

Automated Live Cell Imaging System

Celloger[®] Nano

Instruction manual

Developed and manufactured by CURIOSIS Inc.

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I. Package contents

Celloger® Nano package includes the following items.

Item	Quantity
Celloger Nano	1
Main Power Cable	1
POE Injector	1
Adapter	1
LAN Cable (5M, 4.8M)	2
USB memory	1
- Instruction Manual	1
- Celloger Nano Scan App	1
- Celloger Nano Analysis App	1

When receiving the package,

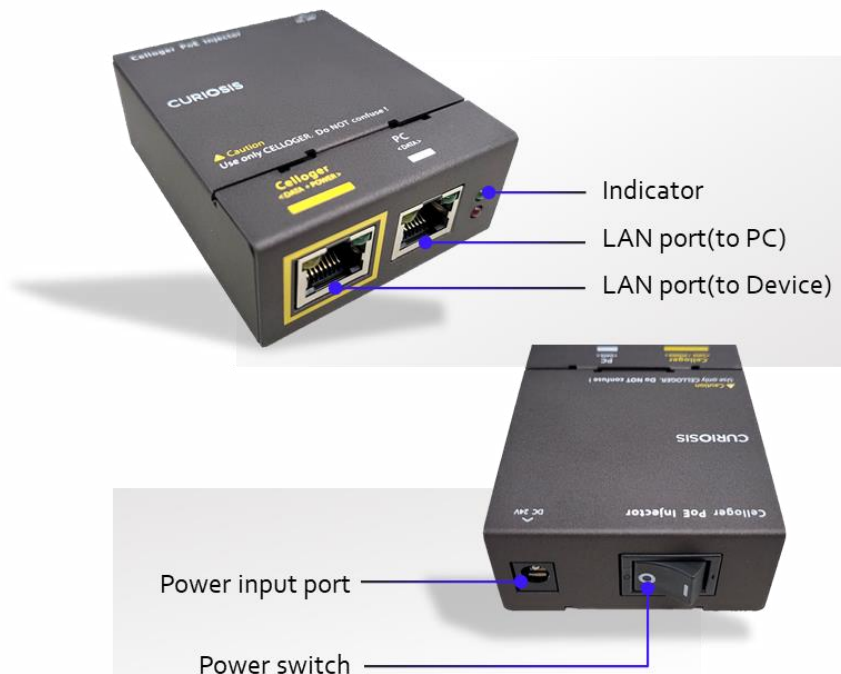
- Check that all items listed above are included in your package.
- Examine the device carefully for any damage during shipping.
- Contact your local distributor or info@curiosis.com if any items are missing or damaged.
- Any loss or damage claims must be filed with the carrier.

II. Device layout

Front-left side



POE Injector



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[®] Nano 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[®] Nano.
- 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 (Refer to page 46)

- Celloger[®] Nano 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. If there is no LAN port, LAN cable adapter should be prepared.

2. Installation

2.1.Unpacking

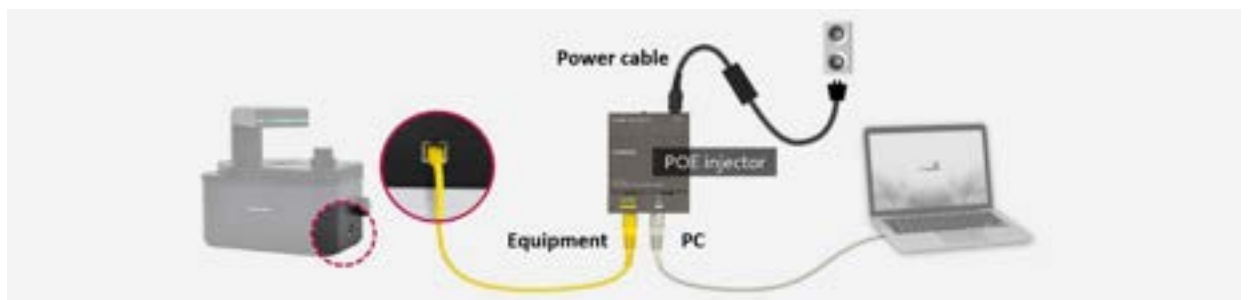
1. Lift the Celloger® Nano from the box, remove all the covers, and place it on a clean and level surface.
2. Make sure all accessories are present by checking “Package Contents” (p.4). If any items are missing or damaged, please contact your distributor or Curiosis at info@curiosis.com.

2.2.Connect the device and computer

1. Prepare a computer that meets the specifications (refer to p.46 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 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

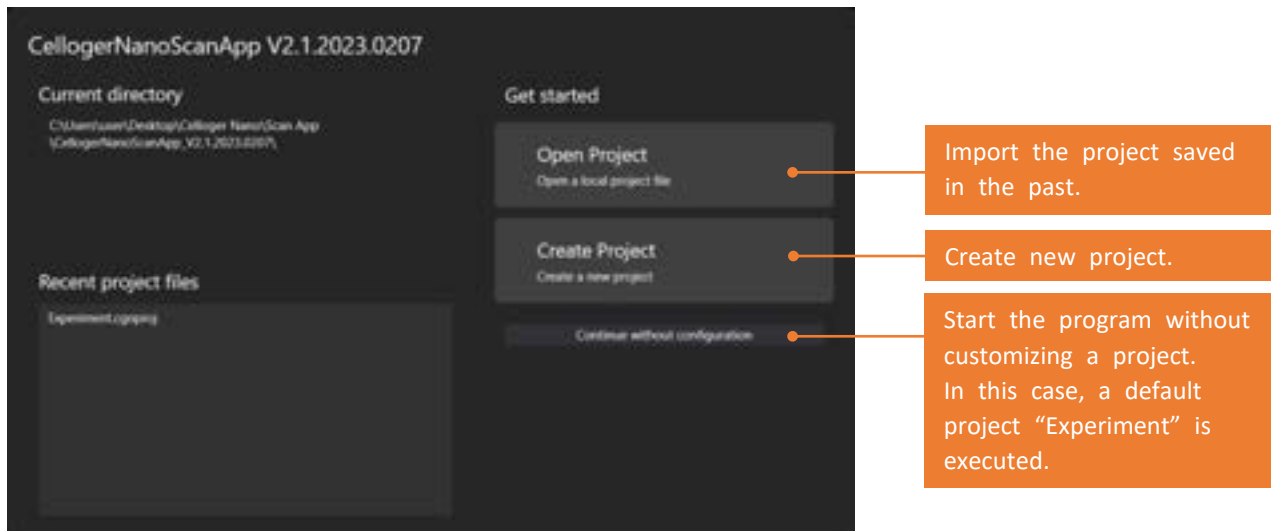
1. Place the device inside the incubator.
* Before using the device, make sure to clean the device with soft dampened with 70 % ethanol.
2. Place the sample on the stage.
3. Check the connection between the PC and the device. To connect the device and PC, refer to “**Connect the device**”(p.9)



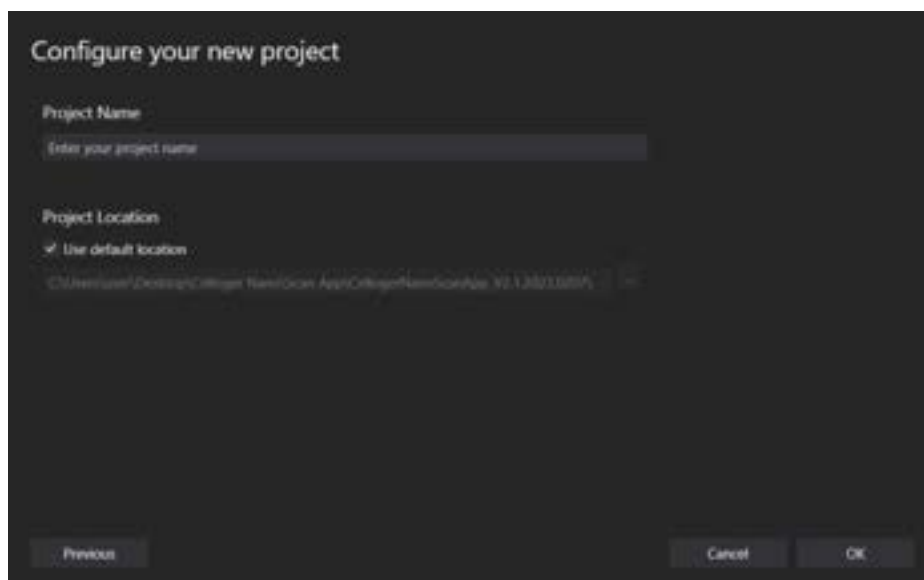
3. Create or open a project

Upon executing the **Celloger Nano Scan App**, popup screen for project selection is displayed. With Celloger® Nano, 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 Nano Scan 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.



4. Connect the device



1. Open **Celloger Nano 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 **Refresh 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 **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 device IP address directly from.

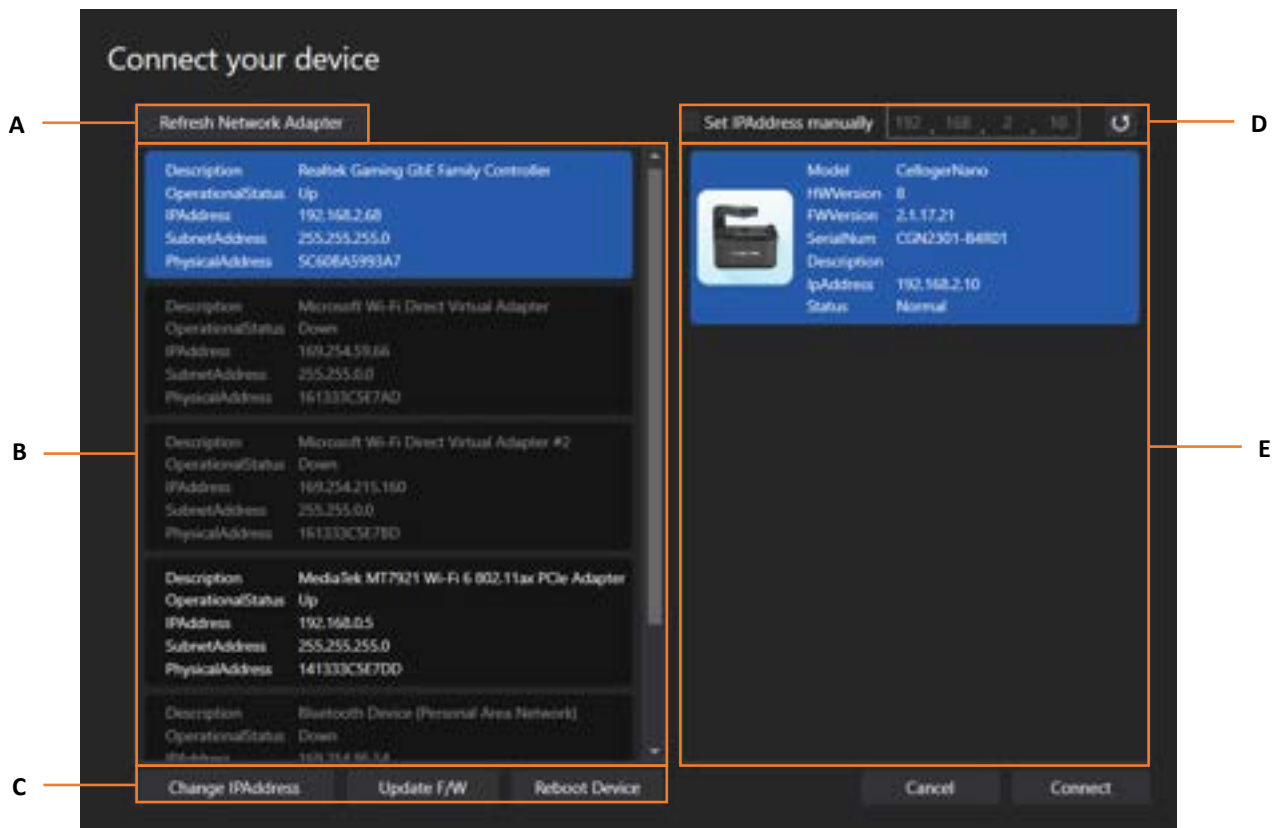


Case 4. When using multiple devices in one PC, each device has the same IP.

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.48 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>



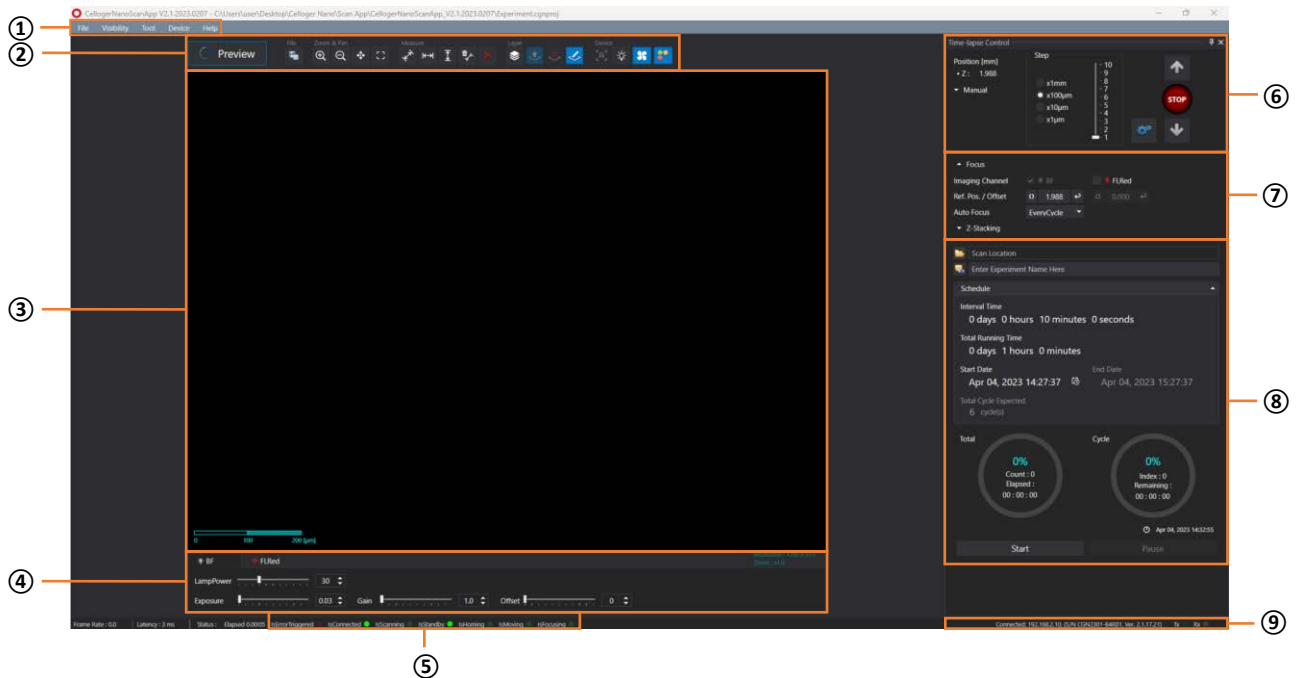
- A. **Refresh Network Adapter** reads the connected network adapter from the PC.
- B. Network adapter list. **Operational Status** shows the network connection status where **Up** means network is enabled, while **Down** means disabled.
- C.
 - ✓ **Change IP Address:** Change the IP address of selected 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.

5. Software overview

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

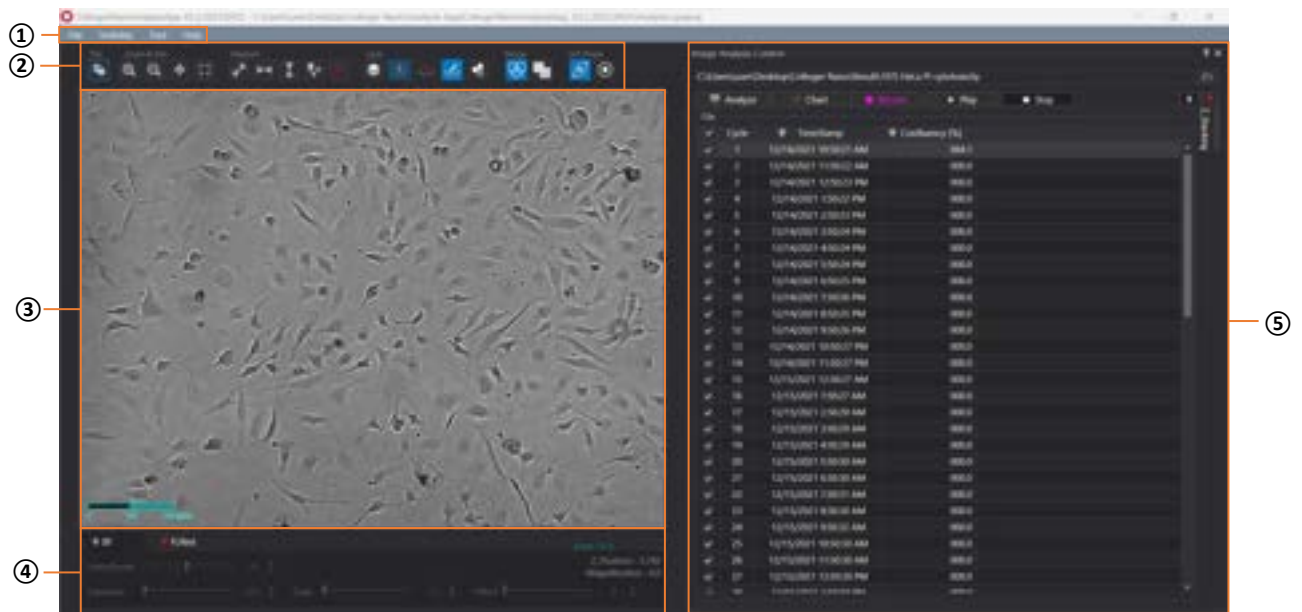
Note. Refer to p.32 for full software descriptions.

5.1 Celloger Nano Scan App



- ① Menu bar
- ② Toolbox
- ③ Display (Preview)
- ④ Light source control
- ⑤ Device status
- ⑥ Z-stage control
- ⑦ Position control
- ⑧ Time-lapse control
- ⑨ Connection status

5.2 Celloger Nano 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)

- ① Place **Celloger® Nano** 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 Place the cell sample on the stage of **Celloger® Nano**.

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.

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

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

2.1 Location adjustment

Celloger® Nano has mechanical X, Y and Z stages while Z-stage can also be manipulated using the software.

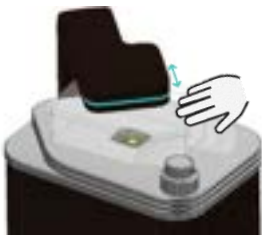
1) Adjust X, Y position

X, Y stage of **Celloger® Nano** uses X, Y stage controller(knob) to move X, Y axis by +/-6 mm respectively. To adjust further, the vessel must be manually moved. In order to designate the scan point, first, move the vessel by hand to see the desired location in preview stage, and then adjust the X, Y location using X, Y stage controller.

- ① Press **Preview** for streaming while BF is selected in the **Light Source Control**.



- ② Check the **Display** while moving the vessel. (If it is too dark or too bright to see the cells, perform “2.2 Light intensity adjustment” in p.16 first.)




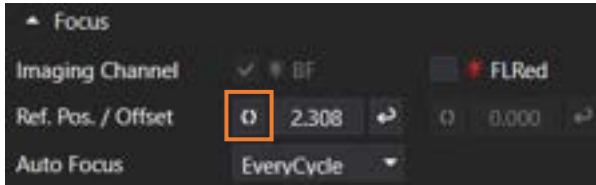
- ③ If the target cells become visible, use X, Y stage controller for precise adjustment.



2) Set BF Focus

➤ Manual adjustment [using Z-knob]

- ① In the Preview state, adjust the focus by rotating the Z-knob.
- ② Upon finding the best focal point for scanning, designate the location by pressing  in **Focus**.



Note. Since the focus of the **FL** channel may be different from that of the **BF**, it is recommended to set the focus position individually. For Z positioning of the **FL** channel, check the **Display** while **FL** channel is selected in **Light Source Control**, and move the **Jog** to find a clear point like **BF**.

➤ Manual adjustment [using Celloger Nano Scan App program]

- ① In the Preview state (“BF” tab selected on **Light Source Control**), select the **Step** in Z-stage control as “X1mm” 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.

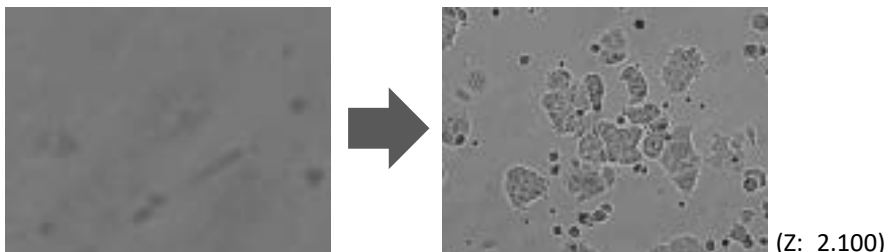
Note. Celloger® Nano’s Z-stage absolute coordinate error is 0.001 mm.

- ② Please refer to the **Manual adjustment using Z-knob** step 2 above.

➤ Auto focusing

Autofocus function of **Celloger® Nano** 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 autofocus parameter for more successful autofocusing. Therefore, set Z position roughly(manually) and use the autofocusing function.

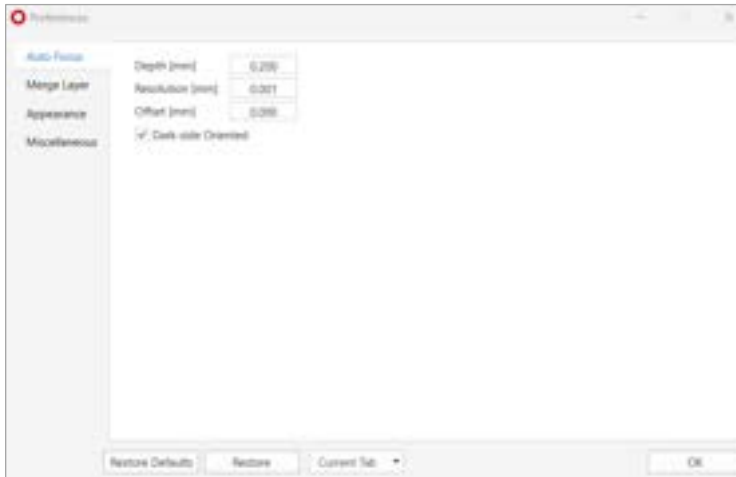
- ① Move the focal plane using **Jog** button or Z-knob.



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



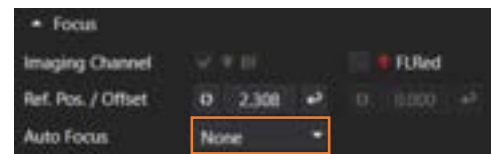
- ③ Designate parameter(**Depth, Resolution, Offset**) in the **Auto Focus** and press **OK**.



- ✓ **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® Nano**, input the difference between the point and Z position coordinates from autofocusing.
- ✓ **Dark-side Oriented**: 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 and autofocusing time.

- ④ Set the value except for **None** in **Auto Focus** to set the method of autofocusing (refer to p.37 for **Auto Focus**).




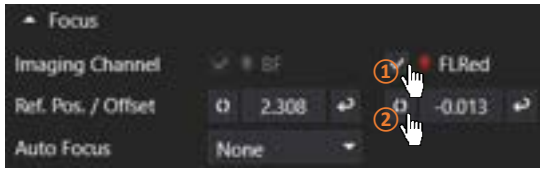
3) Set FL Focus Offset

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

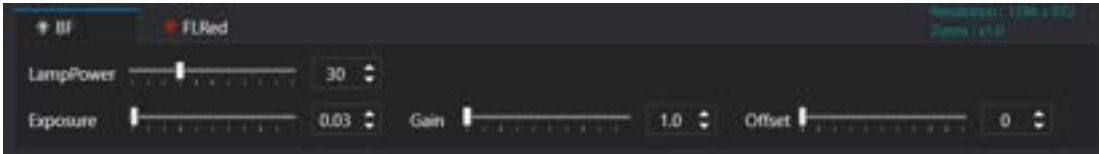


- Check that the **FL channel** checkbox in **Focus** is checked and click on  to specify the focus.



2.2 Light intensity adjustment

Light source option for **Celloger® Nano** 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 the Light Source Control in the App connected to Red FL Celloger® Nano





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

2.3 Z-stacking

1) Manual Z-stacking

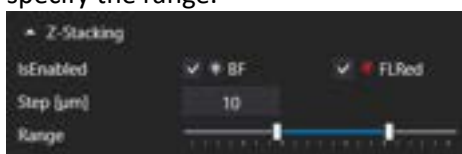
- By using **Jog** or Z-knob, find the position to start stacking.
- Designate the stacking unit in the **Step**.
Ex) Step setting for stacking by 10 μm



- Press  once and save it by pressing .
- Repeat step ③ until it reaches the desired range of stacking.
- Press  once and save it by pressing .
- Repeat step ⑤ until it reaches the desired range of stacking.

2) Automated Z-stacking


- Select whether or not to execute the Z-stacking function in BF and FL in **Z-stacking**. (refer to p.37 for Z-stacking)
- After specifying the interval (= **Step**) to execute the Z-stacking function, adjust the scroll bar under **Range** to specify the range.





<Z-stacking>

3. Image analysis [in Scan App]

3.1 Capture

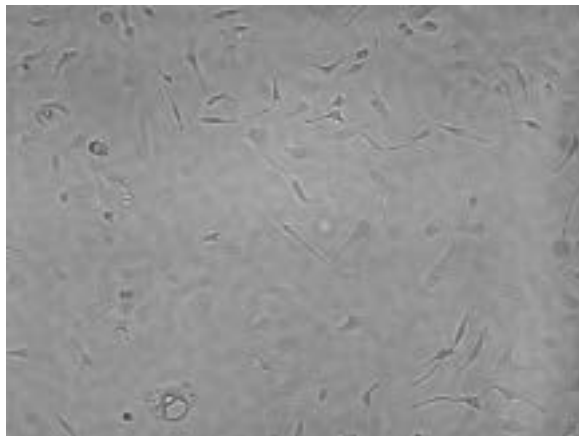
 icon in **Toolbox** captures the image on **Display**. (Use “Ctrl + I” as a shortcut)

1) An image with features

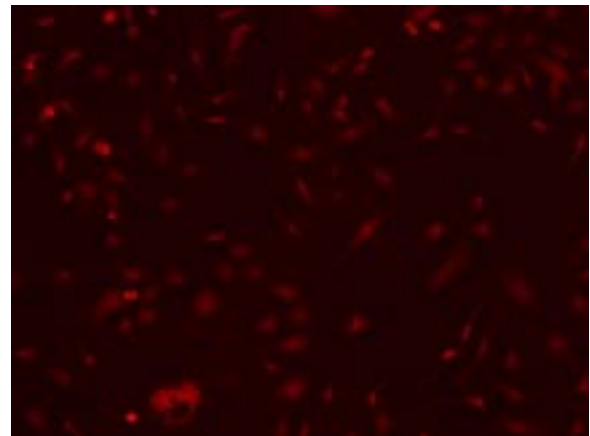
It is possible to generate and manipulate the desired features, show it on **Display** and save it as it is. To obtain without features, press  (**Features layer**) for deactivation and then press .

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 .





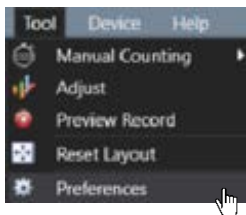
<BF channel image>



<FL (red) 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® Nano conducts thresholding for **FL** channel image based on intensity and merge it with **BF** image. There are two methods for intensity thresholding.

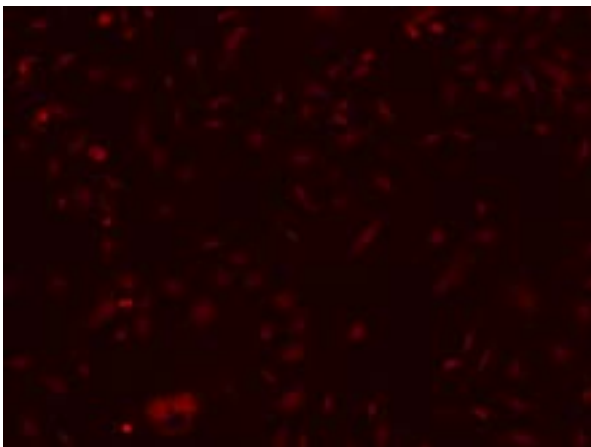
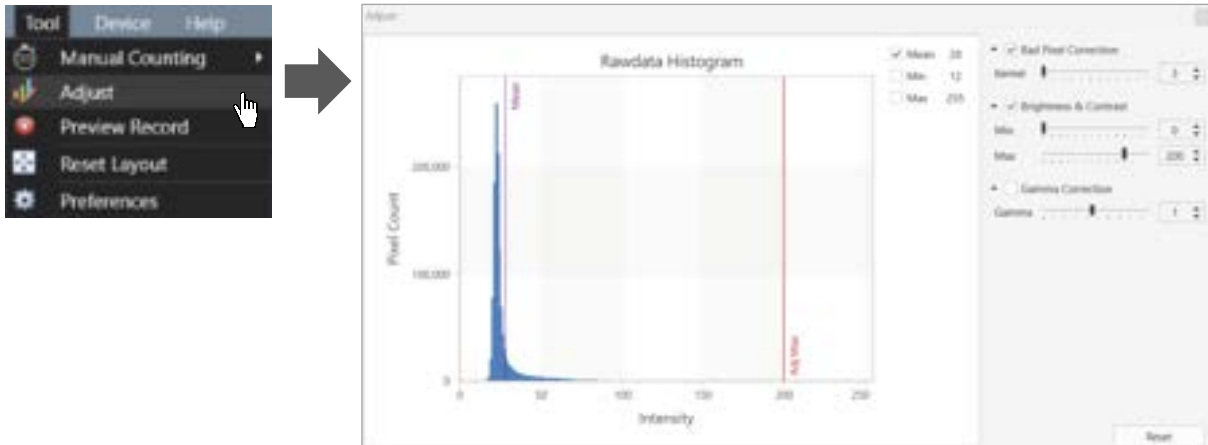
Method 1) Global thresholding: Thresholding is executed based on the overall intensity of the image. Adjust a parameter of **Threshold** while looking at the mask of an image after unchecking the **Adaptive Algorithm** box.

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.)

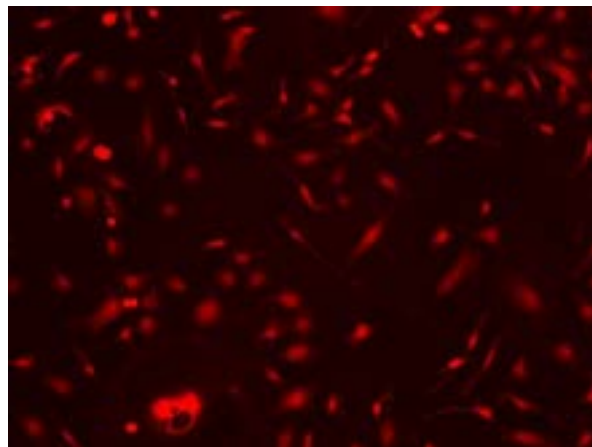
Note. When the fluorescence areas are distorted or clustered in one place, **Global thresholding** function is recommended; and when the fluorescence areas are evenly distributed, **Local adaptive thresholding** function is recommended.

3.3 Image adjustment

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

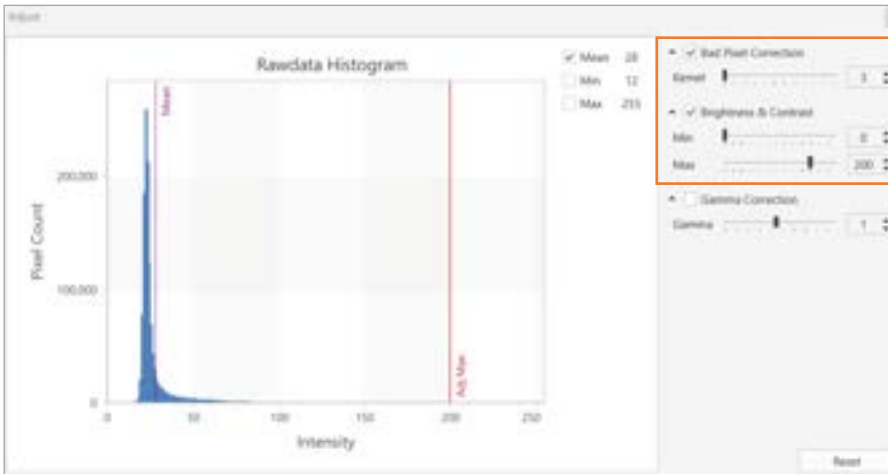


Before adjustment



After adjustment

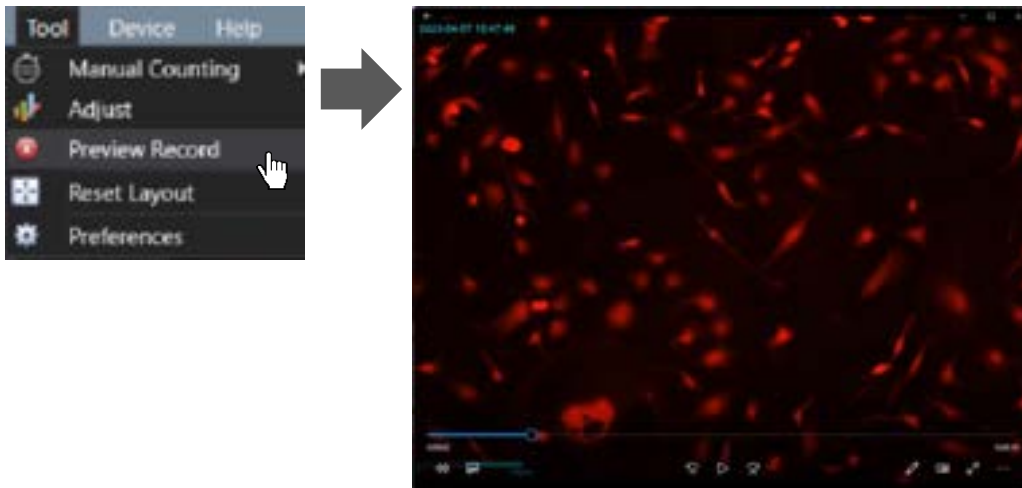
Note. In case of fluorescence as shown in the image below, **Bad Pixel Correction** and **Brightness & Contrast** are set by default, therefore it should be adjusted.



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

3.4 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.

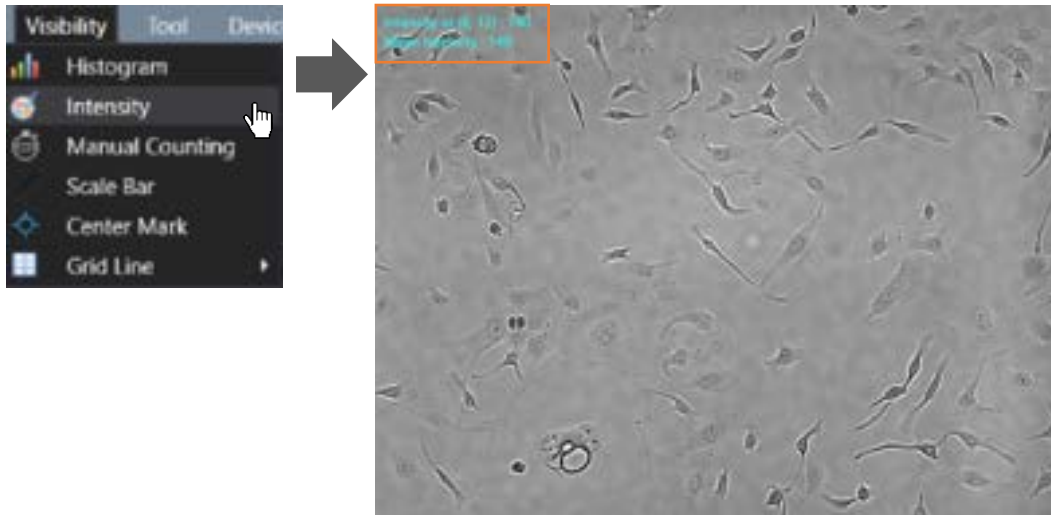


Note. When **Preview Record** is activated, recording continues even **Preview** is turned off and on. To end the recording, you must deactivate it in **Preview Record**.

3.5 Intensity

1) Point intensity & Mean intensity

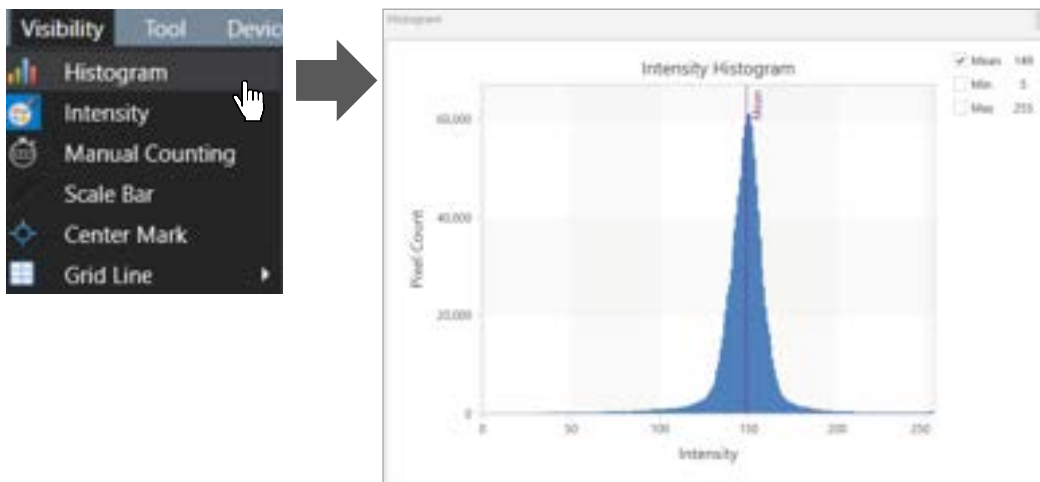
Upon activating **Intensity** in **Visibility** menu, **Point Intensity** and **Mean Intensity** information is displayed at the upper left of **Display**.



- ✓ **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**.






3.6 Manual Counting

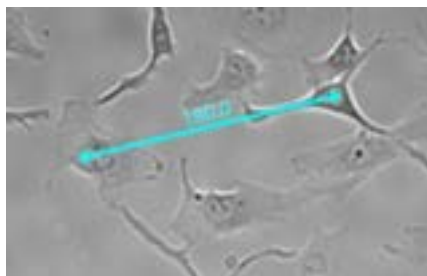
Select **Count** in **Manual Counting** in **Tool** menu and click the desired area to count with cursor, clicked area is checked and the counted number is shown in **Counting Result**.



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.

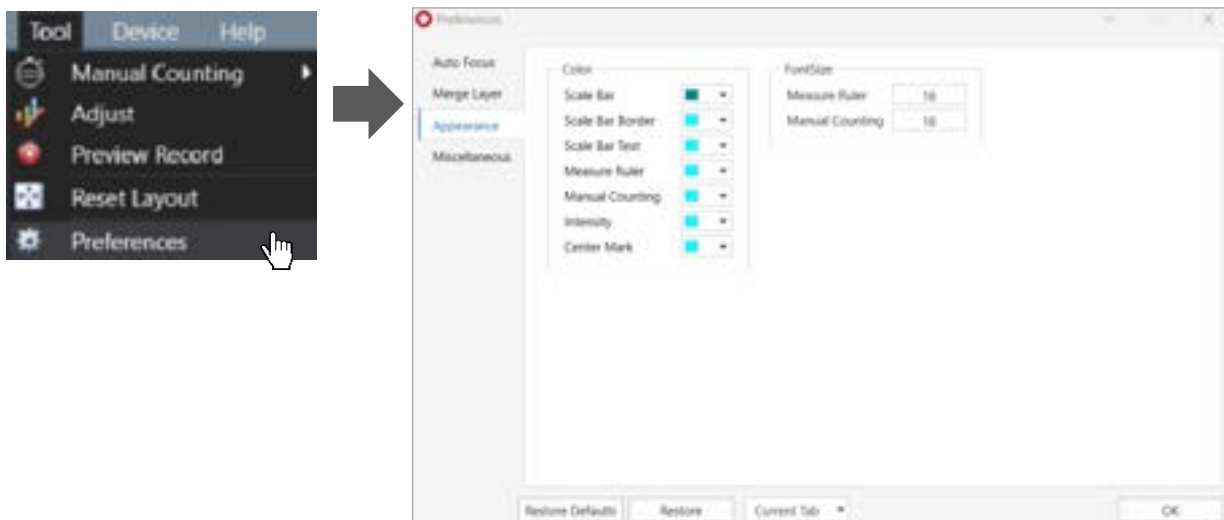
3.7 Ruler

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).



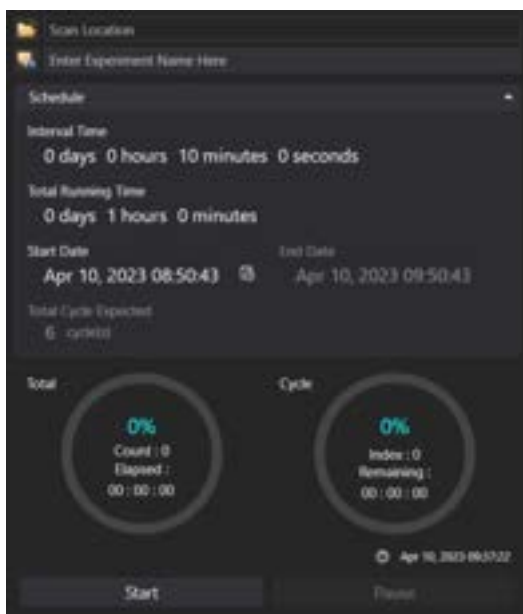
Note. To undo the selected point (after selecting one point), press “*Esc*” on the keyboard. Be sure that the “*Num Lock*” is turned on.

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



4. Time-lapse imaging [in Scan App]

- ① Click on **Preview** and adjust the light (p.16) and focus (p.14) by referring to the **Display**. If fluorescence is also scanned at this time, the quantity of light of fluorescence should also be adjusted.
- ② Specify the position and focus to be scanned. At this time, select whether or not to execute the autofocusing function (p.14) and Z-stacking function (p.16). If fluorescence is also scanned, the focus of fluorescence should also be adjusted.
- ③ 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 Nano Scan App** folder and if the project name is not entered, a folder will be automatically created under the scan start date.
- ④ Set the schedule (**Interval Time**, **Total Running Time** and **Start Date**) (p.38). 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.

- ⑤ Click on **Start** to begin scanning.

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

- ⑥ Once the scanning is completed, check the saved images, make videos, or perform additional analysis using **Celloger Nano 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.

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

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

5.1 Import the original data taken by Celloger® Nano

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

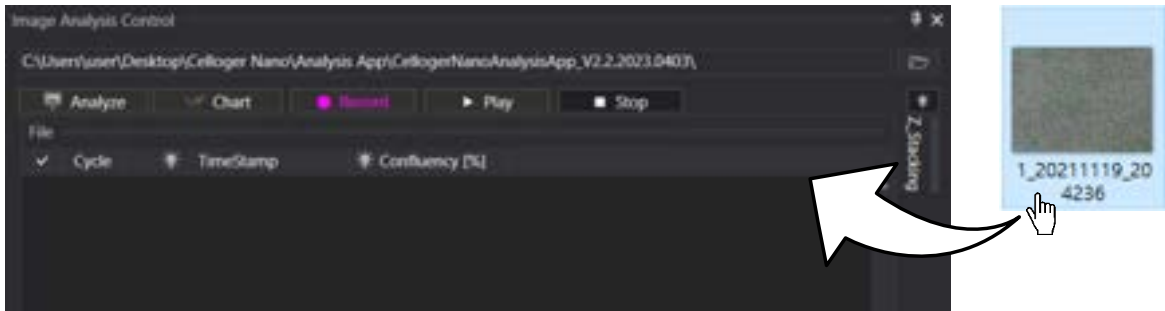
1) Open the time-lapse folder.

Select the time-lapse folder taken by **Celloger® Nano**. Upon opening the selected folder, the images in the folder are listed following the order of cycle.



2) Open the image file.




Drag the image file.





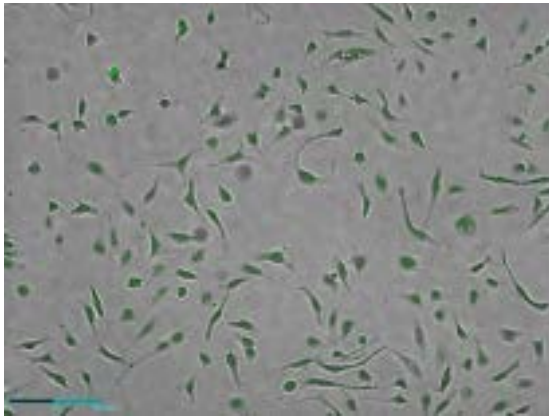
Note. A folder can be dragged.

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 Nano Analysis App**. If it fails to import the file, check whether the file name and location address is written in languages other than English.

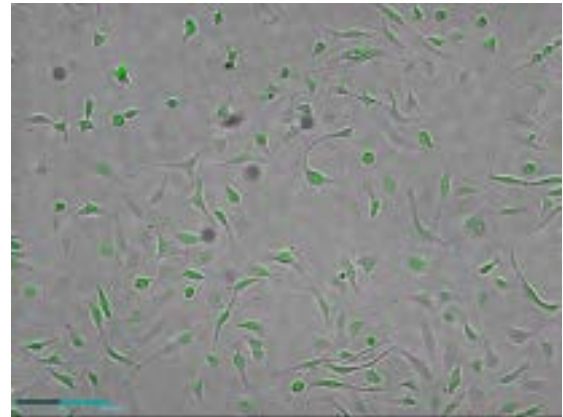
5.2 Merge FL image

Press  (**Merge layer**) for activation , and select the merge method () , merged image is shown on **Display**. Merge setting can be changed by adjusting the **Merge layer** in **Preferences** of **Tool** menu as shown below.

- 1)  (**Alpha Blending**): A method of combining images with transparent effect. It is the same merge method as **Celloger Nano Scan App**, so refer to **Celloger Nano Scan App's** merge parameter(refer to p.17).
- 2)  (**Channel Merge**): A method of combining images without transparent effect. The parameters are the same as for **Alpha Blending**.


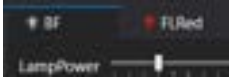

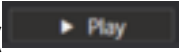


<Alpha Blending>



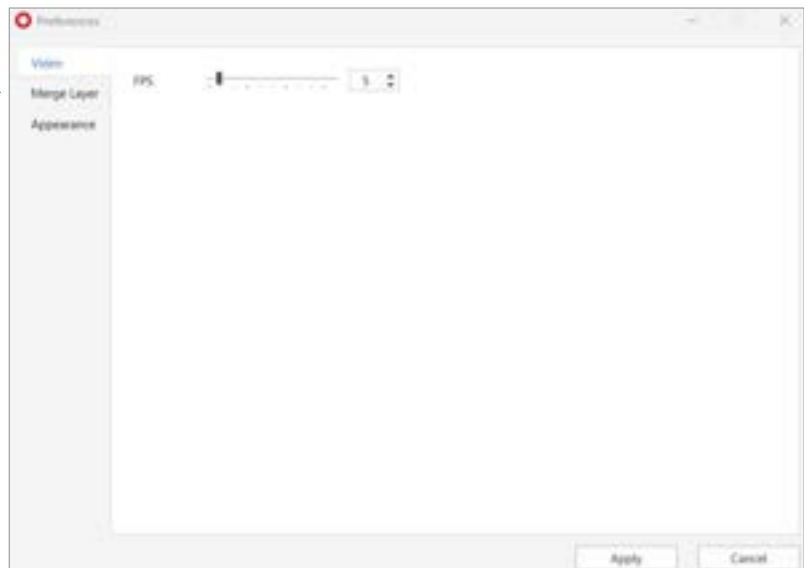
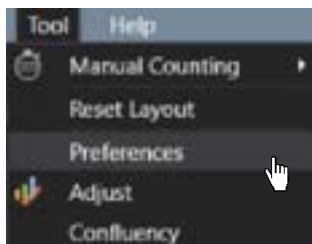
<Channel Merge>

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**.



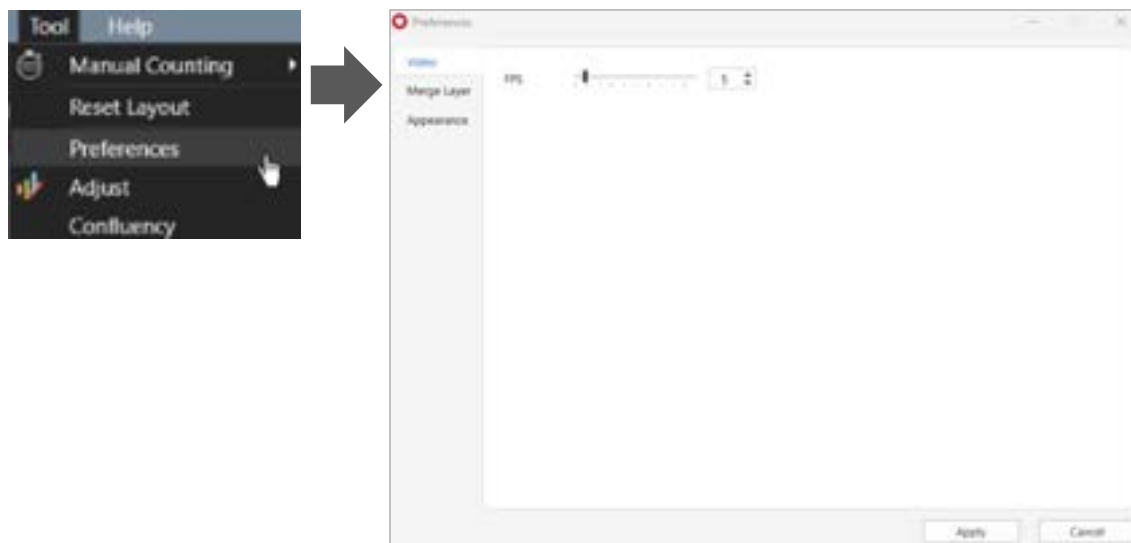
5.4 Make video

- 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.

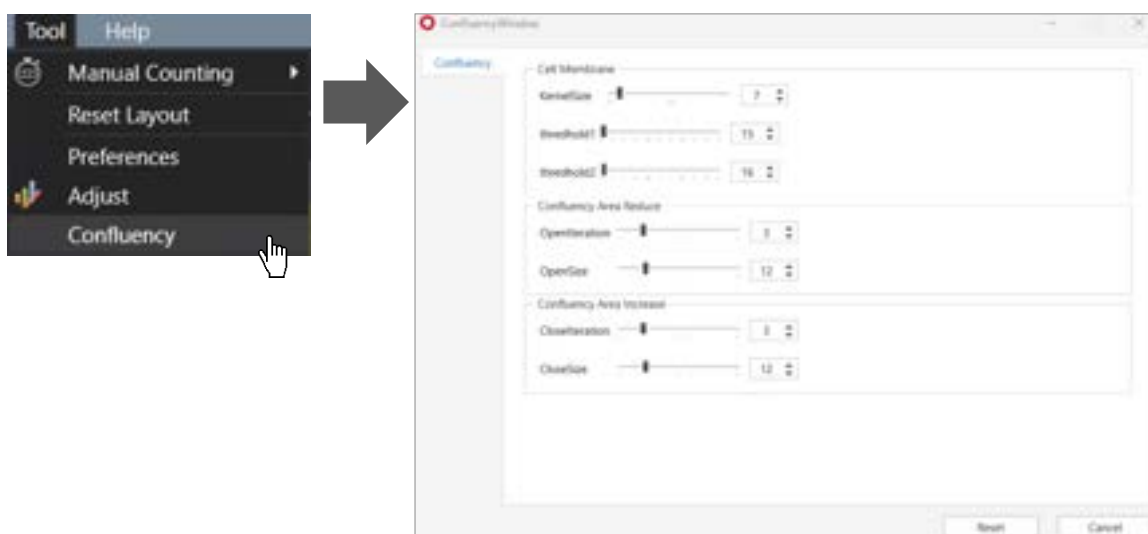
5.5 Estimate confluency (coverage)



① Open the time-lapse folder or images captured with **Celloger® Nano**.

② Select the cell shape between **Normal cell** () and **Spheroid cell** ().

③ Press **Analyze**.

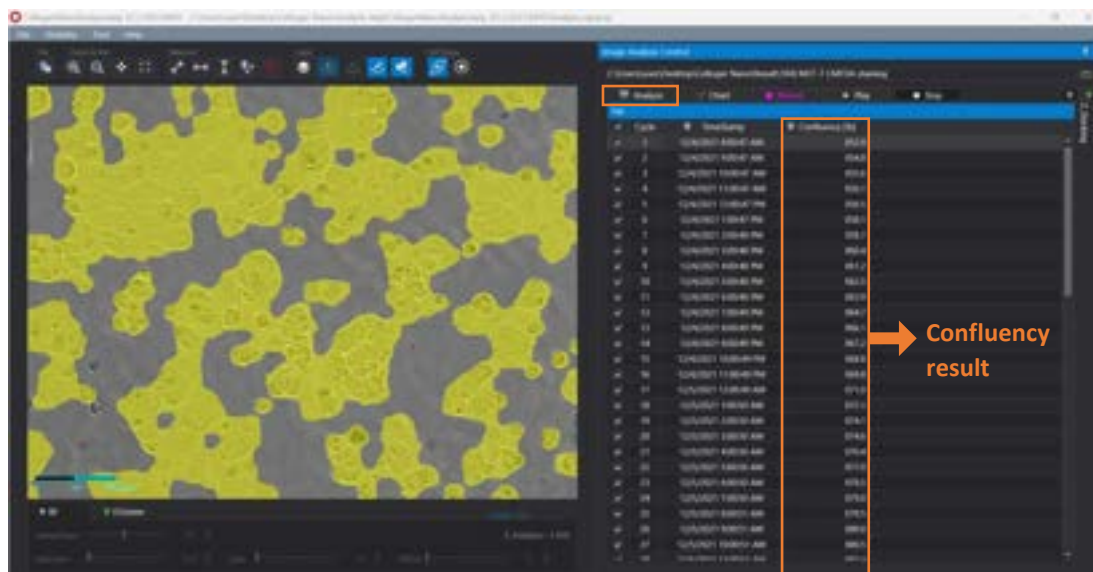
Note. You can change the confluency parameters in **Confluency** of **Tool** menu.



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

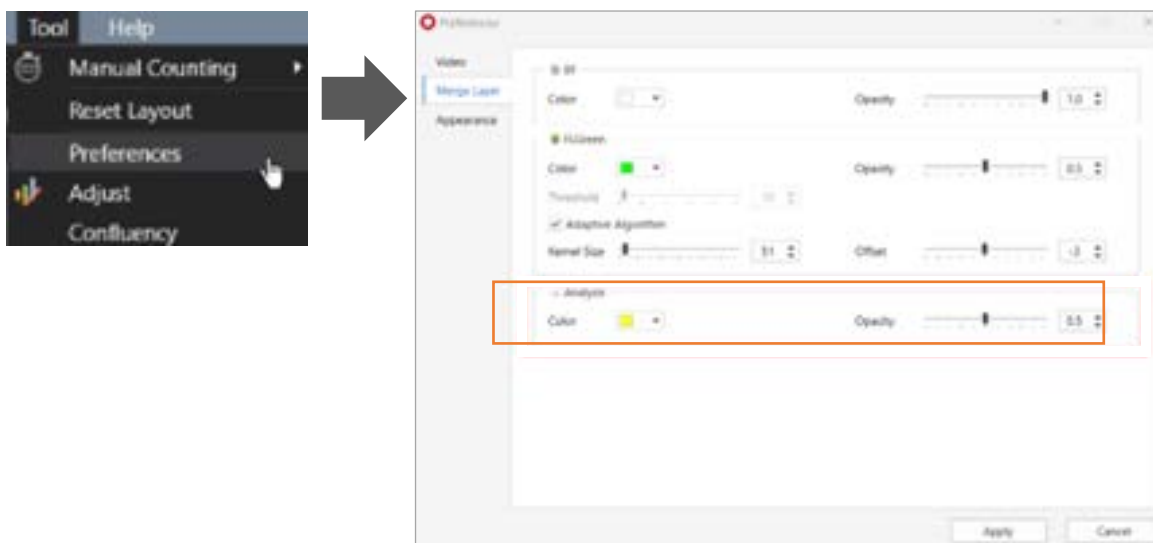
parameter. Therefore, to accurately calculate the coverage of fluorescence area, adjust the appropriate thresholding parameters (refer to p.17) before executing the coverage analysis.

- ④ The 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>

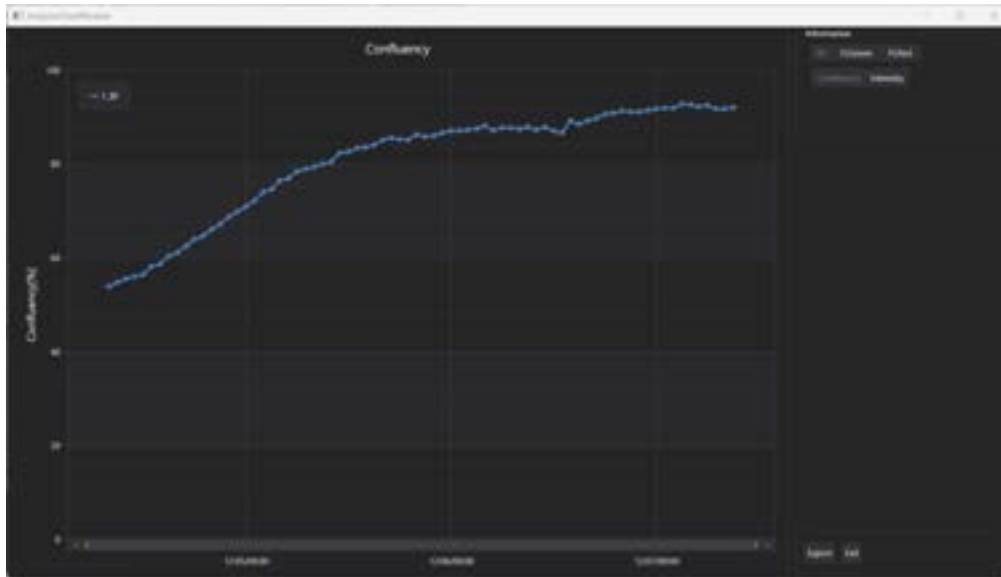
Note. If you wish to adjust the confluency masking color or transparency, select **Preferences** under the **Tool** menu, change the parameters in **Analysis** under **Merge Layer** and click **Apply**.



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

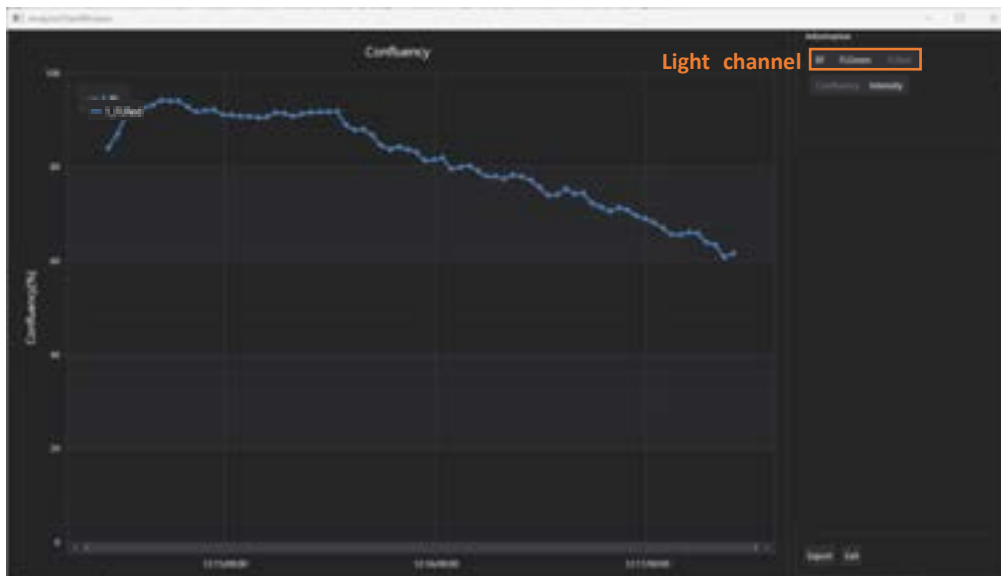
5.6 Create confluency graph

- ① Open the time-lapse folder or images captured with **Celloger Nano Scan App** and press **Analyze** to analyze the confluency.
- ② Create the confluency graph by pressing **Chart**.



<Confluency graph>

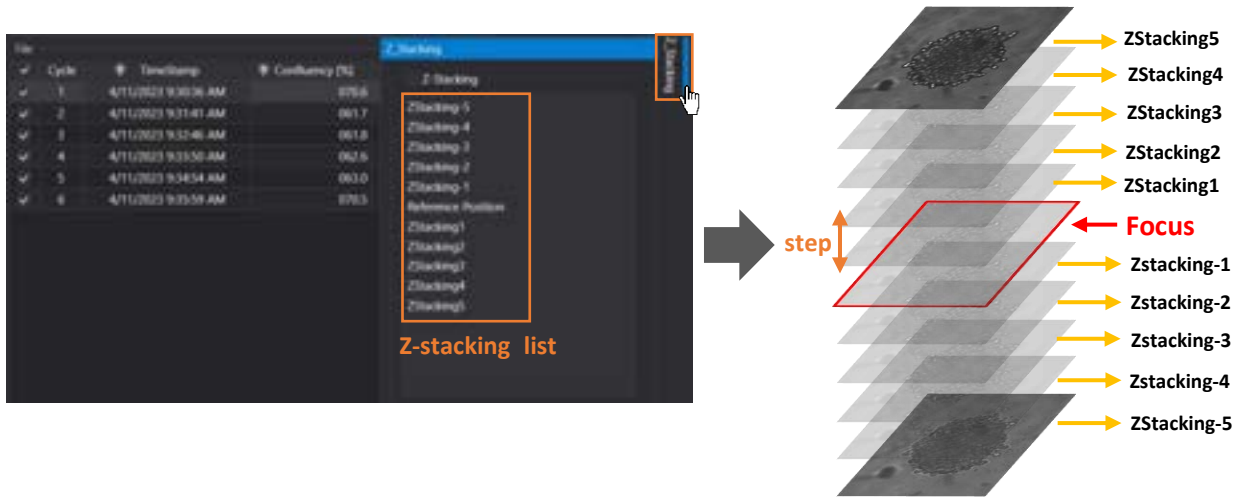
Note. Upon selecting the light channel in the information of the chart, FL green or FL red graph can also be seen.



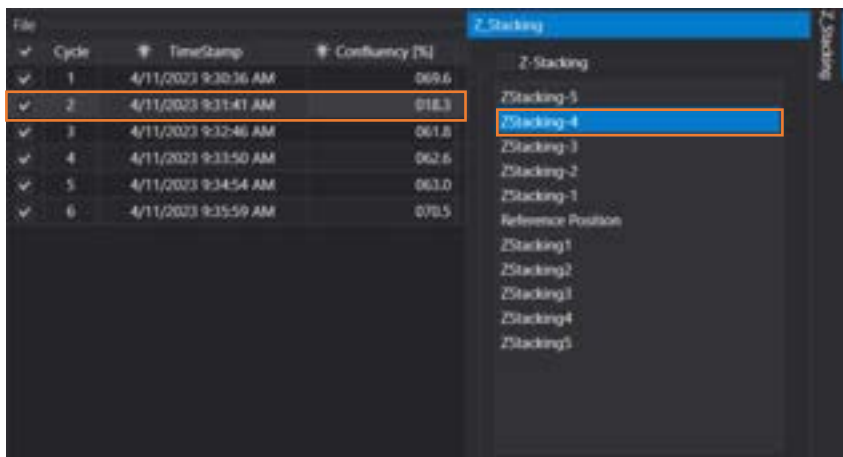
Note. Intensity graph can also be seen in **Chart**.

5.7 Show Z-stacking image

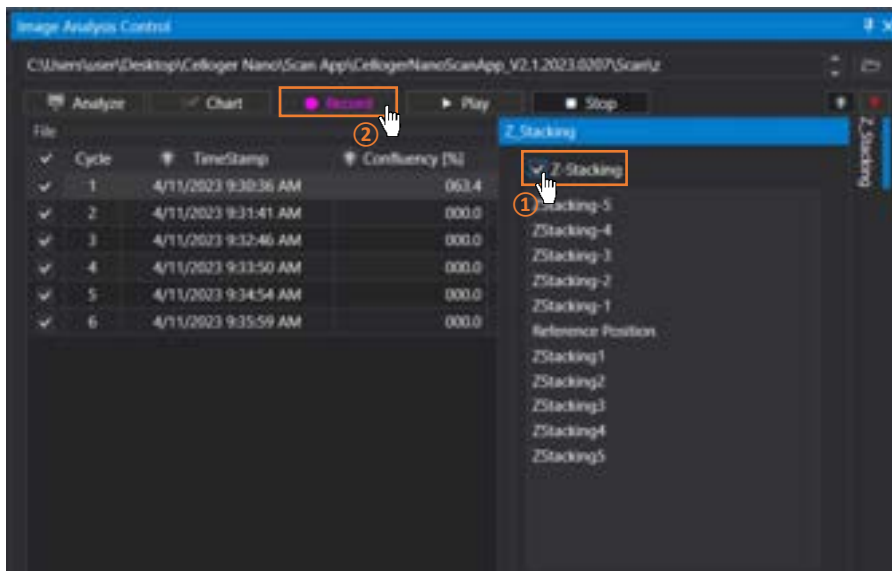
- ① Open the time-lapse folder or images captured with Z-stacking function.
- ② Click the **Z-stacking** tab to see the **Z-stacking list**. The image below shows the **Z-stacking list** when the **Range** of z-stacking function is set to (-5,5).



- ③ Select one from the **Z-stacking list** and check the image on **Display**. For example, the image below shows that the second cycle, and “ZStacking-4” is selected in the **Z-stacking list**. In this case, you can see the “ZStacking-4” image of the second cycle on **Display**. “ZStacking-4” means the image taken four **steps** below the reference position(=Focus). For instance, if the **Step** is set to 10 μm , “ZStacking-4” is taken 40 μm below the reference position.



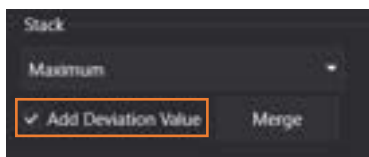
Note. If you want to make a video of the images in the **Z-stacking list**, ①check the Z-stacking checkbox above the **Z-stacking list** and ②click **Record**. The video appears in the order of the **Z-stacking list** (from low to high focus). The **FPS** of the video can be adjusted in **Properties** of **Tool** menu (p.26).



5.8 Merge Z-stacking image

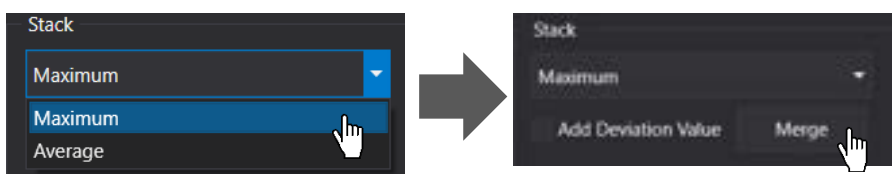
- ① Open the time-lapse folder or images captured with Z-stacking function.
- ② Click the **Z-stacking** tab (on the right side of the analysis software).
- ③ Select the merge parameters. Celloger's Z-stacking merge parameters offer two options for merging multiple images captured at different focal planes: **Maximum** and **Average**. **Maximum** is a method of merging the highest intensity value, while **Average** is a method of merging the average intensity value in each focal plane.

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



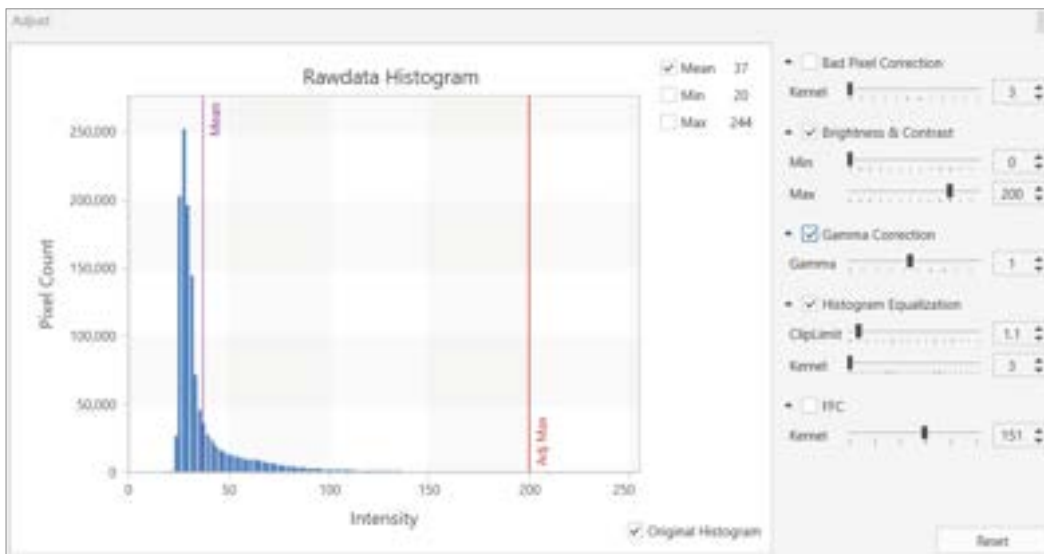
Note. This function only can be activated in the fluorescence image.

- ④ Click **Merge**. The merged image appears on **Display**.



5.9 Image adjustment

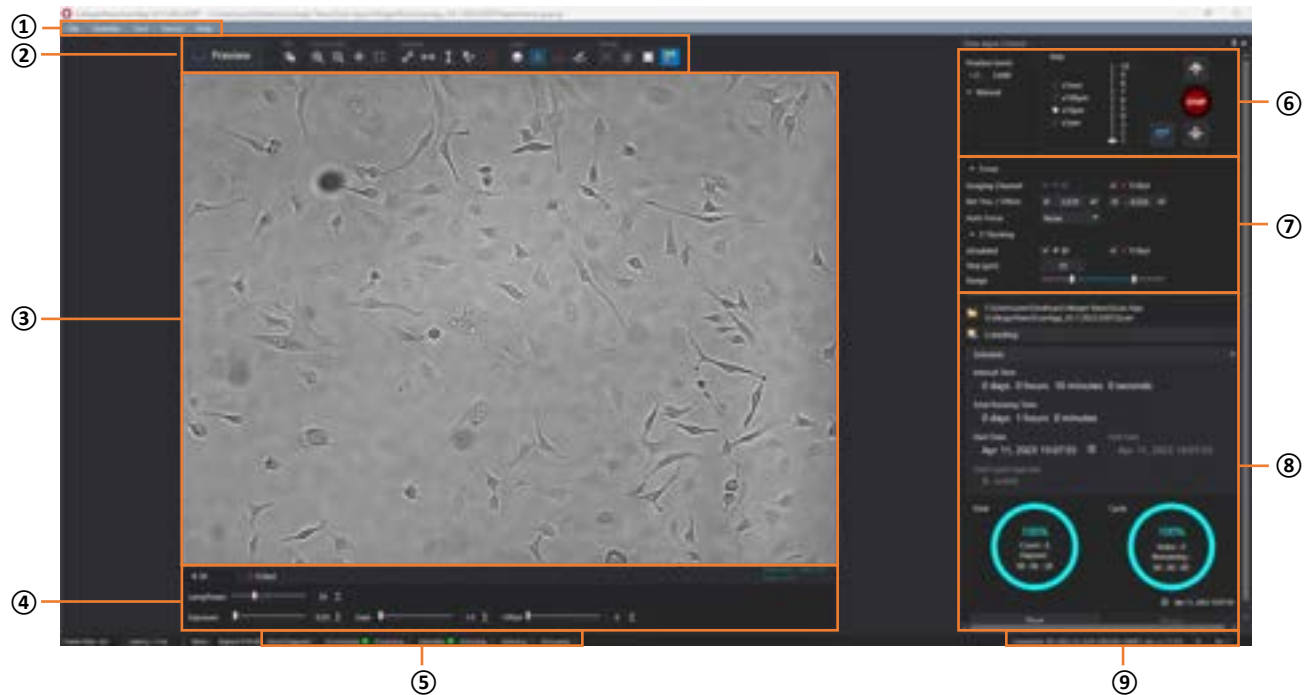
Adjust the parameters in the same way as “Image Adjustment” of **Celloger Nano Scan App** (refer to p.18) and obtain the desired image.



V. Software description

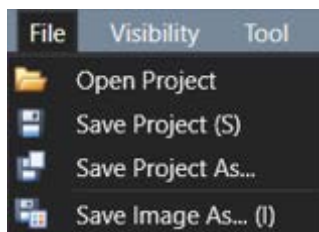
1. Celloger Nano Scan App

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



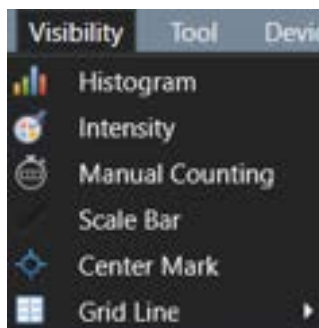
① Menu bar

[File]



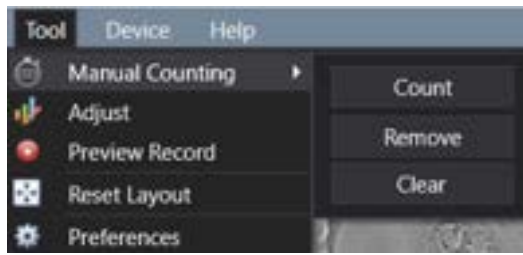
- ✓ **Open Project:** Import a previously saved project.
- ✓ **Save Project:** Save settings (Light intensity, Focus position, Schedule, etc.) on current project. Keyboard shortcut “*Ctrl + S*” can be used.
- ✓ **Save Project As...:** Create a new project with current settings.
- ✓ **Save Image As...:**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

[Visibility]



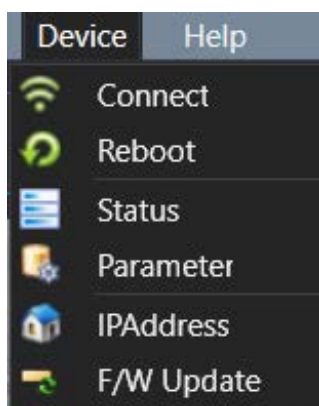
- ✓ **Histogram (p.20):** Show/hide intensity histogram of an image.
- ✓ **Intensity (p.19):** Show/hide point and mean intensity of an image.
- ✓ **Manual Counting:** Show/hide “Manual Counting” function.
- ✓ **Scale Bar:** Show/hide scale bar on the left bottom of **Display**.
- ✓ **Center Mark:** 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.21): 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.18): Adjust an image.
- ✓ **Preview Record** (p.19): Record the streaming image.
- ✓ **Reset Layout** (refer to **Note** in p.39): Return to “User Interface” before change.
- ✓ **Preferences**: Set **Auto Focus** (p.14), **Merge Layer** (p.17), **Appearance**, and **Miscellaneous** 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.
- ✓ **IPAddress**: Change the IP address of the connected device.
- ✓ **F/W Update**: Update the firmware of connected device.

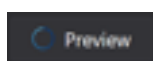
[Help]



- ✓ **About CellogerNano**: Display app information.

② Toolbox

[Preview]



: On/off the streaming. The live image is shown based on the selected channel such as brightfield (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. Drag the display in zoom-in status. It can be used by clicking and dragging with the mouse while pressing the “Shift” key.
- ✓ : 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.17).

[Device control]

- ✓ : Execute autofocusing depending on the autofocusing settings in streaming status (refer to p.14 for autofocusing).
- ✓ : Turn on the white light in LED status indicator of the connected device.
- ✓ : On/off the fan.
- ✓ : Turn on/off the LED status indicator.

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 **Cellgoer® Nano**.



- ✓ **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.

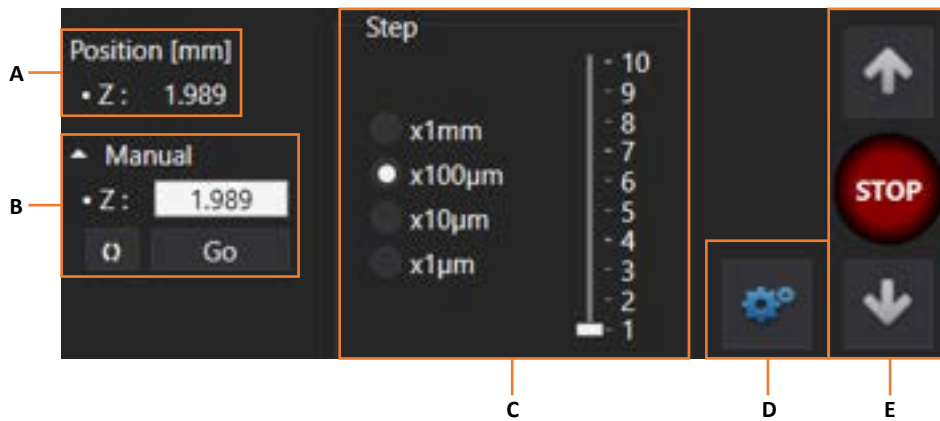
Note. For better results, it is recommended to adjust the parameters as follows. It is not recommended to increase the **LampPower** to high value as phototoxicity may occur. Moreover, it is not recommended to leave the **Preview** on state for a prolonged period as photobleaching may occur.

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 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).

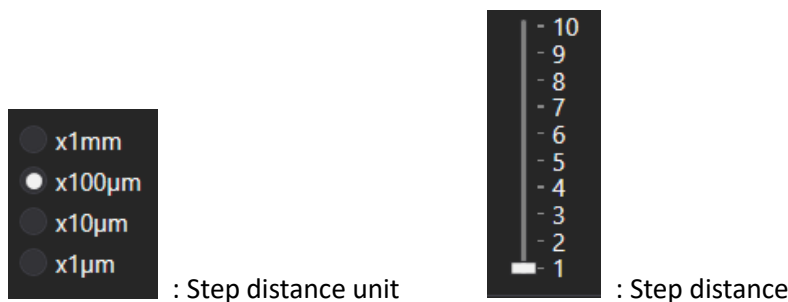
⑥ **Z-stage Control**



A. Current 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 **0**, current coordinates are entered in the boxes.


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






D. Button to activate/deactivate the Z-stage using **Jog button** (E).

✓ : Z-stage using **Jog button** activated

✓ : Z-stage using **Jog button** deactivated

Note. Upon focusing using the **Jog button (E)**, the Z-knob is deactivated. Therefore, if you want to use the Z-knob, make sure to deactivate () the **Jog button** and then use it.

E. Jog button


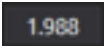


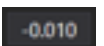

- ✓  : Move Z-stage up. Adjustments can also be done using “+” on the number pad of the keyboard when “Num Lock” is off.
- ✓  : Move Z-stage down. Adjustments can also be done using “-” on the number pad of the keyboard when “Num Lock” is off.
- ✓  : Stop the stage on move.

⑦ Position Control

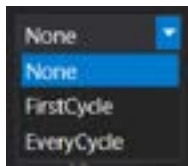
[Focus]

Select the channel used for time-lapse imaging and set the focus position (Z coordinate) for each channel.



- ✓ **Imaging Channel:** Channel used for time-lapse imaging. **BF** is always checked.
- ✓ **Ref. Pos./Offset**
 - **Ref. Pos.** (=Reference position): Position of **BF** channel for time-lapse.
 - A.**  : Upon clicking, the current coordinate is entered on the box next to it.
 - B.**  : The location of Z-axis of **BF** channel when **Auto Focus** function is off (**None**) or median coordinate when **Auto Focus** is on **First Cycle** or **Every Cycle**.
 - C.**  : 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 1.988 position.
 - **Offset** (from BF reference position): Position of **FL** channel for time-lapse.
 - D.**  : Upon clicking, the current coordinate is entered on the box next to it.
 - E.**  : The location of Z-axis of **FL** channel captured while performing time-lapse. The value shown is the difference from the location of Z-axis captured in the **BF** channel (B). To capture the **FL** channel on the same coordinates of **BF** channel, enter “0”.
 - F.**  : 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, the Z-axis of **BF** channel is 1.988, and the Z-axis of the **FL** channel is 0.010 below 1.988; as such, fluorescence imaging is performed at 1.978 (=1.988 - 0.010).


✓ **Auto Focus**



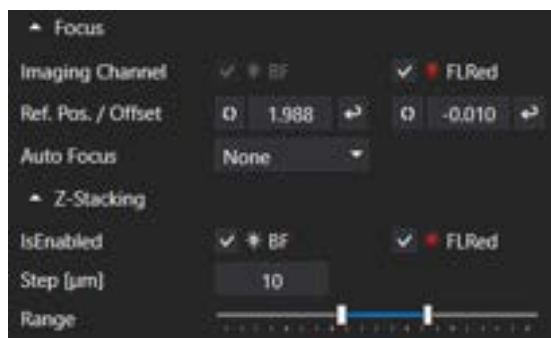
- ✓ **None:** During the time-lapse imaging, autofocusing function is not executed and captured based on the set coordinates.
- ✓ **FirstCycle:** During the time-lapse imaging, autofocusing function is executed only for the first cycle while the remaining cycles are imaged based on the Z coordinates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

[Z-Stacking]



- ✓  : Select whether or not to run the Z-stacking function in **BF** and **FL** 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 * **Steps**” µm to “10 * **Steps**” µm based on **Focus**.

<Example>




The image above is when the **Range** of Z-stacking is set to (-2, 3) and **Step** of Z-stacking is set to 10 µm. This means the images will be taken from below 20 (=2 x 10) µm to above 30 (3 x 10) µm every 10 µm based on the reference position **Focus** (BF: 1.988 mm, FL: 1.978 mm). As such, **BF** images are taken with the Z-axis of 1.968, 1.978, 1.988, 1.998, 2.008, and 2.018 mm and **FL** images are taken with the Z-axis of 1.958, 1.968, 1.978, 1.988, 1.998, and 2.008 mm.

- ✓ **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.)

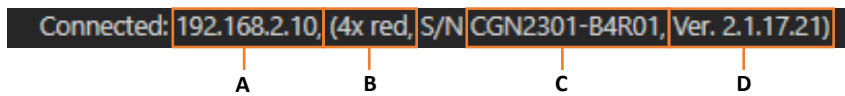
Note. The **Total Cycle Expected** is calculated by simply dividing **Total Running Time** by **Interval Time** and it does not consider the data processing time. Therefore, **Total Cycle Expected** could be different from the actual scanning cycles.

- 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 (with **Celloger® Nano**, **Index** is always "0").
- E. Current date and time
- F. Upon clicking **Start**, scanning starts based on the set schedule.

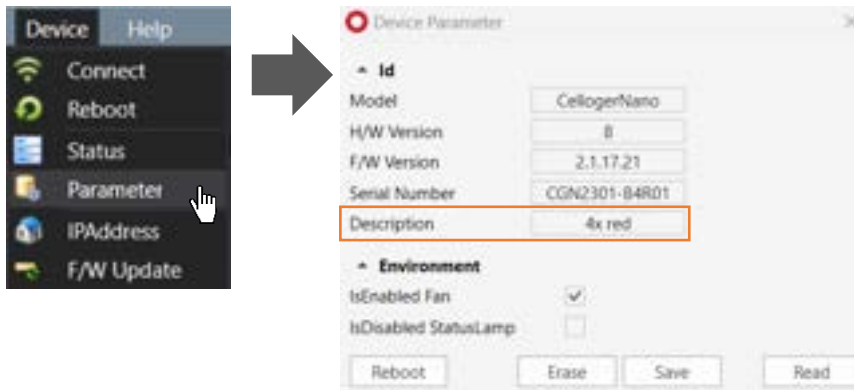
Note. Window can be separately floated so that it can fit on the monitor. It is possible to change the window by pressing  (Autohide) for floating or hiding. The layout is automatically saved upon terminating the program. To go back to the original state, press **Reset Layout** in **Tool** menu before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

⑨ Connection status

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

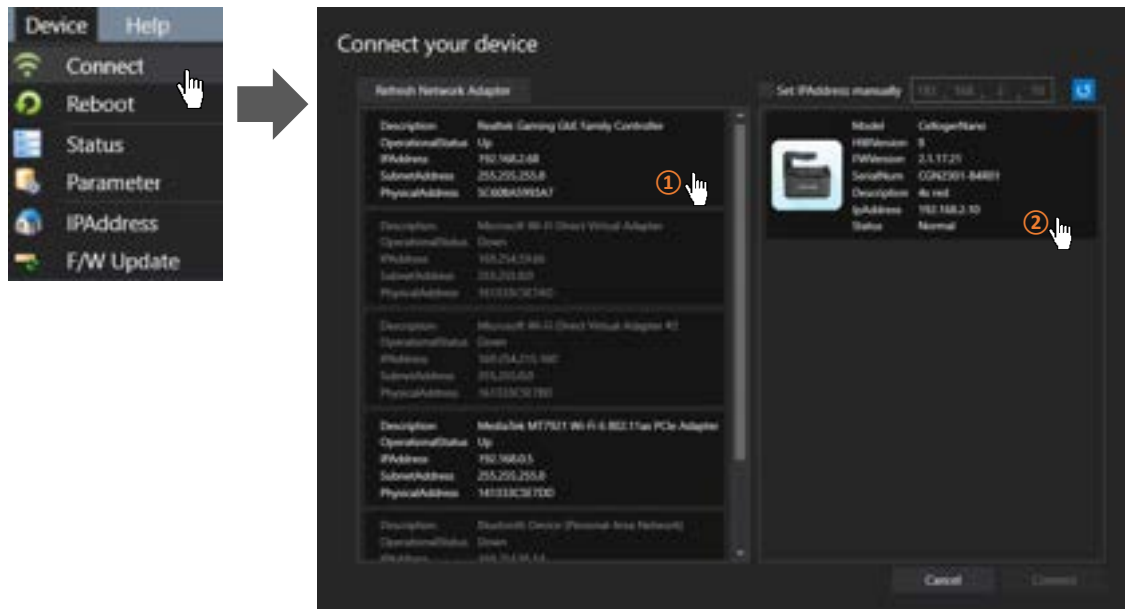


- A. IP address of connected device
- B. Description of connected device (it can be changed in **Parameter** in **Device** menu.)



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

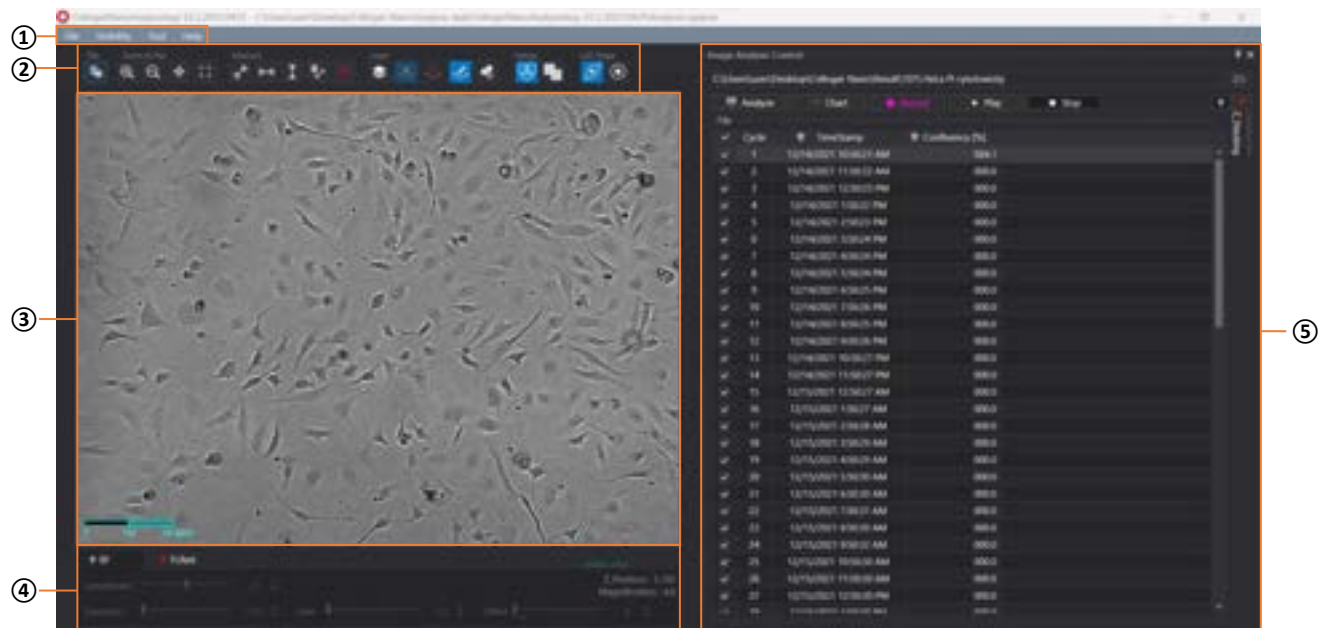
Note. If the device is not connected, **Disconnected: 0.0.0.0 Tx Rx** shows up. Click **Connect** in **Device** menu for reconnection. If the connection window does not show the device icon, refer to p.9.



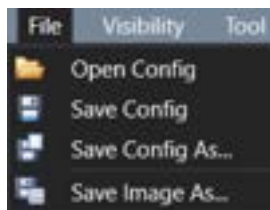
2. Celloger Nano Analysis App

Upon opening **Celloger Nano Analysis App** and selecting **Config file**, the following main page is displayed.

Note. **Config file** is a file that pops up when **Celloger Nano 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 Nano Scan App**.

② Toolbox

Except for the below functions, all other tools and functions are the same as those of **Celloger Nano Scan App**.

[Layer]



: Mask the area recognized as confluency.

[Merge]

It is a way to combine images captured from different channels, Celloger® provides two methods.



(Alpha Blending): A method of combining images with transparent effect. It is the same as the merge method of **Celloger Nano Scan App**.



(Channel Merge): A method of combining images without transparent effect.

③ Display

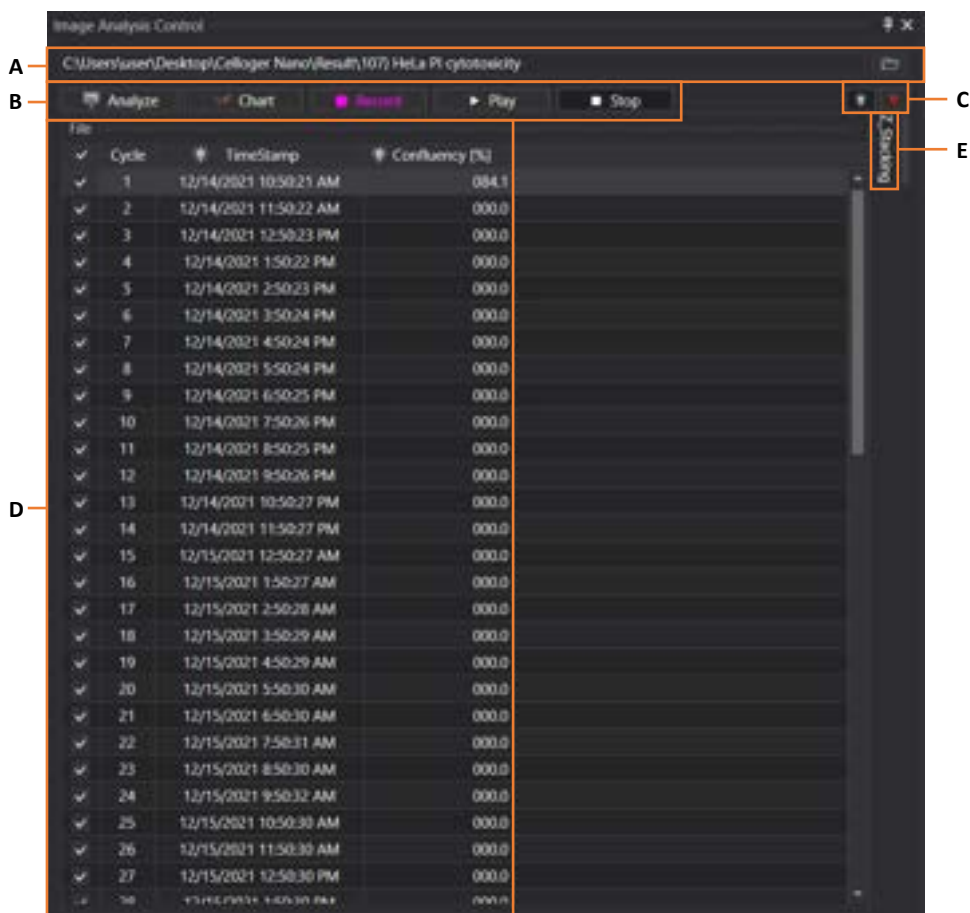
Display the image selected from **Image Analysis Control** (⑤) list. The image is displayed based on the activated channel that can be selected in **Light Source Control** (④). Upon completing analysis, coverage mask is shown and function such as measure (= **Ruler**), **Intensity**, and **Manual Counting**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

④ Light Source Control & Z coordinates

Display the light source settings and Z coordinates of **Celloger Nano 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 video (refer to p.24~31).



A. Click  to load the image file taken with **Celloger® Nano** (refer to p.24).

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 (C) (refer to p.26).
- ✓ **Chart:** Show the confluency graph and intensity graph (refer to p.28).
- ✓ **Record:** Make video (refer to p.25).
- ✓ **Play:** Show the images in the list in order (refer to p.25 for slideshow).
- ✓ **Stop:** Stop playing.

C. It is light source setting of the image taken. Once clicked, the image of selected channel appears on the display.

D. **Image list:** It is the cycles of scan position. The image of selected cycle is shown on the display. The time when the images was taken for each cycle is shown and result of confluency in percentage is shown after undergoing calculation using **Analyze**.

E. It shows the list of Z-stacking (refer to p.29).

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.

2. Operating condition

Operating power	100~240 VAC
Electrical input	24 VDC, 2.5 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 lens with organic solvents.
- Lightly wipe the working surfaces of the **Celloger® Nano** 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 alters 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. 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® Nano device

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

Focus

Focus is irregular

- ✓ Make sure the sample thickness is uniform.
- ✓ Do not touch the stage or z-knob while the imaging is in progress.

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.

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 Nano Scan App**.

Cannot analyze the confluency

- ✓ Check if the images are obtained from **Celloger Nano Scan**; Analysis can be performed only with the images obtained from **Celloger Nano 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 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.).
- ✓ 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.

IX. Product specifications

Dimension	211 x 146 x188 mm
Weight	3.2kg / 7.0lb
Objective Lens	4X / 10X
Fluorescence	Green : Excitation (470/40x) / Emission (510lp) Red: Excitation (525/30x) / Emission (570lp)
Light source	LED
Camera	1.25MP CMOS
Focus	Auto / Manual
maging positions	1
File export format	TIFF, AVI
Culture vessels	Flask, dish, well plate, slide
Operating environment	10~40°C, 20~95% humidity
Power requirements	100-240V, ~50/60Hz
Output ports	Ethernet
Accessories	PoE adapter, ethernet cable, USB memory

Warranty	1 year
Computer	External PC
O/S required	Window 10
Processor (Recommended)	CPU 3G
Storage (Recommended)	1TB
Monitor (Recommended)	1920*1080mm

Ordering information

Cat. No.	Description
CRCLG-NB02	Live cell imaging system (Bright field, 2X)
CRCLG-NB04	Live cell imaging system (Bright field, 4X)
CRCLG-NB10	Live cell imaging system (Bright field, 10X)
CRCLG-NBG04	Live cell imaging system (Bright field + Green Fluorescence, 4X)
CRCLG-NBG10	Live cell imaging system (Bright field + Green Fluorescence 10X)
CRCLG-NBR04	Live cell imaging system (Bright field + Red Fluorescence 4X)
CRCLG-NBR10	Live cell imaging system (Bright field + Red Fluorescence 10X)
CRCLG-NTS	Tablet set (PoE, Tablet, SW)

Appendix A. Connecting multiple devices

With **Celloger® Nano**, 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.

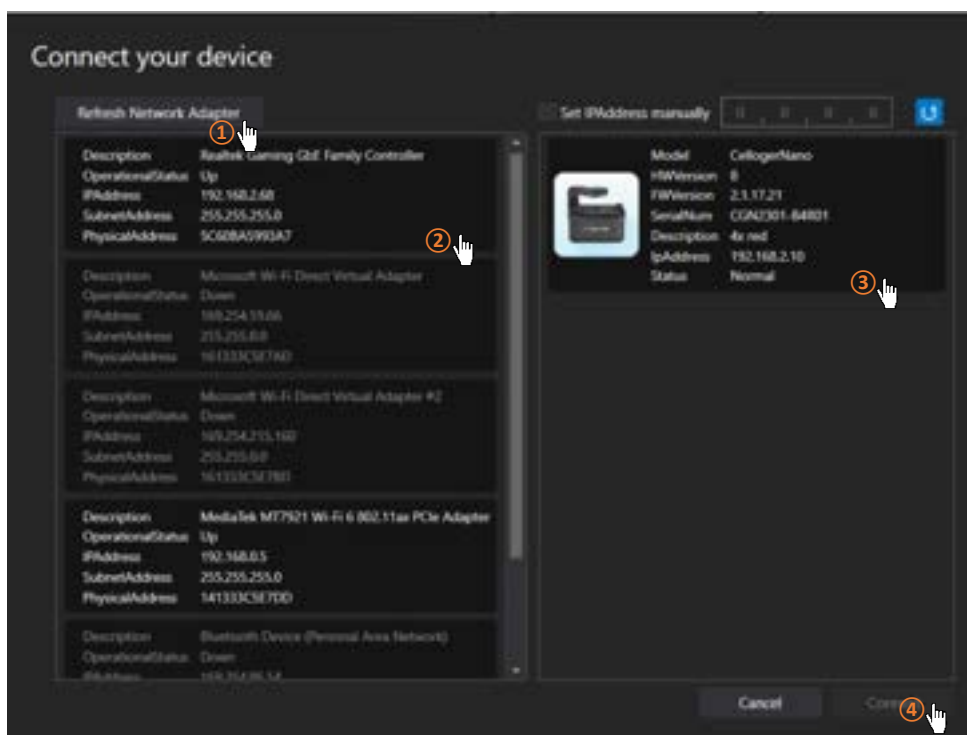
Note. Be careful as system works abnormally if the same device is connected using two programs.

1. Installation

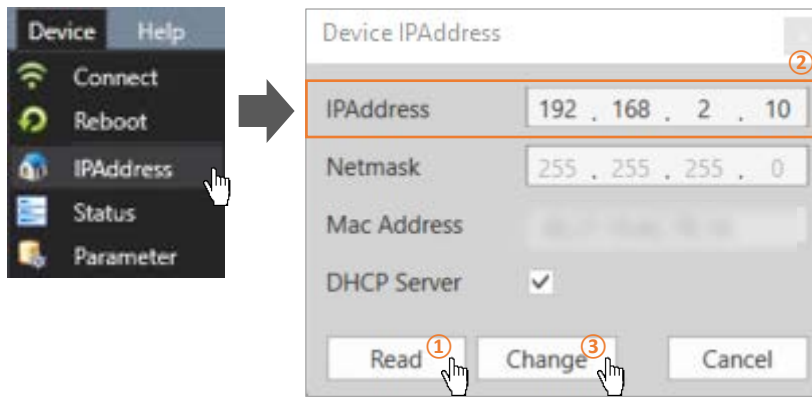
1.1 Device IP setup

Celloger® Nano 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® Nano** units with the same IP address, it is necessary to set the IP address of the devices. It is possible to change the IP address of a device in the **Celloger Nano Scan App**.

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



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



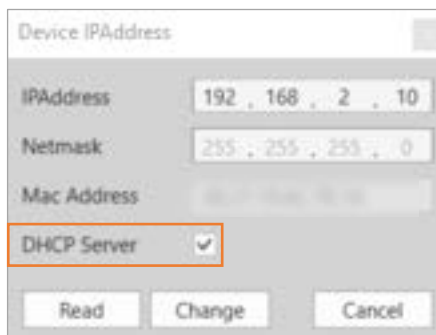
- ④ Change the IP address and click **Change** button.

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.

Ex) IP address setting for connecting 3 Celloger® Nano 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

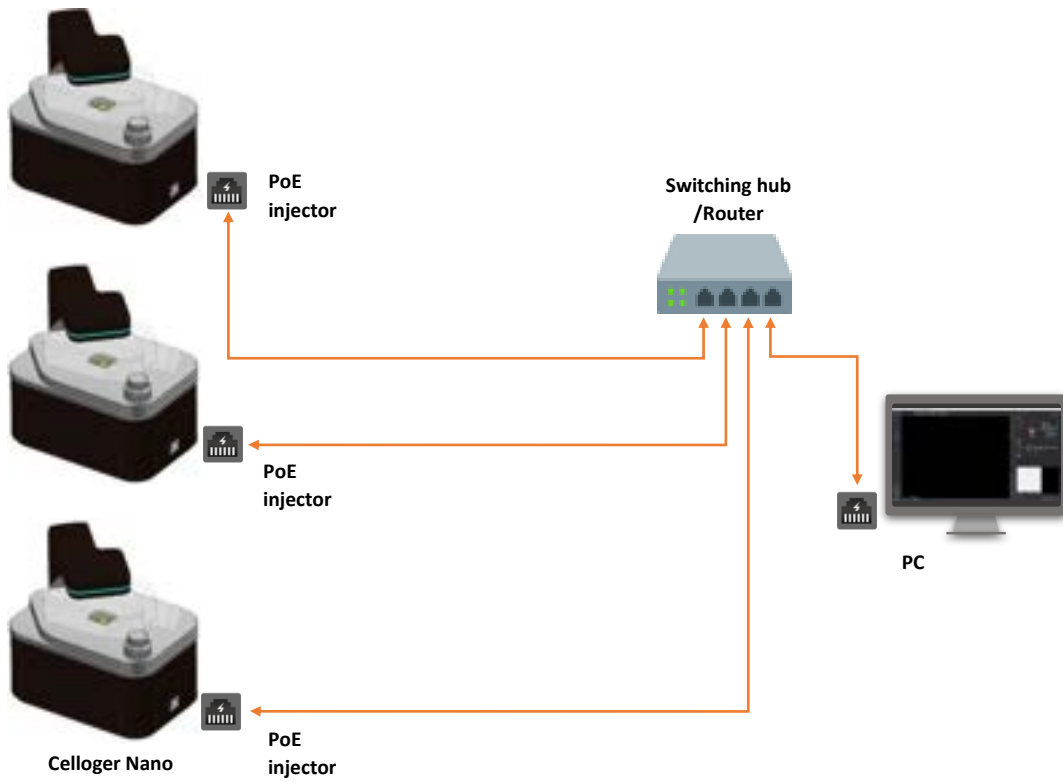
Note. When setting the IP address of the network adapter in **Celloger Nano Scan App**, only one of two or more devices must be checked in the **DHCP Server** and other devices must be unchecked. In case of setting the IP manually on PC (p.52), connection can be done regardless of the **DHCP Server**.



- 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 Nano 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.39 ⑨).
- 3) Click **Identify**() and check white LED 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.54).

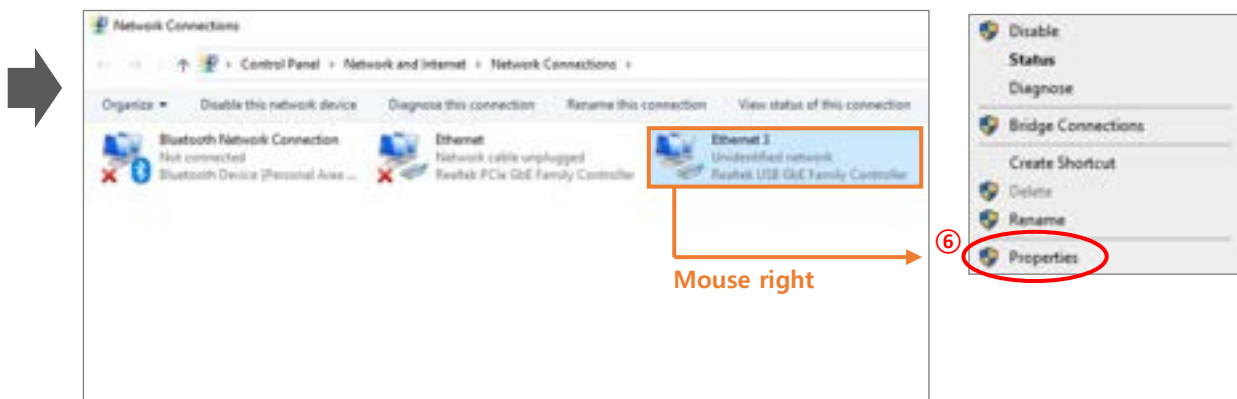
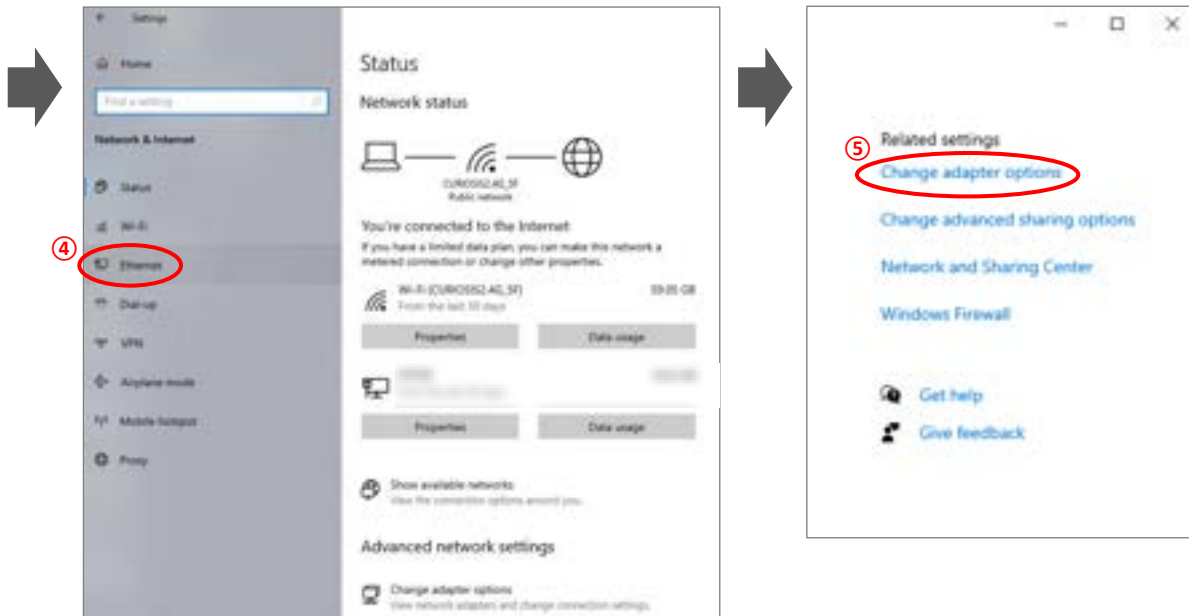
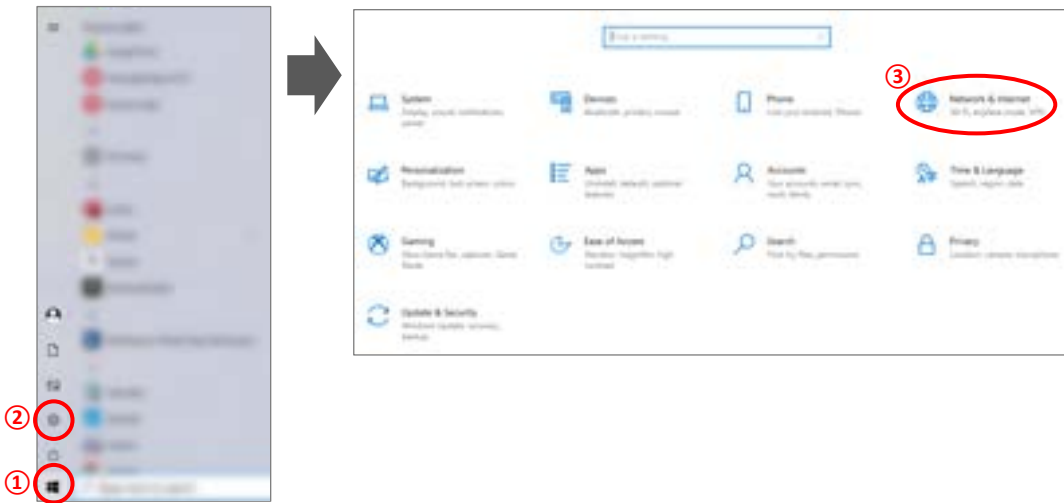
- ① Turn on the computer and click “**Start**” or “**Window**” icon.
- ② Click “**Setting**”.
- ③ Click “**Network and Internet**” in Control panel.
- ④ Click “**Ethernet**”.
- ⑤ Click “**Change adapter options**”.
- ⑥ Select “**Ethernet**” (this name can vary according to PC.) and right-click the mouse 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.

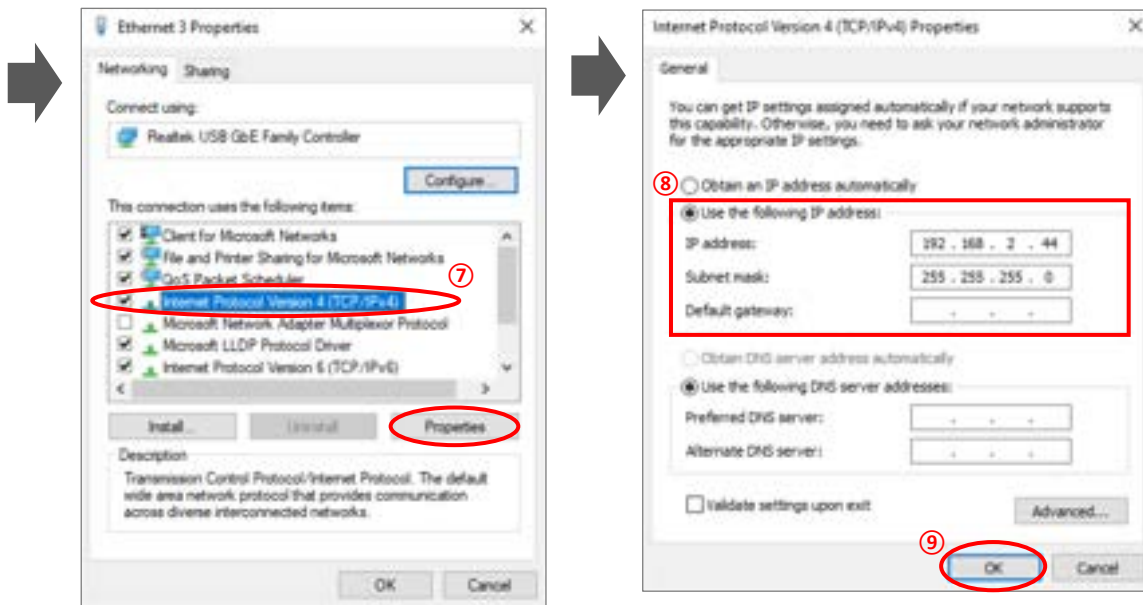
Note. Fill in any numbers from 2~254 except 10 in XX fields.

- 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 Nano Scan App**, first, connect the USB drive provided with device into the PC, then copy app folder to PC and execute “**Celloger Nano Scan App.exe**” in the app folder.

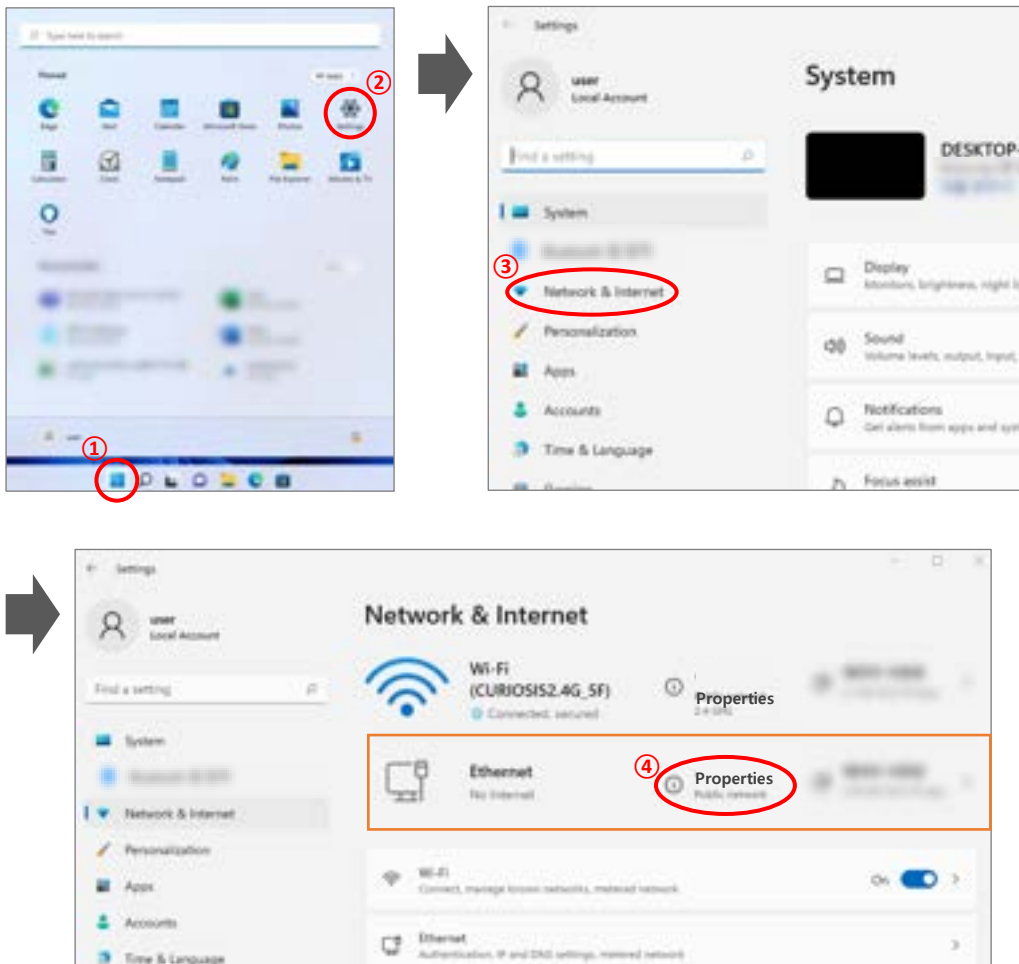
< Image Descriptions of PC network setting >

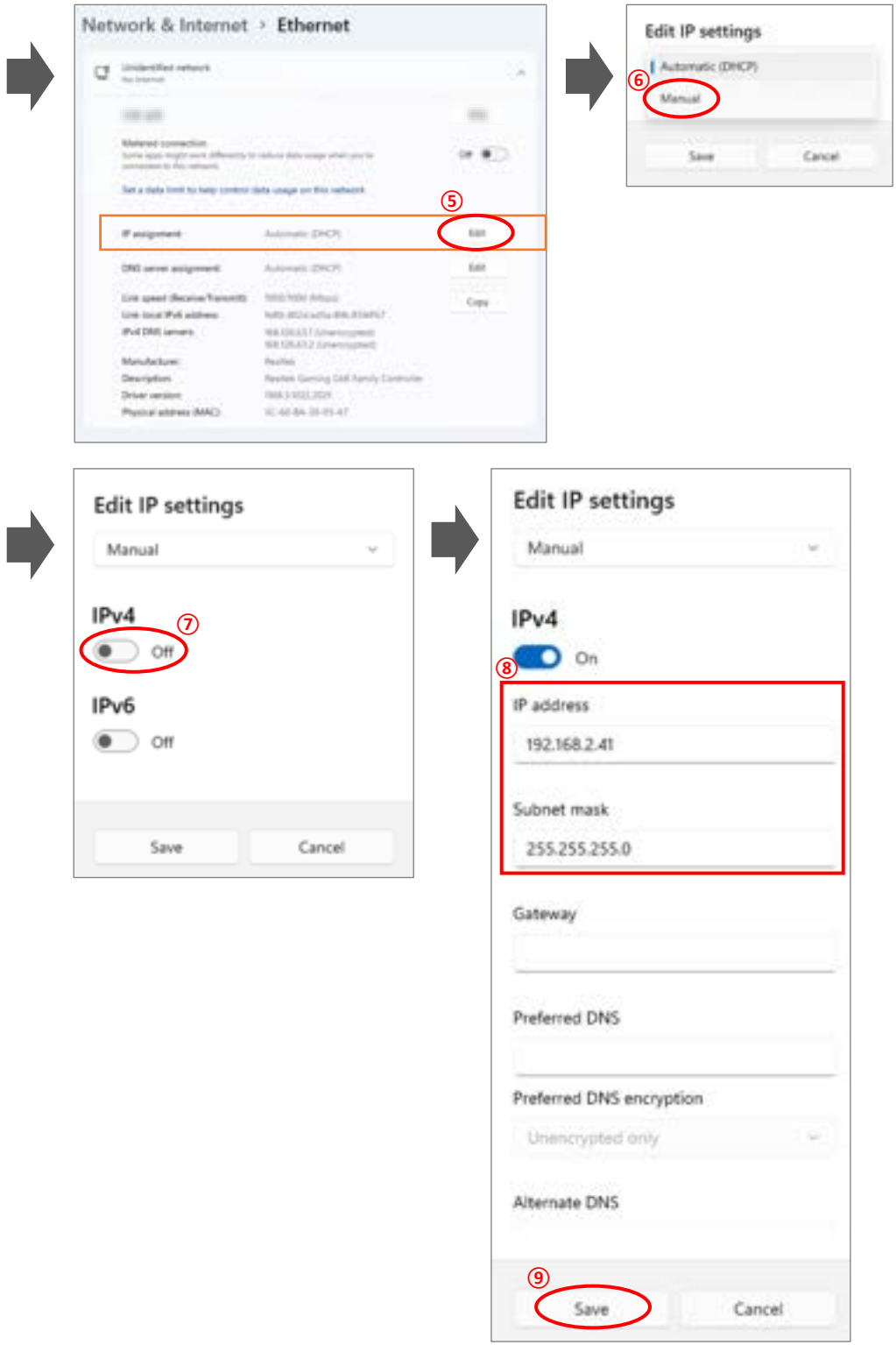
1) Window 10





2) Window 11





Revision History

Date	Version	Description
2021/08/15	CR-IFU-006(Rev.0)	Initial issue
2022/06/09	CR-IFU-006(Rev.1)	Specification modified
2023/05/18	CR-IFU-006(Rev.2)	<ul style="list-style-type: none">- Added contents (Image adjustment, Preview record, Z-stacking)- Revised contents (Table of contents, Device Layout, Getting started, Chart, Merge image, Trouble shooting, Appendix B, Product specification)- Revised UI (Celloger Nano Analysis App)



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