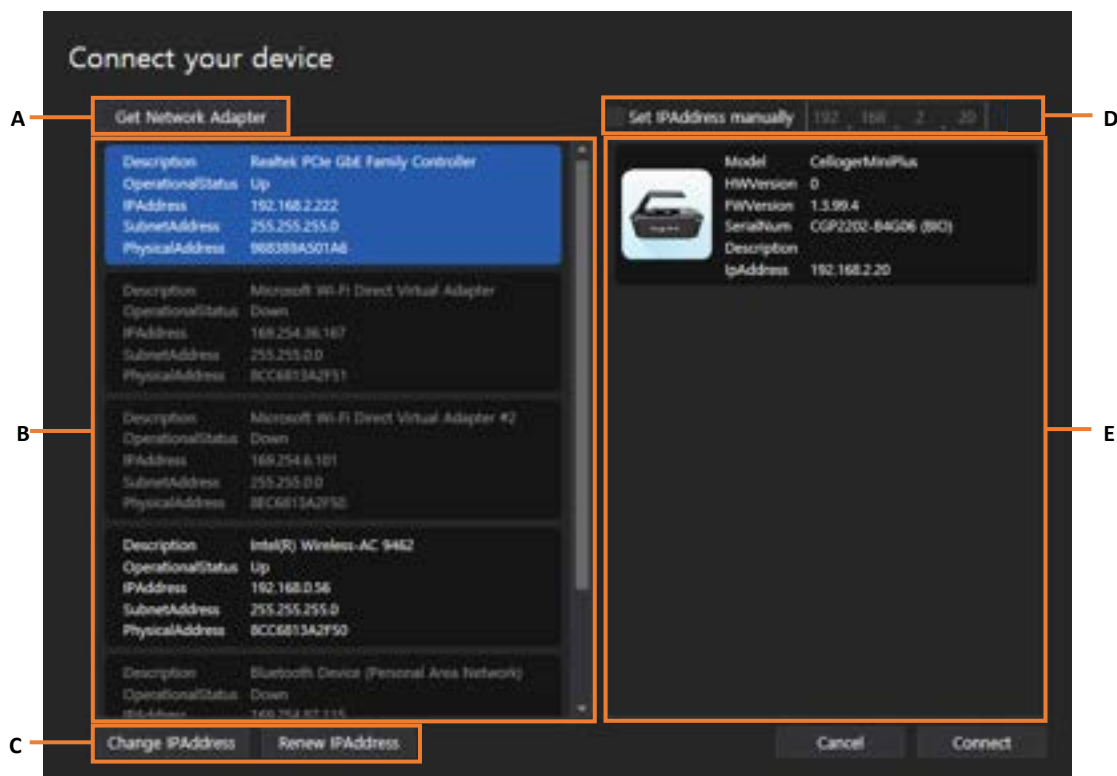


Case 4. When using multiple devices in one PC, each device has the same IP.

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.57 for device IP setting)

7. Click **Connect**. When the connection has successfully completed, the LED status indicator lights in green (from yellow)

Device connection pop-up description



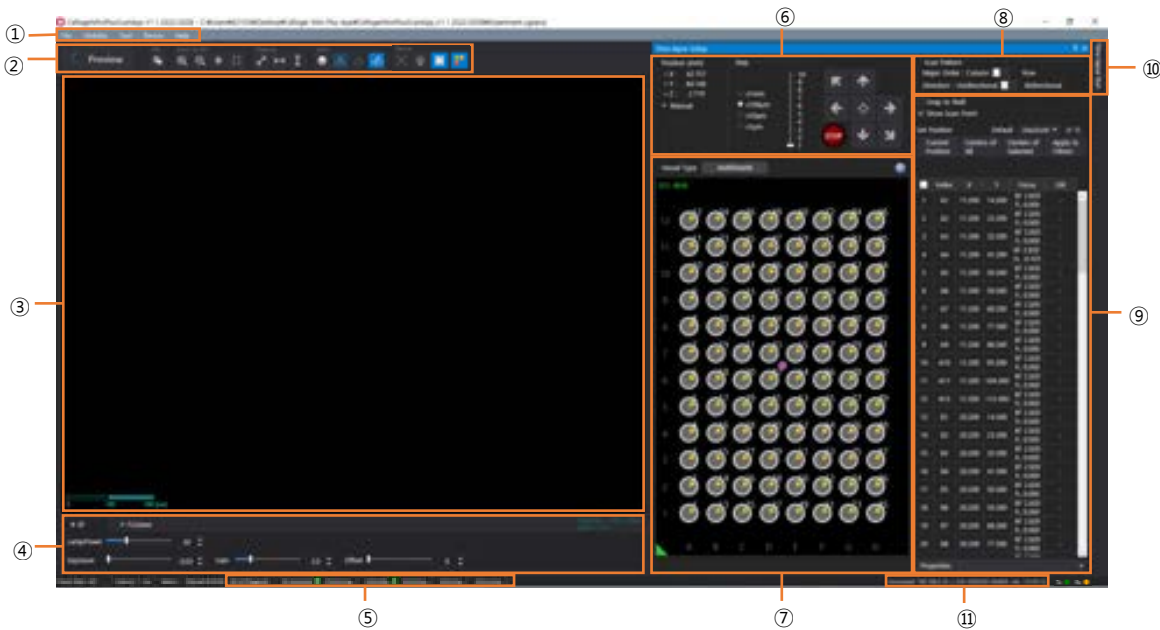
- Get Network Adapter** reads the connected network adapter from the PC.
- Network adapter list. **OperationalStatus** shows the network connection status where **Up** means network is enabled, while **Down** means disabled.
- Change the IP address of the selected network adapter.
- Manually search for the IP address of the device.
- The list of connected devices.

6. Software overview

Celloger® Mini Plus provides two software: **Celloger Mini Plus Scan App** and **Celloger Mini Plus Analysis App**. **Celloger Mini Plus Scan App** is a program for actual operation of **Celloger® Mini Plus** device which includes real-time cell monitoring and time-lapse imaging function. **Celloger Mini Plus Analysis App** is a program for analyzing and post-processing the images obtained from **Celloger® Mini Plus** and it can be operated even without the device connection.

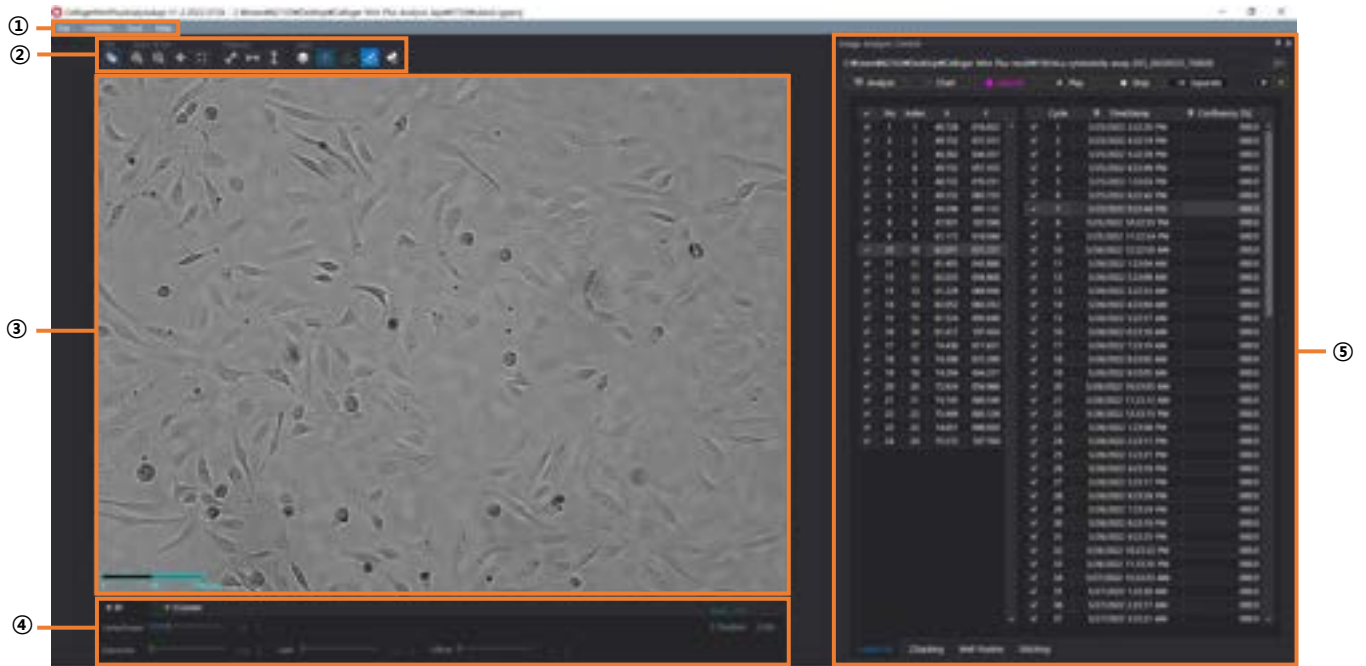
Note. Refer to p.35 for full software descriptions

6.1. Celloger Mini Plus Scan App



- 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

6.2. Celloger Mini Plus Analysis App



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

IV. General Operation

1. Preparation (cell/device preparation)

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

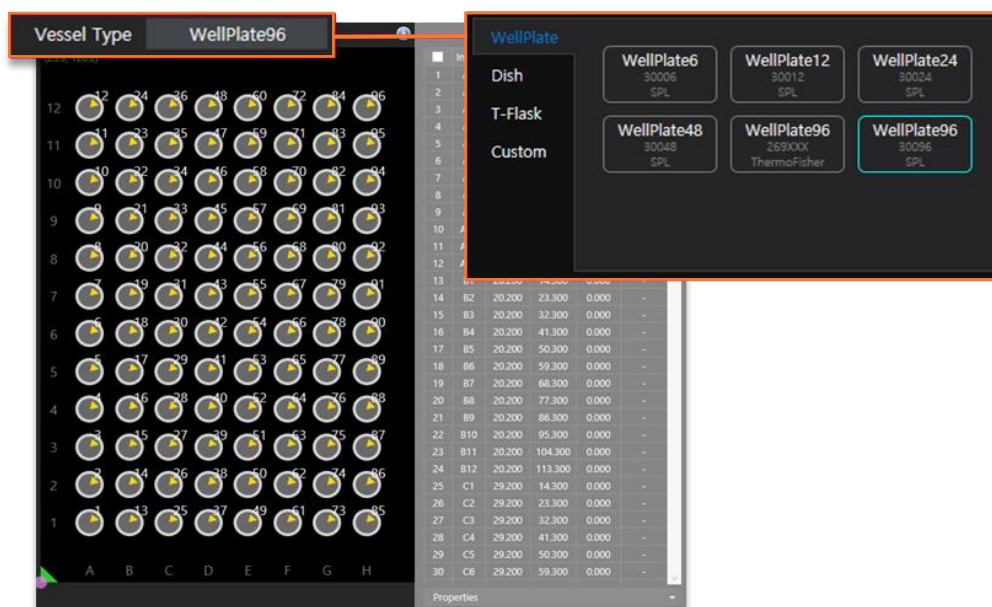
1. Before culturing the cell, place **Celloger® Mini Plus** in the incubator for prewarming.
Note. Install the system as far as possible from the incubator door and place the device around 10 cm away from the surroundings for proper air flow of rear cooling fan.
2. Prepare pre-warmed medium and cells on a plate.
3. Discard the medium on vessel and wash the cells with PBS buffer.
4. Discard PBS buffer and treat trypsin-EDTA solution to detach the cell from vessel.
5. Incubate for a few minutes and check cell detachment by Celloger® Mini Plus or microscopy.
6. Remove cell and Trypsin-EDTA to conical tube and add fresh medium to neutralize the Trypsin reagent.
7. Centrifuge the suspension cells on conical tube.
8. Discard the supernatant and add fresh medium into cells.
9. Gently pipette the cell several times.
10. Transfer the appropriate amount of cells into new vessel and dilute the cell concentration by fresh medium.
11. Cover the plate with a lid and gently shake the plate to spread the cells evenly.
Note. Before covering the plate, warm the lid by slightly passing the lid over fire of alcohol lamp to prevent condensation. This step is not necessary, but it will prevent trapping the condensation on a lid.
12. Place the plate on Celloger® Mini Plus.
Note. Both plate and device should be pre-warmed for 30 minutes prior to time-lapse imaging to prevent condensation.T

2. Cell monitoring and image setting [in Scan App]

2.1 Location adjustment

1) Adjust X/Y position

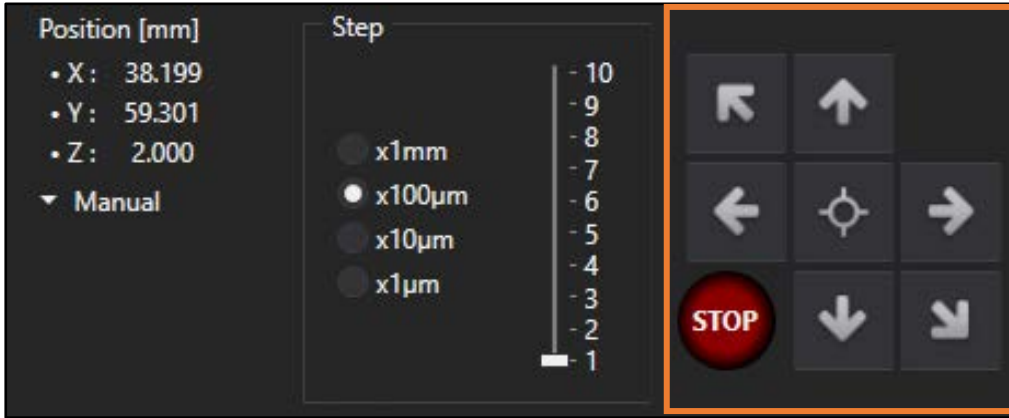
- ① Select vessel type and desired scan location on the **Vessel & Plate panel**.



- ② Press **Preview** for streaming while **BF** is selected in the **Light Source Control**.



- ③ Move to the desired scan position using the **Jog button**. If the display is too dark or bright, preventing the cells to be seen, run **2.2 Light intensity adjustment** in p.17 first.



2) Set BF Focus

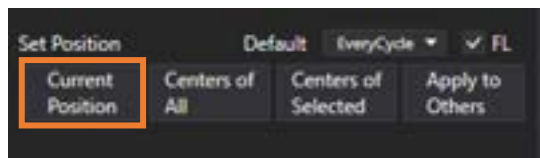
➤ Manual adjustment


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

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

Note. Celloger® Mini Plus’ Z stage absolute coordinate error is 0.001 mm.

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



Note. If you want to change the focus, select the position you wish to change in the scan table, click  under **Properties** and click on **Apply** or **Apply All** to apply the change. (refer to p.45 for **Properties**)

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

➤ Auto Focus

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

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

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

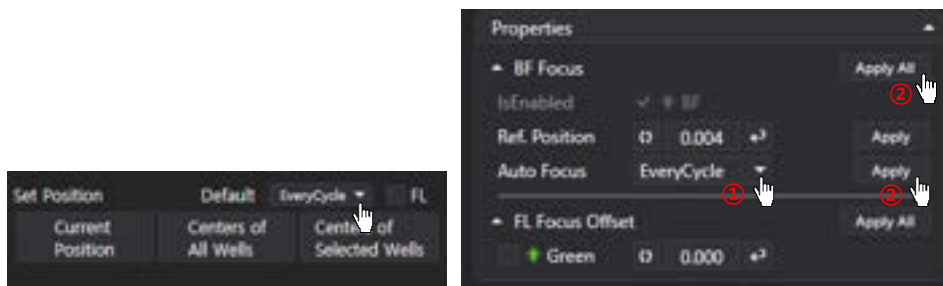


- ③ Designate parameter (**Depth, Resolution, Offset**) in the **Auto Focus** and press **OK**.



- ✓ **Depth:** Screening scope for autofocusing
 - ✓ **Resolution:** The interval of autofocusing calculation within screening scope (0.010 mm or less for 4X optic and 0.005 mm or less for 10X optics)
 - ✓ **Offset:** If you want to select the point different from focal plane recognized by **Celloger® Mini Plus**, input the difference between the point and Z position coordinates from autofocusing.
 - ✓ **IsDarkSideOriented:** Select whether to focus on the black side when performing autofocus. Focus is on black side when checked, if not, focus is on the clearer side of either black or white.
- Note.** The larger **Depth** and the smaller **Resolution**, the higher autofocusing accuracy. Meanwhile, the longer the screening time, the longer autofocusing time.

- ④ Set the value except for **None** in **Auto Focus Frequency** or **Auto Focus** in **Properties** to set the frequency of autofocusing function (refer to p.42 for **Auto Focus Frequency**). If selecting it from **Properties**, click **Apply** or **Apply all** after selecting.



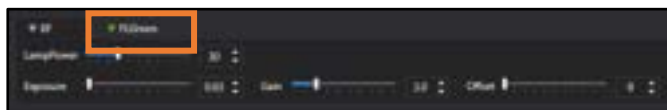
3) Set FL Focus Offset


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

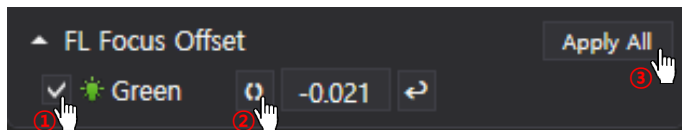
- ① Select the desired scan position on the **Scan table**. It is indicated in green once selected.

Index	X	Y	Focus	USB	
1	A1	11,200	14,300	BF 2.890	-
2	A2	11,200	23,300	BF 2.890	-
3	A3	11,200	32,300	BF 2.890	-
4	A4	11,200	41,300	BF 2.890	-
5	A5	11,200	50,300	BF 2.890	-
6	A6	11,200	59,300	BF 2.890	-
7	A7	11,200	68,300	BF 2.890	-
8	A8	11,200	77,300	BF 2.890	-
9	A9	11,200	86,300	BF 2.890	-
10	A10	11,200	95,300	BF 2.890	-
11	A11	11,200	104,300	BF 2.890	-
12	A12	11,200	113,300	BF 2.890	-
13	B1	20,200	14,300	BF 2.890	-
14	B2	20,200	23,300	BF 2.890	-
15	B3	20,200	32,300	BF 2.890	-
16	B4	20,200	41,300	BF 2.890	-
17	B5	20,200	50,300	BF 2.890	-
18	B6	20,200	59,300	BF 2.890	-

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



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



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

Index	X	Y	Focus	USB	
1	A1	11,200	14,300	BF 2.890	-
2	A2	11,200	23,300	BF 2.890	-
3	A3	11,200	32,300	BF 2.890	-
4	A4	11,200	41,300	BF 2.890	-
5	A5	11,200	50,300	BF 2.890 FL -0.021	-
6	A6	11,200	59,300	BF 2.890	-
7	A7	11,200	68,300	BF 2.890	-
8	A8	11,200	77,300	BF 2.890	-
9	A9	11,200	86,300	BF 2.890	-
10	A10	11,200	95,300	BF 2.890	-
11	A11	11,200	104,300	BF 2.890	-
12	A12	11,200	113,300	BF 2.890	-
13	B1	20,200	14,300	BF 2.890	-
14	B2	20,200	23,300	BF 2.890	-
15	B3	20,200	32,300	BF 2.890	-
16	B4	20,200	41,300	BF 2.890	-
17	B5	20,200	50,300	BF 2.890	-
18	B6	20,200	59,300	BF 2.890	-

2.2 Light intensity adjustment

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



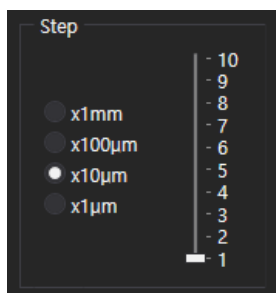
<Shows Light Source Control in the App connected to Green FL Celloger® Mini Plus>


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

2.3 Z stacking

1) Manual Z stacking

- ① By using **Jog**, find the position to start stacking.
- ② Designate the stacking unit in the **Step**.
Ex) Step setting for stacking by 10 μm



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

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 whether or not to execute the z-stacking function in BF and FL in **Z-stacking** under **Properties**. (refer to p.46 for Z-stacking)
- ③ After specifying the interval (= **Step**) to execute the z-stacking function, adjust the scroll bar under **Range** to specify the range.



Properties (Z-Stacking)

- 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	2,453
2	A2	11,200	23,300	2,453
3	A3	11,200	32,300	2,453
4	A4	11,200	41,300	2,453
5	A5	11,200	50,300	2,453
6	A6	11,200	59,300	2,453
7	A7	11,200	68,300	2,453
8	A8	11,200	77,300	2,453

2.4 Stitching

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

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 whether or not to execute the stitching function in BF and FL in **Stitching** under **Properties**. (refer to p.46 for Stitching)
- ③ Set the overlapping area between the images using **Overlap** scroll bar and designate the range to be scanned in **Range**.



Properties (Stitching)


- 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 stitching is performed under autofocus and fluorescence setup, be reminded that it takes approximately 2 minutes to scan 3 x 3, and 7 minutes and 30 seconds to scan 7 x 7 with the light conditions of BF (Lamp: 30, Exposure: 0.06, Gain: 1) FL (Lamp: 30, Exposure: 0.5, Gain:1).

3. Image analysis [in Scan App]

3.1 Capture

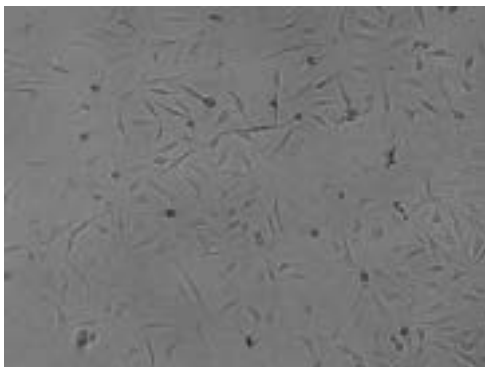
 icon in **Toolbox** captures the image on **Display**. (Use Ctrl + I as a shortcut)

1) An image with features

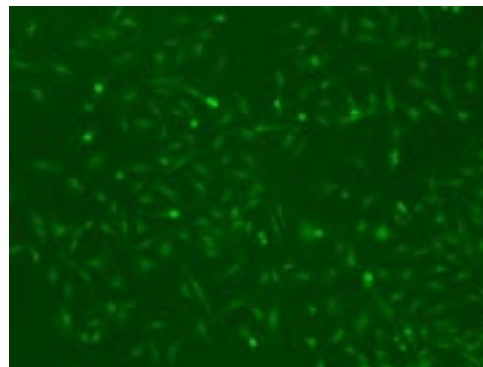
It is possible to generate and manipulate the desired features, show it on **Display** and save it as it is. To obtain without features, press  (**Features layer**) for deactivation and then press .

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 .





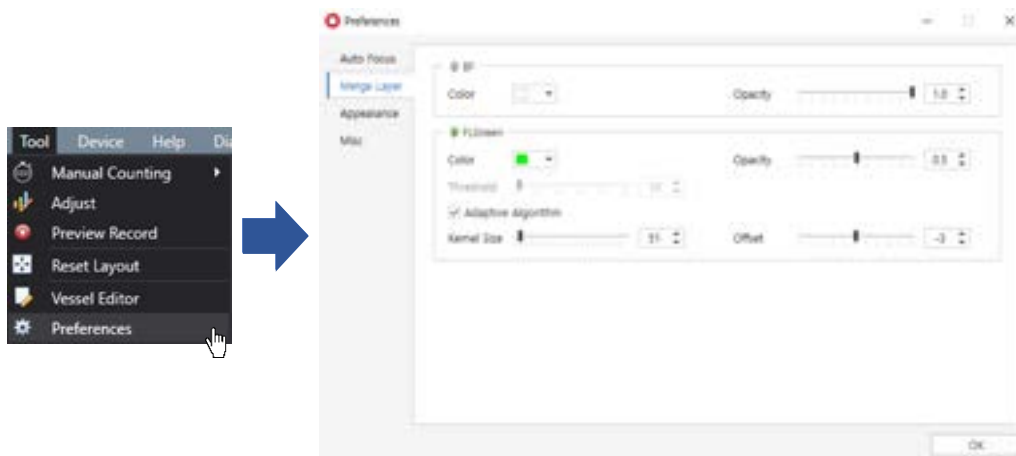
<BF channel image>



<FL channel image>

3.2 Merge image

Press  (**Merge layer**) for activation , merged image is shown on **Display**. Adjusted image as follows can be obtained upon changing **Merge Layer** setting (**Opacity** and **Threshold**) in **Preferences** of **Tool** menu.



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 mask of an image after unchecking the **Adaptive Algorithm** box.

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

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

3.3 Image Tile

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

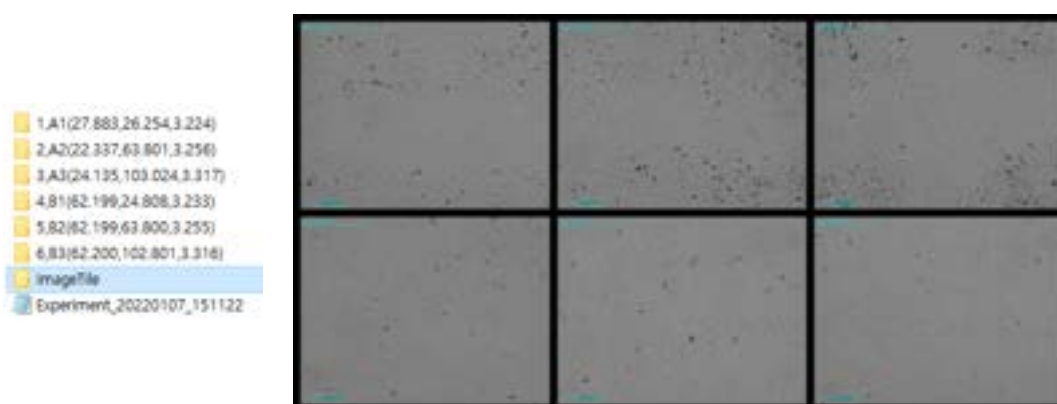
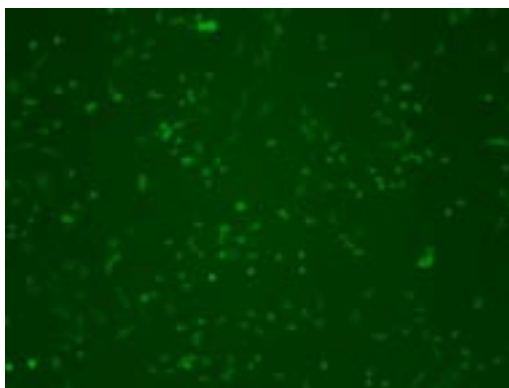
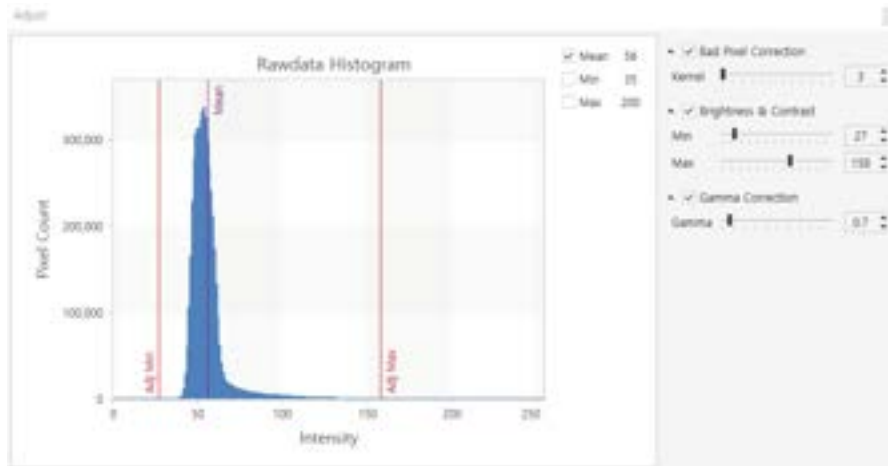


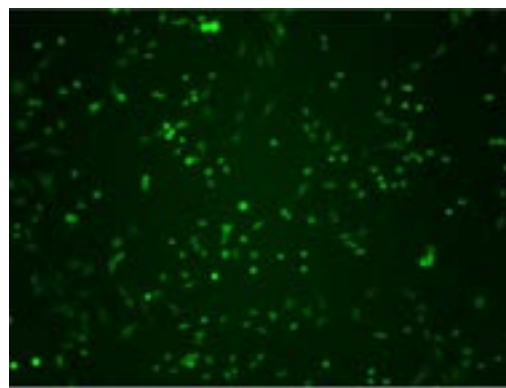
Image Tile

3.4 Image adjustment

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

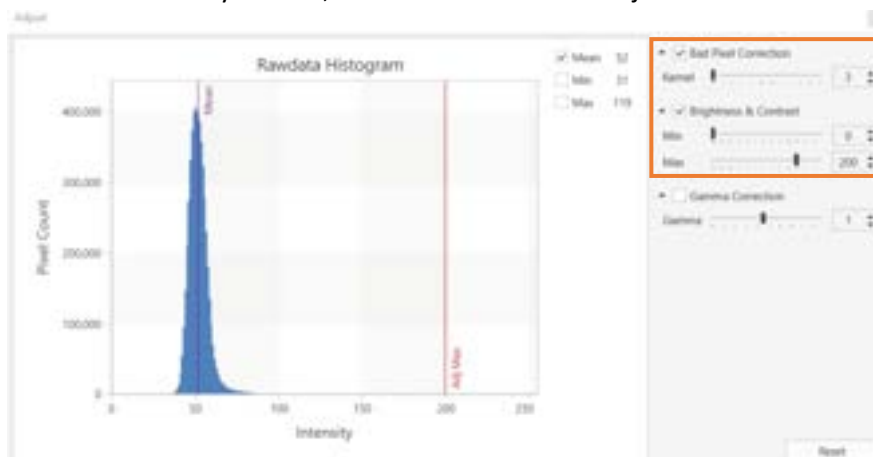


Before adjustment



After adjustment

Note. In case of fluorescence as shown in the image below, **Bad Pixel Correction** and **Brightness & Contrast** are set by default, therefore it should be adjusted.



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

3.5 Preview record

Upon activating **Preview Record** in **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 of the recorded video.

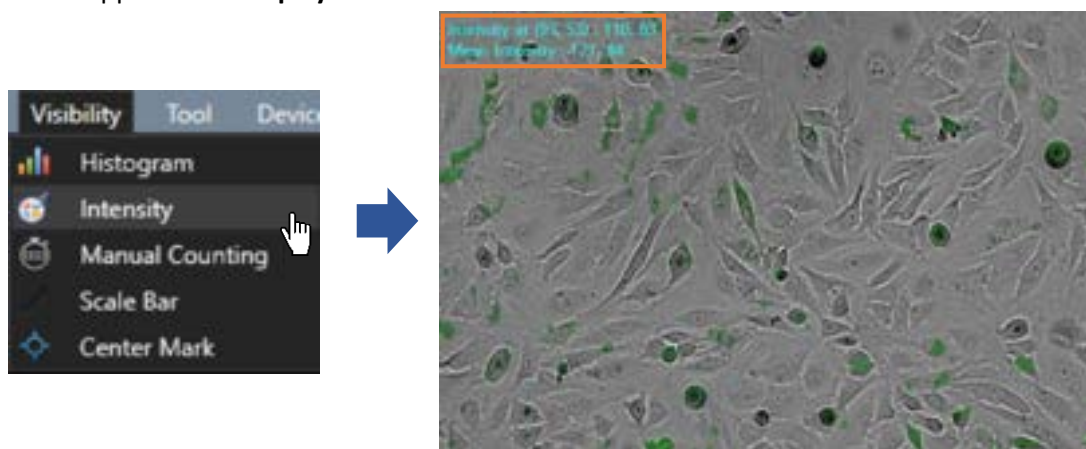


Note. When **Preview Record** is activated, recording continues even **Preview** is turned off and on. To end the recording, you must deactivate it in **Preview Record**.

3.6 Intensity

1) Point Intensity & Mean Intensity

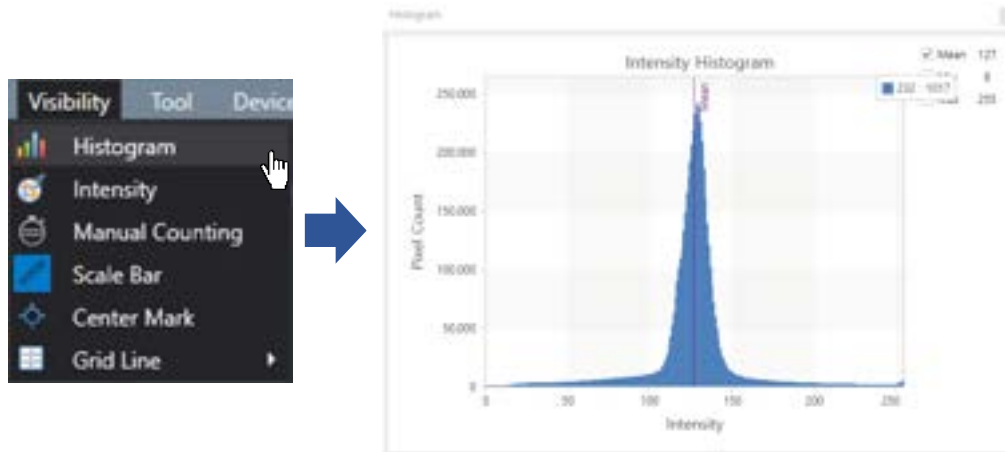
Upon activating **Intensity** in **Visibility** menu, **Point Intensity** and **Mean Intensity** information is displayed at the upper left of **Display**.



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

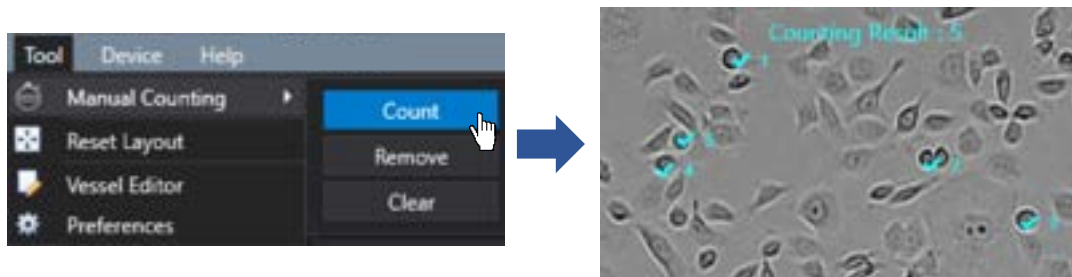
2) Histogram

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



3.7 Manual Counting




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



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

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

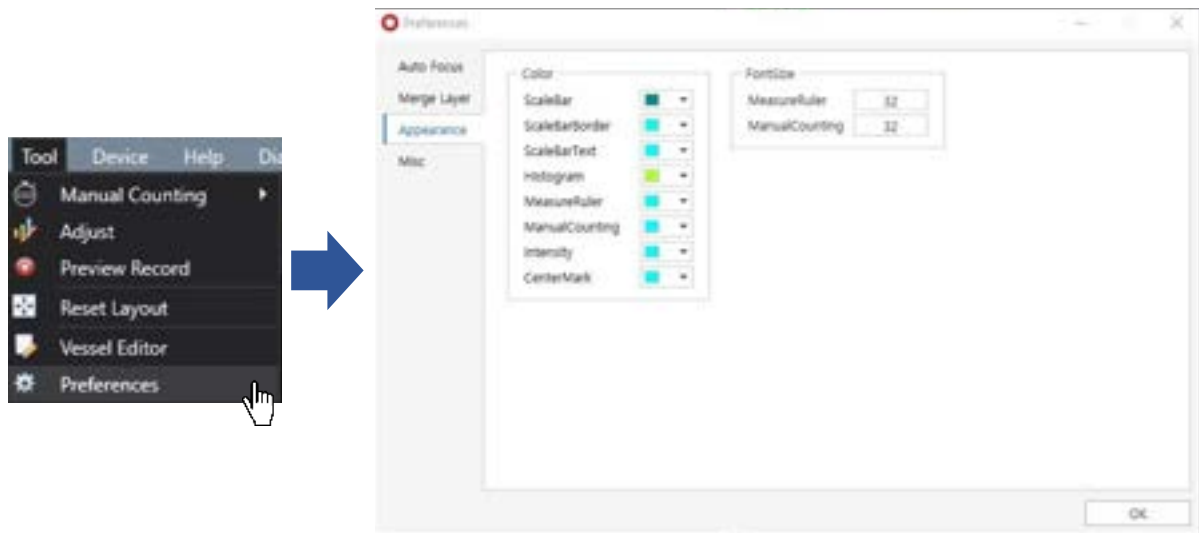
3.8 Ruler

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 (μm).



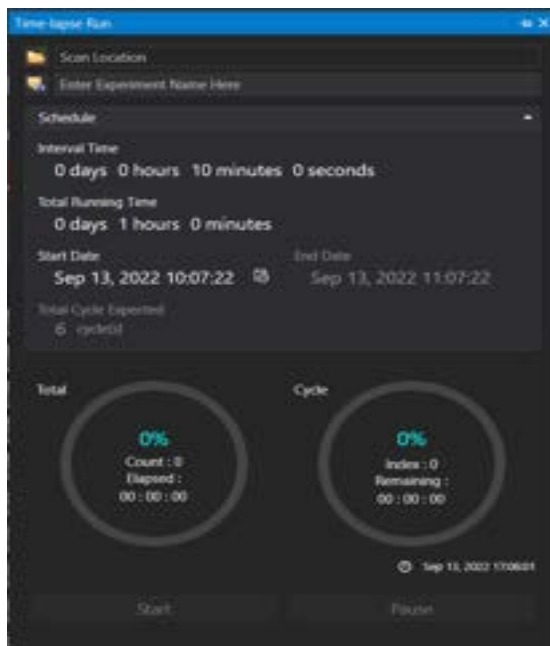
Note. To undo the selected point (after selecting one point), press *Esc* on the keyboard. Be sure that the *Num Lock* is turned on.

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



4. Time-lapse imaging [in Scan App]

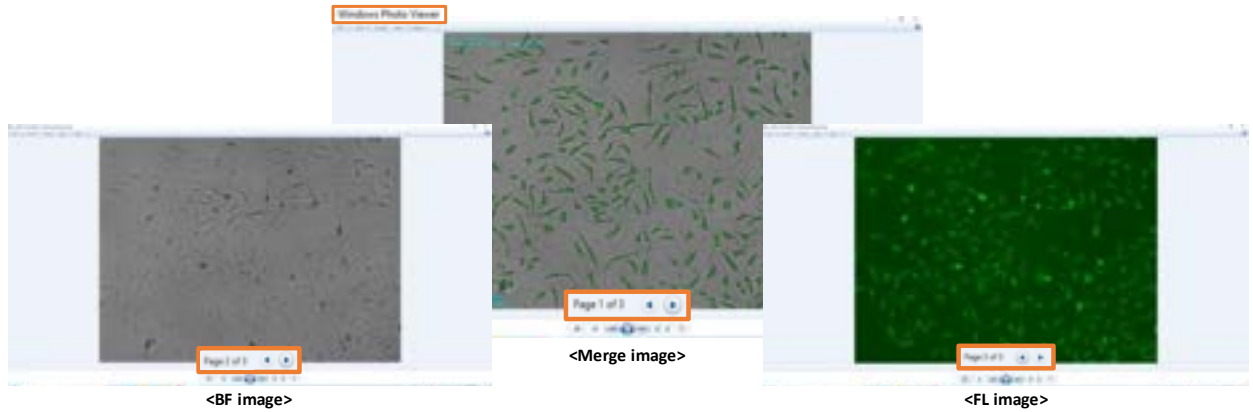
- ① Select the vessel type (p.39).
- ② Click on **Preview** and adjust the light (p.17) and focus (p.13) by referring to the **Display**. If fluorescence is also scanned at this time, the quantity of light of fluorescence should also be adjusted.
- ③ Specify the position and focus to be scanned. At this time, select whether or not to execute the autofocusing function (p.14), z-stacking function (p.17), or stitching function (p.18). If fluorescence is also scanned, the focus of fluorescence should also be adjusted.
- ④ Open **Time-lapse Control** and select the location to save and enter the project name. If the image storage location is not specified, the file will be saved in the **Scan** folder under the **Celloger Mini Plus Scan App** folder and if the project name is not entered, a folder will be automatically created under the scan start date.



Time-lapse Control

- ⑤ Set the schedule (**Interval Time**, **Total Running Time** and **Start Date**) (p.48). If you want to start immediately, **Start Date** does not need to be adjusted.
Note. If **Start Date** is set as past, it is updated automatically to the present time.

- ⑥ Click on **Start** to begin scanning.
Note. The LED indicator lights up blue while performing time-lapse.
- ⑦ Once the scanning is completed, check the saved images, make videos, or perform additional analysis using the **Celloger Mini Plus Analysis App**.
Note. Images are saved as “*Tiff files*” by default. If fluorescence was also imaged, the saved images are stacked file of merged image and raw images of each channel. Stack image can be viewed with the Windows basic image viewer “*Windows Photo Viewer*”.



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

[Stitching] When image is captured with only BF channel or 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. 47 for the stitching scanning order)

5. Process single image or time-lapse images [in Analysis App]

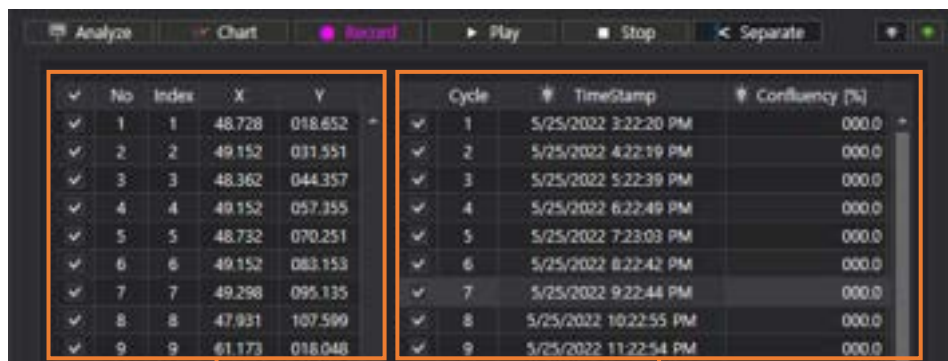
It is possible to conduct confluency analysis and make videos in **Celloger Mini Plus Analysis App** using time-lapse images taken by **Celloger® Mini Plus**. In addition, intensity measurement, length measurement and manual counting are available in **Cellger Mini Plus Analysis App** and the methods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

5.1 Import the original data taken by Celloger® Mini Plus

Celloger Mini Plus Analysis App can only import the original data taken by **Celloger Mini Plus Scan App**.

1) Open the time-lapse folder.

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

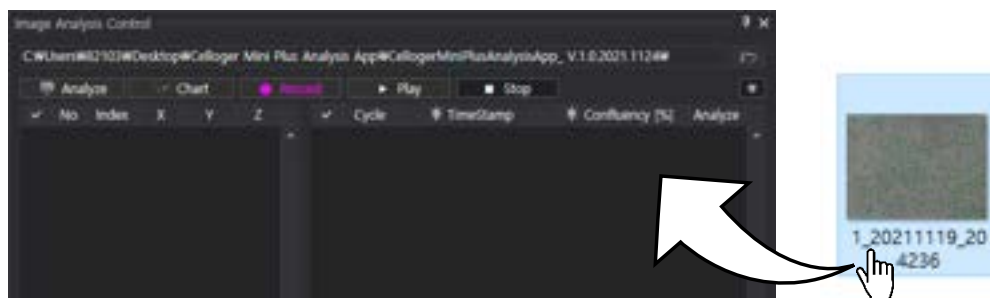


Scan positions

Images

2) Open the image file.

Drag the image file.



Note. Folder can be dragged.


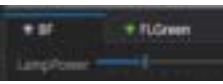
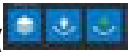
Note. The file location, address and file name should only be composed of English alphabets, number, and some special symbols (a file name shouldn't contain any of the following characters: /, :, *, ?, ", <, >, |) to import the file correctly in **Celloger Mini Plus Analysis App**. If it fails to import the file, check whether the file name and location address is written in languages other than English.

5.2 Merge FL image

The same as **Celloger Mini Plus Scan App**. (refer to p.20)

5.3 Slide show

- ① Open the time-lapse folder.


- ② Select the light channel ( or ) or press **Merge Layers** ().
- ③ Press **Play** ().

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

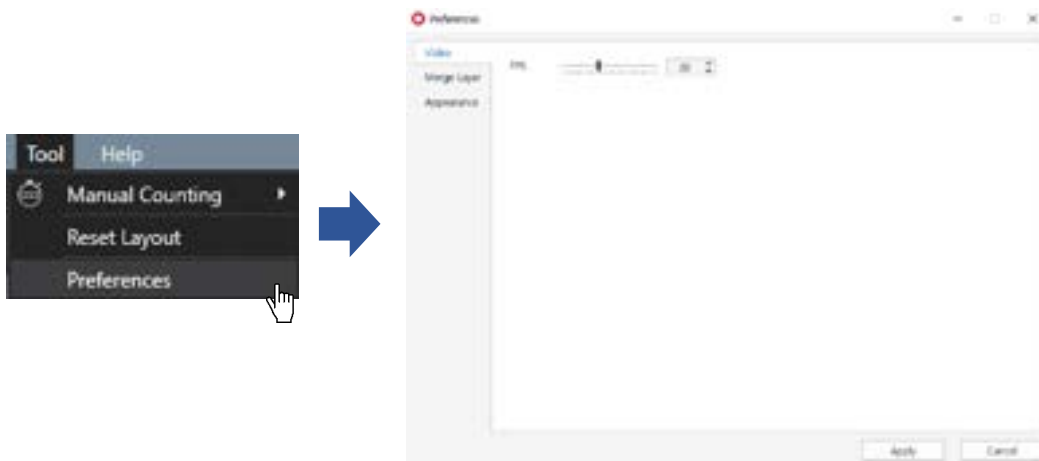
Note. **FPS** is a parameter indicating the speed of slide show and video. However, the speed of slide show and video could be different even with the same **FPS**.



5.4 Make video

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



Note. In order to change the speed, change the parameter in **Preferences** of **Tool** menu. (Recommended value: 5~13)



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

5.5 Estimate confluency (coverage)

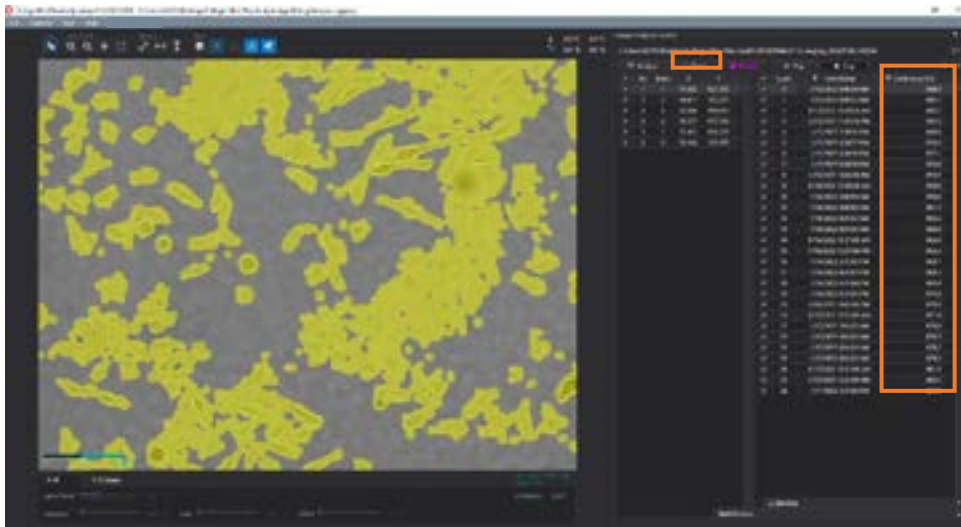
- ① Open the file and images captured with **Celloger® Mini Plus**.
- ② Press **Analyze** (To analyze certain images from the retrieved file, use check function on the left.)

Note. Upon selecting right icon  (Green FL device) or  (Red FL device), the coverage ratio of fluorescence area is calculated. At this point, coverage is calculated after thresholding according to merge

parameter. Therefore, to accurately calculate the coverage of fluorescence area, adjust the appropriate thresholding parameters (refer to p.20) before executing the coverage analysis.

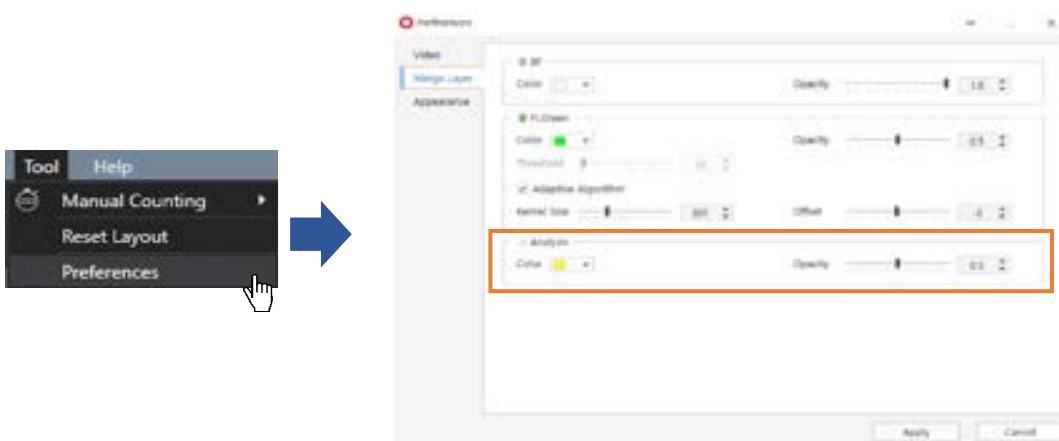
- ③ Confluency values and confluency mask (yellow) are indicated.


When a cycle is selected from the list, the corresponding cycle's image and confluency mask can be seen.



<Calculated confluency values and confluency mask>

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

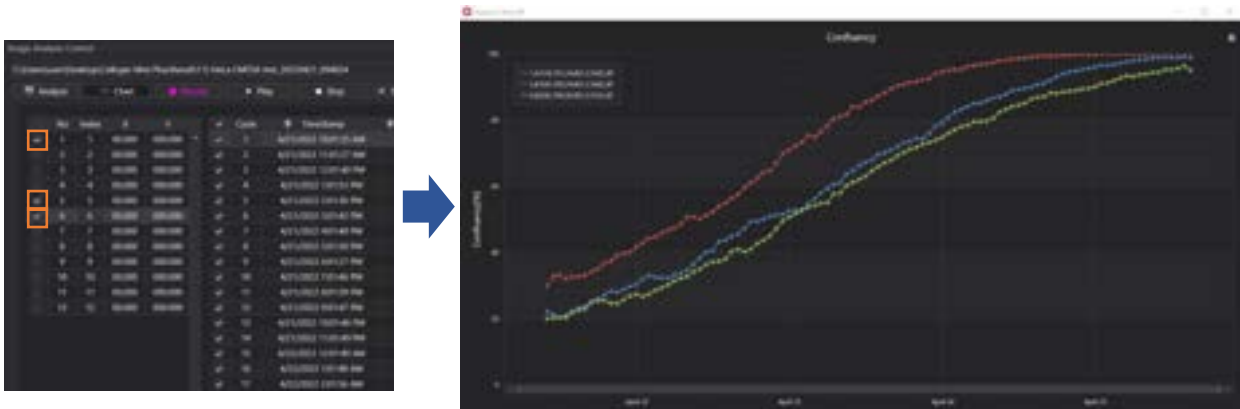


Note. If you do not want to see the confluency mask, deactivate it by clicking on  under **Toolbox**.

5.6 Create confluency graph

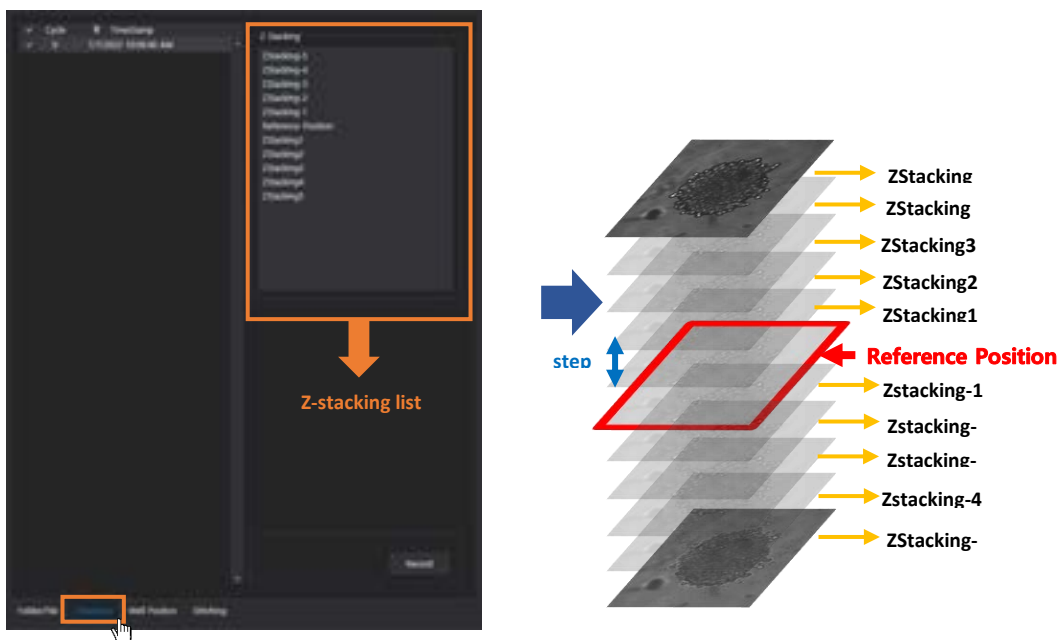
- ① Open the file and images captured with **Celloger® Mini Plus** and press **Analyze** to analyze the confluency.
- ② Create the confluency graph by pressing **Chart**.

Note. If you want to create the confluency graphs of only some positions from the scan position list, check the desired position and press **Chart**. Upon selecting all scan positions, the confluency graphs of all positions are displayed at the same time. The image below shows the confluency graph when selecting 3 positions among 12 scan positions.

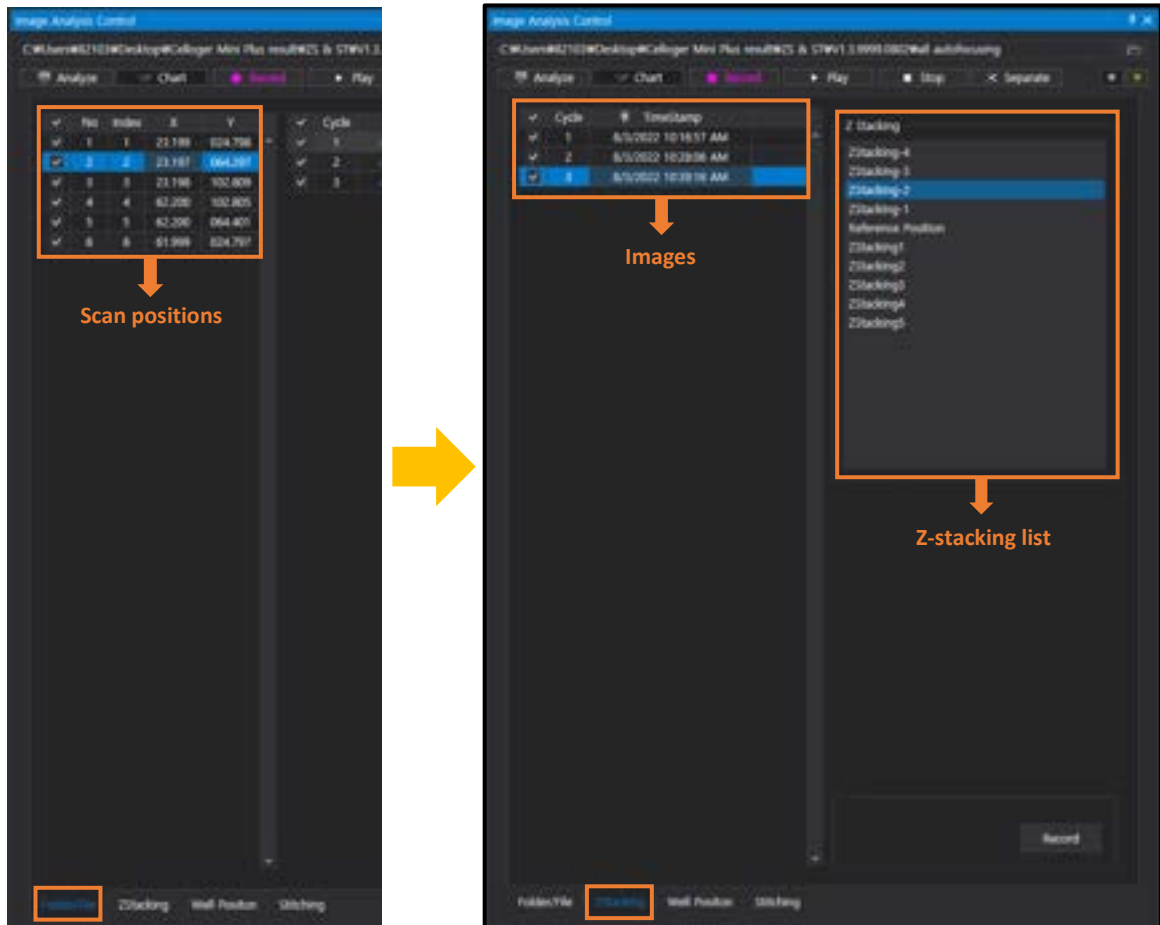


5.7 Show Z-stacking image

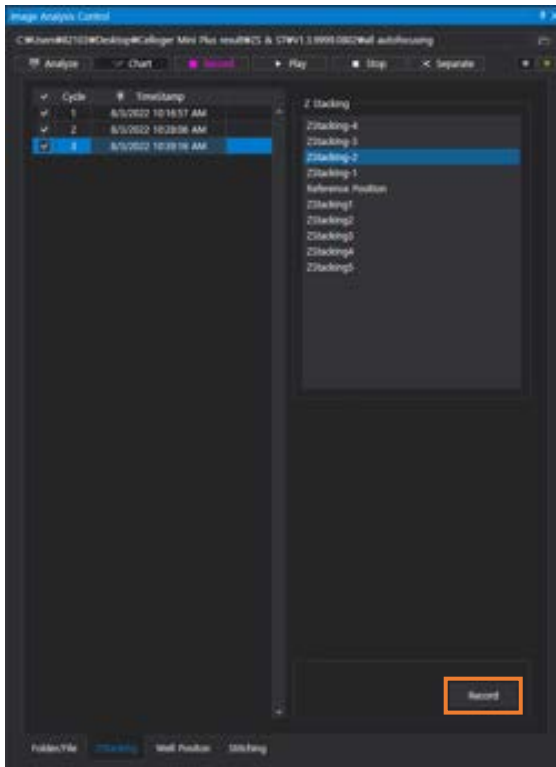
- ① Open the time-lapse file or images captured with Z-stacking function.
- ② After selecting the scan position with Z-stacked images, click the **Z-stacking** tab under **Image Analysis Control** to see the **Z-stacking list**. The image below shows the **Z-stacking list** when the **Range** of z-stacking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



- ③ Select one from the **Z-stacking list** and check the image on **Display**. For example, the image below shows that the second image in **Scan Positions**, the third cycle in **Images**, and "**ZStacking-2**" are selected in the **Z-stacking list**. In this case, you can see the "**ZStacking-2**" image of the third cycle captured at the second scan position on **Display**. "**ZStacking-2**" means the image was taken two **steps** below the reference position. (= **Focus**). For instance, if the **Step** is set to 10 μm , "**ZStacking-2**" is taken 20 μm below the reference position. (refer to p.17 for more information of Z-stacking setup.)

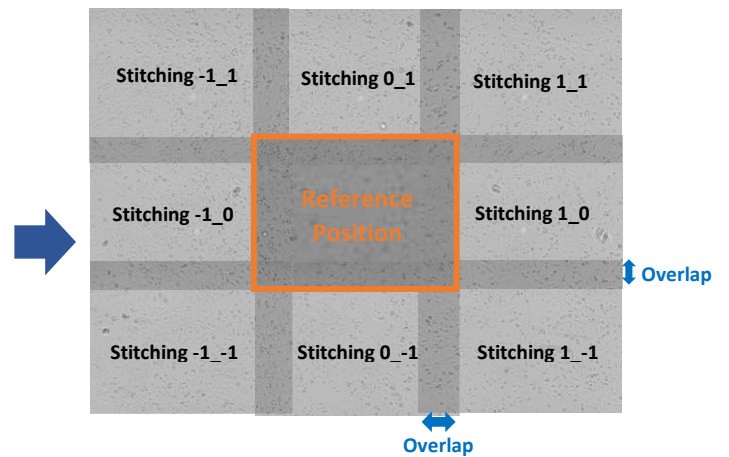
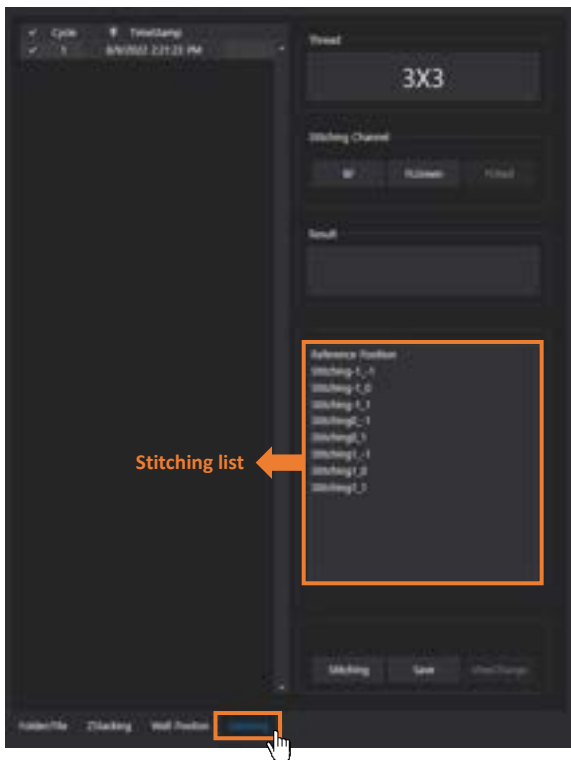


Note. If you want to make a video from the images of the Z-stacking list, click **Record** on the **Z-stacking** tab. The video appears in the order of the Z-stacking list (from low focus to high focus). The FPS of the video can be adjusted in **Properties** of **Tool** menu (p.27).

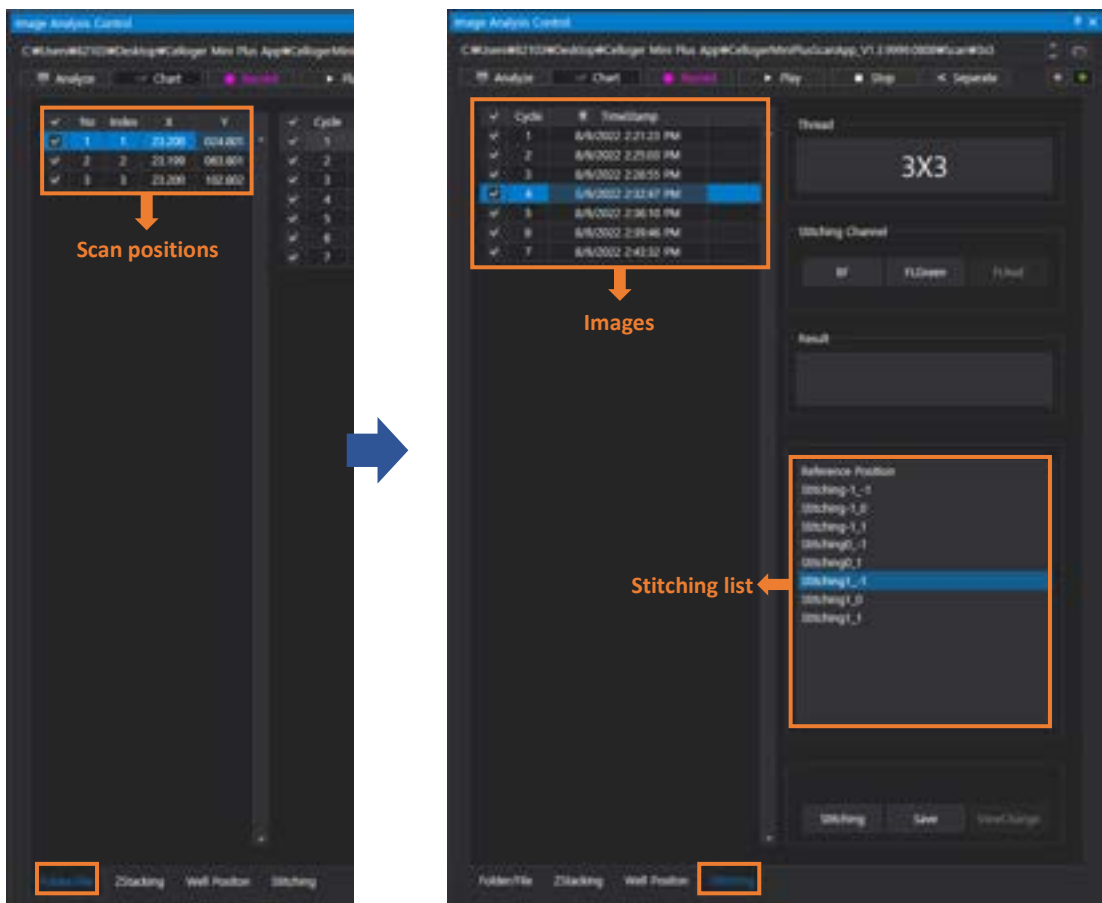


5.8 Stitch images

- ① Open the time-lapse file or images captured with stitching function.
- ② After selecting the scan position with stitching images, click the **Stitching** tab under **Image Analysis Control** to see the **Stitching list**. The image below shows the **Stitching list** when the **Range** of stitching function is set to (1,1,1,1).



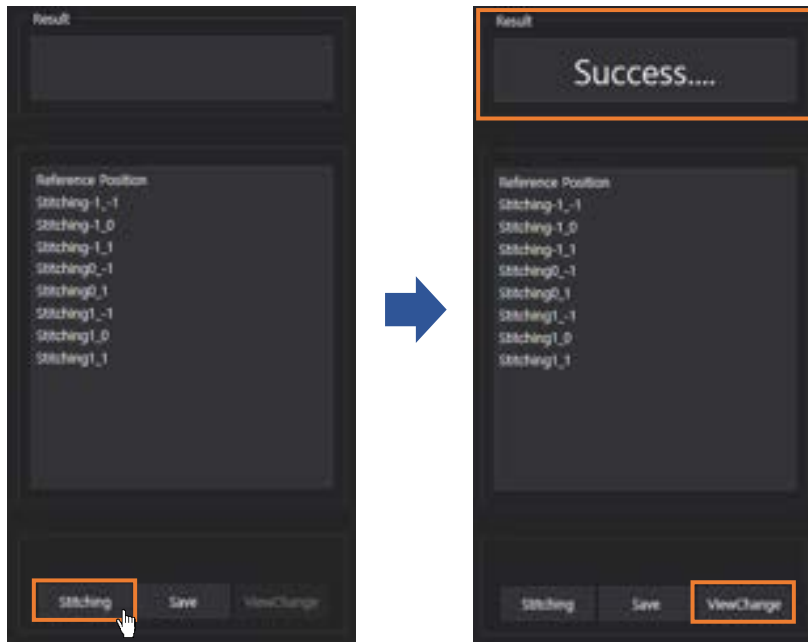
- ③ Select one from the **Stitching list** and check the image on **Display**. For example, the image below “**Stitching1_-1**” is selected in the fourth cycle of the first **Scan Positions**. In this case, you can see the “**Stitching1_-1**” image of the fourth cycle captured at the first scan position on **Display**. “**Stitching1_-1**” means the image was taken one step right and one step down from the reference position, overlapped by the value set in **overlap**.



Note. **Thread** refers to the number of images taken to perform the stitching function (horizontal x vertical). **Stitching Channel** shows the light channel selected when stitching was performed.

- ④ Click **Stitching** under the **Stitching list** to stitch the captured images. When stitching is completed, **ViewChange** is activated, and **Success** is displayed in the **Result**. When **ViewChange** is clicked, stitched image appears on the **Display**.

Note. When stitching fails, **Fail** appears in the **Result**.



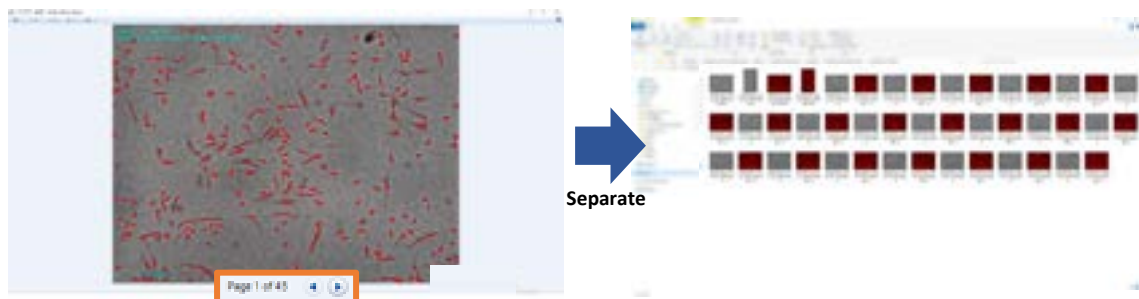
- ⑤ Click **Save** to save the stitched image.



Note. Be reminded that the image shown on the **Display** will be saved so be sure to check the display image before clicking **Save**.

5.9 Separate the image

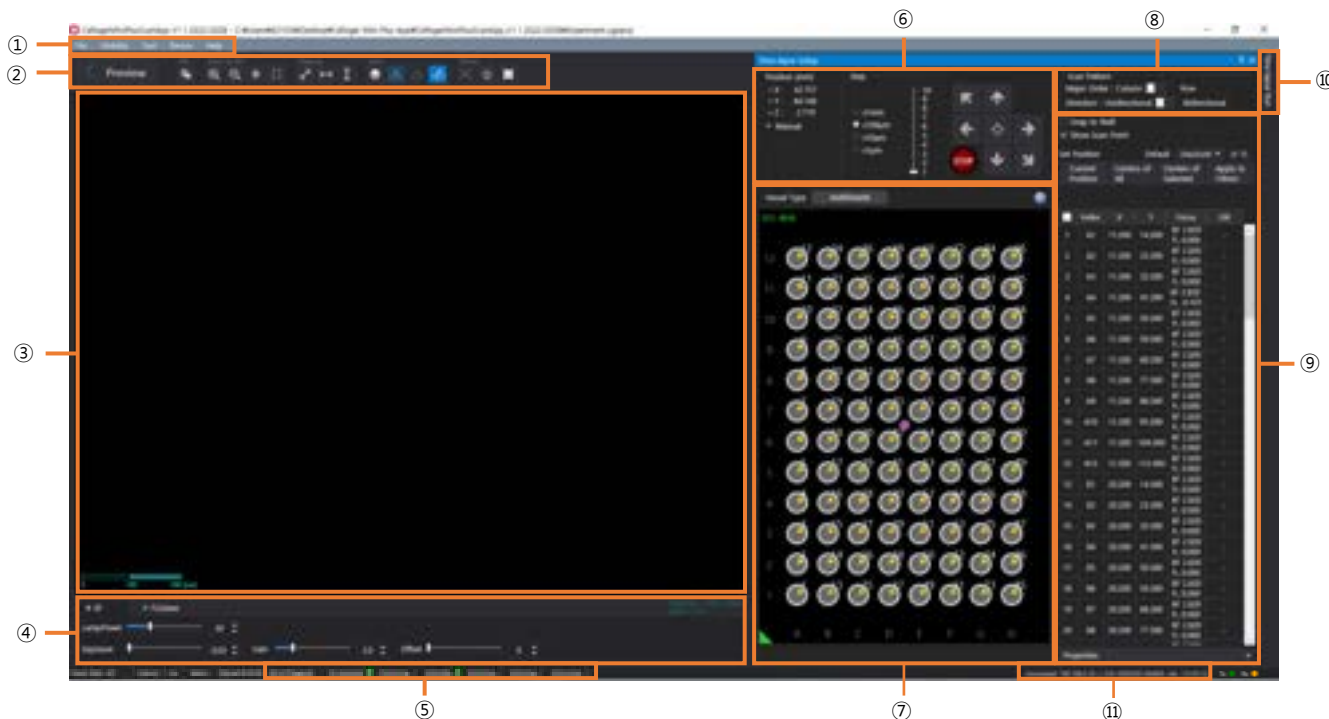
Celloger Mini Plus Scan App stores images by stacking them with raw images when the scan is performed with Fluorescence, Z-stacking, and Stitching. Using **Separate** function, stacked images are converted and saved as individual image files. When **Separate** is selected from **Folder/File** tab, the raw images (BF, FL) of the reference position is separated and saved. When clicked from the **Z-Stacking** or **Stitching** tab, images from each list will be separated and saved. For example, if you click **Separate** on the **Stitching** tab, the images in the **Stitching list** are made into individual image files and saved (refer to the image below).



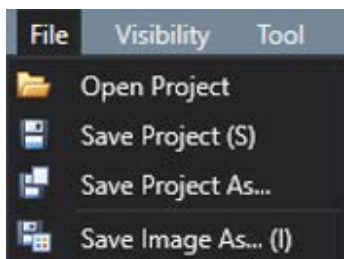
V. Software description

1) Celloger Mini Plus Scan App

Upon opening **Celloger Mini Plus Scan App** and completing pre-setup (refer to p.8~10), the following main page is displayed.

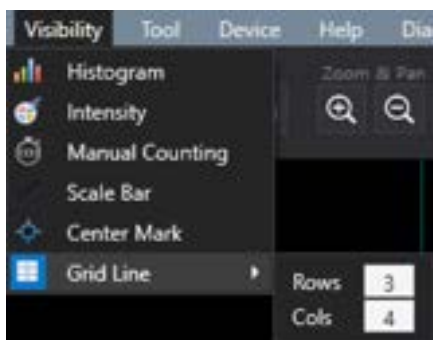


① Menu Bar [File]



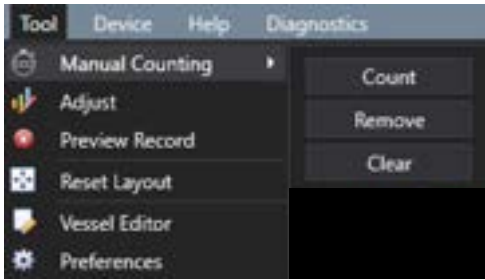
- ✓ **Open Project:** Import a previously saved project.
- ✓ **Save Project:** Save settings (Light intensity, Focus position, Schedule, etc.) on current project. Keyboard shortcut '*Ctrl + S*' can be used.
- ✓ **Save Project As...:** Create a new project with current settings
- ✓ **Save Image As...:**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

[Visibility]



- ✓ **Histogram (p.23):** Show/hide intensity histogram of an image.
- ✓ **Intensity (p.22):** Show/hide point and mean intensity of an image.
- ✓ **Manual Counting:** Show/hide "Manual Counting" function.
- ✓ **Scale Bar:** Show/hide scale bar on the left bottom 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.23)**: 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.21)**: Adjust image
- ✓ **Preview record (p.22)**: Record the streaming image
- ✓ **Reset Layout** (refer to **Note** in p.49): Return to “User Interface” before change.
- ✓ **Vessel Editor (p.64)**: The specifications of the well plate are customizable.
- ✓ **Preferences**: Set **Auto Focus** (refer to p.14), **Merge Layer** (refer to p.20), and **Appearance** to change the color and font size of the feature.

[Device]



- ✓ **Connect**: Connect a new device or change the connection to another device.
- ✓ **Reboot**: Reboot the connected device.
- ✓ **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.

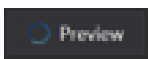
[Help]



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

② Toolbox

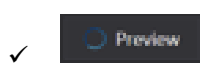
[Preview]



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



✓ : 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.
Keyboard shortcut is '*Shift + mouse scroll wheel*'
Note. Keyboard shortcut is '*Shift + mouse scroll wheel*'
Note. If you double-click a location within the display while pressing *Shift* key, the camera moves to center the 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/FL Red) image layer
- ✓  : Features [Ruler(Measure), scale bar, manual count, etc.] layer
Note. Once activating **Merge Layers**, the displayed image is an image processed by setting **Opacity** and **Threshold** in the **Preferences** (refer to p.20)

[Device control]

- ✓  : Execute autofocusing depending on the autofocusing settings in streaming status (refer to p.32 for autofocusing).
- ✓  : Turn on the white light in LED status indicator of the connected device.
- ✓  : Fan on/off
- ✓  : Turn on/off LED status indicator

③ Display

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

④ Light Source Control

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

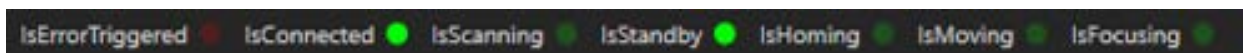


- ✓ **LampPower (%)**: Controls the lamp (illuminator) power that is adjustable from 0 to 100 %.
- ✓ **Exposure (second)**: The duration of exposure of cells to light, adjustable from 0.01 to 2 seconds. Be careful as photobleaching occurs when exposure value is high.
- ✓ **Gain**: The amount of signal 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 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 recommend to adjust the parameters as follows.

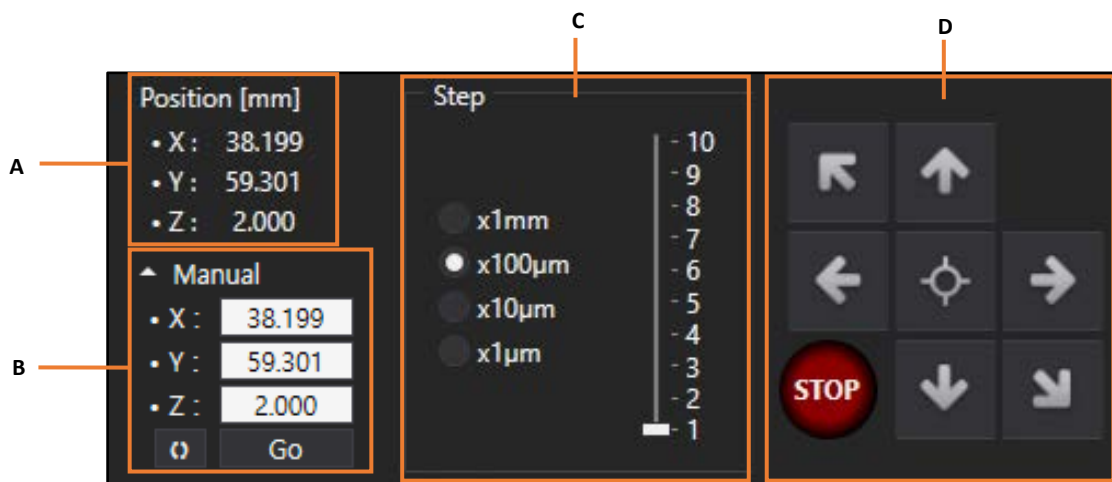
It is not recommended to increase the **LampPower** to high value as phototoxicity may occur. Moreover, it is not recommended to leave the **Preview** on for a prolonged period as photobleaching may occur.

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 above image, the green lights on **IsConnected** and **IsStandby** which means the device is connected to PC and is in stand-by state (not scanning).


⑥ Jog Control




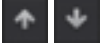
- Current X, Y, Z stage coordinate
- Upon pressing **Go** after entering the desired coordinates in the box, the stage is moved according to the set coordinates. Upon pressing **0**, current coordinates are entered in the boxes.
- Distance moved upon pressing **Jog button** (D) (Hereafter referred to as **Step distance**). Adjustments can also be done using “*”, “/” when ‘Num Lock’ is off on the number pad of the keyboard.





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.

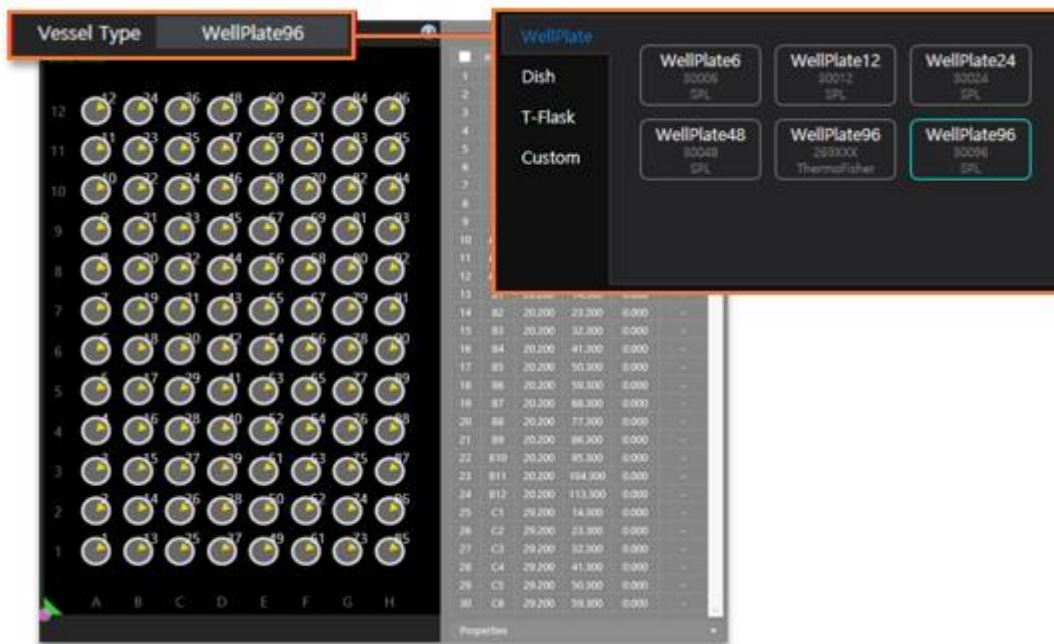
 : Stop the stage on move.

⑦ Vessel & Plate panel

[Vessel type]

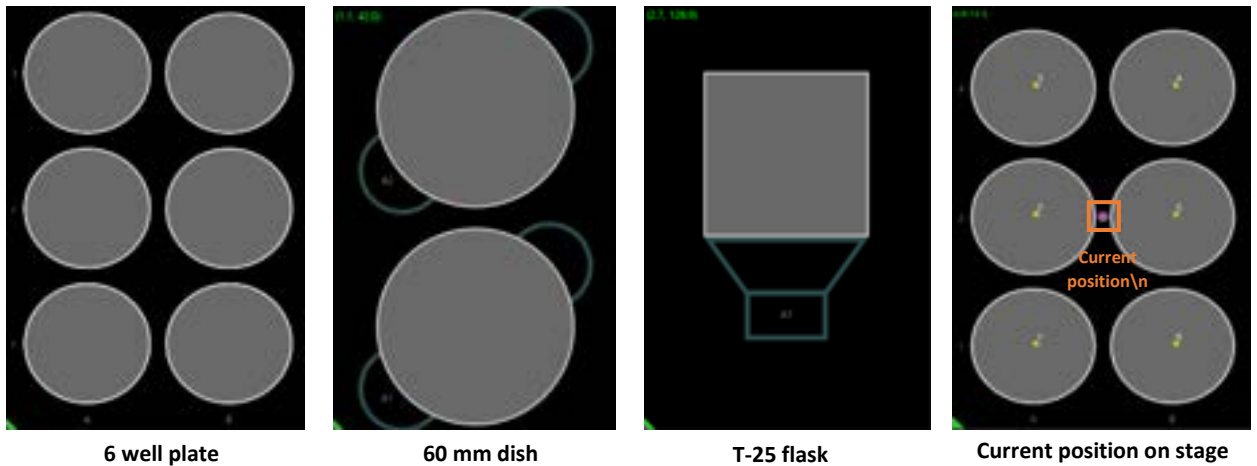
This is the tab where the vessel type is selected. Well plate (6, 12, 24, 48, 96 wells), Dish (35 mm, 60 mm, 90 mm), and T-flask(25 cm², 75cm²) can be selected.

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 plates is used, it is recommended to change the vessel specifications in the **Vessel Editor** under the **Tool** menu. (refer to p.61 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.42 for **Show Scan Point**). The image below is an example of vessels that appear on the **Plate panel**, and the pink dot indicates the camera's current position.



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

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 displays the Position Control software interface, which is divided into several sections. Callouts A, B, C, and D point to specific features:

- A:** Points to the "Snap to Well" and "Show Scan Point" checkboxes at the top left.
- B:** Points to the "Set Position" section, which includes a dropdown menu (currently set to "Default") and a "FL" checkbox.
- C:** Points to a table listing well positions. The row for well A7 is highlighted in green.
- D:** Points to the "Properties" section, which contains various configuration options for BF Focus, FL Focus Offset, Z-Stacking, and Stitching.

Index	X	Y	Focus	Util	
4	A4	11,200	41,300	BF 2.453 FL 0.000	-
5	A5	11,200	50,300	BF 2.453 FL 0.000	-
6	A6	11,200	59,300	BF 2.453 FL 0.000	-
7	A7	11,200	68,300	BF 2.453 FL -0.020	Z5 (-2,3)
8	A8	11,200	77,300	BF 2.453 FL 0.000	-
9	A9	11,200	86,300	BF 2.453 FL 0.000	-
10	A10	11,200	95,300	BF 2.453 FL 0.000	-

Properties

- BF Focus** (Apply All)
 - isEnabled:
 - Ref. Position: 0 2.453
 - Auto Focus: EveryCycle
- FL Focus Offset** (Apply All)
 - isEnabled: FLGreen 0 -0.020
- Z-Stacking** (Apply All)
 - isEnabled: BF FLGreen
 - Step: 10 μm
 - Range:
- Stitching** (Apply All)
 - isEnabled: BF FLGreen
 - Overlap: 20
 - Range:

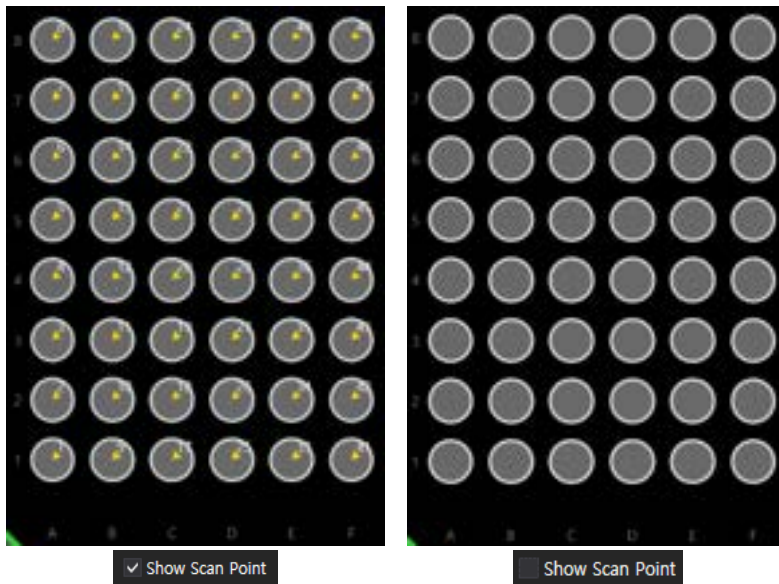
A. Snap to Well & Show Scan Point

[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]

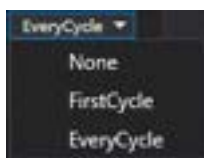
If checked, the position specified for scanning is indicated on the **Plate panel** as shown in the image below.



B. Auto Focus Frequency & Set Position

[Auto Focus Frequency]

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



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

[FL mode]

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

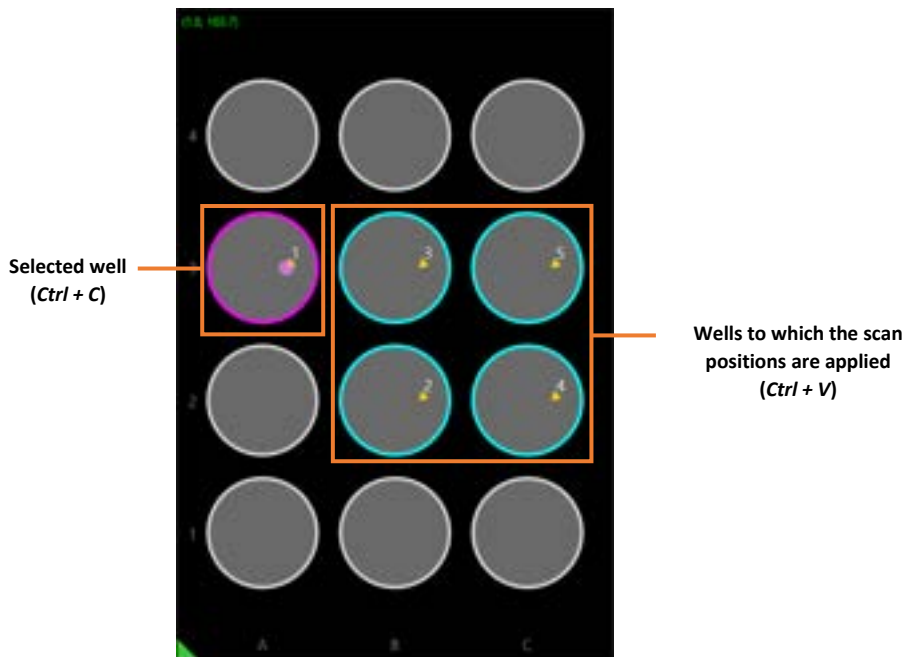
Note. The selected **Auto Focus Frequency** and **FL mode** are set as default, and the set scan position is imaged based on the default settings. If you want to execute the autofocusing function at an **Auto Focus Frequency** other than the default value at some scan positions, the desired position can be selected and changed from the **Scan table (C)** (Refer to **D** on how to change the position). **FL mode** 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:** Set the center of all wells as the scan position (Can only be used when the vessel type selected is a well plate).
- ✓ **Centers of Selected Wells:** Set the centers of the selected wells as the scan position. At this time, the wells are selected by dragging the mouse (Can only be used when the vessel type selected is a well plate).

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



C. Scan table

It shows the scanning order and the corresponding coordinates and Z-stacking values.

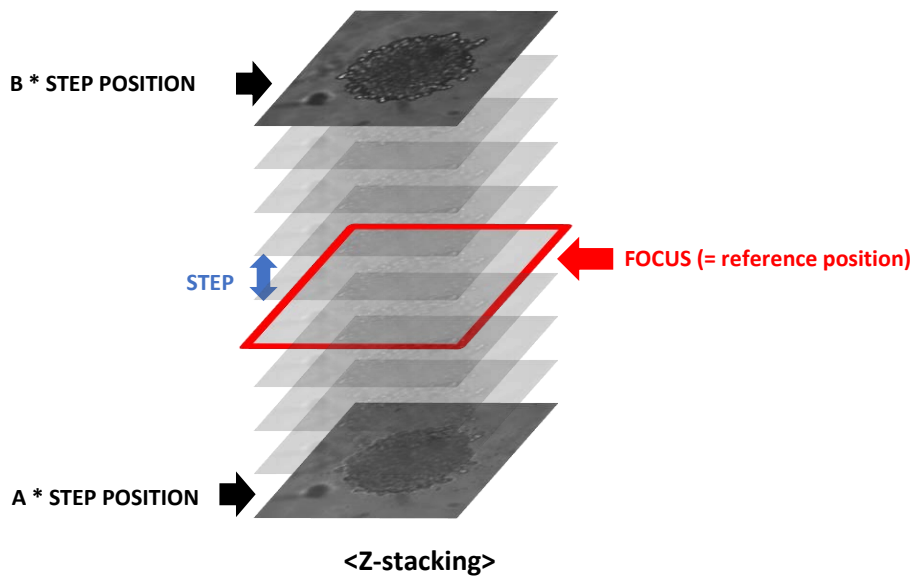
Index	X	Y	Focus	Util	
1	A1	11.200	14.300	BF 2.453 FL 0.000	-
2	A2	11.200	23.300	BF 2.453 FL 0.000	-
3	A3	11.200	32.300	BF 2.453 FL 0.000	-
4	A4	11.200	41.300	BF 2.453 FL 0.000	-
5	A5	11.200	50.300	BF 2.453 FL 0.000	-
6	A6	11.200	59.300	BF 2.453 FL 0.000	-
7	A7	11.200	68.300	BF 2.453 FL -0.020	ZS (-2,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

- ✓ **Index:** 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 coordinate of BF and FL channels.
BF channel: shows the focus of Z coordinates when **Auto Focus** function is off (= **None**) or it is the median coordinate when **Auto Focus** function is on (= **FirstCycle** or **EveryCycle**).

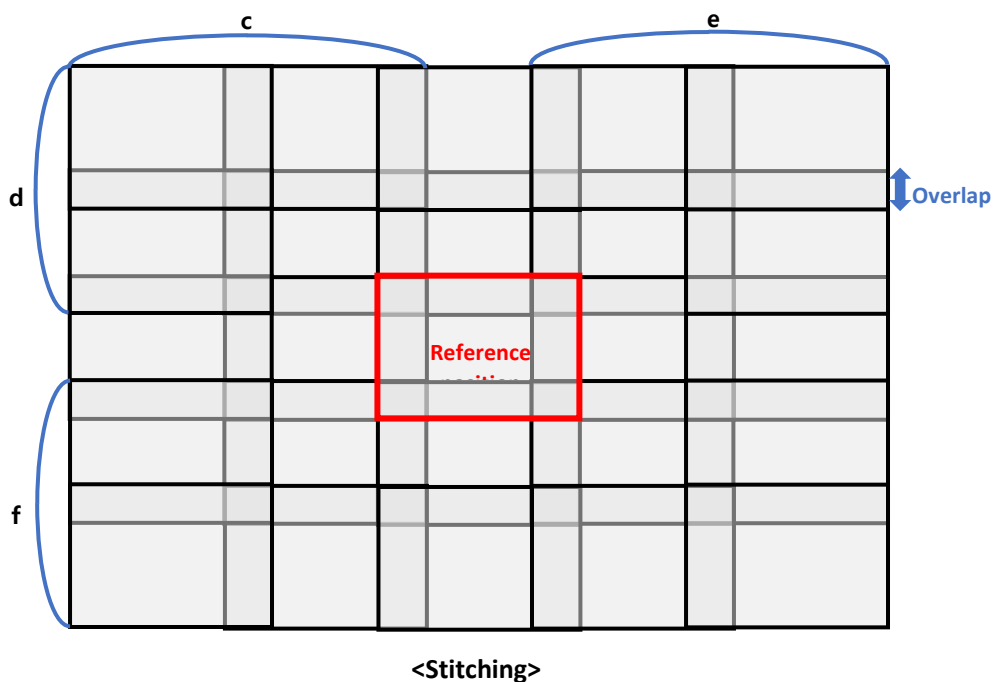
FL channel: shows the focus difference from BF channel. When FL channel has lower focus than BF channel, ' - ' is added, '0.000' appears when with same focus, and only numbers are displayed when FL channel has higher focus than that of BF channel.

- ✓ **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** (refer to **Z-Stacking** of p.46) 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 **Z-Stacking** of p.46).



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, downwards respectively from the reference position that are set in **Range**.

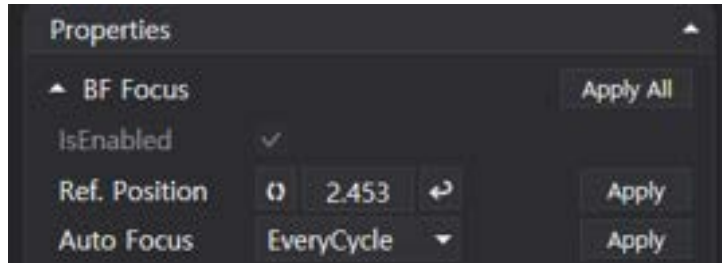




Note. For a detailed description of z-stacking and stitching, refer to the example in Z-Stacking and Stitching on p.46~47.

D. Properties

It shows the **Focus**, **Auto Focus Frequency**, and **FL mode** 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 (= if checked in the **FL mode**). Furthermore, the z-stacking settings can be adjusted after selecting whether or not to perform the z-stacking.

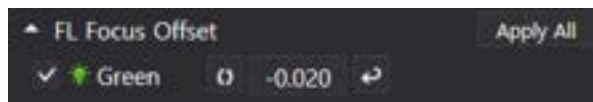
➤ BF Focus






- ✓ **Ref. Position:** Position of BF channel for time-lapse. **2.453** is the location of z axis of BF channel when **Auto Focus** is **None** or the median coordinate when **FirstCycle** or **EveryCycle** is selected in **Auto Focus**.
- ✓  : Upon clicking, the current coordinate is entered on the box next to it.
- ✓  : Upon clicking, z position moves according to the coordinates shown on the left box. Click on the button in the state shown above to move to 2.453 position.
- ✓ **Auto Focus:** It shows the **Auto Focus Frequency** selected in **Position Control** (p.42) and it can be changed.

Note. After changing the **Ref. Position** and **Auto Focus**, the **Apply** button must be clicked on for each to apply the changes. Click the **Apply All** button to apply both functions at once.

➤ FL Focus Offset




- ✓ **FL Focus Offset:** Position of FL channel for time-lapse. **-0.020** is the location of z axis of FL channel captured while performing the time-lapse imaging. The value shown is the difference from the location of Z axis captured in the BF channel. To capture the FL channel on the same coordinates of BF channel, enter 0.
- ✓  **Green** : It shows the **FL mode** selected in the **Position Control** (p.42), and if **FL mode** is checked (= if fluorescence imaging is performed at the same time), the fluorescence focus can be set. When unchecked, fluorescence imaging is not performed during the time-lapse.
- ✓  : Upon clicking, the current coordinate is entered on the box next to it.
- ✓  : Upon clicking, it moves from the position of the BF channel to the position recalculated based on the value written in the left box. For example, referring to the image above (in **Scan table** of p.21), the z-axis position of the BF channel is 2.453, and the z-axis of the FL channel is 0.020 below 2.453; as such, fluorescence imaging is performed at 2.433 (= 2.453 – 0.020).

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

➤ Z-Stacking



- ✓  : Select whether or not to run the z-stacking function in BF and FL by checking the boxes.
- ✓ **Step**: When executing the z-stacking function, it sets the interval of the z-axis to be imaged and it can be set up to 1,000 μm.
- ✓ **Range**: It sets the range for executing the z-stacking function by moving the scroll bar, and it can be adjusted from -10 to 10. The z-stacking function can be executed from $-10 * \text{Step}$ μm to $10 * \text{Step}$ μm based on **Focus**.

<Example>

Index	X	Y	Focus	Util	
1	A1	11,200	14,300	2,453	-
2	A2	11,200	23,300	2,453	-
3	A3	11,200	32,300	2,453	ZS (-2, 3)
4	A4	11,200	41,300	2,453	-
5	A5	11,200	50,300	2,453	-
6	A6	11,200	59,300	2,453	-
7	A7	11,200	68,300	2,453	-
8	A8	11,200	77,300	2,453	-

Scan table




Properties (Z-Stacking)

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

➤ Stitching



- ✓  : Select whether or not to run the stitching function in BF and FL by checking the boxes.
- ✓ **Overlap**: The area of overlap between the images being stitched, which can be set from 20% to 50%.
- ✓ **Range**: The range where stitching function is performed, and can be set from 1 to 3 (up, down, sideways respectively). When **Symmetric** is selected, the symmetrical range is set automatically.

<Example>

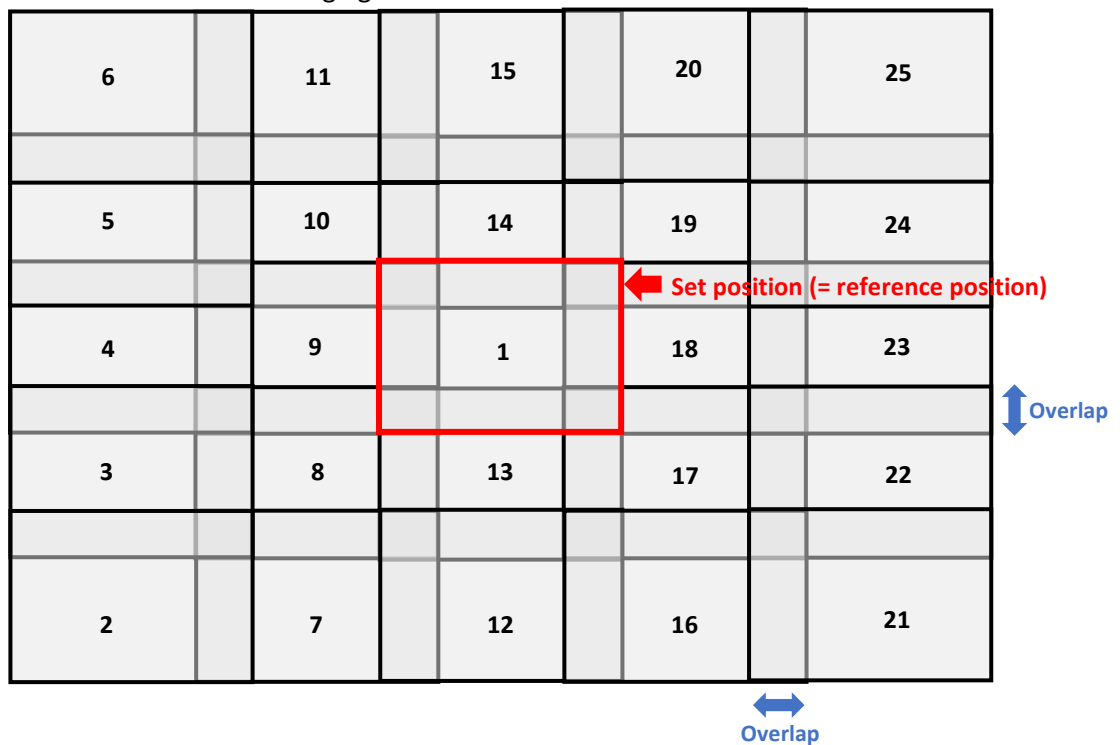
Index	X	Y	Focus	Util	
4	A4	11.200	41.300	BF 2.453 FL 0.000	-
5	A5	11.200	50.300	BF 2.453 FL 0.000	-
6	A6	11.200	59.300	BF 2.453 FL 0.000	-
7	A7	11.200	68.300	BF 2.453 FL -0.020	ST (2,2,2,2)
8	A8	11.200	77.300	BF 2.453 FL 0.000	-
9	A9	11.200	86.300	BF 2.453 FL 0.000	-
10	A10	11.200	95.300	BF 2.453 FL 0.000	-

Scan table





Properties (Stitching)

In the above figure, the **Util** of the location selected in the **Scan table** is ST (2,2,2,2) and the **Overlap** set in **Properties** is 20%. This means that based on the selected position (reference position of X: 11.200, Y: 68.300), two images are taken 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 Reference position (#1) is imaged first, then the order of imaging is from bottom left (#2) to top left (#6) in vertical 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**. The selected folder location will be shown on **Scan Location** and 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** field before saving. If a file name is not entered, the file will be automatically saved as the start time of the scan settings. Click on  to directly open the folder where the file is saved.



Browse For Folder



B. Schedule

Set the schedule for time-lapse imaging. Time except for **End Date** and **Total Cycle** can 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 the past, 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. The time elapsed (**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. The progress rate (%) within a cycle and remaining time until the next cycle (**Remaining**). **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.

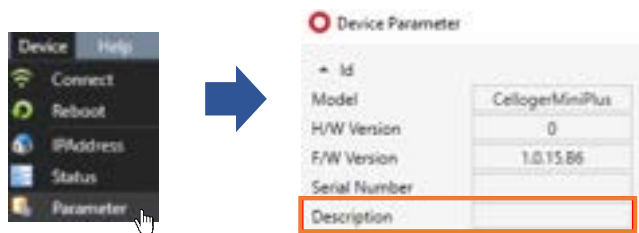
Note. Window can be separately floated so that it can fit on the monitor. It is possible to change the window by pressing   (**Autohide**) for floating or hiding. The layout is automatically saved upon terminating the program. To go back to the original state, press **Reset Layout** in **Tool** menu 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.

⑩ **Connection status**

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

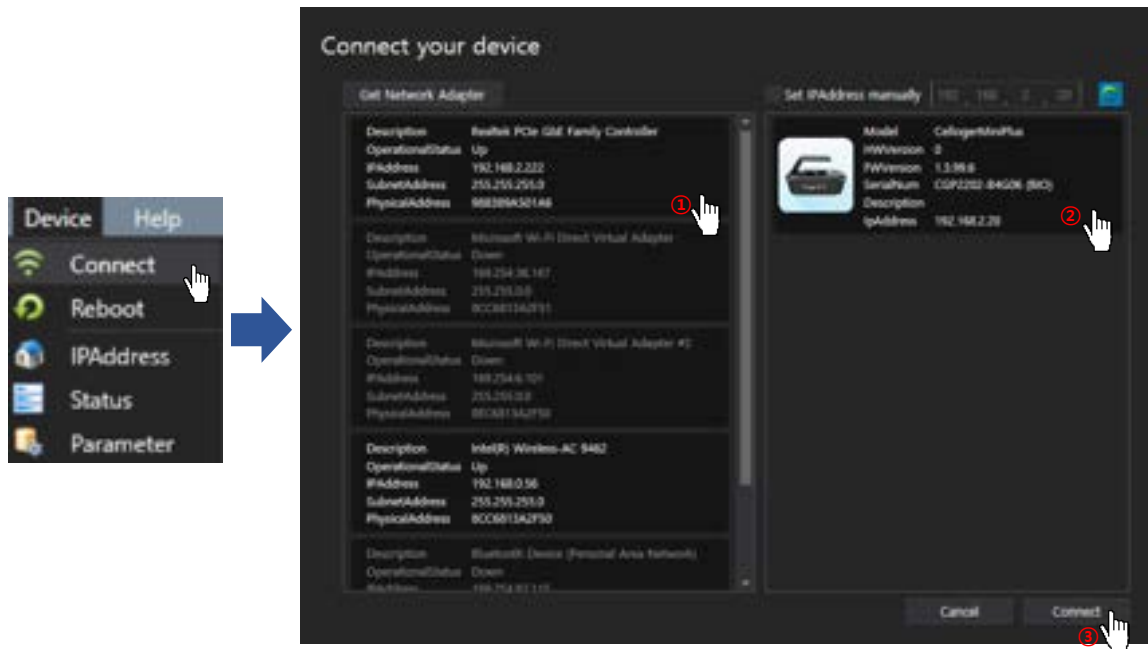


- A. IP address of connected device.
- B. Description of connected device (can be changed in **Device -> Parameter**).



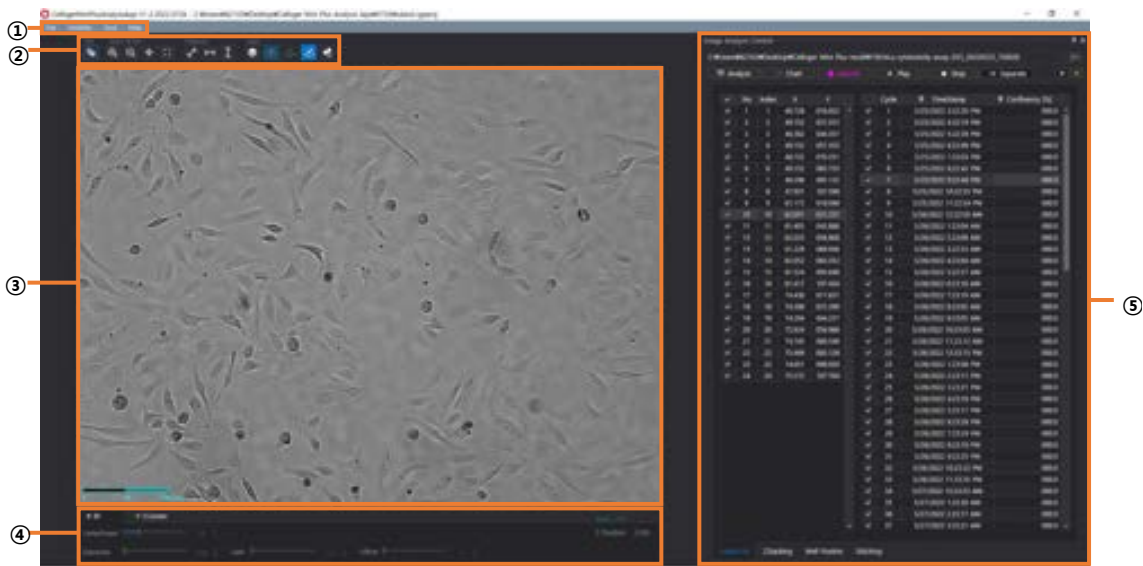
- C. Serial number of connected device.
- D. Firmware version of connected device.

Note. If the device is not connected, **Disconnected: 0.0.0.0 Tx Rx** shows up. Click **Device -> Connect** for reconnection. If the connection window does not show the device icon, refer to p.10.

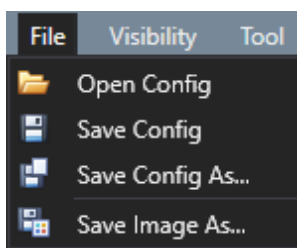


2) Celloger Mini Plus Anaysis App

Upon opening **Celloger Mini Plus Analysis App** and selecting **Config file**, the following main page is displayed.
 * **Config file**: It is a file that pops up when **Celloger Mini Plus Analysis App** starts. It is for saving and managing parameters set in the **Preferences** of **Tool** menu.



① Menu bar



It is managed by “config file” instead of a project.

Other menus are the same as those of **Celloger Mini Plus Scan App**.

② Toolbox [Layer]



: Mask the area recognized as confluency. All other tools and functions are the same as those of **Celloger Mini Plus Scan App**.

③ **Display**

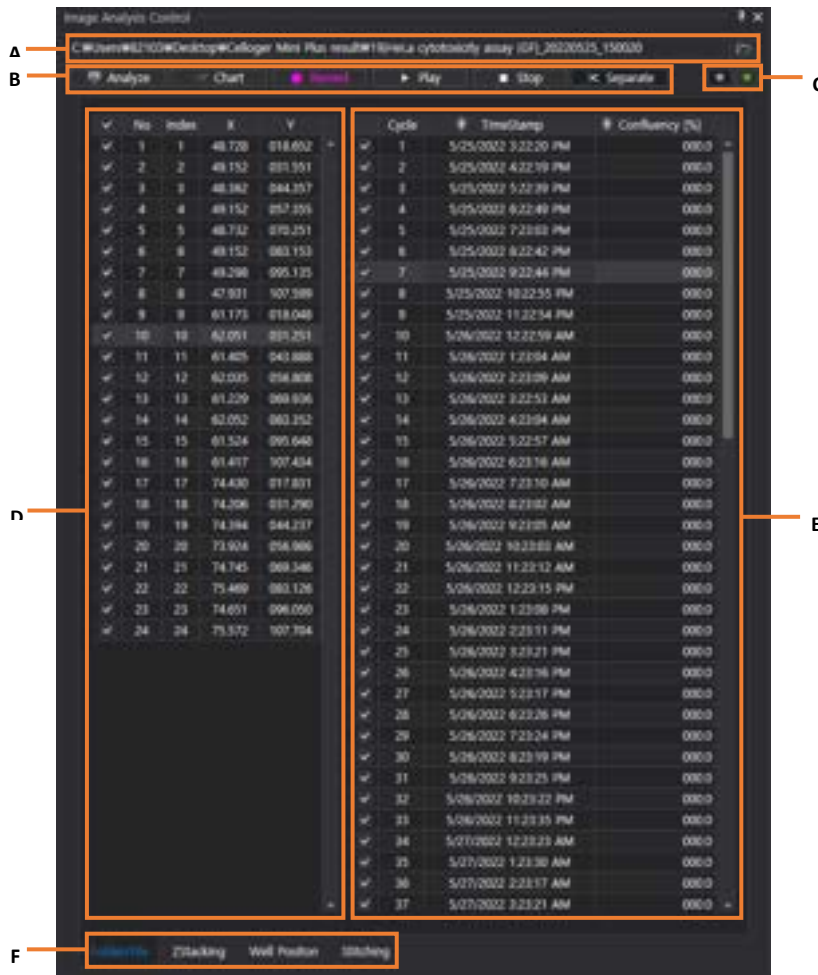
Display the image selected from **Image Analysis Control** (⑤) list. The image is displayed based on the activated channel that can be selected in **Light Source Control** (④). Upon completing analysis, coverage mask is shown and function such as measure (**Ruler**), **Intensity**, **Manual Counting** can be performed on the screen.


④ **Light Source Control & Z coordinates**

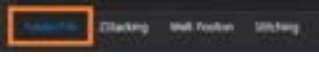


Display the light source settings and Z coordinates of **Celloger Mini Plus Scan App** and it cannot be changed.

⑤ **Image Analysis Control**

Upon importing a folder, file list is displayed. When a file is selected, the image is shown on **Display**. It is possible to analyze images or make a video. (refer to p.25~34)



- A. Click  to load the image file taken with **Celloger® Min Plus** (refer to p. 26).
- B. Analyze images or make time-lapse videos.
 - ✓ **Analyze:** Calculate the confluency of the files checked on the list. Analyze the image of selected light channels on the right (refer to p.27).
 - ✓ **Chart:** Show the confluency graph.
 - ✓ **Record:** Make video (refer to p.27).
 - ✓ **Play:** Show the images in the list in order (refer to p.27 for slideshow).
 - ✓ **Stop:** Stop playing.
 - ✓ **Separate:** The staked images (BF, FL, stitching, z-stacking) are divided into individual image files and saved.
- C. It is the light source setting of the image taken. Once clicked, the image of selected channel appears on the display.

- D. Scan positions: In **Folder/File** tab  , the scan positions specified in **Celloger Mini Plus Scan App** are shown in order as well as the X and Y coordinates of those positions.
- E. Images: In **Folder/File** tab  , it is the cycles of selected scan positions. The image of selected cycle is shown on the display. The time when the image was taken for each cycle is shown (TimeStamp) and result of confluency in percentage is shown (Confluency %) after undergoing calculation using **Analyze**  .
- F. Tabs showing the Z-stacking, stitching list, and well positions.
- ✓ **Folder/File**: Shows the scan position and cycles of the loaded image.
 - ✓ **Z-stacking**: Shows the list of Z-stacking images (refer to p. 29).
 - ✓ **Well Position**: Shows the well position at the time of imaging.
 - ✓ **Stitching**: Shows the list of Stitching images (refer to p. 31).

VI. Safety Instruction

* Read all instructions before use.

1. General guidelines

- Install the device on a rigid and level place.
- Operate the device in conditions described in the operating condition.
- 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.
- Do not insert any metallic objects into the device through bottom and side air vent to avoid electrical shock causing 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, the warranty is not provided even though 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.

2. Operating condition


Operating power	100~240 VAC
Electrical input	48 VDC, 1.04 A
Frequency	~50/60Hz
Operating temperature	10~40°C
Relative humidity	20~95%
Installation site	Indoor use only

3. Safety standards

European standards 

US standards 

UK Standards 

Korean standards 

VII. Cleaning and Maintenance

- When cleaning optical elements, use only a damp cloth to avoid scratching soft lens coatings. Do not clean the lenses with organic solvents.
- Lightly wipe the working surfaces of the **Celloger® Mini Plus** with soft cloth dampened with 70 % ethanol or hydrogen peroxide (H₂O₂). Do not pour or spray liquids anywhere on the instrument.
- If liquid spills on the instrument, turn off the power and wipe dry immediately.
- Make sure that liquid or foreign matters do not enter fan holes or lens holes.
- In case of storage at a room temperature after taking it out of the incubator, store it in a dry and well-ventilated place.
- Never disassemble the instrument yourself. Do not remove any covers or parts that require use of tool to obtain access to moving parts.
- Operators must be trained before being allowed to perform the hazardous operation.
- Unauthorized repairs may damage the instrument or alter its functionality, which may void your warranty. Contact your local distributor to arrange for service.
- Do not place the POE injector inside in the incubator.
- Do not block the fan hole of the device.
- When not using the equipment for a long period of time, turn on the fan and dry it before storing. Store it at a room temperature on a flat place without humidity and vibration.

VIII. Troubleshooting

Installation

Device does not power up

- ✓ Check the 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

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

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 **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 space of the hard disk.

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 **Celloger Mini Plus Scan App**.

Cannot analyze the confluency

- ✓ Check if the images are obtained from **Celloger Mini Plus Scan App**; Analysis can be performed only with the images obtained from **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 form on the well plate, dish lid or top of the flask (= Condensation occurs)

- ✓ Warm up the device 30 minutes before start monitoring.
- ✓ Check for and remove any condensation on the lid of the culture dish. (In order to prevent condensation from occurring, it is better to warm up the plate or dish lid slightly when preparing the sample.)
- ✓ Check if 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.

IX. Product specifications

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

X. Ordering information

Celloger® Mini Plus

Cat. No.	Description
CRCLG-MPB04	CLG Mini Plus, Live cell imaging system (Bright Field, 4X)
CRCLG-MPB10	CLG Mini Plus, Live cell imaging system (Bright Field, 10X)
CRCLG-MPBG04	CLG Mini Plus, Live cell imaging system (Bright Field+Green Fluorescence, 4X)
CRCLG-MPBG10	CLG Mini Plus, Live cell imaging system (Bright Field+Green Fluorescence, 10X)
CRCLG-MPBR04	CLG Mini Plus, Live cell imaging system (Bright Field+Red Fluorescence, 4X)
CRCLG-MPBR10	CLG Mini Plus, Live cell imaging system (Bright Field+Red Fluorescence, 10X)
CRCLG-MPWPS	Vessel holder, Well plate 6~96 (Single)
CRCLG-MPTFS25	Vessel holder, T-Flask A25cm2 (Single)
CRCLG-MPTFD25	Vessel holder, T-Flask A25cm2 (Dual)
CRCLG-MPTFS75	Vessel holder, T-Flask A75cm2 (Single)
CRCLG-MPPDD35	Vessel holder, Petri dish 35mm (Dual)
CRCLG-MPPDD60	Vessel holder, Petri dish 60mm (Dual)
CRCLG-MPPDS90	Vessel holder, Petri dish 90/100mm (Single)

Appendix A. Connecting multiple devices

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

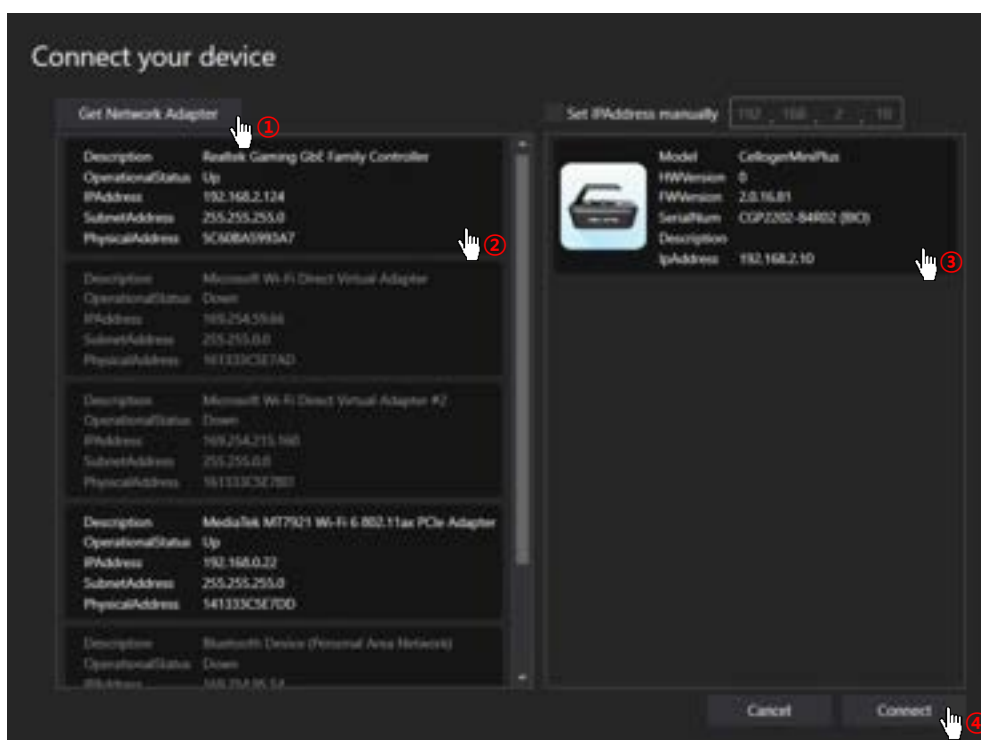
Note. Be careful as system works abnormally if the same device is connected using two programs.

1. Installation

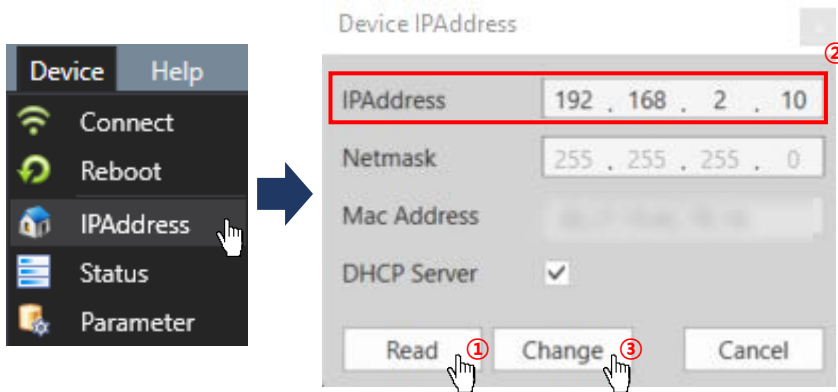
1.1 Device IP setup

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

- ① After connecting one device to PC, open the **Celloger Mini Plus Scan App**.
- ② Start the App after selecting the device. (If device does not show up, refer to p.9 for **Device Connection**).



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



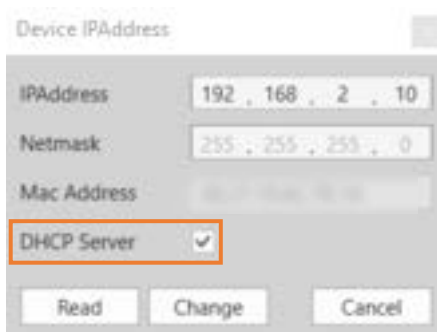
- ④ Change the IP address and click **Change** button.

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

<Example. IP address setting for connecting 3 Celloger® Mini Plus devices and PC>

- PC IP address: 192.168.2.200
- Device 1. IP address: 192.168.2.10
- Device 2. IP address: 192.168.2.11
- Device 3. IP address: 192.168.2.20

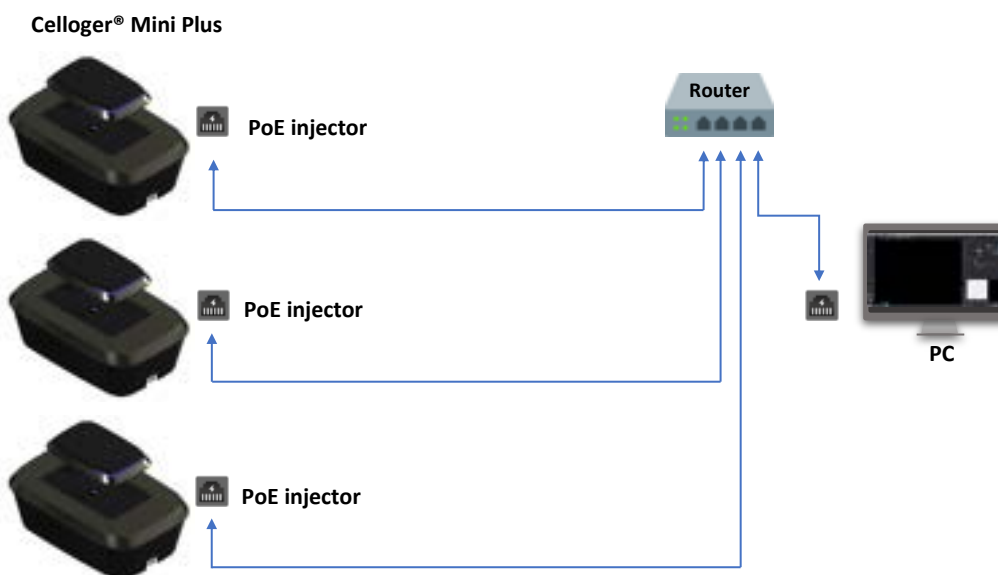
Note. When setting the IP address of the network adapter in **Celloger Mini Plus Scan App** (p. 9), only one of two or more devices must be checked in the **DHCP Server** and the other devices must be unchecked. In case of setting the IP manually on PC (p. 60), connection can be done regardless of the **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 **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 IP address at the bottom of the program (refer to p.49 ⑩).
- 3) Click **Identify**  and check white LED blinking.

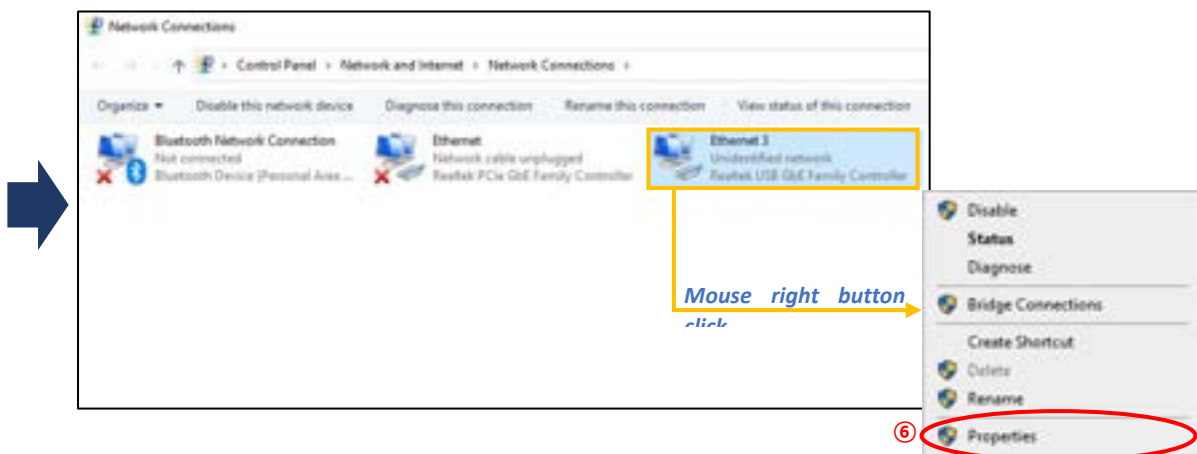
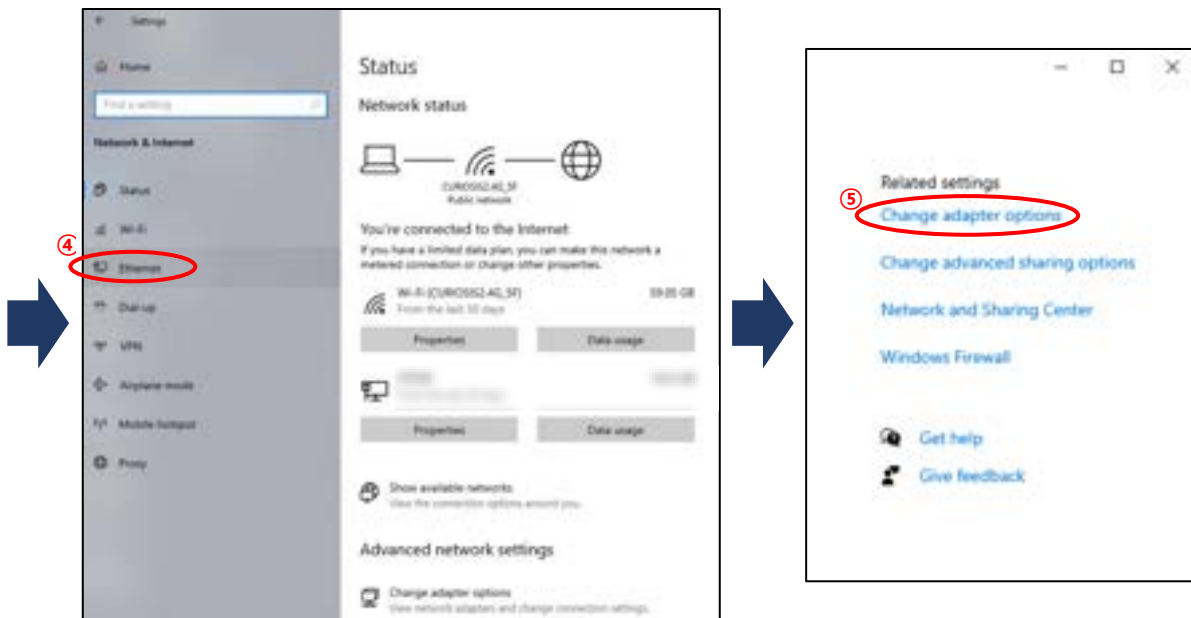
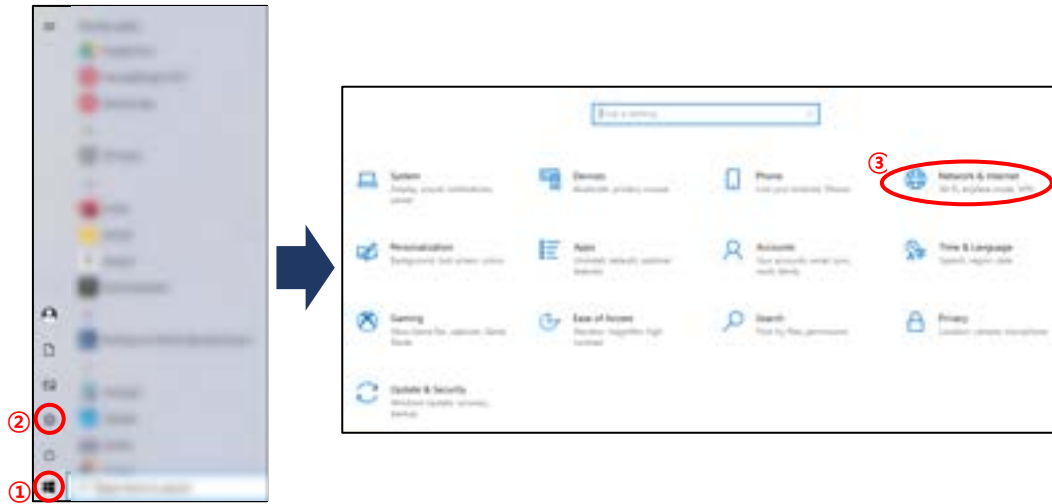
Appendix B. Alternative PC network setup

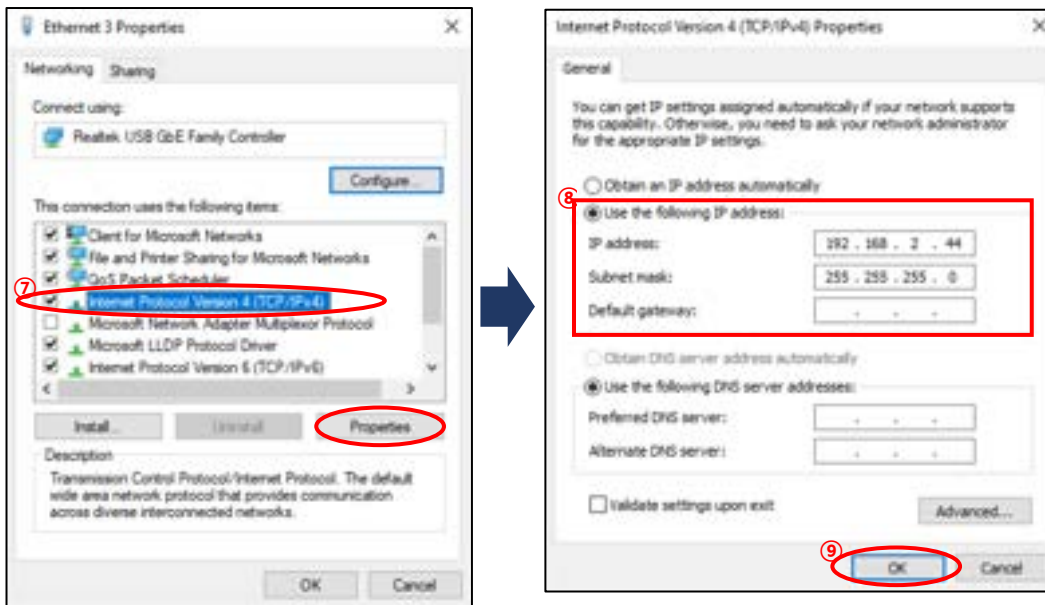
Perform the PC network settings as follows to connect the device with PC. The steps below are based on Windows 10. For Windows 11, refer to the images below (p. 62~63).

- ① Turn on the computer and click “**Start**” or “**Window**” icon.
- ② Click “**Setting**”.
- ③ Click “**Network and Internet**” in Control Panel.
- ④ Click “**Ethernet**”.
- ⑤ Click “**Change adapter options**”.
- ⑥ Select “**Ethernet**” (This name can vary according to 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 **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 IP address should be set differently from the device IP address or the network adapter such as a router. The default device IP is 192.168.2.10 and to set up the PC IP address, enter 192.168.2.XX (in XX field, input 2~254 except 10).
 - This IP setting guideline may not be applicable to some PC depending on the PC network environment. In case of connection failure, contact us via info@curiosis.com or your local distributor.
- ⑨ Click “**OK**”, then network configuration is completed.
- ⑩ To start **Celloger Mini Plus Scan App**, first, connect the USB drive provided with device into the PC, then copy APP folder to PC and execute “**Celloger Mini Plus Scan App.exe**” in the App folder.

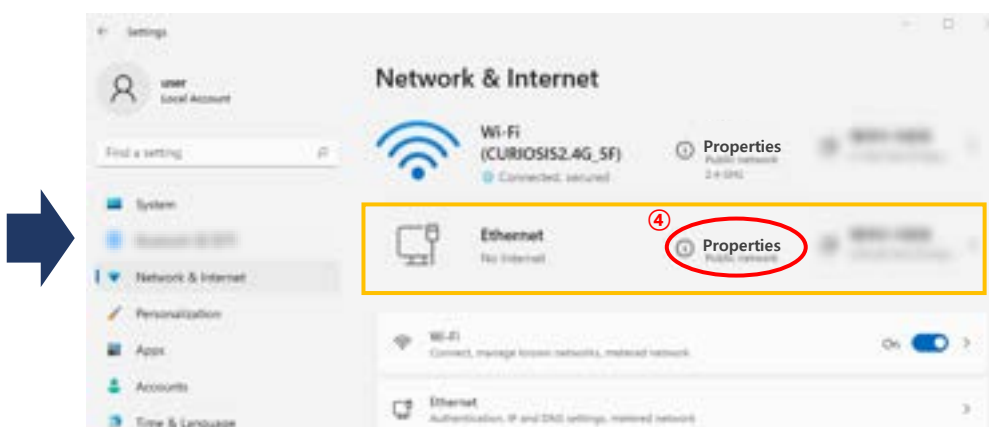
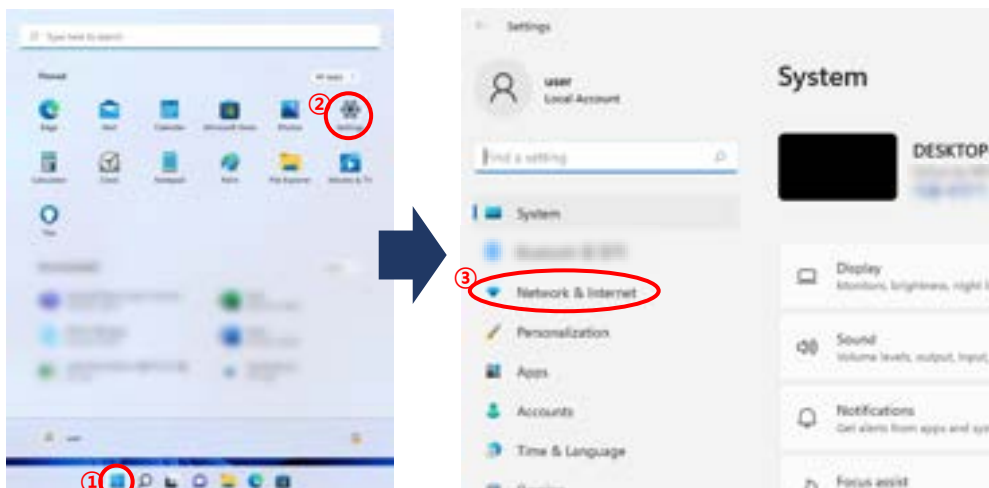
<Image descriptions of PC network setting>

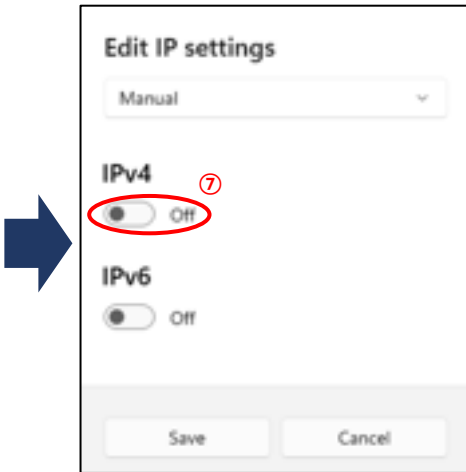
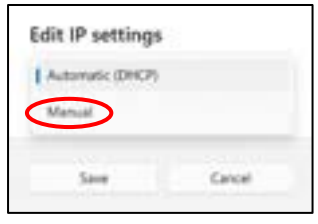
1) Window 10





2) Window 11

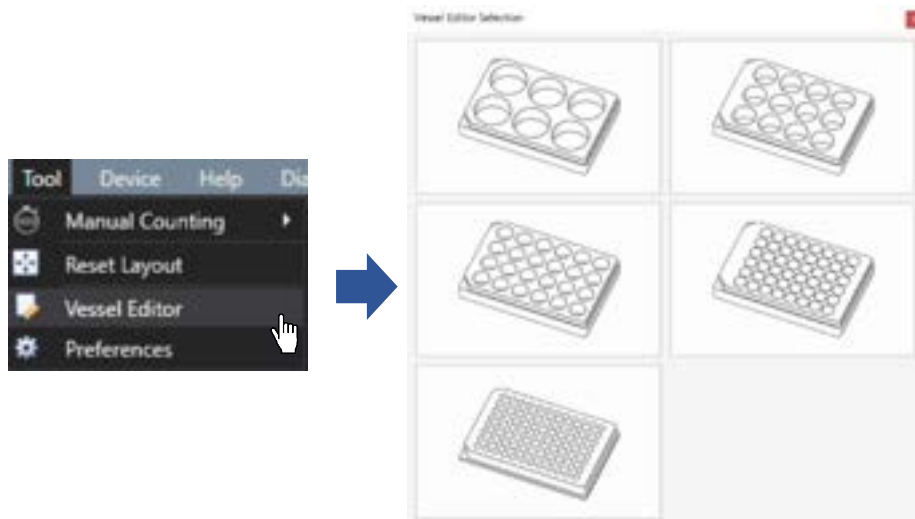




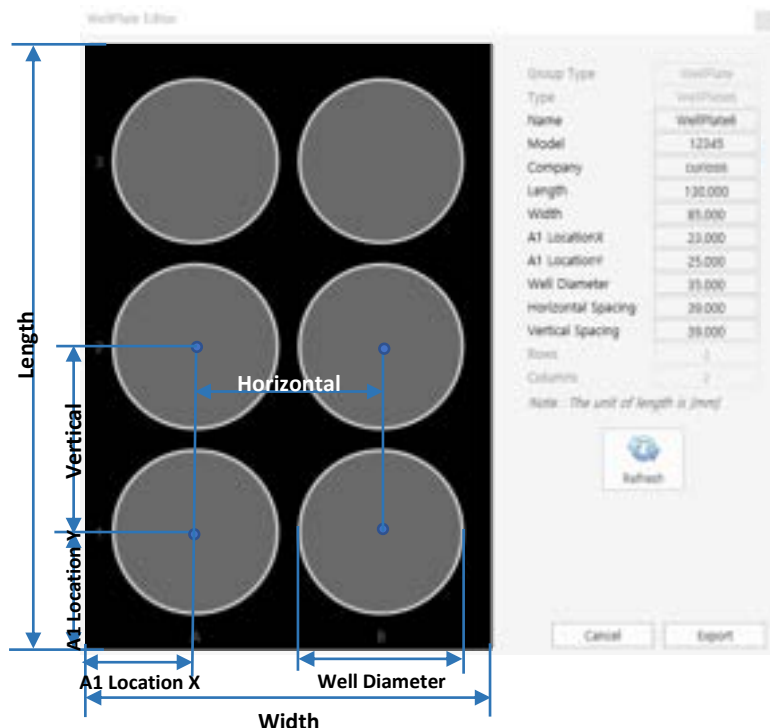
Appendix C. How to use vessel editor

The **Celloger® Mini Plus** 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**.

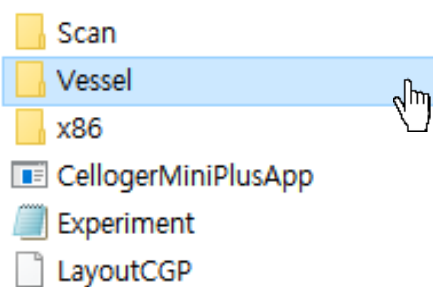
- ① Select **Vessel Editor** from the **Tool** menu.
- ② Select the type of well plate (6/12/24/48/96 well) to be used from the **Vessel Editor Selection** window.



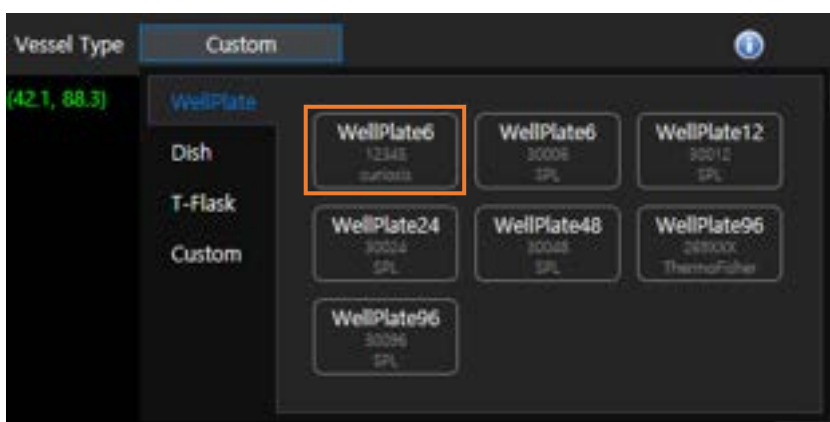
- ③ Change the specifications (**Length**, **Width**, **A1LocationX**, **A1LocationY**, **Well Diameter**, **Horizontal Spacing**, **Vertical Spacing**) of the well plate to be used after entering the **Model** and **Company** in the **WellPlate Editor** window. Refer to the 6 well plate image below 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.

Revision History

Date	Version	Description
2022/03/03	CR-IFU-008	Initial issue
2022/09/15	CR-IFU-008(Rev.2)	- Added contents (Grid line, Image adjustment, Preview record, Stitching, Chart) - Revised contents (Table of contents, Getting started, Z-stacking, Image analysis control, Autofocus, Cleaning and Maintenance, Troubleshooting, Appendix B) - Revised UI (Image analysis control)

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