RWD

RWD Life Science Co.,Ltd

C200FL
Fluorescent Automated Cell Counter

Инструкция по эксплуатации A

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Preface

First of all, thank you for choosing the fluorescent automated cell counter RWD!
Before installing and using this product, make sure that you read all the attached schedules carefully for better future reference.
RWD is committed to continuously improving product functions and service quality, and reserves the right to change any products described in this user manual and the contents of this user manual without prior notice.
If the latest product information is needed, please feel free to contact us or visit our website (*www.rwdstco.com*). In the case of inconsistency between the actual condition and this user manual in the application of the instrument, we appreciate your comments about this user manual. Please contact RWD for any further questions.
This user manual is applicable to the follow product manufactured by RWD：

■C200FL Fluorescent Automated Cell Counter

This device should be operated and managed by trained professionals!

1- Overview

In cell culture, it is often necessary to understand the living state of cells and identify whether cells are live or dead, determine the inoculation concentration and quantity of cells, and understand the survival rate and proliferation degree of cells. In some more complex cell experiments or samples, it is also necessary to distinguish and count a particular type of cells with the help of fluorescence. For example, the number of cells expressing GFP fluorescence after successful transfection is counted in the transfection experiment.
The fluorescent automated cell counter can detect the concentration of cells in the sample by collecting and analyzing the image of cells in the slide using a camera. After trypan blue staining, the proportion of live cells can be calculated, and specific fluorescence analyses can be performed by fluorescence counting.
The device is suitable for accurate measurement and further fluorescence analysis of the cell number and the cell viability of cell lines, stem cells and primary cells, which solves the problems of time and labor consumption and poor accuracy of the traditional manual counting.

1.1 Advantages

- Focusing-free design and more stable imaging
- The preset parameter can be directly called without manual intervention, with high repeatability
- Options from 1-3 views, fast and accurate counting
- The self-developed algorithm is accurate, and the agglomerated cells can be

intelligently recognized.

- High-performance fluorescent components, clearer fluorescent imaging
- App type counting program, grouped management, and one-click call
- 6-channel slide, one-click automatic sample feeding
- 500 G storage, one-click export of concentration/viability/agglomeration rate/growth curve
- The system design complies with FDA 21CFR PART11

1.2 Requirements for instrument environment

Please prepare the operating environment as the conditions listed in the table below to ensure the operability and safety of the device.

	Description
Operating environment	Temperature: 10℃~40℃
	Humidity: 10%~75% (non-condensing)
	Altitude: <2000 m
Storage and transport temperature	-20℃~60℃
Thermal operation	≤40℃ 75%RH (non-condensing)
Thermal environment storage and transportation	≤40℃ 93%RH (non-condensing)
Storage environment	Temperature: -20℃~60℃
	Humidity: 10%~93% (non-condensing)
	Altitude: <2000 m
Operating voltage	1) AC100V-240V, 50/60Hz 2) DC24V, 152.4VA

1.3 Product parameters

Parameters	**Description**
Length ×width ×height	254 mm×303 mm×453 mm
Weight	≤13kg
Screen size/type	10.1-inch LVDS touch screen
Screen resolution	1280×800
Imaging magnification	5×optical magnification
Imaging pixel	≥1.4 megapixels
Optical resolution	5 μm
Counting area	1.72 mm×1.3 mm

1.4 Product list

Note: *This product list is for reference only due to the differences between the different versions of the Manual. When receiving the goods, please compare the delivered parts with the accompanying packing list. If any inconsistency is found, please contact the after-sales service personnel of RWD immediately.*

Attachment	**Name**	**Quantity**	**Description**
Standard	Main unit	1	Cell counting
Standard	Power cord	1	Device power supply
Standard	Power adapter	1	Device power supply
Standard	Cell slide	1	50 pcs/box
Standard	USB flash drive	1	Data import/export, software upgrade (only support FAT32 U disk)
Standard	AO/PI dye	1	1 mL, premix dye Uniformly mix with a cell suspension in a ratio of 1:1 when in use
Standard	Trypan blue dye	1	1 mL, mix with cell suspension at 1:1 when used

1.5 Intended use

The intelligent fluorescent automated cell counter C200FL manufactured by RWD is a cell analysis system that combines intelligent image recognition and advanced optical imaging technology for accurate counting. The self-developed intelligent algorithm can effectively recognize live and dead cells, and achieve 5s rapid imaging and accurate counting. App type counting program can be called with one click, the samples can be automatically loaded and counted, and the total number of cells, viability, agglomeration rate, growth curve and other information can be obtained with one click. It can be used in combination with multiple fluorescence channels to perform fluorescence analysis on cells in suspension, and simultaneously display bright field (BF) and fluorescence images, clearly presenting the count results and cell morphology.

The data management and control capabilities of the C200FL software system are fully compliant with FDA 21 CFR Part 11 and are suitable for cell analysis in the fields of cell therapy, bio-pharmaceuticals, immunology and vaccine development, oncology research, stem cell and metabolic research.

2- Important information and security

2.1 Safety symbols

Hazards in flammable environment
Do not operate the device in the presence of flammable gases.

Electromagnetic interference hazards
To avoid the danger of device fault, always operate the device in a controlled electromagnetic environment.

Radiation hazards
When handling radioactive samples, follow all applicable procedures for radiation safety. When handling radioactive contaminants, take proper disinfection and safety measures. Wear protective clothing (e.g., particle masks, gloves, and shoe covers) in accordance with the rules and regulations of the respective laboratory for handling radioactive contaminants. Radioactive contaminants must be disposed of in accordance with the relevant regulations.

Biological infection hazard
The samples used during the expected operation of the instrument may be infectious. Therefore, it is recommended to comply with the general laboratory regulations on infection control procedures. For information on decontamination media, their use, dilution, and effective application range, refer to the latest Laboratory Biosafety Manual of the World Health Organization (WHO). Pleae comply with all applicable safety procedures when disposing infectious samples. Make sure that appropriate disinfection and safety measures are taken when disposing infectious materials. Make sure that protective clothing (e.g., particulate masks, gloves, and protective shoe covers) is always worn according to the infection control procedures of respective laboratories. Infectious waste must be disposed of in accordance with corresponding regulations.

Waste disposal
All debris, waste, infectious and radioactive contaminants generated during the operation must be disposed of in accordance with the appropriate laboratory regulations. Disinfectants, cleaning solutions and section waste must be disposed of in accordance with the special waste disposal regulations! Reagents must be disposed of in accordance with the manufacturer's safety data sheet.

2.2 Safety Note

2.2.1 General safety

Individuals shall be trained by professionals before using this instrument. This instrument is only allowed to be operated within its application range, and only components and accessories suitable for this instrument shall be used. Damage to the instrument or injury to individuals

including the third party caused by nonconforming procedures is out of the extent of liabilities of RWD. Any changes not authorized by the manufacturer may cause operational failure of the instrument.

2.2.2 Electrical safety

In order to safely use the built-in electrical appliances, the following provisions shall be observed:

a) Before connecting the main power supply of the instrument, check whether the running voltage settings of all components are correct. A power supply must be chosen for the instrument according to the model label of the instrument. If you are unsure of the model of the power supply, please contact the authoritative power supplier or local power company for consultation.
b) Use a standard grounded three-prong outlet made in China to connect the instrument with the peripheral equipment, and use a grounded branch power supply with fuse. Make sure that the peripheral equipment is connected with the protective ground lead like this instrument, and that the ground lead is unblocked. If the fuse is missing or not installed, or the ground lead is not connected, there may be an electric shock hazard.
c) Make sure that the power cord is clean.
d) Once a potential unsafe operation is detected, immediately power off the instrument and pull out the plug to isolate it from the main power supply.
e) If liquid comes into contact with the circuit of the instrument, immediately power off the instrument, pull out the plug to isolate it from the main power supply, and wipe up the dipped part of the instrument in time.
f) Make sure that the operating board is dry during the operation of the instrument.
g) Types of fuse (actual voltage, actual current and model) must comply with the manufacturer's regulations. Do not use a second-hand fuse, and the fuse line cannot be short circuit.
h) The instrument must be grounded to prevent accidental electric shock.

2.2.3 Chemical waste safety

Caution! *Hazardous waste. Refer to the Safety Data Sheet (SDS) and local regulatory requirements when handling and disposing of waste.*

To minimize the risk of chemical waste:

a) Before storage, handling or disposal of chemical waste, read and understand the SDS in the waste container provided by the chemical manufacturer.
b) Provide primary and secondary containers for waste. (Primary containers are used to directly store generated wastes. The secondary containers are used to store waste spilled or leaked from the primary containers. Both containers must be compatible with the chemical characteristics of the waste and must comply with local regulations for waste storage.)
c) Try to avoid exposure to chemicals. Wear appropriate personal protection products (e.g. safety glasses, gloves or protective clothing) when handling chemicals. Refer to the SDS for detailed safety guidance.

d) Minimize the risks of inhaled chemicals. Do not open containers for chemicals. Use chemicals only in well-ventilated environments (e.g. fume hoods). Refer to the SDS for detailed safety guidance.
e) Handle chemical waste in a well-ventilated environment.
f) After emptying the waste container, seal the container with the included cover.
g) Waste trays and bottles should be discarded in accordance with good laboratory practices and local environmental and health regulations.

2.2.4 Biosafety

Biological samples (such as tissue, body fluid and blood samples from humans and other animals) have the potential to spread infectious diseases. All applicable local regulations should be complied with. Wear appropriate protective glasses, protective clothing and gloves.

3- Introduction to product structure

3.1 Cell counter main unit

Figure 3-1

Figure 3-2

Figure 3-3

No.	Component/interface	Description
1	System switch button (with status indicator)	Turn on/off (**long press for off**) cell counting system Status indicator: ● Orange - Device powered up, counting system not turned on ● Green - Counting system in operation
2	Slide socket	To insert slide
3	Power switch	Device power on/off
4/5/6	USB port	3 USB 3.0 ports. Can be used for USB storage, upgrades, mouse and keyboard connections (only support FAT32 USB)
7	Network port	Connect to the internet
8	HDMI interface	Connect HD display
9	Locking handle	Figure 3-3 shows the unlocked state, rotate to the opposite direction to lock. Please make sure to tighten handle when transporting the device
10	Power interface	Connect the power adapter

3.2 Cell slide

Note: *For best results, do not touch the optical surfaces of the cell slide and wear gloves when handling samples.*

Note: *Reuse of disposable slides may affect count results.*

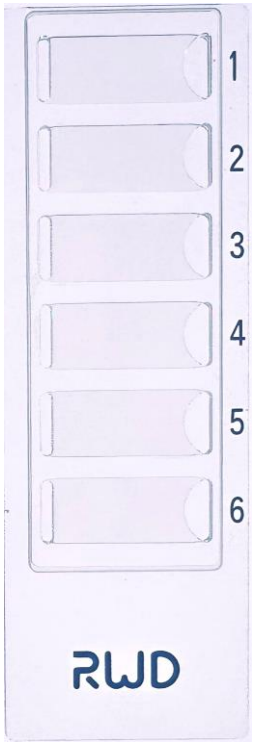


Figure 3-4

4- Preparation before operation

4.1 Sample preparation

For best results, follow these recommendations:

1) Before aspirating the cell sample, it is necessary to ensure that the cell suspension is evenly mixed. After the cell sample has been standing for a long time, it is needed to gently flick the centrifuge tube or gently pipette the cell suspension. It is generally recommended to aspirate the sample from the middle.
2) The detection range is 1×10^4-3×10^7 cells/mL, with an optimal range of 1×10^5-4×10^6 cells/mL.
3) To obtain accurate analysis results of cell viability, ensure that the counting area is covered with cell suspension and count cells immediately after staining according to the protocol.
4) Do not touch the optical surface of the slide. Hold the edge of the slide.
5) Be careful not to form bubbles in the sample.
6) Minimize debris in cell samples. Regardless of the counting or staining method used, it is difficult to count and assess viability of the samples containing large amounts of debris. Debris may come from trypan blue dye or cell samples. Possible handling methods for different sources of debris:
    - Centrifuge the trypan blue dye and harvest the supernatant.
    - Centrifuge and resuspend the cells.

4.2 Staining method

Tips: If you only need to count the total concentration, staining is not needed but directly load the sample.

4.2.1 Trypan blue staining

Recommended staining methods:
Mix 10μL of cell suspension with 10 μL of trypan blue and load 10μL onto the slide.

4.2.2 AO/PI staining

Recommended staining methods:
Mix 10μL of cell suspension with 10 μL AOPI dye and load 10μL onto the slide.

4.3 Slide loading

1) Pipette and mix the sample with a pipette, and load the sample into the chamber of the slide.
2) Allow the sample mixture to stand in the slide for 30 s.

5- Startup and login

Note: *Before the first use, please turn the "Locking Handle" at the bottom of the machine to the unlocking position on the left，show as figure 5-1..*

Figure 5-1

When the device is properly connected, press the power switch to power on the device, and then click the system switch button to turn on the cell counter. After the system initialization is completed, automatically enter the login page, as shown in Figure 5-1.
Click "Language" here or Refer to Section 8.10 for switching the system language.
Initial login account of the device: Admin Password: RWD2002

Note: *The system will force the user to modify the initial password when logging in for the first time. Please refer to* ***Section 8.7 Change Password****.*

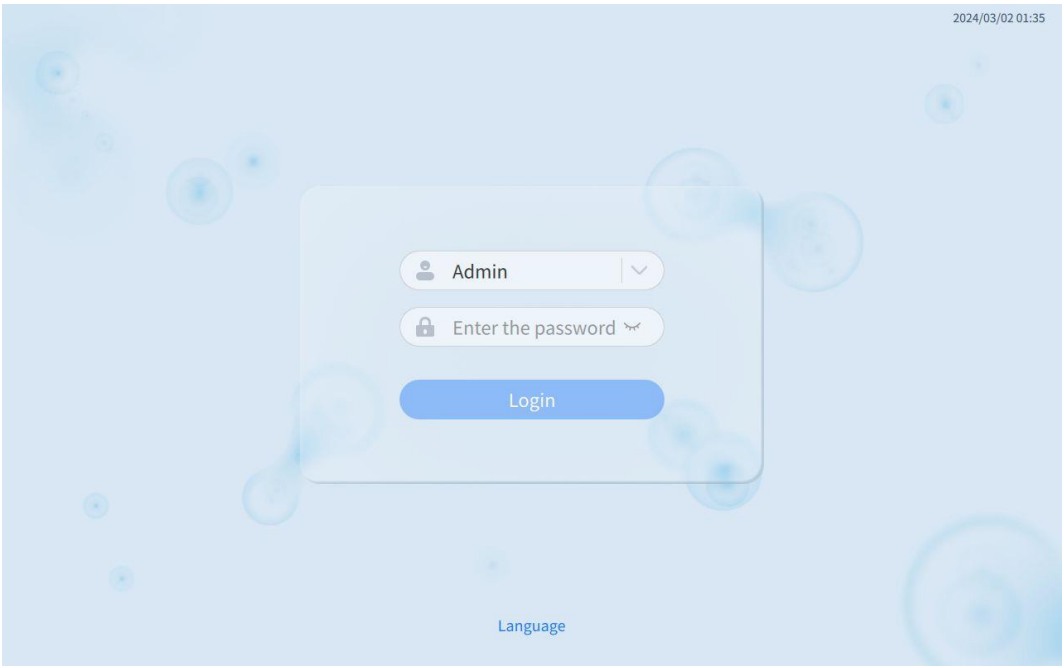


Figure 5-2

After entering the correct account and password, click [Login] to enter the main interface as shown in Figure 6-1.
The system consists of three main modules: Count, data, and setting.
Enter the [Count] module by default.
Note: *The interface images and parameters in this manual are for example only, and the display during actual operation should prevail.*

6- Counting

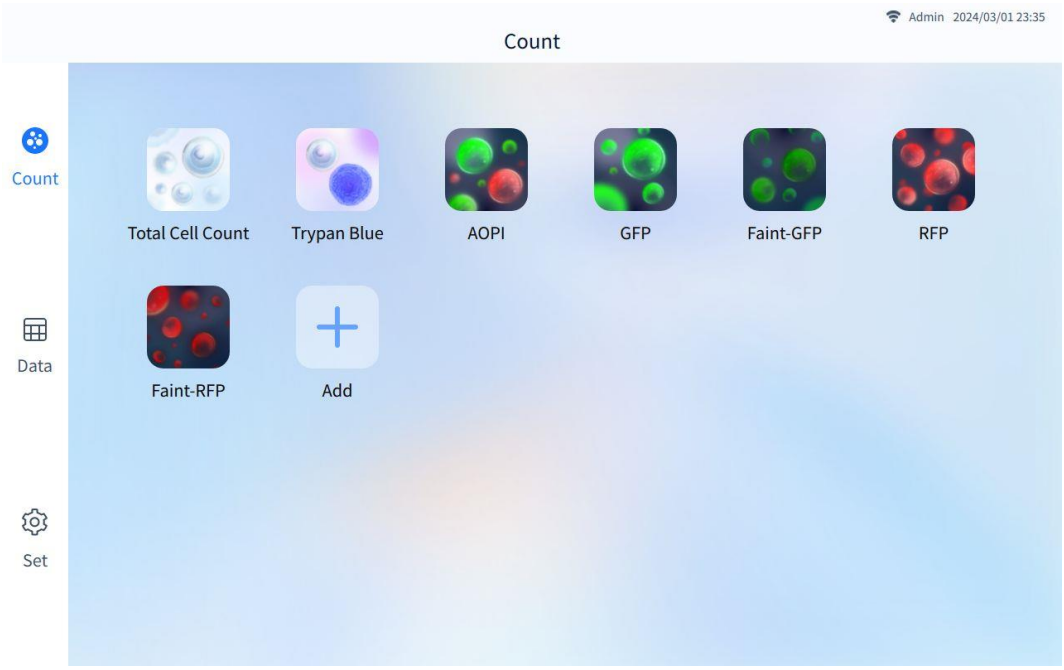


Figure 6-1

6.1 Fast counting

As shown in Figure 6-1, seven commonly used count types are preset in the [Count] interface: Total cell count, trypan blue, AOPI, GFP transfection rate, faint-GFP transfection rate, RFP transfection rate, faint-RFP transfection rate.

Select the count type, and “Please insert slide” will pop-up, as shown in Figure 6-2.

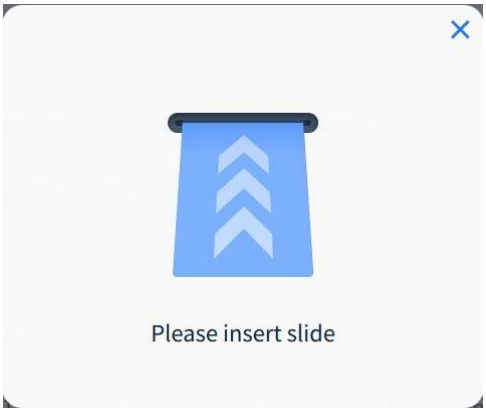


Figure 6-2

Insert the slide according to the prompt, and “Edit Counting Information” will pop-up, as shown in Figure 6-3.

This interface displays the current count type and experiment name. CH1 editable counting information by default, including sample name, cell t ype and dilution factor.

Click to cancel the selection of CH1 channel and then light up other channels as you need. When CH3 is selected, editable CH3 counting information will be displayed synchronously. Refer to Figure 6-3.

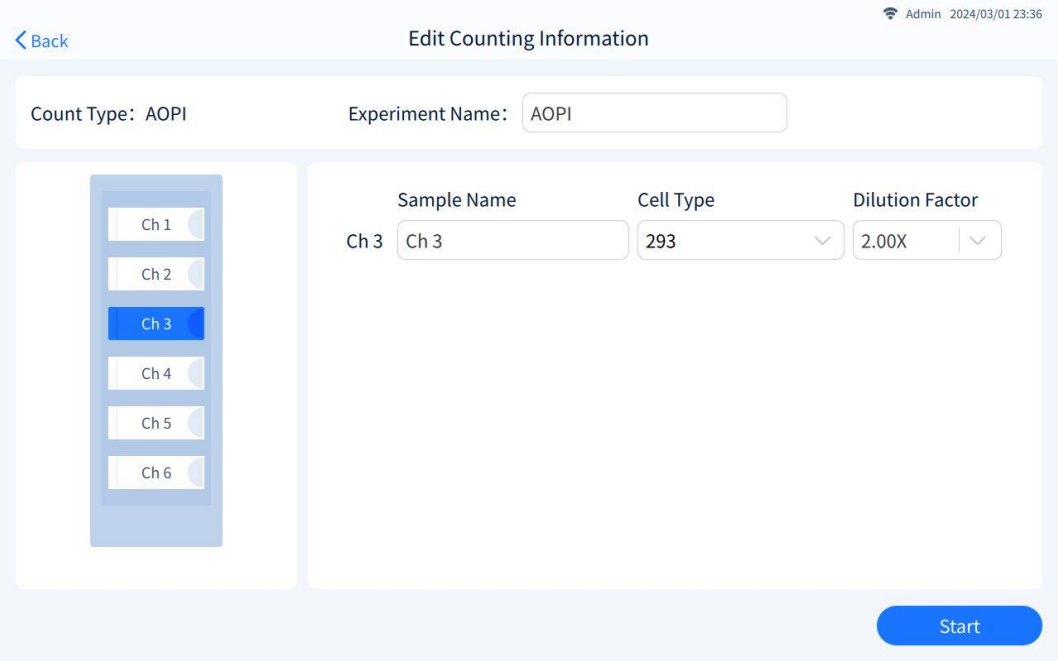


Figure 6-3

The number of the sample chamber on the slide corresponds to the number of the interface channel, and the slide is inserted as indicated by the red arrow.

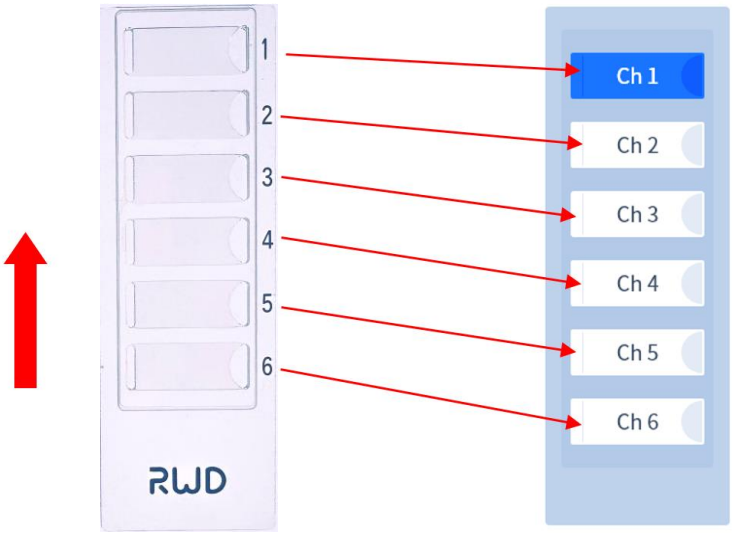


Figure 6-4

1) Experiment Name/Sample Name: User-defined, 0-20 characters.
2) Cell Type: Click the drop-down arrow to select all preset cell types under this count type. To add cell type, refer to Section 8.2.
3) Dilution Factor: Click the drop-down arrow to select the preset dilution factor (1.00X, 2.00X, 10.00X, and 100.00X), or click the box to enter the dilution factor on the numeric keypad, range: 1.00 ~ 10000.00.

After the counting information is set, click [Start] to enter the page “Counting…” as shown in Figure 6-5. Please wait for the completion of counting. Click [Stop] to stop this counting. After counting, automatically enter the page “Result”, as shown in Figure 6-6.

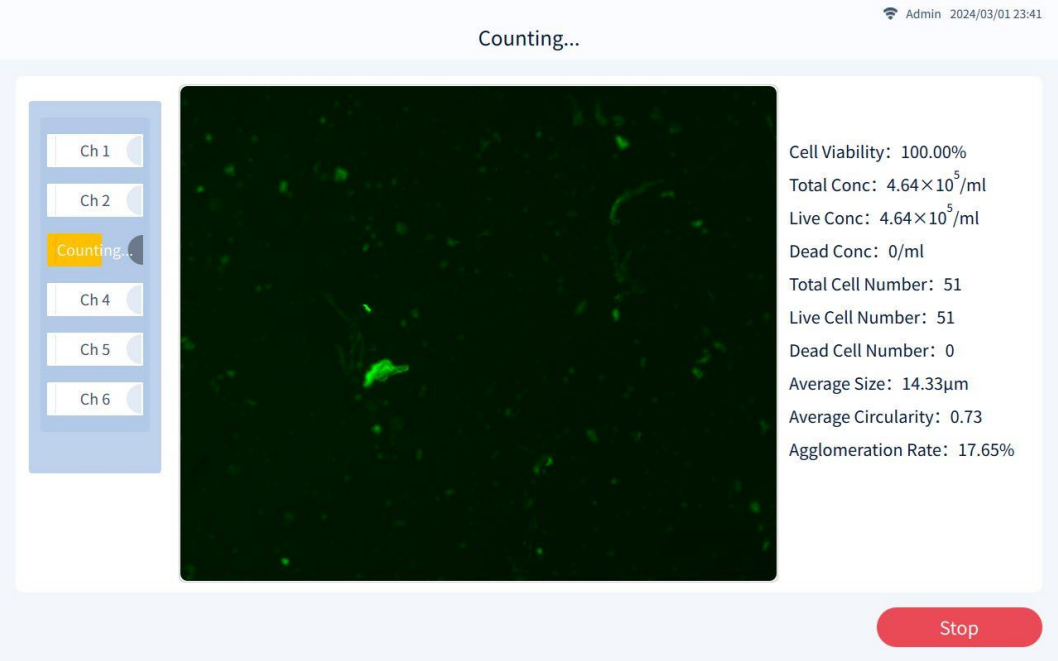


Figure 6-5

6.2 Count results

Based on the count type selected, the count results will present different channel combinations. If AOPI count type is selected, four channels of BF, AO, PI and Merge will be displayed.

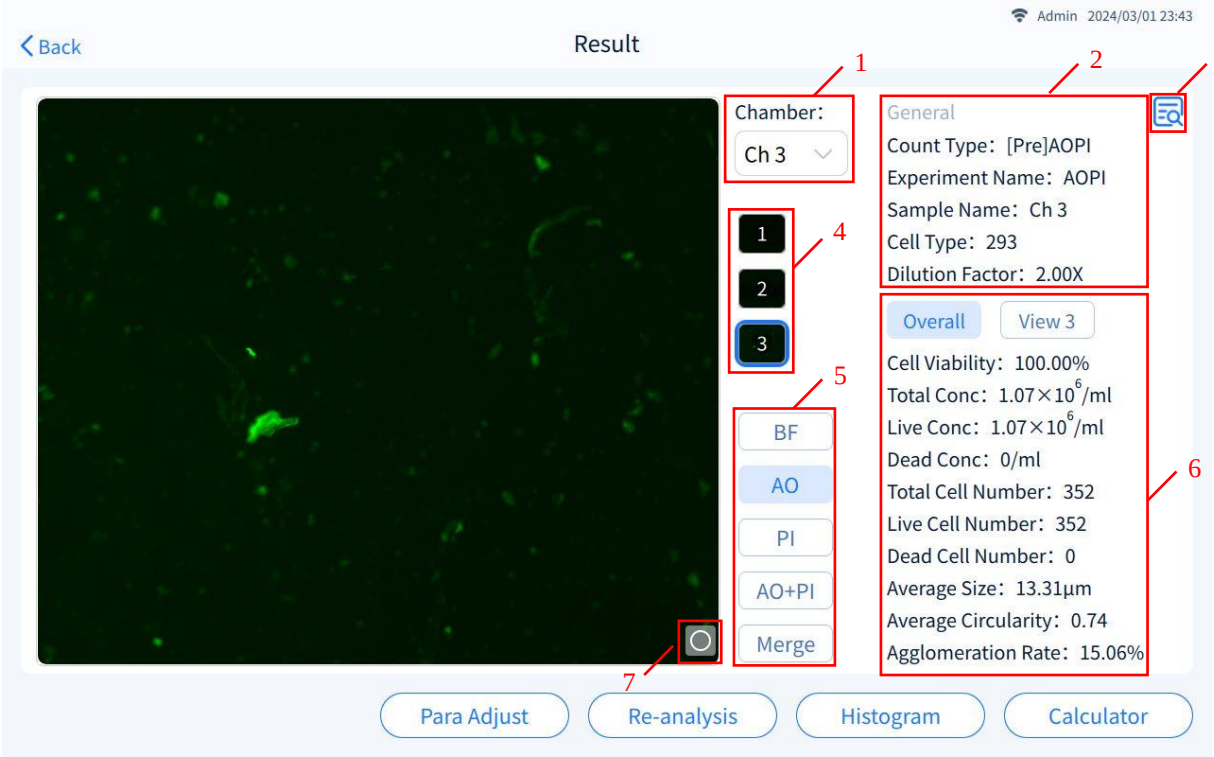


Figure 6-6

No.	Description
1	Switch between different sample chambers
2	Display the general parameter
3	Observe the detailed parameters

4	Switch between different views
5	Switch between different fluorescence
6	Count results of current view
7	Switch original/marker view

Common count types correspond to the following channels:

Total cell count	BF	/	/
Trypan blue	BF	/	/
AOPI	BF	AO	PI
GFP transfection rate	BF	GFP	/
Faint-GFP transfection rate	BF	GFP	/
RFP transfection rate	BF	/	RFP
Faint-RFP transfection rate	BF	/	RFP

Count results - Overall

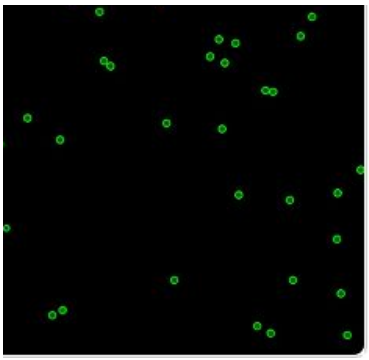
Cell viability = sum of live cell number in all views/sum of total cell number in all views.
XXX proportion = XXX cell number in all views/sum of total cell number in all views.
Average RFU = sum of RFU of all fluorescent cells in all views/sum of total cell number in all views.
Total concentration = sum of total concentration in all views/number of views.
Cell size = sum of cell sizes in all views/sum of total cell number in all views.
Average circularity = sum of cell circularity in all views/sum of total cell number in all views.

Count results - View N

Cell viability = live cell number /total cell number.
XXX proportion = XXX cell number/total cell number.
Average RFU = sum of RFU of all fluorescent cells/total cell number.
Total concentration = calculated by the algorithm based on the total cell number in the current view and the view volume.
Cell size = sum of cell sizes/total cell number.
Average circularity = sum of cell circularity/total cell number.

6.2.1 Cell marker

Marker enabled



Marker disabled

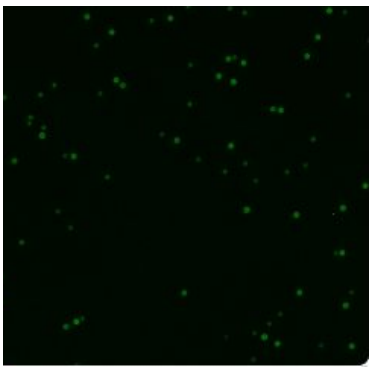


Figure 6-7

6.2.2 Image zoom/restore/move

Image zoom: Pinch the screen with two fingers to zoom out. Otherwise, spread the fingers to zoom in.
Zoom in/out: Double-click the image area to zoom in/out the image.
Move: When zooming in, long press to drag the image display area.

6.2.3 View parameters

Click [View] to enter the interface as shown in Figures 6-8.
This interface shows the following parameters: Current count type, cell type, number of views, dilution factor, exposure parameters of the current count type, etc.

Figure 6-8

Main parameter items and their definitions:

Parameters	Meaning
MinSize	BF channel parameters, minimum cell size. Cells smaller than this diameter will be deleted.
MaxSize	BF channel parameters, maximum cell size. Cells bigger than this diameter will be deleted.
Alive/Dead	BF channel parameters, cell viability threshold. If the viability of a single cell is lower than the threshold, it will be judged as dead cell, and if it is higher than the threshold, it will be judged as live cell.
Filter Radius	BF channel parameters, smoothness. This parameter is used to remove overlapping cells, that is, to form a circular area with this parameter as the radius, and the centers of all cells within this area will be combined into a single center. When there are overlapping cells, the greater the smoothness, the more cells merge, and the smaller the smoothness, the fewer cells merge. The effect of adjusting this parameter is obvious in cluster splitting.
Vote	BF channel parameters, circle similarity threshold. The rounder the cell boundary, the higher the response value for circle, and the flatter the cell boundary, for example, non-curved boundaries such as squares, rectangles and so on, the lower the corresponding circle response value. This parameter is used to remove objects in non-circular shapes (the corresponding circular response value is lower than normal cells).
BorderPix	BF channel parameters, cell boundary threshold. This parameter is used to determine the cell boundary. The larger the parameter, the thicker the cell boundary, and the smaller the parameter, the finer the cell boundary.
Edge	BF channel parameters, cell boundary detection parameter. The smaller the value, the stronger the virtual focus edge detection, and the larger the value, the weaker the virtual focus edge detection.
Boost	BF channel parameters, background impurity removal coefficient. The larger the value, the greater the background removal capability, and the smaller the value, the weaker the background removal capability.
MinArea	FL channel parameter, the minimum fluorescence area of the cell. Cells smaller than this fluorescence area will be deleted.
MaxArea	FL channel parameter, the maximum fluorescence area of the cell. Cells bigger than this fluorescence area will be deleted.
FlouTH	FL channel parameter, fluorescence threshold. This parameter is used to select targets with average fluorescence intensity higher than this value, as a way to filter target cells.

6.2.4 Parameters Adjust

Click on the [Para Adjust] to enter the interface as shown in Figure 6-9.

Note: *Parameter adjustment will not modify the existing parameters and data. Click [Save As] to save as a new cell type (Invalid for the preset count type), and then re-analyze the data. Refer to Section 6.2.5.*

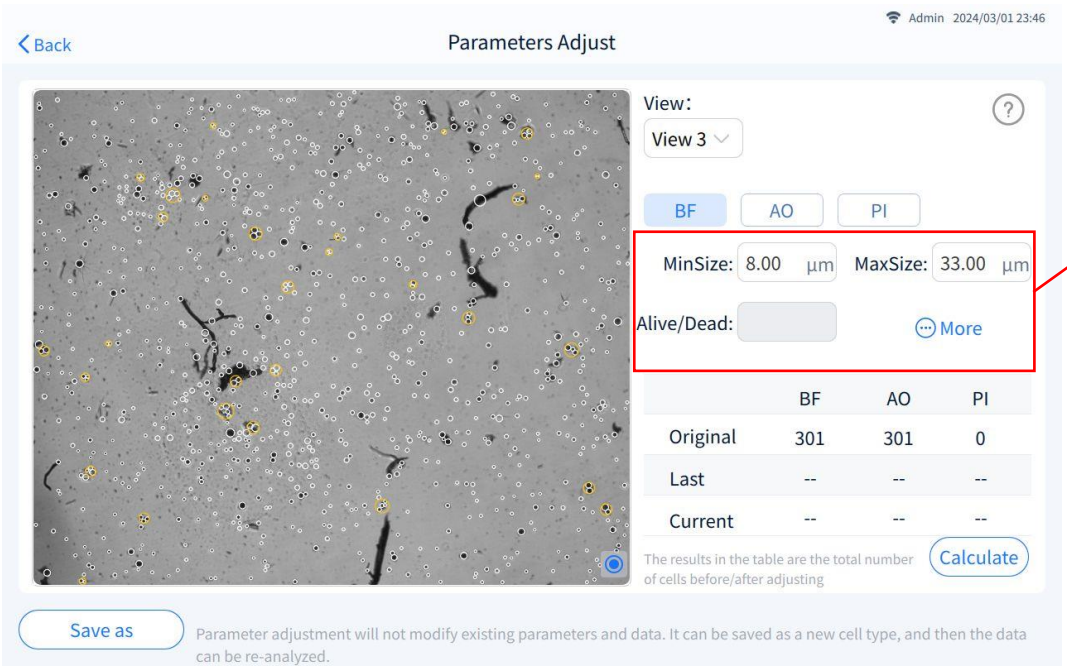


Figure 6-9

Note： *[More] is only available in BF channels of BF type and fluorescence type, as shown in Figure 6-10.*

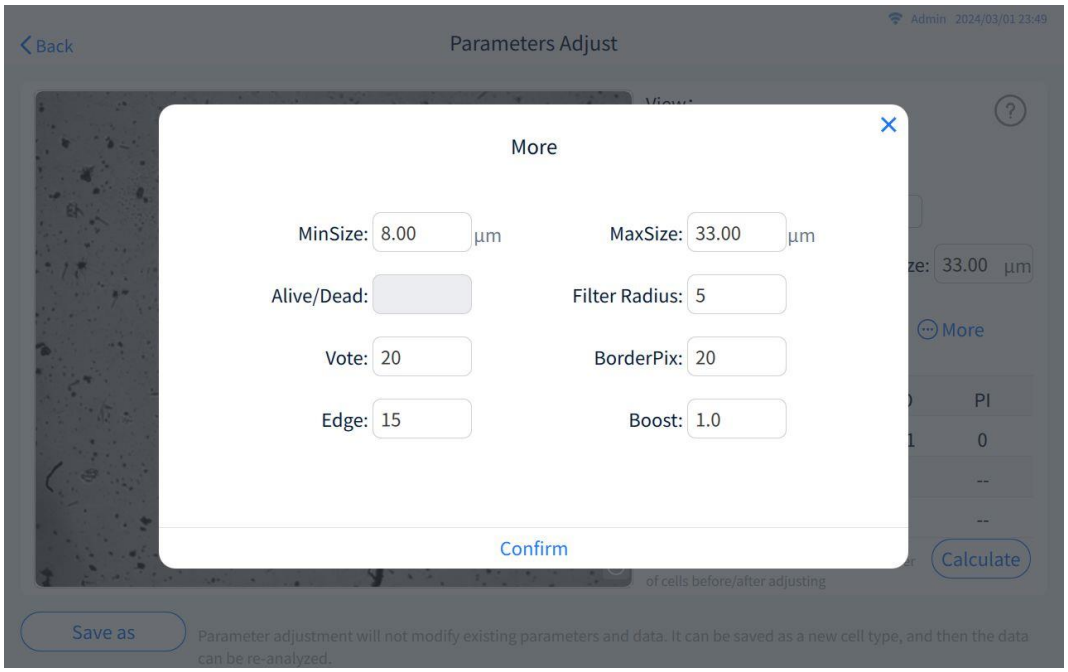


Figure 6-10

Click to switch to other channels (such as AO channel) for parameters adjustment, as shown in Figure 6-11.

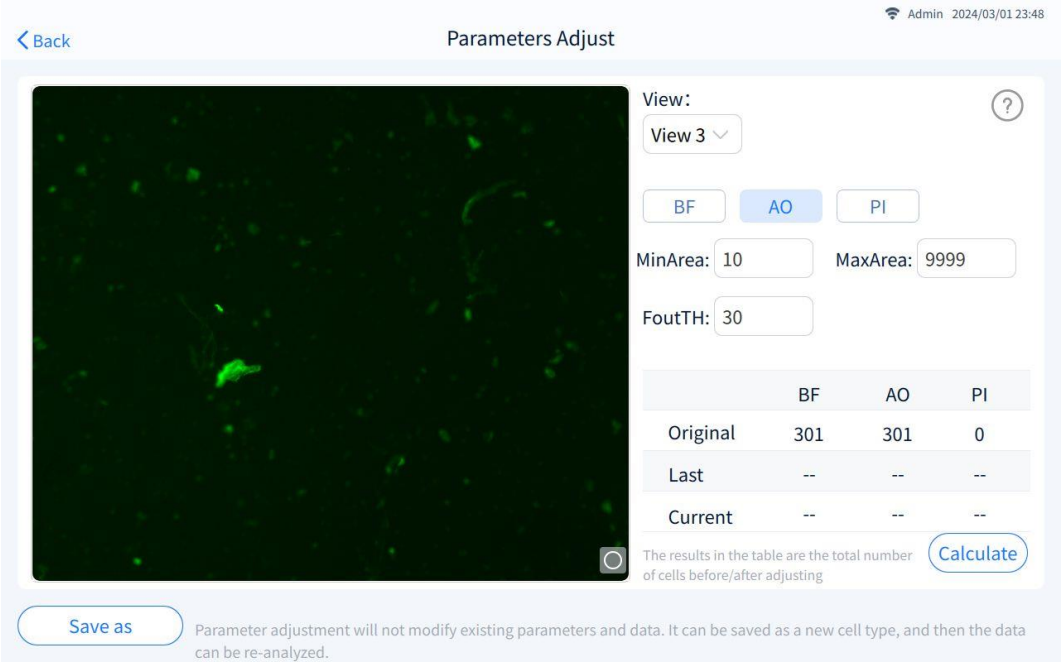


Figure 6-11

Click on the help ⓘ to enter the “Help” pop-up window as shown in Figure 6-12 to view the relevant interpretation of parameters.

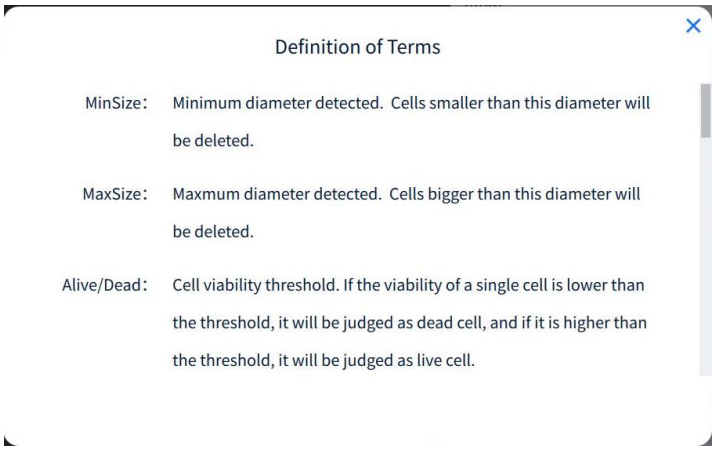


Figure 6-12

Main parameter items and setting range:

Parameters	**Setting range**
MinSize	3-180 μm
MaxSize	3-180 μm
Alive/Dead (Only the trypan blue mode is active)	0-255
Filter Radius	1-255
Vote	1-999
BorderPix	1-255
Edge	1-999
Boost	1.0-10.0
MinArea	1-9999

MaxArea	1-9999
FlouTH	1-255

6.2.5 Re-analysis

The user can re-select the parameters of other cell types from the saved data for re-analysis, and the re-analyzed data will be saved as new data without overwriting the original data, and can be traced back to the original data through the unique sample ID in the remark column at the end of the “Data” interface.

Click [Re-analysis], and enter the login password to enter the interface as shown in Figure 6-13. Click the drop-down arrow to reselect the cell type, click [Confirm] and wait for the count result.

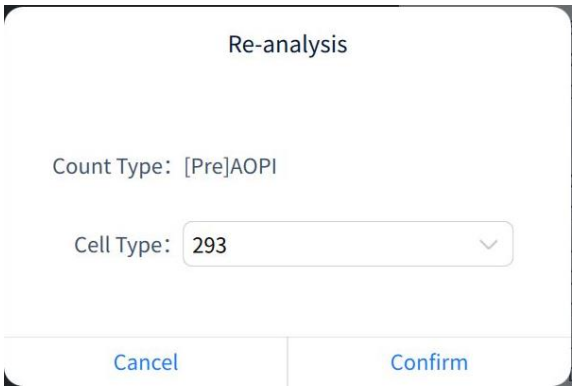


Figure 6-13

6.2.6 Histogram

Click [Histogram] to view the diameter distribution and RFU distribution, as shown in Figure 6-14 and 6-15.Histograms are displayed differently depending on the count type.

Figure 6-14 Diameter distribution

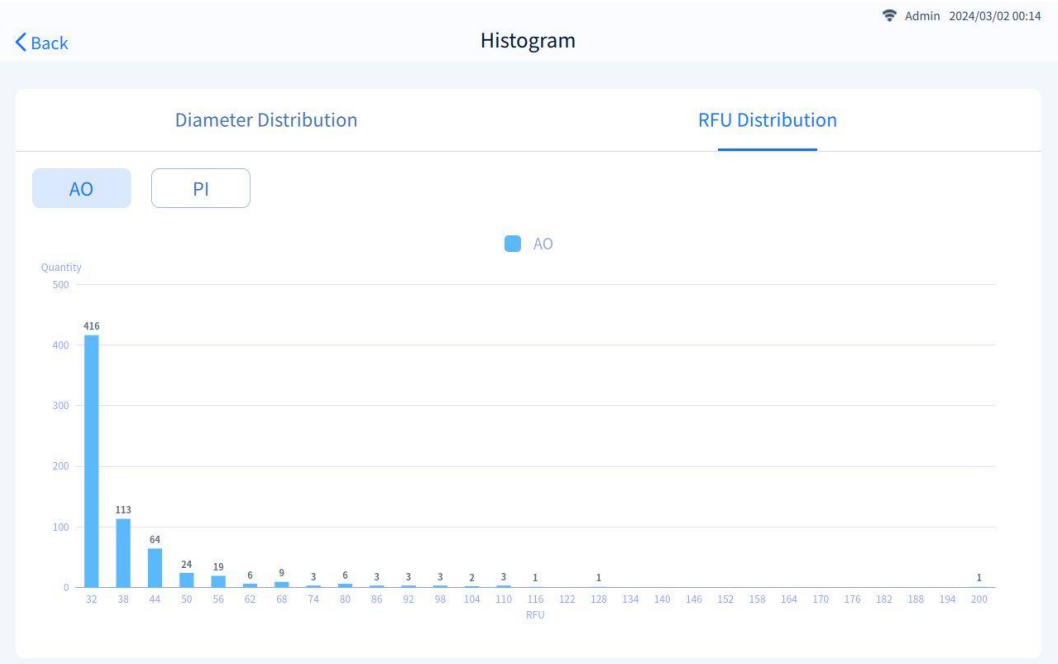


Figure 6-15 RFU distribution

6.2.7 Calculator

Click [Calculator] to enter the interface as shown in Figure 6-16. [Calculator] is used to calculate how much volume of existing cell solution needs to be mixed with buffer when diluted to the target volume and target concentration.

Manually enter the target concentration and target volume, and the system will automatically suggest values.

Click [Close] to close the window.

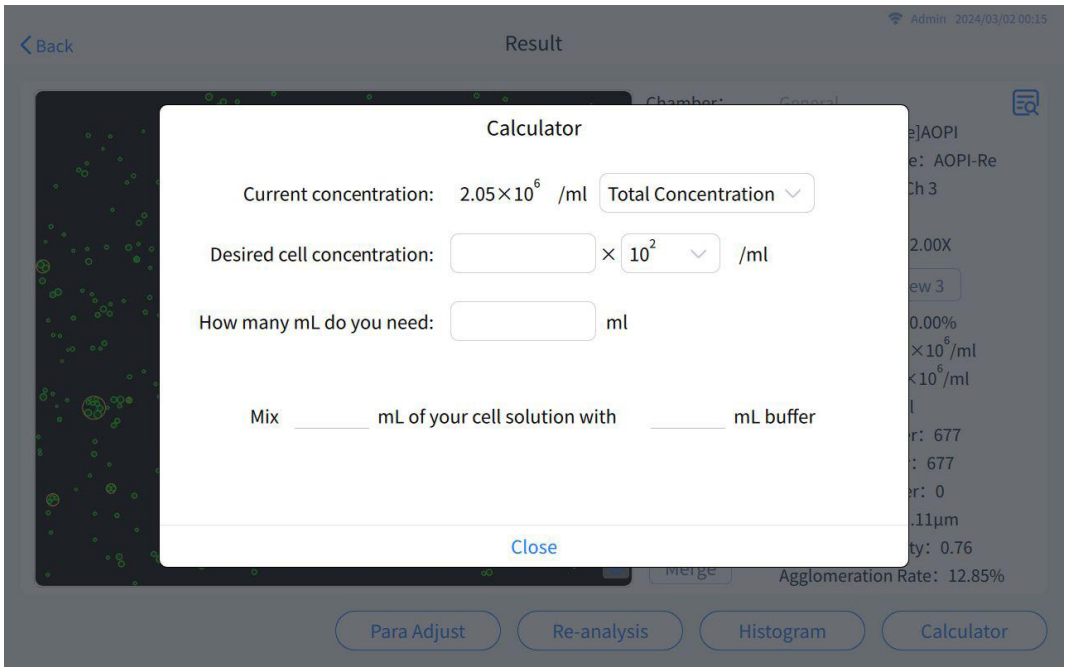


Figure 6-16

6.3 Add count type

Count type needs to be added from external device, and make sure the external device and the counter are connected before clicking 

key to add the count type. When the system recognizes that there is a valid count type in the external device, the password entry interface will pop up, and enter password of current user, and the system will automatically enter the interface as shown in Figure 6-17.

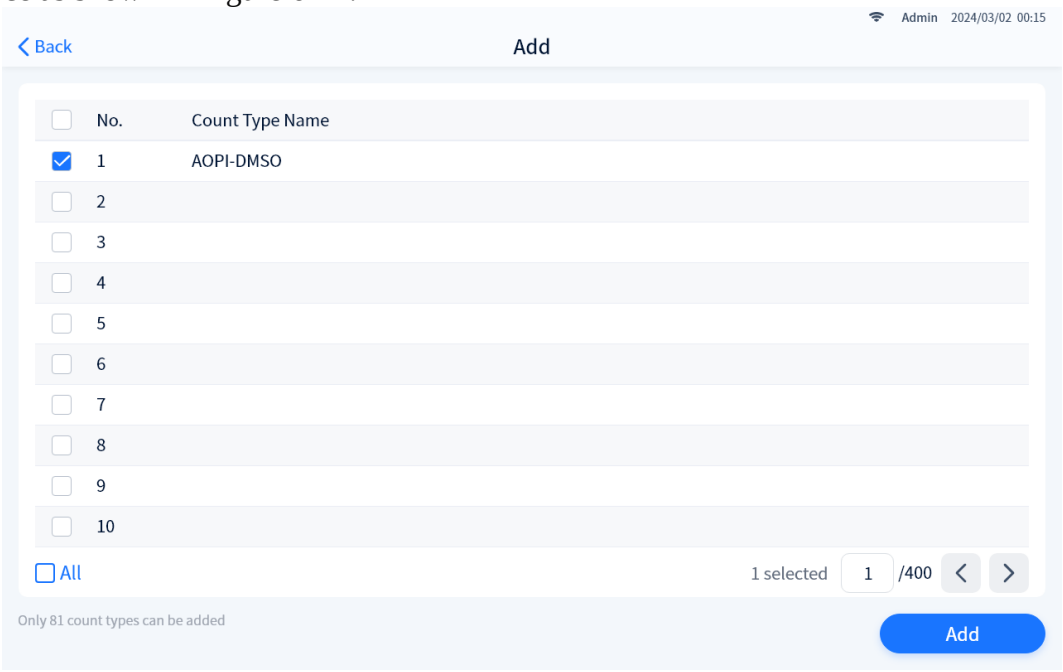


Figure 6-17

The system supports up to 89 count types (including the preset types). According to the number of existing count types, the system will prompt the user for the number of count types still able to be added at the bottom of the interface in Figure 6-17.

Check the count type to be added, click [Add], and the system prompts “Add completed”, that is, the count type is added successfully. The newly added count type will be automatically displayed in the counting main interface, and a random icon will be generated, such as “AOPI-DMSO” as shown in Figure 6-18.

Figure 6-18

6.4 Copy/ delete/export/edit count type and icon groups

As shown in Figure 6-19, long press the icon to copy/delete/export/edit count types.

Note: *7 preset count types can only be copied, and cannot be edited, deleted or exported.*

(a) Long press the preset type icon

(b) Long press the custom type icon

Figure 6-19

Drag one count type onto another to automatically group them, as shown in Figure 6-20.

Figure 6-20

A maximum of 12 count types can be placed in a group. When there are only two count types left in the group, the group will be automatically broken if one of them is dragged out of the group.

Long press the group icon only to delete and export.

Click on the group name "Group" to customize the name.

- **Copy**

Click [Copy] to enter the interface as shown in Figure 6-21.

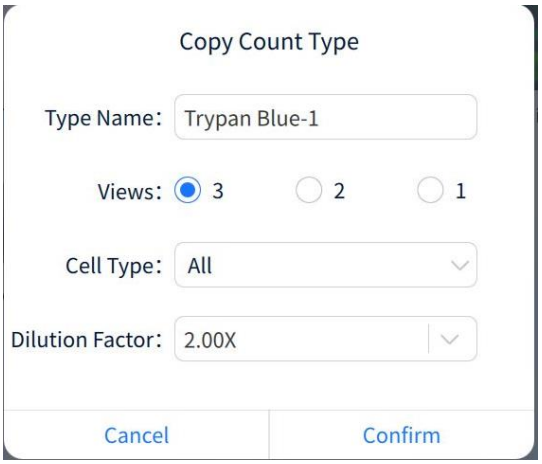


Figure 6-21

The user can customize the name of the count type, number of views, cell type and dilution factor after copying. Click [Confirm] to complete the setting.

- **Delete**

Click [Delete] to enter the interface as shown in Figure 6-22. Click [Confirm] and enter password of current user to delete the count type.

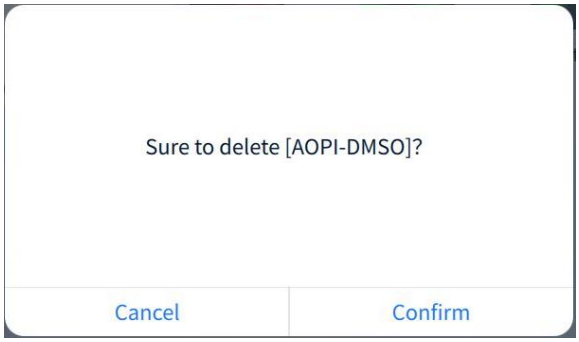


Figure 6-22

- **Export**

Click [Export] to enter the interface as shown in Figure 6-23. Select to export to external storage device or server, and click [Confirm].

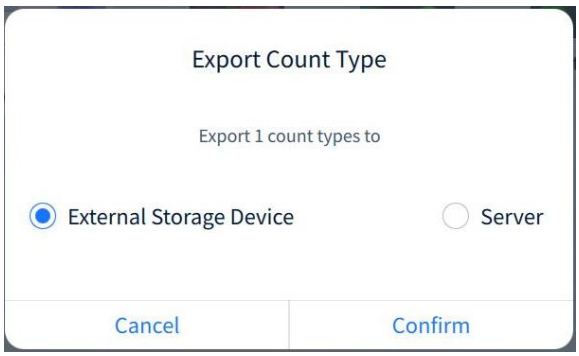


Figure 6-23

- **Edit**

Click [Edit], enter password of current user, insert the slide according to the screen tip, and enter the interface as shown in Figure 6-24.

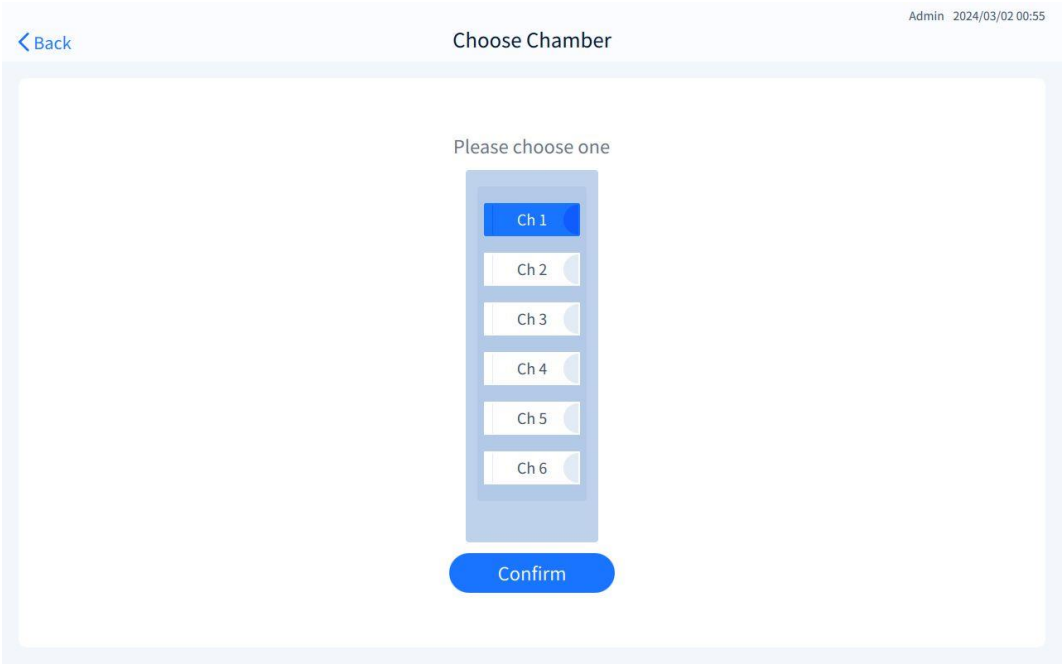


Figure 6-24

Select the chamber, click [Confirm] to enter the interface “Edit Count Type”, and set the general information and exposure parameters of the count type, as shown in Figure 6-25. Click [Save] to complete editing.

Note: *The content displayed on the interface varies according to the count type.*

Edit General Information:

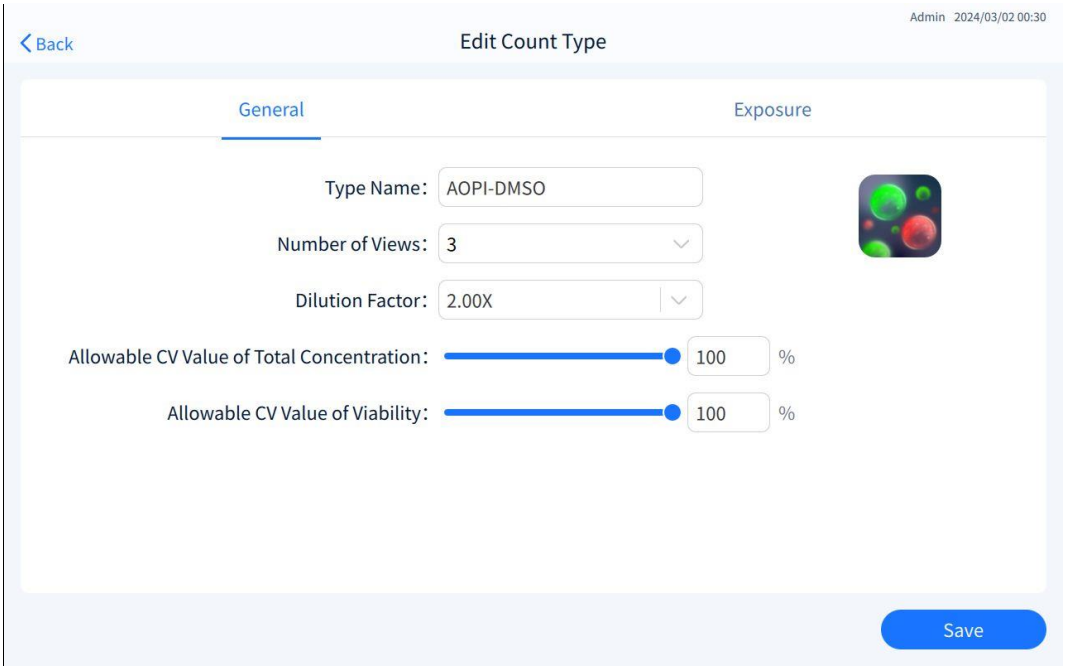


Figure 6-25

Parameters	**Description**
Type Name	Custom, enter 1-20 characters
Number of views	Options from 3, 2 and 1

Dilution Factor	Options from 1.00X, 2.00X, 10.00X, and 100.00X). Custom entry range: 1.00 ~ 10000.00
Allowable CV value of total concentration	0-100%, resolution 1%
Allowable CV value of viability	0-100%, resolution 1%. **Trypan blue, AOPI count type**
Allowable CV value of transfection rate	0-100%, resolution 1%. **GFP, RFP, faint-GFP, faint-RFP count type**

Description of calculation formula:

Allowable CV value of total concentration/viability/transfection rate

$$RSD = \frac{S}{\overline{x}} \times 100\% = \frac{\sqrt{\frac{\sum_{i=1}^{n}(x_i - \overline{x})^2}{n-1}}}{\overline{x}} \times 100\%$$

$\overline{x}$ is the average of 1-3 views in the sample.

x_i is the actual value of 1-3 fields of the corresponding sample.

In [Edit Count Type]: “CV value of total concentration” & “CV value of viability” & “CV value of transfection rate” are the CV in 3 views.

When the number of views is 1 or 2, it is not needed to display/use this calculation formula.

Note: *The CV value in the table is the CV value among 3 views. Click the current icon as shown in*  *above to enter the interface “Select Icon” and reselect the display icon. Click [Confirm] to complete icon change.*

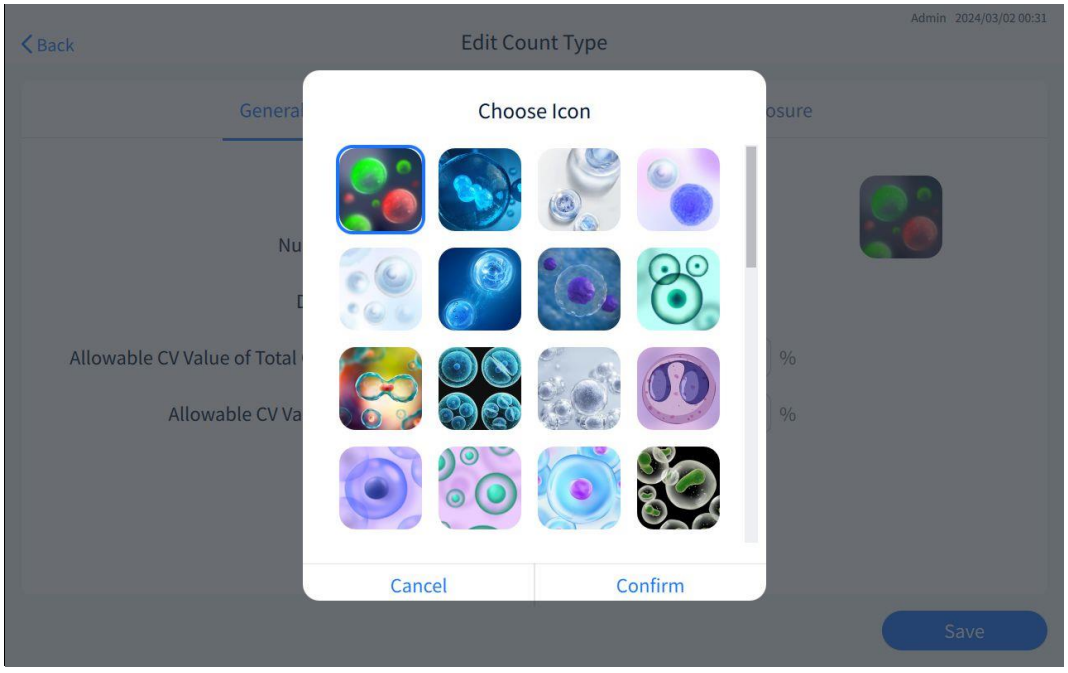


Figure 6-26

Edit exposure parameters:

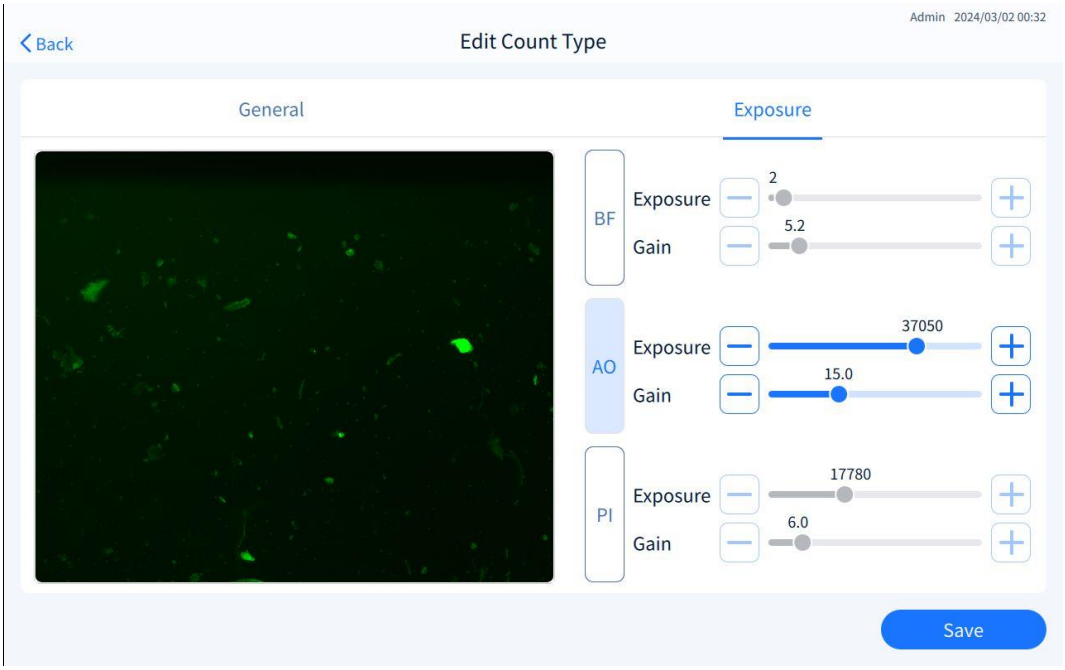


Figure 6-27

Parameters	**Entry range**
Exposure of BF	0-72
Exposure of fluorescence	0-51870
Gain	0-48.0

7- Data

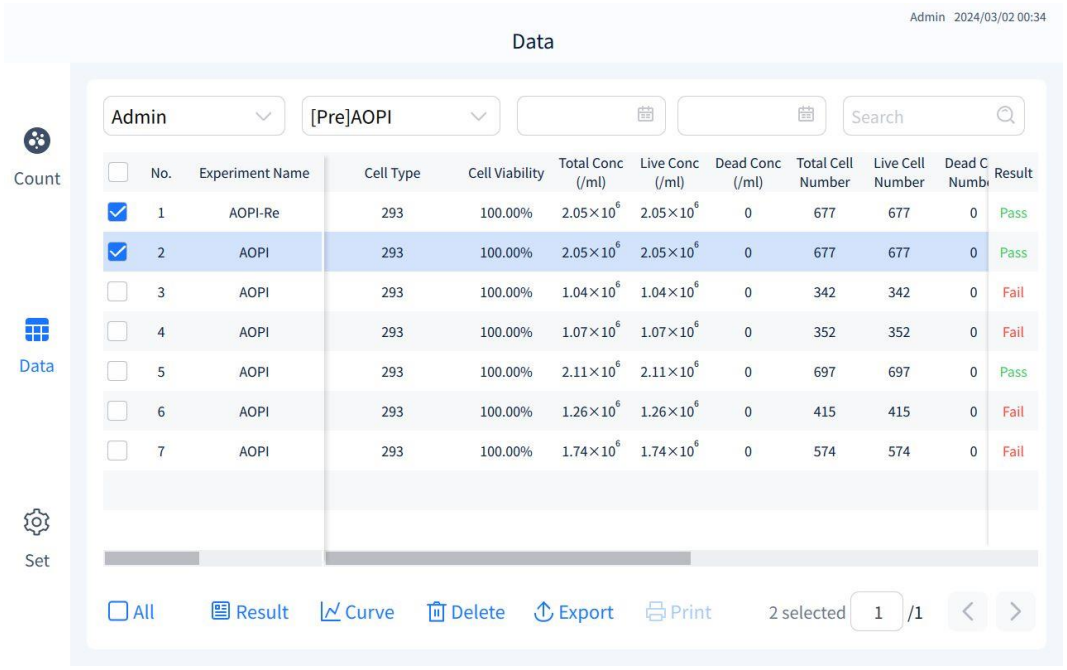


Figure 7-1

The interface “Data” displays all experiment data under the current user by default. Click the drop-down arrow of Account  to switch to display all accounts and experiment data that the current user has access to.

Click the drop-down arrow of count type  to switch and select the count type with count data of the current user.

Click to set the start and end time of experiment data and screen the experiment data in this time period.

Click the search box  and enter characters to perform fuzzy search, including the experiment name, sample name, and cell type.

Click a single piece of data, and the data in blue shading are in a selected state, as shown in Figure 7-1. Click “Results” to view the detailed count results of this piece of data. Please refer to Section 6.2. You can view the count results for only one piece at a time.

Click √ before the number to check a single or multiple data, as shown in Figure 7-1, to view the “growth curve” of the checked data. Refer to Section 7.1. Data can be deleted, exported, and printed (make sure the printer is connected) as prompted.

Results: Pass or Fail. If the count results meet the requirements, it is displayed as Pass, or it is displayed as Fail. Click Fail to display the [Results Information] pop-up window, as shown in Figure 7-2.

There are three reasons for Fail:

① The concentration/viability/agglomeration rate exceeds the setting range of the total concentration/viability/agglomeration rate in the corresponding cell parameter under its count type.

(Comparison is performed only when the cell parameter [Concentration] of the cell type has a set value.)

② When “Instrument Calibration” is on, and instrument calibration is not performed or calibration is expired, the count result is Fail.
(If “Instrument Calibration” is turned off, this item is not judged.)
③ Count results for each chamber: The concentration/viability bias among the 3 views exceeds the upper limit of the allowable CV value of total concentration/viability (if any)/transfection rate (if any) as set in Count type-General (Figure 6-24).
(When the count type [Number of Views] is 1 or 2 in the count result, the set value is not compared.)

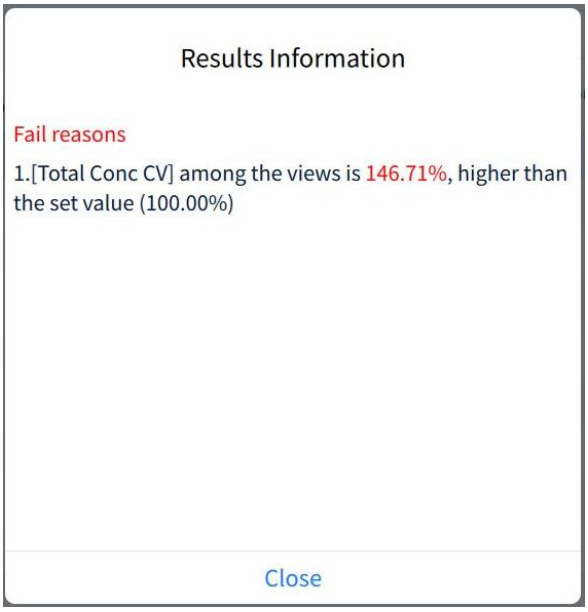


Figure 7-2

7.1 Growth curve

After checking the result data of the growth curve to be generated in the interface [Data], click [Curve] to display the “Curve Name” pop-up window, as shown in Figure 7-3. Users can customize the curve name.

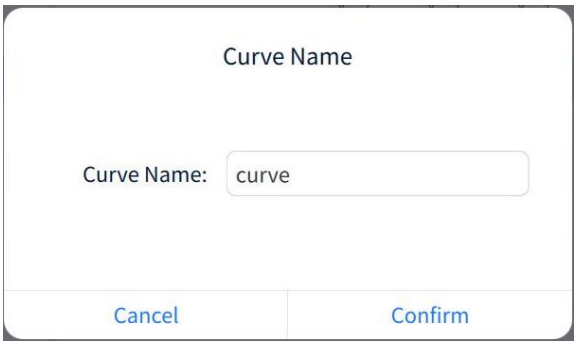


Figure 7-3

Click [Confirm] to enter the interface as shown in Figure 7-4.

Figure 7-4

By default, X-axis is displayed according to the “Sequence” arrangement. Click the drop-down arrow to switch to the “Time” order display.

The drop-down options on the Y-axis are related to the current count type, for example, when using the total cell count type, the drop-down options show the total concentration, total cell number, average size, and agglomeration rate.

Click [Export Curve] to export the curve with the file name.jpg to an external storage device or server according to the prompt.

7.2 Delete Data

After checking the result record to be deleted in [Data], click [Delete] to enter the interface as shown in Figure 7-5. Click [Confirm] and enter password of current user to delete the records.

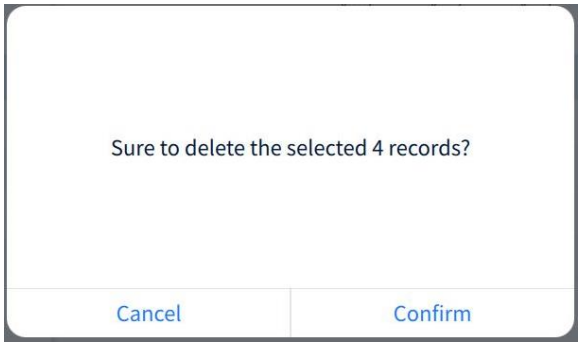


Figure 7-5

7.3 Export data

To export data to an external storage device, make sure you insert a USB flash drive in the correct format (FAT32).
After checking the result data to be exported in [Data], click [Export] to enter the interface as shown in Figure 7-6. Select the data format to be exported and storage path, click [Confirm] and enter password of current user.

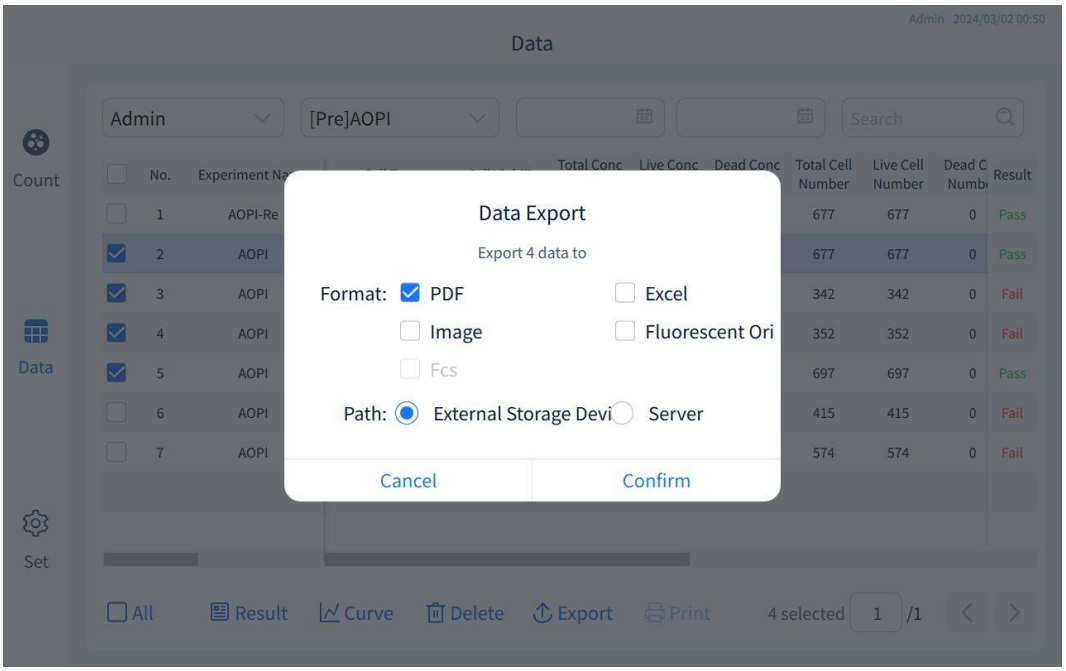


Figure 7-6

8- Set

Click [Set] to enter the setting interface. The Admin account setting interface is shown in Figure 8-1, and the non-Admin account setting interface in Figure 8-2.

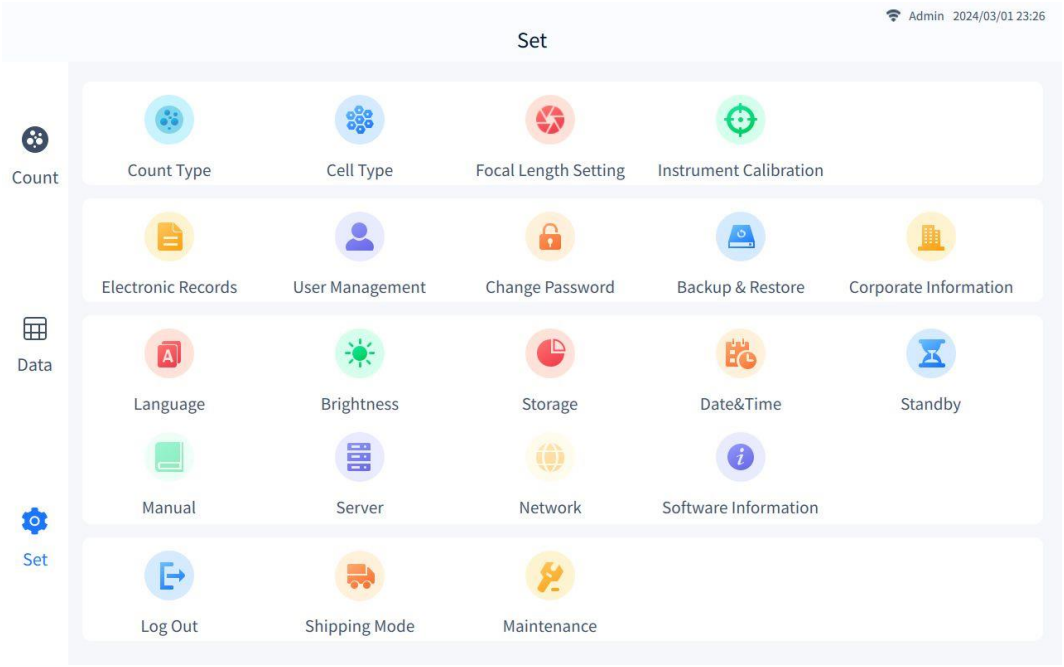


Figure 8-1

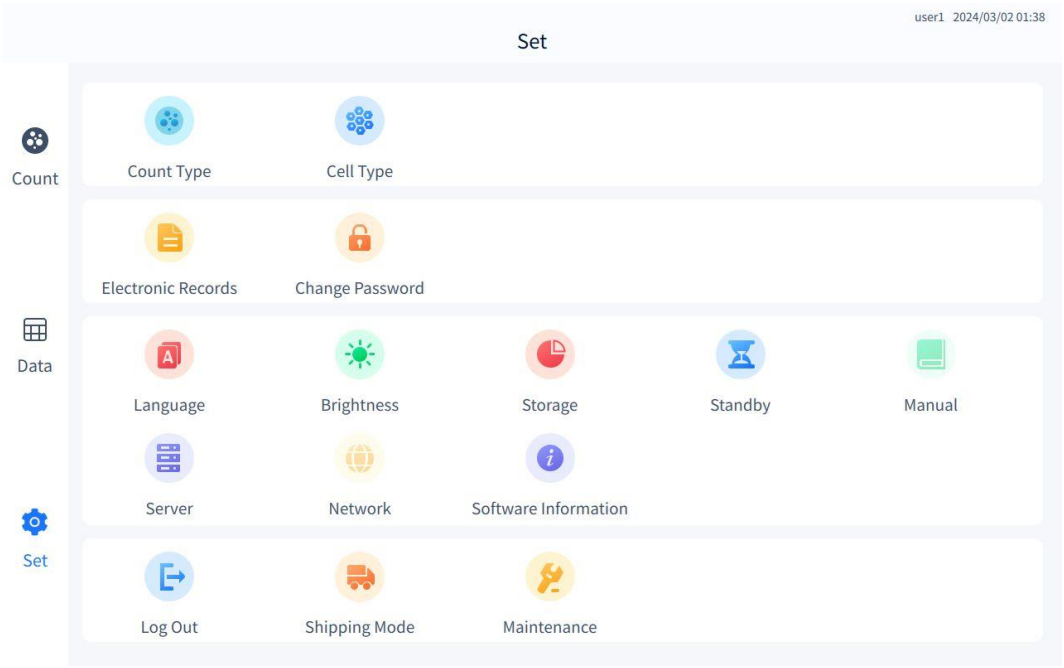


Figure 8-2

8.1 Count type

Click [Count type] to enter the interface as shown in Figure 8-3. Default display system preset count type.

Please refer to Sections 6.3 and 6.4 for descriptions of **Add/Copy/Delete/Export/Edit** count types. Multiple or all count types can be checked for the above operations. **Preset count types cannot be checked/All selected**.

When the current login account is selected in the drop-down box, the operations related to

count type in this interface are displayed synchronously with the count type in the main interface.

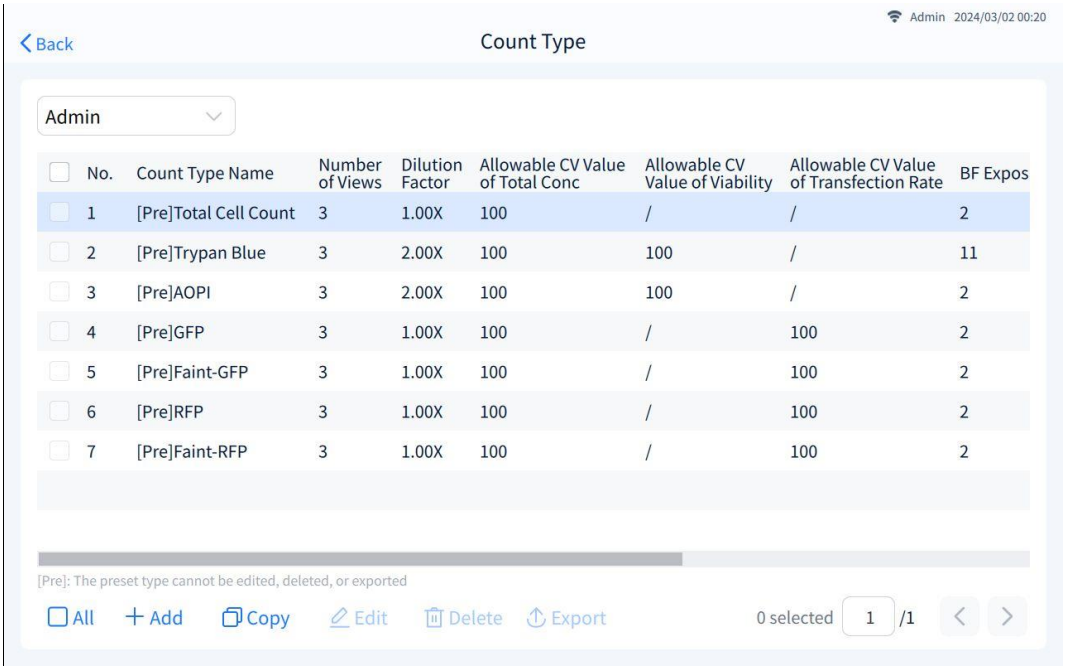


Figure 8-3

8.2 Cell Type

Click [Cell Type] to enter the interface as shown in Figure 8-4 to display cell types under different count types.

Click 

to switch all accounts that the current login account has access to.

Click 

to switch the display of other count types.

Note: *The cell types under the preset count type cannot be copied, added, edited, deleted, checked or all selected.*

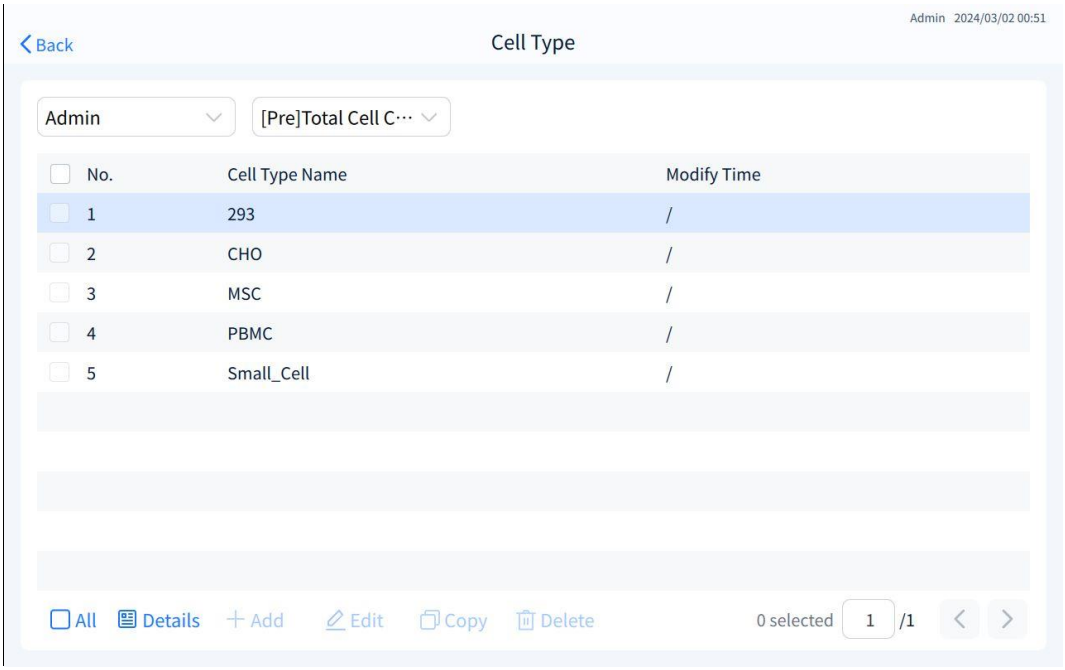


Figure 8-4

Click [Details] to view the details of the current cell type, refer to Figure 8-5.

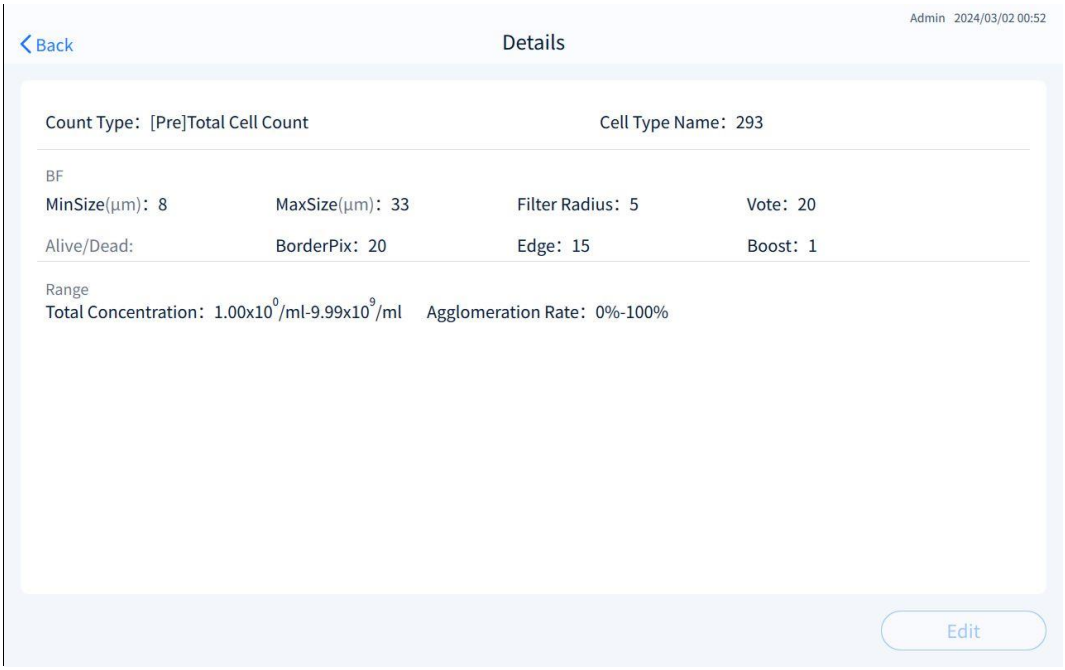


Figure 8-5

For the cell type under user-defined count type, add, copy and edit can be performed after blue shading is selected. Such as switch the count type to the “AOPI-DMSO” and select the cell type name of “293”, shown in Figure 8-6.

After checking/selecting all, deleting operation can be performed by following the prompts.

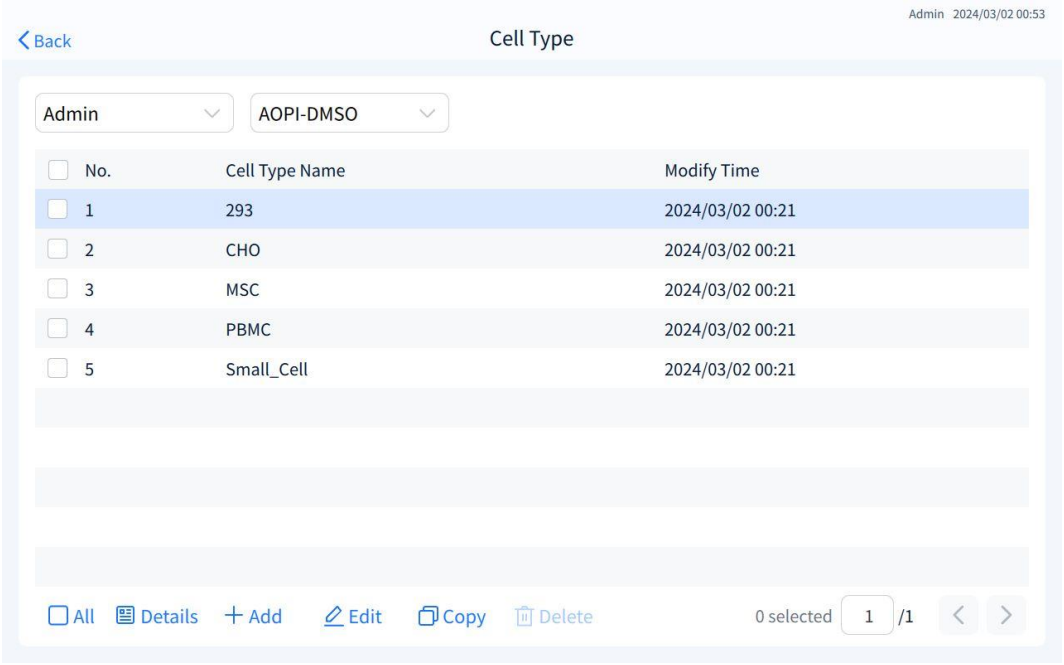


Figure 8-6

Selection recommendations for cell type:

1) [Small_Cell] is recommended for immune cells smaller than 10 μm, such as mouse spleen cells.
2) [293] or [CHO] can be selected for conventional cell lines larger than 10 μm.
3) Please select [PBMC] for human PBMC.

8.2.1 Add/Copy/Delete/Edit Cell Type

- **Add**

Note: *A count type supports up to 50 cell types (including presets).*

Click [Add], enter the login password of the account, and enter the interface as shown in Figure 8-7.

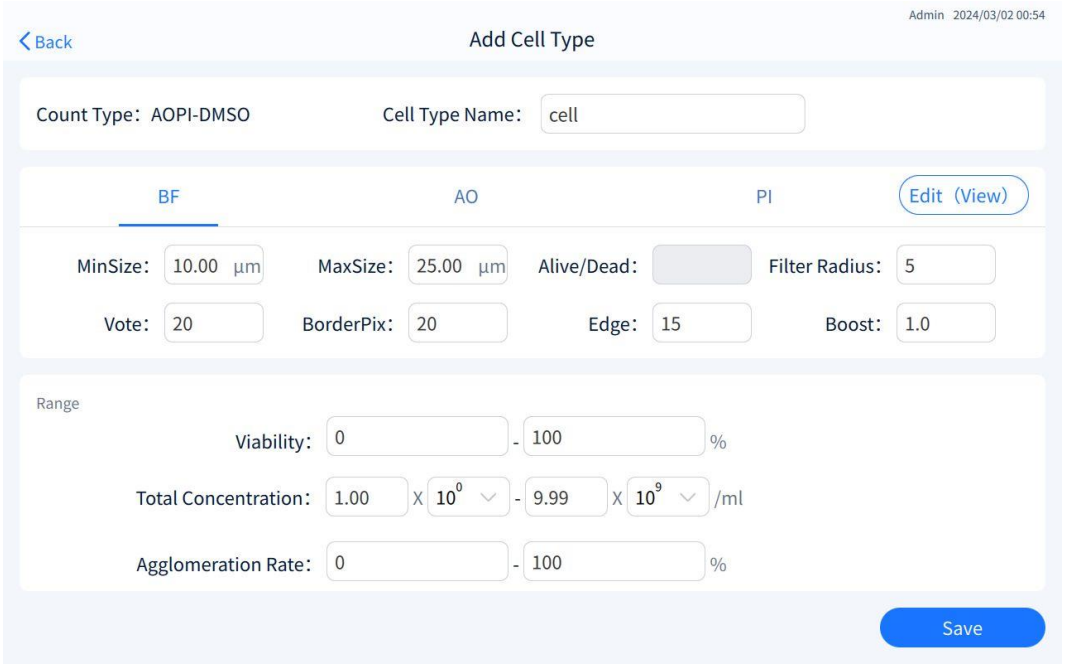


Figure 8-7

The default cell type name is “cell”, which can be customized.

Click to switch the channel and enter the corresponding parameters.

Click [Edit (View)] to enter the interface as shown in Figure 8-8, select the chamber where the target sample has been added and click [Confirm] to enter the counting state.

Note: It is needed to insert the slide and ensure that the sample that requires cell parameter adjustment has been added into the chamber.

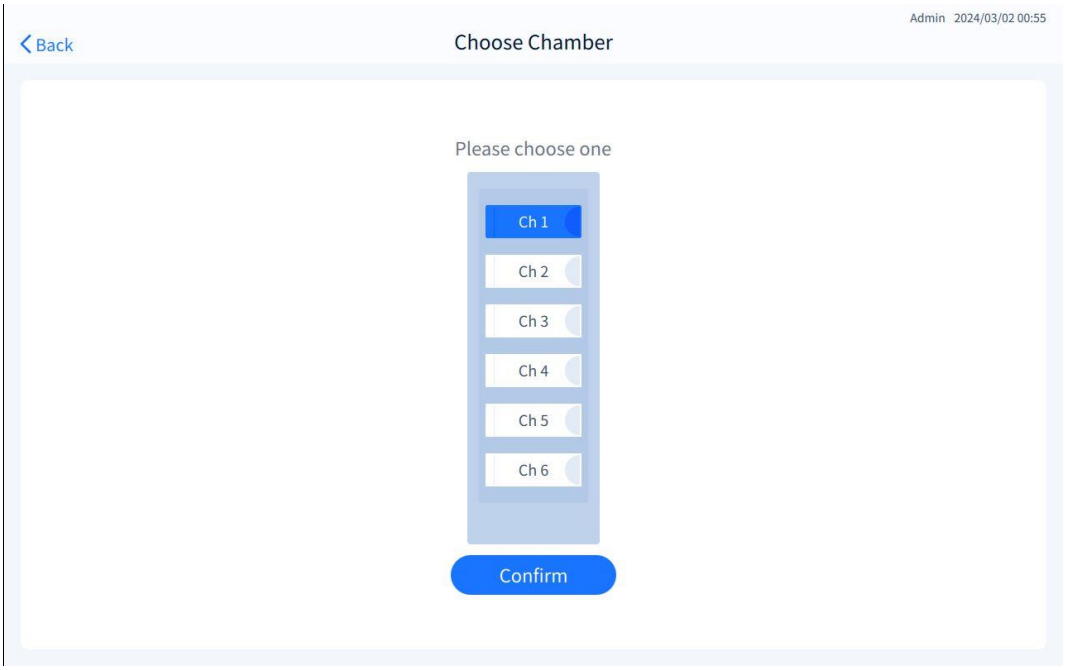


Figure 8-8

After counting, enter the interface “Edit Cell Profile”, as shown in Figure 8-9. Refer to ***Section 6.2.4*** for setting channel parameters and calculation.

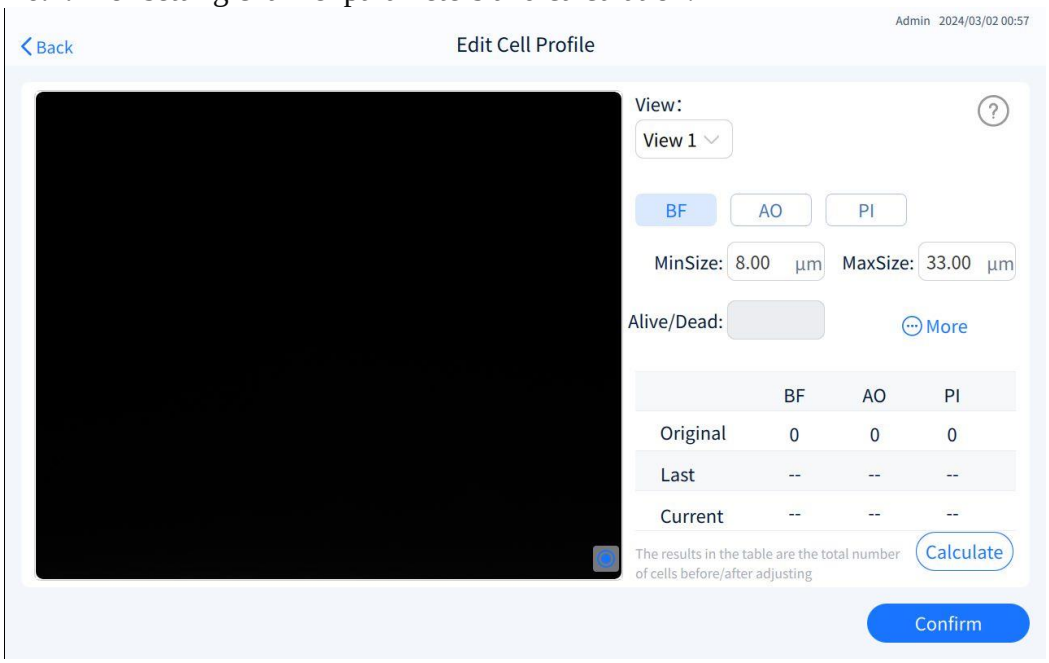


Figure 8-9

Click [Confirm] to complete the editing of cell parameters, and return to the interface as shown in Figure 8-7. Click [Save] to complete the adding of cell types.

- **Copy**

Check one of the cell type data and click [Copy] to enter the interface as shown in Figure 8-10. Customize the cell type name and click [Confirm] to complete the copy.

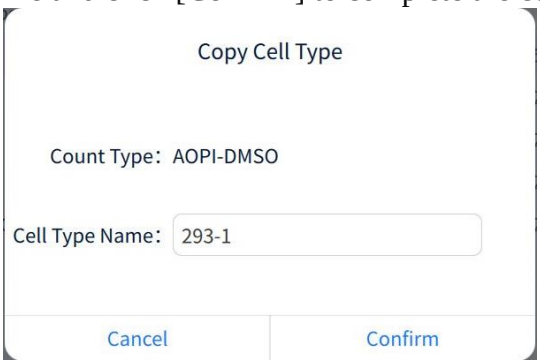


Figure 8-10

- **Delete**

Check the cell type data to be deleted, and click [Delete] to enter the interface as shown in Figure 8-11. Click [Confirm] and enter password of current user to delete the cell type.

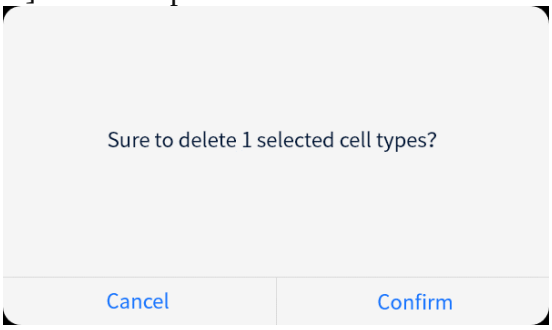


Figure 8-11

- **Edit**

The “Edit” function operates similarly to the “Add” function.

8.3 Focal length setting

Click [Focal Length Setting] to enter the interface as shown in Figure 8-12.
Click [Auto] to adjust the focal length automatically.
If autofocus fails, manual fine adjustment can be made. Click ⊞/⊟ button or slider bar to manually adjust focal length.
Click [Save] in the lower right corner and enter password of current user to save the current calibrated focal length.
Users can adjust the focal length according to their needs. For example, in case of imaging blurring and defocus, 8 μm standard beads provided with the main unit can be used for focal length calibration.
Focal length calibration method: Fully invert and mix the 8 μm standard beads, load 10 μL of samples into the third sample chamber of the slide (only the third chamber is available), allow to stand for 30 s, and insert into the slide interface.

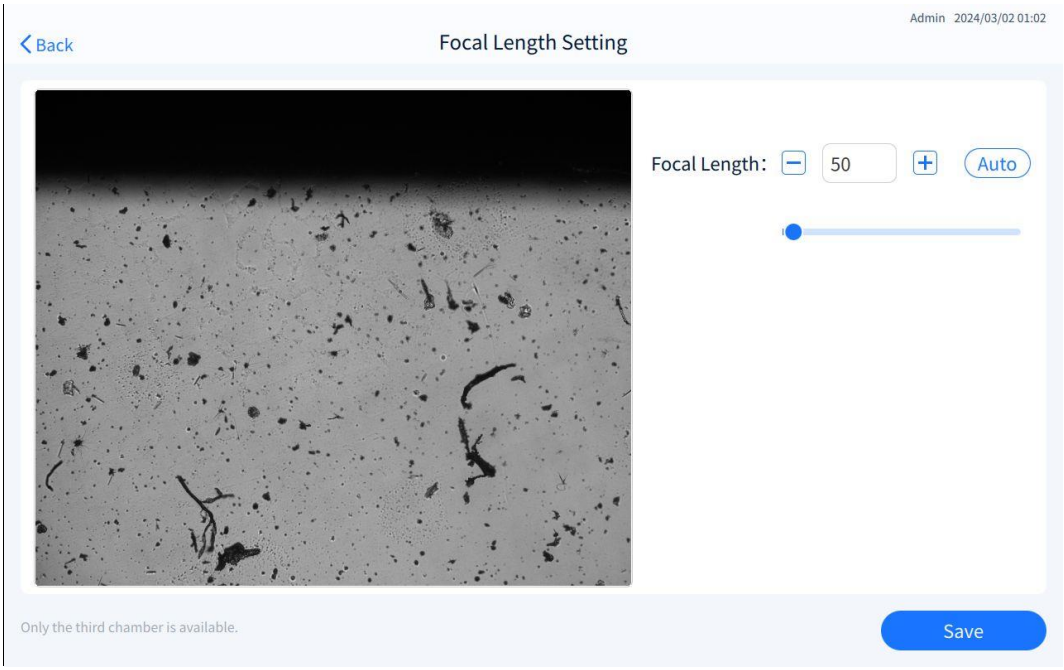


Figure 8-12

8.4 Instrument calibration

The “Instrument Calibration” function is used to calibrate cell concentration, viability, and cell size, as shown in Figure 8-13.

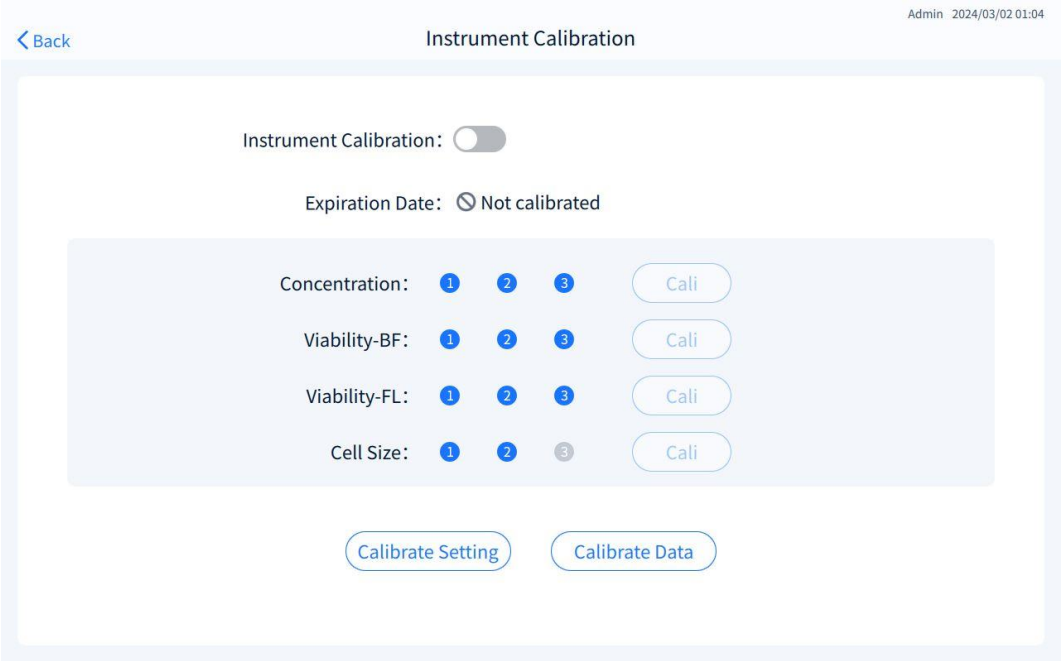


Figure 8-13

Shift to the enabled state Instrument Calibration: , shown as figure 8-14.

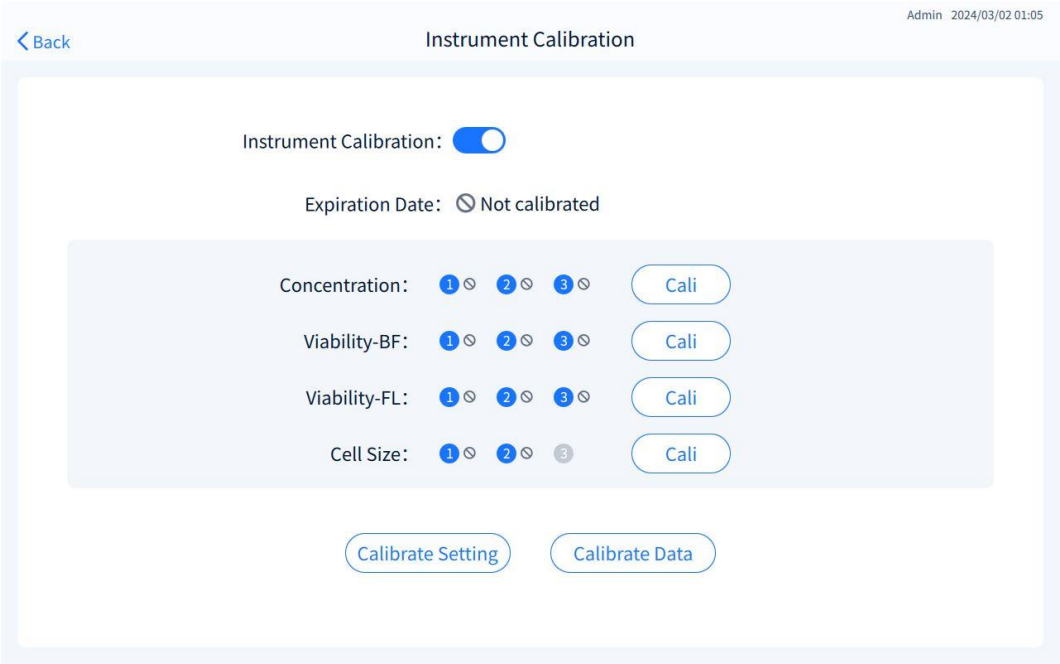


Figure 8-14

Select the item to be calibrated, such as “Viability-BF”, click the corresponding [Cali] button, insert the slide according to the screen tip, and then automatically enter the “Edit Calibration Information” interface, customize the sample name, select the standard value and dilution factor, and click [Start].

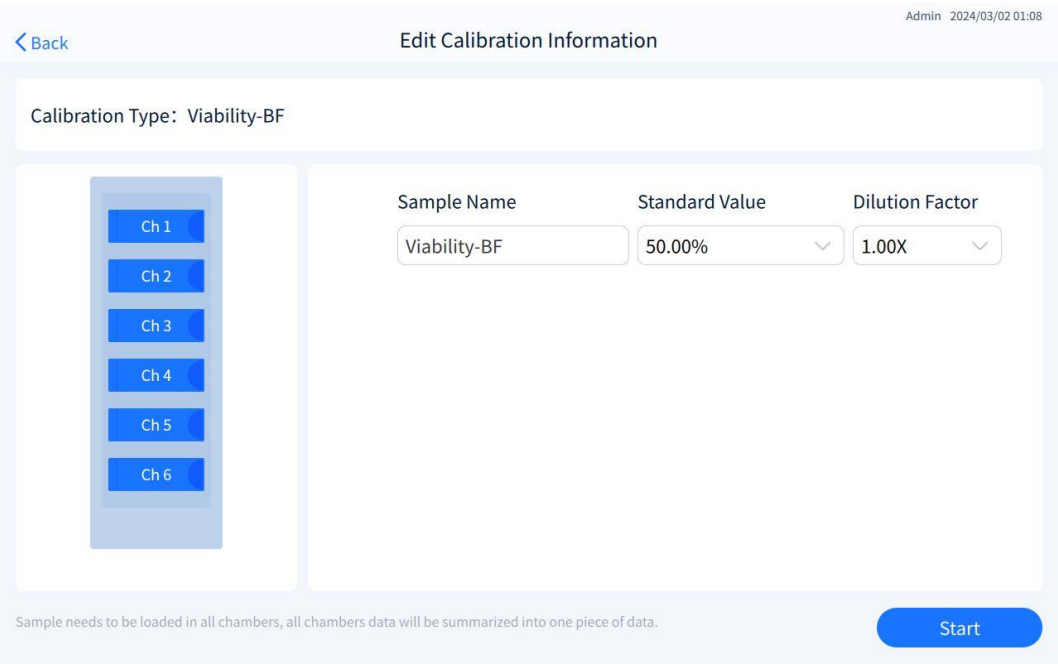


Figure 8-15

Note: *Before starting calibration, make sure that the same standard has been loaded into all chambers of this plate and matches the calibration type and standard value selected, or calibration will fail.*

Display calibration results after calibration is complete

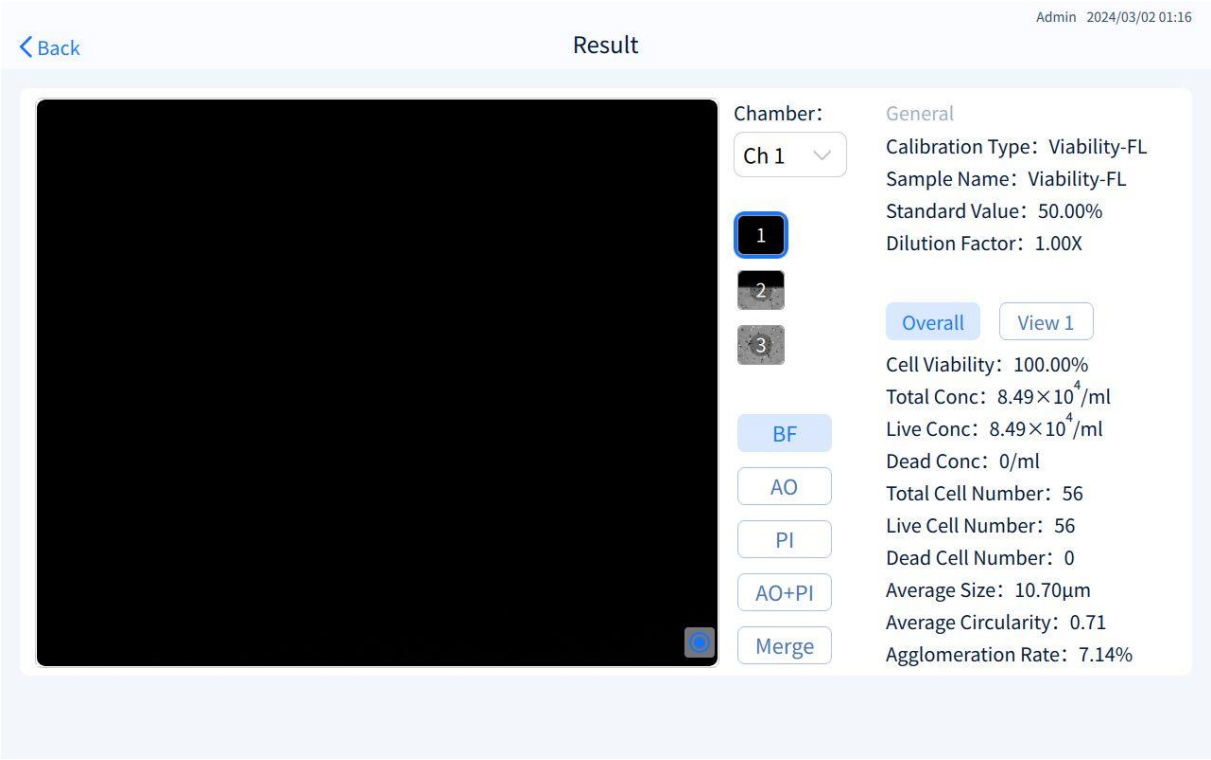


Figure 8-16

8.4.1 Calibrate setting

Click [Calibrate Setting] to enter the interface as shown in Figure 8-17, and set the calibration parameters of “Concentration/Viability-BF/Viability-FL/Cell Size”.

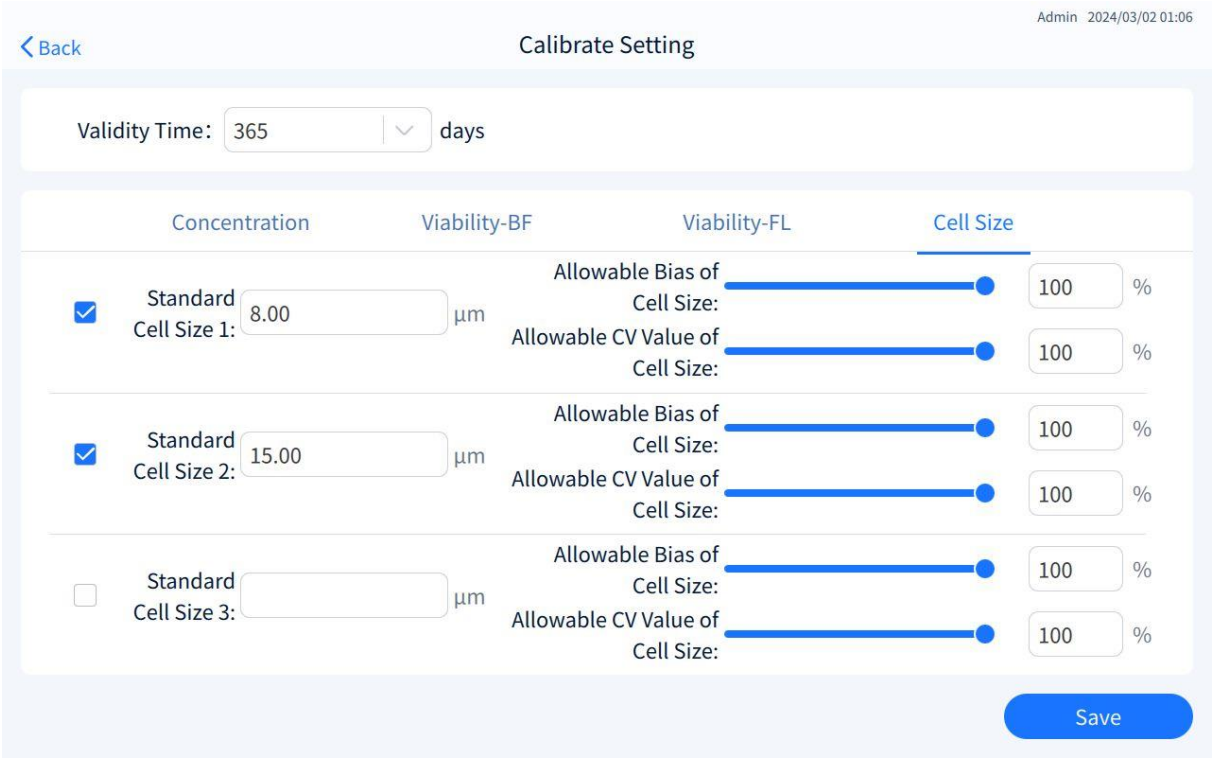


Figure 8-17

Parameters	**Description**
Expiration Date	1-365
Standard concentration 1-3 (concentration)	1.00×10^{0}-9.99×10^{9}/ml
Standard viability 1-3 (viability-BF, viability-FL)	0-100%
Standard cell size 1-3 (cell size)	3-180 μm
Allowable bias of total concentration	0-100%
Allowable bias of viability	0-100%
Allowable bias of cell size	0-100%
Allowable CV value (concentration, viability, and cell size)	0-100%

Description of calculation formula:

1) Allowable CV value

$$RSD = \frac{S}{\overline{x}} \times 100\% = \frac{\sqrt{\frac{\sum_{i=1}^{n}(x_i-\overline{x})^2}{n-1}}}{\overline{x}} \times 100\%$$

$\bar{x}$ is the average of 1-6 chambers of the sample.

x_i is the overall values for 1-6 chambers calibrated.

"CV value" in [Calibrate setting] is the CV value of 6 chambers corresponding to Concentration, Viability-BF, Viability-FL and Cell size.

2) Calibration setting - Allowable bias of viability

a) Overall viability of chamber N = sum of live cell number in 3 views/sum of total cell number in 3 views.
b) Mean viability = (overall viability of chamber 1+…+ overall viability of chamber 6)/6.
c) Bias of viability =|(mean viability - standard viability)/standard viability| *100%.

3) Calibrate setting - Allowable bias of total concentration

a) Overall concentration of chamber N = sum of total concentration of 3 views/3.
b) Mean total concentration =(overall concentration of chamber 1+…+ overall concentration of chamber 6)/6.
c) Bias of total concentration =|(mean total concentration - standard concentration)/standard concentration| *100%.

4) Calibration setting - Allowable bias of cell size

a) Overall cell size of chamber N = sum of all cell sizes in 3 views/sum of total cell number in 3 views.
b) Mean cell size = (overall cell size of chamber 1+…+ overall cell size of chamber 6)/ 6.
c) Bias of cell size = |(Mean cell size-standard cell size)/standard cell size| *100%.

Additional: The calculation formula in [XX Bias] of 2) 3) 4) is obtained after following the formula steps of 1) 2) 3). After the instrument calibration is completed, the [XX Bias] must be ⩽ [Allowable XX Bias] and the "CV value" of 6 chambers must be ⩽ "Allowable CV value", so that the calibration is considered to be passed.

8.4.2 Calibrate data

Click [Calibrate Data] to view historical calibration data, as shown in Figure 8-18.
Select a single calibration data in blue shading, and click [Result] to view the calibration results of the corresponding data.
Select a single or all to **export/delete** data.
Click the drop-down arrow to switch between different accounts and different sample names.

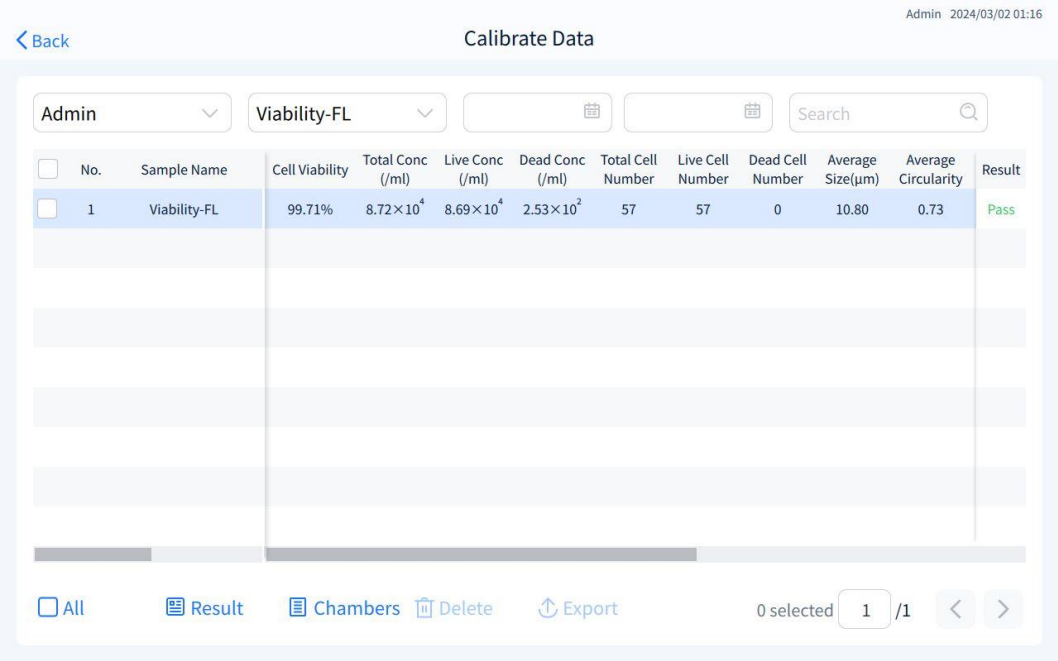


Figure 8-18

Click [Chamber Information] to view the results information of all chambers of this data (the display parameters depend on the calibration type), as shown in Figure 8-19.

Drag the slider bar left or right to browse more parameters.

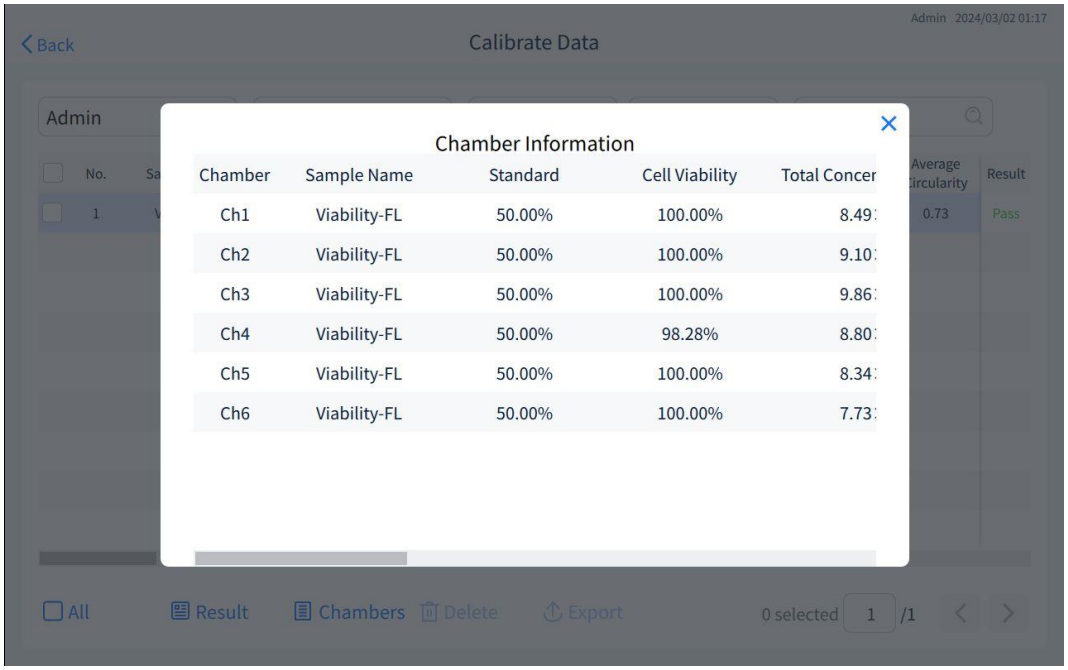


Figure 8-19

Export calibration data: Export formats include Excel, image formats include Bmp (original) and marker (jpg). Export paths include external storage device or server.

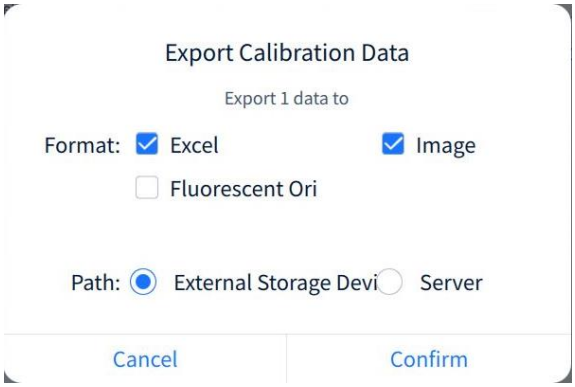


Figure 8-20

8.5 Electronic Records

The interface [Electronic Records] displays the account, operation time, operation type and operation details of this instrument, which can be filtered and viewed according to the account name and operation time range.

Click [Export] to select the export path as external storage device or server, and follow the prompts.

Click the search box to enter key words to perform a fuzzy search.

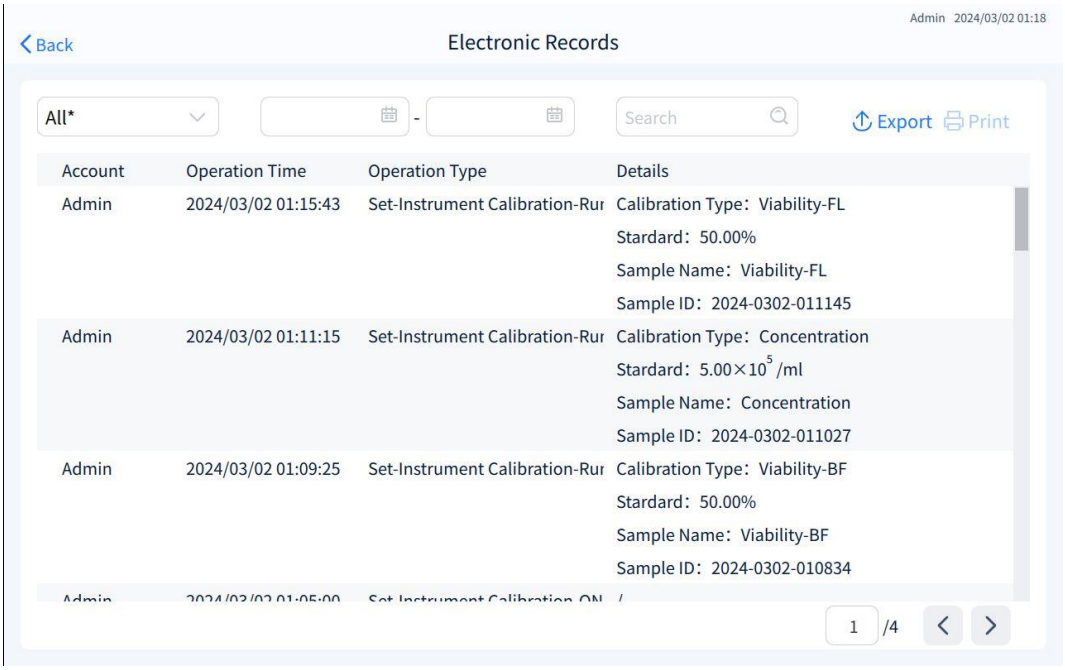


Figure 8-21

8.6 User management

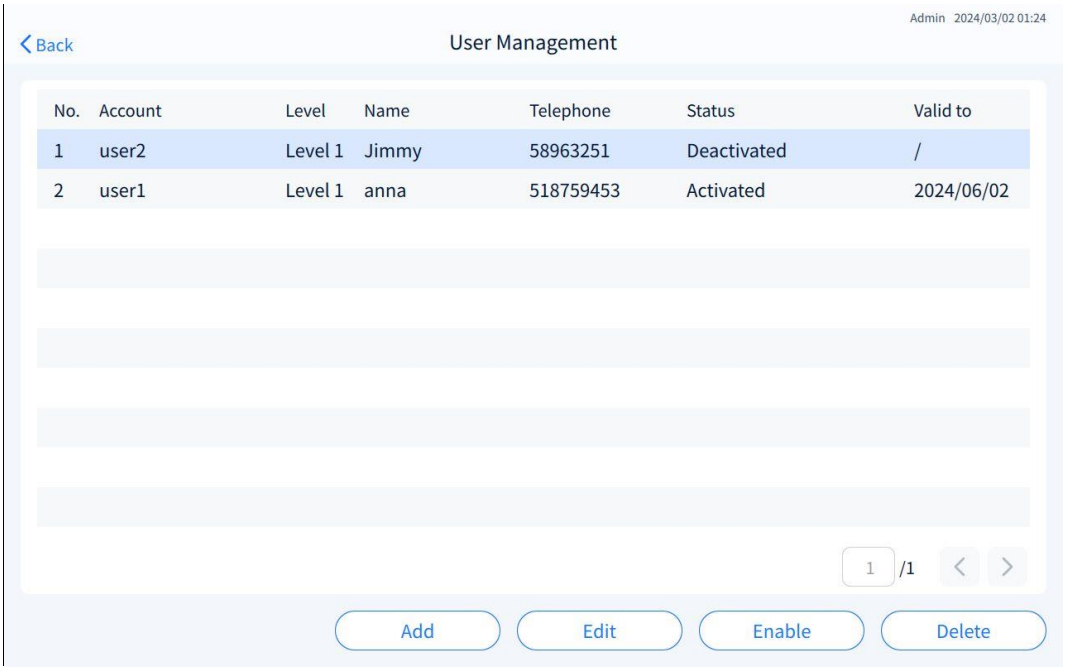


Figure 8-22

In the interface [User Management], add, delete, edit, deactivate/activate accounts, etc.

Note: *Account can only be configured by the Admin account, and has all authorizations of this instrument.*

Note: *Deleting an account will delete the count type, cell type, historical data, etc. corresponding to the user. Please use this function carefully.*

Click [Add] to enter the interface [Add Account] as shown in Figure 8-23. In this interface, you can configure account authorization, define the level, account name, user name, telephone, job number, and validity time of new users, click [Save] to complete the setting.

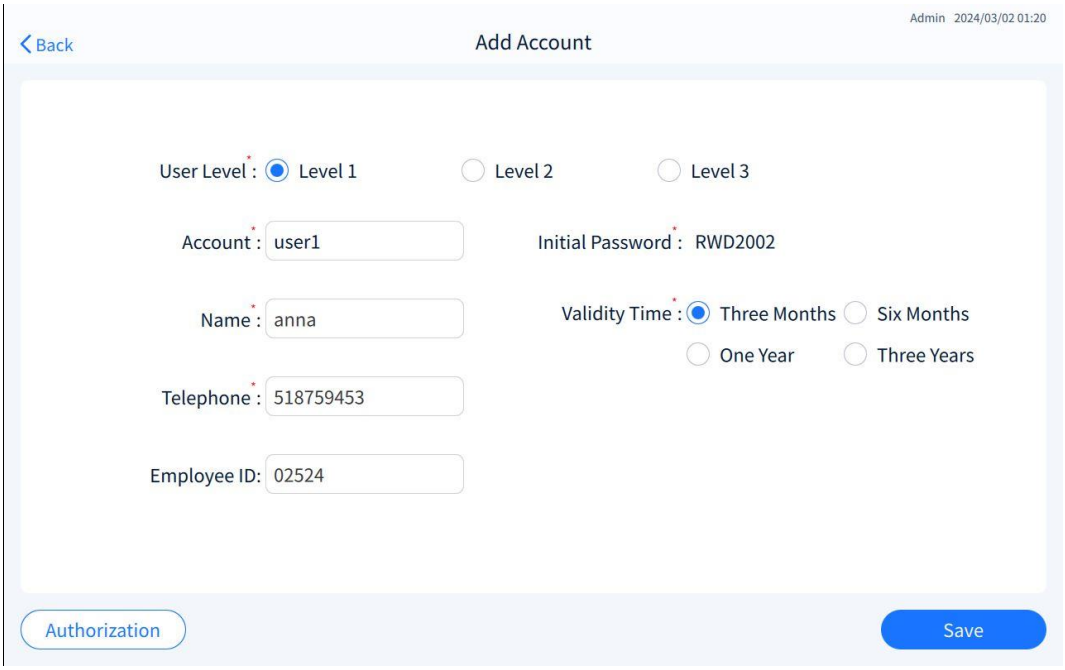


Figure 8-23

Edit Account/Reset password

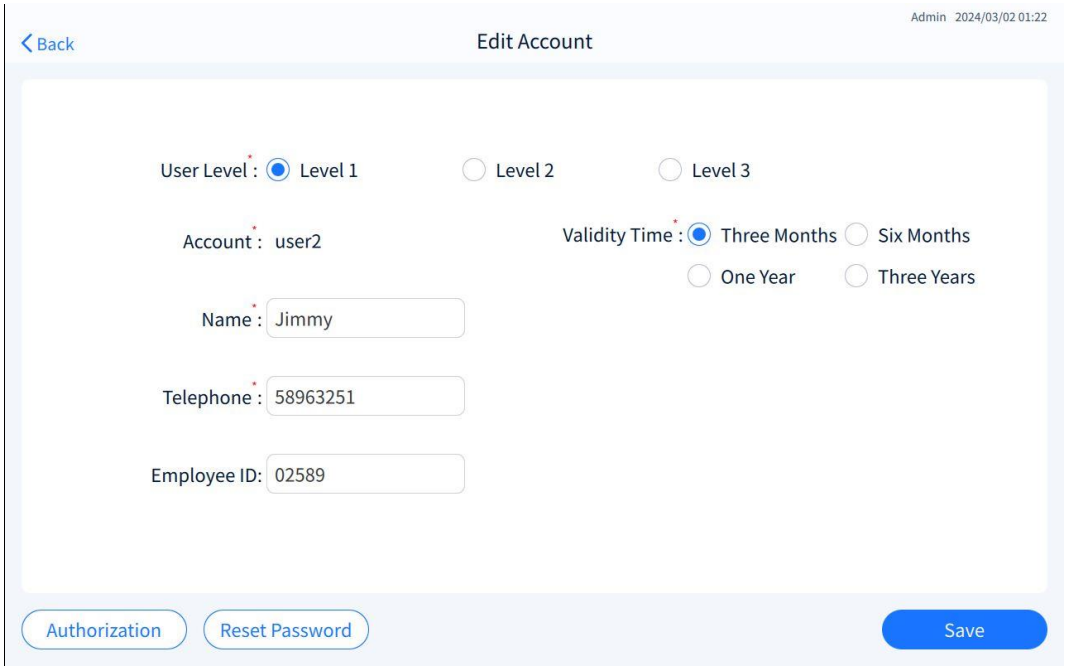


Figure 8-24

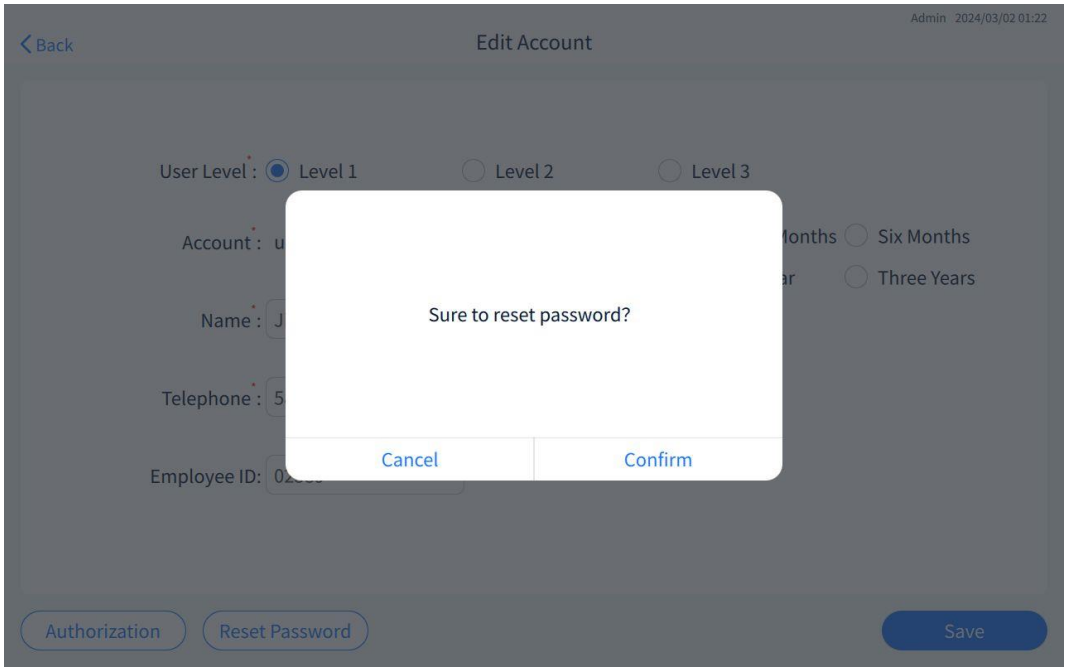


Figure 8-25

Click [Authorization] to enter the authorization interface as shown in Figure 8-26. You can set authorization for the new account. Mark √ on the authorization required for the account. Click [Confirm] to complete the authorization for the account.

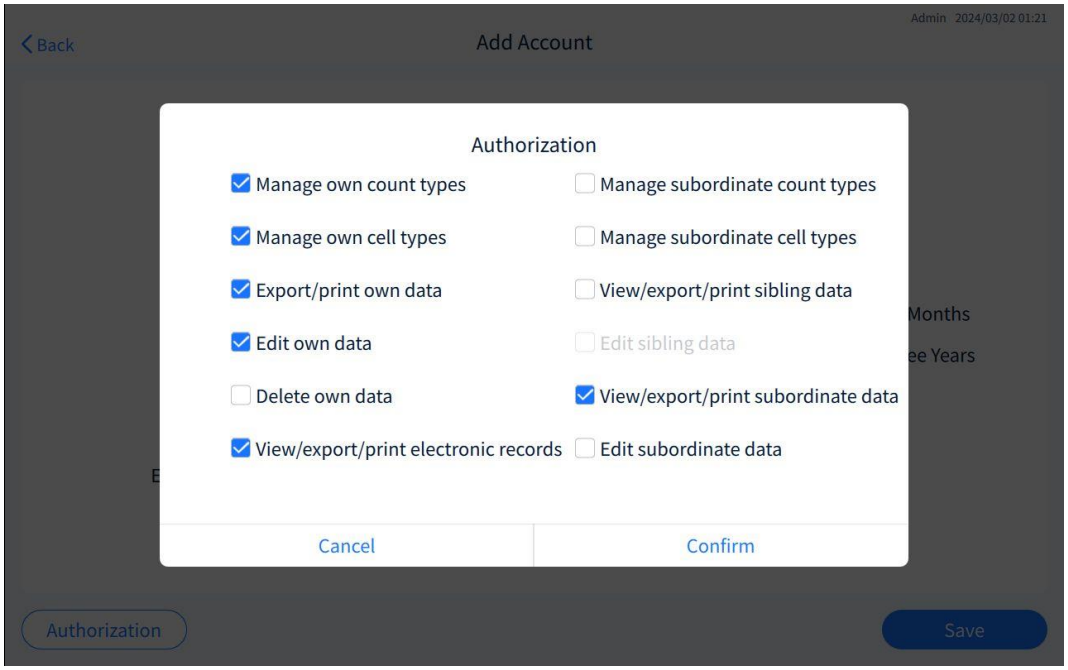


Figure 8-26

8.7 Change password

Click to enter the interface [Change Password], enter the current password, enter the new password twice, and click [Save].

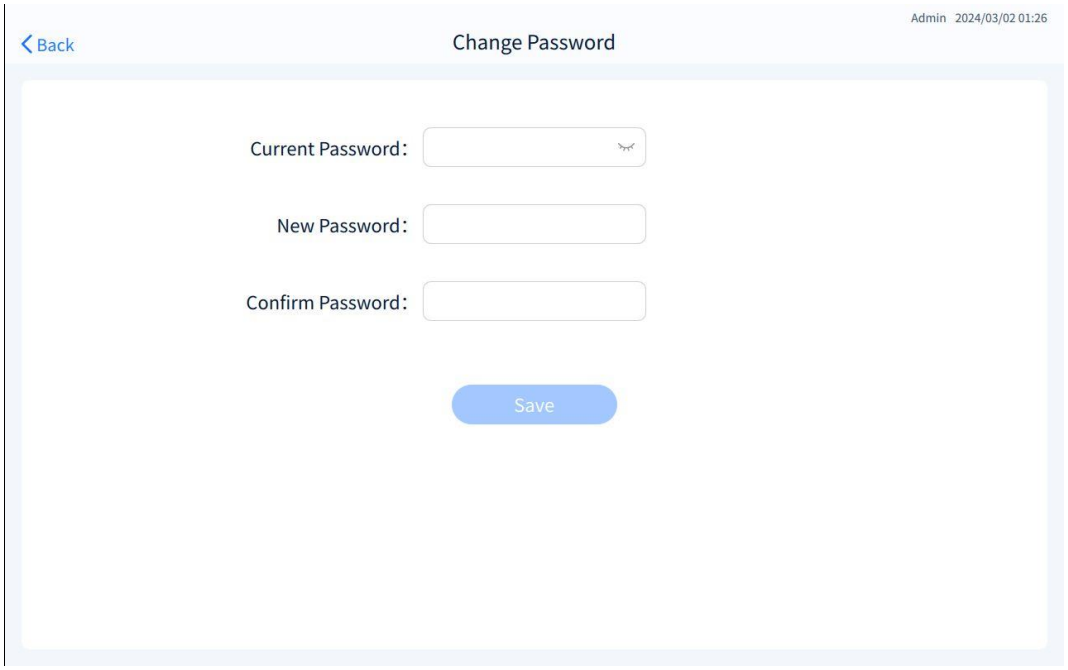


Figure 8-27

8.8 Backup&restore

Click [Reset to Factory] and enter the Admin password according to the pop-up window prompt. After the system verification passes, all sets of the device will be restored to factory settings and all user data will be cleared. Please use this function carefully.

If you need system backup, please ensure that the instrument has been connected to the external storage device, click [System Backup], enter the Admin password according to the

pop-up window prompt, and export the count type, cell type, counting data, calibration data, account, electronic records, and all system settings with one click.
If system restore is required, please ensure that the instrument has been connected to the external storage device, click [System Restore], enter the Admin password according to the pop-up window prompt, and import the count type, cell type, counting data, calibration data, account, electronic records, and all system settings with one click.

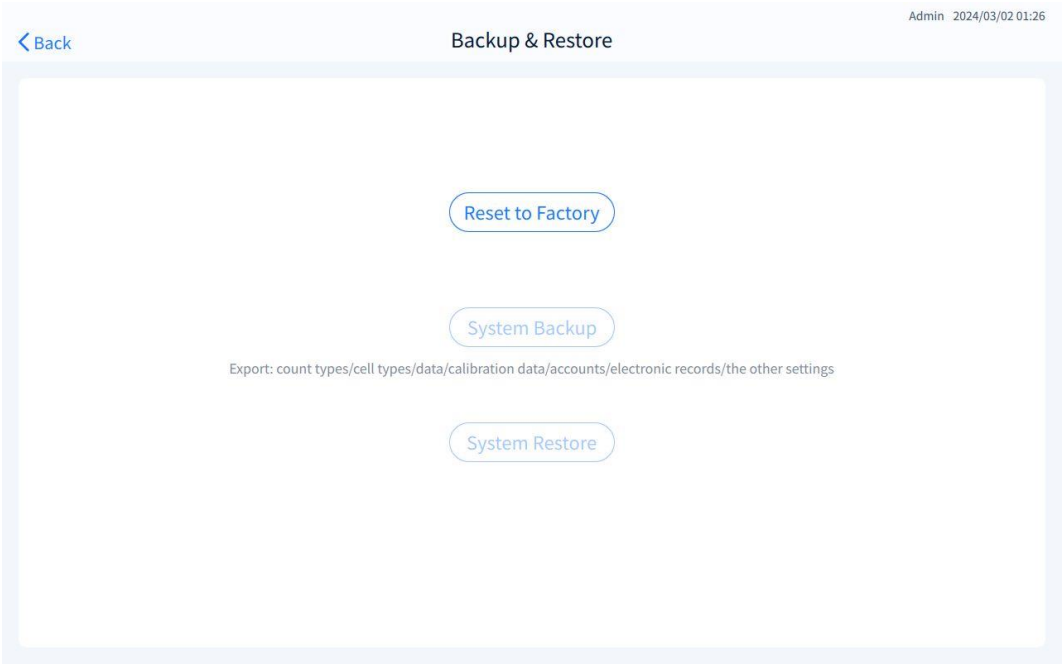


Figure 8-28

8.9 Corporate information

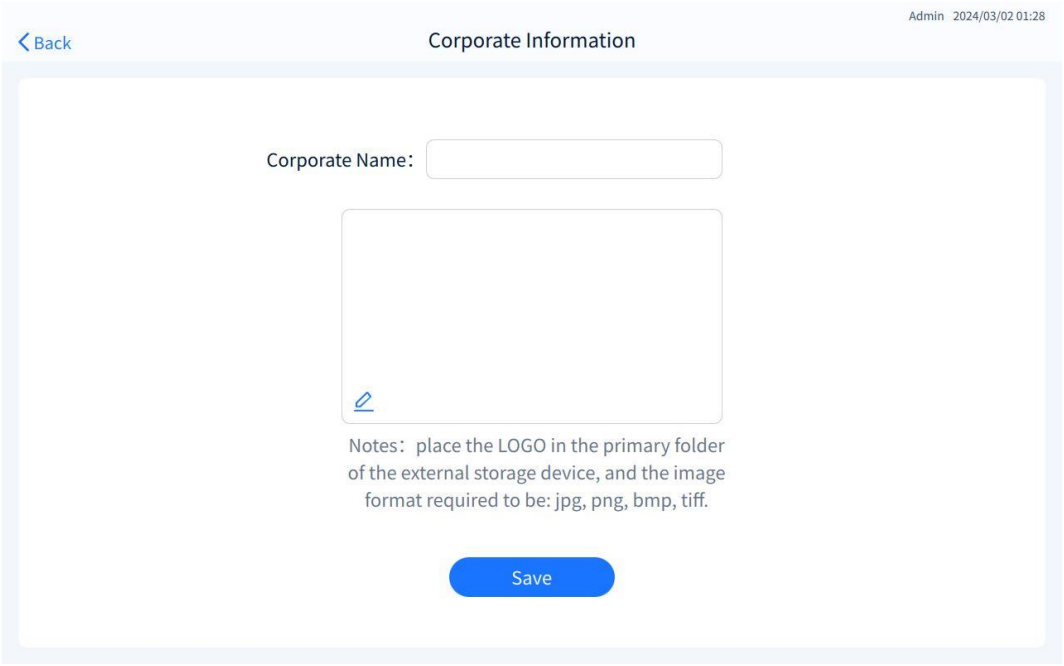


Figure 8-29

Click [Corporate Information] to enter the interface as shown in Figure 8-29. User can customize the enterprise name: Click the input box to modify the enterprise name (limited to 0-30 characters).
If you want to change the interface display image, please first make sure that the instrument

has been connected to the external device. Click  to enter the interface “Change Image”, as shown in Figure 8-30. The interface displays all the images in the external device that meet the format requirements (jpg, png, bmp, and Tiff). Select the image according to the displayed file name, and then click [Confirm] to complete the image replacement.

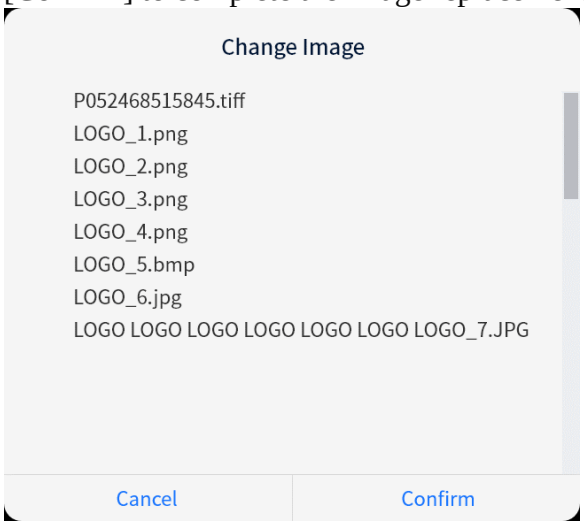


Figure 8-30

8.10 Language setting

As shown in Figure 8-31, drag the slider bar to increase/decrease the screen brightness. Adjustable range: 1-100%.

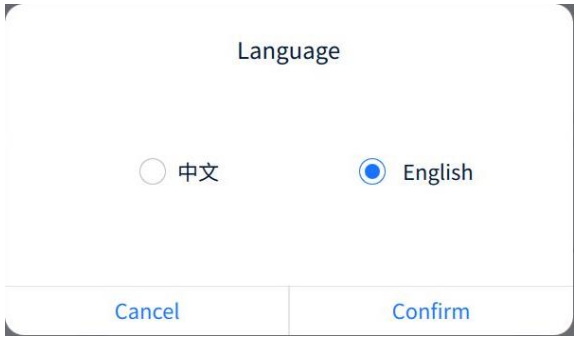


Figure 8-31

8.11 Brightness setting

As shown in Figure 8-32, drag the slider bar to increase/decrease the screen brightness. Adjustable range: 1-100%.

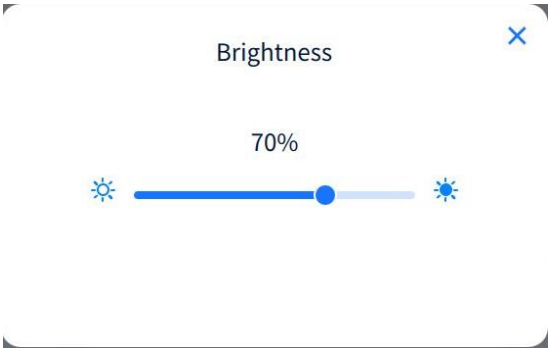


Figure 8-32

8.12 Network setting

Network connection: Wired connection and WiFi connection are available.

8.12.1 Wired connection

Select from “Auto-acquisition” or “Manual setting”. If the address automatically acquired is unsuccessful or incorrect, the user can select manual setting.

- **Auto-acquisition**

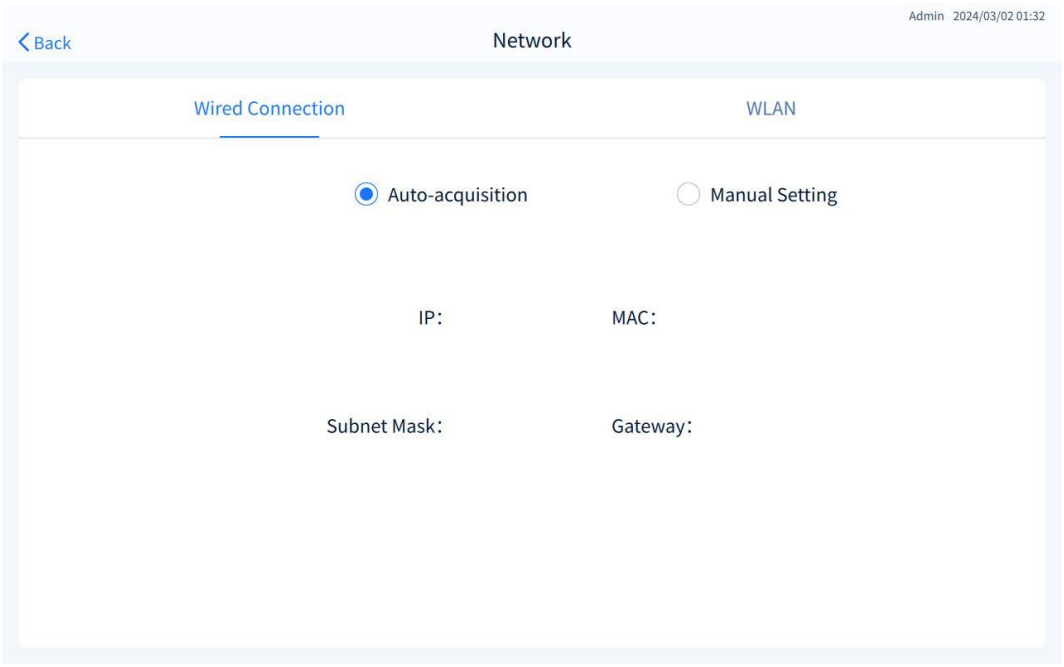


Figure 8-33

- **Manual Setting**

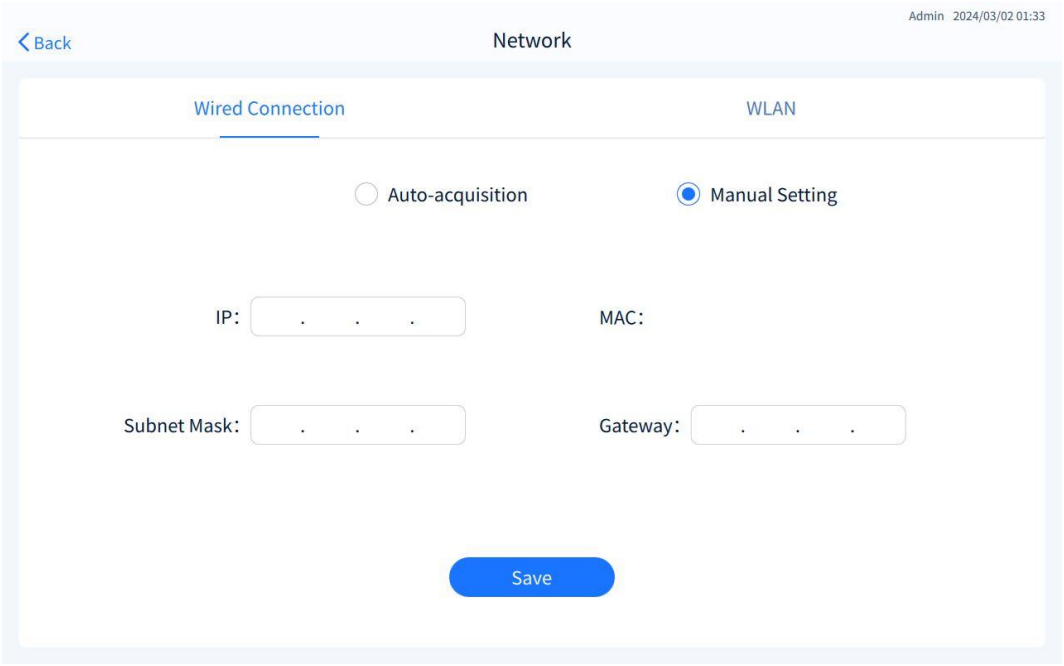


Figure 8-34

8.12.2 WiFi connection

Click on “WiFi connection”, then manually select the WiFi account name and enter the password.

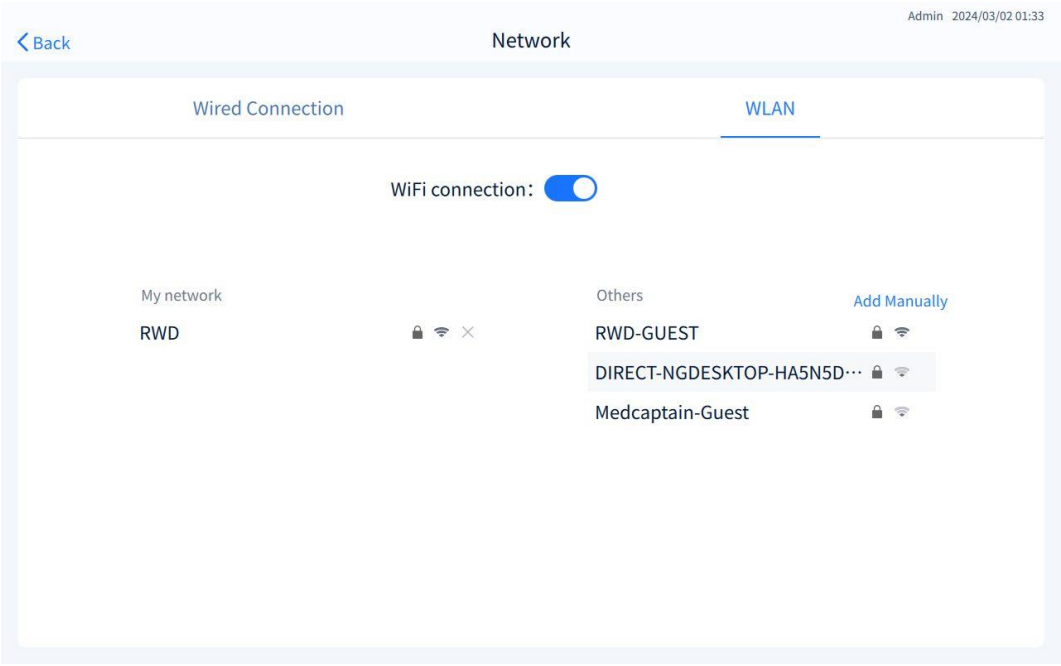


Figure 8-35

8.13 Server setting

Click [Server] to enter the interface as shown in Figure 8-36. The interface displays the current server connection status.

Real-time data upload can be enabled/disabled by click , and when enabled, the system will automatically upload the data to the server in the selected format. A single or multiple choices are available for the data format.

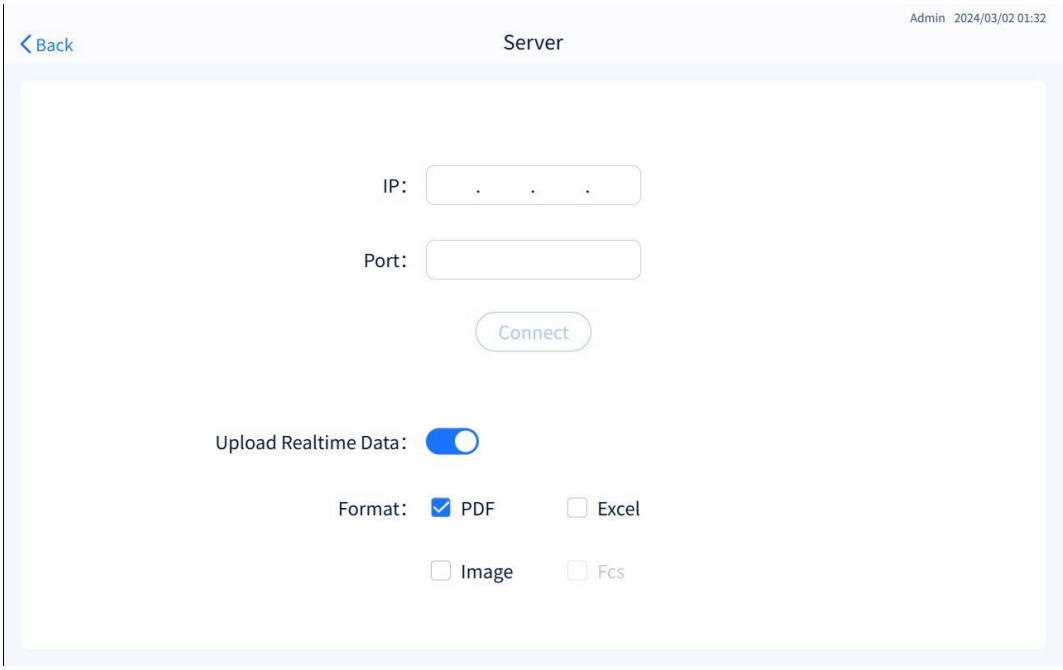


Figure 8-36

8.14 Date&time

Only administrator accounts can modify the date/time of the system. Enter the login password according to the prompt and enter the interface as shown in Figure 8-37.

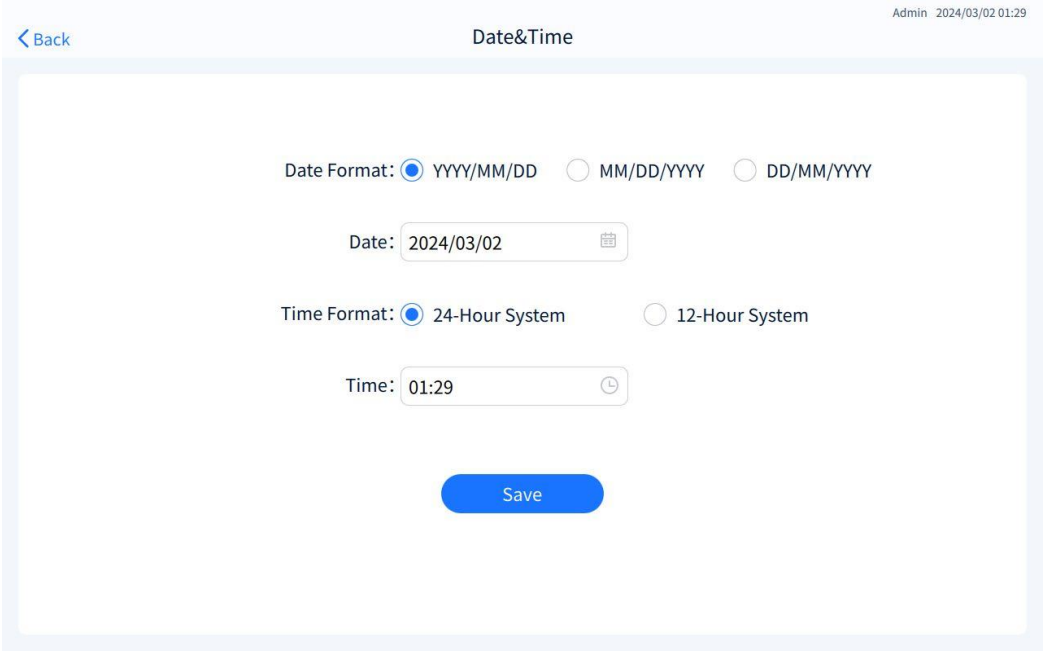


Figure 8-37

Optional date format:
YYYY/MM/DD, MM/DD/YYYY, DD/MM/YYYY

Optional time format:
24-hour and 12-hour system

8.15 Standby setting

When the user does not operate the device for a long time, the system will automatically enter the standby state. The user can customize the standby time. Click [Standby] to enter the interface as shown in Figure 8-38. The automatic standby time includes 30 minutes, 1 hour, 2 hours, 6 hours and 12 hours.

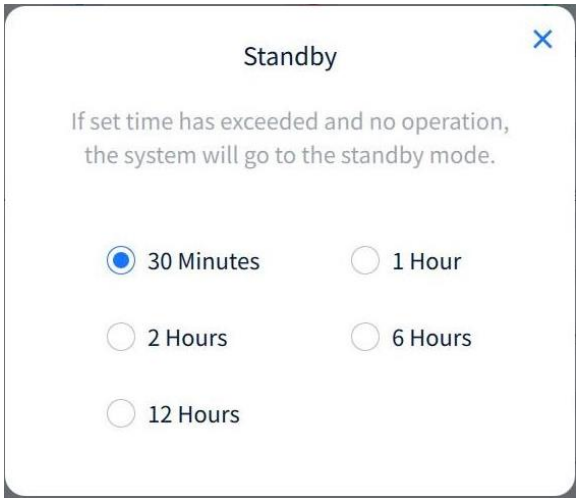


Figure 8-38

8.16 Storage space

Click [Storage] to view the storage status of the current device. Display the total device storage space, available space, and used space.

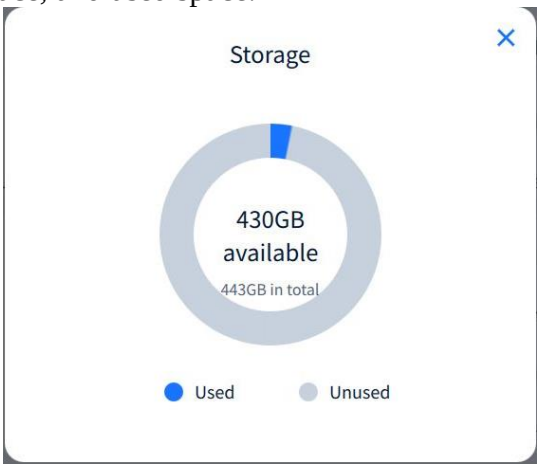


Figure 8-39

8.17 Manual

Click [Manual] to view the instrument manual and operate according to the manual.

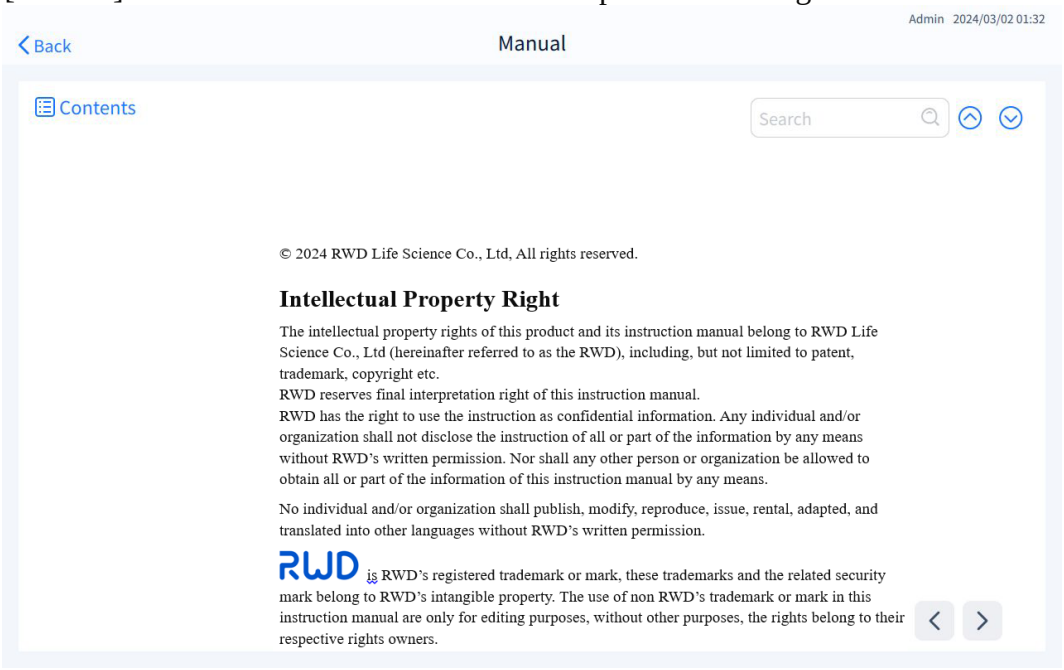


Figure 8-40

8.18 Software information

Click [Software Information] to view the current software version and upgrade the software (the upgrade function is only available to administrator accounts).

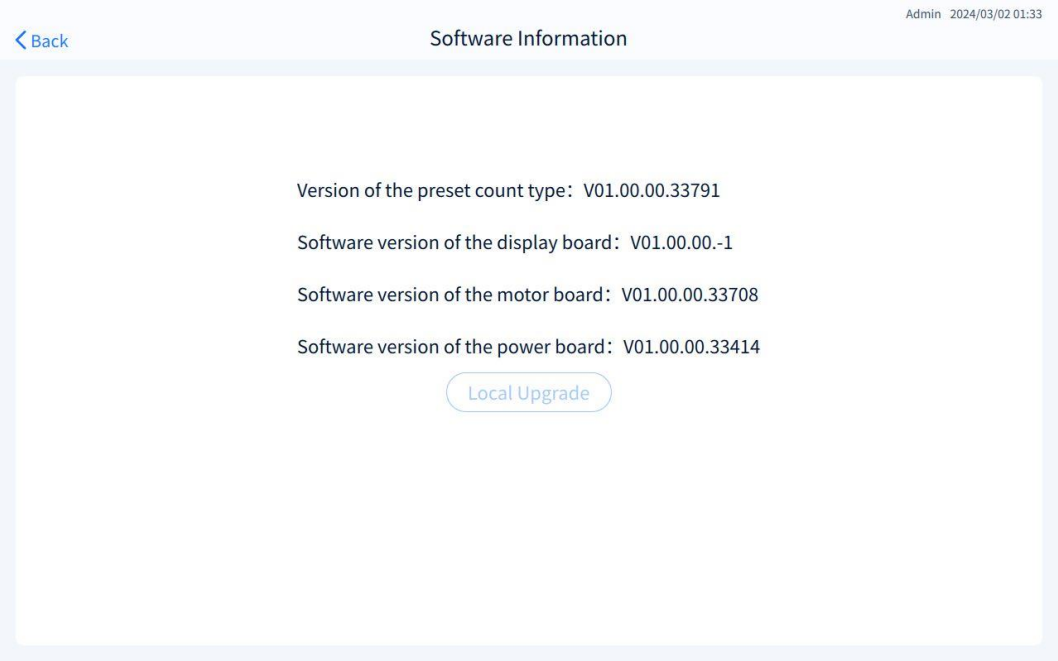


Figure 8-41

8.18.1 Local upgrade

Upgrade the latest software version of fluorescent automated cell counter to the instrument by a USB flash drive. First, copy the upgrade package of the latest software to the USB flash drive, insert the USB flash drive into the USB port. When the system successfully recognizes the USB flash drive, click [Local Upgrade]. When the system recognizes the software program in the USB flash drive, the window “Upgrade Password” as shown in Figure 8-42 will pop up. Enter the upgrade password: 00000. Click [Confirm] to enter the software upgrade interface. Please operate according to the prompt.

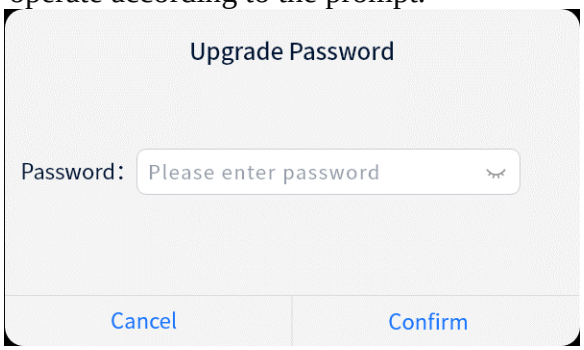


Figure 8-42

8.19 Log out

Click [Log out] to enter the interface as shown in Figure 8-43, and click [Confirm] to log out of the current login account.

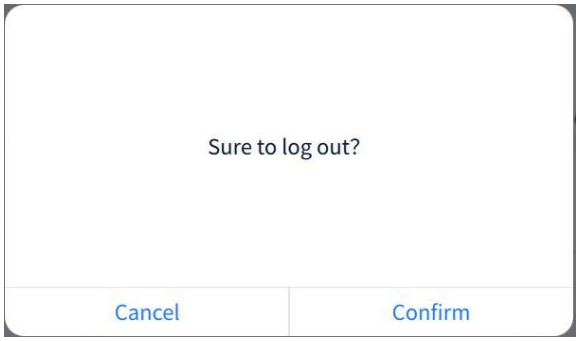


Figure 8-43

8.20 Shipping mode

To activate the shipping mode, first remove the slide and rotate the back locking handle to the locked position. Please make sure to tighten handle, otherwise the device may be damaged during transportation.

Click [Shipping mode] to enter the interface as shown in Figure 8-44, and click [Shutdown].

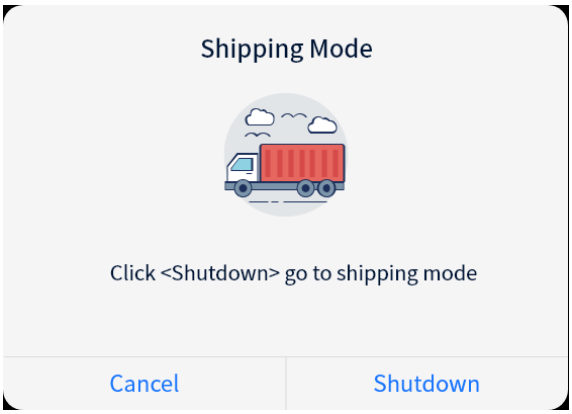


Figure 8-44

If the locking khandle on the rear of the device is not locked, a pop-up message is displayed, as shown in Figure 8-45.

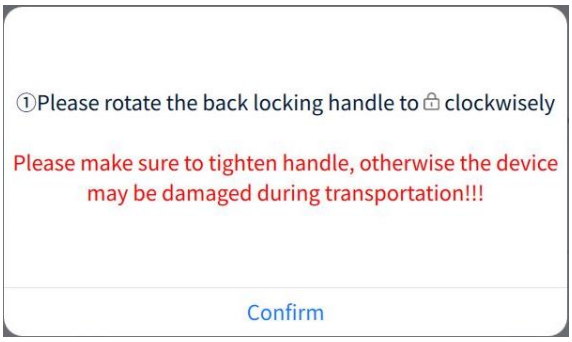


Figure 8-45

In addition to starting the shipping mode in the setting interface, long press the system switch 3s to enter the shipping mode/shutdown interface. Refer to ***Section 8.22***.

8.21 Maintenance

For after-sales maintenance personnel only.

8.22 Shutdown

In the running status, long press the system switch 3s to enter the interface as shown in the figure below. Click [Shutdown] to shut down the system.

Figure 8-46

Note: *Please make sure to shut down according to the correct method, that is, long press the system switch 3s until the system is turned off, and then press the power switch to disconnect the power supply. Do not disconnect the power supply directly, or it may affect the use of the instrument.*

9- Alarm imformation

Refer to the table below for handling alarm list.
Note: *When an alarm list appears, counting will be disabled, and the current count results will be abnormal and cannot be used as correct data.*

Error code	Alarm level	Alarm imformation	Possible cause	Solution
001	High	Feed motor failure!	1) Encoder harness not connected. 2) Encoder failure. 3) Motor failure. 4) Motor stuck by foreign matters. 5) Drive circuit failure. 6) Motor harness not connected. 7) Photomicrosensor failure. 8) Photomicrosensor harness not connected.	Please contact after-sales service.
002	High	Optical filter motor failure!		
003	High	Focusing motor failure!		
004	High	Light source motor failure!		
005	High	Camera abnormality!	1) Camera not connected. 2) Camera failure.	1) Please restart. 2) Please contact after-sales service.
006	High	Communication abnormality!	1) Serial communication cable not connected. 2) Serial communication circuit abnormality. 3) Power cord not connected.	Please contact after-sales service.
007	High	Fan fault!	1) Fan stuck by foreign matters. 2) Fan damage. 3) Fan harness abnormality. 4) Motor board fault.	Please contact after-sales service.
00	High	Please rotate the back locking handle to the end counterclockwise, and restart the device!	1) Locking handle not unlocked. 2) Lock detection sensor damage.	1) Please make sure to restart after unlocking. 2) Please contact after-sales service.

10- Troubleshooting

This chapter describes the possible failures encountered when operating the product, the possible causes of the failure, and the measures to be taken.

Failure phenomenon	**Troubleshooting**
Count results cannot be saved	1) After the program upgrade, restore to factory settings. 2) Back up the data before restoring to factory settings.
Image blurring	1) Power on the device again. 2) Refocus.
USB drive not recognized	1) The USB flash drive need to be formatted in the FAT32 file system. 2) Replace it with a USB flash drive formatted in the FAT32.
Software cannot be upgraded	1) Ensure that the USB flash drive is formatted in the FAT32 file system, and transfer the file to the USB flash drive for software upgrade. 2) The upgrade file must be located on the top layer of the USB flash drive, and cannot be placed in a folder or subfolder. 3) The file must be uncorrupted during transmission and have a.zip suffix.

11- Maintenance

11.1 Product cleaning

- **Cleaning of touch screen**

  1) Wipe the touch screen of the instrument with a soft lint-free cloth soaked in LCD cleaning solution. Do not use excessive force during cleaning. Dry the touch screen immediately after cleaning.

  2) Ensure that the cleaning solution does not enter the power button, power outlet, slide interface, or USB port.

  3) Do not pour or spray any liquid directly on the instrument to avoid electric shock when plugged into the power supply.

  4) Do not use aggressive cleaning solutions or materials to avoid scratching the touch screen.

- **Cleaning of housing**

  1) Wipe the housing with a soft lint-free cloth soaked in distilled water. Wipe dry immediately after cleaning.

  2) Ensure that water or other cleaning solutions do not enter the power button, power outlet, slide interface, or USB port.

  3) Do not pour or spray any liquid directly on the instrument to avoid electric shock when plugged into the power supply.

- **Decontamination**

  1) Wipe the instrument housing with a soft lint-free cloth soaked in 70% ethanol. Wipe dry immediately after cleaning.

  2) Avoid the use of bleach solutions, which may leave bleach crystals on the instrument.

  3) Ensure that water or other cleaning solutions do not enter the power button, power outlet, slide interface, or USB port.

  4) Do not pour or spray any liquid directly on the instrument to avoid electric shock when plugged into the power supply.

12- Warranty

The warranty of this instrument starts from the date of delivery. During the warranty period, if the instrument cannot be used normally due to material and process defects, the Company should provide after-sales services such as instrument maintenance and parts replacement.
Any damage to the instrument caused by improper use or beyond the scope of use is not within the scope of warranty. If repair or parts replacement is required, the expenses incurred should be borne by the user.
If the repaired instruments are found to have been disassembled without authorization of RWD upon arrival, the Company will not provide after-sales services such as warranty, free maintenance and parts replacement.
This warranty statement (including its limitations) is issued exclusively by RWD and covers all other warranty conditions.

Контактная информация сервисных центров

Сервисный центр Диаэм в Москве:
Адрес: 129345, г. Москва, ул. Магаданская, д.7, стр.3
Тел.: +7 (495) 745-05-08 (многоканальный), 8 (800) 234-05-08
E-mail: service@dia-m.ru
www.dia-m.ru

Сервисный центр Диаэм в Новосибирске:
Адрес: 630090, Новосибирск, Академгородок, пр. Ак. Лаврентьева, 6/1, офис 100А
Тел.: +7 (495) 745-05-08 (многоканальный), 8 (800) 234-05-08
E-mail: service@dia-m.ru
www.dia-m.ru

Сервисный центр Диаэм в Казани:
Адрес: 420111, Казань, ул. Профсоюзная, д.40-42, пом. № 8
Тел.: +7 (495) 745-05-08 (многоканальный), 8 (800) 234-05-08
E-mail: service@dia-m.ru
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Сервисный центр Диаэм в Санкт-Петербурге:
Адрес: Адрес: 197022, Санкт-Петербург, ул. Профессора Попова, д. 23, лит. Д, офис 614 (БЦ «Гайот»)
Тел.: +7 (495) 745-05-08 (многоканальный), 8 (800) 234-05-08
E-mail: service@dia-m.ru
www.dia-m.ru