RWD

RWD Life Science Co.,Ltd

C100-SE Automated Cell Counter

User Manual **A**

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CONTENTS

1-Introduction

1.1 Overview

Thank you first for choosing the C100-SE Automated Cell Counter manufactured by the RWD Life Science Co., Ltd (hereinafter referred to as the RWD)!

For better use of this product, please read the supplied instructions carefully before the initial installation and use of this product.

RWD Life Science Co., Ltd. is endeavored to improve the product function and service quality. RWD reserves the right to change or alter the contents of user manual without prior notice.

If you would like the latest product information, you are welcome to call us or visit our website (http://www.rwdstco.com/). If you find any discrepancy between the instruments and this manual during the practical use of our product, or if you have any questions or suggestions, you are welcome to contact us.

This user manual is applicable to the following Automated Cell Counter products manufactured by RWD:

- C100-SE Automated Cell Counter

1.2 Safety

In order to avoid the harm to the operator and the damages to the system, please refer to Chapter "*2-System Safety*".

If you have any questions or suggestions related to safety, please contact our company for after-sales service support.

The instrument should be operated and managed by trained professionals!

1.3 Comprehensive Description

Because of necessity to understand the living conditions of cells and identify dead and living cells during the cell culture, and to confirm the inoculation concentration and quantity of cells, and gain the cell survival rate and growth rate.

The automated cell counter collects and analyzes cell images from the count slide by the camera to detect the concentration of the cells in the sample. After trypan blue staining, the proportion of living cells can be obtained.

This equipment is suitable for relatively accurate measurement of cell quantity and survival rate for cell lines, stem cells and primary cell samples, which solves the problem of time-consuming and poor accuracy in traditional manual counting.

1.4 Product Features

- Light-weight and compact design and easy-to-operate user interface
- Accurate results of cell count and survival rate are available within 15 seconds.
- Threshold values are adjustable for cell analysis
- Disposable cell count slides are used to eliminate cleaning procedures and avoid cross infection of samples
- Each count slide consists of 2 closed chambers for loading samples, so that one slide can be used for 2 different samples or for the same sample as duplicates for data validation purpose
- The cell count data can be saved and exported

1.5 Environmental Requirements

The instruments operating environment is prepared according to the conditions listed below to ensure the operability and safety of the laser speckle imaging system.

	Description
Working environment	Temperature: 10℃~40℃
	Humidity: 10%~75%　(non-condensing)
	Altitude:＜2000m
Storage environment	Temperature: -20℃~60℃
	Humidity: 10%~93%　(non-condensing)
	Altitude:＜2000m
Working power supply	1) Use the supplied RWD AC adapter Input voltage: 90 V~264 V, 50/60 Hz, 2 A Output voltage: 24V DC; output currency: 1.5 A 2) Voltage and currency fluctuations should be within 5% of the working voltage.

1.6 Product Parameter

Parameter	**Description**
Cell size range	Recommended 7~60 μm, allowable 4~60 μm
Cell circularity range	1~100 (min./ max. size)
Cell concentration range	10^4~10^7 cells/mL
Count time	< 15 s
Accuracy of replicates	CV<5%
Stored data size	1,000 entries
Touch screen resolution	1024*600
Operation response time	100 ms
Magnification factor	2.5x optical magnification
CMOS image resolution	5 mega pixel
Digital magnification	8X magnification

1.7 User setting Parameter

Parameter	Description
Cell size	4~60 μm
Cell circularity	1~100
Cell brightness	1~255
Bright field luminance	0~100
Magnification (%)	100~400
Focus	0~2,400
Profile options	Up to 18 Profiles

1.8 Product List

Configuration	Items	Quantity	Description
Standard	Automated Cell Counter	1	Cell count
Standard	AC adapter	1	Power supply
Standard	Cell count slide	1	1 box of 50 pcs
Optional	Standard concentration beads	1	Count calibration

2- System Safety

2.1 Safety symbols

Flammable environment hazard
The instrument should not be operated in an environment with flammable gases

Electromagnetic interference hazard
Make sure to operate the instrument in a controlled electromagnetic environment to avoid any risk associated with instrument failure. Never use any transmitters such as mobile phones near the instrument. In case the instrument breaks down or requires servicing, shut down the instrument and contact RWD after-sales service.

Radiation hazard
Always follow all applicable radiation safety procedures when handling radioactive samples. Make sure to take appropriate disinfection and safety measures when disposing of radioactive pollutants. According to the relevant rules and regulations of the respective laboratories for the disposal of radioactive pollutants, operators must always wear protective clothing (such as particle protective mask, gloves, protective shoe covers). Radioactive pollutants must be disposed of in accordance with the relevant regulations.

Biological infection hazard
Samples used in the intended operation of the instrument may be infectious. For this reason, it is recommended that general laboratory regulations on infection control procedures be observed. For information on decontamination agent, its use, dilution and effective application, please refer to the *Laboratory Biosafety Manual* issued by the World Health Organization in 1984. Always follow all applicable safety procedures when handling infectious samples. Make sure to take appropriate disinfection and safety measures when disposing of infectious substances. According to the infection control procedures of the respective laboratories, operators must always wear protective clothing (such as particle protective mask, gloves, protective shoe covers). Infectious pollutants must be disposed of in accordance with the relevant regulations.

Waste disposal
All debris, wastes, infectious and radioactive pollutants generated during operation must be disposed of in accordance with corresponding laboratory regulations. Disinfectant, cleaning solution and section waste must be disposed of in compliance with special waste disposal regulations. Reagents must be disposed of as described in the manufacturer's Safety Data Sheet (SDS).

2.2. Safety Precautions

2.2.1 General safety requirements

a) Please follow the instructions of all safety warnings and labels in the manual and on the instrument. The instrument can only be used by trained professionals. The instrument is only

allowed to operate within the scope of application, and only the components and accessories suitable for the instrument can be used. The manufacturer will not be held liable for any damage to the instrument caused by any improper operation, including damage to the third party.

b) The maintenance of the instrument is allowed, but only to the extent of recommended maintenances stated in this manual. In order to ensure safe operation of the instrument and the instrument works properly, it is necessary to perform the manufacturer recommended maintenance on a regular basis. Trained and qualified authorized maintenance personnel or engineers must be able to complete the instrument maintenance services not mentioned in this manual. Any change to the instrument without the manufacturer's authorization may cause failure of the instrument.

2.2.2 Chemical safety

In order to use the electrical devices supplied with the instrument safely, the following provisions should be followed:

a) Before connecting the instrument to the mains, check whether the working voltage settings of all components are correct. A power supply compatible with the instrument must be used. See the model label of the instrument for the compatible power supply.
b) Use a national standard three-hole grounding socket to connect the instrument and peripheral apparatuses, and use a branch power with fuse and ensure it is properly grounded. Make sure that the peripheral apparatuses are connected with protective ground wires, and that the ground wires are unblocked. If the fuse is missing or not installed, or the ground wire is not connected, there may be a risk of electric shock.
c) Make sure the power cord is clean.
d) If any potential unsafe operation is detected, turn off the power and remove the plug immediately to disconnect the mains.
e) If any liquid comes into contact with the circuit of the instrument, turn off the power and remove the plug immediately to disconnect the mains, and wipe the moistened parts of the instrument dry in a timely manner.
f) Always keep the bench dry during operation.
g) The fuse type (rated voltage, rated current and model) must comply with the manufacturer's specifications. Do not use any unserviceable or used fuse, and avoid short circuit of the fuse.
h) The instrument must be grounded to prevent accidental electric shock

If potentially hazardous waste is generated during operation of the instrument, you must:

a) Determine the nature of the waste (analyze if necessary) according to the specific use, reagents and substrates used in the laboratory.
b) Protect the health and safety of all staff in the laboratory.
c) Always store, transfer, transport and dispose of instrument waste in accordance with local regulations.

2.2.3 Chemical waste safety

Caution! Hazardous waste. Please refer to the SDS and relevant local regulations when handling and disposing of waste.

To minimize the risk of chemical waste, you must:

a) Read and understand the SDS provided by the chemical manufacturer in the waste container before storing, handling or disposing of chemical waste.
b) Provide main and auxiliary containers for storing waste. (The main container is used to store generated waste. And the auxiliary container is used to store waste spilled or leaking from the main container. Both containers must be compatible with the chemical properties of the waste materials and comply with local regulations on waste storage.)
c) Minimize exposure to chemicals. While handling chemicals, always wear appropriate protective articles (such as protective glasses, gloves or protective clothing). For detailed safety guidelines, please refer to SDS.
d) Minimize the risk of inhalation of chemicals. Do not let chemical containers open. Use chemicals only in a well-ventilated environment (e.g. fume cupboard). For detailed safety guidelines, please refer to SDS.
e) Handle chemical waste only in a well-ventilated environment.
f) After the waste containers are emptied, use the attached cover to seal the containers.
g) Waste in trays and bottles should be disposed of in accordance with good laboratory practices and local environmental and health regulations.

2.2.4 Electrical safety

a) Do not disassemble the instrument to prevent electric shock.
b) When replacing fuses, use fuses of the correct type and rating specified for the instrument.
c) The continuity of the grounding circuit is critical to the safe operation of the instrument. Do not operate the instrument when the ground wire is disconnected.
d) Connect the instrument to a properly grounded outlet with appropriate current.

2.2.5 Biosafety

Biological samples (such as samples of human and other animal tissues, body fluids and blood) have the potential risk of transmitting infectious diseases. Always follow all applicable local regulations. Wear suitable protective glasses, protective clothing and gloves.

2.2.6 Electromagnetic compatibility

This section provides the following information:

European security and EMC standards

The CE sign indicates that the instrument conforms to all applicable European Community provisions for which this marking is required. DO follow the conditions described in this manual for operating the instrument.

3-Product structure

The C100-SE Automated Cell Counter is composed of the cell counter host and cell count slide.

- **Automated Cell Counter**

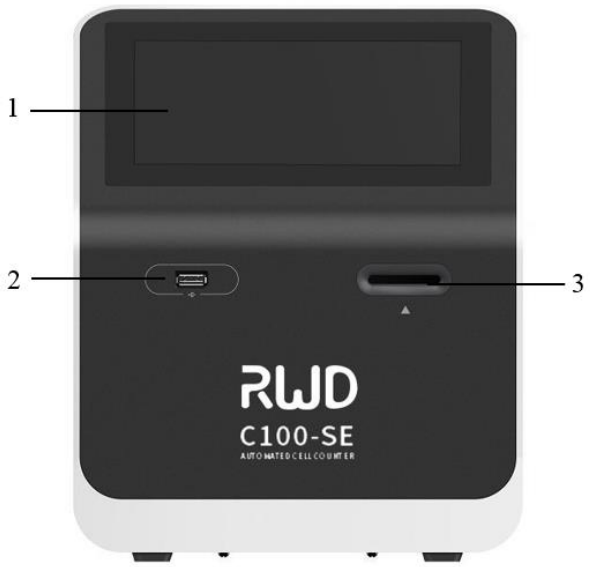


Fig. 3-1

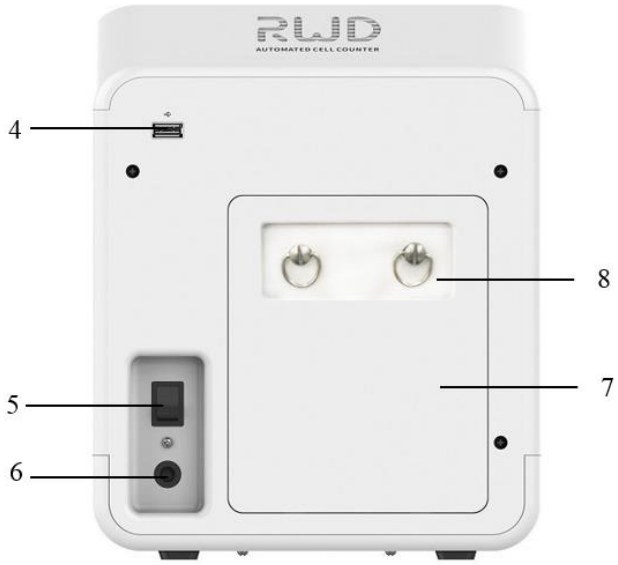


Fig. 3-2

① Touch screen display: 7 inch capacitive screen.

② USB port 1: the count data and image can be downloaded onto an external device with the USB port for storage. USB drive format: FAT32.

③ Count slide port: used for inserting the count slide loaded with samples.

④ USB port 2： The function is the same as USB port 1.

Note: two USB ports are provided at the front and rear of the instrument for transferring data. And the instrument cannot read two USB at one time.

⑤ Power button: main switch.

⑥ Power outlet: uses must connect the power outlet of the instrument to an electrical outlet with the supplied power cord and an appropriate plug by checking the electrical outlet configuration in your country.

⑦ Rear plate.

⑧ Locking screw: the locking screw of the back cover panel that can be loosened by counterclockwise rotation.

- **Cell count slide**

The plastic disposable closed count slide consists of 2 chambers for loading samples, which can be used for loading the same sample as duplicates into 2 chambers for reproducibility validation. Cell count is performed in the center of each chamber. The total volume of cell for count is 20μL, which is equivalent to two small squares in a standard blood cell counter.

Note: In order to achieve the best results with the counter, do not touch its optical surface and always wear gloves when handling samples.

4-Preparation

4.1 Start-up

When the instrument is properly wired, the cell counter can be started. Press the power switch to start the instrument and enter the main interface as shown in the picture below.

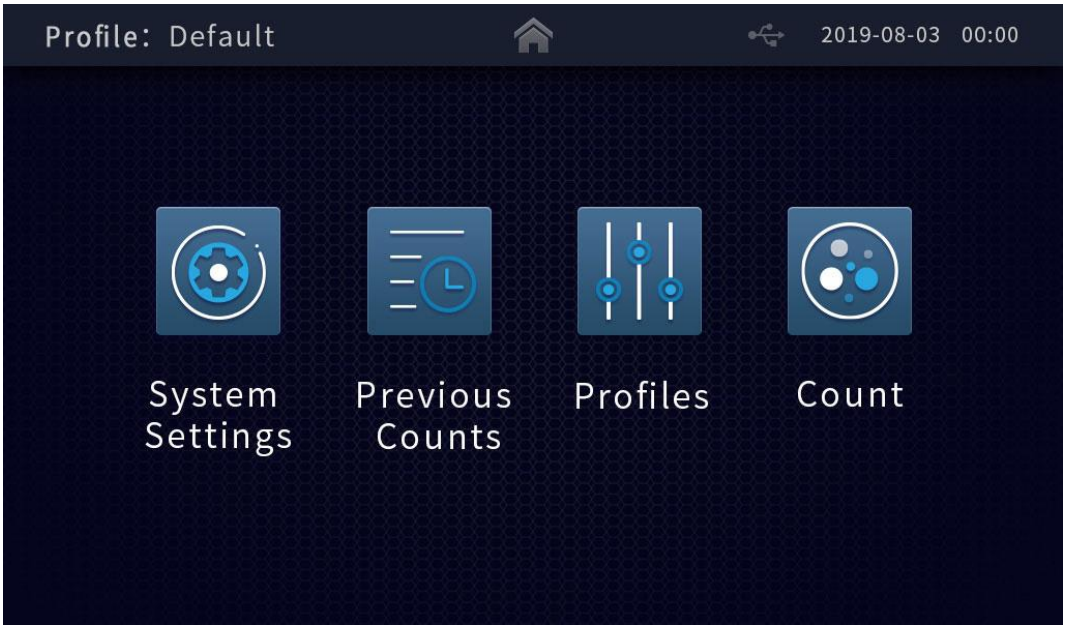


Fig. 4-1

The main interface contains four parts: System Settings, Previous Counts, Profiles, and Count.

Note: If directly inserts the count slide while in this interface, the system will automatically enter the Count page, on which operator can start count immediately according to the default settings and get the count results. You can also set the cell size, brightness and circularity on the Profiles page as needed to filter the count results.

4.2 Profiles Setting

In the BF assays, set the threshold values of the count results based on the size, brightness and circularity by the Profiles slider.

The Profiles page allows to adjust the threshold values of the Profiles, rename and save them, so that the adjusted Profiles can be called in the next cell count.

Bright field (BF) Mode

Click [Profiles] on the main interface to enter the Profiles setting page. The instrument is in Non-Trypan Blue mode by default. In this mode, the total cell concentration, histogram of total cell size distribution histogram and average size are available for view.

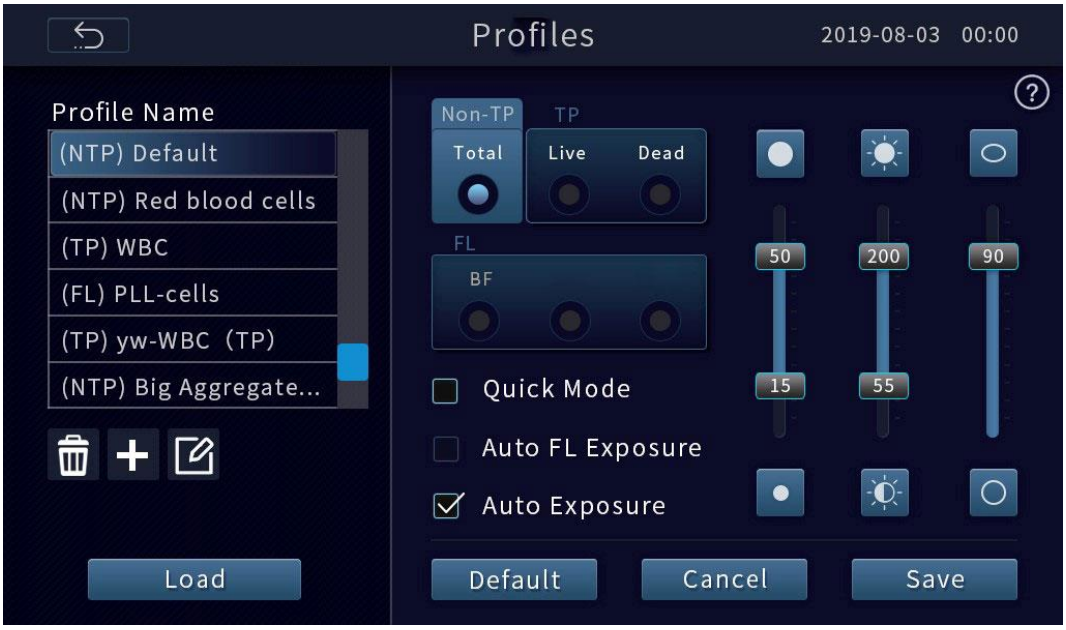


Fig. 4-2

Click [Trypan Blue] to switch to the Trypan Blue mode. In this mode, the total cell and dead/ live cell concentrations, histograms of dead / live cell size distribution and average size are available for view.

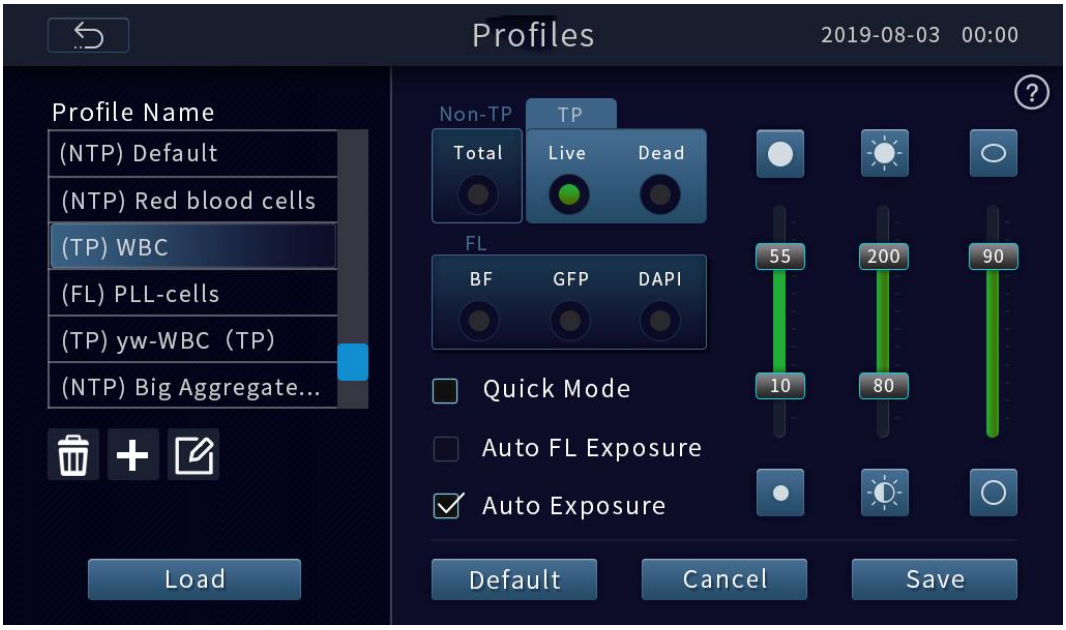


Fig. 4-3

4.2.1 Adjusting parameter

Size, brightness and circularity sliders

Size: Drag the slider to cause the system to screen the cells according to their size while counting.

= Larger target = Smaller target

Size screening: the device default range of 7-60 μm, the lower limit of identification can be up to 4 μm;

When there are many fragments in the sample, the interference of cell debris can be eliminated by adjusting the recognized size range and setting the threshold value.

Brightness: Drag the slider to cause the system to screen the cells according to their brightness while counting.

= Target with higher brightness =Target with lower brightness

Circularity: Drag the slider to cause the system to screen the cells according to their circularity while counting.

= Target with lower circularity = Target with higher circularity

The circularity slider only sets a single threshold value; cells within the set value range are counted, and cells beyond the range are excluded. To adjust the circularity threshold, drag the slider toward the target.

Click the [help] symbol in the upper right corner of the interface to learn more about the concepts of size, brightness, circularity, and so on. As shown in figure 4-4

Fig. 4-4

Quick Mode

By checking [Quick Mode], you will perform count in a quick mode; otherwise, you will perform count in a standard mode. After selecting the count Profiles in the Quick mode, while the count slide is recognized, the device will calculate the cells and output the results; in the normal mode, the focusing results will be presented first, and then the count results will be output after manually clicks the [Count] button.

The fast mode is suitable for cell samples with no debris and moderate concentration. It can automatically focus and count after inserting the count slide, which can greatly reduce the counting time. Even if the imaging effect in the counting results is not ideal, you can click the back key to enter the Count interface again for manual fine-tuning.

Auto Exposure

Choose whether to activate [Auto Exposure]. The Auto Exposure features allows you to adjust the brightness respectively depending on the characteristics of cells for count, and then choose whether to restore the system to the default brightness after count.

Restore Default

Click [Default] to restore part of the initial settings, including Auto Exposure, Quick Mode, Standard Mode, threshold value of each Profiles, etc.

4.3 Load Profiles

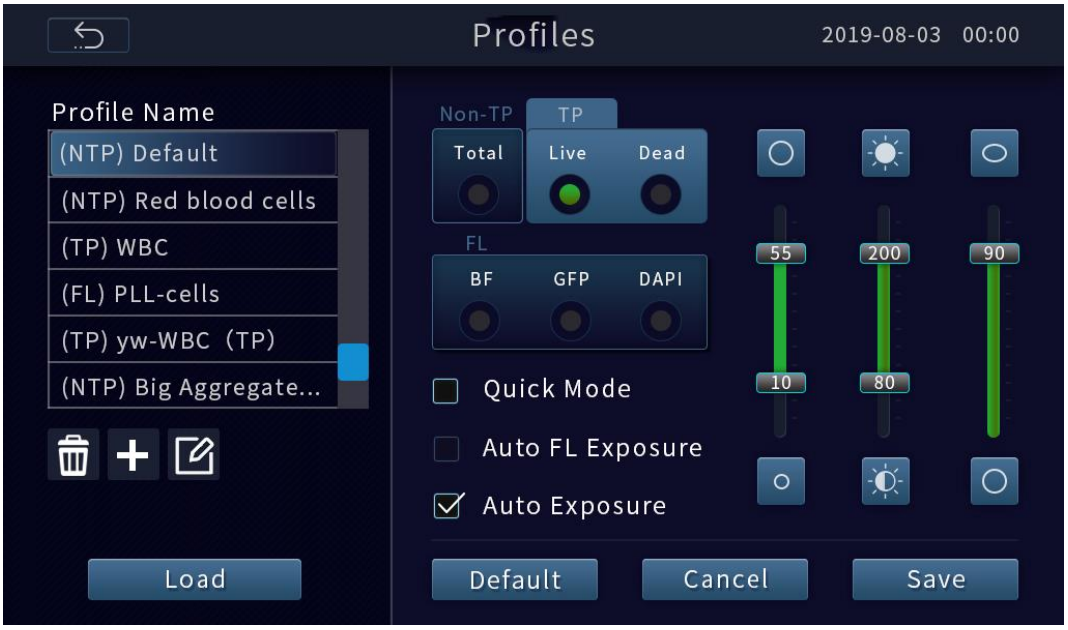


Fig. 4-5

In the left column of the Profiles page, you can delete/ add/ rename Profile protocols, corresponding to  icons respectively. Here, in addition to the Default and Red Blood Cells profiles, up to 16 profiles can be added, which can be called directly before and during count. Each Profile protocol defines the count Profiles (size, brightness, circularity) to simplify the workflow. After selecting a protocol, click [Load], then the specified count Profiles will be applied to all new cell counts. If you have already completed count, by applying new data from the Results page, you can apply the new count settings to the current count results (total cells, survival rate, etc.) and all new counts.

4.4 Sample Preparation

For best results, the instructions below should be followed:

1) Make sure the cell suspension is well mixed before taking the cell sample. After the cell sample is left standing for a long time, it is necessary to flick the tube or gently blow the cell suspension and draw the sample from the center of the tube rather than the bottom of the tube.
2) Cell concentration range: 1×10^4–1×10^7 cells/mL, optimally 1×10^5–4×10^6 cells/mL.
3) In order to get accurate cell survival rate analysis results, make sure that the count area is covered with cell suspension, and start count immediately after staining according to the analytical protocol.
4) Do not press the optical surface of the count slide. Hold the count slide by the edges.
5) Be careful not to produce bubbles in the sample.
6) As far as possible to minimize the amount of debris in the cell sample. No matter which counting or staining method is chosen, it is difficult to count and assess the survival rate of samples containing large amounts of debris. The debris may come from trypan blue stain or cell samples. Possible treatments for different sources of debris:

- The trypan blue stain was centrifuged to obtain the supernatant;
- The cells were centrifuged and then re-suspended.

4.5 Loading Samples into Slide

1) Mix 10 μL of the cell suspension with 10 μL of 0.4% Trypan Blue Stain to prepare the sample. Pipette up and down repeatedly to mix well.
2) Gently draw 10 μL of sample and load into the upper semilunar chamber. The sample is then loaded onto the count slide under the action of capillary effect.
3) Wait for 30 seconds, and insert the sample-loaded slide into the slide port. When completely inserted, a soft click can be heard.
4) To remove the count slide, just push it gently into the slide port until it clicks and is pushed out under spring pressure. Hold the count slide by the edges to take it out.

Note: Do not reuse the disposable count slide, which may influence the count results.

5-Cell count and cell survival rate assays

If the system recognizes insertion of the count slide, the Count page will pop up to show the cell view. This page allows you to perform such actions as magnification, focused fine-tuning and light source brightness adjustment, so as to capture optimized images for accurate count of cell concentration or dead/ live cells.

5.1 Bright Field Assays

Count page of Trypan Blue mode / Non-Trypan Blue mode

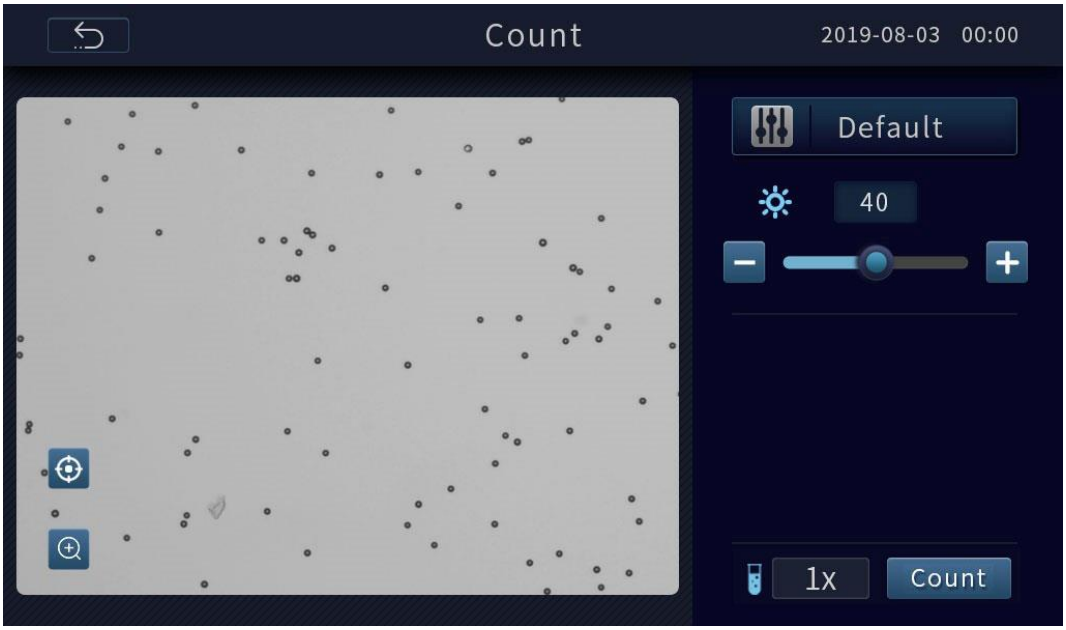


Fig. 5-1

in the figure above shows the active Profiles protocol name. Click this icon to enter the Profiles page.

 is where you can adjust the light source brightness. If you check [Auto Exposure], the results are auto adjustment values by default. You can also manually adjust the brightness of light source by clicking  &  . Adjustment range: 0-100.

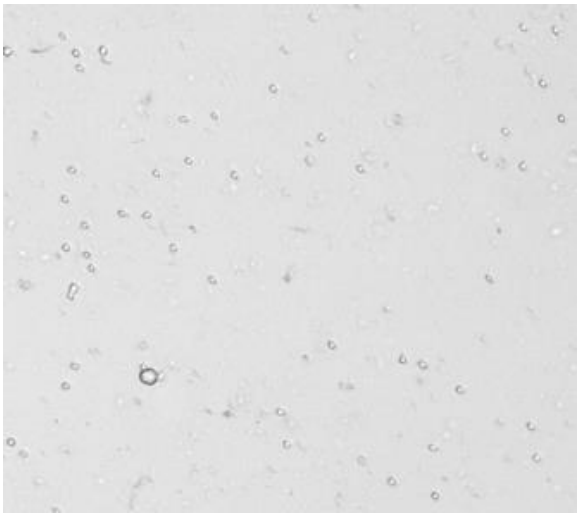
For setting the dilution factor of the sample. Click “1×” to pop up a numeric keypad. The range of setting value: 1~999.

Dilution factor of sample:

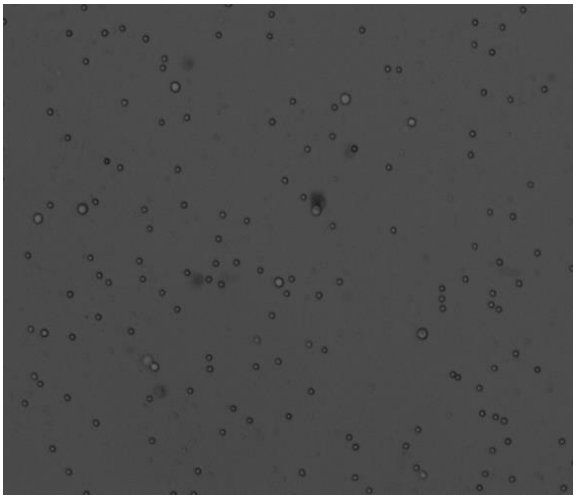
The cell concentration counted by cell counter is the actual concentration of the cells on the current count slide, which may be obtained by dilution of the original solution, so you need to convert the current concentration into the concentration of the original solution to calculate the volume of the original solution that required for dilution to the target concentration.

Too high or too low brightness has an effect on imaging quality. In general, when automatic exposure is turned on, better bright field white light illumination can be obtained automatically when

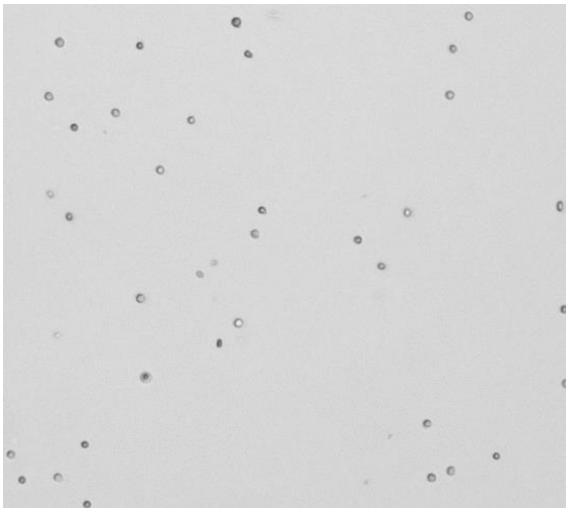
entering the imaging interface. The overexposed, moderate and underexposed effects of the bright field are shown in Figure 5-2 (A~C) below



A



B



C

Fig. 5-2

A-overexposure: the light source is too bright, and the background is white, and the cell details are covered by light, which affects cell recognition.

B-underexposure: the light source is too low, and the background is too dark, and the cells as a whole are too dark, which affects the judgment of the live and the dead.

C-normal exposure: moderate brightness of light source, clear outline of cells, live and dead cells show significantly different transmittance characteristics.

5.1.1 Focusing

The  icon in the lower left corner is for focusing. Click the icon for auto focus, or click it to get the regulator slide 

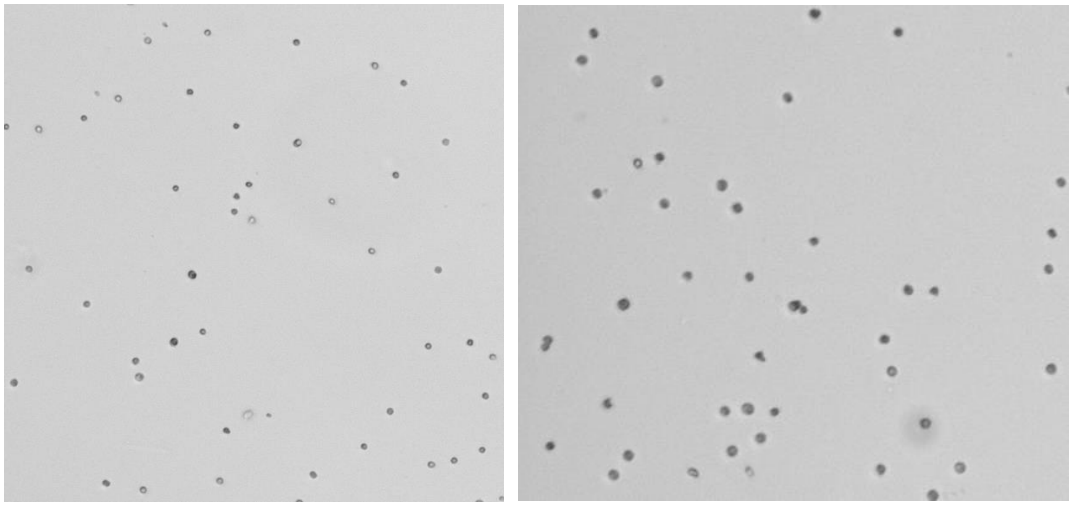
, and then click  and  to focus. Adjustment range: 0-2400.

The mean of 

icon: customize the focus value. The next time the instrument is used, the system defaults to focus fine tuning based on the last set point in order to obtain good picture quality that can be used for accurate counting.

Note: the point of set will be influential to autofocus.

Note: Ensuring proper focus is the key to accurately identifying cells and distinguishing dead or live. Cell images with correct and incorrect focus are shown in Figure 5-3（A~B）



A B

Fig. 5-3

A Normal focus: cells are well defined, with a clear aperture in the middle of live cells, and dead cells are opaque due to trypan blue staining.

B. Abnormal focus: the cell contour is blurred, and they all appear as opaque as dead cells.

Incorrect focusing can result in the following:

- Due to the spherical structure of the cell, when the focal length of the device deviates, the cell center presents the opaque state after Trypan blue staining, which leads to the

misjudgment of live cells as dead cells by the device.

- The focal length of the device was deviated, and the cells presented a fuzzy halo phenomenon, which caused the device to misjudge the dead cells as living cells.

How to determine if the focus is correct:

- After the completion of automatic focusing, observe the imaging state of the cells in the field of vision. If you can see that some cells show clear outline and the middle is transparent, and some cells are overall clear but the middle is opaque, then the cell focus is correct at this time.
- If there are no obvious features mentioned above, appropriately enlarge the image and select the area with a large quantity of cells, and fine tune the focal length from top to bottom to clearly observe the changes of cell imaging when the focal length changes, and select the state with the clearest cell edge as the focus.

By clicking  , the slide regulator  will show up for you to zoom the active view/ image.

Upon completion of operations on the [Count] page, the [Result] page will pop up, where you can zoom in, mark cells, or view histograms, adjust profiles and count for the captured images. The results page of trypan blue mode as shown below.

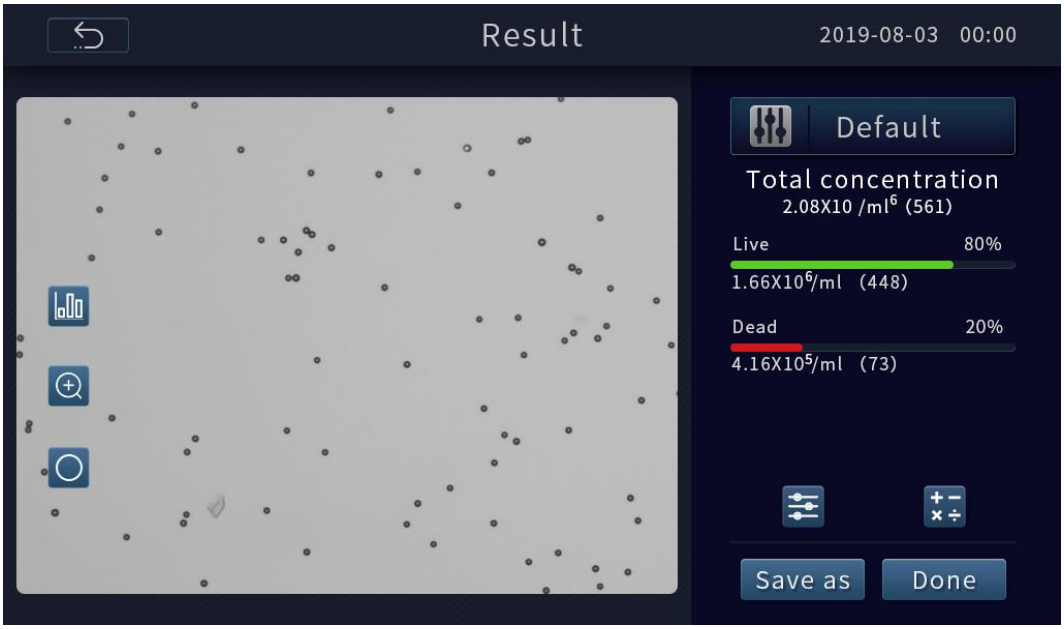


Fig. 5-4

Click  to get a histogram, as shown in the figure below, and click again to back.

Fig. 5-5

Click  to mark cells, as shown in the figure below.

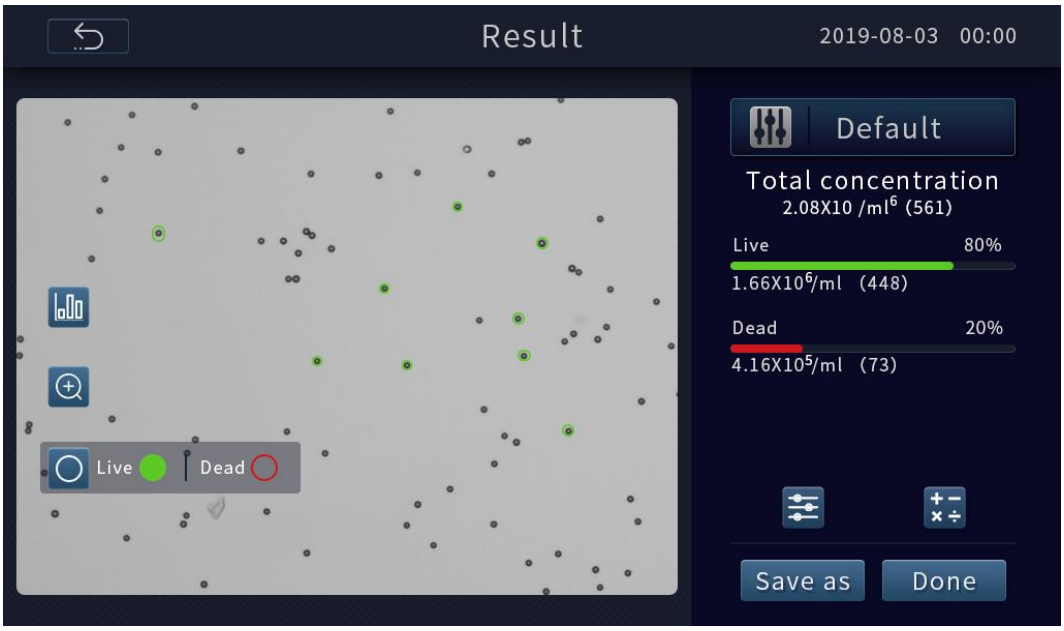


Fig. 5-6

Click  to enter the Adjustment page to re-adjust the Profiles.

Click  to enter the Dilution Calculator page, as shown in the figure below, where you can calculate the volume of existing cell stock solution and the volume of buffer solution to be mixed for diluting to the target volume and concentration.

is a “Help” symbol, click it to get more about calculation method.

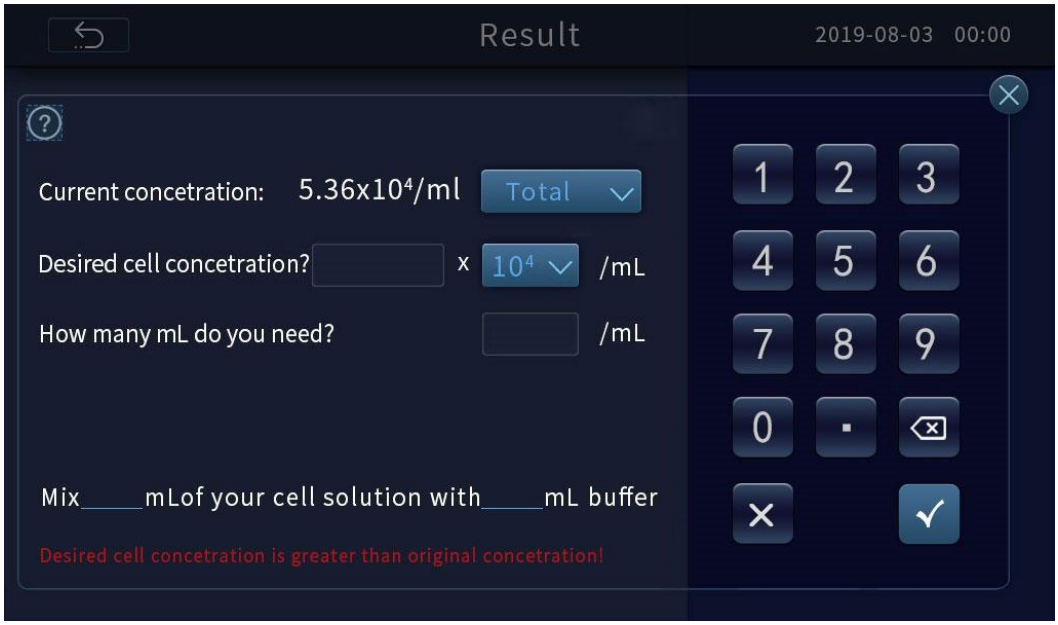


Fig. 5-7

5.2 Saving Results

After count, you can choose to save/ export the original image, cell marking image, count Profiles, cell count report and other data, and also Profile the default naming rules for results exporting.

Click [Save as] to save the results to the local computer in various forms or/ and to an external storage device that meets the requirements. Click [OK] to enter the Home page and automatically save the results to the local computer or/ and external storage device under the system default name.

Note: up to 1000 entries can be stored in the system. If you continue to save new data beyond this limit, the count results saved at the earliest time will be deleted by default.

Fig. 5-8

5.3 Previous Counts

Click [Previous Counts] on the Home page to enter the following interface, where you can view the operation Previous data of the instrument, delete and export data to the external storage device.

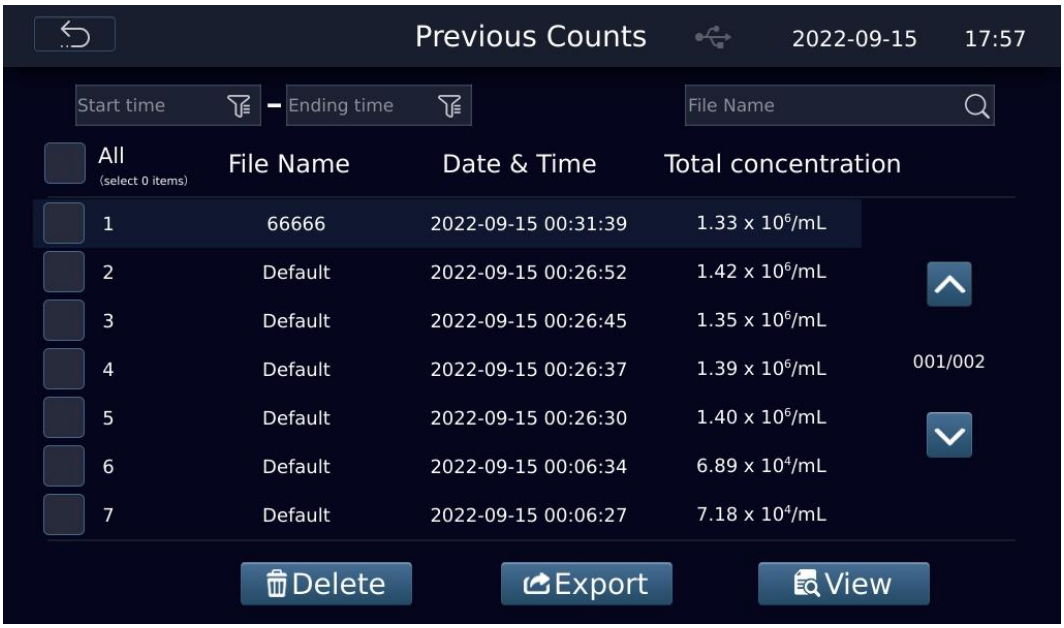


Fig. 5-9

5.4 System Settings

Click [System Settings] on the Home page to enter the following interface. It allows you for performing such actions as software update, date / time setting, language shift and administrator.

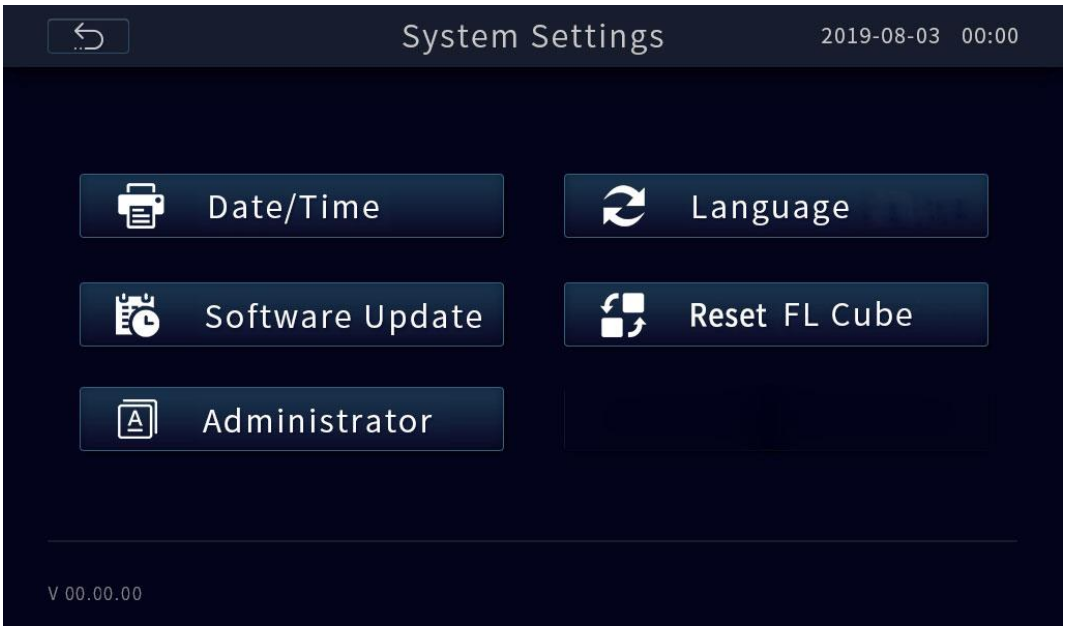


Fig. 5-10

5.4.1 Software Update

The latest version of the software can be updated to the instrument by the USB.

The method: First of all, copy the latest software to the USB and insert it into the USB port. When the system successfully identifies the software program in the USB, it will enter the upgrade state as shown below. Click [Update Now] at the interface and enter the correct password and operate according to the prompts.

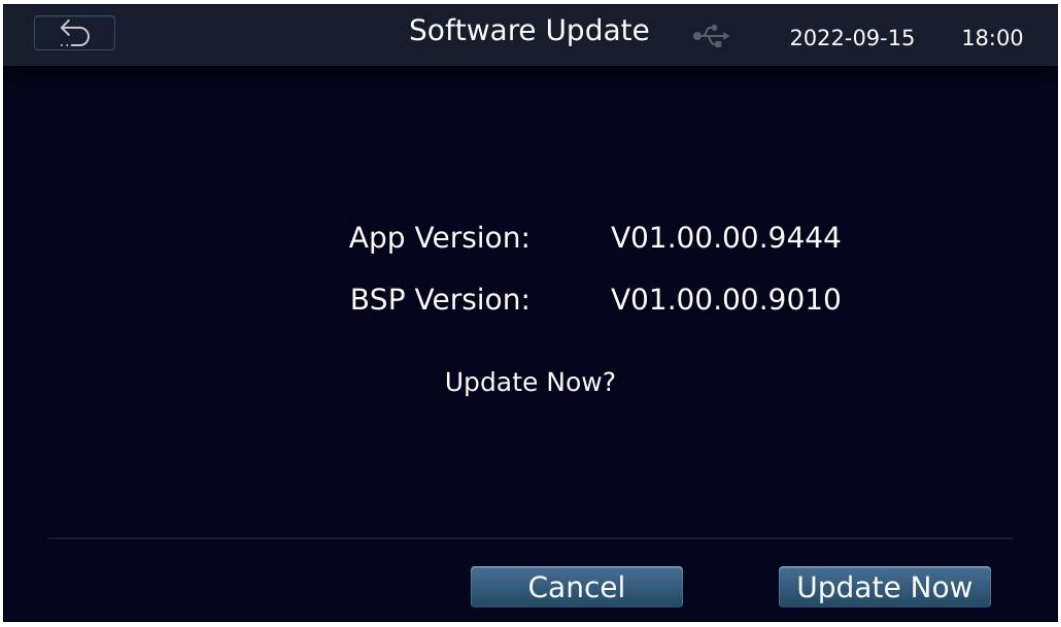


Fig. 5-11

6-Maintenance

6.1 Annual inspection

RWD recommends a comprehensive inspection of the Automated Cell Counter every year, as the annual inspection can ensure a good operating state of instrument. For details, please contact RWD. It is recommended to clean the instrument regularly to prevent dust buildup from affecting its performance and causing contamination. See the following for cleaning procedures.

6.2 Cleaning

- **Cleaning the touch screen**
  - Gently wipe off the touch screen with a soft lint free cloth moistened with an LCD cleansing detergent. Be gentle and cautious during cleaning. Wipe the touch screen dry immediately after cleaning.
  - Prevent ingress of cleaning solvent into the Power button, power socket, count slide port or USB port.
  - Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.
  - Do not use a corrosive cleaning solution that can cause scratches on the touch screen.

- **Cleaning the case**
  - Use a soft lint free cloth moistened with distilled water to wipe the case. Wipe the case dry immediately after cleaning.
  - Prevent ingress of water or other cleaning solvent into the Power button, power socket, count slide port or USB port.
  - Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.

- **Decontaminating the instrument**
  - Use a soft lint free cloth moistened with 70% alcohol to wipe the outer case. Wipe the case dry immediately after cleaning.
  - Do not use a bleach solution that can cause bleaching liquid crystal on the instrument.
  - Prevent ingress of water or other cleaning solvent into the Power button, power socket, count slide port or USB port.
  - Never pour or spray any liquid onto the instrument in order to avoid electric shock when the instrument is powered on.

7-Troubleshooting

This section introduces the problems (faults) that are common to the product and their possible causes and solutions.

Problems	**Solution**
Autofocus cannot focus cells well	Make sure there is no bubble or debris on the screen to interfere with autofocus.
USB drive recognition fails	1) The USB is not in FAT32 format. Format it into FAT32 format; 2) Replace a USB in the correct format
Firmware update fails	1) Make sure the USB is of FAT32 format, and upload the file to the USB for software update. 2) The file must be saved into the root directory of the USB instead of saving it in a folder or subfolder. 3) The file must be intact with the extension .zip.
Motor jammed， please contact the after-sales personnel!	Turn off the power and contact RWD aftersales personnel

8-Warranty

The warranty of this equipment starts from the date of leaving the factory. During the warranty period, the equipment cannot be used normally due to problems such as materials and process defects. RWD undertakes after-sales service such as equipment maintenance and parts replacement.

Any damage caused by improper use or over-range use is not covered by the warranty. If repair or replacement of parts is required, the cost will be borne by the user.

If the reworked equipment was found to have been unauthorised disassembly, RWD will not provide after-sales service such as warranty, free maintenance and parts replacement.

The warranty statement (including its restrictions) is exclusively issued by RWD and covers all other warranties.

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