

Countess™ 3 Automated Cell Counter

USER GUIDE

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| B.0 | 18 January 2021 | Update Technical specifications |
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Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Contents

The Countess™ 3 Automated Cell Counter is shipped with the components listed below.

| Component | Quantity |
|--|----------|
| Countess™ 3 Automated Cell Counter | 1 each |
| Power cord with 4 adapters (for U.S./Canada/Taiwan/Japan, Europe, or UK) | 1 each |
| Countess™ Cell Counting Chamber Slides (50 slides/box) | 1 box |
| Countess™ 3 Disposable Slide Holder | 1 each |
| Countess™ 3 Reusable Slide Holder | 1 each |
| Countess™ 3 USB Drive | 1 each |
| Countess™ 3 Automated Cell Counter Quick Reference Card | 1 each |

Product description

The Countess™ 3 Automated Cell Counter is a fully automated cell counter and assay platform that state-of-the-art optics and image analysis algorithms to analyze trypan blue stained samples in suspension.

- The Countess™ 3 Automated Cell Counter offers an intuitive user interface with the option to save data and generate a report, which can then be transferred to a PC using the USB drive supplied with the instrument or available separately.
- The cells to be counted are loaded into the instrument in disposable Countess™ Cell Counting Chamber Slides. Each chamber slide contains two enclosed chambers to hold the sample and allow measurement of two different samples or perform replicates of the same sample.
- The instrument takes 10-20 seconds per sample for a typical cell count in the brightfield channel and is compatible with a wide variety of eukaryotic cells. In addition to cell count and viability, the Countess™ 3 Automated Cell Counter also provides information on cell size.

Upon receiving the instrument

Examine the instrument carefully for damage incurred during transit. Ensure that all parts of the instrument, including accessories listed above, are included with the product. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

See “Install the instrument” on page 8 for instructions on installing the instrument.

Register your instrument

Visit www.thermofisher.com/registercountess to register your instrument. You will be asked to supply the serial number, your name, and your contact details. Registering your instrument ensures that you will receive notifications of software upgrades and information on new assays for use with the Countess™ cell counter.

Exterior instrument parts



- ① **Touchscreen display:** The 7-inch capacitive display is the main user interface of the Countess™ 3 Automated Cell Counter. It contains the buttons for all instrument functions and displays data from the cell count.
- ② **Front USB port:** Allows you to transfer and save the cell count data and image to an external computer for record keeping and printing purposes. You can use any standard FAT32-, exFAT- or NTFS-formatted USB drive for data transfer. If desired, you can plug in a USB mouse into the rear USB port for instrument control.
- ③ **Slide port:** Used to insert the analysis slide containing the sample into the counter.
- ④ **Power switch:** ON/OFF rocker switch is the main power switch. It is not necessary to use the power switch for day-to-day operation of the instrument.
- ⑤ **Rear USB port:** An additional USB port to perform functions such as plugging in a USB mouse for instrument control.
- ⑥ **Power input jack:** Connects the instrument to an electrical outlet through the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.

2

Installation

Operating environment

- Place the instrument on a level surface away from vibrations emanating from other pieces of equipment.
- Allow at least 5 cm (2 in) free space at the back of the instrument to allow for proper ventilation and prevent overheating of electronic components.
- Set up the instrument away from direct light sources, such as windows. Ambient room lighting can enter the imaging path and affect the image quality.
- Operating temperature range: 4°–32°C (40°–90°F).
- Relative humidity range: <80%.

IMPORTANT! Do not position the instrument so that it is difficult to turn off the main power switch located on the back of the instrument.

In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the instrument from the wall outlet.



Install the instrument

1. Unpack the instrument and place the instrument on a flat, level, dry surface.
2. Remove the thin plastic protector film from the touch-screen display.
3. Plug one end of the power cord appropriate for your region into the instrument.
4. Plug the power cord into the electrical outlet. Be sure to use only the power cord supplied with your instrument. Powering the instrument with an unapproved power cord may damage the instrument.

Turn ON the instrument


After unboxing, upon switching on the instrument for the first time, it will run through a series of set-up screens.

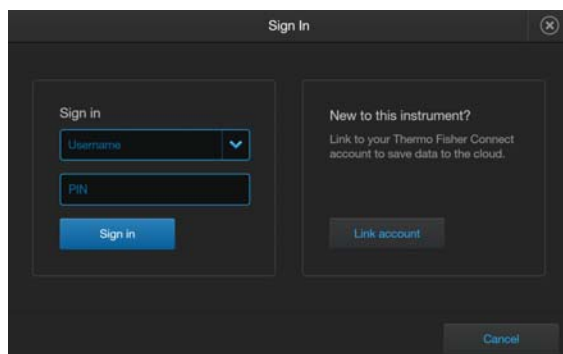
- Read the End User License Agreement and press **Agree** to proceed with start up.
- Set the **Date** and **Time** using the buttons (see “Set the date and time” on page 36). Press **Next**.
- Upon completion, press **Done**. You will proceed to the Home screen and can begin using the instrument as described below.

1. Turn on the instrument by flipping the power switch on the back of the instrument to the **ON** position. See “Exterior instrument parts” on page 7.
The instrument will walk you through an initial Out-of-Box Experience to set up the instrument for first use.
2. From the **Home** screen, you can proceed immediately to the assays by inserting a slide. See Chapter 5, “Cell count and cell viability assays”.
Alternatively, you can change or add a template in step 3 below or change instrument settings in step 4 below.
3. To change the current template or add a new template to the instrument, press  **Templates**.
Templates allows you to create customized count preferences (i.e., gate counts based on cell size, brightness, and/or circularity). See “Load a template” on page 14.
4. To change instrument settings, press  **Settings**.
Settings allows you to update the Countess™ 3 instrument software, customize instrument settings, view instrument details, and export error logs. See “Settings screen” on page 35.

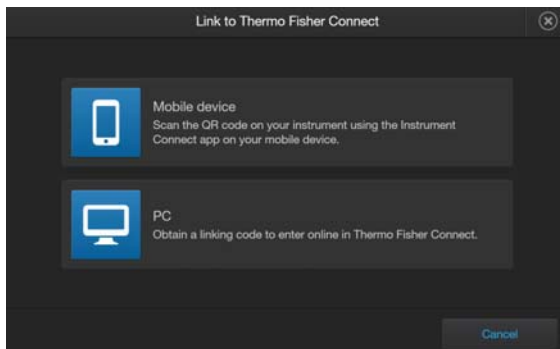
Sign in to the instrument with a new Connect account

Note: A Wi-Fi adapter needs to be installed and a Thermo Fisher Connect account is required for linking. See <http://thermofisher.com/connect> for details on using Connect.

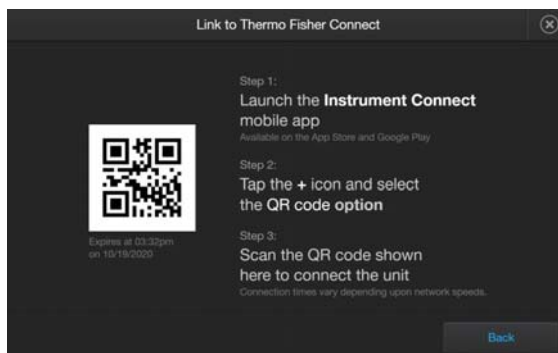
1. Press  **Sign in** from the **Home** screen.
2. Press **Link account**.



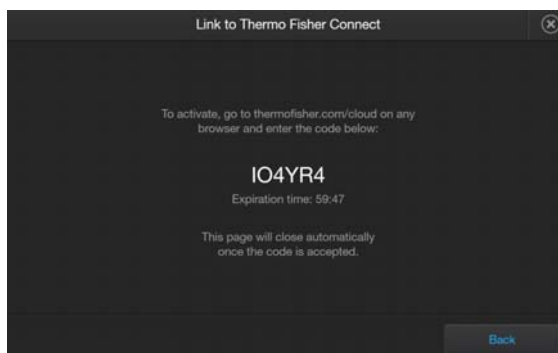
3. Choose a method to connect.



- Press **Mobile device**. Scan the QR code on your instrument using the Instrument Connect app on your mobile device. Follow the steps shown on the page.




- Press **PC** to obtain a linking code to enter online in your Thermo Fisher Connect account.

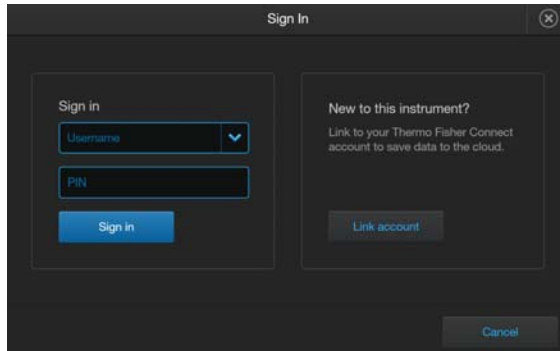


4. A completion screen will show. Press **Done**. You then return to the **Home** screen and are logged into your account.

Once set up, you will then only need to sign in to your instrument account using your **Username** and **PIN**.

Sign in to the instrument with an existing Connect account

1. Press  **Sign in** from the **Home** screen.
2. Select your **Username** from the drop-down box. Enter your **PIN**.
3. Press **Sign in**.

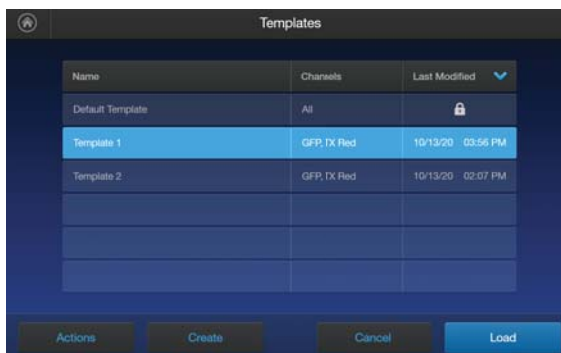


3

Templates

Templates screen

Templates allows you to create and save customized templates. Each custom template defines the count parameters (size, brightness, and circularity) for a consistent and streamlined workflow.



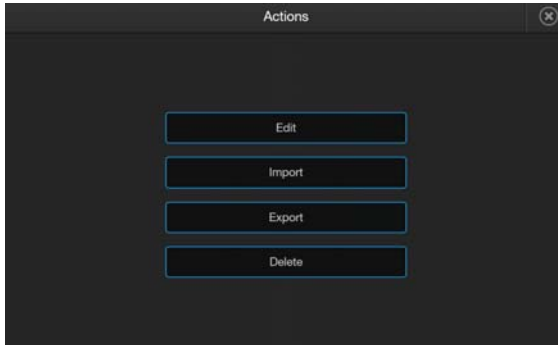
| Name | Channels | Last Modified |
|------------------|-------------|-------------------|
| Default Template | All | |
| Template 1 | GFP, Dk Red | 10/13/20 03:56 PM |
| Template 2 | GFP, Dk Red | 10/13/20 02:07 PM |
| | | |
| | | |

Actions Create Cancel Load

- Templates can be accessed from the **Home** and **Gating** screens.
- The currently selected template is displayed at the bottom of the **Home** screen.
- Automatic instrument functions and count parameters are defined in the **Edit template** screen. See “Create a template” on page 14 or “Edit a template” on page 14.
- The Default template contains default count settings and cannot be edited.
- The count parameters specified in the selected template are applied to all new cell counts.
- Templates can be sorted by pressing **Name**, **Channels**, or **Last Modified** headers.
- If you have already performed a count, loading a new template from the **Results** screen applies the count preferences to the current count results (total cells, viability, etc.) and to all new counts.

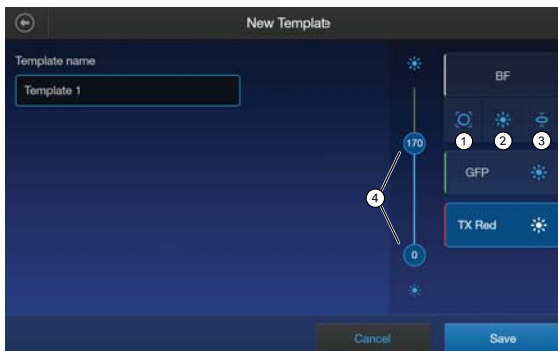
Template functions

The **Actions** screen allows you to edit, import, export, and delete templates. Edit can be used to modify the currently selected template, while Import, Export, and Delete can be used on multiple templates.



Count parameters

Count parameters are adjusted in the **Edit template** screen using the Gating sliders. Moving the top and bottom gate boundaries adjusts the range of size, brightness, and circularity parameters.



- ① **Size Gating** : Moving the top gate down removes larger objects; moving it up includes larger objects. Moving the bottom gate up removes smaller objects; moving it down includes smaller objects.
- ② **Brightness Gating**: Moving the top gate down removes brighter objects; moving it up includes brighter objects. Moving the bottom gate up removes dimmer objects; moving it down includes dimmer objects.
- ③ **Circularity Gating**: Moving the top gate down removes more circular objects; moving it up includes more circular objects. Moving the bottom gate up removes less circular objects; moving it down includes less circular objects.
- ④ **Gating Boundary Sliders**

Live or **Dead** can be selected only on a brightfield count. For a fluorescence count, select the individual channels.

Load a template

1. Press **Templates**.
2. Press the desired template to select, then press **Load**.
3. To return to the previous screen without loading the new template, press **Cancel** or **Back**. The instrument will keep the saved template, but will return to the previous screen without loading it.

Create a template

1. Press **Templates**.
2. Press **Create**. To assign a name to the new template or to change the name of the existing template, press the Template name text box to open the keypad.



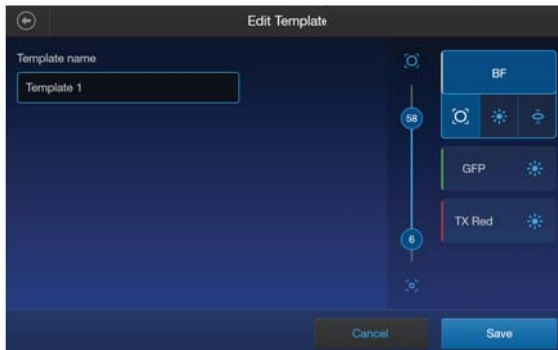
3. Type in the desired template name. To enter symbols, press the symbol (@%&) key. To return to the alpha-numeric keypad, press **ABC**.
4. Press **Enter** to save the name and return to the **New Template** screen. Press **X** to return to the **New Template** screen without saving the name.
5. Press **Save** to save the new template. Press **Cancel** or **Back** to return to the **Templates** screen without saving.

Edit a template

1. Press **Templates**.
2. Select an existing template, then press **Actions**.

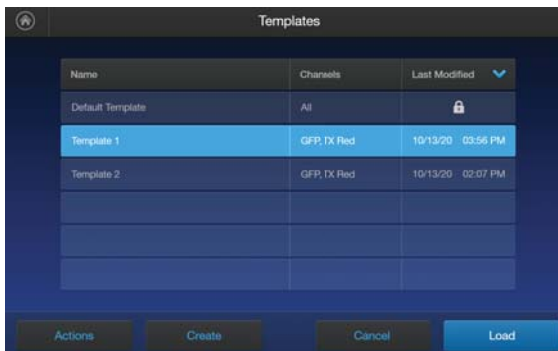
Note: The default template contains default count settings and cannot be edited.

3. Press **Edit**.



The **Edit** screen for the selected template opens.

4. Press the appropriate channel (BF or other dye) and edit the desired parameters (see “Count parameters” on page 13) to create a custom template.
5. Press **Save**.

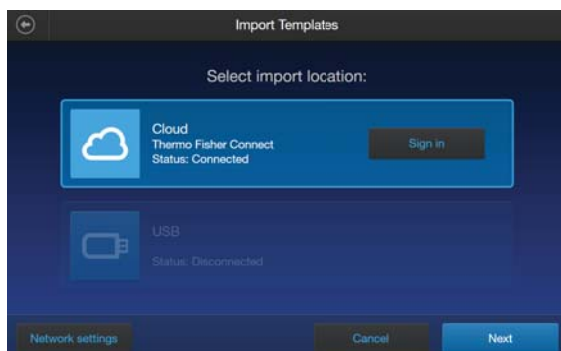


From the **Templates** screen, select the desired template and press **Load** to use that template to read the sample.

Import a template


1. Press **Templates**.
2. Press **Actions**.
3. Press **Import**.
4. Press the import location **Cloud** or **USB**.
 - a. For **Cloud**, press **Sign in** to log in to your Thermo Fisher Connect account. If the **Cloud** is not active and disconnected, press **Network settings** to connect to a network, then press **Back** to try and sign in again.

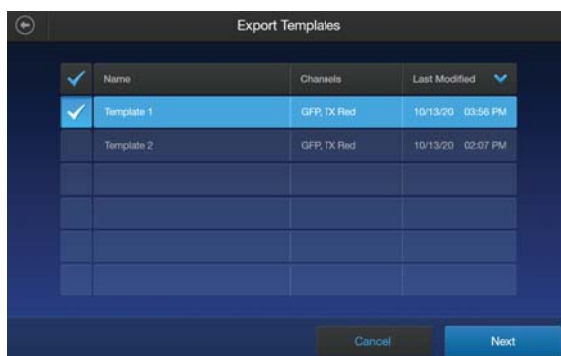
- b. For **USB**, insert your USB drive into the slot on the front of the instrument.




5. Press **Next**.
6. Select one or more templates from the list.
7. Press **Import**.
8. Once complete, press **Done**.

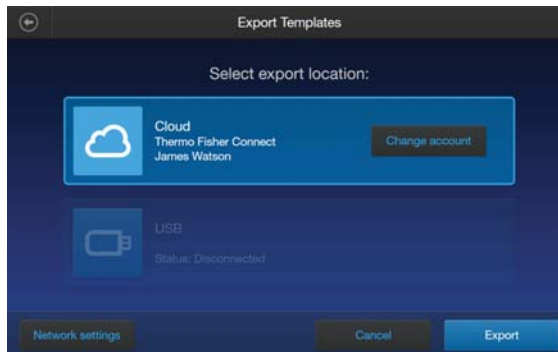
Export a template

1. Press  **Templates**.
2. Press **Actions**.
3. Press **Export**.
4. Select one or more templates from the list.



5. Press **Next**.
6. Press the export location **Cloud** or **USB**.
 - a. For **Cloud**, press **Sign in** to log in to your Thermo Fisher Connect account. If the **Cloud** is not active and disconnected, press **Network settings** to connect to a network, then press  **Back** to try and sign in again.

- b. For **USB**, insert your USB drive into the slot on the front of the instrument.



7. Press **Export**.
8. Once complete, press **Done**.

4

Slide preparation and operation

The Countess™ 3 and 3 FL cell counters accept disposable Countess™ Cell Counting Chamber Slides and glass Countess™ Reusable Slides on interchangeable, slide-specific carriers.

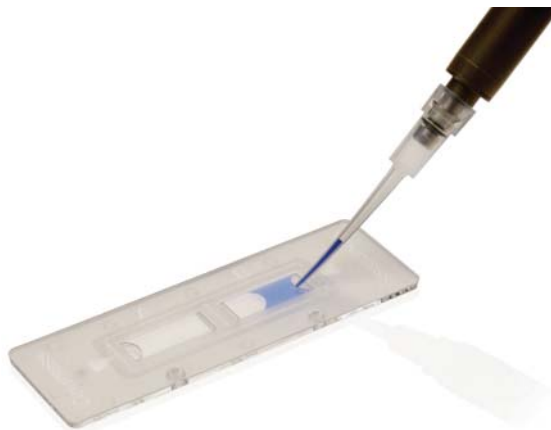
Recommendations

To obtain the best results, follow these recommendations:

- Ensure that the cell sample is homogeneously mixed.
- The measurement range extends from 1×10^4 to 1×10^7 cells/mL, but the optimal range is 1×10^5 to 4×10^6 cells/mL.
- For accurate results in cell viability assays, ensure that the counting area is covered with the cell suspension and count the cells immediately after staining per the assay protocol.
- Do **not** press the optical surfaces of the chamber slides. Hold the slides by the edges.
- Take care to avoid forming bubbles in the sample.

Load Countess™ Cell Counting Chamber Slide

1. Prepare the sample by adding 10 μ L of your cell suspension to 10 μ L of 0.4% trypan blue stain (for brightfield only). Mix the sample mixture well by pipetting it up and down a few times.
2. Gently pipet 10 μ L of the sample into the half moon-shaped sample loading area. The sample is loaded into the chamber through capillary action.



3. Let the sample mixture settle in the chamber for 30 seconds, and then insert the slide into the slide adapter. You will hear a soft click once the slide is pushed in correctly.

Note: The instrument reads one side of the slide at a time, so insert the sides appropriately.

Note: The instrument is shipped with the slide adapter already installed.



4. Use the touchscreen to run the sample count. See Chapter 5, “Cell count and cell viability assays”.
5. To remove the slide, push the slide gently into the instrument until it clicks and the slide springs out. Grasp the slide and pull it out the rest of the way.

Note: After using the Countess™ Cell Counting Chamber Slides, appropriately dispose of them as biohazardous waste. **Do not reuse the disposable chamber slides.**

6. (Optional) To remove the slide adapter, gently squeeze the tabs and pull the adapter completely out of the instrument.

Load the Countess™ Reusable Slide

1. Before loading your sample into the Countess™ Reusable Slide, place a cover slip on the counting chamber, making sure the cover slip is clean and free of grease.
2. Gently pipet 10 μ L of the sample into the sample inlet, allowing capillary action to draw the sample into the counting chamber. A properly loaded counting chamber should have a thin, even film of fluid under the cover slip.



Note: Each chamber in the Countess™ Cell Counting Chamber Slide or the Countess™ Reusable Slide has a 10- μ L sample capacity. Do not overfill the slide chambers.

3. Load the reusable glass slide into the reusable slide holder.



4. Insert the reusable slide holder containing the slide into the slide port and gently push into the instrument until it clicks into place.
5. Use the touchpad to run the count.
6. To remove the reusable slide holder, push the slide gently into the instrument until it clicks and a spring pushes the slide out. Grasp the slide and pull it out the rest of the way.

7. *(Optional)*: To count the second sample present on the reusable slide, simply remove the slide from the holder, rotate, and reinsert the slide into the holder so that the second sample is aligned with the sample viewing hole.
8. After using the Countess™ Reusable Slide, rinse the glass slide and cover slip with water, and then clean with 70% ethanol. Use Kimwipes™ laboratory tissues to clean and dry the slides, as needed.

5

Cell count and cell viability assays

Count cells in brightfield

Capture and count

To bypass adjusting individual parameters before the brightfield count, you can select **Rapid capture** at the top of the **Home** screen. This will automatically set the parameters and perform a count as soon as the slide is inserted into the instrument. To adjust parameters prior to the count, follow the procedure below.



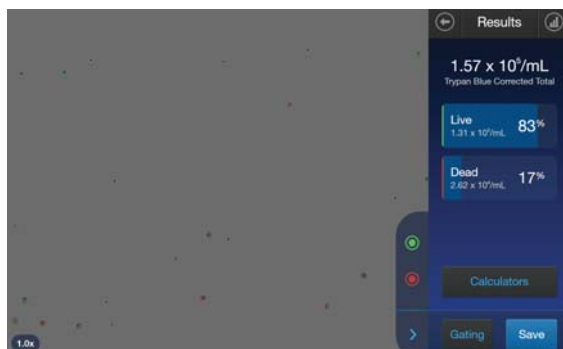
1. Load the samples into the instrument as described in Chapter 4, “Slide preparation and operation”.
2. (Optional): Press **Templates** and load the desired gating parameter settings as described in “Load a template” on page 14.
3. Insert the sample slide into the slide port, making sure that the sample side is inserted completely into the instrument. You will hear a soft click if the slide is pushed in correctly.
4. When the slide is inserted, the instrument automatically illuminates the sample, sets the intensity of brightfield illumination, and auto focuses on the cells.

Note: Auto lighting is optimal for producing accurate results, but if needed, can be disabled by adjusting the lighting slider.

5. (Optional): Set exposure using the light source slider.
The light source slider controls the LED intensity, camera gain and exposure time, and allows you to adjust the image brightness.

6. Press **Count**.

The instrument captures the image and displays the results (total concentration, percentage, and concentration of live and dead cells). For more information, see “View results” on page 24.



Next steps

- To identify the objects (i.e., cells) counted as Live, press . To identify objects counted as Dead, press . See “Identify cells counted in cell count and cell viability assays” on page 25.

Note: The **Live** and **Dead** buttons are enabled by default after the image has been counted.

- To see the distribution of live and dead cells in a graphical format, press **Histogram**. See “Histogram count results” on page 26.
- To gate the results by object size, brightness, or circularity, press **Gating** to go to the **Gating** screen. See “Gate count results” on page 26.

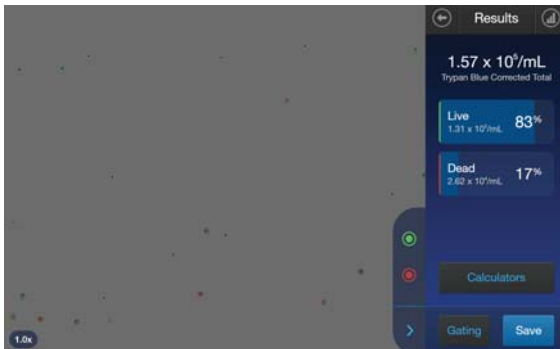
Note: You can save the changes you make to the size, brightness, or circularity parameters in the **Gating** screen to the current template or as a separate template. See “Save as new template” on page 27.

- To calculate the volume of cell sample and buffer needed to reach a desired concentration based on the count results, press **Calculators** to open the Calculator applications. See Chapter 6, “Calculators”.
- To permanently save the results, press **Save**. See Chapter 7, “Save results”.
- To perform a new count, push the slide to eject, then insert a new sample slide.

View results

Results screen for brightfield

The Results screen for cell count and cell viability assays performed using the brightfield channel displays a composite image of the objects counted and the results of the cell count and cell viability calculations (total concentration, percentage and concentration of live and dead cells).



Note: When performing cell counts and cell viability assays in brightfield, the counting algorithm assumes that you have diluted your cells 1:1 in trypan blue and takes this dilution into account when calculating the total cell concentration. The cell concentration displayed in the Results screen is the original cell concentration before dilution into trypan blue.



Identify objects counted

Results screen

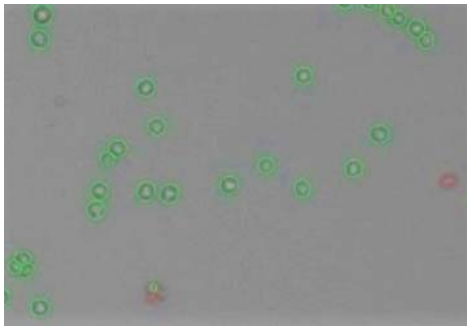
The **Results** screen allows you to identify the objects (i.e., cells) counted in each channel and included in the count results for further review. After reviewing the marked objects, you can adjust the threshold for size, brightness, and/or circularity as desired for your application. See “Gate count results” on page 27.

Identify cells counted in cell count and cell viability assays


On the Results screen, Live or Dead cells can be selected.

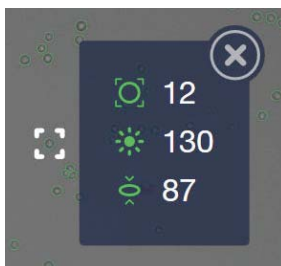
1. To view Live cells, press  on the right panel. This marks all Live cells in green.
2. To view Dead cells, press  on the right panel. This marks all Dead cells in red.

Note: You may select either or both options. The detailed image below shows both Live (green) and Dead (red) cells are selected.



3. To unmark the cells, press the appropriate colored button again.

Individual cells can be selected to view their specific size, brightness, and circularity gating settings. Zooming in on the image will allow for easier selection of individual objects. Click on the desired cell and a pop-up displays the parameters. Press  to close the pop-up.




Histogram count results







View histogram

For cell count and cell viability assays performed in the brightfield channel, you can view the distribution of cells (live and/or dead) based on size in a graphical format.

Note: You can view the histogram on the **Results** and **Gating** screens.

1. To view the histogram showing the distribution of live and/or dead cells based on cell size, press  **Histogram**.




2. To view the distribution of only the live or dead cells, press the corresponding  **Live** (green) or **Dead** (red) button. The graph will automatically update and display the distribution of cells based on size only in the selected population.
3. (Optional): Press **Gating**. Using the  **Size**,  **Brightness**, and  **Circularity** sliders, adjust the count parameters. As you adjust the count parameters, the count results and the graph will be automatically updated.
4. To close the histogram, press  **Histogram** or .

Gate count results

Gating screen

The **Gating** screen for cell count and cell viability assays in the brightfield channel contains the controls for gating results based on size, brightness, and circularity. You can adjust the count parameters before or after performing a count, and save these changes to the current profile or as a separate profile (“Save as new template” on page 27).

Gate count results

1. On the Results screen, press **Gating** in the bottom right corner to open the **Gating** screen.
2. (Optional) Press  **Histogram** to view the distribution of cells (live and/or dead) based on size as you gate the count results. See “Gate count results” on page 27.
3. Select the channel (**Live** or **Dead**) you wish to gate.
4. Select the count parameter (size, brightness, or circularity) to be changed.
5. Using the start and end gate sliders, adjust the count parameters.

Note: For a description of the count parameters and count parameter controls (i.e., parameter sliders), see “Count parameters” on page 13.

6. When finished, press **Apply** to set the changes to the parameters and return to the **Results** screen.
7. To save the parameter changes to the current template or to create a template with the new parameters, see “Create a template” on page 14.

Save as new template



Edit and save a new template

If you have made changes to the count parameters after performing a count, you may save your settings to a new template.

1. Press **Templates**.
2. To create a new template with the edited parameters, press **Create**. The new template screen opens and displays the edited count parameters from the **Gating** screen.
3. To update an existing template with the edited parameters, select a template from the list, then press **Actions** ▶ **Edit**.

Note: You cannot edit the Default template.

The **Edit Template** screen opens. To import the current settings from the **Gating** screen, press **Import settings**.

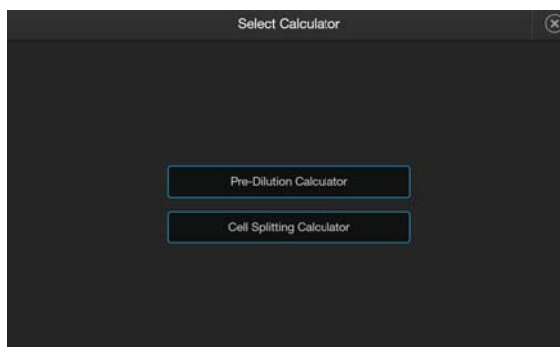
4. To change the name of the selected template, press the **Template name** text box and enter the desired name using the alphanumeric keypad.
5. (Optional) If desired, make additional changes to the template and the count parameters.
6. Press **Save** to save the new template settings and return to the **Templates** screen. Press **Load** to apply the template to your current and future counts OR press **Cancel** or  **Back** to return to the **Templates** screen, then **Cancel** or  **Back** again to return to the **Results** screen without saving the changes to the template.

Pre-Dilution Calculator

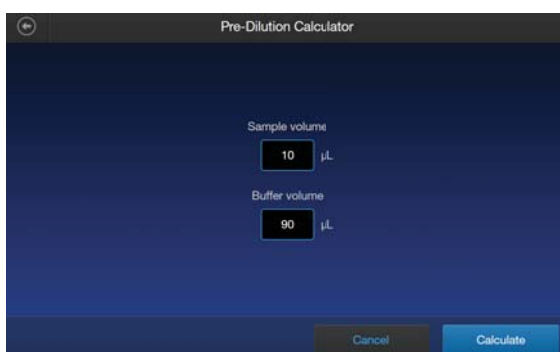
The **Pre-Dilution Calculator** is used to account for diluting a very concentrated sample before loading it into a sample slide for counting.

For a brightfield count, this calculator also has an option to account for trypan blue use.

1. On the **Results** screen, press **Calculators** then select **Pre-Dilution Calculator**.



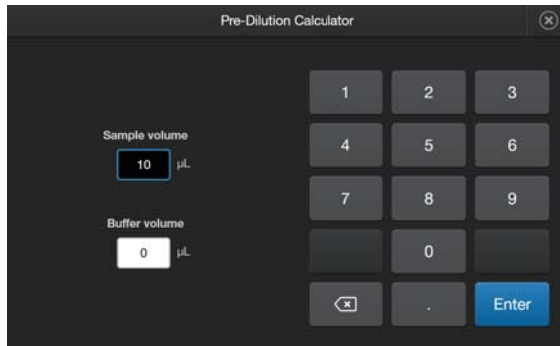
2. Click in one of the text fields.



Note: For BF (brightfield) counts using trypan blue, you can select the checkbox at the bottom of the screen to correct for a 1:1 trypan blue dilution.



- Using the keypad, enter the appropriate volumes and concentrations needed for your experiment. Press **Enter** after filling in both text boxes.



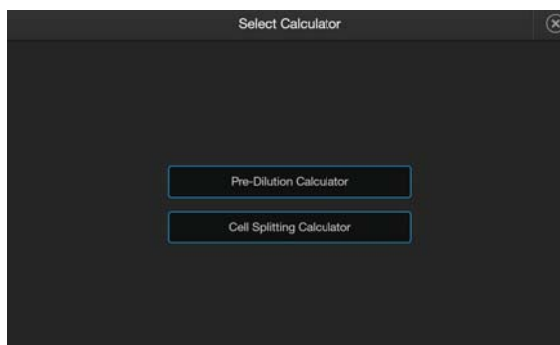
- Press **Calculate**. The result will appear at the top of the **Results** screen.



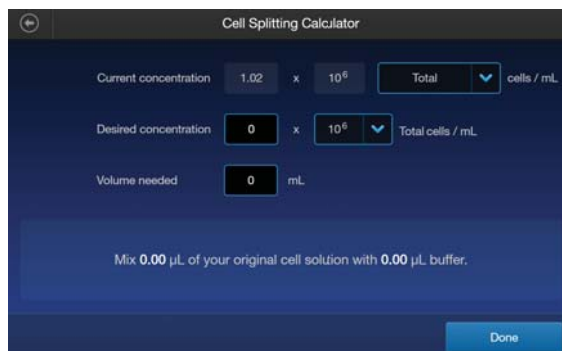
Cell Splitting Calculator

The **Cell Splitting Calculator** is used to determine the amounts of sample and buffer needed to reach a desired concentration.

- On the **Results** screen, press **Calculators** then select **Cell Splitting Calculator**.

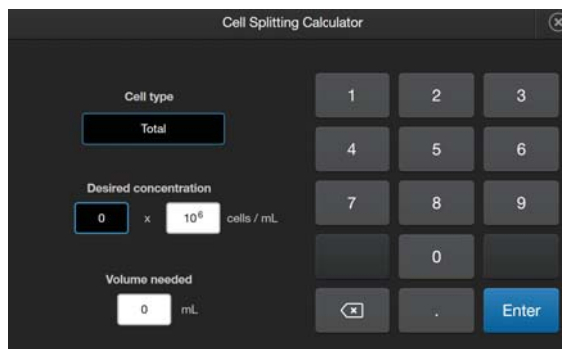


2. Click in one of the text fields.



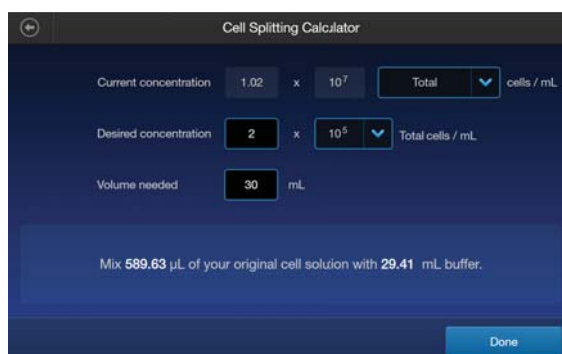
The screenshot shows the 'Cell Splitting Calculator' interface. It has three input fields: 'Current concentration' with a value of 1.02 x 10⁶ cells/mL, 'Desired concentration' with a value of 0 x 10⁶ cells/mL, and 'Volume needed' with a value of 0 mL. Below these fields, a blue box contains the instruction: 'Mix 0.00 µL of your original cell solution with 0.00 µL buffer.' A 'Done' button is located at the bottom right.

3. Using the keypad, enter the appropriate volumes and concentrations needed for your experiment. Press **Enter** after filling in the text boxes.



The screenshot shows the keypad interface of the 'Cell Splitting Calculator'. It features a numeric keypad with buttons for digits 1-9, 0, a decimal point, and an 'Enter' button. The 'Cell type' dropdown is set to 'Total'. The 'Desired concentration' field shows '0' x 10⁶ cells/mL. The 'Volume needed' field shows '0' mL.

4. The calculation will automatically update below the text fields. When completed, press **Done**.



The screenshot shows the 'Cell Splitting Calculator' interface with updated values. The 'Current concentration' is 1.02 x 10⁷ cells/mL, the 'Desired concentration' is 2 x 10⁵ cells/mL, and the 'Volume needed' is 30 mL. The blue instruction box now reads: 'Mix 589.63 µL of your original cell solution with 29.41 mL buffer.' The 'Done' button remains at the bottom right.



Save results

Save screen

The Countess™ 3 and 3 FL cell counters allow you to save your data and images using a USB flash drive or link to a Thermo Fisher Connect account.

To save your experiment, choose from the following options, in any combination:

- **Result:** Saves the **Results** screen as it is displayed on the instrument, with or without the graph, in the selected image format (JPEG, PNG, or TIFF).
- **Images:** Saves the raw captured image as well as underlying channel images in the selected image format (JPEG, PNG, or TIFF).
- **Report:** Saves a printer-friendly report of the results, graph(s), and image in PDF. For more information, see “Report file” on page 34.
- **CSV:** Saves the data from current and previous counts as a CSV file (comma separated values). The CSV format allows for processing or re-displaying results with any third party software or spreadsheet program. For more information on the CSV file format, see Appendix E: CSV file format.

Note: A dropdown list will provide options for how many counts to save. You can choose from **Current Capture**, **Session**, **Today**, **Yesterday**, **Past 7 days**, **Past 30 days**, or **All**.

- **FCS:** Saves the data from the experiment as a Flow Cytometry Data File Standard. The FCS format allows for processing or re-displaying results with FlowJo™ or flowCore commonly used tools for flow cytometry.

Note: If you wish to save your results with the graph showing the distribution of cells based on cell size or fluorescence intensity, make sure that the desired graph is displayed on the **Results** screen.

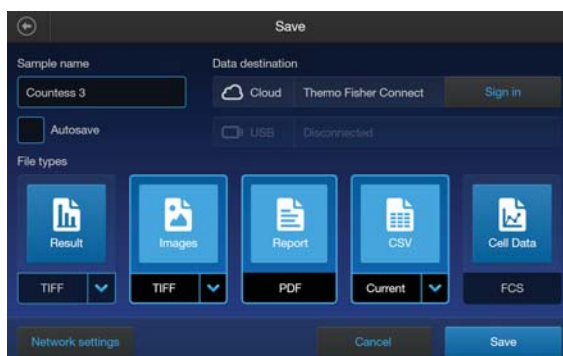
Save procedure

1. To save your data, insert the Countess™ USB drive (or equivalent) into an available USB port on the instrument or confirm the instrument is connected to a Thermo Fisher Connect account.

Note: If the instrument is not connected to a network, then use **Network settings** at the bottom of the screen to reconnect.

Note: The USB ports located in the front and the back of the instrument function the same. However, the first USB drive connected will be the preferred saving location.

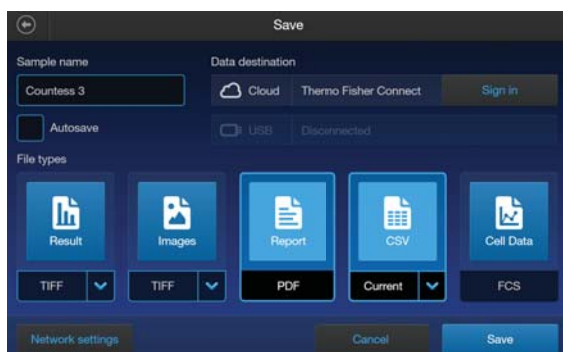
2. On the **Results** screen, press **Save** to view the **Save** screen.



3. To assign a name to your count, press the **Sample name** text field and enter the name. To enter symbols, press the **symbol (@%&)** key. To return to the keypad, press **ABC**.




4. Press **Enter** to save the name and return to the **Save** screen. To return to the **Save** screen without saving the name, press **Close**.
5. Select the desired file type to save your experiment (**Result**, **Images**, **CSV**, **Report**, **Cell Data**). You can select an individual mode (e.g., **Result only**) or any combination of modes (e.g., **Result**, **Images**, **Data**, and/or **Report**). In the example below, **Report** and **CSV** are selected.



6. By default, **Result** and **Images** are saved as TIFF files, and **CSV** uses the current capture data.

7. To choose a different file format, press the desired file type. Available options are **JPEG**, **PNG**, and **TIFF**. For CSV files, you can choose from the selections of **Current Capture**, **Session**, **Yesterday**, **Past 3 days**, **Past 7 days**, or **All**.

After you make your selection, the instrument returns to the **Save** screen.

To return to the **Save** screen without changing the file format, press  **Back**.

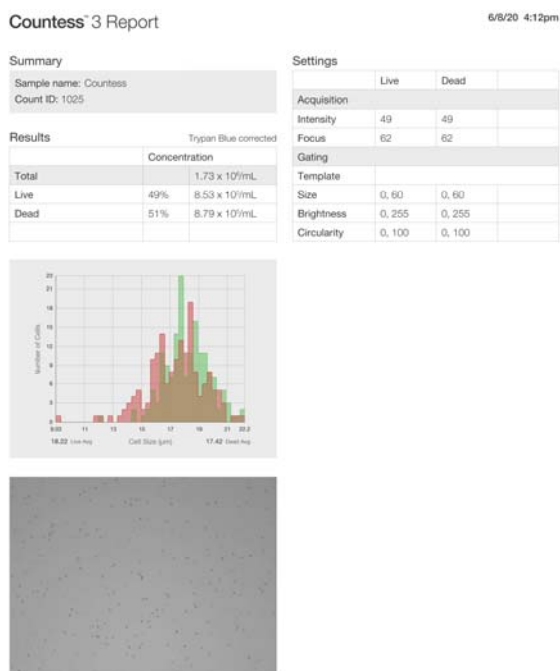
8. Press **Save** to save your experiment to the selected mode(s) in the USB drive or Connect account.
9. Press **Done** and then transfer the USB drive to the desired location.

Report

Report file

The **Report** function allows you to save a printer-friendly report of the results, graphs, and images in PDF format.

Report from brightfield count



Countess³
Automated Cell Counter

invitrogen
by Thermo Fisher Scientific

- The top section of the report contains a table with the results as displayed on the **Results** screen, showing the concentration of the sample and the percentage and number for the total, Live, and Dead channels.
- Below the results table, the report contains the “number of cells vs. cell size” graph
- Under the graph, the report contains the brightfield count image, with the live and dead cells identified by the green and red circles, respectively.
- At the top, the report displays the template information used to gate these images.



Settings overview

Settings screen

To access the **Settings** screen, press  **Settings** on the **Home** page.

Settings allows you to perform various functions:

- Perform software updates (see “Update the Countess™ 3/3 FL Cell Counter software” on page 36)
- Set the date and time (see “Set the date and time” on page 36)

Connect to a wireless network

| Network connecting options | |
|--|--|
| <ol style="list-style-type: none">1. From the Home screen, press Settings.2. Press Instrument settings.3. Press Network settings. A list of available networks will display.4. Select the desired network and press Connect. | <ol style="list-style-type: none">1. From the Home screen, press Save options.2. Press Network settings.3. Select the desired network and press Connect. |
| Follow either set of steps to also disconnect the current network and connect to a new one. | |

Software update

Guidelines for software update

- Software updates can be done via your Connect account. When connected to the internet, the system will automatically check for and download software updates to the instrument.

Note: Using the Cloud will require a Wi-Fi dongle be installed in the instrument.

- The USB drive used for transferring the software update file must be FAT32 formatted; verify this before proceeding. If necessary, reformat the USB drive to FAT32 following the recommended procedure for your operating system.

Note: Reformatting the USB drive will result in the loss of all files. Back up the files in the USB drive prior to reformatting.

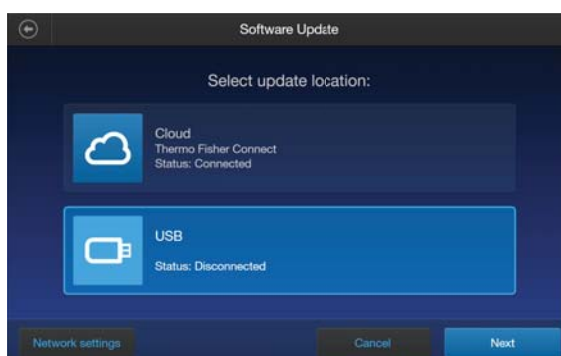
- The software update file must be saved on the top level of the USB drive, not within a folder or a subfolder.
- The software update file must be uncorrupted during transfer. Do not rename, zip, or compress the software update file.

Update the Countess™ 3/3 FL Cell Counter software

1. Go to www.thermofisher.com/countessupdate, and download the latest Countess™ 3/3 FL cell counter software version to your desktop.

Note: The software update file has a version-specific name followed by the extension .exe (e.g., Countess_xxxx for software version 2.0.202).

2. Copy the software update file onto the USB drive, making sure that it is saved on the top level and not hidden within a folder.
3. Insert the USB drive into one of the USB ports of the instrument.
4. Press **Settings** on the **Home** page to open the **Settings** screen.
5. Press **Software Update**.



6. Select the update location. Press **Next**.
If the **Cloud** option is disconnected, press **Network settings** to proceed to reconnect to your network.

Note: Using the Cloud will require a Wi-Fi dongle be installed in the instrument.

7. If an update is available, press **Update**. Once the update has completed, the **Home** screen will appear to resume normal operation.

Set the date and time

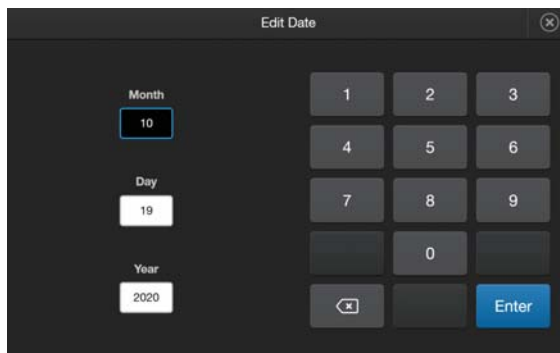
1. Press **Settings** on the **Home** page.
2. Press **Instrument Settings**.

3. Press **Date and time**.



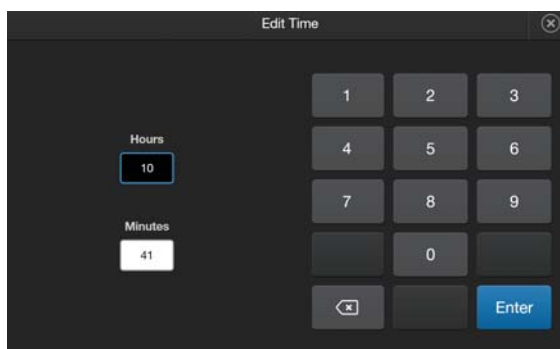
4. Select the date format (**Month/Day/Year**, **Day/Month/Year**, or **Year/Month/Day**).

5. Press any **Date** text box (**Month**, **Day**, or **Year**) to open the **Edit Date** keypad.



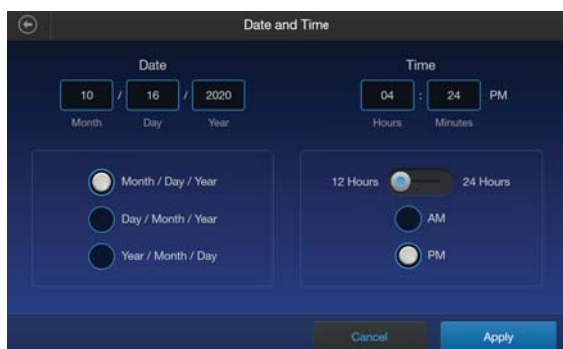
6. Using the keypad, enter the date into the **Month**, **Day**, and **Year** text boxes. Press **Enter**.

7. Press any **Time** text box (**Hours** or **Minutes**) to open the **Edit Time** keypad.



8. Using the keypad, enter the time into the **Hours** and **Minutes** text boxes.

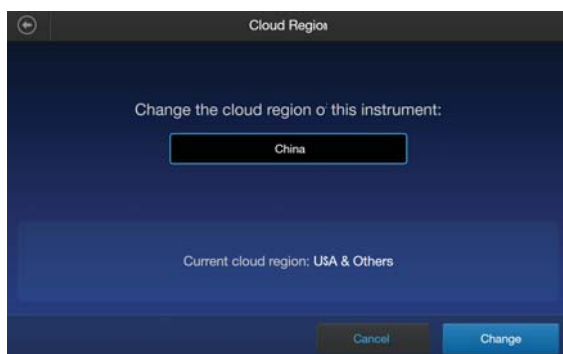
9. Select the time format you wish to use (**12 Hours** with **AM** or **PM** or **24 Hours**).



10. Press **Apply** to set the Time and Date and return to the **Instrument Settings** screen. Press **Cancel** or **Back** to return to the **Instrument Settings** screen without saving your changes.

Change your cloud region

1. Press **Settings** on the **Home** page.
2. Press **Instrument Settings**.
3. Press **Cloud region**. Your current cloud region is displayed at the bottom of the screen.



4. Select the desired cloud region from the available choices. Press **Change**.
5. Once the change is complete, press **Done**.

Note: Changing the cloud region will require relinking of all instrument accounts.



Maintenance

Instrument care

General guidelines for care

- Use the appropriate cleaning solutions for each component, as indicated in the cleaning procedures in “Clean the cell counter” on page 40.
- If liquid spills on the instrument, turn off the power immediately and wipe dry.

Power supply

Always use the correct power supply. The power adapter specifications appear on the serial number label (bottom of the instrument) and in the "Technical specifications" section of this user guide (“Technical specifications” on page 45). Damage due to an incompatible power adapter is not covered by warranty.



CAUTION! Never disassemble or service the instrument yourself. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts. Operators must be trained before being allowed to perform the hazardous operation. Unauthorized repairs may damage the instrument or alter its functionality, which may void your warranty. Contact your local distributor to arrange for service.

IMPORTANT! If you have any doubt about the compatibility of decontamination or cleaning agents with parts of the equipment or with material contained in it, contact Technical Support or your local distributor for information.

Clean the cell counter

Introduction

Clean the Countess™ Automated Cell Counter periodically to prevent buildup of dust and dirt that might reduce its performance and cause contamination.



CAUTION! To avoid electrical shock, always turn OFF the Countess™ Automated Cell Counter and unplug the power cord before cleaning or decontaminating the instrument.



CAUTION! All biological samples and materials that come into contact with them have the potential to transmit infectious diseases and are considered biohazardous. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves.

IMPORTANT! Using a cleaning or decontaminating method other than that specified by the manufacturer may result in damage to the instrument.

Clean the touchscreen

- Wipe the touchscreen of the Countess™ Automated Cell Counter using a soft, lint-free cloth moistened with an LCD cleaning solution. Do not apply excessive force during cleaning. Wipe the touchscreen dry immediately after cleaning.
- Ensure that the cleaning solution does not enter the power button, the power inlet, the slide port, or the USB ports.
- Never pour or spray any liquids directly on the instrument to avoid electrical shock when the instrument is plugged in.
- Do not use abrasive cleaning solutions or material to prevent the touch-screen from getting scratched.

Clean the instrument case

- Wipe the instrument case of the Countess™ Automated Cell Counter using a soft, lint-free cloth moistened with distilled water. Wipe the instrument dry immediately after cleaning.
- Ensure that water or other cleaning solutions do not enter the power button, the power inlet, the slide port, or the USB ports.
- Never pour or spray any liquids directly on the instrument to avoid electrical shock when the instrument is plugged in.

Decontaminate the instrument

- Wipe the instrument case of the Countess™ Automated Cell Counter using a soft, lint-free cloth moistened with 70% alcohol. Wipe the instrument dry immediately after cleaning.
- Avoid using a bleach solution, because it may leave a residue of bleach crystals on the instrument.
- Ensure that water or other cleaning solutions do not enter the power button, the power inlet, the slide port, or the USB ports.
- Never pour or spray any liquids directly on the instrument to avoid electrical shock when the instrument is plugged in.



Troubleshooting

| Observation | Possible cause | Recommended action |
|--|--|--|
| Abnormally high percentage of dead cells or live cells counted as dead | Focus was not correct for the sample. | Ensure that the cells are focused correctly so that live cells have bright centers and dead cells are dark throughout. If the cells are not well focused and look dark on the screen, the Countess™ 3 FL cell counter will count them as dead cells. |
| | Live cells vs dead cells were difficult to distinguish. | If cells are well focused, have bright centers, and are being counted as dead, confirm that they are within the appropriate cell size range and try adjusting the settings. |
| | Cells were exposed to trypan blue for too long. | If cells are exposed to trypan blue for a long period of time, viability could be affected so slide should be prepared and counted fresh each time for best results. |
| | Capture settings were not optimal. | Gate out the debris using the size, brightness, and circularity sliders. |
| Autofocus does not focus properly | Debris or other material interfered with the focus. | Make sure there are no bubbles or debris visible on the screen that could interfere with the autofocus and make it more difficult to get the sample in the correct focal plane. |
| | Live cells vs dead cells were difficult to distinguish. | If cells are well focused, have bright centers, and are being counted as dead, confirm that they are within the appropriate cell size range and try adjusting the settings. |
| Incorrect concentration for the Countess™ test beads | Beads were not suspended in solution or mixed correctly. | The beads can settle quickly in solution, which will affect the concentration reading. |
| | | Vortex the bead stock on high for a full 30 seconds to resuspend, and add 10 µL of the bead suspension to 10 µL of trypan blue without delay. |
| | | Pipet the bead and trypan blue mixture up and down several times to make sure it is well mixed and immediately load 10 µL into the slide. |

| Observation | Possible cause | Recommended action |
|--|--|--|
| Some cells appear in the image but are not included in the count | Brightfield or fluorescence settings were not optimal. | For cell count and cell viability assays performed in brightfield, adjust the size, brightness, and circularity gates for both live and dead cells to include all of the cells in the count. |
| | | For fluorescence assays, adjust the size, brightness, circularity, and fluorescence intensity gates in all available channels to include all of the cells in the count. |
| | | After including all of the cells in the count, you can narrow the count criteria, if you wish to exclude cells of a certain size or certain brightness. |
| | | When the gates are fully maximized, the CSV should indicate 0–70 for cell size and 0–255 for brightness. |
| | | Be sure channels are optimally illuminated in both brightfield and fluorescence (only in Countess™ 3 FL Automated Cell Counter) modes. |
| Unable to update the Countess™ software | USB drive did not function properly. | Make sure the USB drive is formatted to FAT32. If it is not, reformat the USB drive to FAT32 before transferring the files onto the USB drive for software update. |
| | | Make sure the update file is at the top (root) of the USB drive, not in a folder or subfolder. |
| | | File cannot be renamed in any way. |
| | | File cannot be zipped or compressed during distribution. It must be uncorrupted during transfer and have a .lft suffix. |
| | | If needed, check that the USB port is functional by testing a USB mouse. |
| USB drive not recognized by the instrument | USB drive was not correctly formatted. | The USB drive must be FAT32 formatted to be recognized by the instrument. If it is not, reformat the USB drive to FAT32. |
| | | Try another correctly formatted USB drive. |
| Variable counts for the same sample of cells | Pipetting was done incorrectly. | If you are pipetting different samples from the same cell sample, the variability could be due to pipetting or mixing. |
| | | Use recently calibrated pipettors and make sure that the cells are well suspended by pipetting up and down several times before adding trypan blue. |



| Observation | Possible cause | Recommended action |
|--|--|--|
| Variable counts when performing replicate counts of the same slide | Slide was mishandled or viewed differently multiple times. | If you are counting replicates of the exact same slide, visually inspect that all cells are counted correctly in the image. |
| | | There may be a slightly different field of view each time a slide is inserted. Depending on the concentration and uniformity of the cells, this will cause some variability when performing replicate counts of the same slide, although it should be less than 10%. |
| | | When counting fewer cells, a small field of view change for only a small number of cells can have a larger affect. Count cells at a higher concentration to reduce variability |
| | | Make sure you do not shake or agitate the slide between counts. |



Technical specifications

Physical characteristics

| | |
|-----------------------|---|
| Instrument type | Benchtop cell counter and suspension cell-based assay platform |
| Instrument dimensions | 9.6 (W) × 6.7 (D) × 9.4 inches (H) |
| Weight | 8 lbs |
| Operating power | 100–240 VAC, 1.0 A MAX |
| Frequency | 50/60 Hz |
| Electrical input | 12 VDC, 3 A |
| Installation site | Indoor use only, Class A Environments (i.e., non-residential or light industrial); Pollution degree 2. |
| Operating temperature | 10°–40°C (50°–104°F) |
| Operating humidity | <80% (non-condensing) |

Technical specifications

| | |
|------------------------------|---|
| Processing time | <30 seconds |
| Sample concentration range | 1×10^4 – 1×10^7 cells/mL |
| Particle/cell diameter range | 4–60 μ m |
| Required sample volume | 10 μ L |
| Firmware | Countess™ Automated Cell Counting Platform Software |
| USB drive | 32 Gigabytes (FAT32-, exFAT-, or NTFS-compatible) |

Optics

| | |
|---------|---|
| Optics: | 1 channel (brightfield) |
| Camera: | 5 Mega pixels, 2.5× Optical Magnification |



Analysis slide

| | |
|----------------|------------------------------------|
| Material | Poly(methyl methacrylate) (PMMA) |
| Dimensions | 25 mm (W) × 75 mm (D) × 1.7 mm (H) |
| Chamber volume | 10 μ L |



Countess™ 3 Automated Cell Counter and accessories

The following Countess™ 3 instruments and instrument accessories are available from Thermo Fisher Scientific. For more information, visit www.thermofisher.com or contact Technical Support.

| Product | Quantity | Cat. No. |
|---|----------|-----------|
| Countess™ 3 Automated Cell Counter | 1 each | AMQAF1000 |
| Countess™ 3 Power Adapter with four adaptor cords | 1 each | A48207 |
| Countess™ 3 USB Drive | 1 each | A26774 |
| Countess™ 3 FL Light Cube Removal Tool | 1 each | AMEP-4747 |
| Countess™ 3 Disposable Slide Holder | 1 each | AMEP-4745 |
| Countess™ 3 Reusable Slide Holder | 1 each | A48208 |

Accessory products

The following products can be used with the Countess™ 3/3 FL Automated Cell Counters and are available separately from Thermo Fisher Scientific. For more information, visit www.thermofisher.com or contact Technical Support.

| Product | Quantity | Cat. No. |
|---|--------------------------|----------|
| Countess™ Cell Counting Chamber Slides, 50 Slides (100 counts) | 1 box ^[1] | C10228 |
| Countess™ Cell Counting Chamber Slides, 500 Slides (1000 Counts) | 10 boxes ^[1] | C10312 |
| Countess™ Cell Counting Chamber Slides, 1250 Slides (2500 Counts) | 25 boxes ^[1] | C10313 |
| Countess™ Cell Counting Chamber Slides, 2500 Slides (5000 Counts) | 50 boxes ^[1] | C10314 |
| Countess™ Cell Counting Chamber Slides, 5000 Slides (10,000 Counts) | 100 boxes ^[1] | C10315 |
| Countess™ Reusable Slide | 1 each | A25750 |



(continued)

| Product | Quantity | Cat. No. |
|---|----------|----------|
| Countess™ Test Beads (1 × 10 ⁶ beads/mL) | 1 mL | C10284 |
| Trypan blue stain (0.4 %) | 2 × 1 mL | T10282 |

[1] Each box of Countess™ Cell Counting Chamber Slides contains 50 slides and 2 × 1 mL vials of trypan blue (0.4%), sufficient for 100 counts.



CSV file format definition

Overview

A comma-separated values (CSV) file stores tabular data (numbers and text) in plain-text form. Plain text means that the file is a sequence of characters, with no data that has to be interpreted as binary numbers. A CSV file can be opened with any third party software or spreadsheet program. The table below describes the categories of the Countess™ 3 data saved as a CSV file and opened with a spreadsheet program.

| Category | Column | Name | Description |
|-------------------------|------------------------|---|---|
| General | A | Count ID | ID number for current count |
| | B | Session ID | ID number for current session |
| | C | Sample name | Name of sample |
| | D | Date & Time | Date and time of sample run |
| | E | Type | BF-Brightfield or FL-Fluorescence |
| Trypan Blue/Brightfield | F | Trypan blue corrected | Sample with trypan blue correction applied |
| | G | Pre-Dilution corrected | Sample with pre-dilution correction applied |
| | H | Total concentration | Concentration of the entire sample |
| | I | Total cells counted | Total number of cells counted in the sample |
| | J | Live concentration | Concentration of just the "live" portion of the sample |
| | K | Live cells counted | "Live" cells counted in sample |
| | L | Dead concentration | Concentration of just the "dead" portion of the sample |
| | M | Dead cells counted | "Dead" cells counted in sample |
| | N | Viability (%) | Percent viability of the sample based on trypan blue staining |
| | O | Live average size (µm) | Average size of "live" cells in microns |
| P | Dead average size (µm) | Average size of "dead" cells in microns | |
| General | AD | Focus value | Focal position number |
| | AE | Focus motor value | Motor position in relation to focal position number |

(continued)

| Category | Column | Name | Description |
|----------------------------|--------|----------------------|---|
| Trypan Blue/Brightfield | AF | BF light intensity | Brightfield light intensity value from 0-100% |
| | AG | BF LED intensity | LED intensity in brightfield mode |
| | AH | Live size min | Minimum size of "live" cells in microns |
| | AI | Live size max | Maximum size of "live" cells in microns |
| | AJ | Live brightness min | "Live" adjustment slider value for minimum brightness |
| | AK | Live brightness max | "Live" adjustment slider value for maximum brightness |
| | AL | Live circularity min | "Live" adjustment slider value for minimum circularity |
| | AM | Live circularity max | "Live" adjustment slider value for maximum circularity |
| | AN | Dead size min | Minimum size of "dead" cells in microns |
| | AO | Dead size max | Maximum size of "dead" cells in microns |
| | AP | Dead brightness min | "Dead" adjustment slider value for minimum brightness |
| | AQ | Dead brightness max | "Dead" adjustment slider value for maximum brightness |
| | AR | Dead circularity min | "Dead" adjustment slider value for minimum circularity |
| | AS | Dead circularity max | "Dead" adjustment slider value for maximum circularity |
| Trypan Blue/Brightfield | AX | BF size min | Minimum size of "brightfield" cells in microns |
| | AY | BF size max | Maximum size of "brightfield" cells in microns |
| | AZ | BF brightness min | "Brightfield" adjustment slider value for minimum brightness |
| | BA | BF brightness max | "Brightfield" adjustment slider value for maximum brightness |
| | BB | BF circularity min | "Brightfield" adjustment slider value for minimum circularity |
| | BC | BF circularity max | "Brightfield" adjustment slider value for maximum circularity |
| General | BH | Template name | Template used for count |
| | BI | Software revision | Current software version used for count |



Safety



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, see the “Documentation and Support” section in this document.

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
<https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf>
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf



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 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

