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Countess™ 3 FL Automated Cell Counter

USER GUIDE

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A.0	09 November 2020	New user guide.

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1 Product information

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

Contents

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

Component	Quantity
Countess™ 3 FL 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 FL Disposable Slide Holder	1 each
Countess™ 3 FL Reusable Slide Holder	1 each
Countess™ 3 FL Light Cube Removal Tool	1 each
Countess™ 3 USB Drive	1 each
Countess™ 3 FL Automated Cell Counter Quick Reference Card	1 each
Wi-Fi Dongle	1 each

Product description

The Countess™ 3 FL Automated Cell Counter is a fully automated, 3-channel cell counter and assay platform that uses EVOS™ light cube technology, state-of-the-art optics, and image analysis algorithms to analyze fluorescently labeled cells or trypan blue stained samples in suspension.

- The Countess™ 3 FL 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 either in disposable Countess™ Cell Counting Chamber Slides or in glass Countess™ 3 FL Reusable Slides (page 19) . Each chamber slide contains two enclosed chambers to hold the sample to allow you to measure 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 FL Automated Cell Counter also provides information on cell size.
- In addition to the brightfield channel, the Countess™ 3 FL Automated Cell Counter can accommodate two interchangeable EVOS™ fluorescent light cubes (page 64) , enabling it to be used for multiple-fluorescence research applications.
- When equipped with EVOS™ light cubes, the Countess™ 3 FL Automated Cell Counter can be used to perform fluorescence assays for cells in suspension, including simultaneous counts of cells stained with two different fluorescent dyes, GFP and RFP expression, apoptosis, and cell viability (live, dead, and total cells).

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 10 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 FL 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 the USB drive supplied with the instrument or any other 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.
The Countess™ 3 FL Automated Cell Counter accepts both the disposable Countess™ Cell Counting Chamber Slides and the glass Countess™ 3 FL Reusable Slides via interchangeable, slide-specific carriers. For more information, see Chapter 4, “Slide preparation and operation”.

④ **Back panel**: Allows access to the optional EVOS™ light cubes and provides storage for the light cube tool and the reusable slide carrier. The back panel is secured to the instrument by two captive ¼-turn fasteners.

⑤ **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.

⑥ **EVOS™ light cubes**: Allow the Countess™ 3 FL Automated Cell Counter to analyze fluorescently labeled samples. Can accommodate two fluorescent light cubes. For more information, see “EVOS™ light cubes” on page 62.

⑦ **Rear USB port**: An additional USB port to perform functions such as plugging in a USB mouse for instrument control.

⑧ **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 48). 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 9.
   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 protocol in step 3 below or change instrument settings in step 4 below.

3. To change the current protocol or add a new protocol to the instrument, press **Protocols** .

   Protocols allows you to create customized count preferences (i.e., gate counts based on cell size, brightness, circularity and/or fluorescence intensity). See “Load a protocol” on page 16.

4. To change instrument settings, press **Settings** .

   Settings allows you to update the Countess™ 3 FL instrument software, customize instrument settings, view instrument details, export error logs, and change up to two EVOS™ light cubes. See “Settings screen” on page 46.

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 Protocols

Protocols screen

Protocols allows you to create and save customized protocols. Each custom protocol defines the count parameters (size, brightness, circularity, and fluorescence intensity) for a consistent and streamlined workflow.

- Protocols can be accessed from the **Home** and **Gating** screens.
- The currently selected protocol is displayed at the bottom of the **Home** screen.
- Automatic instrument functions and count parameters are defined in the **Edit protocol** screen. See "Create a protocol" on page 16 or "Edit a protocol" on page 16.
- The Default protocol contains default count settings and cannot be edited.
- The count parameters specified in the selected protocol are applied to all new cell counts.
- Protocols can be sorted by pressing on the **Name**, **Channels**, or **Last Modified** headers.
- If you have already performed a count, loading a new protocol from the **Results** screen applies the count preferences to the current count results (total cells, viability, etc.) and to all new counts.

Protocol functions

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

Count parameters

Count parameters are adjusted in the **Edit Protocol** 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 using the Countess™ 3 FL instrument, select the individual channels.

- **Setup** – Press **Setup** to select the required channels (e.g., BF, GFP, TX Red) for capture. BF (brightfield) is checked by default and cannot be deselected.
- **Calculators** – Press **Calculators** to access the Pre-Dilution and Cell Splitting Calculators and store default calculator values. For detailed protocols, see Chapter 7, “Calculators”.
- **Save** – Press **Save** to save default save settings and your data to the cloud or a USB. For details, see “Save screen” on page 41.

Load a protocol

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

Create a protocol

1. Press **Protocols**.
2. Press **Create**. To assign a name to the new protocol or to change the name of the existing protocol, press the Protocol name text box to open the keypad.

3. Type in the desired protocol 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 Protocol** screen. Press ✕ to return to the **New Protocol** screen without saving the name.
5. Press **Save** to save the new protocol. Press **Cancel** or **Back** to return to the **Protocols** screen without saving.

Edit a protocol

1. Press **Protocols**.
2. Select an existing protocol, then press **Actions**.

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

3. Press **Edit**.

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

4. Press the appropriate channel (BF or other dye) and edit the desired parameters to create a custom protocol. Additionally, use the **Setup**, **Calculators**, and **Save** buttons to set up any of the needed parameters to complete the custom protocol. For details, see “Count parameters” on page 15.

5. Press **Save**.

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

Import a protocol

1. Press **Protocols**.

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 protocols from the list.
7. Press **Import**.
8. Once complete, press **Done**.

Export a protocol

1. Press **Protocols**.
2. Press **Actions**.
3. Press **Export**.
4. Select one or more protocols 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**.

Delete a protocol

1. Press **Protocols**.

2. Press **Actions**.

3. Press **Delete**.

4. Select one or more protocols from the list.

5. Press **Delete**.
   The system will prompt you to confirm the deletion, then press **Delete** again.

6. 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 µL of your cell suspension to 10 µL 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 µL 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. Prepare the sample by adding 10 µL of your cell suspension to 10 µL of 0.4% trypan blue stain (for brightfield only). Mix the sample mixture well by pipetting it up and down a few times. For a fluorescence sample, only add 10 µL of your sample neat.

3. Gently pipet 10 µL 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™ 3 FL Reusable Slide has a 10-µL sample capacity. Do not overfill the slide chambers.

4. To use the Countess™ Reusable Slide, unlatch the back panel of the Countess™ 3 FL Automated Cell Counter with the two captive ¼-turn fasteners that secure the back panel on the rear of the instrument.

5. Remove the reusable slide holder from inside of the back panel.

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

7. Insert the reusable slide holder containing the slide into the slide port and gently push into the instrument until it clicks into place.

8. Use the touchscreen to run the sample count. See Chapter 5, “Cell count and cell viability assays”.

9. 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.

10. *(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.

11. 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.

12. Store the reusable slide holder again in the panel on the back of the instrument.

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 **Protocols** and load the desired gating parameter settings as described in “Load a protocol” on page 16.
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.

Note: For the Countess™ 3 FL Automated Cell Counter equipped with an EVOS™ light cube, first press **Adjust**, and then select **BF** (brightfield) as the light source. Set the exposure, then press **Apply** to return to the **Setup** screen.

6. Press **Count**.

Note: If your instrument is equipped with an EVOS™ light cube, make sure that only the **BF** (brightfield) checkbox is selected before capturing the image.

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 26.

Autosaving will occur when the count is completed if you have **Autosave** enabled from the **Save Options** screen.

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 27.

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 28.
- To gate the results by object size, brightness, or circularity, press **Gating** to go to the **Gating** screen. See "Gate count results" on page 28.

Note: You can save the changes you make to the size, brightness, or circularity parameters in the **Gating** screen to the current protocol or as a separate protocol. See "Save as new protocol" on page 29.

- 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 7, "Calculators".
- To permanently save the results, press **Save**. See Chapter 8, "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 29.

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 protocol” on page 29).

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 29.
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 15.

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 protocol or to create a protocol with the new parameters, see “Create a protocol” on page 16.

Save as new protocol

Edit and save a new protocol

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

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

   Note: You cannot edit the Default protocol.

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

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

6 Fluorescence assays

Count cell fluorescence

Overview

Countess™ 3 FL Automated Cell Counter equipped with the optional EVOS™ light cubes can be used for a variety of fluorescent applications, including simultaneous counts of cells stained with two different fluorescent dyes, GFP and RFP expression, and apoptosis and cell viability assays.

For instructions on installing EVOS™ light cubes to your Countess™ 3 FL Automated Cell Counter, see "Install or change an EVOS™ light cube" on page 52.

Count procedure

1. Ensure that your fluorescent cell sample is homogeneously mixed.
2. Load 10 µL of the fluorescent sample mixture per chamber into the sample slide as described in "Load Countess™ Cell Counting Chamber Slide" on page 20. Let the sample mixture settle for 30 seconds.
3. *(Optional)*: Press **Protocols** to open the **Protocols** screen and load the desired gating parameter as described in "Load a protocol" on page 16.
4. Insert the sample slide into the slide port ("Exterior instrument parts" on page 9), 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.
5. 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.

6. To view your sample under a different light source, press the desired light source button (**BF** (brightfield) or a source provided from an installed light cube (e.g., **GFP**, **DAPI**, **TX Red**, etc.)). The instrument displays the sample in the selected channel (brightfield or fluorescent).

   In the example below, the sample is displayed in the DAPI channel.

Note: The light source buttons select the light channel (brightfield and/or fluorescence) for sample illumination and are used when setting the exposure for the selected channel (Steps 9-11); they do not determine which channels are used for capturing the image.

7. To set exposure, press **Adjust** to go to the **Adjust** screen.

8. Press the light source button for the channel you wish to set exposure and adjust the exposure using the light source slider. Repeat the procedure for the remaining channels, if desired.

9. Press **Apply** to keep the current setting and return to the **Setup** screen OR press **Cancel** or ⊕ **Back** to return to the **Setup** screen without saving changes .

10. On the **Setup** screen, select the check boxes to the right of the channels you wish to capture. **BF** (brightfield) is checked by default and cannot be deselected.

11. Press **Count**.

    The instrument captures the images and displays the results (total concentration, percentage and concentration of cells counted in each fluorescence channel). For more information, see “View results” on page 33.

    Autosaving will occur when the count is completed if you have **Autosave** enabled from the **Save Options** screen.

Next steps

- To identify the brightfield objects (i.e., cells), press ◎. For identifying fluorescence objects, press the appropriate colored icon for the specific EVOS™ light cube (e.g., press ◉ for GFP, press ◉ for TX Red).
- To see the distribution of cells counted through each channel in a graphical format, press **Histogram**. See "View histogram for cell fluorescence assays" on page 34.
- To gate the results by object size, brightness, circularity, or relative fluorescence intensity, press **Gating**. See "Gating screen" on page 36.

  Note: Changes made in the current or a separate template to size, brightness, circularity, and relative fluorescence intensity can be saved directly from the **Gating** screen.

- 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 application. See Chapter 7, "Calculators".
- To permanently save the results, press **Save**. See "Save screen" on page 41.
- To perform a new count, push the slide to eject, then insert a new sample slide.

View results

Results screen for cell fluorescence assays

The **Results** screen for cell fluorescence assays 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 cells counted through each fluorescence channel).

Note: The total cell concentration displayed after a fluorescent count does not take any dilution into account. Therefore, the results reflect the actual cell concentration in the sample slide, which must be multiplied by any dilution factor present to calculate the original cell concentration. See Chapter 7, "Calculators".

This is in contrast to the cell counts in brightfield, where the counting algorithm assumes a 1:1 dilution of the sample in trypan blue and displays the original cell concentration (i.e., before the dilution) in the **Results** screen.

Identify objects counted

Identify cells counted in fluorescence assays

1. On the **Results** screen, select the appropriate icon/s to turn on or off the layer/s viewed.

   For example, select .

2. To identify the cells that are counted in a specific channel, press the corresponding contours icon. Cells counted in the selected channel will be circled on the screen with the same color as the selected channel.

   For example, select .
   In the example below, both the DAPI and TX Red boundary buttons are selected, and the cells counted in the DAPI and TX Red channels are marked with blue and red circles, respectively.

3. To unmark the cells counted in a specific channel, press the corresponding boundaries button again.

Histogram count results

View histogram for cell fluorescence assays

For fluorescence assays, you have the option of viewing the distribution of the cells based on size or based on relative fluorescence intensity 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 cells based on size, press **Histogram** and then select **BF** (brightfield).
   The histogram displays the size distribution of the total cell count (number of cells vs. cell size in µm) and the average size of the cells counted in each available fluorescence channel.

2. To view the distribution of cells based on relative fluorescence intensity, press **Histogram**, then select **FL** (fluorescence).
   The histogram displays the distribution of cells based on fluorescence intensity.

3. To remove the cells counted in a specific channel from the histogram, press the corresponding channel button on the histogram.
   The histogram automatically updates and displays the distribution of cells based on relative fluorescence intensity only in the selected (i.e., checked) channel.

4. To add the cells counted in a specific channel to the histogram, press the corresponding channel button.

5. To close the histogram, press **Histogram** or **Close**.

Gate count results

Gating screen

The **Gating** screen for cell fluorescence assays contains the controls for gating count results based on size, brightness, circularity, and fluorescence intensity. You can adjust the count parameters before or after performing a count, and save these changes to the current protocol or as a separate protocol (“Save as new protocol” on page 29).

Gate count results

1. On the **Results** screen, press **Gating** to open the count parameters screen containing the controls for adjusting the count parameters in the selected channel.

   Note: For a description of the count parameters and count parameter controls (i.e., parameter sliders) see “Count parameters” on page 15.

2. *(Optional)*: Press **Histogram** to view the distribution of cells based on size or fluorescence intensity. See “View results” on page 33.

3. Press **BF** (brightfield) to adjust the parameters for size, brightness, and circularity using the gating sliders.

4. Select the desired fluorescence channel (e.g., DAPI, TX Red, etc.) to adjust the threshold for fluorescence intensity using the fluorescence intensity slider.

   Note: The fluorescence channels available depend on the EVOS™ light cubes installed in the instrument.

5. When finished, press **Apply** to save the changes to count parameters and return to the **Results** screen OR press **Cancel** or **Back** to return to the **Results** screen without saving the changes.

6. To save the parameter changes to the current protocol or to create a protocol using the new count parameter, see “Edit and save a new protocol” on page 37.

Save as new protocol

Edit and save a new protocol

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

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

   Note: You cannot edit the Default protocol.

   The **Edit Protocol** screen opens. To import the current settings from the **Gating** screen, press **Import settings**.
4. To change the name of the selected protocol, press the **Protocol name** text box and enter the desired name using the alphanumeric keypad.
5. *(Optional)* If desired, make additional changes to the protocol and the count parameters.
6. Press **Save** to save the new protocol settings and return to the **Protocols** screen. Press **Load** to apply the protocol to your current and future counts OR press **Cancel** or ⊖ **Back** to return to the **Protocols** screen, then **Cancel** or ⊖ **Back** again to return to the **Results** screen without saving the changes to the protocol.

7 Calculators

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.

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

4. 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.

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

2. Click in one of the text fields.

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

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

8 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 44.
- **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

Countess™ 3 Report

6/8/20 4:12pm

Summary

Sample name: Countess
Count ID: 1025

Results — Trypan Blue corrected

	Concentration	
Total		1.73 x 10⁶/mL
Live	49%	8.53 x 10⁵/mL
Dead	51%	8.79 x 10⁵/mL

Settings

	Live	Dead	
Acquisition			
Intensity	49	49	
Focus	62	62	
Gating			
Protocol			
Size	0, 60	0, 60	
Brightness	0, 255	0, 255	
Circularity	0, 100	0, 100	

Countess™ 3
Automated Cell Counter

invitrogen

- 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.

Report from fluorescence count

Countess™ 3 Report

10/15/20 4:49pm

Summary

Sample name: Countess
Count ID: 1025

Results — Pre-dilution corrected

	Concentration	
Total		5.09 x 10^6/mL
GFP	100%	5.09 x 10^6/mL
TX Red	53%	2.68 x 10^6/mL
GFP + TX Red	53%	2.68 x 10^6/mL

Settings

	BF	GFP	TX Red
Acquisition			
Intensity	65	92	100
Focus	37		
Gating			
Protocol	Protocol 1		
Size	11, 54		
Brightness	0, 255	0, 255	0, 255
Circularity	0, 100		

Countess™ 3 FL
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 of cells for the total, FL1, FL2, and FL1 + FL2 channels.
- Below the results table, the report contains the “number of cells vs. cell size” graph on the left, and “number of cells vs. RFU (relative fluorescence units)” graph on the right.
- Under the graphs, the report contains the count images, with the brightfield image on the left and the fluorescence images on the right.

  In the brightfield image, the cells counted in the brightfield channel are identified by the white “total count” circles.

  In the fluorescence image (overlaid, if there are two channels), the cells counted are identified by the circles with the same color as the fluorescence channel in which they were counted (in two colors, if there are two cubes installed).
- At the top, the report displays the profile information used to gate these images.

9 Settings overview

Settings screen

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

Settings allows you to:

- Perform software updates (see “Update the Countess™ 3/3 FL Cell Counter software” on page 47)
- Set the date and time (see “Set the date and time” on page 48)
- Change or install EVOS™ light cubes (see “Install or change an EVOS™ light cube” on page 52)

Connect to a wireless network

Network connecting options	
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**.	**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 inside the **Date** text box 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 the **Time** text box to open the **Edit Time** keypad.

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

9. Press the drop-down menu under Time to select your time zone.

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

11. Press **Apply** to set the Date and Time 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.

10 Change EVOS™ light cubes

Install or change an EVOS™ light cube

The Countess™ 3 FL Automated Cell Counter can accommodate up to two EVOS™ light cubes. Each user-interchangable, auto-configured EVOS™ light cube contains an LED, collimating optics, and filters for fluorescence applications. EVOS™ light cubes do not come standard with the device and must be purchased separately (“EVOS™ light cubes” on page 62). To install or change a light cube:

1. Press ⚙ **Settings** on the **Home** page.
2. Press **Instrument Settings**.
3. Press **Change light cube**. The instrument positions the light cube tray to enable light cube installation.
4. When prompted, power off the Countess™ 3 FL Automated Cell Counter using the power switch on the back of the instrument (“Exterior instrument parts” on page 9).
5. Unplug the power cord from the Countess™ 3 FL Automated Cell Counter.
6. Unlatch the back panel with the two captive ¼-turn fasteners (indicated by black arrows below) that secure the back panel on the rear of the Countess™ 3 FL Automated Cell Counter and remove the back panel.

7. Place the light cube into one of the empty slots in the back of the device.

8. Using the tool provided on the inside of the back panel (red arrow in Figure A), secure the light cube by tightening the two screws on the end of the cube (red arrows in Figure B).

A

B

Note: Figure B shows two light cubes installed.

9. To remove a light cube, unscrew both screws that secure it to the instrument.

10. Thread the light cube removal tool into the central hole in the cube (white arrows in Figure B) and gently pull the light cube out of the device.

Note: Always store the cube removal tool in the back panel for easy access.

11. Install the back panel and secure it in its place with both ¼-turn fasteners.

12. Plug the power cord back into the Countess™ 3 FL Automated Cell Counter.

13. Turn on the Countess™ 3 FL Automated Cell Counter by flipping the power switch on the back of the instrument to the ON position.

11 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 55.
- 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 60). 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.

A Troubleshooting

Observation	Possible cause	Recommended action
Uneven screen illumination (screen is dark on one side, bright on the other side)	Light cubes misaligned.	Reset the light cube tray by selecting Change light cube from the Instrument Settings screen. See Appendix C, “EVOS light cubes”.
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.
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.
Fluorescence is extremely bright and bleeding through into other filters	Light intensity was not set correctly.	Decrease the fluorescence light intensity before counting the cells.

Observation	Possible cause	Recommended action
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.
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.
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.
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.

Observation	Possible cause	Recommended action
Abnormally high percentage of dead cells or live cells counted as dead *(continued)*	Capture settings were not optimal.	Gate out the debris using the size, brightness, and circularity sliders.
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.
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.

B 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℃ (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 µm
Required sample volume	10 µL
Firmware	Countess™ Automated Cell Counting Platform Software
USB drive	32 Gigabytes (FAT32-, exFAT-, or NTSF-compatible)

Optics

Optics	3 channels (brightfield and 2 slots for EVOS™ LED light cubes)
Camera	5 megapixels, 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 µL

C EVOS light cubes

LED illumination

The Countess™ 3 FL Automated Cell Counter utilizes an adjustable intensity LED light source provided by the proprietary, user-interchangeable LED light cube (see “EVOS™ light cubes” on page 62). Because the LED light source is as close as possible to the objective, the number of optical elements in the channel is minimized. High-intensity illumination over a short channel increases the efficiency of fluorophore excitation, providing better detection of weak fluorescent signals.

EVOS™ light cubes

Each user-interchangable, auto-configured EVOS™ light cube contains an LED, collimating optics, and filters. In addition to the brightfield channel dedicated to cell count and cell viability assays using trypan blue, the Countess™ 3 FL Automated Cell Counter can accommodate two fluorescent light cubes for multiple-fluorescence research applications

The following table lists some of the common fluorescent and specialty EVOS™ light cubes available from Thermo Fisher Scientific. For a complete list, go to **www.thermofisher.com/evoslightcubes** or contact Technical Support. For instructions on changing the LED light cubes, see Chapter 10, “Change EVOS™ light cubes”.

Light Cube	Dyes
DAPI	DAPI, Hoechst™, BFP
TagBFP	TagBFP
CFP	ECFP, Lucifer Yellow, Evans Blue
GFP	GFP, Alexa Fluor™ 488, SYBR GREEN™, FITC
YFP	EYFP, acridine orange + DNA

(continued)

Light Cube	Dyes
RFP	RFP, Alexa Fluor™ 546, Alexa Fluor™ 555, Alexa Fluor™ 568, Cy3™, MitoTracker™ Orange, Rhodamine Red, DsRed
Texas Red	Texas Red™, Alexa Fluor™ 568, Alexa Fluor™ 594, MitoTracker™ Red, mCherry, Cy3.5™
Cy5	Cy5™, Alexa Fluor™ 647, Alexa Fluor™ 660, DRAQ5™
Cy5.5	Cy5.5™, Alexa Fluor™ 660, Alexa Fluor™ 680, Alexa Fluor™ 700
Cy7	Cy7™, IRDye 800CW

Note: The EVOS™ light cubes are available only for the Countess™ 3 FL Automated Cell Counter. The Countess™ 3 Automated Cell Counter uses only brightfield illumination and does not support the EVOS™ light cubes.

D Countess™ 3 FL Automated Cell Counter and accessories

The following Countess™ 3 FL 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 FL Automated Cell Counter	1 each	AMQAF2000
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 FL Disposable Slide Holder	1 each	AMEP-4745
Countess™ 3 FL 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.

E 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 FL 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	Concetration of just the "live" portion of the sample
	K	Live cells counted	"Live" cells counted in sample
	L	Dead concentration	Concentration of jus 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
Fluorescence	Q	Cube 1 name	EVOS™ light cube name in the first (top) position

(continued)

Category	Column	Name	Description
Fluorescence	R	Cube 1 concentration	Concentration of cells showing fluorescence in the first cube position
	S	Cube 1 (%)	Percentage of the total cells in brightfield that show fluorescence in the first cube position
	T	Cube 1 cells counted	Total number of cells counted in the first cube position
	U	Cube 2 name	EVOS™ light cube name in the second (bottom) position
	V	Cube 2 concentration	Concentration of cells showing fluorescence in the second cube position
	W	Cube 2 (%)	Percentage of the total cells in brightfield that show fluorescence in the second cube position
	X	Cube 2 cells counted	Total number of cells counted in the second cube position
	Y	Cube 1+2 concentration	Concetration of cells showing fluorescence in the first and second cube positions combined
	Z	Cube 1+2 (%)	Percentage of the total cells in brightfield that show fluorescence in the first and second cube positions combined
	AA	Cube 1+2 cells counted	Total number of cells counted in the first and second cube positions combined
	AB	Cube 1 average Size (µm)	Average size in microns of cells counted using the first (top) cube
	AC	Cube 2 average Size (µm)	Average size in microns of cells counted using the second (bottom) cube
General	AD	Focus value	Focal position number
	AE	Focus motor value	Motor position in relation to focal position number
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

(continued)

Category	Column	Name	Description
Trypan Blue/Brightfield	AL	Live circularity min	"Live" adjustment slider value for minimum circularity
	AM	Live circularity max	"Live" adjustment slider value for minimum 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
Fluorescence	AT	Cube 1 light intensity	First (top) light cube light intensity value from 0-100%
	AU	Cube 1 LED intensity	LED intensity of first (top) light cube
	AV	Cube 2 light intensity	Second (bottom) light cube light intensity value from 0-100%
	AW	Cube 2 LED intensity	LED intensity of second (bottom) light cube
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
Fluorescence	BD	Cube 1 brightness min	First (top) light cube adjustment slider value for minimum brightness
	BE	Cube 1 brightness max	First (top) light cube adjustment slider value for maximum brightness
	BF	Cube 2 brightness min	Second (bottom) light cube adjustment slider value for minimum brightness

(continued)

Category	Column	Name	Description
Fluorescence	BG	Cube 2 brightness max	Second (bottom) light cube adjustment slider value for maximum brightness
General	BH	Template name	Template used for count
	BI	Software revision	Current software version used for count

F 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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

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  - User guides, manuals, and protocols
  - Certificates of Analysis
  - 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**.