Countstar Automated Cell Counter
User Manual

(Model: IC 1000)

Shanghai Ruiyu biotech Co., Ltd.

Content

Disclaimer

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Countstar® is a registered trademark of Shanghai Ruiyu-Biotech co. Ltd.

Countstar® BioTed has no approval as a medical device and shall therefor not be used for IVD.

Safety Instructions

It is important to follow all safety instructions and guidelines in order to ensure a safe and reliable operation of Countstar® IC1000:

Operation enviroment

1. Place the device on a dry and stable surface.
2. Avoid vibrating sources placed close to the Countstar® analyzer
3. Avoid humidity or water.
4. Avoid dust or smoke.
5. Avoid direct sunlight.
6. Guarantee air circulation around the Countstar® system during operation (particularly around its linked computer and monitor).
7. Operate the analyzer within a temperature range from +50°F to +100°F (+10°C to +40°C).
8. Operate the Countstar® within a range of 10% to 70% air humidity.

9. The device shall be plugged to an earth connected power outlet.
10. The Countstar® shall only be used in the described configuration (Countstar® Cell counter, Countstar® Software, and defined computer).
11. Only trained personnel in a laboratory environment shall operate the Countstar® system.
12. All services and repairs shall only be performed by authorized service technicians.
13. Always observe the mandatory safety regulations when handling Trypan Blue. Follow all safety instruction when using Trypan Blue.

    (Waste contaminated with biological material such as tissue or cells, e.g. Countstar® chamber slides, pipette tips, etc. must be disposed according to the compulsory rules for handling biohazard materials.)
14. Only use the Countstar® chamber slides from Shanghai Ruiyu Biotech co. Ltd. or from an authorized Countstar® distributor to avoid unexpected damages. Other slides will cause irreversible damages to Countstar® equipment!
15. The Countstar® chamber slides were designed for single use only.
16. Always wear protective clothing, particularly gloves, when handling samples.
17. Avoid dirt or dust particles entering the device.
18. Ensure that all consumables required for the analysis, e.g. Countstar® chamber slides, reaction vials, pipette tips, etc. are stored in a dust-free environment since dust particles / fibers may cause poor quality images.
19. Make sure the slide is free of observable contamination before use. Only clean slides can provide an optimal functioning of the Countstar® analyzer.
20. Ensure that the Trypan Blue solution is free of contamination and agglutinated particles, otherwise filter the suspension prior to use.
21. Avoid looking too closely into the front panel blue LEDs when they are glowing. Extended and close distance to the LEDs may be harmful to your eyes!
22. In case of a maintenance or repair at Ruiyu Biotech co. Ltd., please decontaminate the analyzer, and fill out the **Declaration of Harmlessness** and pack together with the device.

Decontamination and packing:

1. Please use **75%±5(v/v%) ethanol** solution to clean the surface of **the device, the electrical and USB cables and any accessories**.
2. Pack the device, cables and accessories in a clean **plastic bag** and seal the bag with **scotch tape**.
3. The **original box** and **cellular plastic pad** shall be used for transportation in case of any damage. Cover the top and the bottom part with **cellular plastic pad** and then put them into the box.

   Notice: The **Positioning Knob** shall face the bottom of the box when packing. The **accessories** can be placed on the top of the box.
4. Put the **Declaration of Harmlessness** in the box then seal the box tightly with **scotch tape.**

Unpacking and installation

1. Open the top side scotch tape with a knife. Make sure the knife enters no more than 5mm to avoid any damage.
2. Open the top cover of the box and find the Item List on the top, check each item according to this list. If any part is missing, contact your local supplier.
3. Take out the Countstar device by double hands. If Countstar device fallen on the floor, any damage will be irreversible!
4. Install Countstar device on a place according to the Safety Instruction.
5. Connect the power cable and the USB cable.

Countstar® – Quick Start Guide

Start the analyzer and software

1. Make sure the USB cable is correctly connected with the computer.
2. Switch on the computer.
3. Login to the MS Windows operating system.
4. Start the Countstar® software by double clicking on the **Countstar** icon on your desktop or in the designated program folder.
5. Switch on the Countstar® cell counter.

Start a test

1. Click **Cell Mode** and choose **Cell or Object** according to the sample's composition.
2. Click **Count Cells** to open the measurement window.
3. In the measurement window, enter a clearly defined **Sample ID, containing at maximum 15 alphanumeric characters.**
4. Enter the **Dilution** of the cell sample to be analyzed
5. Select the **Cell Type** parameter settings for the sample.

Sample preparation and Start the Measurement

1. Add 20µL of 0.2% Trypan Blue solution to 20µL of cell sample and gently mix it. After 5 sec of incubation time at room temperature carefully pipette 20µL into an empty sample chamber of the Countstar® chamber slide. The volume of the Trypan Blue solution can be increased due to the final concentration wanted in the experiment.

   Note: The final concentration of Trypan blue in the sample shall be 0.1%, otherwise it may cause inaccurate results.
2. Insert The Countstar® chamber slide into the slide port of the Countstar® analyzer.
3. Turn the **Positioning Knob** on the right side to position the slide chamber

containing the sample to its correct position. The blue LEDs from top to bottom correspond to the chambers 1-5 (starting with chamber 1 = top LED that is closest to the Countstar® lettering on the slide. A soft click can be sensed when positioned correctly, and the chamber position indicator light will show the correct position.

4. Click the **Start** button for the measurement.

   Multiple Measurements: If multiple samples on one Countstar® chamber slide will be tested, click **Next Sample** button and turn the **Positioning Knob** to move the chamber slide to its next position. When the image is stable, press the **Start** button again. The new sample will be measured subsequently.

   Multiple records: If more than one vision field will be counted, click the **Next Record** button and turn slightly the positioning knob to move to another field of vision. When the image is stable, press **Start** button again. The result will be stored and analyzed automatically.

5. Measurement results will be displayed automatically.

Shut down the Countstar® analyzer

1. Click on the **Back** icon.
2. Close the software: click Exit under the **Main Menu Bar** or click on the X-icon top of the User graphical interface window.
3. Switch off Countstar® analyzer.

Chapter1 Introduction

Countstar® automated cell counters are benchtop instruments designed for the accurate, reproducible and simple measurement of cell concentrations and viability (live/dead cells). Featuring the standard Trypan Blue dye exclusion method, the autofocus technology and the Countstar® sophisticated digital image analysis algorithm, Countstar® analyzers can rapidly provide cell counts, viability, morphology, aggregation rates, and average cell sizes in less than 20 seconds. The system is compatible with a wide variety of eukaryotic cells. The Countstar® can analyze samples at a concentration from 1×10^4 to 3×10^7 cells/mL and with a cell diameter of 5 to 180μm. Cells either grown as adherent monolayers or in suspension, the Countstar® needs only 20μL of cell sample to provide accurate and reproducible results.

Countstar® automated cell counter software offers a graphical user interface that includes functions such as saving data and images, exporting and printing data. By its sophisticated image recognition analysis, Countstar® offers results including viable cell concentration, total cell concentration, viability, roundness, aggregation rate, and average diameter. The acquired images of the analyzed cell samples can be viewed on screen or can be exported in JEPG format. Diameter histograms, aggregate histograms and cultivation time charts will be shown in graphical distribution format. The cultivation time charts combine the analysis of single measurements of a growing cell culture over time, the resulting growth curves allows for calculating the doubling time [T] and specific growth rate [μ]. The overlay option helps researchers to compare single analysis results in one graph. This data can be exported to PDF for data bank storage.

The Countstar® automated cell counter is limited to use Countstar® chamber slides. Each slide contains five single chambers enabling users to measure either five different samples or to perform replicates of the same sample. Only 20μl of a stained cell suspension is required for each cell count.

System Overview

Instrument Overview

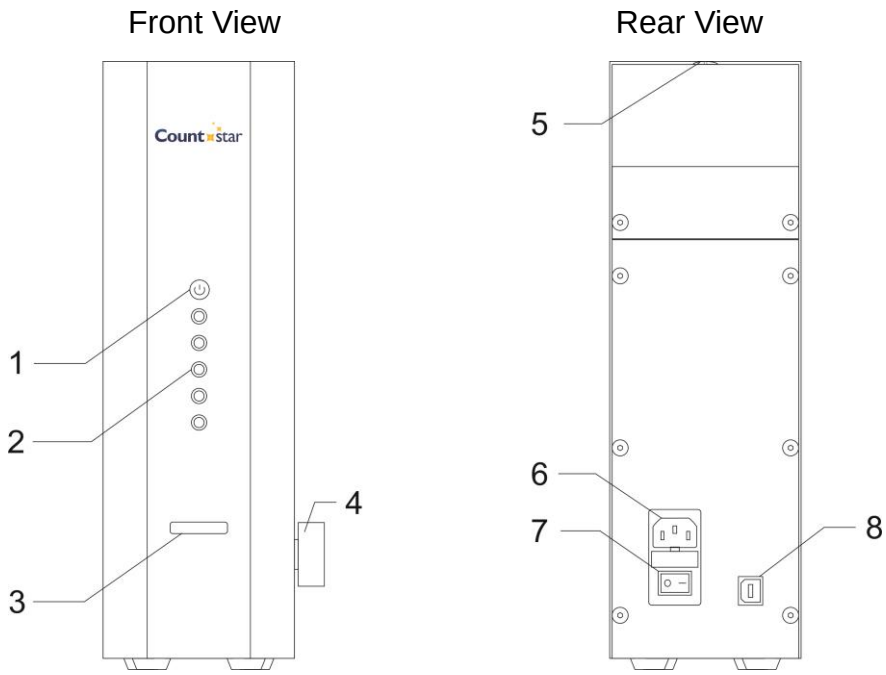


Figure 1 The Countstar® Automated cell counter IC1000 overview

Countstar automated cell counter components (Figure 1) include:

Front panel:

1. Power indicator light –Light off indicates that the instrument is off; The red light indicates that the instrument is on.

2. Sample chamber position indicator lights – Five lights indicate the chamber position separately, the top light indicates the first chamber, and the bottom LED indicates the fifth chamber.

3. Slide port –The slide port is the position for inserting the Countstar® chamber slide.

Side (right) panel:

4. Positioning knob – The Positioning knob is used to vary the position of the Countstar® chamber slides.

Top panel:

5. Focus lock –The focus lock is used to adjust the optimum focus to obtain the best contrast between living (bright centers, dark object border) and dead (dark centers and object borders) cells, which is critical for accurate cell numbers and viability measurement.

Rear panel:

6. Power button –The power button is used to turn the instrument on and off.

7. Power socket – The power socket is used for connecting the Countstar® to the electrical power supply with the supplied power cable. Prove in advance, if the plug, fits to the electrical outlet type in your country.

8. USB socket – The USB socket is used to connect the instrument via a USB 2.0 Male to Male data transfer cable to the computer. Take care of the right orientation of the sockets, when plugging in the cable.

Software Overview

The main menu of the Countstar® software is splitted into two elements:

The Login window

The Main Menu Bar

Figure 2 Countstar® software Login window

- **Login window:**

Login with your user name and correct password. You will be able to perform all those functions in the control system that fit to your personal user privileges.

Notice: Different operators may have limited access to several menu functions.

- **The Main Menu Bar**

The Main Menu Bar is automatically activated when the login to the control system was successful. The Main Menu Bar contains the following icons:

Figure 3 Countstar® software Home page and Menu Bar

- 

Cell Type

Modify existing cell type parameter settings or set-up a new one.

- 

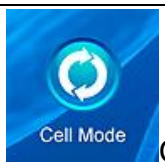
User Interface

User Password management

- 

System Setting

Checking and calibrating the hardware components

- 

Cell Mode

  Select Cell/Object analysis mode

- 

Count Cells

  Defining and executing measurements counting

- 

Data Library

  Access to acquired analysis data

- 

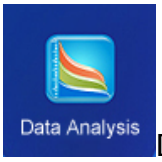
Data Export

  Export experiment data to readable files

- 

CTC (Cultivation Time Chart)

  Generating the growth curves out of single cell counts

- 

Data Analysis

  Analyze and compare growth curves and/or histogram

Product Specifications

Table 2 Countstar® Automated Cell Counter Specifications

Countstar® Automated Cell Counter IC1000 Specifications	**Environmental Conditions** Power Supply: 100–230 VAC, 5W Frequency: 50/60 Hz Installation site: for indoor use only Operating Temperature: 10–40℃(50℉-100℉) Humidity: 20–80% Altitude: <2,000m Pollution Degree: 2 Degree of Protection: IP20 **Instrument Specifications** Type: Benchtop cell counter Processing Time/single measurement: <20sec. Cell Concentration Range: 1×10^4–3×10^7/mL Cell Diameter Range: 5-180μm Dimensions: 206 mm (W) × 123 mm (D) × 346mm (H) Weight: 8.5 kg **Slide Specifications** Material: Polymethyl methacrylate (PMMA) Dimensions: 75 mm (W) × 25 mm (D) × 1.8 mm (H) Chamber Depth: 190±3μm Chamber Volume: 20μL **recommended computer configuration:** Memory: ≥2GB Free hard disk drive memory : ≥20GB

	Hard disk partitions: 2 partitions (1 partition is no problem for Countstar to run properly. But it is still recommended to make 2 partitions: one for operation system and the other one for Counstar® software and data. If only 1 partition is available, the operation system might getting slower when data base increasing.) CPU: ≥2GHz Monitor: ≥1024 x 768 pixels, 24 bit color, 60 Hz. CD-ROM: required USB port: USB 2.0 or USB 3.0, at least 1 port is required. Key board and mouse: standard Operating System: 32/64bit Microsoft Windows, all version of Windows XP/Vista/7/8/8.1/10

Chapter 2 Countstar® operation guide

Install the Countstar® automated cell counter

Package contents

- Countstar® automated cell counter IC1000
- Power cable
- Countstar® USB drive / Dongle *
- Countstar® Hex key
- User manual
- Countstar® software CD-ROM or USB memory key.

  * Optional

Device Installation

1. Unpack the box carefully and keep them for optional reshipment. Check the instrument and accessories for damages during transportation. Make sure all parts as listed above are inside the box. If any item is missing or damaged, please contact your sales representative promptly.
2. Place the Countstar® IC1000 cell counter on a dry, even, and stable surface.
3. Plug the one end of the power cable into the instrument.
4. Connect the power cord to your power supply.
5. Insert the B-plug of the USB 2.0 cable in the right orientation carefully into the USB socket of the Countstar® analyzer at the rear panel and link the analyzer with the A-plug to one of the free USB ports of your PC / laptop / tablet.
6. After installation Countstar® software please turn on the Countstar® after login to the software on PC. Please follow the instructions “**Installing the Countstar software**” as listed below.

Installing the Countstar® software

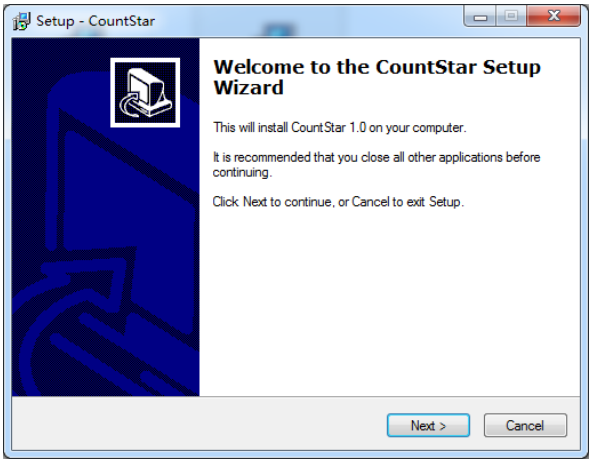
The Countstar® software is compatible with Windows XP, Windows Vista, Windows 7 (32bit or 64bit) or Windows 8, 8.1, 10 (32bit or 64bit version). 2GB memory or above is recommended to guarantee an optimum function of the Countstar® software.

Please close all other applications on your PC before starting the installation of the Countstar® application software.

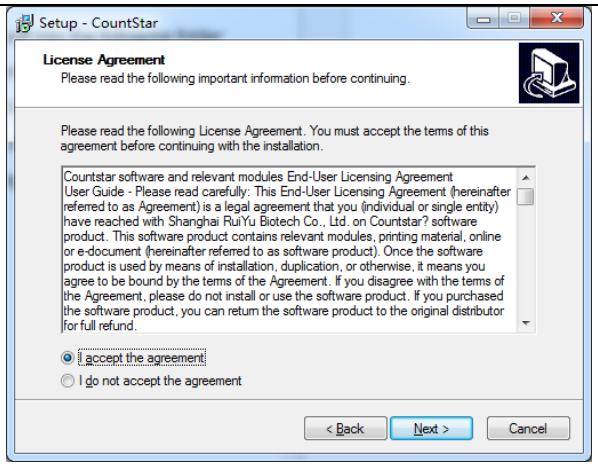
Installing the Countstar® software:

1. Insert the software CD-ROM into the disc drive of your PC or insert the USB memory.
2. Double-click on the installation software icon. If the computer is installed with 64bit Windows, please choose “Countstar1.0Setup-64bit.exe”, otherwise choose “CountStar1.0Setup.exe”. The following instruction will use 64bit system as an example.

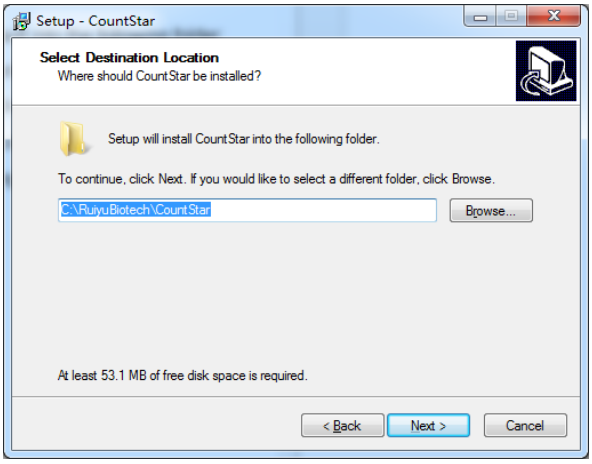
3. The installation wizard will guide you through the installation of the Countstar® software 1.0 on your computer.



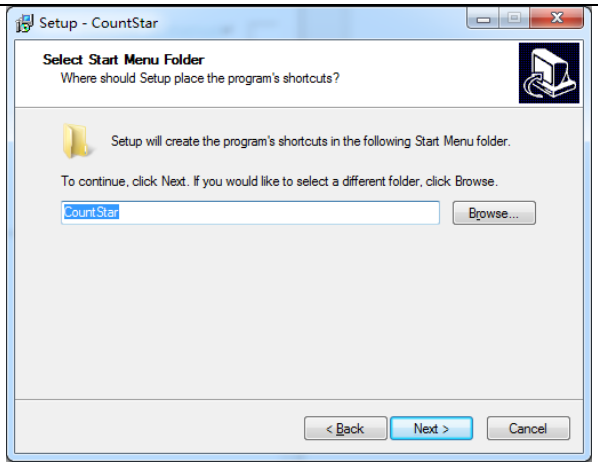
4. Please read the following license agreement carefully. You’ll have to accept the terms of this agreement before continuing with the installation.



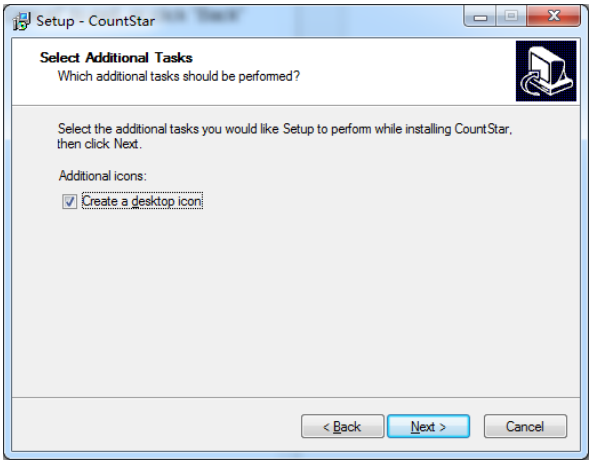
5. The setup will install the Countstar® software by default into the following folder: C:\Countstar. To continue, click “Next”. If you want to install the software in another folder, click “Browse” to select another directory, select the designated folder, click “Next” to continue, or click “Cancel” to exit, or click “Back” if you want to review or change any settings.



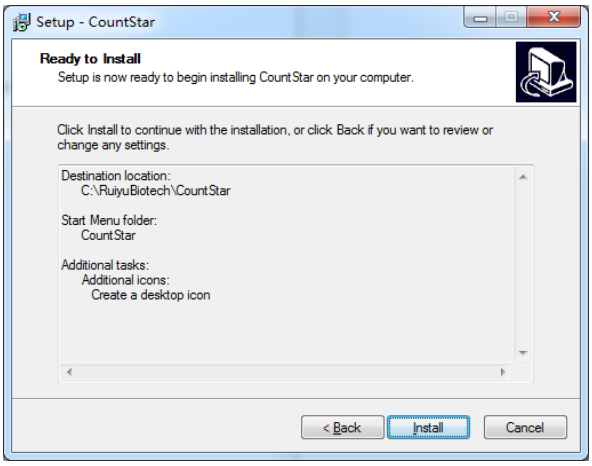
6. During setup the software will create shortcuts for the program in the Start Menu folder. To continue, click “Next”. Click “Browse” to select another folder for the short cuts, click “Next” to continue, or click “Cancel” to exit, or click “Back” if you want to review or change any settings.



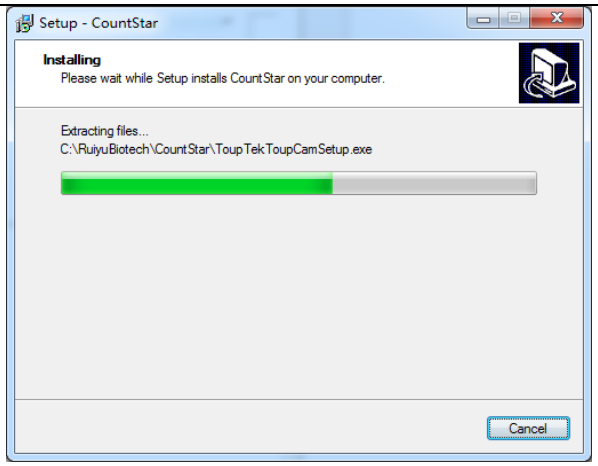
7. Select “Create a desktop icon” if you would like to setup while installing the Countstar® software. Then click “Next”, or click “Cancel” to exit, or click “Back” if you want to review or change any settings.



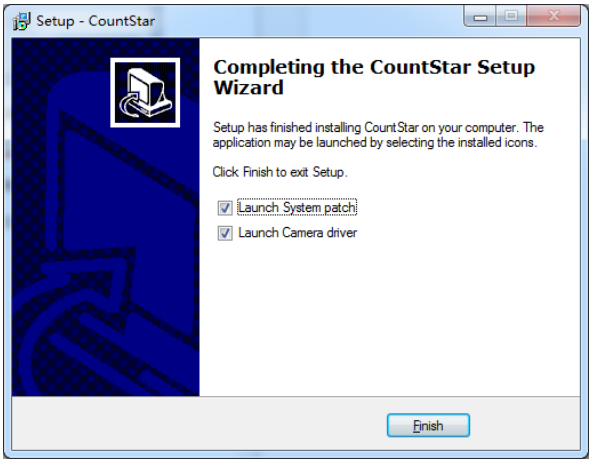
8. The setup program is now ready to install Countstar® software on your computer. Click “Install” to continue, click “Cancel” to exit or click “Back” if you want to review or change any settings.



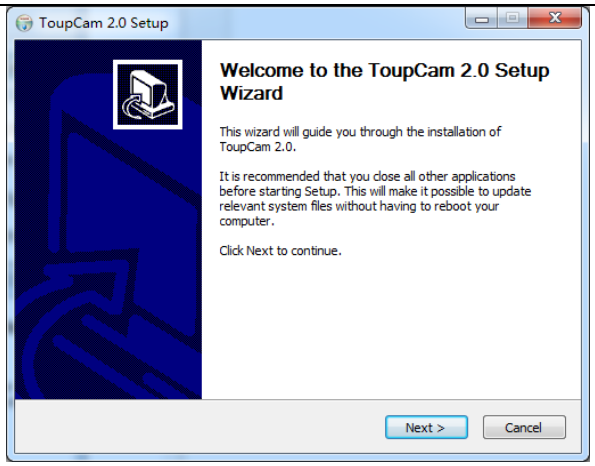
9. Please wait until the installation of the Countstar® software is completed on your computer, or click “Cancel” to exit.



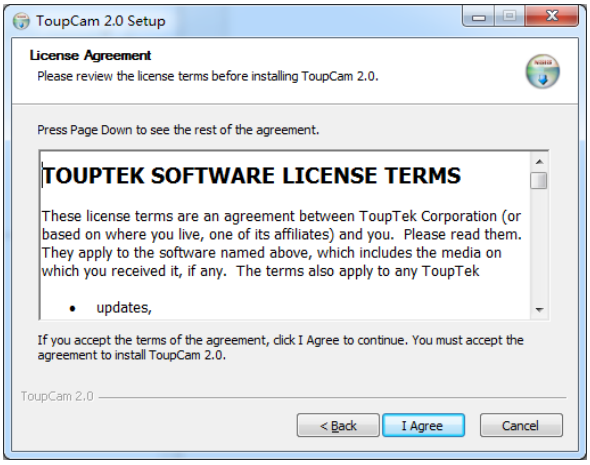
10. After the wizard has successfully installed the Countstar® on your computer, select “Launch camera driver” if it will be the first time you install a version of the Countstar® software on this computer. And select “Launch System Patch” additionally to install Microsoft Visual C++ 2008 for system patching. The camera will not function without both of these apps installed. Click “Finish” to complete the installation.



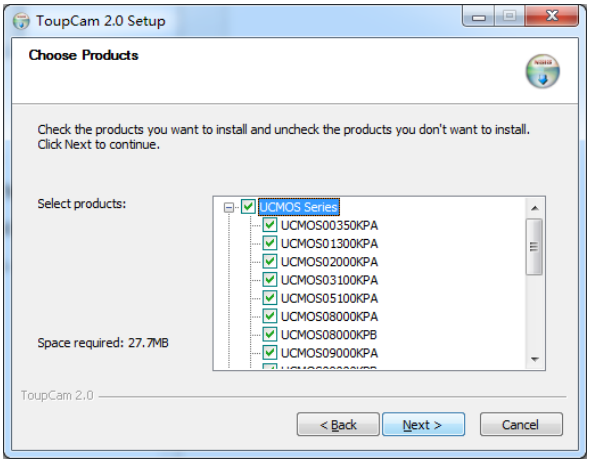
11. This wizard will guide you through the installation of the ToupCam 2.0. It is recommended that you still have all other applications closed before starting this setup. The program will update relevant system files without rebooting computer. Click “Next” to continue or click “Cancel” to exit.



12. You should precede installation by clicking “Next”. Click “Cancel” to exit or click “Back” if you want to review or change any settings. Then a license agreement window will pop up. User shall choose **I Agree** to continue.

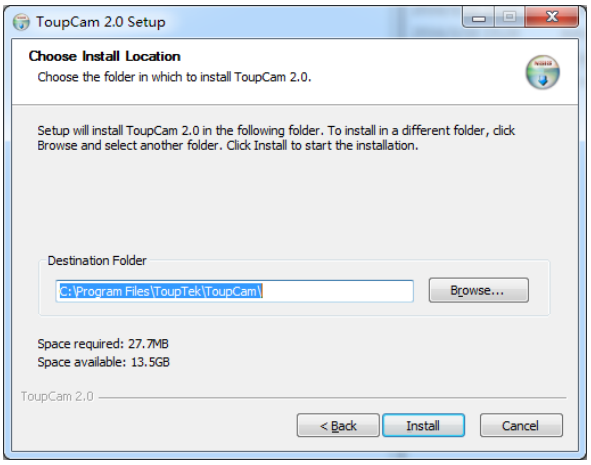


13. User shall click on “Next” to proceed installation. Click “Cancel” to exit or click “Back” if you want to review or change any settings.

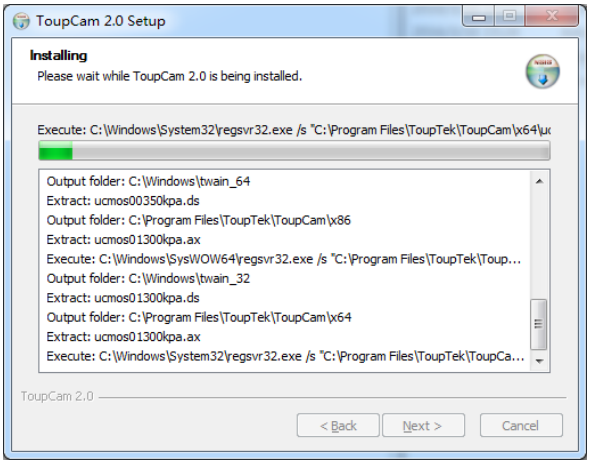


14. The installation wizard will install ToupCam 2.0 in the following folder: C:\program files\ToupTek\ToupCam”. To continue, click “Next”. Click “Browse” to select

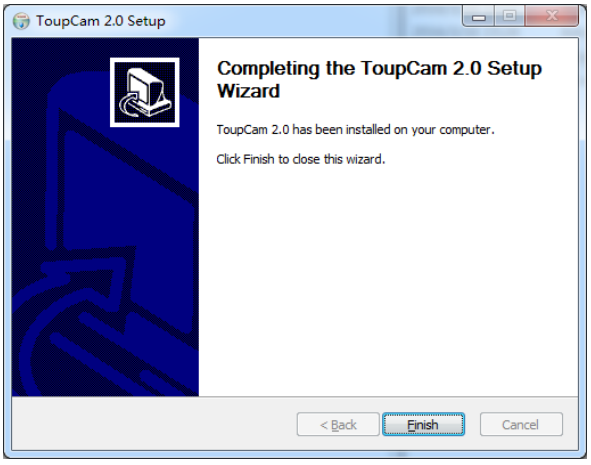
another folder, click “Next” to continue, or click “Cancel” to exit or click “Back” if you want to review or change any settings.



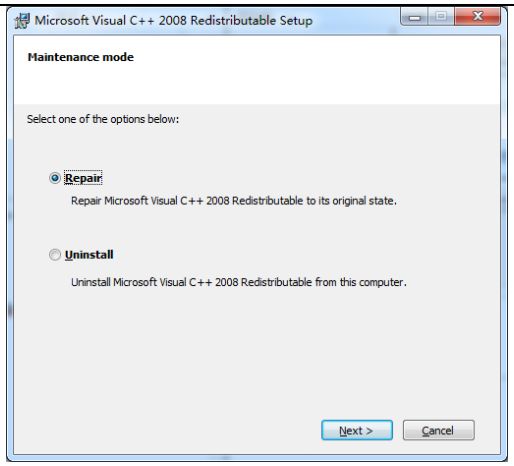
15. Please wait while installing ToupCam 2.0 on your computer.



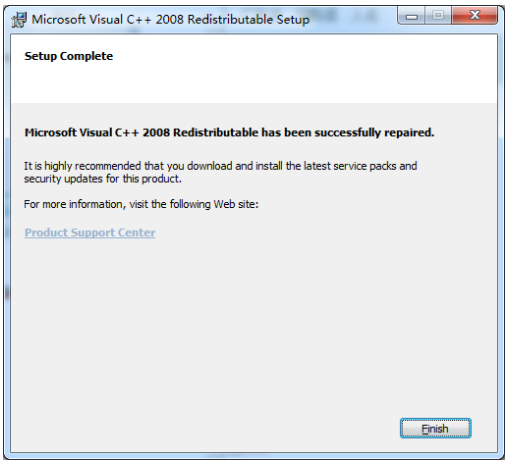
16. ToupCam 2.0 has been installed on computer. Click “Finish” to close this wizard.



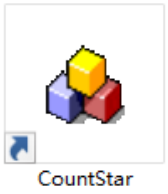
17. Now the system will start to install Microsoft Visual C++ 2008 on your computer. Select **Repair** and then click on **next** to continue the installation guide. Click on **Cancel** will terminate the installation.



18. Now Microsoft Visual C++ 2008 is successfully installed on your computer. Click on **Finish** to complete the installation.



19. Now you should find the shortcut icon for the Countstar® software on desktop of your PC.



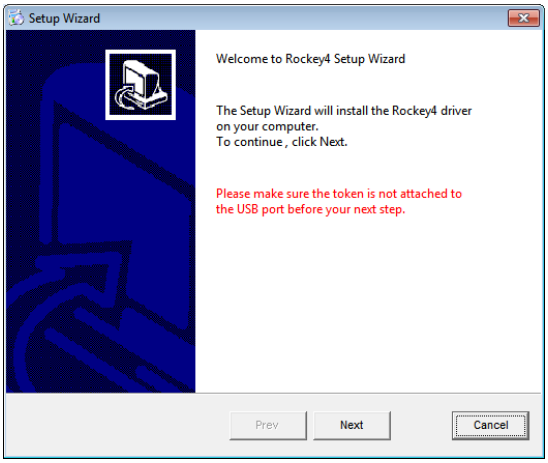
20. Rebooting the computer is recommended before activating the Countstar® software for the first time. Before starting the software, please insert the Countstar® dongle (if software is dongle protected), into your computer when using Countstar®.

WARNING

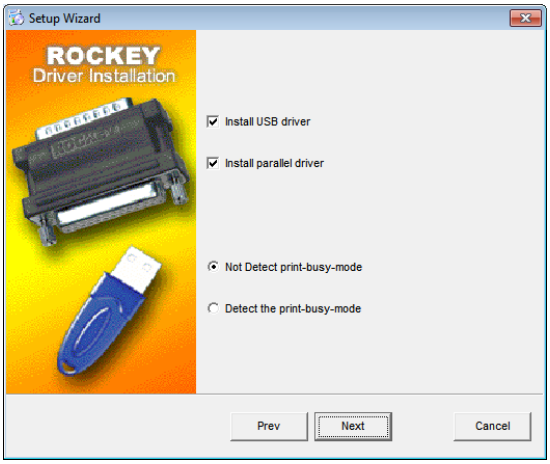
The Countstar® software shall be installed following the installation guide. Otherwise, the system will not work properly.

Rockey4 dongle driver software installation (Only if a dongle is purchased and not functioning properly)

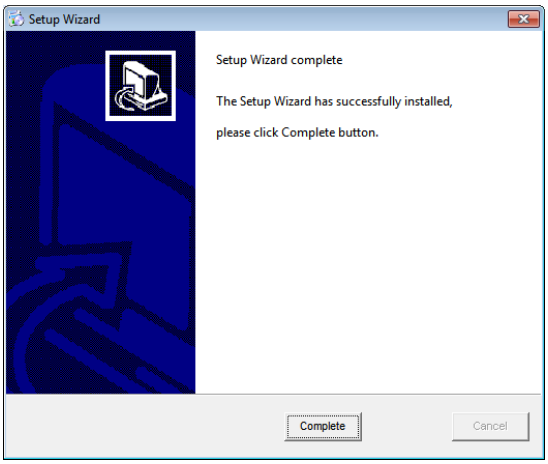
1. Double click on InstDrv-64bit or InstDrv-32bit according to your Windows configuration.



2. Click on “next” to continue, “Prev” to review your previous settings or “Cancel” to quit. Please click on “Next” to proceed.



3. After approximately three seconds, the setup wizard will finish the installation. Click on “Complete” to finish the installation.



4. Finally the software will require a reboot of the computer to finish the installation. Click on “Yes” to restart now or “No” to restart later.

Switch on and shut down the system

Switch on the Countstar® cell counter:

Instruction:

1. Connect the Countstar cell counter to the PC or laptop.
2. Switch on the PC or laptop.
3. Switch on the **Countstar® software**. Double-click the Countstar icon on the desktop to launch the software.
4. Switch on the Countstar® cell counter.

WARNING

The Countstar® IC1000 analyzer has to be connected with the computer. Otherwise, the program will not be initialized correctly.

Shut down the Countstar® cell counter:

1. Shut down the software by clicking the close (X) button.
2. Switch off the cell counter.

Count Cells

1. Double click the **Countstar** icon on the desktop, login to the system with your username and password, then the menu bar with its functions is activated.
2. Before analyzing a sample containing cells or particles, the user may create a new or modify an existing Cell Type if the default cell type parameter settings does not meet your analysis expectations. The single parameters shall be adjusted according to the individual characteristics of cells and/or objects and have to be stored. The new Cell Type will be available when counting cell/objects. Please find below the functions of the single parameter settings:

Min_Size

Range: [0, 200], Default: 7

This parameter determines the minimum cell size. It can seperate debris from cells or will exclude smaller cells in a mixed cell culture, when focussing in analysis on bigger cells only (e.g. analysis of feeding cell cultures).

Max_Size

Range: [0, 200], Default: 20

This parameter determines the maximum size of single cells. It can help to distinguish large dirt particles from cells, and is important for correct aggregate analysis

Prm_grdthres

Range:[1,9], Default: 7

Background contrast, Target feature coefficient, used for target objects fine adjustment. Increase this value when target objects show great differences on the gray scale to the background; otherwise decrease this value. Usually the value is 7, 8 or 9, but the best value relies on the single measurement test result of this cell type.

Alive_dead_param

Range:[0,2], Default: 1

It is a cell refractive index to distinguish between living cells and dead cells. If the value increases more cells will be counted as dead cells; otherwise they will be counted as live cells. The default value is 1. No function will be shown if this value is set to 0.

Sep_param

Default: 0.7

Separation connection factor. This parameter is used to determine if two cells form a cluster. If the cells margins are too close to each other, then increase this value to make sure the cells will not be counted as one aggregate; otherwise decrease this value. The recommended value is 0.7 or 0.8.

Overlap_param

Default: 0.5

Aggregated cells overlapping factor, used to identify the single cells inside aggregates. Increase the value to recognize more cells in an aggregate, otherwise decrease it. This parameter will only function if “Overlap” mode is activated. The recommended value is 0.5.

Big_block_flag

Default:1,000

Macro-aggregated area coefficient used to determine the minimum area of aggregates. It will function only when the target area exceeds this value. This parameter will be activated in “Overlap” mode. The recommended value is 1000.

Eject_dis

Range: Default: 3

Overlapping circle-center span parameter, used for determining the cell numbers inside aggregates. If the value increased, the extrapolated numbers of cells will increase, otherwise decrease this value to count less cells inside aggregates. This parameter will only be activated in “Overlap” mode. The recommended value is 3.

Reg_in_param

Cell separation critical confident, used to identify elongated cells from normal cells. For elongated cells increase the value, otherwise decrease it. This parameter will only be activated in “Big cell” mode. The recommenced value is 0.5.

Big cell

Default: not activated

Combination mode, used for elongated cells and big cells from aggregated cells. Select this mode when cells either too big or have an elongated shape. In the default parameter settings, this algorithm is not activated.

Overlap

Default: not activated

Overlap mode used for when the cells are adhering into aggregates. If this mode is selected, simulated separation will be carried out for the aggregate; otherwise, it

is not necessary to select it. The default parameter settings is not activated.

NOTICE

After modification of the cell type parameters, the new cell type parameter will be added “-M1” in the end of the original cell type name. If the same original file name is modified and saved 2 times then the name will be add “-M2”, and so on. If the modification is applied on “-M1” parameter, then the name of the file will show “-M1-M1” in the end of the original parameter name.

original parameter name	Modified parameter name
XXX	XXX-M1
XXX	XXX-M2 (the 2nd time of modification)
XXX-M1	XXX-M1-M1

3. The default counting mode on Countstar® cell counter is the **Cell** mode. If users want to count beads/particles, please switch **Cell Mode** into **Object** before counting.
4. Click on the **Count Cell** icon (Figure 4) and type in an appropriate Sample ID. The correct dilution factor has to be selected according to the final sample dilution ratio prior to the analysis in the Countstar®.

NOTE: All of the characters, numbers, and symbols can be used for generating the **Sample ID**.

5. Choose a Cell Type from the list. The cell type should be selected according to the cell line analyzed.
6. Add 20 µL sample to 20 µL of Trypan Blue staining solution. Then mix gently by pipetting six times up and down slowly to avoid disruption of cells. Incubate the cell suspension with the staining solution for 5 seconds.

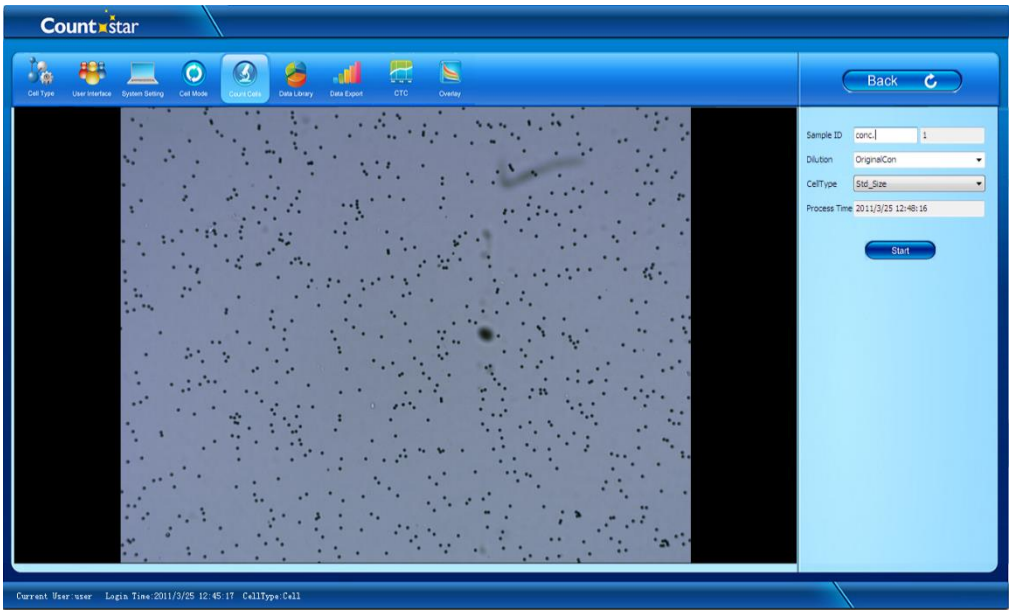


Figure 4 Countstar® Count Cell menu's window

NOTE: Cell samples should be suspended in PBS or serum free media, since serum proteins can be stained by Trypan Blue too, and may cause misleading results.

7. Pipette 20 μL of the mixture to one chamber of a Countstar® cell counting slide. You can measure only one chamber or up to five chambers according to your choice on one Countstar® chamber slide.
8. Insert the Countstar® chamber slide with the Countstar® logo readable pointing towards you into the slide port. Push the slide into the port, until you'll sense a slight resistance. Each chamber will be analyzed separately. Users can switch to the next chamber by turning the position knob (Figure 5).

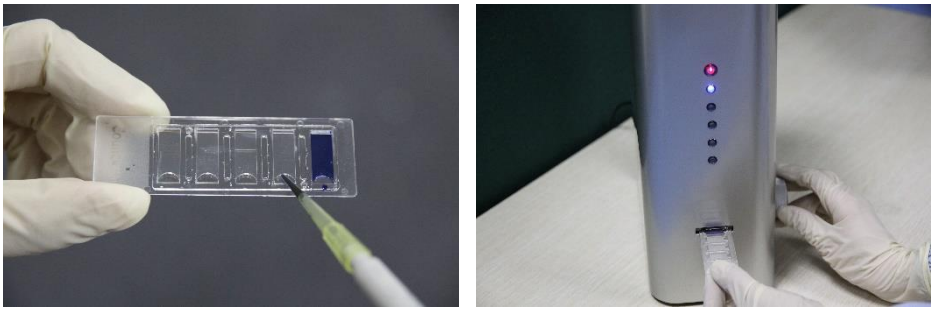


Figure 5 count cell

9. Press the **Start** button to analyze the sample.

NOTE: ■ The Countstar® can determine cell concentrations within a range of 1×10^4 to 3×10^7 cells/ml. Higher concentrations will require a dilution in advance. The

performance of object recognition lies within a range of 5 μm to 180μm.

■ Inserted chamber slides shall be analyzed immediately! If the sample liquid stays for more than 10 minutes inside the chamber of the chamber slide, evaporation may cause image situations with air bubbles, and this can end in inaccurate results.

10. It takes approximately 20 seconds to analyze each single sample. The results include the cell concentrations for total, living, and dead cells, the viability, the average diameter and compactness, and the percentage of aggregation of the cells will be displayed on the result screen (Figure 6).

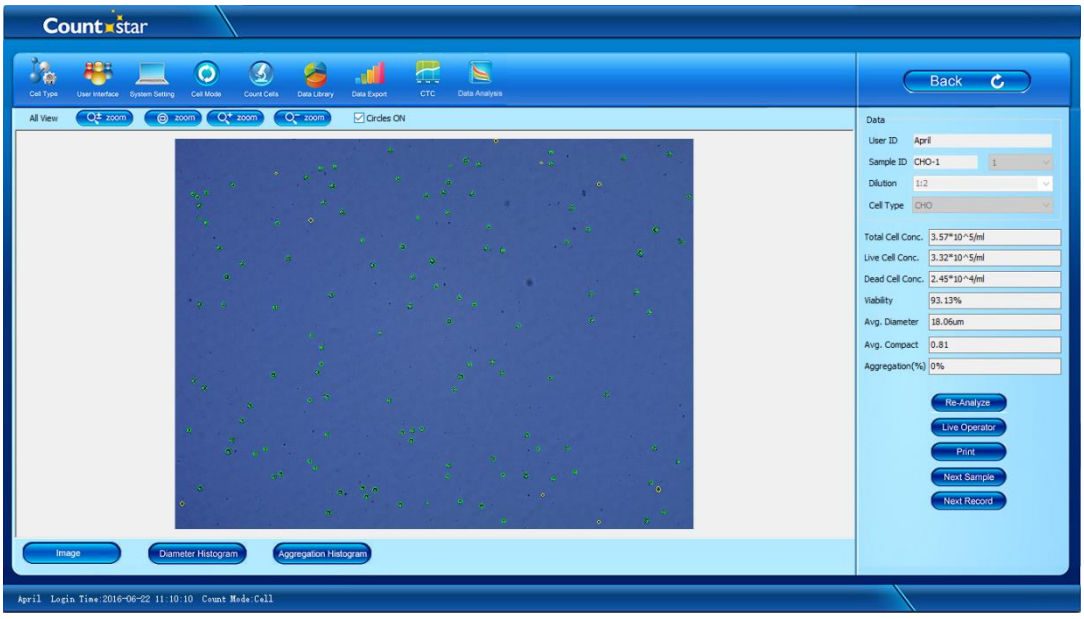


Figure 6 Measurement results window

11. The results of the analysis are displayed on the screen as soon as the measurement is finished. Then Countstar® cell counter is ready for another measurement by pressing **Next Record/Next Sample**. Press the **BACK** button to return to the main menu functions if you don't want to analyse more samples.

Analyzing the results

Results are automatically displayed in the same window (Figure 6) after analysis is completed. Previous results can be viewed in the menu **Data Library**.

The **Measurement Results** window is divided into 5 areas:

- The Menu Bar:

  Switch to other functions of the Countstar® analyzer or go back to the Main Menu Page

- Image tool area:

  Zoom in/out image and activate / deactivate result labeling

- The Image and Graph Area:

  View the images and the result graphs

- The Result Data Area:

  Displays sample parameters and counting results

- Processing area:

  Access to processing functions, such as: re-analyzing, re-starting or exporting results.

Result Data Area

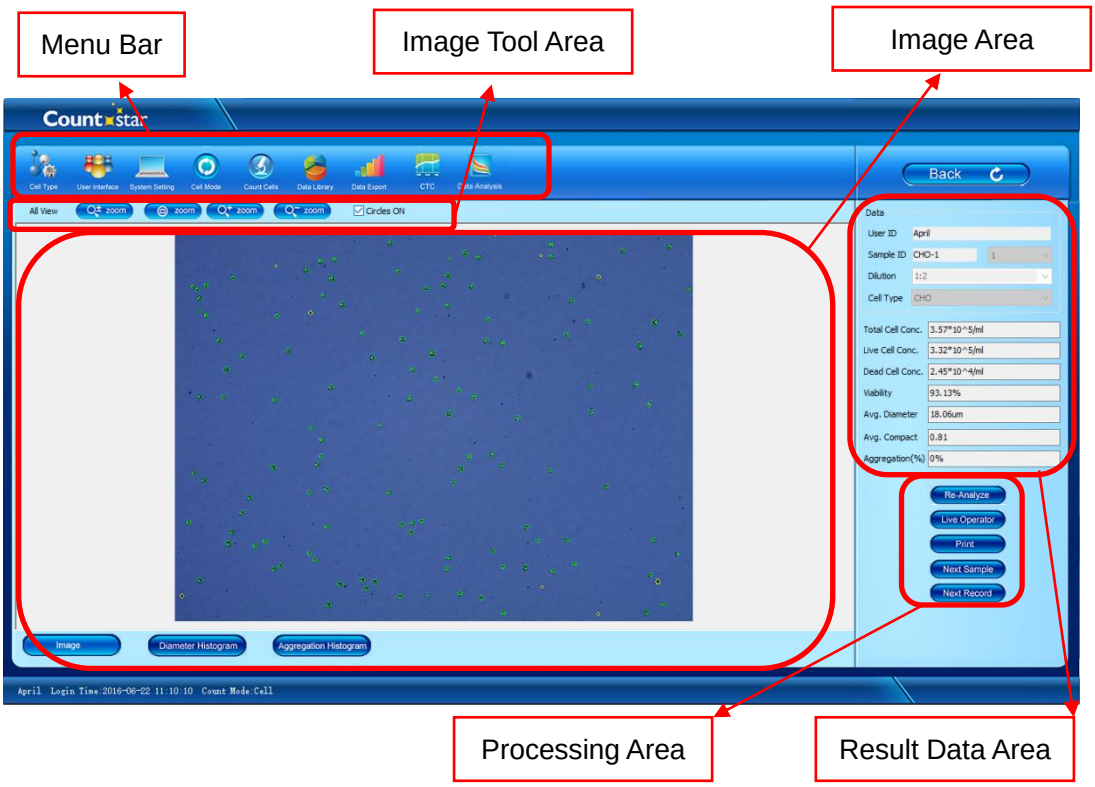


Figure 7: Result Data Area

The results of the sample analysis will be displayed on the Result Data Area which is on the right side of the user interface.

The following data are shown in the Result Data Area:

Total Cell Conc.: Total cell concentration**,**

Live Cell Conc.: Living cell concentration.

Dead Cell Conc.: Dead cell concentration.

Viability: The proportion of viable cells (%) in relation to the total number of cells.

Avg. Diameter: The average diameter [μm] of viable cells.

Avg. Compact: The average deviation of viable cell shape from an ideal sphere.

Aggregation (%): The percentage of aggregated viable cells in relation to total viable cell numbers.

Image and Graph Area

The Countstar® software allows users to take a detailed look on the original, acquired cell image, to zoom in/out regions of interest, activate/inactivate recognition marks (circles). Histograms can be displayed in the image window after clicking **Diameter Histogram** (Figure 8) or **Aggregation Histogram** (Figure 9).

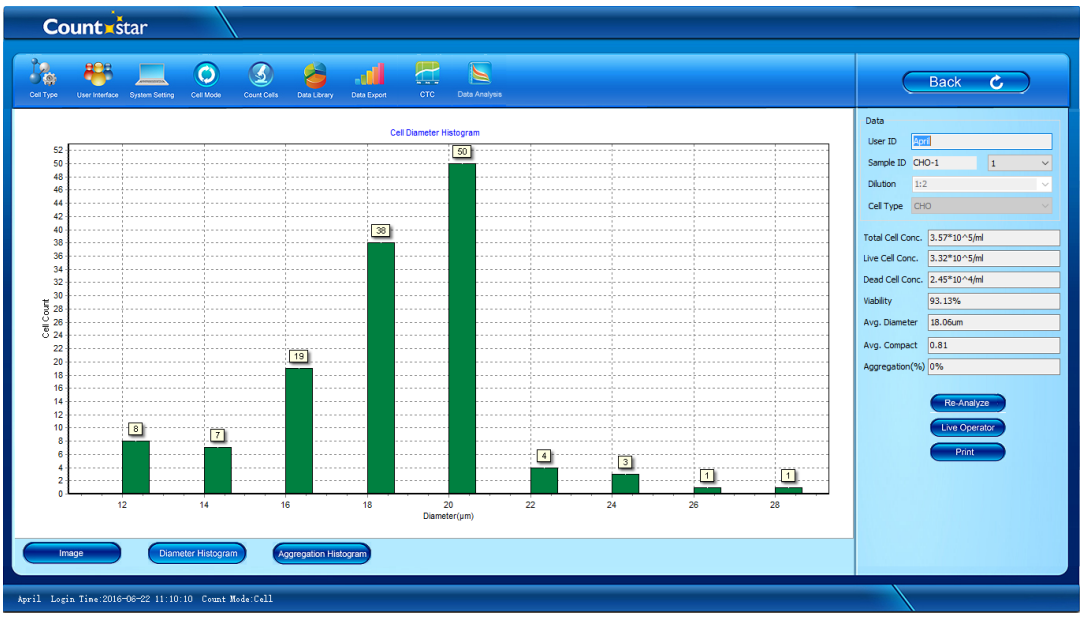


Figure 8: Cell Diameter Histogram chart

Cell Diameter Histogram

Click the **Diameter Histogram** button to display the Cell Diameter Histogram (Figure 8). The Cell Diameter Histogram shows the diameter distribution of living cells which

is determined from all living cells visible in the image.

Aggregation Histogram

The Countstar® offers a detailed evaluation of aggregates in cell cultures. Click the **Aggregation Histogram** button at the bottom to open the **Aggregation Histogram** (Figure 8). It shows the number of cells aggregates, as well as the cell counts in each aggregate. The **Number of Cells Aggregates** is displayed on the y-axis and at the top of the bars. The **Aggregate Size,** in terms of the number of cells per aggregate, is displayed on the x-axis.

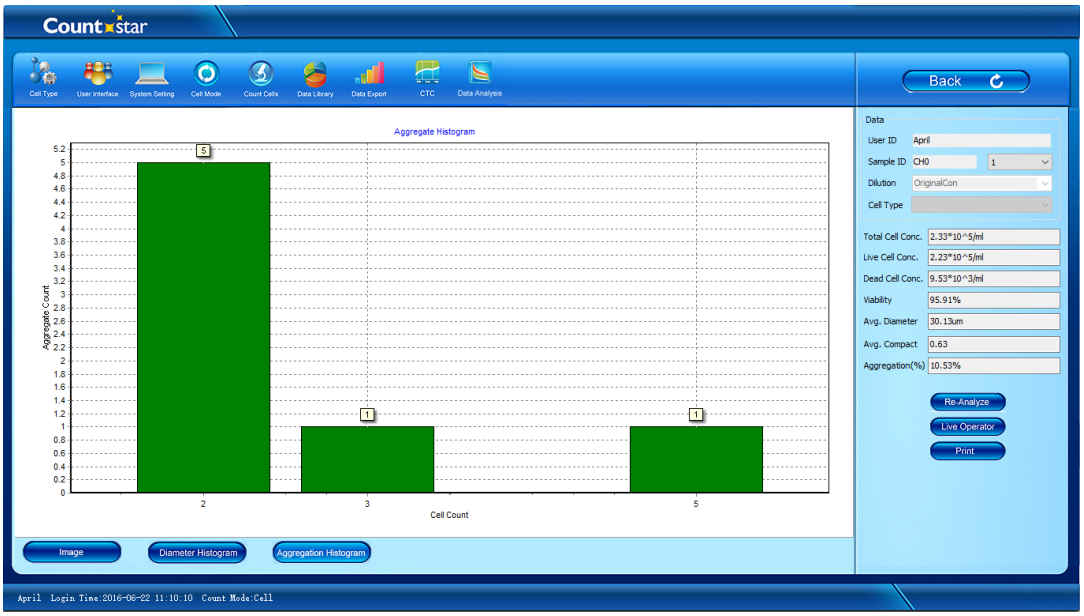


Figure 9: Cell Aggregation Histogram chart

The **Image** button guides you back the acquired images of the Countstar® analyzer.

The Image Area provides the following options:

Circles On check-box: Activate /inactivate the mark of live/dead recognition.. Live cells are marked by a green circle and dead cells are marked by a yellow circle. The diameter of circle corresponds to the cell diameter. Non-cell objects will also be marked by a yellow circle.

Zoom: “+Zoom” or “-Zoom” is for enlarging or shrinking image sections. “+/- Zoom”: When user want to zoom in a certain area of the image, click on “+/- Zoom” then select the desired area. Certain sections of the images can be enlarged by

executing the following:

1. Place the mouse arrow at the top left of interested area.

2. Click and hold the left mouse button while moving it to the lower right edge of the area of interest.

3. Release the mouse button. The selected area will now fill the whole window.

All View: Activating the All View button allows to move to other areas of the image in the enlarged view by holding the left mouse key and moving the virtual hand.

Scroll bars: Glance over an enlarged image (scroll bars will appear at the lower and right edge of the image when an image is enlarged).

Processing Area

Users can make use of the following functions in **Processing Area**: re-analyzing, optimization or exporting results. Users may re-analyze a result by adjusting the Cell Type parameters or by changing labeling in the image via “Live operator”. Countstar® software offers multiple options to adjust and optimize the image analysis for unique cell lines or a specific counting mode. After adjusting or changing, the user can proceed to a new analysis by clicking the **“Next Sample”** or **“Next Record”** button.

Data Library

All previous measurement data can be opened via the **Data Library** menu. Open the Data Library by clicking the **Data Library** button in the Main page or by selecting **Data Library** button in the menu bar at the top. A series of operations can be carried out in the **Data Library** window including search, result details, re-analyze, print and export.

Search

To find a result, use the filter functions including process time, Sample ID, User ID, and Cell Type at the upper right corner of the **Data Library** window. Find a result through **Processing Time**:

1. Click on the drop-down icon in the upper window near **Test Time**. A calendar

will appear and the start time of search period can be selected.

2. Click on the drop-down icon near the lower check box to select the end time of the search period.

3. Click **Search** button.

4. All of the results that match the defined time window will be displayed in the list.

Results can also be searched based on **User ID, Sample ID** and/or **Cell Type** (drop-down menu in **Cell Type**) by choose these search attributes.

Check for new results

Double click on the result in the list or select it then click the **View** button. A detailed result window will open.

Processing new results

Depending on the user access levels, new results can be processed such as: re-analyzing, deleting or exporting results, pressing Modify/Delete/Printing button

Reanalyze existing data.

The Countstar® software also allows users to reanalyze existing results. These changes are carried out on base of the original acquired images and analysis results, but will have no impact on the basic software and hardware settings of the Countstar® cell counter.

- **Re-analysis by Modifying Cell Type Parameters**

Reprocessing existing measurement results with new analysis parameters can be achieved via the **Re-Analysis** button. The **Re-Analysis** function offers the following options: Change the dilution ratio and adjust single Cell Type Parameters. The new data will be available when the re-analysis with the modified parameter settings is completed.

NOTICE

Any re-ananlysis will automatically generate a new record, the “-R1” will be added in the end of the original file name. If the same original data is modified and saved 2 times then the name will be added “-R2”, and so on. If re-analysis is applied on “-R1” file, then the name of the data will show “-R1-R1” in the end of the name.

Example:

original data name	Re-analyzed data name
XXX	XXX-R1
XXX	XXX-R2 (the 2nd time of reanalysis)
XXX-R1	XXX-R1-R1

- **Re-analysis by Live Operator**

The Live operator is another method for result reanalysis. It can be applied when debris or impurities significantly affect the result data. There are three function buttons in the live operator menu:

New Cells: Add unidentified cells to the counts.

Delete Cells: Delete obvious debris, impurities or improperly identified cells according to the image.

Delete Aggregate: Delete false cell clusters.

Export Cell Data

There are different formats for the data export. User can export data by clicking the **Data Export** button in the main page or the **Export** in the result window. The acquired images can be saved in the .JPG format and data in .XLS readable files and/or in a PDF format.

Export results as follows:

1. Select the **Data Export** in the main menu of the software. Then click **Export** button to export .XLS format file .

2. Click the **Export** button in the Result/CTC/Overlay Windows will export .PDFformat files.

3. Export the acquired images (without labels) by a right click on the mouse, when the

mouse arrow is positioned on the image to be exported. The image will be exported in .JPG format.

The Cultivation Time Chart

The **Cultivation Time Chart** (CTC) is used to evaluate cell cultures over time. Click the **CTC** button in the Main Page or Menu Bar on each page to open the CTC window. The **Chart List** and **Sample List** are two parts on the left side of the CTC window. In the **CTC** dialogue window on the right**,** charts can be opened, exported, created and deleted (Figure 10).

Previously created cultivation time charts can be found via the **Search** section located at the right top of the window. The user can search by name, or by creation/ modification date. Type the name and click the **Search** button. When searching by creation/modification time, click the drop-down menu on the right, then choose the date in the calendar.

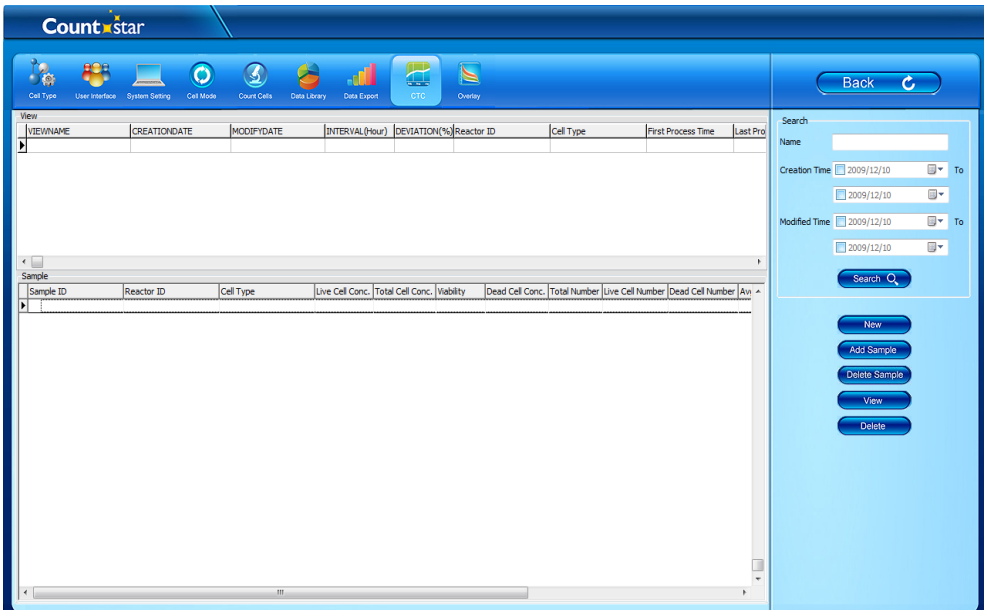


Figure 10: Countstar® CTC dialogue windows

If the filter settings contain no data, all generated CTC will be displayed in the **CTC list**. If the filter is set, only those results will be shown that match to the settings. Select a chart then click **View** button to open the chart. To create a new chart, click the **New** button. Press the **Delete** button to delete a chart.

Creating a new CTC View

Click the **New** button then select samples from the “select sample” window. Then type name, time interval and time deviation for the new chart.

Time interval is used to define a fixed length of test time circle.

Time deviation is used to define an acceptable variation of test time circle, any test accomplished beyond the defined deviation time will not be accepted by CTC function to avoid untrustworthy data point.

The new **chart** will appear highlighted in the **Chart list** area. Then add samples by pressing **Add Sample** button at the right half of the **CTC** dialogue window. After samples are added, click view to check the chart. (Figure 11).

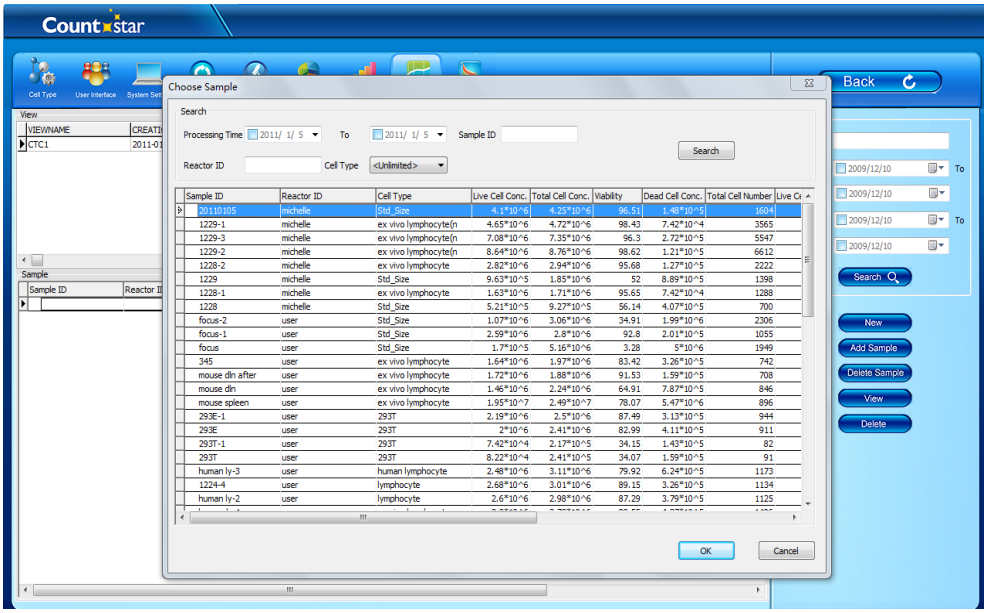


Figure 11: sample selected window

Delete CTC

Entire CTC Views can be deleted by selecting the CTC and click the **Delete** button. If one sample of a specific view shall be deleted, highlight the relevant sample in the sample area, then click the **Delete Sample** button.

Viewing and Exporting CTC Charts

When viewing the CTC curves, the curves to be displayed in the diagram are defined in the **Data Selection** area of the **Cultivation Time Chart** window (Figure 12). Click the appropriate checkbox to select the desired values. The following values can be selected for display:

Dead Cell Conc.: Dead cell concentration [cells/mL]

Live Cell Conc.: Live cell concentration [cells/mL]

Total Cell Conc.: Total cell concentration [cells/mL]

Dead Cell Count: The total number of dead cells

Live Cell Count: The total number of viable cells

Total Cell Count: Total number of all the cells

Viability [%]: Percentage of viability

Avg. Diameter: Average diameter of live cells [µm]

Aggregation Rate [%]: Percentage of cells in aggregates

Avg. Compact: Average compactness of living cells [without unit]

Total Object: The total number of objects counted

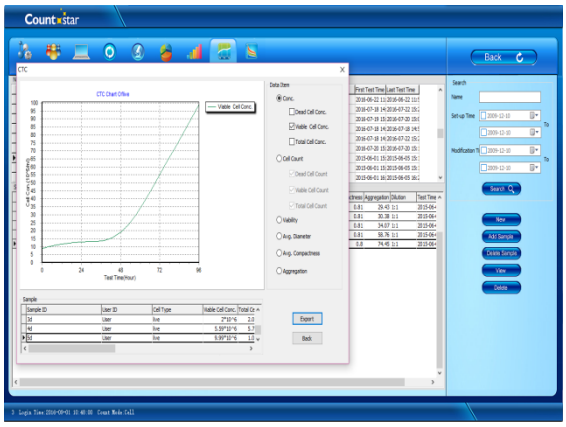


Figure 12: The selected CTC chart

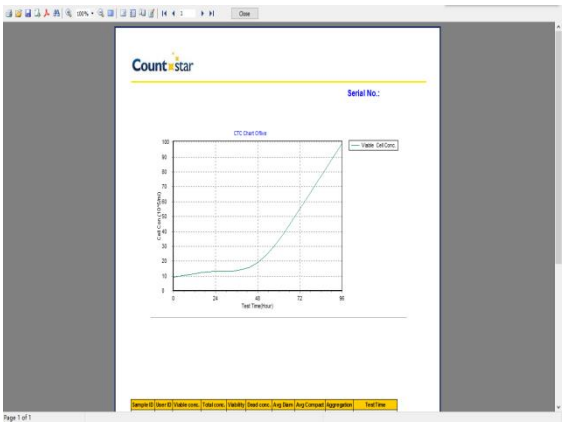


Figure 13: The .PDF-file of the CTC chart

To export a chart, click the **Export** button. A preview window will be opened, then this view can be either saved as a .PDF file or can be printed out, if a printer is connected to the Countstar® computer. Click the **Print** button to open the preview window (Figure 13).

Data Analysis

The diameter distribution of viable cells inside different samples can be evaluated in the Overlay. Click the **Diameter Distribution Overlay** button at the bottom of the Countstar® homepage. In the Overlay window (Figure 14), charts can be searched, created, edited, viewed, exported or deleted.

Three possibilities of overlay functions can be generated: Histogram Overlay, Diameter Distribution Overlay, and CTC Overlay. Users can select the desired option by clicking on the icons at the bottom of the Overlay window.

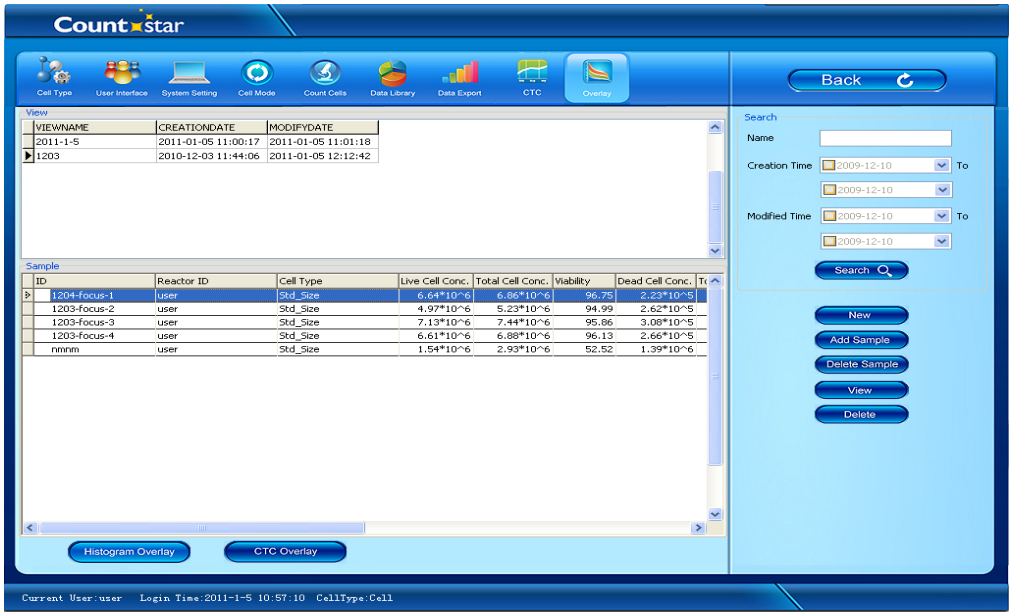


Figure 14: Data Analysis window

Histogram Overlay

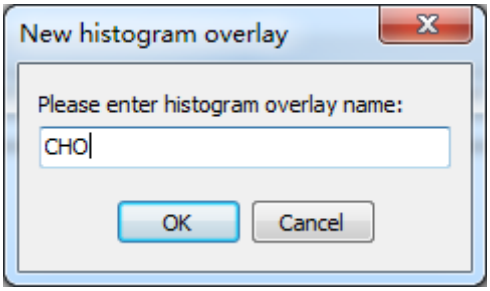
Histogram Overlay is used for comparing sample concentrations, cell counts and cell viabilities of different samples to each other. Each overlay can be composed of up to 10 separate results.

An existing chart can be searched by its name, set-up time or modification date in the Overlay window.

Set up an Histogram Overlay

User can setup a new overlay graph by clicking the **NEW** button on the right side of Data Analysis Menu window, or **Edit** an existing Overlay by select the Overlay from the list.

1. Click on **Histogram Overlay**.
2. Click on **New** on the right side of the window, then the following window will pop up. Type in a name for the overlay and then click on **OK** to continue, click on **Cancel** to quit. If OK is clicked, a new name will appear in the overlay list. If user want to **edit** an existing overlay, please go to step 3.



3. Left click on the name to select it, then click on **Add Sample**. The **Select Sample** window(Figure15) will pop up, user shall choose a sample from the list then click **OK** to continue. Then, the first sample is added in the overlay. An overlay is consist of at least 2 samples. Click on **Add Sample** again to add another sample. If multiple samples are required in the overlay, please repeat to add more samples. If a wrong sample is selected, click **Delete Sample** to remove it.

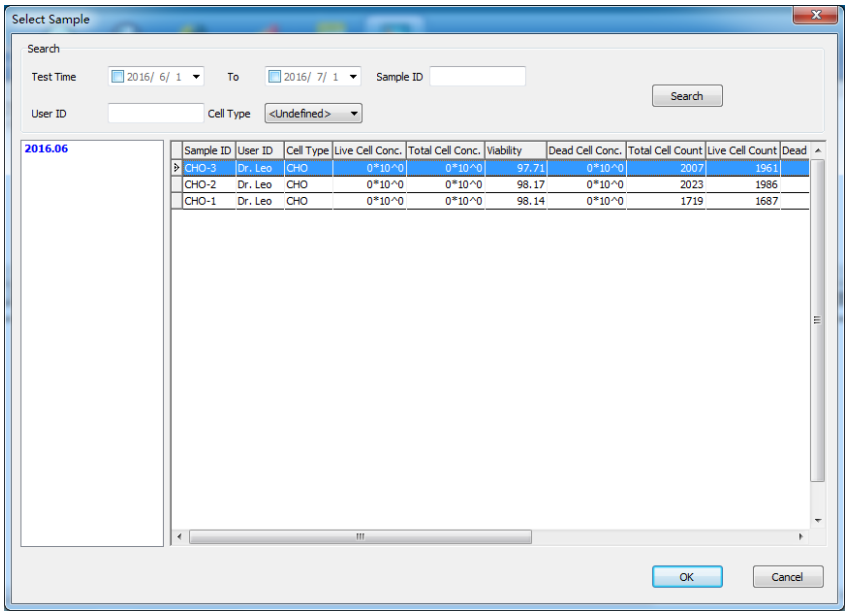


Figure 15: Select Sample window

Note: At maximum 10 separate samples can be added to one overlay. The **Add Sample** button will be automatically deactivated after 10 samples have been overlayed to each other.

4. After finishing add sample, click on **View** to see the overlay result like below. The **Histogram Overlay** (Figure 16) can be exported into .pdf format by clicking on **Export**.

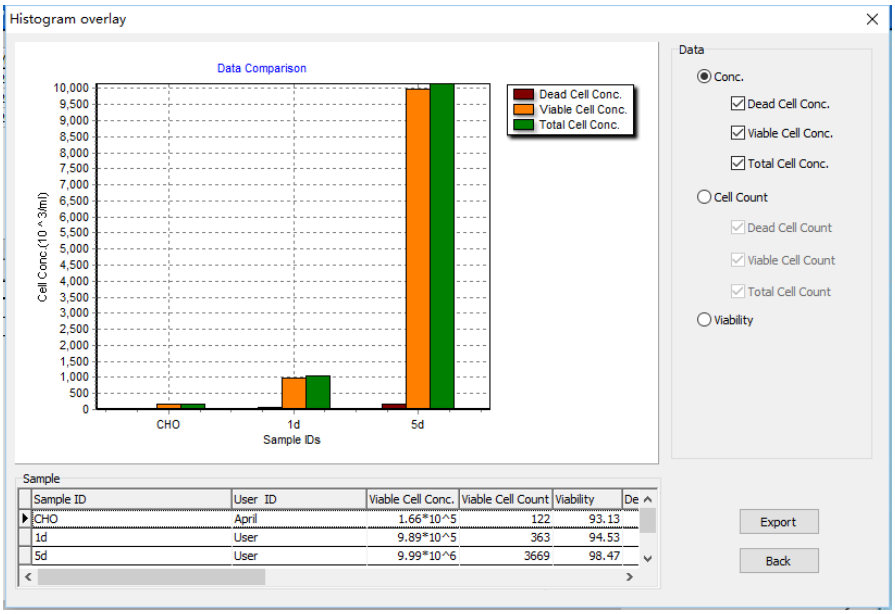


Figure 16: Histogram Overlay

5. Deleted an Overlay: Select the overlay you want to delete and click on the Delete button, then a confirmation window will pop up, click Yes to deleted it or click on No to quit. An Overlay will be deleted and can't be restored.

Diameter Distribution Overlay

It is used for comparing cell diameters of various samples to each other and/or to monitor its change over the duration of a cell culture experiment.

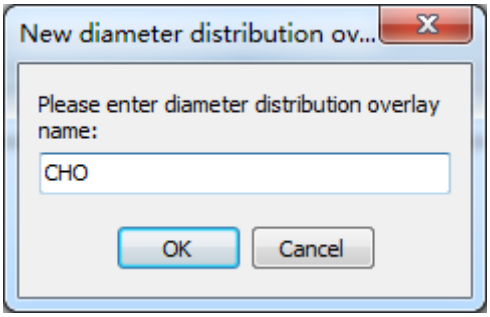
Search for a chart

An existing chart can be searched by its name, set-up time or modification date in the Overlay window.

Set-up an overlay

User can setup a new overlay graph by clicking the **NEW** button on the right side of Data Analysis Menu window or **Edit** an existing Overlay by select the Overlay from the list.

1. Click on **Diameter Distribution Overlay**.
2. Click on **New** on the right side of the window, then the following window will pop up. Type in a name for the overlay and then click on **OK** to continue, click on **Cancel** to quit. If OK is clicked, a new name will appear in the overlay list. If user want to **edit** an existing overlay, please go to step 3.



3. Left click on the name to select it, then click on **Add Sample**. The **Select Sample** window will pop up, user shall choose a sample from the list then click **OK** to continue. Then, the first sample is added in the overlay. An overlay is consist of at least 2 samples. Click on **Add Sample** again to add another sample. If multiple samples are required in the overlay, please repeat to add more samples. If a wrong sample is selected, click **Delete Sample** to remove it.

   Note: At maximum 10 separate samples can be added to one overlay. The **Add Sample** button will be automatically deactivated after 10 samples have been overlayed to each other.
4. After finishing add sample, click on **View** to see the overlay result like below. The **Diameter Distribution Overlay** can be exported into .pdf format by clicking on **Export**.
5. Deleted an Overlay: Select the overlay you want to delete and click on the **Delete** button, then a confirmation window will pop up, click **Yes** to deleted it or click on **No** to quit. An Overlay will be deleted and can't be restored.

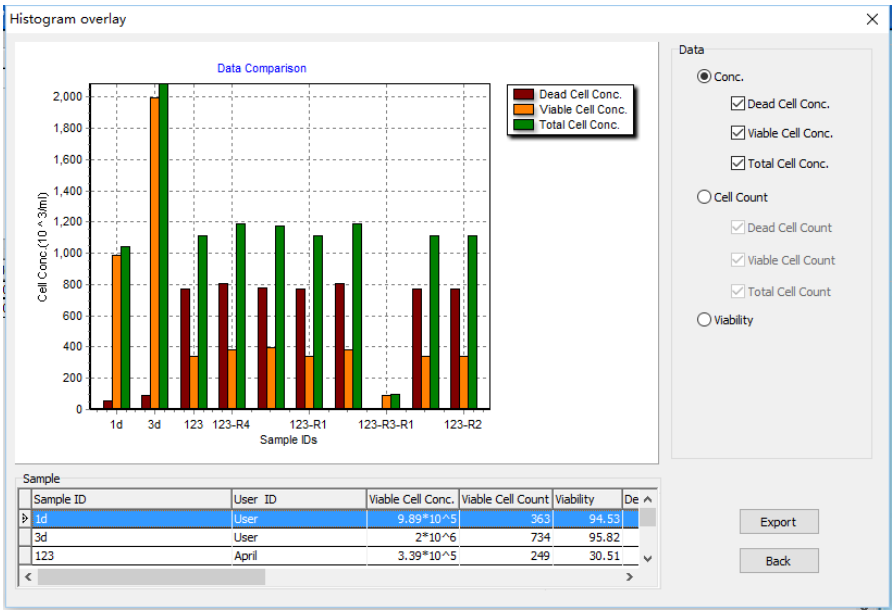


Figure 17: Diameter Distribution Overlay

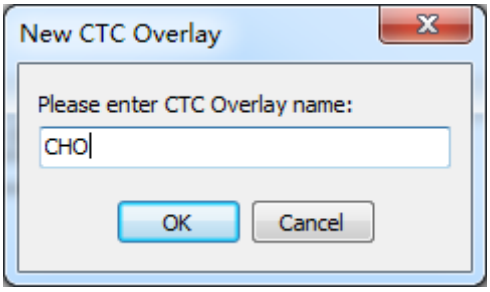
CTC Overlay

CTC overlay is used to compare the cell growth among multiple samples.

Set-up an overlay

User can setup a new overlay graph by clicking the **NEW** button on the right side of Data Analysis Menu window or **Edit** an existing Overlay by select the Overlay from the list.

1. Click on **CTC Overlay**.
2. Click on **New** on the right side of the window, then the following window will pop up. Type in a name for the overlay and then click on **OK** to continue, click on **Cancel** to quit. If **OK** is clicked, a new name will appear in the overlay list. If user want to **edit** an existing overlay, please go to step 3.



3. Left click on the name to select it, then click on **Add CTC**. The **CTC Chart** window will pop up, user shall choose a CTC from the list then click **OK** to continue. Then,

the first sample is added in the overlay. An overlay is consist of at least 2 CTCs. Click on **Add CTC** again to add another CTC. If multiple CTCs are required in the overlay, please repeat to add more CTCs. If a wrong CTC is selected, click **Delete CTC** to remove it.

Note: At maximum 10 separate CTCs can be added to one overlay. The **Add CTC** button will be automatically deactivated after 10 CTCs have been overlayed to each other.

4. After finishing add CTC, click on **View** to see the overlay result like below. The **CTC Overlay** (Figure 18) can be exported into .pdf format by clicking on **Export**.

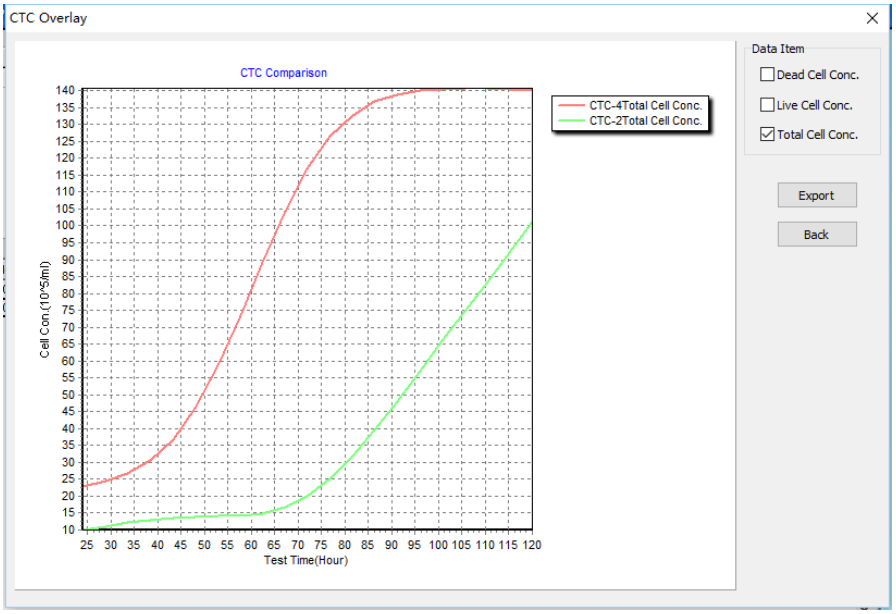


Figure 18: CTC Overlay

5. Deleted an Overlay: Select the overlay you want to delete and click on the **Delete** button, then a confirmation window will pop up, click **Yes** to deleted it or click on **No** to quit. An Overlay will be deleted and can't be restored.

Chapter 3 Troubleshooting and Error Codes

Table 3 Troubleshooting and Error code

Problem	Possible Reasons	Solution
Fail to startup the Countstar® software	The optional dongle is not connected with the computer	➢ Make sure the dongle was purchased and delivered with the system. ➢ Insert the dongle into a free USB hub of the computer, and then restart the computer and the Countstar® software.
	Dongle driver software is not properly installed.	➢ Reinstall the dongle driver following the page 16 of the user manual.
Black screen (Error message at the bottom of the Count Cell window: “The host signal was not detected.”)	USB connection failure	➢ Ensure that the USB cable of the Countstar® is connected with the computer before starting the software. ➢ Remove the USB cable from the computer and re-insert it then restart the software, as data and commands won’t be transmitted without connection.
	The camera driver is not installed successfully or is destroyed.	➢ Remove the USB cable from the computer, and reinstall the software again following the page 9 to 15 of the user manual. ➢ Connect the Countstar and the computer with the USB cable, and try again.
Black screen (Error message at the bottom of the Count Cell	The Countstar device doesn’t match the software serial number	➢ Change the matched software, and try again. ➢ If it doesn’t work, contact with the technical Support of Ruiyu Biotech or your local sales representative.

window: “The host connection failed, …”)		
Poor Image Quality	Slides	➢ Ensure that the slides are clean before using them and prepare the chambers slides in a clean environment.
	Trypan Blue	➢ Do not store Trypan Blue below or above the defined storage conditions. Temperatures below +2°C will cause the irreversible formation of crystals and this will affect the calculating results to much higher dead cell counts -> lower viability values.
	Focus shift	➢ Please contact your local service for focusing adjustment.
	Cell aggregation.	➢ Ensure that cells are not forming aggregates due to an incomplete trypsinisation or due to an insufficient nutrient situation in the cell culture.
	Impurities affection	➢ Avoid impurities or tissue debris by washing the sample with PBS.
Saving and printing problems	Incorrect USB drive	➢ Use the USB drive supplied with the counter (if purchased) or an USB 2.0 drive as some types of USB drive are not detected or installed correctly by the computer. ➢ Too many files in the data library may slow down the counter. If the database of the Countstar® software contains more than 20 GB of image and result data, the performance of the software will be slowed down significantly.

	USB drive removed accidently.	➢ Do not remove the USB drive or turn off the counter while acquiring, saving or reading data as it may damage the function of the software.
	Printer driver is not properly installed	➢ Reinstall the printer driver and reconnect the printer to the computer.
Instrument fails to upgrade to a new firmware version	The firmware file may be corrupted or the transfer to a USB drive was not executed correctly	➢ Download the new firmware version from the download area of the Countstar® website to a USB drive then repeat the upgrade of the firmware again. In case this will not lead to the expected results, contact the Technical Support of RuiYu Biotech or your local sales representative.
Incorrect name	No sample ID is entered.	➢ Type in a sample ID before counting cells.
	Sample ID already exists	➢ Change the name of the sample ID and continue to count cells.
Software is getting slow	Database volume has reached its allowed maximum size.	➢ Transfer (copy) older data folders to your archive on a regular base, then delete these files and folders on the Countstar® computer.
Results PDF exporting problem	Word bank incomplete.	➢ Upgrade the fonts.
	A PDF reader is not installed.	➢ Install Adobe Reader or similar software which is capable of importing and reading .pdf readable files.
Results can't be exported to Excel files.	An Excel software version is not installed.	➢ Install MS-Excel or similar software which is capable of importing and reading .XLS readable files.
Inaccurate cell counts	Sample treatment	➢ Never insert the Countstar slide upside-down, as this may spill liquid into the instrument and

		cause severe hardware damage. ➢ Never reuse a chamber of a Countstar® chamber slide, as the leftovers of dye and cells/particles from the previous test will affect the next test significantly. ➢ Never use any other chamber slides such as the glass hemocytometer or chambers from other companies. As they will cause inaccurate cell counting and will damage the instrument irrevocably. ➢ Ensure when pipetting the sample into the chamber, that the entire chamber volume is filled with liquid and the slide is inserted correctly into the slide port.
	Low or high counts	➢ The Countstar® IC1000 automated cell counter is designed to analyze samples from 1×10^4 cells/mL to 3×10^7 cells/mL. The best accuracy is within 1×10^5 cells/mL to 1×10^7cells/mL. ➢ If the sample is beyond this range, you may need to dilute or concentrate the cell containing samples and test it again.

Appendix

Accessories and Reagents

The following products are recommended to be used together with **Countstar® BioTech** automated cell counters and can be purchased from Shanghai Ruiyu Biotech Co., Ltd., or from your local lab equipment supplier. For more information, contact your regional distributor.

Table 4: Countstar® Automated Cell Counter Accessories and Reagents

Items	**Catalog No.**	**Quantity**
Countstar® Cell Counting Slides	12-0005	50pcs/box
Countstar® reference CD-ROM	14-1000-C	1
Countstar® USB drive / Dongle	15-1000	2
Countstar® User Manual	17-1200-C	1
Countstar® Hex key	18-0001	1
0.2% Trypan Blue staining solution	19-0001	20mL×1
Countstar® Density standard beads	19-0002	1mL×1
Countstar® Diameter standard beads	19-0003	1mL×1
Countstar® Viability standard beads	19-0005	1mL×1

Note: Products No.12-0005, 15-1000, 19-000x can be purchased from your local supplier. Please contact him for more information, prices and delivery.