

Countstar® Mira HT

High throughput Cell Analyzer

User manual

Version: 1.0.4.0



Shanghai RuiYu Biotech Co.,Ltd.

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General Statement

Please read these instructions carefully and operate the Countstar® Mira HT only in accordance with the instructions as described below.

Shanghai RuiYu Biotech Co., Ltd. cannot be held liable for the product's damage and/or consequent losses due to improper use and in the face of disregard the following instructions.

Countstar® is a registered trademark of Shanghai RuiYu Biotech Co., Ltd.

The Countstar® Mira HT Automatic Fluorescence Cell Analyzer is for research use only, not for use in diagnostic procedures.

Safety Instructions

To ensure a safe and reliable operation of the Countstar® Mira HT Automatic Cell Analyzer (named as Countstar® Mira HT in the following context), please note the following safety instructions and operation guides:


Operating requirements

- Voltage input for power supply unit: AC 110-240 V; ~50/60 Hz
- Voltage output of power supply unit: DC 12 V
- Power input Countstar® Mira HT: 90 W
- Grounding: If existing, the power supply unit of the Countstar® Mira HT shall be connected with the appropriate cable to the existing grounding of the local power supply system.
- Allowable temperature range: +10°C to +40°C (+50°F to +100°F) ;
- Optimum temperature range: +18°C to +25°C (+64°F to +77°F)
- Allowable humidity range: 20-80% RH
- Placement of the analyzer: The Countstar® Mira HT shall be positioned on a dry, solid, and even lab bench or table. The user has to ensure that no vibrating or shaking lab equipment are operated close to the analyzer in parallel, when the Countstar® Mira HT is testing.
- Do not place the device permanently on the floor of the lab to avoid damages on the system.

Additional environmental requirements

The Countstar® Mira HT can be operated in some classes of clean rooms (the analyzer contains internal ventilation unit(s), in offices (if non-hazardous samples are used), and of course in standard laboratories with or without air purification, but should not be operated in environments with a high smoke or dust exposure.


The Countstar® chamber slides as well as with 24-well chamber plate consumables are manufactured in a clean room environment, and were sealed singly in a slip cover. After taking the slide out of the protective cover, it is recommended to use the Countstar® chamber slide as well as with chamber consumables as soon as possible to avoid the adhesion of fibers and dust particles.

 **Caution:** The user shall ensure a stable power supply connection, avoiding an abnormal power failure(s) during the operation of the Countstar® Mira HT. Important data should be backed up regularly onto an external USB storage device. Generally, the manufacturer and/or distributor of the Countstar® Mira HT cannot bear any joint liability for a damage or loss of data.

Operation Precautions

All effective safety rules are needed to be followed, when handling hazardous reagents such as staining liquids like Trypan Blue and/or fluorescent dyes.

When handling the Countstar® chamber slides, already containing stained samples, it is highly recommended to wear protective clothing, glasses, and gloves.

 **Caution:** The user shall not look directly into the slide port to avoid harming the eyesight while the Countstar® Mira HT is performing fluorescence experiments.

Requirements for the use of consumables

The Countstar® Mira HT can only be used with the proprietary Countstar® chamber slides as well as with 24-well consumables. Other object slides will cause irreversible damages to the Countstar® Mira HT.

The Countstar® chamber slides as well as with 24-well chamber consumables are a single-used consumables that shall not be reused. Once unpacked, the user shall keep the Countstar® chamber slides as well as with 24-well chamber consumables protected from particles / fibers, using a chamber slide holder.

Waste disposal

Used Countstar® chamber slides, as well as with 24-well chamber consumables pipette tips, and other consumables, used while operating the Countstar® Mira HT, and which are considered to be contaminated with potentially biological hazardous materials, must be treated, according to actual relevant biological waste rules of the laboratories.

After-sales service

In the unlikely event, that a repair of the Countstar Mira HT might be required, an application and decontamination form need to be ordered in advance, filled out, and returned together with the analyzer in the packing box to your local distributor, or to the headquarter of Shanghai RuiYu Biotech.

Address: Building #4, NO.877, Jiuxin Road, Jiuting Town, Songjiang District, Shanghai, P.R. of China

ZIP code: 201615

Email: marketing@ruiyu-biotech.com

Address: 2500 York Road, Suite115 Jamison, PA 18929, USA

Email: contact@alitlifetech.com

<https://alitlifetech.com/countstar-mira/>

The Countstar® Mira HT is classified to be maintenance-free. In case of a preventive maintenance ordered, all services and maintenance need to be carried out by qualified service technicians.

Chapter I System Overview

The Countstar® Mira HT is an image-based, bright-field and fluorescent cell analyzer to detect and analyze suspended cells/particles automatically with advanced optical hardware and algorithms of AI-based image analysis. The Countstar® Mira HT has been developed to detect the concentration, viability, diameters, roundness (morphology factor), and aggregation rates of cells or particles in a single analysis sequence. The Countstar® Mira HT is capable of detecting objectives with diameters on the range of 2µm - 180µm, and concentrations from 1 ×10⁴/mL to 3 ×10⁷/mL. With loading 20µL (Countstar® chamber slides) or 30µL (Countstar® 24-well chamber plate consumables) for loading, the Countstar® Mira HT could take less than 20 seconds to complete the image acquisition and analysis. Up to 24 samples can be tested at one time.

The Countstar® Mira HT is equipped with a data management system, a cGxP compliant four (4)-level user roles access registration, log files and E-record functionality. All features meet the requirements of FDA 21CFR Part11 guidelines.

For the Countstar® Mira HT customized IQ/OQ documents will be designed and provided. Shanghai RuiYu Biotech offers extensive support for validation execution services, and supports a PQ design and execution evaluation. These services shall enable the customer an operation of a Countstar® Mira HT compliant to the actual cGxP regulations of FDA and EMA.

1.1 System composition

The Countstar® Mira HT is an all-in-one analytical device, integrating the patented optical bench, an 8-inch HD touchscreen, a high-speed CPU with an internal 256 GB data storage capacity, a powerful analysis software and multiple applications.

1.1.1 The essential Countstar® Mira HT hardware components

Overview of the single Countstar® Mira HT hardware (see **Fig. 1.1** and **Fig. 1.2** below)

- 1) Touch screen: An 8-inch HD touch screen with resolution 1280*800.
- 2) Slide port: The inlet port for inserting the Countstar® chamber slide and 24-well chamber plate consumables.
- 3) USB 2.0 port 、 USB 3.0 port : Connecting USB devices for data transfer.
- 4) Power switch: Turns on/off the power supply. The switch LED illuminates red, if turned on.

- 5) Ethernet port: 100M/1000M adaptive enabling an ethernet connection to an internal company network.
- 6) HDMI Type-A: video output port.
- 7) Power input socket: Connecting the power supply unit with the analyzer.
- 8) Back fan: Back ventilation outlet.



Fig. 1.1 Front side view of Countstar® Mira HT



Fig. 1.2 Back side view of Countstar® Mira HT

1.1.2 Countstar® Mira HT analysis software

After booting up, the main user interface of the Countstar Mira HT user login screen is displayed. Then enter the main interface of the analysis software, as shown in Figure 1.3:

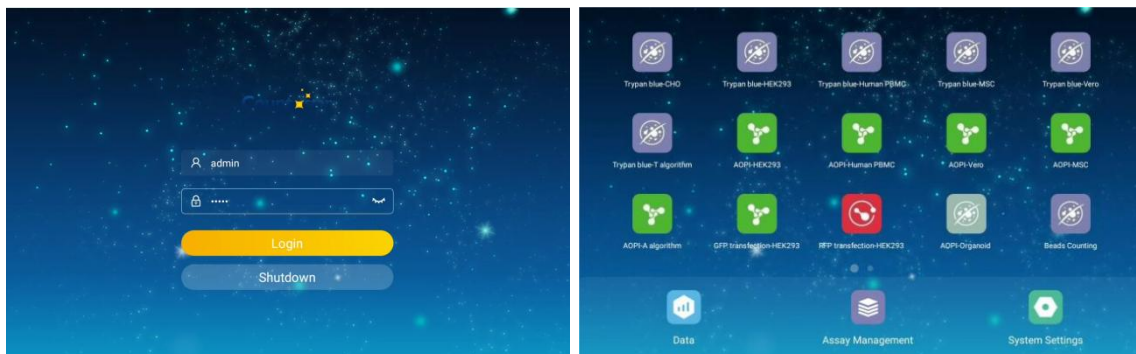


Fig. 1.3 User login and Main user interface of Countstar® Mira HT

In the upper section of the main user interface, 15 pre-installed BioApps (assay templates) for different experiments are shown. The lower section of the main user interface contains the navigation bars with the four functional icons: **Data**, **Manual Analysis**, **Assay Management**, and **System Settings**. A click on one of these icons will automatically guide the user to the corresponding menu:

- ① **Data:** It allows the user to view the results and images after the completion of each experiment.
- ② **Assay Management:** In this menu, the user can import, copy, edit, delete the BioApps, export the assay templates, and determine whether BioApps are visible on the main user interface.
- ③ **System Settings:** Here the user can adjust general aspects like the system's language, user management, Network connection, standby setting, time and date of the device, checking the system's focus, updating the software, and managing the data storage. In the System Settings Menu the user can also get an access to a protection program of transportation and shutdown.

1.2 Consumables for the Countstar® Mira HT

As described above, the user of a Countstar® Mira HT cell analyzer is required to only examine samples on Countstar® chamber slides. As shown in the **Fig. 1.4** below, each slide contains five single chambers to test either up to five different samples consecutively, or five aliquots of the same sample. Only a 20µL liquid sample per chamber is required to fill a chamber. Moreover, Countstar® chamber slide holder has the capacity of detecting 3 Countstar® chamber slides (Total 15 chambers) at once.



Fig. 1.4 Countstar® chamber slide (left) and adapter compatible with 3 count boards (right)

Furthermore, Countstar® Mira HT supports detection of 24 samples at once with using the a Countstar® 24-well chamber plate consumables with 30µL liquid sample per chamber (Shown in **Fig. 1.5**).



Fig. 1.5 Countstar® 24-well chamber plate

1.3 Countstar® Mira HT technical specifications

Table 1 Countstar® Mira HT technical specifications

Detectable cell (object) diameter range	2 µm-180 µm
Measurable cell concentration range	1x10 ⁴ -3x10 ⁷ cells/ml
Optimal cell concentration range	5x10 ⁵ -1x10 ⁷ cells/ml
Optical magnification	5x
Camera	8.3MP CMOS
Fluorescence channel	Ex: 465-485 nm
	Em: 535/40 nm; 600LP
USB connectivity	USB3.0(1x), USB2.0(1x) (only support FAT32 external memory)
Storage capacity	1128 GB
Power input	110-230 V/AC; 50/60 HZ
Screen size	10.1 inch, 1280*800
Product weight	8.6 kg
Product size (W*D*H)	308 mm * 335 mm * 354 mm
Holder	Compatible to Countstar® chamber slides

Chapter II Operation Guide

2.1 Countstar® Mira HT unpacking, transportation and packing

2.1.1 Unpacking

1) Place the carton box of the Countstar® Mira HT in upright position on the floor. Open carefully the carton box, not cutting too deep (<0.5cm) into the adhesive tape sealing the box to avoid scratches / damages of the analyzer. The view into the opened carton box is shown in **Fig. 2.1**.

2) Take out the power adapter and consumable adapter, hold the body of the instrument (~8.6kg) with both hands, as shown in the figure below (Figure 2.1), and put them on the specified experimental table after taking them out. Remove the foam, connect the device to the external power DC socket, and connect the power cord to the laboratory AC power supply.



Fig 2.1 View into the box of Countstar® Mira HT after opening

2.1.2 Transportation

Whenever moving the Countstar® Mira HT (e.g. relocation inside a company, shipping to another facility, or return to the factory for repair), the instrument has to be shut down completely into shipping mode to ensure its intactness during transport and after reactivation.

1) The system needs to be turned on (power switch at the back of the analyzer) and booted up completely, so that the main menu is visible on the screen of the instrument. The user has to enter the System Settings in the user interface, as displayed in **Fig. 2.2**.

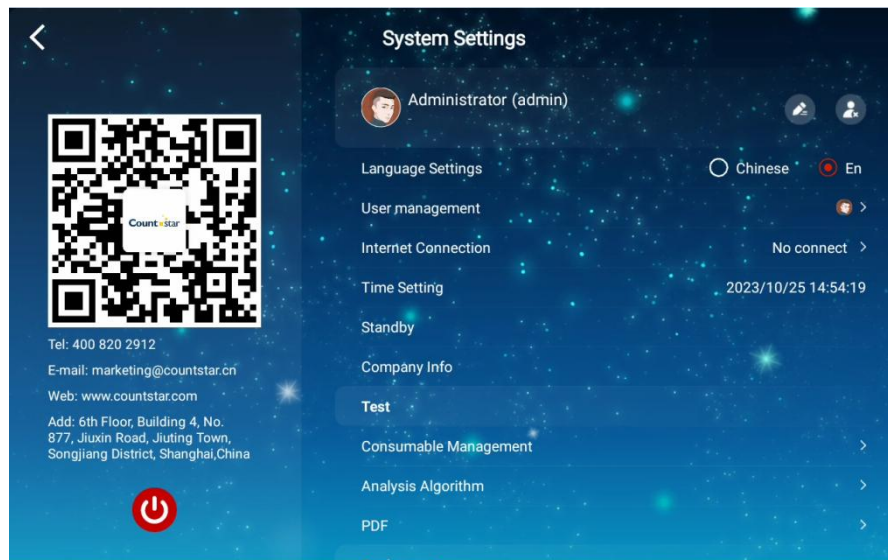



Fig. 2.2 System Settings user interface of the Countstar® Mira HT

2) The user has to click on the "Off  " icon on the left and the following pop-up window will appear, requesting: "Please unplug the consumables and turn off the power after the system is turned off", as shown in **Fig. 2.3**. In case, a Countstar® chamber slide is still inserted in the instrument, it has to be removed completely, followed by a subsequent click on "Confirm". If no chamber slide was inserted, the user can click immediately on the "Confirm" icon. To return to the System Settings menu, the user has to click on "Cancel".

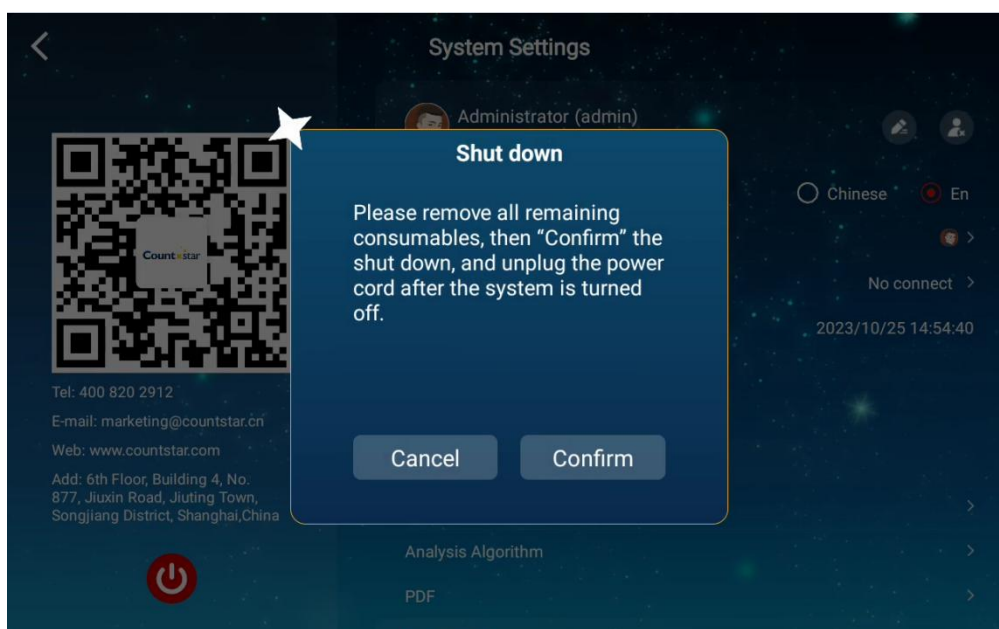


Fig. 2.3 Pop-up window for shutdown procedure

3) After confirmation, the Countstar® Mira HT will shut down completely within 30s. Finally, the user has to turn off the power switch at the rear side of the instrument (see **Fig. 2.6**) and unplug the DC and AC power cables.

2.1.3 Packing

The user shall place the original carton box on the horizontal ground and keep the printed arrow carton on the outside of the box is upwards. First, put two pieces of protective foam into the left and right sides of the instrument body respectively according to the shape (the right side of the foam belt power and adapter jack corresponding to the right side of the body). Pick up the Countstar® Mira HT with both hands and place it vertically into the packing box, as shown in Figure 2.4.

⚠ Caution: If the original packaging material is incomplete or discarded (lost), the user shall contact his regional distribution partners to receive alternative packaging instructions / materials.

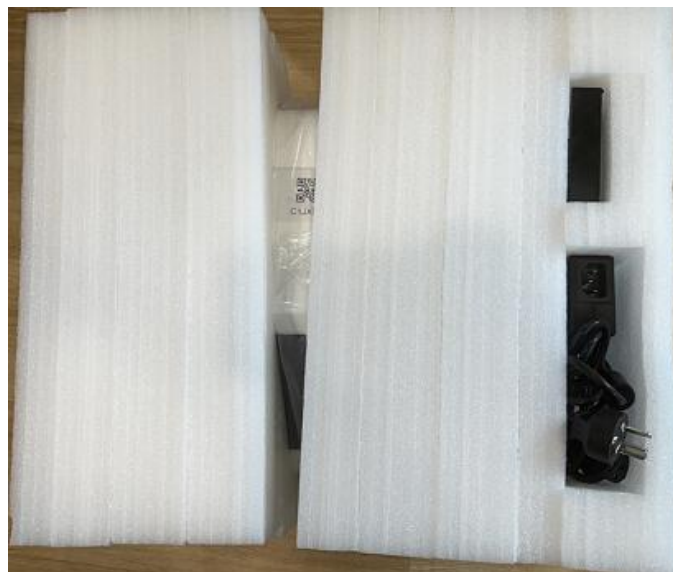


Fig. 2.4 Countstar® Mira HT stands upright in a packing case

- 1) Fold the power cord and put it in the power cord bag.
- 2) Press the protective foam to ensure that the cover of the carton can be smoothly covered with the protective foam.
- 3) Place the power cord bag and adapter in the specified location (Figure 2.5).



Fig. 2.5 View on the open package containing all components of the of Countstar® Mira HT

4) Wrap the top cover of the carton with adhesive tape. Please check the outer packing and wrap at least 3 layers of scotch tape around the bottom and top cover of the carton if necessary.

2.2 The Countstar® Mira HT start-up and shut-down procedures

2.2.1 Starting up the Countstar® Mira HT

1) The user has to turn on the DC power supply by clicking the switch at the lower back side of Countstar® Mira HT. The power switch indicator illuminates red after power-on (**Fig. 2.6**).



Fig. 2.6 Power switch

3) After turning on the power supply, a system startup interface appears (**Fig. 2.7**).



Fig. 2.7 System start-up interface

4) After approximately 30 s to end the animation, the main graphical user interface menu appears automatically, as shown in **Fig. 2.8**.

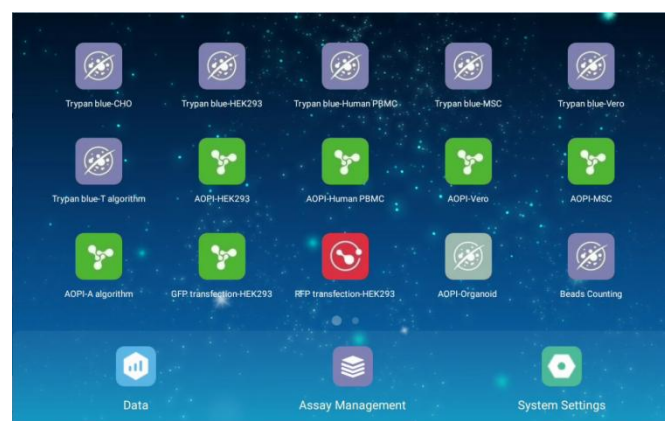



Fig. 2.8 Experimental interface

2.2.2 Shutting down

1) If the user wants to shut down the Countstar® Mira HT, he should click on the "  " icon in the System settings menu (shown in **Fig. 2.2**) to enter a pop-up window with a dialog to determine whether or not shutting down the machine (**Fig. 2.9**).

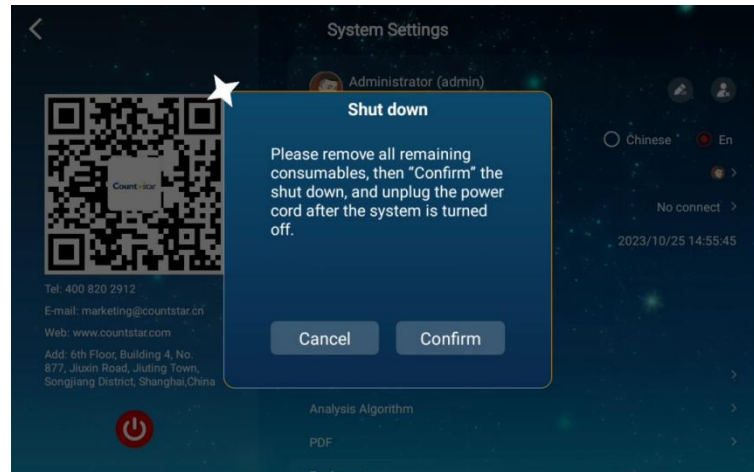



Fig. 2.9 Dialog box of the shut-down menu

2) After removing any remaining chamber slide from the port, the user can click on "Confirm" to shut down the machine. After waiting for 30s approximately while the light touchscreen turns into black, the user can turn off the power by clicking the switch on the backside at the rear side of the housing.

 **Caution:** It will be always mandatory to take out any chamber slide from the port before shutting down the Countstar® Mira HT!

2.3 Executing an experiment

To achieve a successful test, the user shall follow the guides to perform an experiment.

2.3.1 On the main user interface (shown in **Fig. 2.10**) there contains already, as described above, up to multiple conventional BioApps (assay templates) for various applications in bright field and fluorescence analysis. New BioApps can be added to this menu. The operator can choose the corresponding BioApp according to his individual experiment purpose. An example of cell viability determination using an Acridine Orange (AO) / Propidium Iodide (PI) staining kit is described as follows. The experimenter should choose an assay by clicking the BioApp icon named as "AOPI-" + "cell name", such as AOPI-CHO which means that the assay is able to detect and analyze CHO cells strained with AO/PI dyes. By clicking on this icon on the touch

screen, the user will enter the test parameter settings user interface and sample platform moves out (see Fig. 2.12).

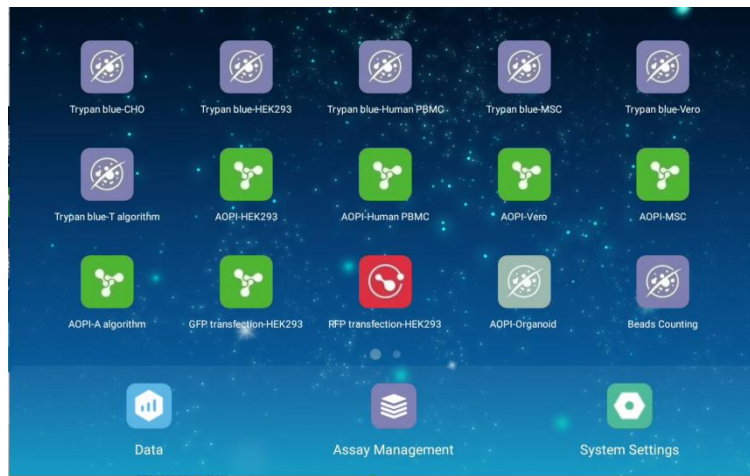


Fig. 2.10 The Countstar® Mira HT main user interface



Fig. 2.11 The Countstar® Mira HT Sample station

2.3.2 Loading the samples into a chamber of the Countstar® chamber slide(s) is performed by applying a micro-pipettor to pipette up 20 μ l or 30 μ l of a homogeneously stained samples which is injected into Countstar® chamber slide and 24-well chamber plate consumables at a constant and low speed (Shown in Fig. 2.12), respectively.

⚠ Caution: The user shall prepare detected samples based on the particular staining SOP, and air bubbles should be avoided in any case, while injecting the sample liquid into the cavity of the Countstar® chamber slide(s).

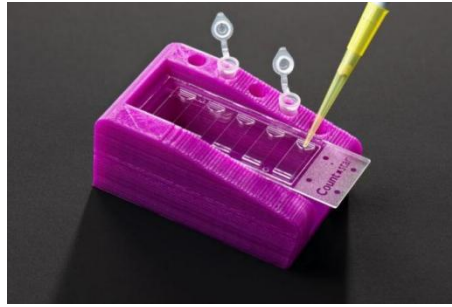


Fig. 2.12 Countstar® chamber slide



Fig. 2.13 Countstar® 24-well chamber plate consumables

2.3.3 Will add good sample Countstar® counting board gently insert Countstar® chamber slide holder (Figure 2.14) then gently flat on the adapter into the Countstar® Mira HT samples in Countstar® 24-well chamber plate does not need the holder and should be placed on the sample platform gently (**Figure 2.14**).



Left: Countstar® chamber slide 5 slots Right: Countstar® chamber slide 24 slots

Fig. 2.14 Different consumables into the sample table

2.3.4 Before performing each test, the user has to select and input the relevant information for the test cycle (composed of chamber selection, Sample ID, dilution rate) in the user interface of Test parameter settings (**Fig. 2.15**).

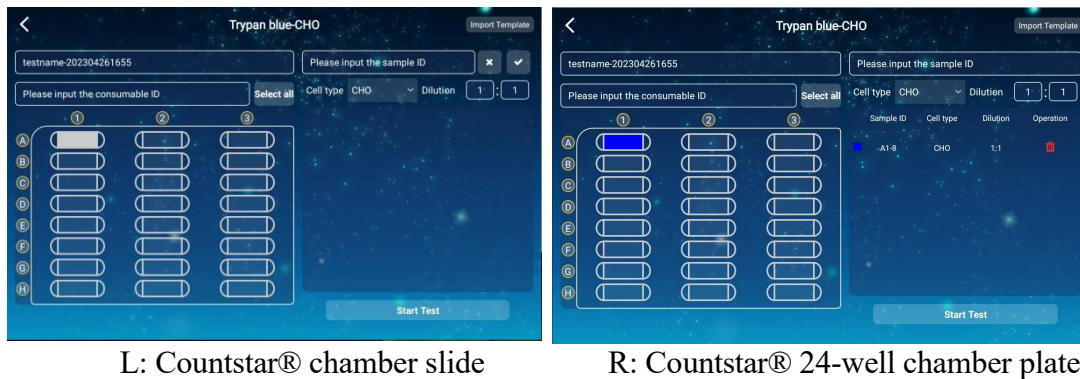


Fig2.15 Test parameter settings user interface

Manual setting:

- 1) Edit sample name: default is test-name-date-time.
- 2) Select slots: Click letters for row selection, numbers for column selection, or click all. The selected slot changes from white to gray.
- 3) Edit sample ID: Default is hole position - number. Experiment again with the same hole position, number +1. If the default sample ID is A1-1, then the default sample ID of the second experiment is A1-2. If you need to edit by yourself, if the ID of the sample in slot 1 is test-1, the sample ID of test-2 will be automatically generated if you continue to select slots, and so on. Then click the "✓" icon to the right of the sample ID. At this time, the selected slot changes from gray to blue. Slot editing is complete.

Note: Select the slot and edit the sample ID in the order that the sample data results will be different.

If the slot is selected first and then the sample ID is edited, the samples are independent data. If the samples are completely different in one experiment, multiple data results will be obtained through independent calculation

If the sample ID is edited first and then the slot is selected, the sample is the combined data. If all the same samples are used in one experiment, one data result will be obtained through average calculation.

- 4) Select cell type: At present, one cell type is bound to the experimental type by default; If you need to add multiple cell types for selection during the experiment, you can add them under Cell type management.
- 5) Dilution ratio: The default is 1:1.
- 6) After editing, click "Start Experiment" and Countstar® Mira HT will start automatic measurement.

Sample ID automatic setting:

Countstar® Mira HT can use a USB flash drive or a server to import a csv file of self-edited sample ID information in a specific format to the device for automatic filling with one click. See Countstar® Mira HT Sample ID Import Template Instructions for details.

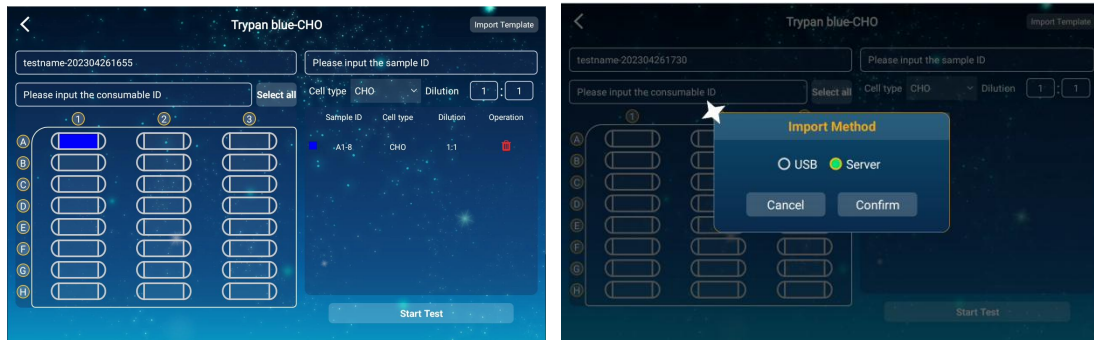


Figure 2.16 Importing a template

2.3.5 The Countstar® Mira HT starts the image recording and analysis of the field of view, displaying a camera symbol on the touchscreen, indicating that the image acquisition and analysis process is in progress. Additionally, thumbnails of already acquired images, and the current measurement information in real time is displayed, as shown in **Fig. 2.17**. The analyzer will automatically move forward the Countstar® chamber slide to its next image acquisition position without any user interaction necessary, until, in all chambers selected, the programmed number of views are photographed and analyzed.

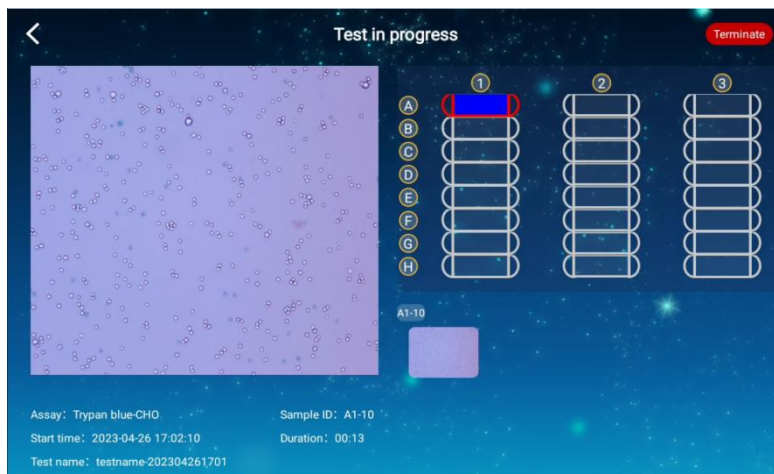


Fig. 2.17 Life view of the measurement progress displaying images and results

2.3.6 To interrupt a running measurement process, the user can click the red "Terminate" button at the upper right of the touchscreen (**Fig. 2.17**) and a system prompt appears, asking: "Do you want to cancel the experiment? " (see **Fig. 2.18**) If the user selects **Confirm**, he definitively terminates the experiment. When clicking on the "Cancel" icon, the Countstar® Mira HT will continue the test sequence up to its defined schedule.



Fig. 2.18 Dialog box of Measurement cancellation

⚠ Caution: As stated in the dialog box, if the experiment is terminated during the measurement, all already acquired images and results will not be saved.

2.3.7 When the user clicks on the "Return" (<) key at the upper left during a sample measurement process, a prompt appears with the note: "Please wait for the current process to be completed before returning to the main menu" (see **Fig. 2.19**). Once the running test is completed, the user has the option to view the images and result data, or to switch to another user interface. The pop-up window will not affect the progress of the currently running experiment. The process will be completed in the background of the system.

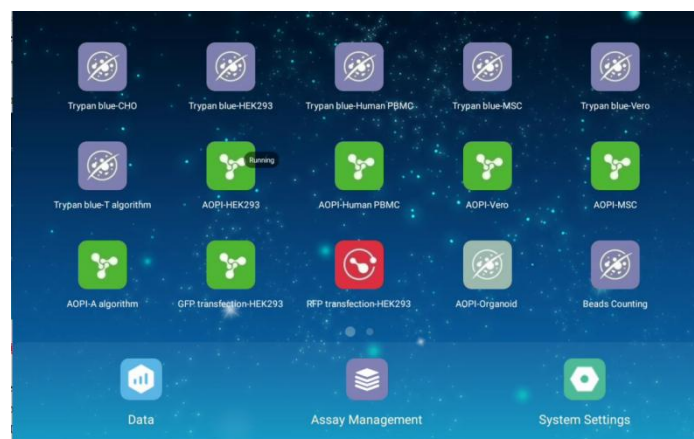


Fig. 2.19 Displayed dialogue box, when clicking on the "Return (<)" key

2.3.8 After the completion of the measurement, the Test results user interface on the touchscreen of the Countstar® Mira HT will automatically appear to display the current test results (**Fig. 2.20**).

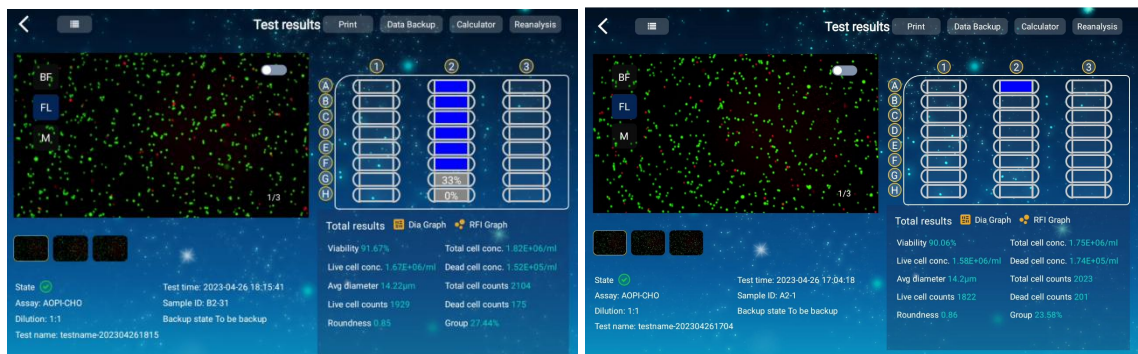






Fig. 2.20 Test results user interface

The result view menu offers various options:

- **The "<" Return icon:** A click on this key in the upper left will guide the user always back to the previously used interface of the menu.
- **The large picture:** The large picture in the menu displays that image, selected from the thumbnail photo list below. To change to another image, the user has to click simply on one of the thumbnails below the large image. The slider at the upper right of the main picture allows the user to activate or deactivate the labelling of objects in the picture. By two fingers spreading and pinching gestures on the touch screen the user can zoom in and out to view images in details. If the experiment BioApp is programmed to acquire only bright field images, these pictures will be displayed in the overview section. In case the BioApp contains bright-field and fluorescent images, the user can switch between a bright field image (**BF**), fluorescent images (**FL**), and a merged (overlay) image of bright field channel and fluorescence channel (**M**), by simply clicking on the corresponding letter icons **BF**, **FL**, and **M** at the upper left of the large image.
- **Thumbnails display:** The thumbnail section gives an overview of all images, acquired under the selected Sample ID. If the number of images acquired exceeds the amounts of mini-images that can be displayed in this section of the menu, the user can view them by swiping to the left or right in this section. To select any thumbnail to be displayed as large image view, the user only needs to double-click on the desired thumbnails.
- : You can jump back to the database interface to view other analysis data in the **Test results** interface after the experiment.
- **Cell Labeling:** When the user moves the slider of the **Cell Labeling** icon to the right, the marked cells (or particles) produced by algorithms of the image-based analysis will be shown. When back to the left, the original image without labeling is displayed.
- **Experiment results:** In the right area of the result view interface, the experiment results of each selected view at the top of the touchscreen of is displaying on the Countstar® Mira

HT, and under **Total results** the average calculated results of samples from all measured views of chambers under the current sample ID. In the middle section, the user can additionally retrieve the diameter distribution histogram (**Dia Graph**), and if measured, the Relative fluorescence Intensity (RFI) distribution histogram (**RFI graph**).

- **Measurement information record:** Below the enlarged images and the thumbnails, the current status of analysis, the BioApp (assay) name, the time of Test, the Sample ID, and the selected dilution ratio are shown.
- The **Analysis status:** The image analysis starts immediately after the recording of the image. Once, the analysis results of this test are completed, a green check mark icon "  " is displayed. If the analysis is completed partly, a red, "Analysis in progress" icon "  " is displayed. In case, the image analysis hasn't started, the yellow icon "  " which means "To be analyzed" is visible.
- The **Calculator** feature: In the next chapter "Data", the function of the Calculator will be explained in details (see 2.4.3).
- **Reanalysis:** The operation of this function is explained below (see 2.4.3).
- **Data Backup:** See "System Settings--Data Backup" for details (see 2.6.14).
- **Print:** The function to print a PDF report from selected data while a network printer is connected by the Countstar® Mira HT.
- The **Diameter Graph:** The diameter distribution histogram of the current sample (example shown in **Fig. 2.21**) can be retrieved by clicking on corresponding icon in the result view on the right. It displays the cell size (μm) range on x axis and numbers (counts) of the specific size on y axis. By changing the size ranges at the upper left side of the graph, the user can have a detailed look on sections of the histogram, showing the proportion of cells in % within the selected size ranges on the upper right side.

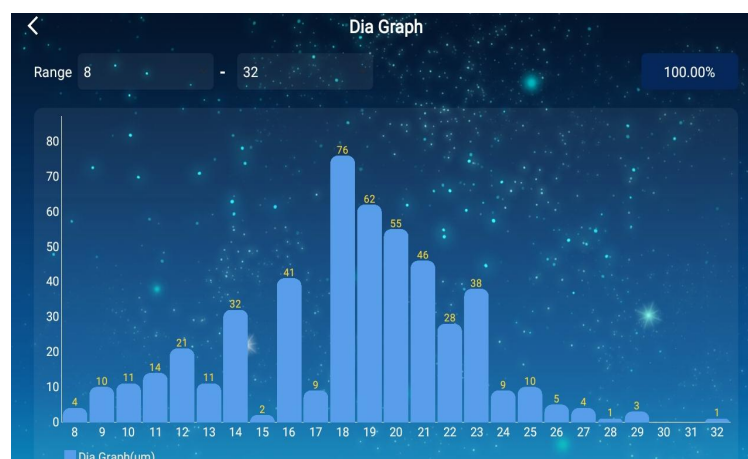


Fig. 2.21 Diameter Graph

- The **RFI Graph**: The user can obtain the Relative fluorescence Intensity (RFI) distribution histogram(s) of the current sample (**Fig. 2.22**) with a simple click on the RFI Graph icon in the Test results menu (mid right section). The graphic shows the relative fluorescence Intensity Units (RFU) on the x axis and corresponding cell numbers (counts) of these RFUs on the y axis. The user can switch between the different fluorescence channel, just clicking on FL1 or FL2. For each histogram the user has the option to define a section of the whole graph. He only has to modify the upper and lower values in the entry fields above the graph. This allows the users to proportionally view on those cells within the pre-defined RFU units.

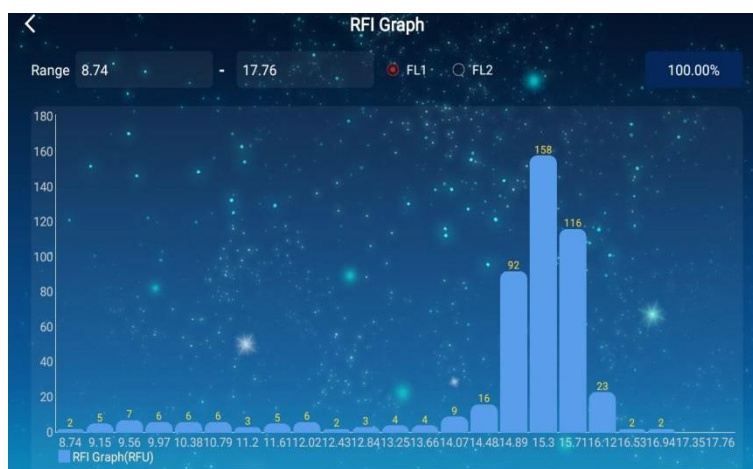


Fig. 2.22 RFI Graph

2.3.9 After the measurement is completed, the sample table does not leave the warehouse. The circular suspension plug-in on the main screen can control the sample table entry and exit to remove the sample.

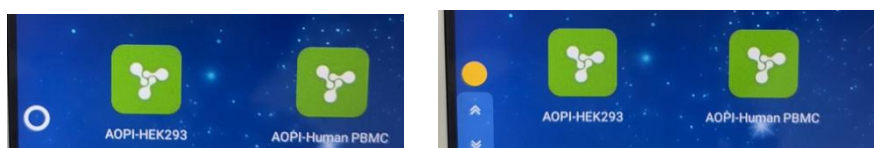


Figure 2.23 Home screen Circular hover plug-in changed from white to yellow when selected

Click the circular suspension plug-in, white turns yellow, and the downward and upward prong icon appears, which can be moved to the left and right sides.

Click the downward tip symbol, sample table out of warehouse; Click the upward symbol, the sample table into the warehouse.

Click the circular hover plugin again, the yellow changes to white, and the downward and upward prongs icon is hidden.

2.4 Data Management

If the user clicks on the "Data Management" icon, as one part of the main menu navigation bar, to open the Countstar Mira HT Data management user interface (Fig. 2.24). The content of the menu includes the Assay (BioApp) selection on the left side, and a table of data on the right side. A selection function dependent on the dates of experiments on the up side of the data chart can help the user to curtail the time frame which the user wants to view.



Fig. 2.24 Data management user interface

2.4.1 Data selection

- ✦ By assay (BioApp): The list below "Assay" displays all stored BioApps in the Countstar® Mira HT. They are listed alphabetically. Per default the correlated results together with a sample thumbnail of the top listed assay are shown on the right. The user can click on any other icon of this list to switch to that BioApp, and the related data will be uploaded to the data area.
- ✦ Subsequently by "Test time" definition: If the time window of measurement results of interest is known, the user can select them easily by defining beginning and end in the "Test time" drop down menu above the table. The data within this time window are displayed in the Data area immediately.

2.4.2 The Data display area

By default, the experiment results of the currently selected BioApp (assay) within a week are displayed in the Data management user interface.

As shown in **Fig. 2.25**, if the user clicks on one of the thumbnails in the list at the right, he can see the images of the selected Sample-ID in a pop-up window in an enlarged view. The register of thumbnails on the right in this window contains each view field of the corresponding sample IDs. In case of only bright field experiments, by swiping up or down in the register of thumbnails on the right side (**Fig. 2.25**), the user can view the bright-field images; if a fluorescence experiment was selected, by swiping up and down on the right side, the user has the option to view all acquired bright field views plus all fluorescence excited views.



Fig. 2.25 Selection of images acquired

If the user clicks on the "Select All" icon in the main menu of Data Management below the list of the single tests (**Fig. 2.21**), all image records of all Sample IDs within the currently selected BioApp (assay) and the pre-defined time range can be accessed. A repeated click on the "Select All" button will cause cancellation of all result items.

If the user selects on one of the circular icons in the list (**Fig. 2.21**), the green-turned circular icon indicates that this current row of data is selected. If the user clicks on the green labelled circle, he cancels his selection of the experiment results. Moreover, selecting multiple sets of data with different Sample IDs is accessible for the user.

The first page on the Data Management menu (**Fig. 2.21**) displays the first five (5) sets of data of the currently selected BioApp. To view more data sets, the user can swipe down the scroll bar at the right side in the list.

Up to three experiment results will be displayed for each row of each sample in the chart; swiping to the right opens the view to further measurement results; swiping back to the left will return to the initial result view of this interface.

2.4.3 View interface for observing more detailed results

If the user has selected a record in the Data Management user interface, he has the access to execute the following operations:

Clicking on the eye-like "👁️" icon will open an interface to view the detailed results (see Fig. 2.26), containing the sample images on the currently selected Sample ID. In this menu, the following operations can be performed for viewing more result information in details.

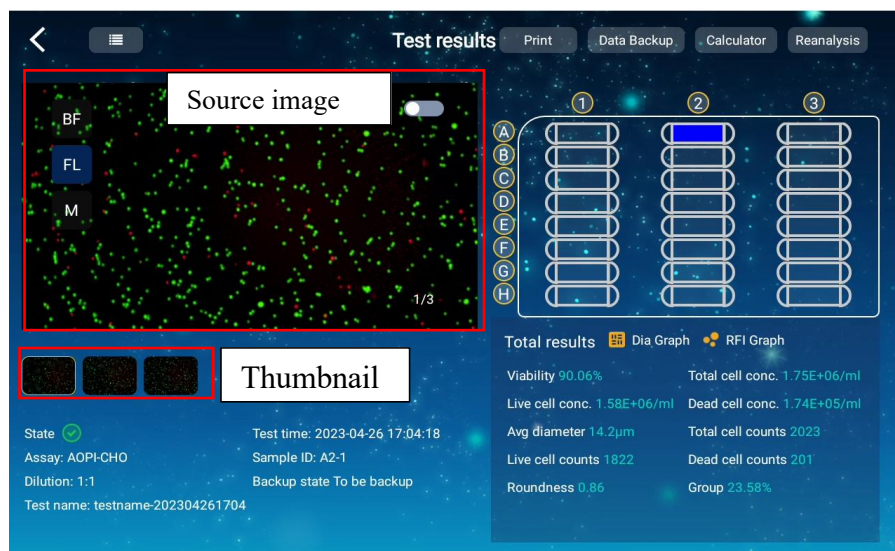


Fig. 2.26 Functional user interface of Test results

- ✦ **Viewing all enlarged thumbnails.** To switch to the next enlarged thumbnails under the same Sample ID, the user has two ways: Either he clicks and swipes on the enlarged thumbnails displayed on the left or he clicks on the number in the lower right corner of the image.
- ✦ **Enlarging an image:** By a click on any area of a selected picture, a new user interface will open (see Fig. 2.27) to show a full view of an image. To zoom in or out the picture, the user needs to move the slider in the upper right of the image. If the mages on this experiment are only taken under bright field channel, the original images acquired will be displayed. In case, sample images under the bright field channel and the fluorescence channels have been taken, a merged picture (M) overlaid from both of the bright field and fluorescent images will be displayed, as well as the brightfield and fluorescent images. By clicking on "BF" or "FL" in the upper left section of the image, the user can switch to the bright field channel (BF) or the fluorescence channel (FL) images, or a

merged picture. A click on the "X" below the image will return to the displayed image with a default size.



Fig. 2.27 Zooming in or out an image

- ✦ **Cell Labeling:** The slider of "Cell Labeling" at the upper right (**Fig. 2.26**) is used to activate / deactivate the cell identification marks.
- ✦ **Reanalysis:** When the user has selected one measurement record, and clicks on the "Reanalysis" icon (**Fig. 2.26**), a new menu window containing multiple parameter settings will open (see **Fig. 2.28**). This gives the user the option to test out alternative cell type parameter settings, if not satisfied with the previously achieved analysis results.

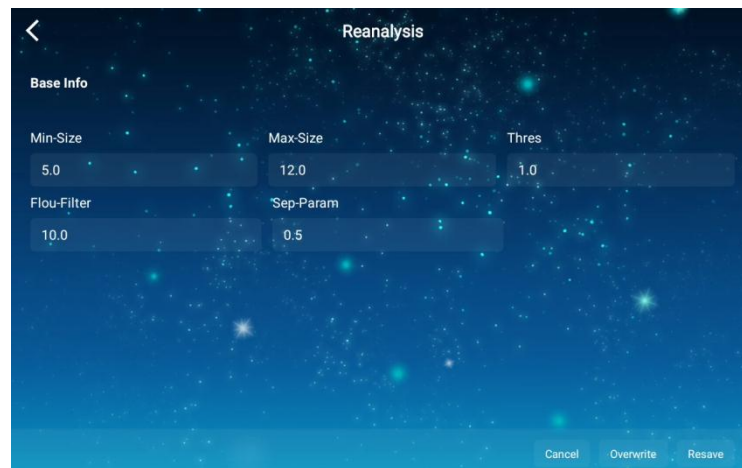


Fig. 2.28 Data Reanalysis user interface

The reanalysis operation is dependent on adjustment under seven (7) single parameters, containing **Prob**, **Nms**, **Min-Size**, **Max-Size**, **Min-Ave-Gray**, **Max-Ave-Gray**, and **Thres**, which the user can vary. The detailed information of these parameters will be shown in the content of "Editing" in the chapter 2.5. In the highlighted menu bar at the bottom of the window, the user has the option to "Cancel" the re-analysis, override the "Primary Data" using changed parameter settings, or "Save as new data" by generating a new result file; If he selects "Overwrite Primary Data", the following prompt (**Fig. 2.29**) will display.

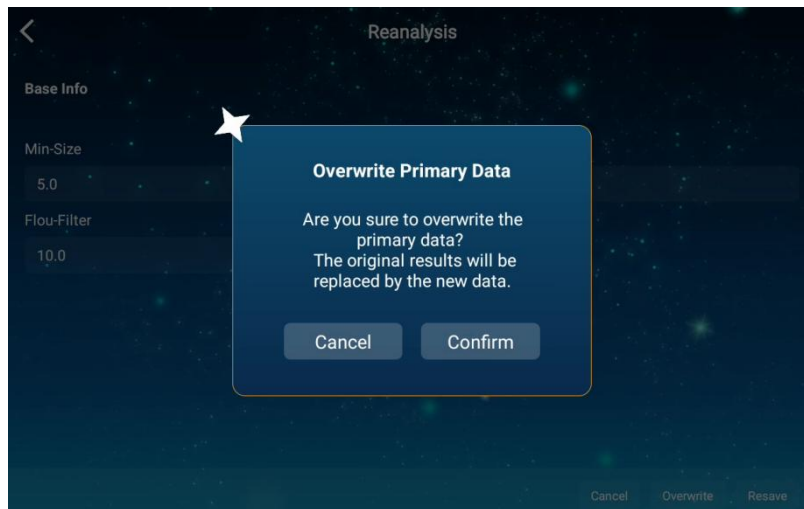


Fig. 2.29 Prompt when selecting "Overwrite Primary Data"

If the user selects "Save as New Data", he has to enter in a dialog box (**Fig. 2.30**) to designate a Sample ID.

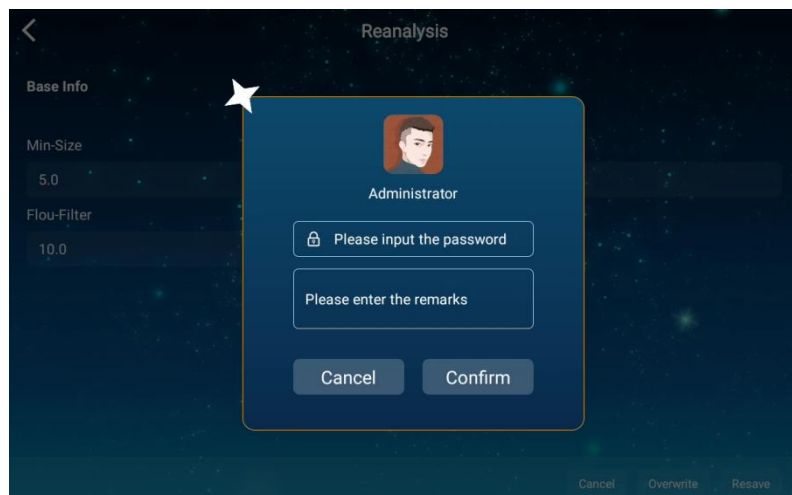


Fig. 2.30 Dialog box for the generation of new Sample ID

With a click on "Confirm", a new result file will be generated, based on the modified parameter settings as shown in **Fig. 2.31**. A progress bar will indicate the status of the analysis.

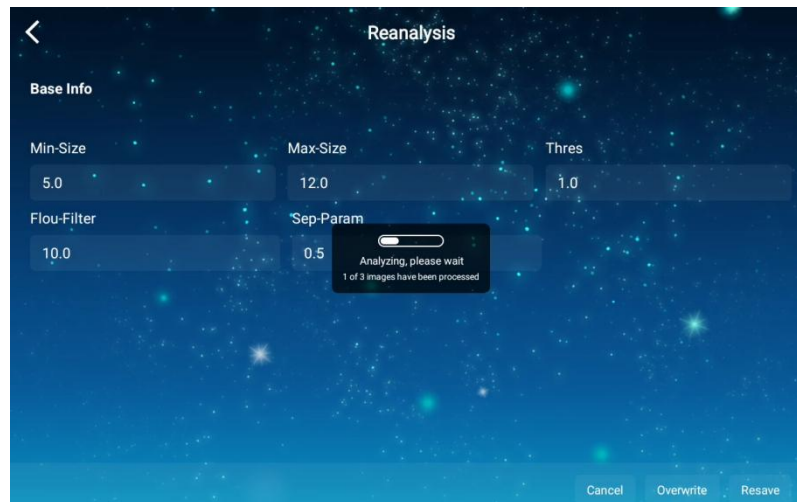


Fig. 2.31 Reanalysis progress pop-up

⚠ Caution: A reanalysis with modified parameter settings will not modify those parameters in a predefined Cell type. But the optimized parameter settings can be noted and used for editing the default settings of the cell type parameters under "Type Management" - "Cell Parameter" (see in Chapter 2.5).

- ★ **Calculator** icon: The "Calculator" function is used to calculate the necessary volumes of the primary cell samples and the diluent ratio to obtain cell samples with a desired concentration.

By a click on the **Calculator** icon at the upper right of the Data user interface (**Fig. 2.26**), a pop-up window will open (**Fig. 2.32**) with a dialog box to show the "Current Sample's Live Cell Concentration" of the selected sample. The fields "Required Target Concentration" and "Required Target Volume" demand for an entry of these values by the virtual keyboard (activation by a double click on the entry field). When clicking on "Confirm" the software will automatically calculate the necessary volumes of the original and diluent cell sample. The calculation results will be shown in the white bar at the bottom of the "Calculator" window. By a click on "Cancel", the software will return to the previous menu.

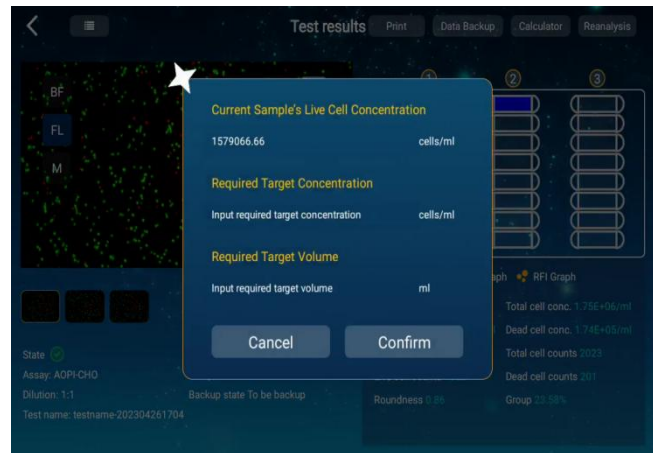


Fig. 2.32 Calculator feature

⚠ Caution: The current living cell concentration of a sample is displayed by default. Optionally, it can be changed to the "Current Sample's Total Cell Concentration".

- ✦ **Backing up to the server:** Backing up the current data to the server.
- ✦ **Print:** Printing the current data as a PDF report.
- ✦ **The Diameter Graph:** When the user clicks on this icon (Fig. 2.26), a diameter distribution histogram (see Fig. 2.33) of the selected sample record will be generated, displaying ranges of the cell size (μm) on x-axis, and the determined cell counts on y-axis. The user can change the sizes beside "Range" to only display a section of the complete histogram for a more detailed analysis.

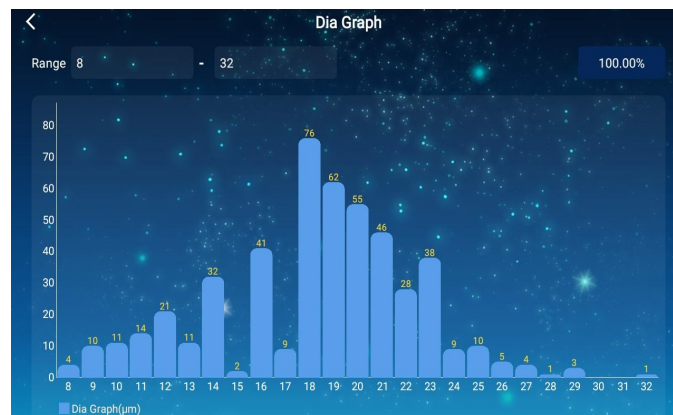


Fig. 2.33 Diameter distribution histogram

- ✦ **RFI Graph:** In case the user has executed an experiment with a BioApp to detect a fluorescent sample, he can view a Relative Fluorescence Intensity (RFI) distribution histogram on currently selected sample ID (see Fig. 2.34). It shows the range of Relative Fluorescent Intensity Units (RFU) on the x-axis and determined cell counts corresponding to the particular RFUs on y-axis. In this diagram, too, the user has the option to modify the range of RFU to have a proportional view on those cells within the pre-

defined RFU range. To switch between the different fluorescence channel data acquired, the user has to click on "FL1", and "FL2".

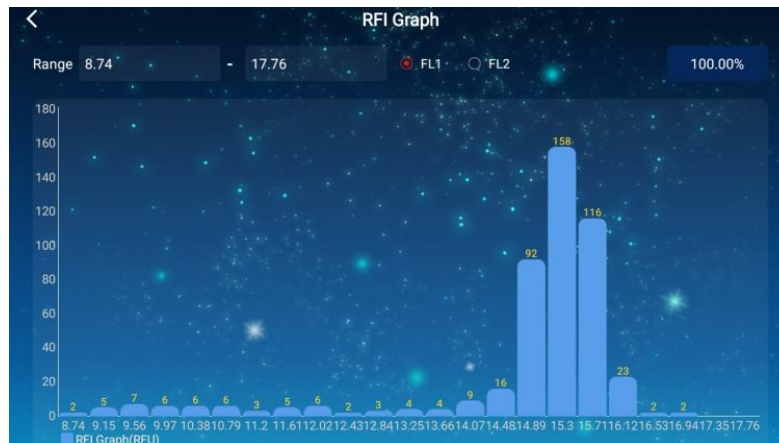


Fig. 2.34 Relative Fluorescence Intensity distribution histogram

2.4.4 Data deletion

In the Data Management user interface, the user can simultaneously delete single or multiple result records acquired by the Countstar® Mira HT. The user needs to select the Sample ID(s) to be deleted in the Data Management area of the software on the touchscreen by a click on the circle icon in the front of the experiment records. Then he could click on the "Delete" icon in the Functional area. A pop-up window displays to ask whether or not to "Delete data" (**Fig. 2.35** below) With a click on "Confirm" the user will irrevocably delete the selected data. Selecting "Cancel" will keep the results and returns to the previous menu.

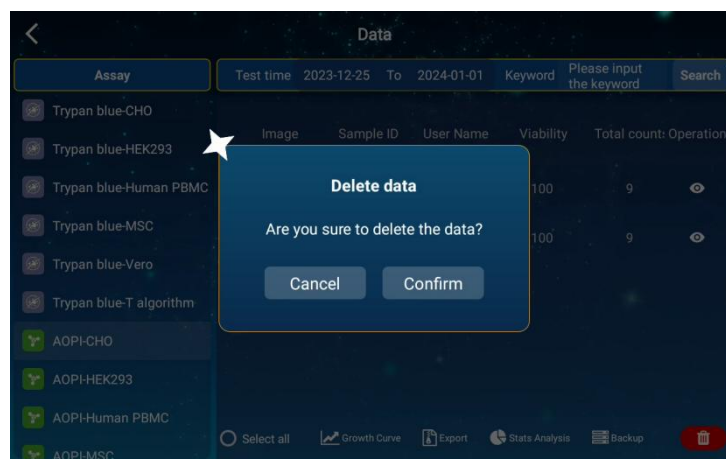


Fig. 2.35 Data deletion dialog box

2.4.5 Generating growth curves

The Countstar® Mira HT offers the function to generate automatically growth curves of the executed experiments. To generate such a chart containing cultivation time and corresponding

cell counts, the user needs to select all corresponding data recorded during the cell growth experiment. When the user clicks on "view growth curve" in the functional area, the growth curve user interface (as shown in **Fig. 2.36**) will be displayed. The selected data will be automatically fitted into a line chart. Different formats of the graphic can be easily generated by changing the scaling of the X-axis and Y-axis.

For the **X-axis**: Change between equal spacing (uniformly spaced) or the real experimental time (Time).

For the **Y-axis**: Under "Curve Dimensions", the user can choose one type of the test results acquired in the experiment(s), such as total cell counts, total cell concentration, live cell counts, live cell concentration, viability, average diameter, average roundness, or group formation.



Fig. 2.36 Growth curve user interface

By a click on the red "Delete", the user can remove the currently generated growth curve.

Click on the icon "Add curve" (**Fig. 2.36**) will open a new user interface (see **Fig. 2.37**) where the user can select data from the result database to add additional graph to the generated growth curve. He can use the search function at the upper right of the user interface (**Fig. 2.37**) beside the magnifier symbol to preselect the data sets by Sample IDs or dates of performing experiments. By a click on "Confirm" at the lower right of the user interface the additional growth curve will instead of the existing graph. At maximum, five (5) growth curves can be displayed in parallel.



Fig. 2.37 User interface for Growth curve data selection

2.4.6 Exporting results Exporting results to an external USB device, SMB or Server

The user can insert the plug of USB storage device with a FAT32 format and sufficient storage capacity into one of the USB ports at the back of the Countstar® Mira HT Analyzer. Subsequently, he needs to select the desired Sample ID(s) in the Data Management menu by a click on the corresponding circle button(s), or using the Select all icon at the lower left of the experiment record list to pick all experiments, followed by a click on the functional button "Export to the USB disk" to open a new dialog box to guide an exporting procedure (Fig. 2.39). This user interface shows the number of data sets selected, and gives the user the option between different export formats: "PDF Report", "Image", "CSV" and "Original Image".

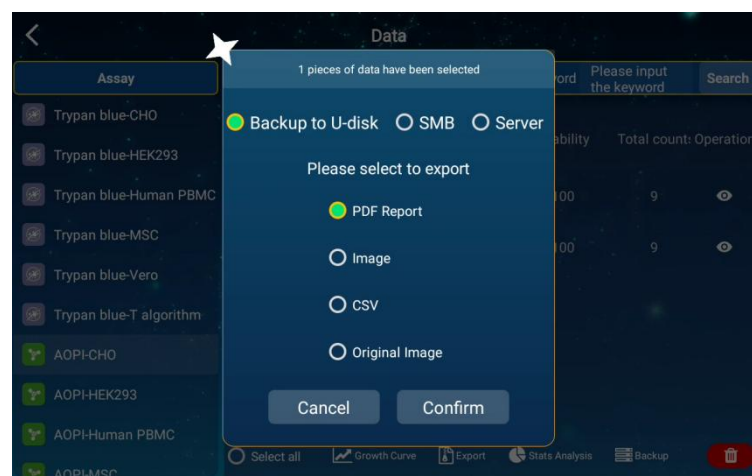


Fig. 2.38 Export data format selection dialog

⚠ Caution: Only a FAT32-formatted USB storage device can be supported to export the data successfully. So, the user needs to connect a USB device that meets this format to guarantee for an issue-free data export.

A click on "Confirm" will start the data export. If the user clicks on "Cancel", the software will return to the previous user interface.

★ PDF Report

When the user selects "PDF report" as a final output format, the Countstar® Mira HT software will automatically generate report(s) as PDF files and save them into the connected USB storage device, with naming the files as "measurement time_SampleID.pdf" (in case, the Sample ID field is left blank, the SampleID will be automatically named as Sample1, Sample2). The path on the USB storage device can be followed through "Machine ID/ assay name/export date/PDF/".

★ Images

When the user selects "Test image" as a final output format, the Countstar® Mira HT software will automatically transfer the images (jpg format) into the USB storage device, with naming the files "measurement time_SampleID.jpg" (in case, the Sample ID field was left blank, the SampleID will be automatically named as Sample1, Sample2). The path on the USB storage device can be found as "Machine ID/assay name/export date/Pic/".

★ CSV

When the user selects "CSV export" as a final output format, the Countstar® Mira HT software will automatically generate an MS-Excel readable csv file and save it on the USB storage device, naming the files "**export** time.xls". The path on the USB storage device can be found as "Machine ID/assay/export date/Excel/**measure** time/".

★ Original Image

Select the data to be exported in the data area (can be selected one by one, multiple or all), pop up the data export content selection window, and then select "Experimental original picture". The system will automatically import the jpg original image results into the USB storage device, and automatically generate the jpg folder named "Measurement time _ sample ID" (if the sample ID is empty. The default Sample ID is displayed, for example, Sample 1, Sample 2..... The path in the USB storage device is Machine ID/experiment type/Export date/OriginPic/".

2.4.7 Backup

Under connection with the network, you can upload the selected data to the server.

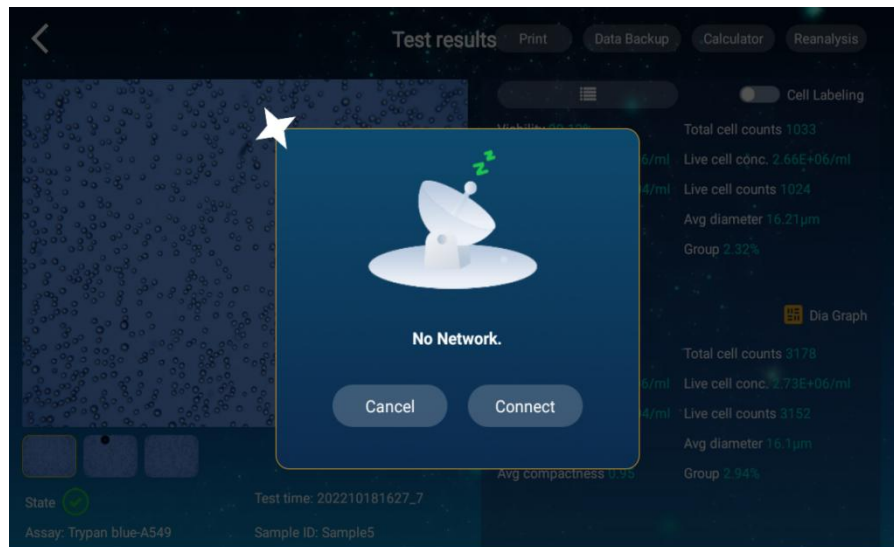




Fig. 2.39 Backing up data to the server

2.4.8 Print

Click "Print" to pop up the print window (Fig 2.40) to modify the printing parameters such as printer, number of copies printed, gray level, etc., and preview a report of the selected data.

Click on the icon "  " to print; Click "  " to cancel printing. If multiple data items are selected, reports of the selected data can be printed at the same time.

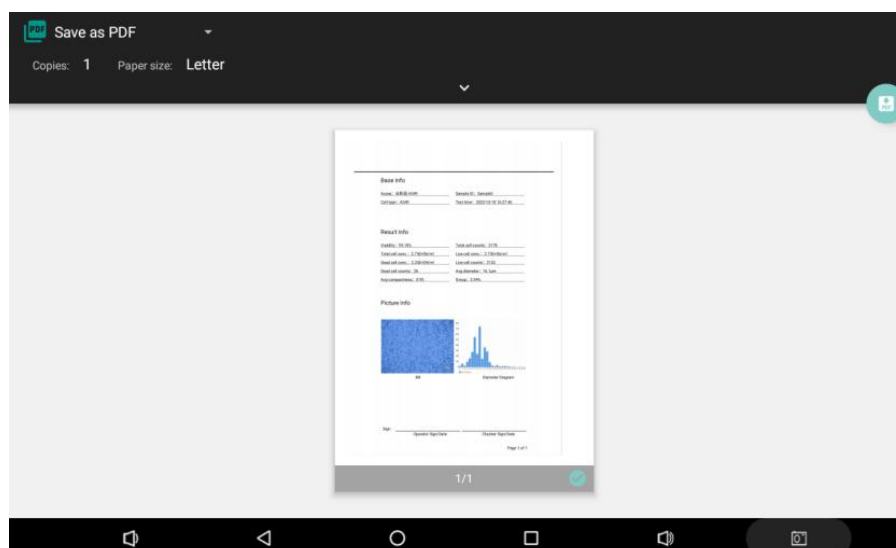


Fig. 2.40 Printing a report as a PDF file

Note: Countstar® Mira HT only support the printer connected with WIFI network

2.4.9 Statistic analysis

Select two or more pieces of data, click "Statistical analysis", and the results of statistical analysis will pop up (**Figure 2.41**), showing Avg values, STD values and CV values of cell viability, total cell concentration, live cell concentration and diameter of the selected data.



Fig. 2.41 Statistic analysis

2.5 BioApp (Assay) management

2.5.1 Importing a BioApp (assay template)

The user of a Countstar® Mira HT can upload conveniently a new, or customized BioApp (test assay template) from an external USB storage device to the internal database of the Countstar® Mira HT, through the "Assay Management" functional modules (see **Fig. 2.11**). In the upper right of this menu interface (see **Fig. 2.42**), a click on the icon **Import Assay** will open a window to display the root directory of the USB drive (see **Fig. 2.43**).



Fig. 2.42 Assay management interface

As an example, the followed steps are described how a BioApp assay template named as "AOPI-Zebrafish" was imported to template database of the Countstar® Mira HT:

- ① User needs to upload the assay template file named "AOPI-Zebrafish" to a folder (named **exp**) on the USB device.
- ② Insert the USB device into one of the USB ports at the back of the Countstar® Mira HT.
- ③ When the user clicks on "Import assay", a user interface, showing the directory of the USB device (**Fig. 2.43**), will pop up to show all assay templates saved in the "exp" folder on the USB device. After the user have clicked on the "AOPI-Zebrafish" BioApp (in this example highlighted by a red spot on the screenshot) user interface, followed with a confirmation, the assay template is started to upload to template database of the Countstar® Mira HT.

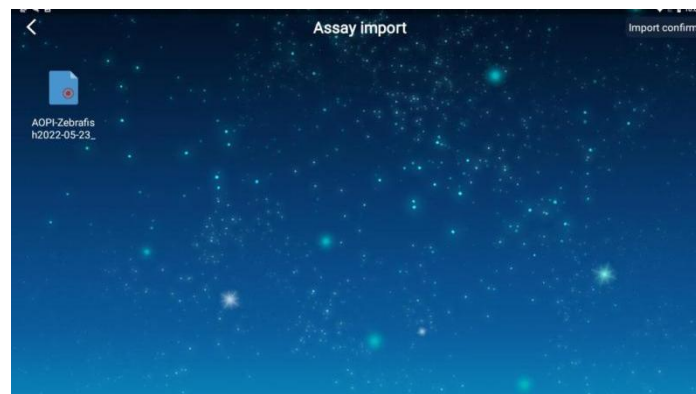


Fig. 2.43 Assay import interface

- ④ Software will automatically return to the "Main Menu" user interface (see **Fig. 2.44**). when the importing process of assay templates, indicated with "Import Success" pop up of completion. Finally, the new icon for the AOPI-Zebrafish BioApp is visible in the user interface of **Assay Management**, which means the BioApp has been imported successfully, and can be used instantly.



Fig. 2.44 Main menu user interface with new imported AOPI-Zebrafish BioApp

2.5.2 Copy/Editing/Deletion/Export/Vision or Invision

All BioApps (Assay templates) of the Countstar® Mira HT can be copied inside the main menu, edited for an optimization of the settings, deleted, if no longer used, or exported to a USB device to be copied to another Countstar® Mira HT. Small icons at the bottom of the BioApp icon can be activated by a longer touch of one of the BioApp icons, and represent these different functions (**Fig. 2.45**).



Fig. 2.45 Activated icons in the Assay Management user interface

2.5.2.1 Copy

By clicking on the "Copy" icon, a replicate of the selected BioApp has been generated, which contains identical parameter settings as programmed in the original version. Subsequently, the user can make parameter adjustments in this copy without overwriting parameters in the original BioApp. As an example, when the user clicks on "Copy" at the bottom of the BioApp "Trypan blue-CHO", a dialog box opens to show whether the copy of the currently selected BioApp has been generated (see **Fig. 2.46**, left). If the name of a new BioApp from a copy is not edited during the procedures, it will be name by adding the suffix "-1" at the end of the original name. In case, the user doesn't want to complete the action, he has to click "Cancel" to return to the Assay management user interface. If the user hasn't changed the name, he has to click "Confirm" to automatically generate the "Trypan blue-CHO-1" BioApp, which icon will appear in the list of all BioApps in the assay management menu (**Fig. 2.46**, right).

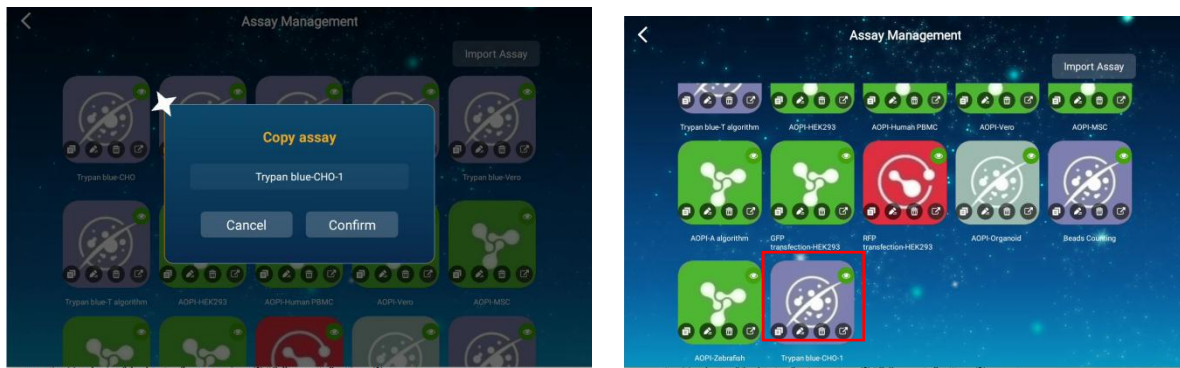


Fig. 2.46 Dialog box for guiding copying the BioApp (left), Assay management menu after completion of the copy process (right)

⚠ Caution: The character numbers for each BioApp names are limited to 16 digits at maximum.

2.5.2.2 Editing

By editing a BioApp, the user has the option to modify this assay template protocol. He can change the more detailed parameters: (1) "Basic information" like the Test name of the BioApp, information about the object slide (consumable) used, adapt image acquisition settings like the exposure time(s) and gain of the detector, fine tune various; (2) "Cell parameter" settings; (3) "Result Display" for the different result settings. As an example, the procedures of editing a BioApp named AOPI-CHO is described in the following. During the editing procedure, it will be inevitable for Countstar® Mira HT with insertion of a Countstar® chamber slide of which at least one of the chambers of the slide are filled, with a cell sample stained with the appropriate dye(s) for this BioApp.

When the user clicks on the "Edit" icon at the bottom of the AOPI-CHO BioApp, the "Assay editing" user interface is opened to show the Basic Information (1) by default (see **Fig. 2.47**).

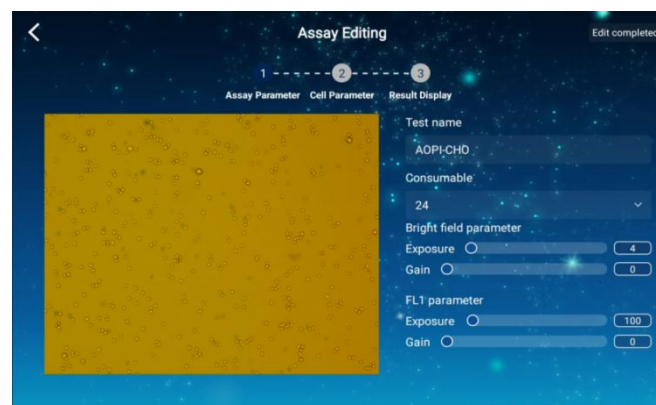


Fig. 2.47 Editing interface for BioApps (assay templates)

1) Basic Information

- ① User should carefully check, if a Countstar® chamber slide filled with AO/PI stained A549 cell sample in Slot 1 and inserted correctly, and "Countstar" is selected under Basic information, consumable. The Countstar® chamber slide will be aspirated to the view in the Slot 1 by the analyzer. And imaging in real time.
- ② User can change the Test name, followed by clicking on "Edit Completed" to save all modified information. The software window will then return to the "Assay management" main interface; A click on the ">" Return icon will lead the current window directly back to the Assay Management user interface without saving the modified information.
- ③ Select under consumables that slide that fits to the pre-adjusted magnification, then click on "Edit Completed" to save the currently modified information. If changed information shall not be saved, clicking on the ">" Return icon will lose the modified settings and software window will be back to the **Assay Management** interface.
- ④ Parameter Settings for acquiring the desired images of bright field and FL1 channel. In this section, the user can test and set the optimal conditions (Exposure time and Gain) on imaging on each light channel (bright field and fluorescence) to obtain the best qualified images.

By default, exposure time of the bright field channel in the AOPI BioApp is fixed to 42ms, with the value (0.0) of a gain factor. The default bright field exposure time for all Trypan blue assays (concentration of Trypan Blue = 0.2%) is set to 100ms, also using a gain factor of 0.0. The brightness of the obtained pictures should be always adjusted to a relative balanced level to work out all details of the objects of interest, as an example shown in the examples with MCF7 cells (**Fig. 2.48**).

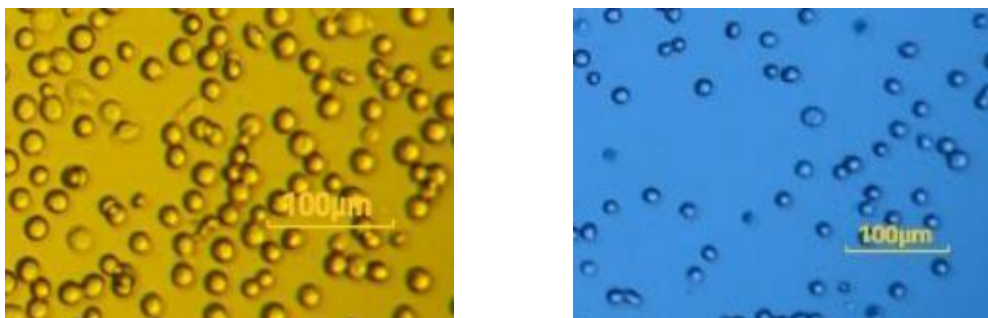


Fig. 2.48 Enlarged image views of MCF7 cells in bright field channel Left: By AOPI BioApp; Right: By Trypan Blue BioApp



Caution: The default exposure parameters during detection of samples stained with Trypan Blue based on the background of a sample stained with Trypan Blue with a

concentration of 0.2%. Shanghai RuiYu BioTech doesn't recommend to use other concentrations of Trypan Blue dye in combination with the Countstar Mira HT analyzer.

The exposure times of images under the fluorescent channel 1 (FL1) (Fig. 2.49, middle) is set to 100ms, with a value (0.0) of the gain factor. But, in most fluorescent based experiments the exposure time needs to be modified due to the variable uptake behavior of the manifold fluorescent dyes into the cells prior to the routine analysis. The best optimal exposure times will be required for producing images with high contrast versus the background, with sharp and detailed information about the distribution of the fluorescent dyes inside or on the surface of the cells. Too short exposure times (e.g. 60ms; Fig. 2.49 left) will produce low-contrast images with lost or weakly labelled cells, while an exposure time of 300ms will generate overexposed images. At this intensive illumination, the difference between the fluorescence emitting cell/particle and the surrounding background obliterates (see Fig. 2.49, right). In this example, the optimum exposure time was iterated to 400ms (see Fig. 2.49, right).

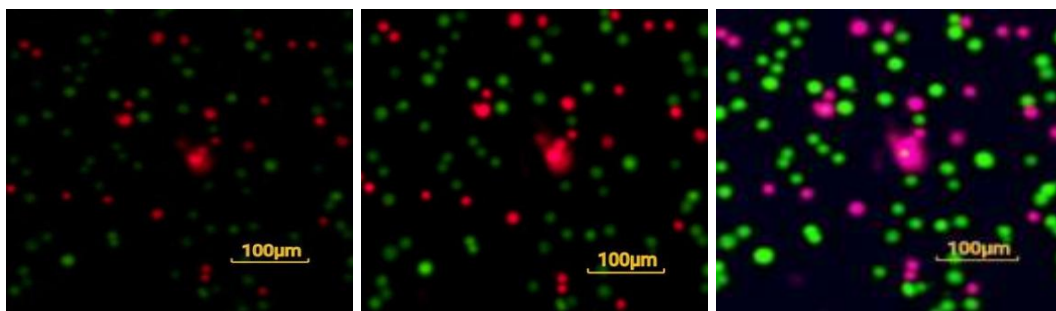


Fig. 2.49 Images of AO/PI-stained MCF-7 cells (5x magnification), acquired at different exposure times of 60 ms (left). 100ms (middle), and 300ms (right)

When the user has found the optimum exposure time for the fluorescence channels, he needs to click on "Edit Completed" to save the final modified settings. The software window will return to the main assay management interface.

A click on the "< Return" icon will get the current window back to the assay management user interface without saving the information.



Caution: The basic information of fluorescent channels for excitation/emission wavelength cannot be edited by the user.

2) Cell parameters

As cell species have the various characteristics of optical appearances in different image-based analysis systems (partly due to their genetical modification), Countstar® Mira HT installs AI-based algorithms of image recognition to intelligently customize imaging parameters to optimize the results of the image analysis, according to cell's individual optical characteristics.

① There are five (5) general variables can be set named **NMS**, **Prob**, **Thres**, **Min_Size**, and **Max_Size**.

NMS and **Prob** is the parameter of AI algorithm: the increase of **NMS** will increase the identification of targets; An increase in **Prob** will reduce target recognition.

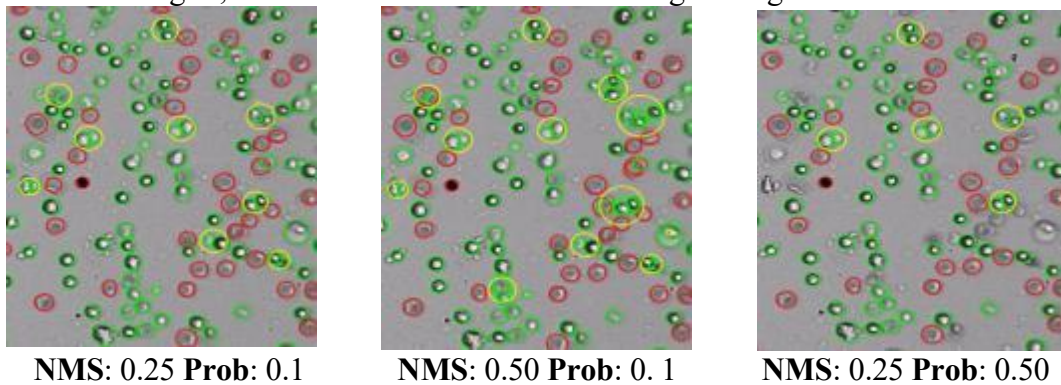


Fig. 2.50 Parameter settings of **NMS** and **Prob**, showing the effect on the labelling of varying configurations

! Caution: The default values of the parameter **NMS**, **Prob** and **Thres** have been applied in many analysis results to be the optimum settings for each pre-installed BioApp. We strongly recommend to abstain from a re-adjustment of these values. If you need to adjust it, contact technical support for reference.

As the user will test cells with the varying sizes, he can change the **Min_Size** values to define the minimum size of objects in the image to be counted as cell, to filter them from smaller cell fragments or unspecified objects during the image analysis process.

The **Max_Size** is used to set the maximum sizes of the single cells, and distinguish it from large impurity particles (or cell clusters) in the analyzed sample.

As shown in **Fig. 2.51**, when the value of **Min_Size** setting is set to 8, all cellular objects are marked correctly, but small impurities and fragments in the image have not be classified as cells.

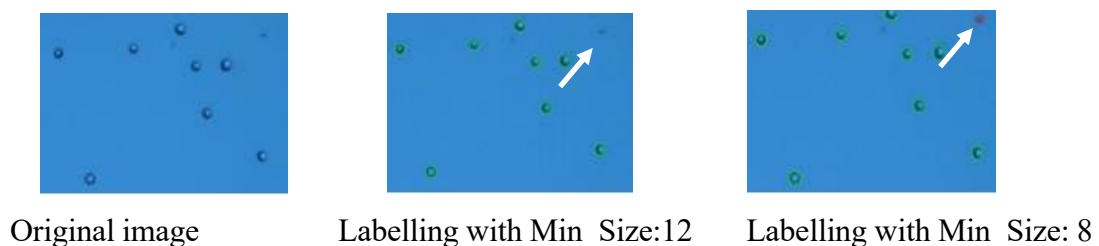


Fig. 2.51 Effect of value of **Min_Size** on the labelling results

As shown in the example (**Fig. 2.52**), when the value of **Max_Size** is 35, viable cells are identified by the green circles, larger particles and impurities will either not be taken into account or marked in red as cells, depending on the **Max_Size** setting.

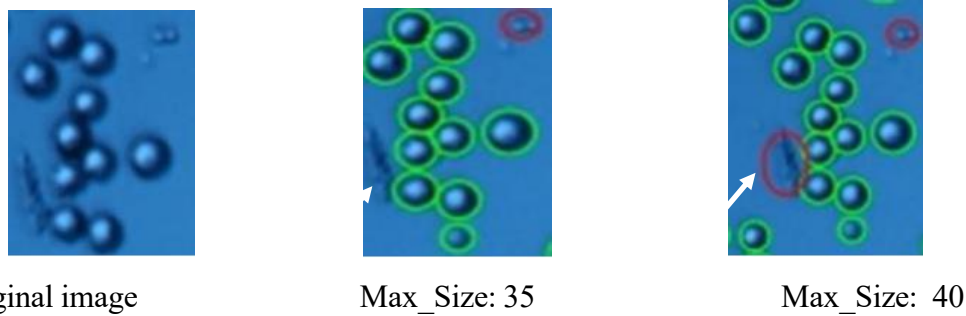


Fig. 2.52 Effect of varying Max_Size settings on the labelling results

②All BioApps with the bright field channel have two specific cellular parameters, named **Min-Ave-Gray** and **Max-Ave-Gray**. These parameters are responsible for the classification of cellular objects from the background, and the discrimination between viable and dead cells.

The lowest Min-Ave-Gray value is 0 and the algorithms will mark all targets with an average of gray scale level higher than 0.

The highest value of Max-Ave-Gray is 255, which is used to identify targets with an average gray level lower than this value. It is mainly used to distinguish and screen for dead cells.

As shown in the example of **Fig. 2.53**, if the Max-Ave-Gray setting is ideally set (130), all dead cells are identified, but impurities with a high gray scale are not taken into account as cells by the image recognition algorithms.

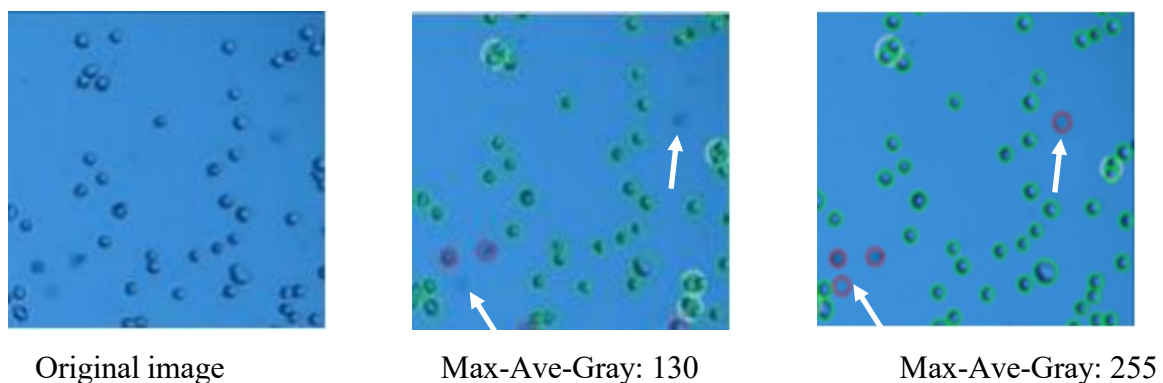


Fig. 2.53 Effect of Max_Gray settings on the labelling results of the algorithms

⚠ Caution: The Min-Ave-Gray and Max-Ave-Gray values, set by default in the pre-installed bright field based BioApps have been optimized to match to a multitude of tested image situations. In case, the user isn't satisfied with the current labeling results of acquired images, he or she may adjust the values to obtain the expected test results. Alternatively, he or she can send the images to our technical supports for image recognition support to get optimum settings for Min-Ave-Gray and Max-Ave-Gray, evaluated on base of the images provided.

③Parameter adjustment of situations of live and dead cells (for Trypan Blue analysis). **Live /Dead Activity.**

Based on the results of the AI algorithms, the "Activity" adjustment mode can be optionally switched on to re-evaluate the results of live and dead cells.

As shown in **Figure 2.54 (right)**, the Live/Dead parameters can be set to 1 and 0.5 respectively, and live cells with a less than translucent center can be identified accurately (White arrow).

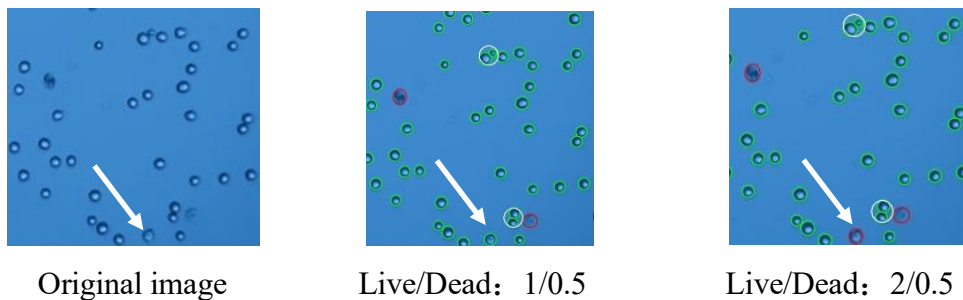


Fig. 2.54 The labelling results affected with different values of Live/Dead parameters.

④All fluorescence-based BioApps have also 4 specific parameters, named **Green-Min-Ave-Gray**, **Green-Max-Ave-Gray**, **Red-Min-Ave-Gray** and **Red-Max-Ave-Gray**, which determining the multiple properties of the photos during the imaging.

Min_Gray and **Max_Gray** are used to mark targets with an average fluorescence intensity higher and less than their values, respectively. The lowest **Min_Gray** value is 0, and the highest **Max_Gray** value is 255. It is used to distinguish and screen target cells.

As shown in **Fig. 2.55**, if the **Red-Min-Ave-Gray** value is reduced to 0, even cells (objects), emitting a very weak signal (**Fig. 2.55**, see arrow), will be identified as targets by the algorithms.

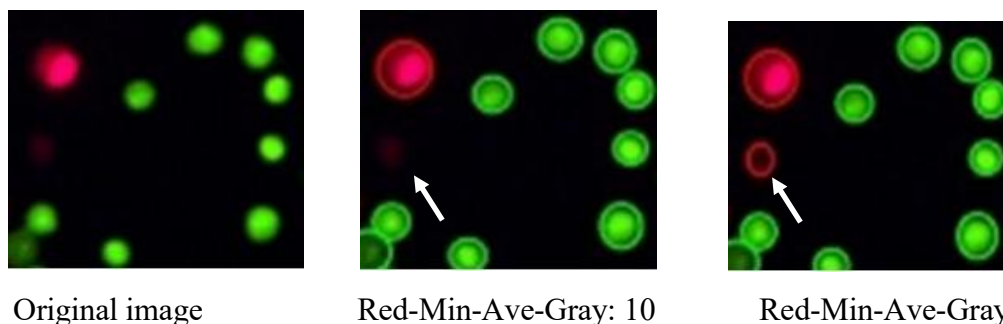


Fig. 2.55 The labelling results affected with different values of **Min_Gray** parameters.

Also, the **Min_Gray** and **Max_Gray** values, set by default in the pre-installed fluorescent based BioApps have been optimized to match to a multitude of tested image situations. In case, the user isn't satisfied with his labelling results of acquired images, he may adjust the values to fit the labelling results of his samples to reach his expected test results. Alternatively, he or she can send the images to our technical supports for image recognition support to get optimum settings for **Min_Gray** and **Max_Gray**, evaluated on base of the images provided.

⑤Ratio of red and green colors can be adjusted as the threshold parameter of living cells (for AOPI viability analysis), and called this parameter as **State-Param**.

As shown in **Fig. 2.56**, while **State-Param** value is reduced to 1 or 0.5(The green component

is greater than the red component), the final results of yellow fluorescent signals or red-green fluorescence signals can be changed according to user requirements.

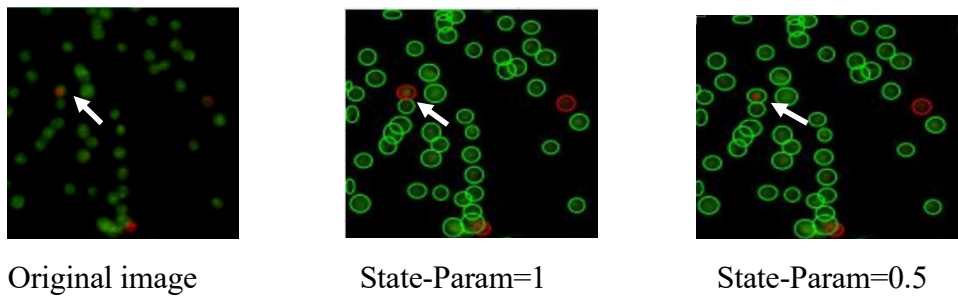


Fig. 2.56 The labelling results affected with different values of State-Param parameters.

3) Result Display

In the submenu of the **Assay edit** user interface, the user can individually manage the orders of the records of the measured and analyzed data from the image recognition algorithms. Moreover, the less important results determined by the user can be hidden (made invisible) in the result display area of the Countstar® Mira HT, (**Fig. 2.57**).

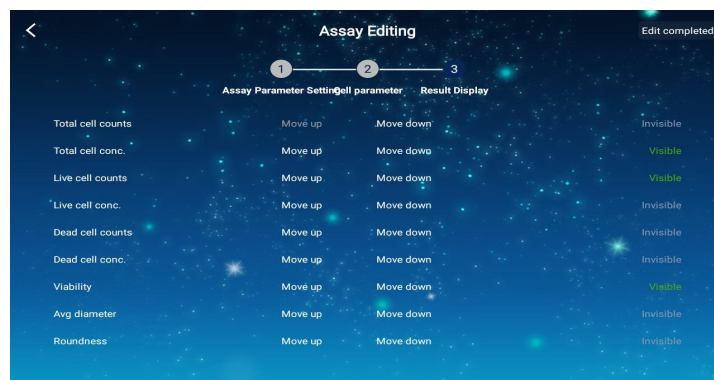


Fig. 2.57 Result Display interface

The user can change the order positions of the displayed results by clicking on the icons **Move up** or **Move down** beside the single result category. The active status of the function is always highlighted in green. By default, all result information generated from a selected assay are set to be **Visible** (green font). If the user clicks on **Visible**, it will switch to **Invisible** instantaneously, displayed in a gray font (**Fig. 2.58**).

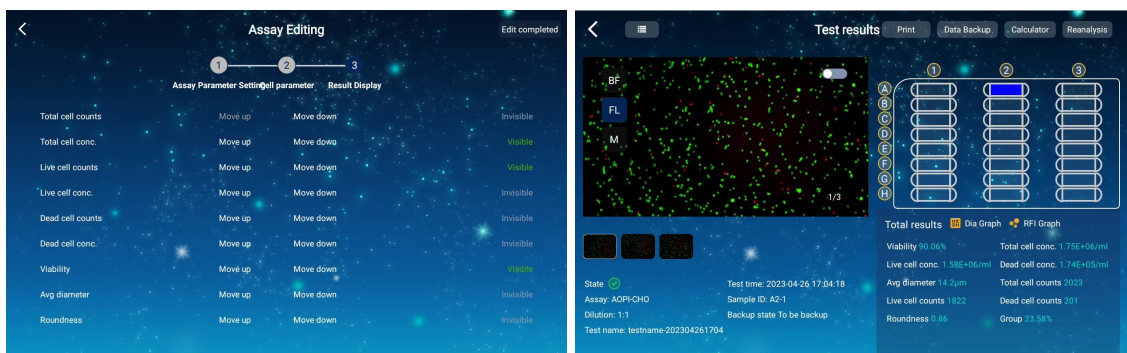


Fig. 2.58 Result setting "invisible" interface

2.5.2.3 Deleting

Although the Countstar® Mira HT has the enough capacity to store different BioApps users required, it can make sense to delete older BioApps in the Assay management menu, while they will not be in use any longer.

⚠ Caution: When deleting a BioApp, not only the assay method itself, but all correlated information, and all associated, already acquired results will be deleted irrevocably from the internal analyzer database as well!

When the user clicks on the **Delete** icon at the bottom of a BioApp in the interface, a popup dialog box appears by asking: "Are you sure to delete the assay? Delete it, the historical data within the assay will be not exist, Cancel/Confirm" (**Fig. 2.59**). When the user clicks on Confirm, the assay icon will disappear from the Assay management user interface, and already acquired data with relationship to this BioApp will be deleted from the internal data base. By a click on **Cancel** the software window will be returned to the previous menu, maintaining the BioApp, and all acquired images and results.

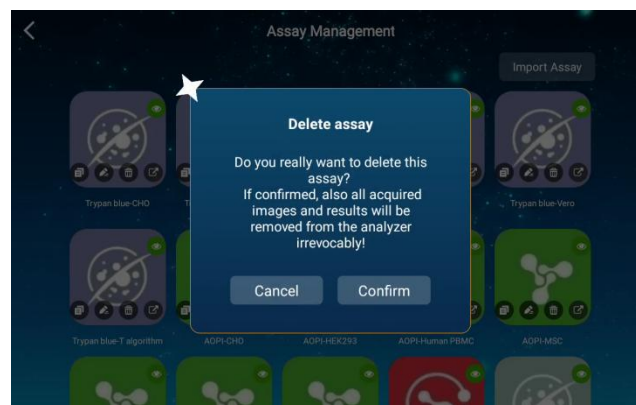


Fig. 2.59 Dialog box of the Deleting BioApp

2.5.2.4 Exporting

In case a BioApp needs to be transferred to another Countstar® Mira HT analyzer, or the BioApp shall be modified (edited) by the experts in the headquarters of Shanghai RuiYu Biotech, the assay template can be easily exported to an external USB storage device. An export folder, named **exp** will be automatically generated, on the USB device. The exported text file is named to: BioApp name plus export date and time (e.g. AOPI-CHO2022-02-15_22-54-39.exp). To start a BioApp export, the user has to insert an external USB storage device with sufficient free data storage capacity into one of the USB ports at the backside of the instrument, first.

Subsequently, the user has to click on the **Export** icon of the selected BioApp to start the export. A dialog box pops up, indicating the progress of the export by a green bar. When the export is finished, the interface prompts "Data export Completed", and showing the location and name of the exported BioApp file. The user needs to click on **Complete** to finish the BioApp export.

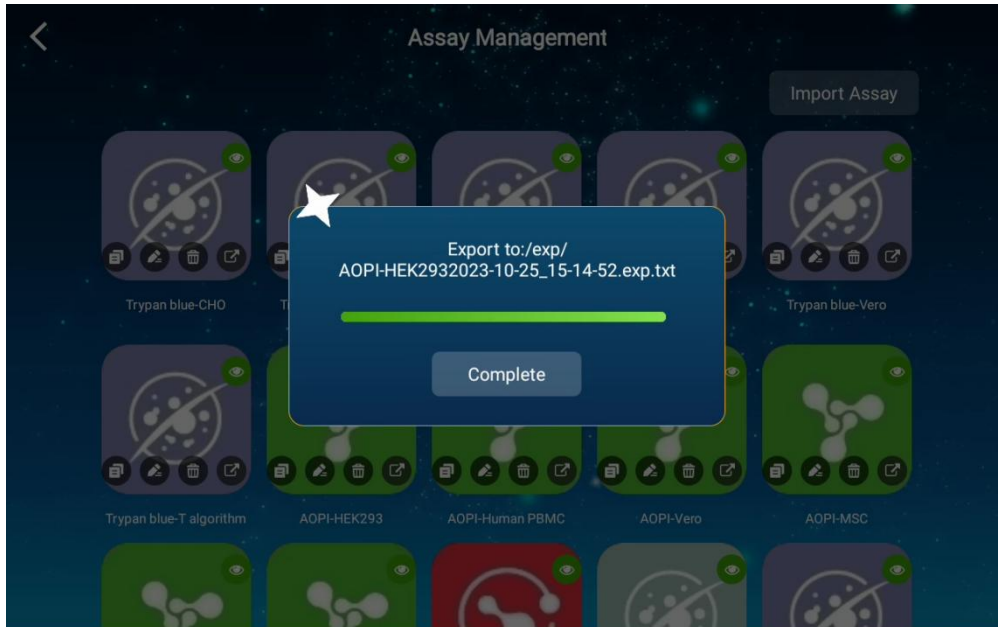


Fig. 2.60 Dialog box BioApp export

To import a BioApp from an external USB storage device, (**check Chapter 2.5.1**).

2.5.2.5 Hiding

In the Assay Management user interface, the visible "👁️" and invisible "👁️" icons can be switched. Users can selectively display the Experimental interface and the types of the assays according to their requirements.

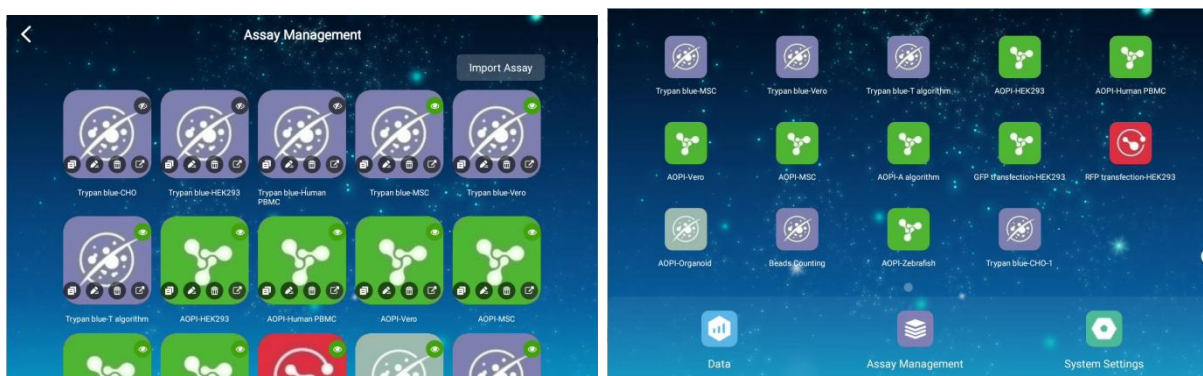


Fig. 2.61 Result setting "invisible" interface

2.6 System Settings

The **System settings** user interface contains the Language Settings, User Management, Network Connection, Time Settings, Standby, Company Information, Test (Consumables Management, Picture library, Analysis Algorithm, PDF), Equipment (Equipment Name, Equipment Test, Software Version, Storage, System Backup, Data Backup, Restoring Factory Settings, Log (Audit trail), and Service (User Manual, About Us, Transport Shutdown).

Note: The transportation shutdown with the red I/O icon, and **Manufacturer information** (including WeChat QR code) (**Fig. 2.62**).

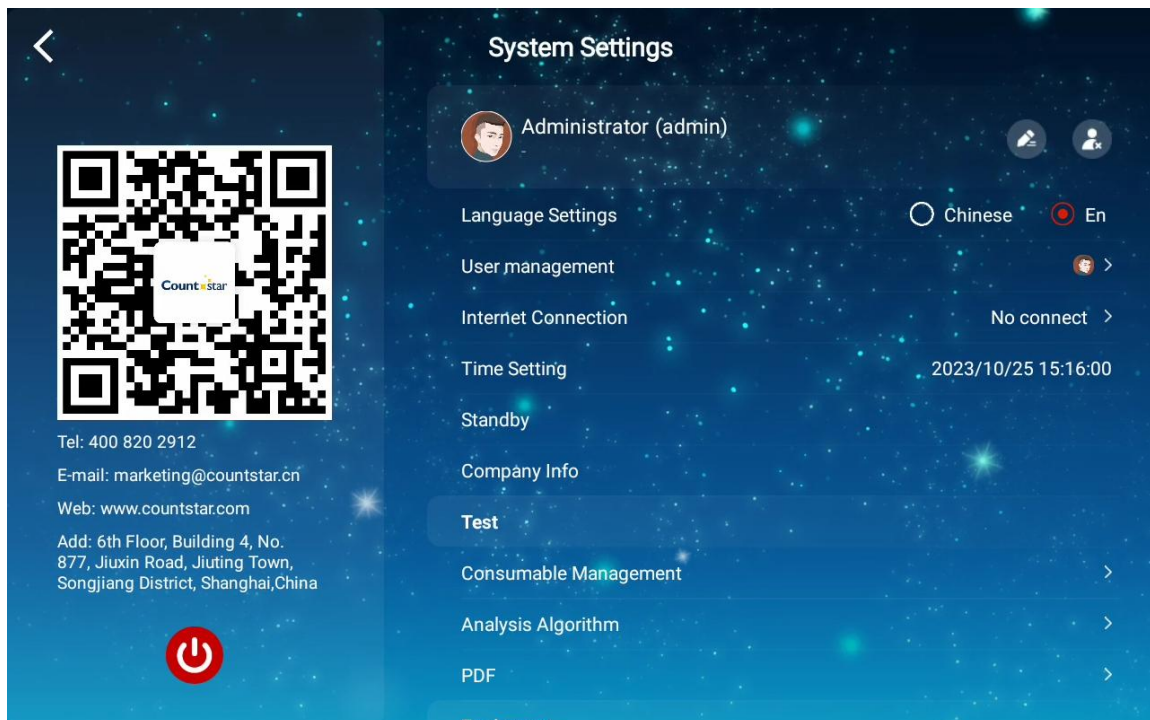


Fig. 2.62 System setting interface

2.6.1 Language settings

The operating language of Countstar® Mira HT can be switch between Chinese and English, based on the users' personal preference, when the user clicks on the check mark beside **EN** or **Chinese**.

2.6.2 User Management

Countstar® Mira HT holds the function of RBAC (Role-Based Access Control) authority model, which is more flexible between user and authority configuration. Once you have set a user role, you can customize the permissions for that user to be set freely. All available permissions are described as follows:

Operations	Permissions	Details
Camera	Photograph	Only taking photos with the machine
Data analysis	Viewing data	Viewing all information on data management user interface
	Exporting data to USB	Exporting data to USB with multiple formats containing reports (PDF), cell photos (JPG) and detailed information (CSV)
	Deleting data	Deleting data
	Backup data	Backup data
Assay management	Viewing	Viewing assays with detailed information
	Copy	Copy an assay as a new
	Editing	Editing an assay
	Deleting	Deleting an assay
	Importing	Importing an assay
	Exporting	Exporting an assay
Consumables management	Editing	Editing the consumables information
Software update	Updating	Updating the software to a new version
Hardware information	Viewing	Viewing the hardware information
User management	deleting, adding or adding	deleting and adding user information, or editing the user information
System backup	Backing up	Backing up the system
Data backup	Backing up	Backing up data
Audit trail	Audit trail	Tracking the records containing the time, user, operation content and other information
Factory setting	Restoring the factory setting	Restoring the software to the factory setting
Assay management	Executing an experiment	Executing an experiment by using this machine

2.6.2.1 Role management

Role inside the User Management menu of the Countstar® Mira HT (**Fig. 2.63**) can be edited for an optimization of the settings, deleted, if no longer used, and viewed for detailed information, as well as adding a new role.

- 1) **Adding a new role:** enter the Role Management interface and subsequently click adding a new role, followed by writing the role name, introduction and permissions. click OK and enter the current user password, and a new role is added.
- 2) **Viewing role information:** role introduction and permission can be viewed inside the Role Management interface.

- 3) **Editing a role:** role introduction and permission can be edited inside the Role Management interface.
- 4) **Deleting a role:** role information can be deleted inside the Role Management interface.

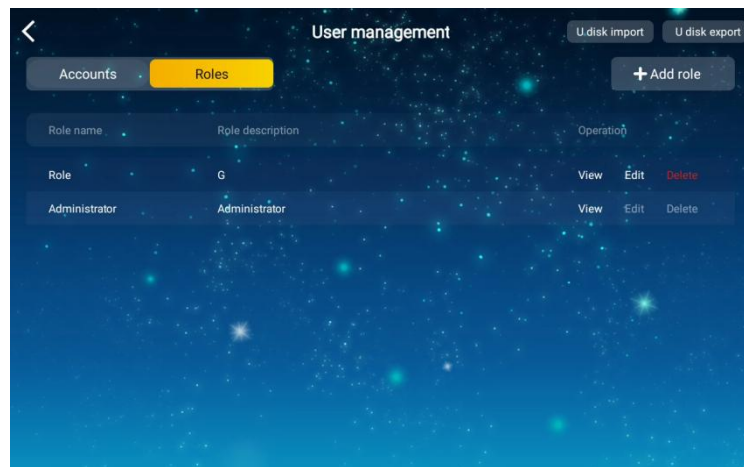


Fig. 2.63 Role management interface

2.6.2.2 Account Management

Accounts inside the User Management menu of the Countstar® Mira HT (**Fig. 2.64**) can be edited for an optimization of the settings, deleted, if no longer used, and viewed for detailed information, as well as adding a new user.

- 1) **Adding a new account:** open the User Management interface and subsequently enter name, password (at least 8 digits, including at least three types of lowercase letters, uppercase letters, numbers and special symbols), and basic information containing true name, phone number, email address, department, job title and avatar. User role and the validity period of the password are selected. Finally, click "OK", and the new account is added.
- 2) **Editing an account:** account information inside the User Management interface can be edited.
- 3) **Freezing an account:** accounts inside the User Management interface can be frozen if currently not used.
- 4) **Deleting an account:** account information inside the User Management interface can be deleted.

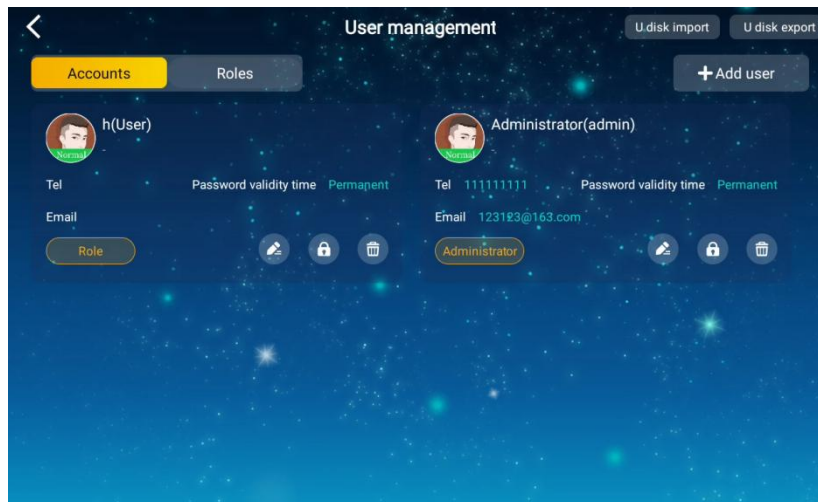


Fig. 2.64 User management interface

2.6.3 Network Connection

Network Connection of Countstar® Mira HT can be supported by "WIFI" and wired network approach. After the network connection is enabled, you can view the information of current WIFI on the screen. To add a "WIFI" network to Countstar® Mira HT, you should select an appropriate WIFI name, then click "Connect". after entering passwords for the current WIFI network, the user can click "OK" button to finish the connection of WIFI network.

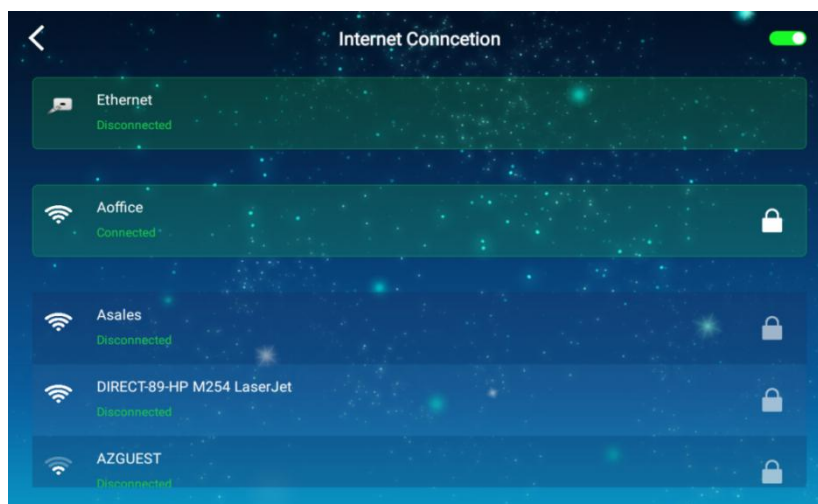


Fig. 2.65 Setting network connections interface

2.6.4 Time Settings

Depending on the date and time zone, where the Countstar® Mira HT will be operated, it will be inevitable for the user to adjust the date and time of the analyzer to the regional date and time zone. When the user clicks on time settings, a calendar and a clock will be displayed (see Fig.

2.66), where he can select the actual date and adjust the time on the clock, moving its fingers to the desired time.

If the instrument is connected to the network, the system can automatically check and update the network time.

⚠️ Caution: It is recommended to calibrate the instrument time every six months.

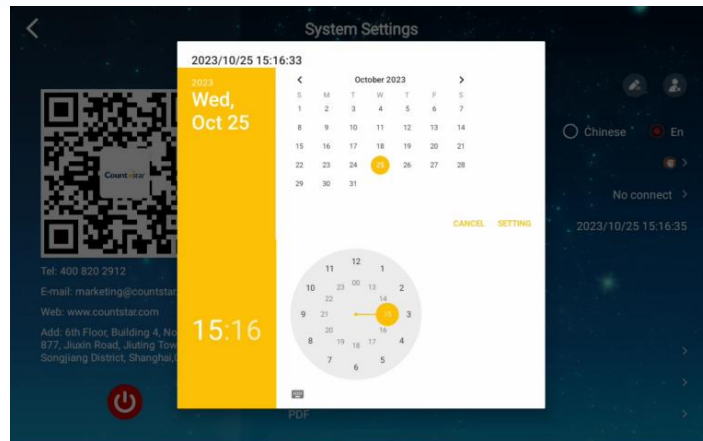


Fig. 2.66 Time setting interface

2.6.5 Standby

The Countstar® Mira HT powered on will automatically enter to the login interface followed by logout or shutdown if the machine is not desired to be used for a certain period of time. Therefore, the user can set a mode between logout and shutdown with remaining time (minutes)(**Fig. 2.67**), which is saved after conformation with entering the current user password.

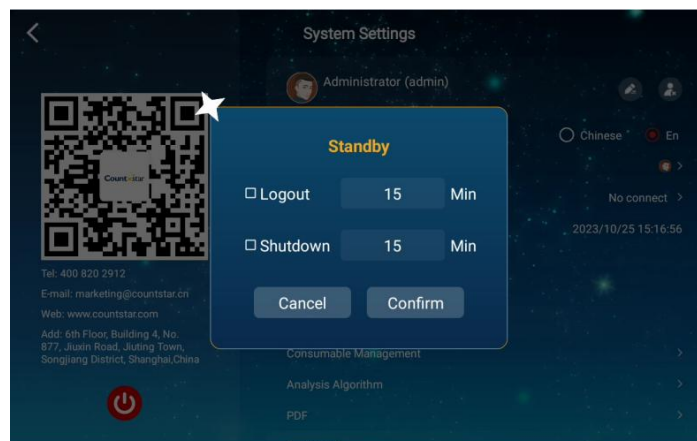


Fig. 2.67 Standby interface

2.6.6 Company Information

You can customize the company logo and name, which will be displayed in the exported reports as PDF files. The procedures of Entering a company logo are shown in **Fig. 2.68**. Firstly, the

user should create a folder on a USB flash drive to save the logo images with JPG/PNG formats and and import the required logo images in JPG/PNG formats and vertical resolution more than. Secondly, the user should connect the USB device to Countstar® Mira HT and the picture at the first position ordered by the first letter of the names is selected as logo image by default (no pictures can be selected), Finally, click "Save" button to finish the logo setting.

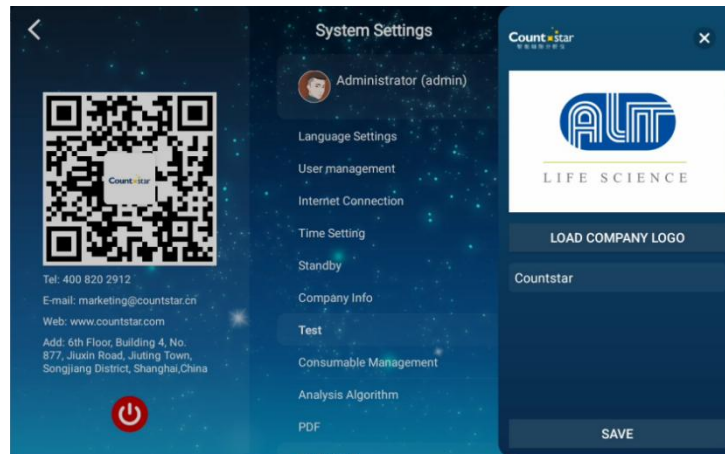


Fig. 2.68 Editing company information

2.6.7 Test

2.6.7.1 Consumables Management

Only both of consumables of Countstar® chamber slides and 24-well chamber plate can be used for sample tests in the Countstar® Mira HT analyzer. According to their requirements, the consumable parameters can be replicated, edited, and or deleted (**Fig. 2.69**).

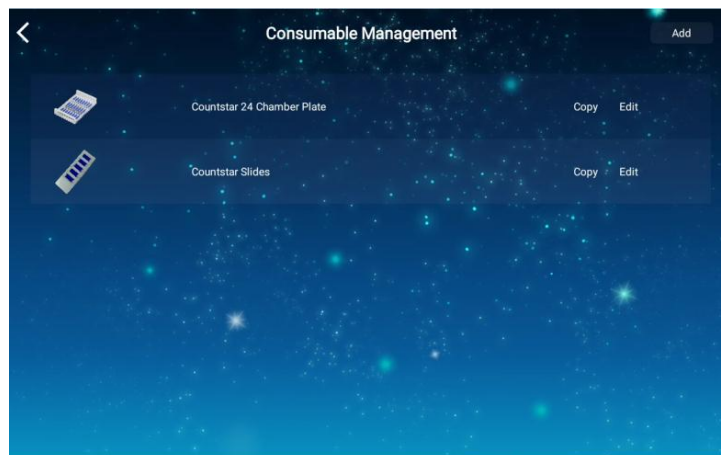


Fig. 2.69 Consumable management user interface

If the user wants to replicate a consumable data set to produce a new identity, he has to select the **Copy** icon along with the data set to open a dialog box and name the new data set (see **Fig. 2.70**):

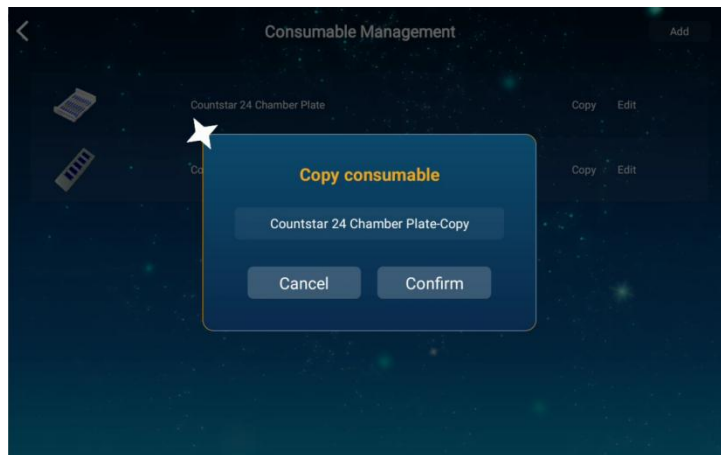


Fig. 2.70 Replicating the consumables

The parameters of an existing consumable data can also be edited (see **Fig. 2.71**) by a click on **Edit** icon beside the consumable data set to open a new submenu where the user can edit the consumable name, consumable geometrical data, the numbers of fields of view during image acquisition, magnification value, and parameters of focal length.

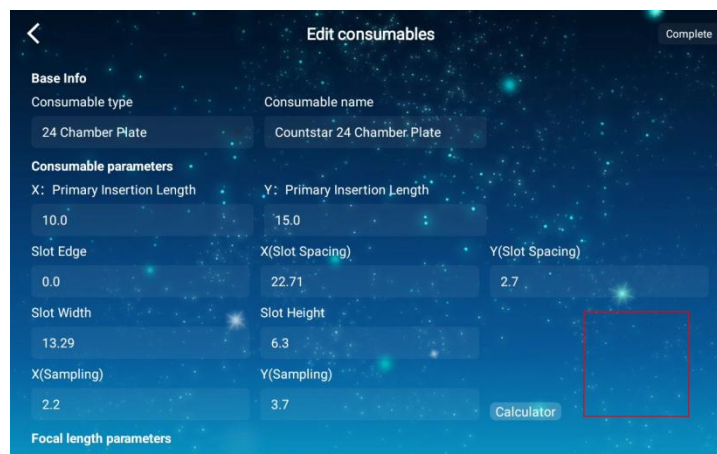


Fig. 2.71 Editing menu of the consumables management

1) Consumable name:

The consumable names can be customized to the user's preferences. A double clicking on the entry field with the current name to open on-screen keyboard to modify the name.

2) Consumable parameters:

Except for the numbers of slide chambers and fields (of view), all other variables in this menu are fixed for the Countstar® chamber slides and 24-well chamber plate. The user cannot modify the other parameters, as he has no access to the geometrical specifications of these consumables.

⚠ Caution: The maximum number of chambers is limited to 5; The default numbers of fields (of view) is set to 3 (up to 5 image recordings per chamber are possible).

3) Focal length parameters

A re-calibration measurement of the focal length on a regular period will document the high stability of the focus system of the Countstar® Mira HT analyzer. The instrument's focus length is initially adjusted during manufacturing and recorded in the focal length settings of the device. To prove the focus system's suitability, beads with proprietary focus calibrations, supplied by Shanghai RuiYu Biotech, can be used to check the focal length. As an example, Images and analyzed results of the test on focal length are shown in **Fig. 2.72** and **Fig. 2.73**.

The detailed operation instructions for the check of the focal length are described here:

- The user has to pipette 20µl of a homogeneously suspended Countstar® Focus Calibration Beads 1 into the center chamber (chamber no.3) of a Countstar® chamber slide;
- The user needs to note the maximum focal length parameters as (shown in **Fig. 2.73** at the lower left①). The value of focal length is **2** by default. After insertion of the Countstar® chamber slide containing the Focus Calibration Beads 1 into the analyzer by pushing the chamber slide into the instrument through the port, until it has reached its designated position.

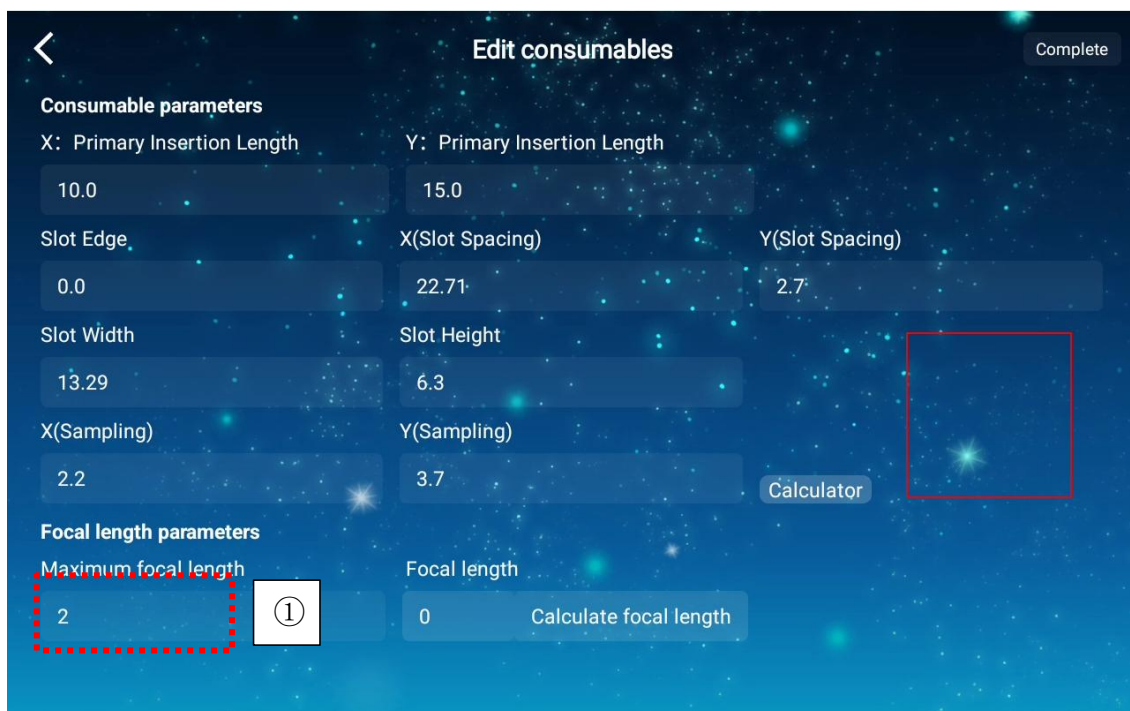


Fig. 2.72 Countstar® Mira HT maximum focal length values that can be entered at different magnifications

- Then the user has to click on "Calculate focal length". The chamber slide is automatically moved to its specified position inside the instrument, where the chamber no.3 is in focus. Once, the image situation on the screen doesn't change significantly, the user needs to click on the Refocus icon below the main image to start the focus calibration. The main, large

picture on the left and the chart on the right with its increasing numbers of thumbnails indicate the progress of the focus calibration process in real time. It will take approximately 1-2 min to finish the whole processes.

- The focal length calibration is finished with a two-step procedure: The focal length calibration of first obtained image are a base for a coarse adjustment of the focus. During this process, the user interface displays the **Coarse best focus** value, as shown in **Fig. 2.74**

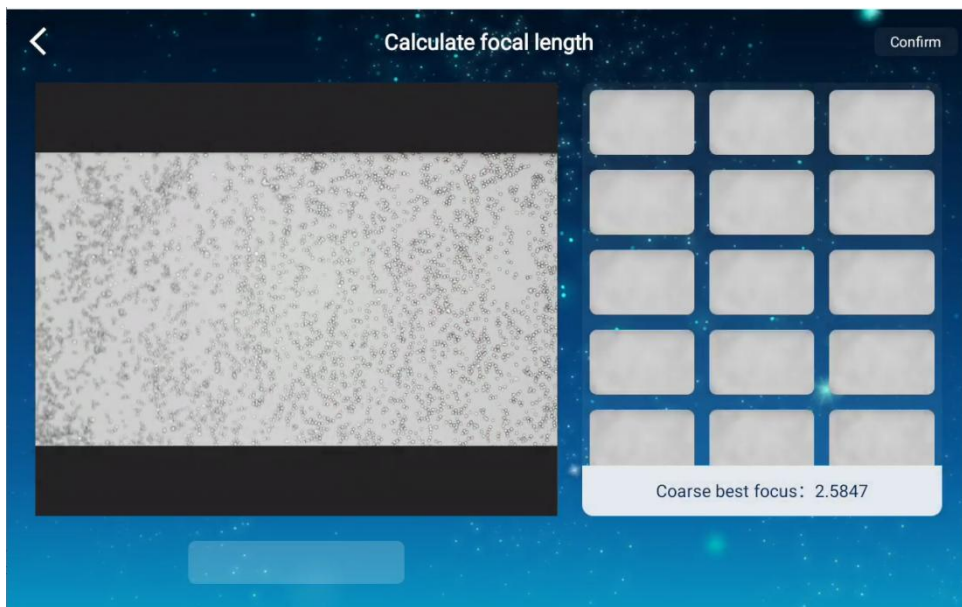


Fig. 2.73 Focus recalibration user interface: Step 1: coarse focusing

- The coarse focus adjustment is immediately followed by the fine focusing calibration. Once this step is finished too, the best focus value will be displayed at the lower right of the touchscreen. The user can click on the thumbnails in the chart on the right to view the images with the best focus value. By clicking on the **Confirm** icon at the upper right of the touch screen, the software window switches back to the **Edit Consumables** user interface. The system can automatically save the new best focus value and display it in the Edit consumables menu.

⚠ Caution: To avoid vibrations to the Countstar® Mira HT during the focusing process, it is recommended to repeat the focus adjustment 3-5 times to demonstrate the stability of the best focus value.

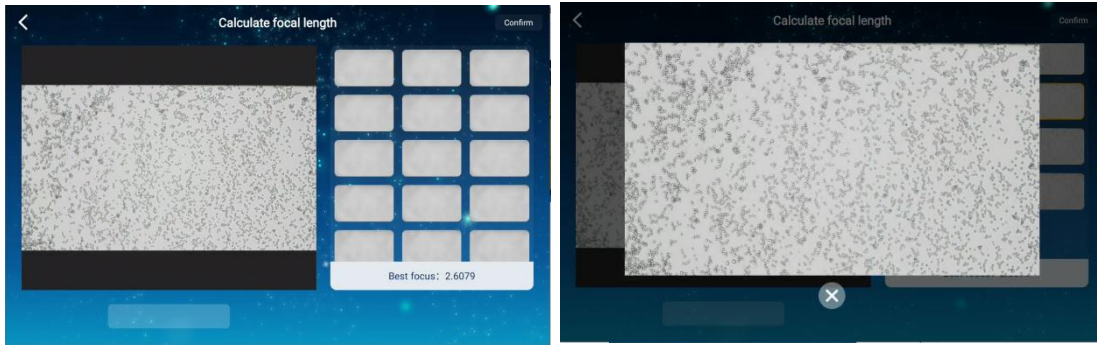


Fig. 2.74 User interface during performing best focus calibration (ti huan)

2.6.7.2 Analysis Algorithm

When a new version of analysis algorithm is released, customers can update the new algorithm through the "Import" function in the analysis algorithm module. If customers need a new individual application, they can also contact Countstar® technical Support and we will customize the analysis algorithm to suit their desired requirement (**Fig. 2.75**).

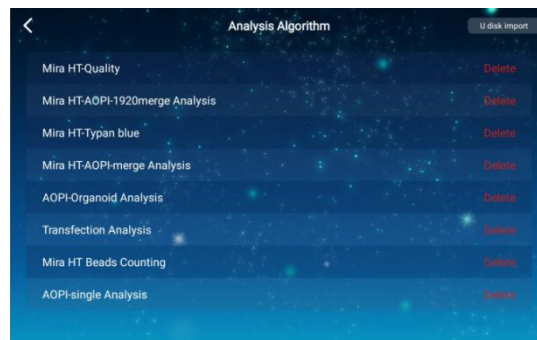


Fig. 2.75 Analysis algorithm import interface

2.6.7.3 PDF

Click on PDF settings to enter the PDF settings interface, where you can set the company name, logo, file signature, and output information for individual experimental types (basic information, sample data, shooting content, chart content, etc.).

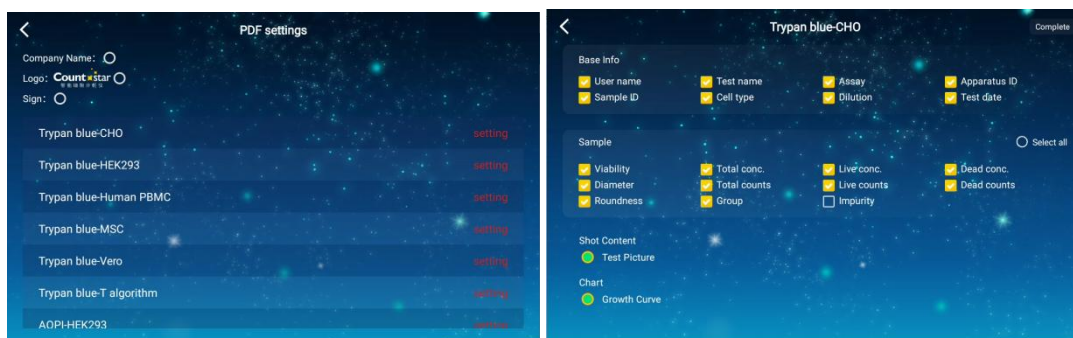


Fig. 2.76 The user interface of the "PDF settings"

2.6.8 Equipment

2.6.8.1 Equipment Name

In this section of the **System settings**, the equipment's name is displayed. When exporting data, the equipment's name will also be used as a unique identity for folders where these data will be stored. Therefore, the equipment name needs to be set in advance, otherwise an error message will occur when trying to export data. The equipment's name is identical to the serial number of the analyzer.

2.6.8.2 Equipment Test

The Countstar® Mira HT offers the possibility to execute a system's self-check of all essential device components at any time. When the user clicks on **equipment test**, he will be asked in a dialog box to pull out any remaining chamber slide prior to **confirming** the start of the equipment test (see **Fig2.77**). In case, the user doesn't want to start the test, he simply needs to click on the **Cancel** icon, and the software window will return to the previous menu.

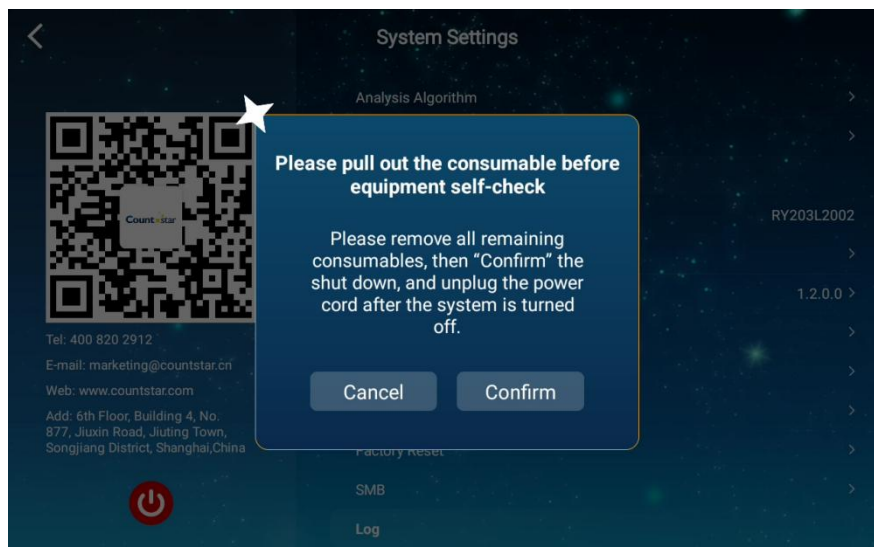


Fig. 2.77 Dialog box for the activation of the equipment self-check

⚠ Caution: It will be absolutely mandatory, before starting the **Equipment test**, to make sure that any remaining chamber slide in the machine has been removed. If the Equipment test procedure is started with a consumable still inserted, the manufacturer and its representing distribution partner cannot be held liable for any damage to the analyzer, caused by ignoring this message!

Once, the **Equipment test** has started, a control board on the right side of the touchscreen pops up, showing the progress of the test of the single analyzer components by rotating clocks (Fig. 2.78). To finish the whole procedures will last approximately 1–2 min.

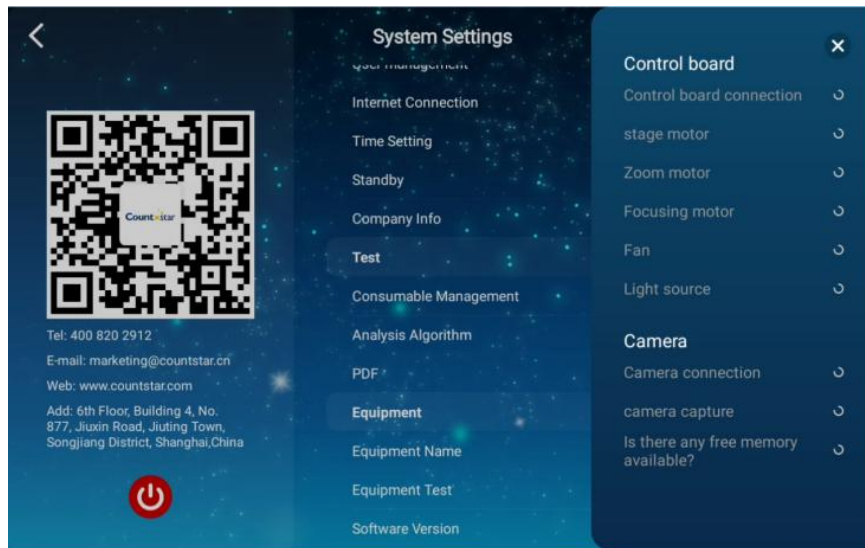


Fig. 2.78 Control board of the Equipment test menu

When the Equipment test is completed, check marks of all components should be displayed in green as shown in Fig. 2.79. The user has to click on the white X at the upper right of the control board to return to the System settings menu. It is recommended to shut down and restart the analyzer, after finishing the Equipment test.

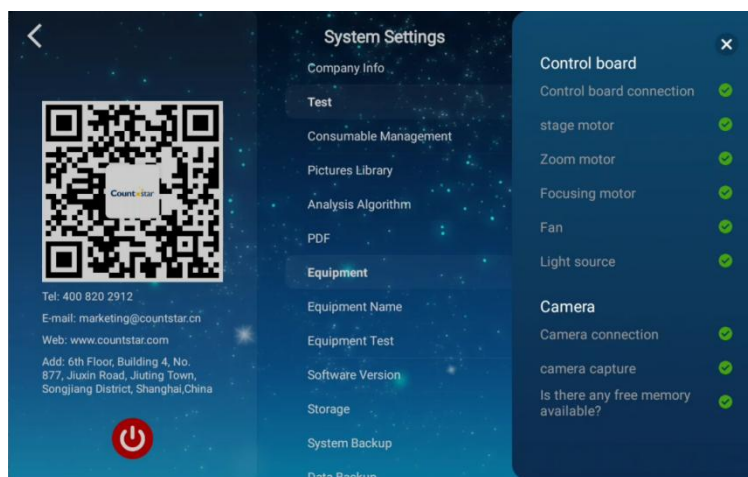


Fig. 2.79 Display of control board after successful Equipment test

⚠️ Caution: If a check of one component fails, it will be indicated by a red cross mark. In such case, the user shall note that component with failed check in the control board, shut down the system immediately, pull out the power supply cable, and contact current regional sales partner or the after sales support of Shanghai RuiYu Biotech to recover the problems.

2.6.8.3 Version information and Upgrade

The current software version number is displayed in the System settings menu. If an upgrade of the software will be available, the user himself can upgrade the analyzer software of the Countstar® Mira HT. The new version will be provided by Shanghai RuiYu Biotech as a zipped folder. The user has to unpack it, and copy it onto an USB storage device. To upgrade the software of the Countstar® Mira HT analyzers, the user has to insert the USB storage device into one of the USB ports at the backside of the analyzer. When he clicks on the small arrow (>) icon beside the software version number, the **Software version upgrade** menu pops up (see **Fig. 2.80**).

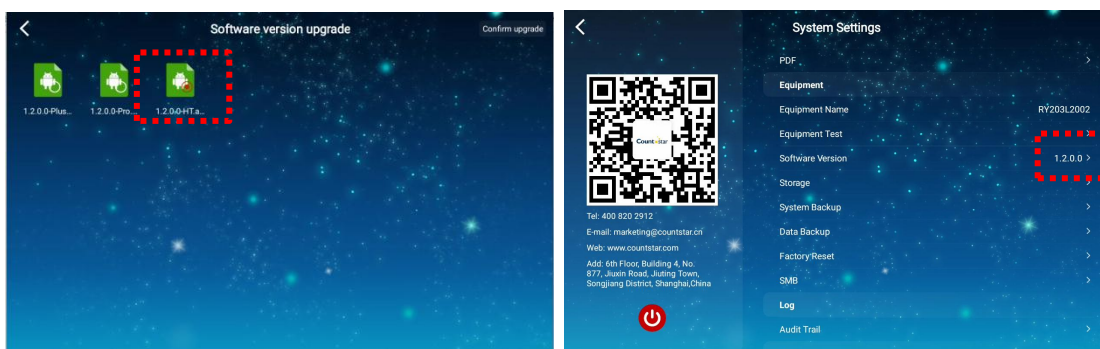


Fig. 2.80 Software version upgrade

In this user interface all available software versions for an upgrade will be listed. The user has to click on that software version with the latest upgrade number, followed by a click on **Confirm Upgrade** at the upper right of the touchscreen to start the upgrade process. The analyzer system will automatically perform the successful installation of the new software version. It will be mandatory to completely shut down the analyzer after the completed upgrade, and restart the Countstar® Mira HT to activate the new version software.

2.6.8.4 Storage Volume

If the user clicks on "Storage Space" in the "System settings" menu, an overview about the total and remaining storage capacity will be shown on the right of this user interface (see **Fig. 2.82**). Below a progress bar documenting the currently occupied storage volume, the sub-interface describes the current consumption of data volume composed of **Pictures**, **Test data** sets, **BioApps (Assay data** templates), and **Other** files (mainly system software relevant content in details).

The database can be backed up and restored (not include images data) .

Database backup: Procedures of automatic backup is performed every time while the device is normally shut down, or when the device is always turned on , automatic backup is also performed when the device time is displayed around 2 AM local time.

Database restore: Manually click Database Restore to restore the device database to keep the latest backup state.

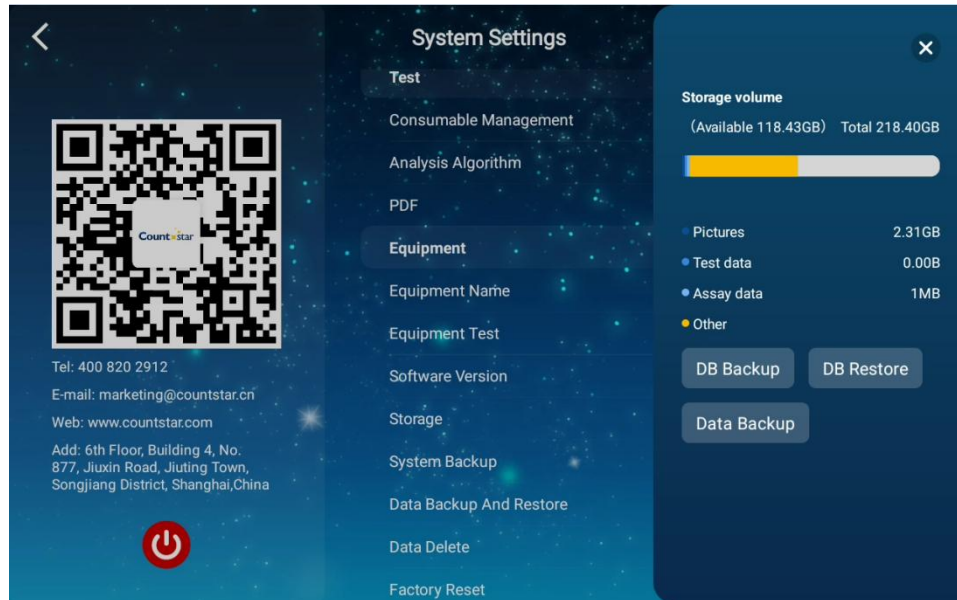


Fig. 2.81 Storage volume user interface

2.6.8.5 System Backup

The system backup function consists of backup and restoration. The contents for backup include system Settings (User mode) files, cell type management data, consumables management data, analysis algorithm data, experiment type files, experiment type data, camera parameters files, experiment parameter files, files on light source parameters under system Settings (Manufacturer mode). The pathways of backup contain local machine, the U disk and the server side, and through the corresponding ways to implement the backup and restore manually.

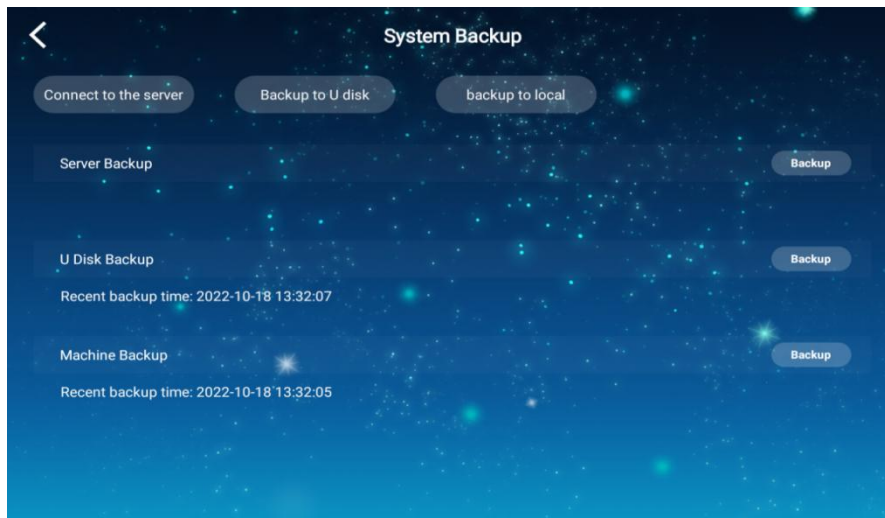

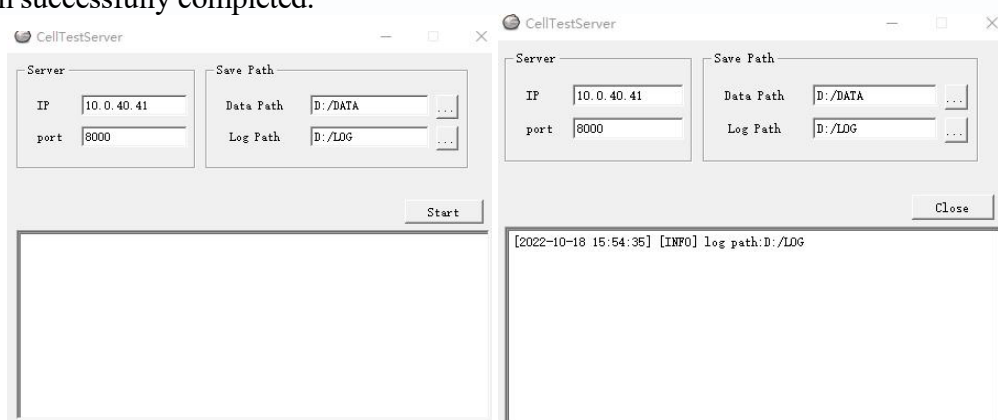


Fig. 2.82 System backup page

2.6.8.6 Data Backup

Protocols for starting the server on the computer: First, open the "CellTestServer.exe" program in the "CellTestServer" folder on the computer; Enter the IP address of the server and the appropriate port number, for example: 8000, 8001...; Click "  " to set the data storage location, and then click " Start ". The server interface displays "startup succeeded", meaning the server construction has been successfully completed.



Computer side server interface

You can upload real-time data through the server path. If function of enable automatic data backup is available, the experimental data will be uploaded to the server in real time after the completion of tests. The routes of storage location of uploaded files named "Test number _ export time.zip" is the "Server DATA Store Location\DATA\datbackup\DEFAULT\admin\Sequential Numeric Number- Experiment type \ Export time to month \ Export time to day \UploadImport" folder on the server.

The detailed information of real-time data upload operation will be displayed on the server interface on the computer (Fig. 2.83).

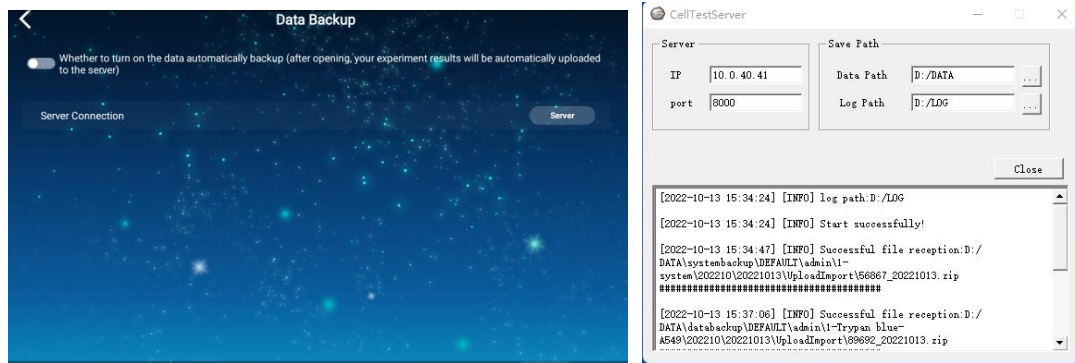


Fig. 2.83 Data Backup interface (left) Real-time data upload server interface display (right)

2.6.8.7 Restoring Factory Settings

Click "Restore Now", the Restore factory Settings window for selection of restoring factory settings appears.

⚠ Caution: Restoring factory Settings will restore the default settings installed for the first time. Please back up the data to be saved, before using the automatic restoration function.

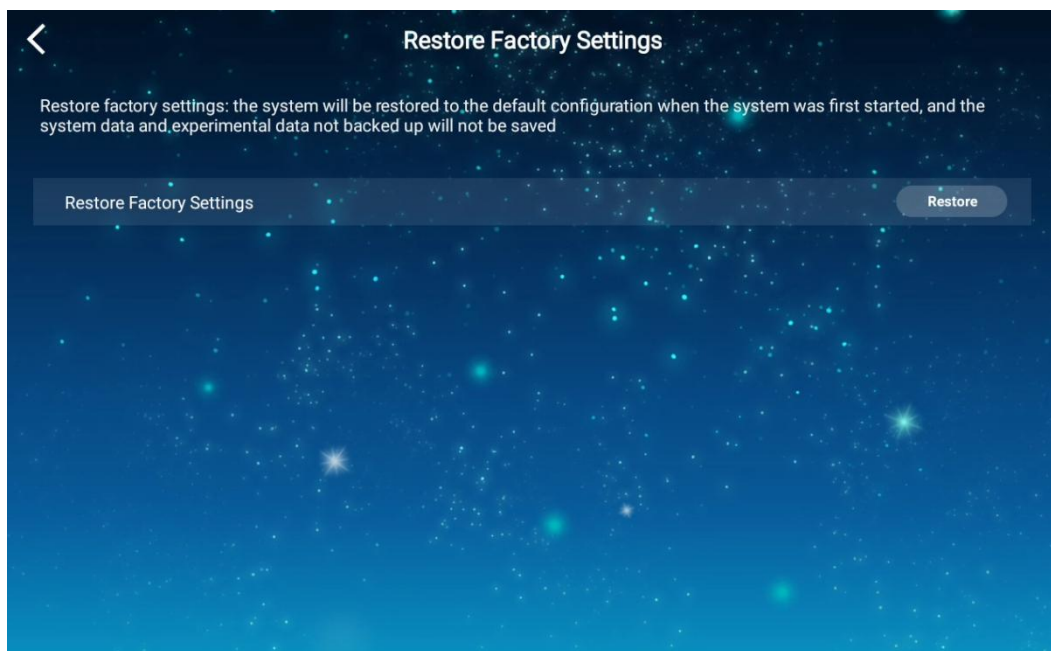


Fig. 2.84 Screen for restoring factory Settings

2.6.8.8 SMB

Server Message Block (SMB) is a software program-level network transmission protocol that is mainly used to enable machines on a network to share resources such as computer files, printers, serial ports, and communications. Before selecting this export method, you need to connect to the network, and enter SMB Settings to input the server, user name, password and folder names for accepted files, before clicking "Save" button.

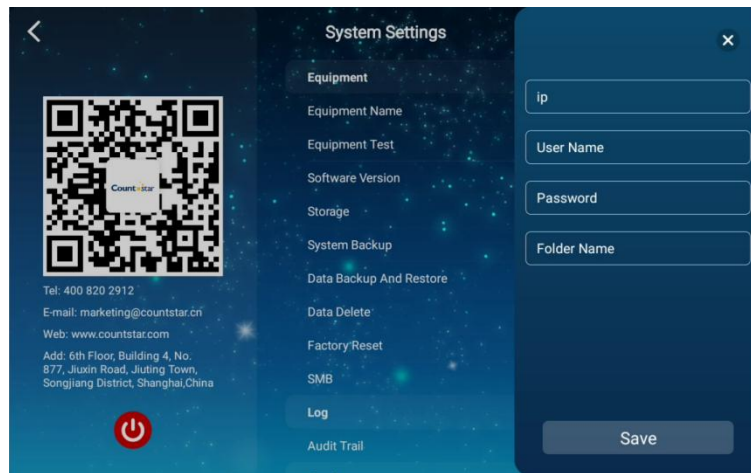


Fig. 2.85 SMB Setting interface

2.6.9 Log

Audit trail

The Countstar® Mira HT can automatically record all detailed information of operations in the system, including time, operator and operation content, etc. And the record information is shown in Audit trail within setting. The log records can be searched by dependence of the operation dates and keywords (Fig. 2.86). Moreover, the record information can be exported to USB storage device with the format of PDF files named "Log_export time.pdf" stored in the folder with the path of "Machine ID/Audit Log_export time".

Time	Operator	Operation	Detail
2023-10-25 15:21	Administrator(admin)	Equipment Test	Test passed! success
2023-10-25 15:14	Administrator(admin)	Export	Export Assay: Export to./exp/AOPi-HEK293/2023-10-25_15-14-52.exp.txt
2023-10-25 15:12	Administrator(admin)	Experiment	Copy experiment type: Trypan blue-CHO-1
2023-10-25 15:11	Administrator(admin)	Experiment	Copy experiment type: AOPi-Zebrafish
2023-10-25 15:11	Administrator(admin)	Experiment	Copy experiment type: AOPi-Zebrafish fail
2023-10-25 15:11	Administrator(admin)	Experiment	Copy experiment type: AOPi-Zebrafish fail
2023-10-25 15:10	Administrator(admin)	Experiment	Delete experimental type: AOPi-Human PBMC success
2023-10-25 15:10	Administrator(admin)	Experiment	Delete experimental type: AOPi-HEK293 success

Fig. 2.86 Audit trail interface

2.6.10 Service

2.6.10.1 User Manual

A digital file of the user manual has been uploaded onto the system of The Countstar® Mira HT software, which can be quickly and conveniently viewed on the "System settings" user interface.

A click on **User Manual** in the System settings user interface opens a new window, containing the detailed operation instructions (see **Fig. 2.87**). The user can view the previous and next page just by swiping to the left and right, respectively. By spreading two fingers and pinching gestures on the touch screen the user can zoom in or out of the current page.



Fig. 2.87 User manual interface

2.6.10.2 About Us

By clicking on the **About us** button at the bottom of the System settings menu, a window at the right pops up, where Shanghai RuiYu Biotech gives a short introduction about the general company's philosophy, and its motivation to develop and manufacture image-based systems, their dedicated accessories, consumables, and services (see **Fig. 2.88**). By a click on the white **X** at the upper right of the touch screen, the software window will return to the System settings menu.

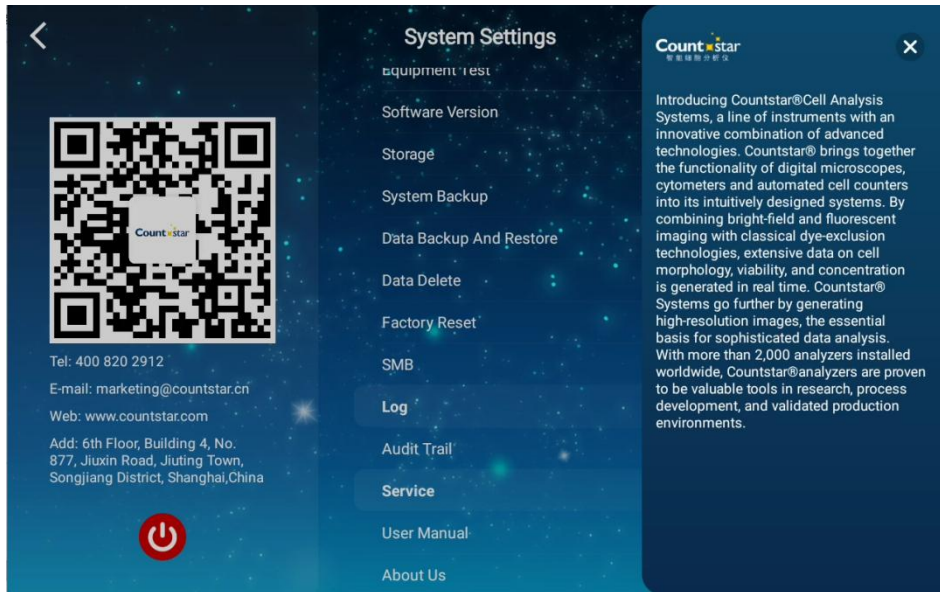


Fig. 2.88 About Us interface

2.6.10.3 Transport Shutdown

In case, the Countstar® Mira HT needs to be moved (for company relocation, transport to another company site, or for workshop repair in the headquarter), the instrument needs to be completely shut down to ensure its integrity and to avoid any damages of the optical systems during transport. There are two steps to complete the shutdown of Countstar® Mira HT.

- 1) To shut down the Countstar® Mira HT, the instrument needs to be turned on, and the user has to enter the **Systems settings** user interface, as shown in **Fig. 2.89**.

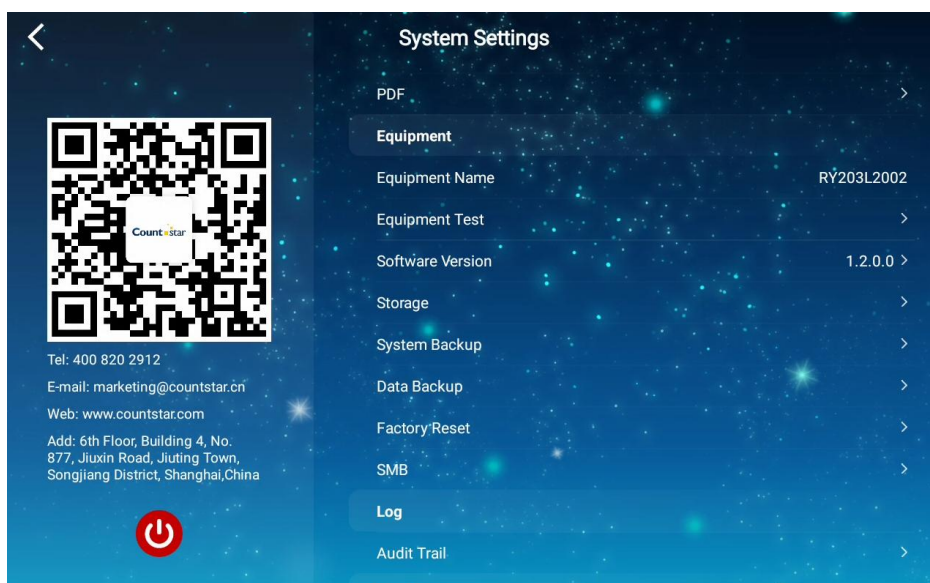



Fig. 2.89 System settings user interface with the red OFF icon below the WeChat QR code

- 2) By a click on the red “” icon, a dialog box appears, stating: “Please remove all remaining consumables, then confirming the shut down, and unplug the power cord after

the system is turned off" (see **Fig. 2.90**). In case a chamber slide consumable is still placed in the instrument, the user has to take it out, before he makes a click on the **Confirm** icon. If no any chamber slide consumable is remained in the analyzer, the user can directly click on **Confirm** to shut down the Countstar® Mira HT, or he can **Cancel** the operation, and the software will return to the previous menu.

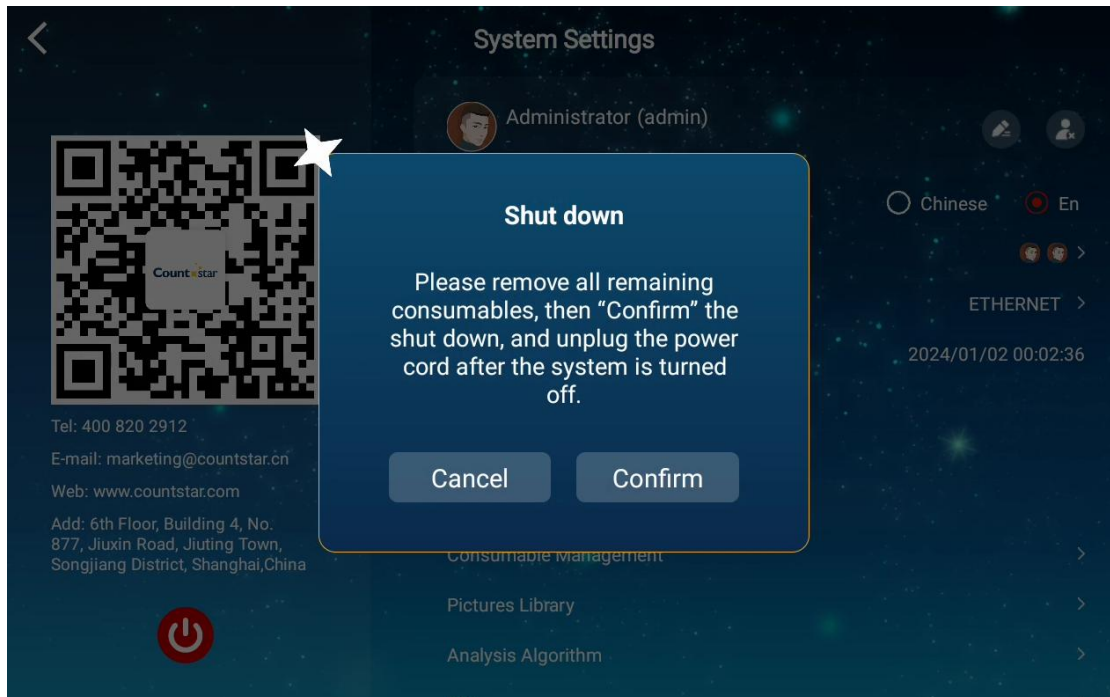


Fig. 2.90 Dialog box to activate the shutdown process

- 3) After confirmation by the user, the Countstar® Mira HT will be automatically shut down in approximately 30 seconds. Finally, to prepare the analyzer for shipping, the user needs to turn off the power by pressing the switch at the bottom of the back side of the instrument, and unplug the power cord from the analyzer just beside the power switch.

2.6.10.4 Manufacturer Information

This section in the System settings user interface on the right is reserved for the entering the logo of the customer's company logo and name. Additionally, in case of a service or support inquiry, the user can refer to one of the offered options on the left side to contact the experts directly in case of a service/support request. The displayed QR code will guide, if scanned, the user to the Countstar website to learn more about other products and services of the company(see **Fig. 2.91**).

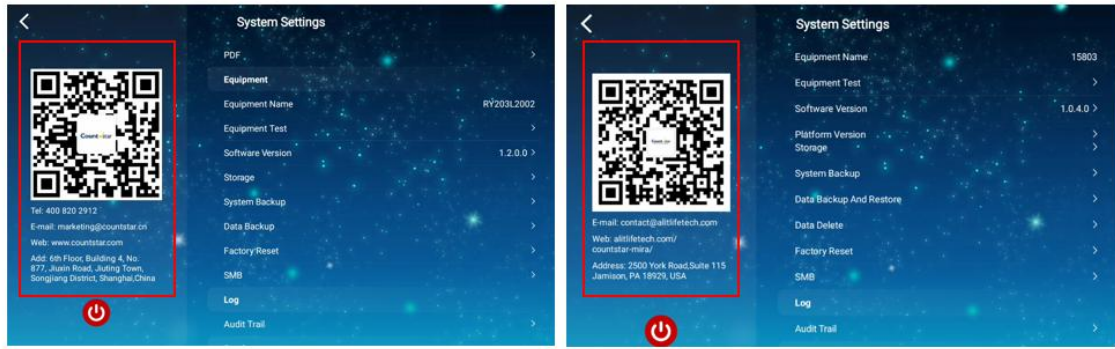


Fig. 2.91 Manufacturer information(US Version)

Chapter III Troubleshooting and Solutions

Table 3 Troubleshooting and possible solutions

Error Classification	Malfunction / operating error	Probable causes	Recommended Solution(s)
Divergences from described process	Unable to view acquired images and results	Insufficient storage volume	Check for remaining storage capacity under "System settings"
	Harsh noise	Fan or motor-stage hardware disturbance	Turn off the power supply immediately at the backside of the analyzer, and contact the regional service engineer / technical support
	Failed to insert a new sample carrier (chamber slide)	Another chamber slide is still inserted	Check, if a chamber slide is visible from the slide port, and wait, until the system ejects the slide. In case, the slide will not move out, turn off the power supply, and contact the regional service engineer / technical support
	Chamber not in designated position during image acquisition (possibly edge of the chamber visible in the image)	Chamber slide wasn't inserted completely prior to start of the measurement	<ol style="list-style-type: none"> 1. Insert the chamber slide completely, until a block at the end of object carrier holder is sensible. 2. If the problem persists, please contact the regional service engineer / technical support.
	Failed to identify an inserted USB storage device	Incorrect insertion of the USB storage device; USB device damaged; Format of USB device not supported or corrupted	Extract and re-insert the USB storage device in one of the USB ports; Check the USB device for FAT32 format; In case no USB storage device is recognized, contact the regional service engineer / technical support.
	Test preparation errors	Incorrect insertion of the Countstar® chamber slide into the slide port	<p>Never insert the Countstar® chamber slide into the instrument in its wrong orientation to avoid serious damages, resulting in liquid spilling into the instrument and mechanical damages of the motor stage.</p> <p>Never reuse a Countstar® chamber slide. (Dried) residues of dye and cell particles in the chambers will seriously affect the results of the upcoming analysis.</p>

		Use of non-compliant consumables with similar dimensions like those of the Countstar [®] chamber slides	Never use other object carriers than the Countstar [®] chamber slides. The focus layer of chamber slides from another manufacturer will vary and may cause unsharp images plus, potentially, mechanical damages to the Countstar [®] Mira HT.
	Cancelled result export	Insufficient capacity of the external USB storage device; removal of device from USB port of the analyzer	1) Choose an alternative USB storage device with higher capacity and repeat the result export again. 2) Keep connection between the USB storage device and analyzer through the USB port of the Countstar Mira HT, until the completion of the result export is confirmed by the analyzer software.
Software exceptions	Reduced speed of the application software	Overflow of database due to insufficient remaining data storage capacity on system's micro SD card	Check in System settings the remaining data storage capacity; Delete unneeded data from the system's database. Use the data export functions, to archive all data of the different BioApps on a regular base. Remove or archive gratuitous BioApps to increase the operation speed of the analyser software
	Freeze of the application software during analysis	Overpassing cell density beyond the allowed maximum; irrational calculation,	Click on the "OK" or "Confirm" icon of the error message to restart the software, returning to the main menu user interface.
	Failure when importing a new BioApp (assay template)	BioApp with identical name already exists in the analyzer database	Either performing the analysis with the original BioApp, or renaming, or deleting this app, prior to importing the new BioApp consecutively.
	Failure to export a BioApp to an external storage device	Incorrect BioApp export file path, or corrupted file	Check, if the file path for the exp. folder in the directory of the USB device is correct; If the issue still persists, contact the regional service engineer / technical support.
Anomalies during image acquisition	Obvious dirt spots on all acquired images at the same position	Impurities on optical components of the analyzer	Contact the regional service engineer / technical support for a preventive maintenance visit to clean the optics.
	Uneven distribution of	Impurities on optical components of the	Contact the regional service engineer / technical support for a preventive

	brightness of the background illumination	analyzer; illumination out of alignment	maintenance visit to check, clean and adjust the optics.
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