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Countess™ 3 Automated Cell Counter

USER GUIDE

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For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0019566 F (English)

Revision	Date	Description
F	28 January 2026	• New section, "Preset Protocols" was created under Chapter 3 - Protocols. • Chapter 5 - Cell count and cell viability assays was changed to Cell counting in brightfield. • New Over-view section added to Chapter 5. • Content, images and tables were edited and formatted in the manual.
E.0	20 January 2024	Update latest draft of product manuals with latest screens and user workflow for SW#3.
D.0	16 September 2022	Adding network-drive saving and Operational Qualification (OQ) functionalities.
C.0	9 June 2021	Changing "Templates" functions to "Protocols". Adding additional settings in protocol editing/creation to include Setup, Calculators, and Save.
B.0	28 January 2021	Add component to Contents table. Update technical specifications.
A.0	9 November 2020	New user guide for Countess™ 3 Automated Cell Counter.

The information in this guide is subject to change without notice.

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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 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
Wi-Fi Dongle	1 each
Trypan Blue Stain	1 each
SafeCount™	1 each

Product description

The Countess™ 3 Automated Cell Counter is a fully automated cell counter and assay platform that uses exceptional optics, and image analysis algorithms to analyze fluorescently labeled cells or colorimetric viability stained samples (for example, Trypan Blue) 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 either in disposable Countess™ Cell Counting Chamber Slides or in glass Countess™ 3 Reusable Slides (Refer to Load Countess™ Cell Counting Chamber Slide on page 23). 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 Automated Cell Counter also provides information on cell size.
- For laboratories operating under 21 CFR Part 11 requirements, the Countess™ 3 and Countess™ 3 FL instruments can be configured with the SAE console. This console enables controlled user access, comprehensive electronic record auditing, and the use of electronic signatures. The user interaction with the SAE Admin Console is described in the SAE Admin Console user guide for use with Countess™ 3 and 3 FL Automated Cell Counters (Pub. No. MAN0025371)

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 9 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 45). 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 8.
   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 counting in brightfield”.

   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 (for example, gate counts based on cell size, brightness, and circularity). See “Load a protocol” on page 15.

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

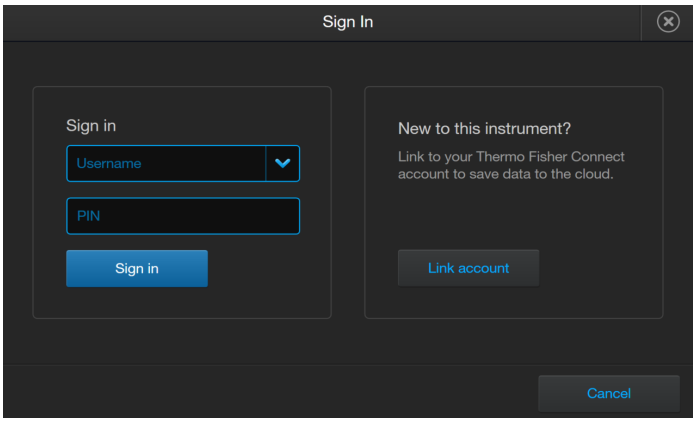
   Settings allows you to update the Countess™ 3 instrument software, customize instrument settings, view instrument details, and export error logs. See “Settings screen” on page 43.

Sign in to the instrument with a new Thermo Fisher™ Connect Platform account

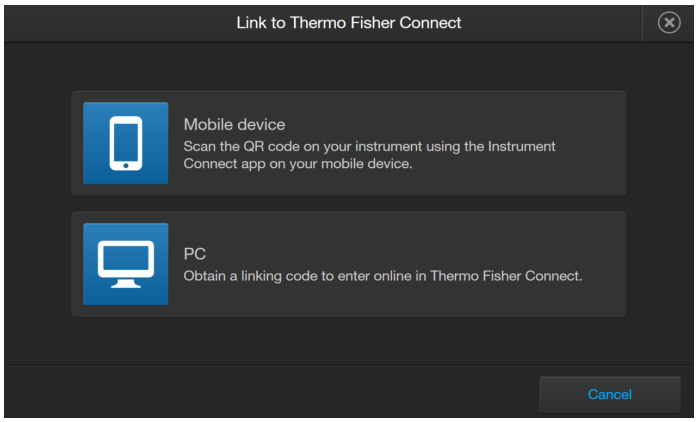
Note: A Wi-Fi adapter needs to be installed and a Thermo Fisher™ Connect Platform account is required for linking. See **http://thermofisher.com/connect** for details on using the Connect platform.

1. Press **(Sign in)** from the **Home** screen.

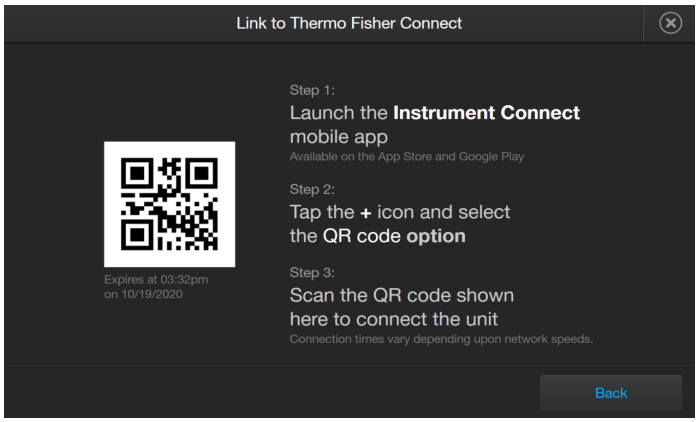
2. Press **Link account**.



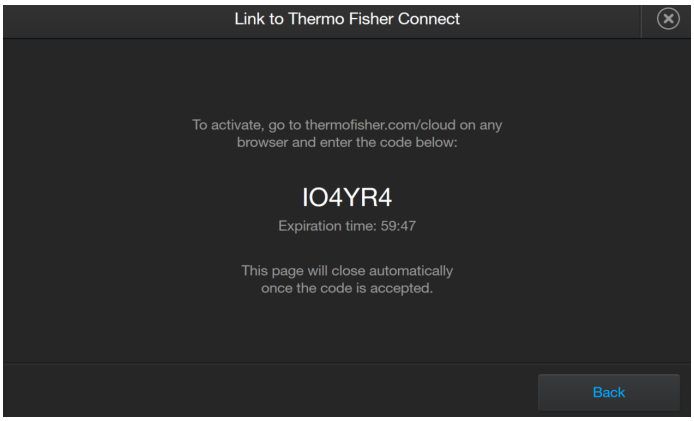
3. Choose a method to connect.



- 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 Connect platform account.

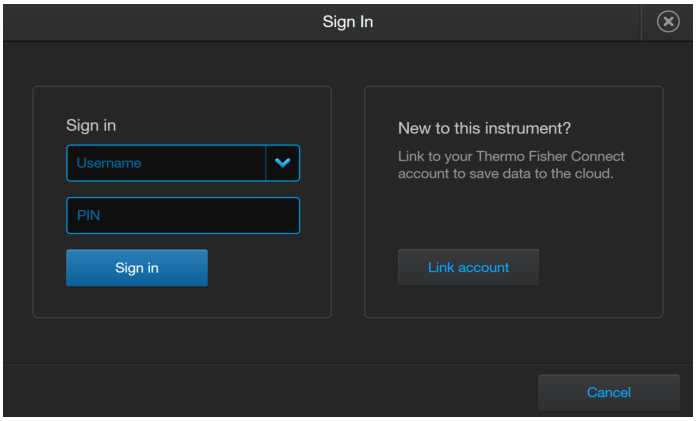


4. A completion screen will show. Press **Done**. You then return to the **Home** screen and are logged into your account.

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 Thermo Fisher™ Connect Platform account

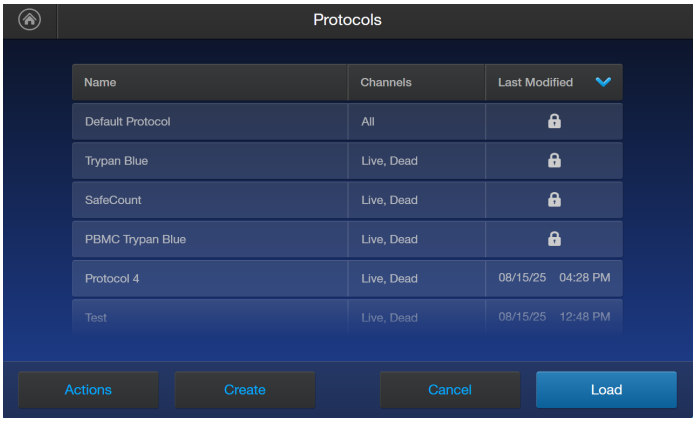
1. Press **(Sign in)** from the **Home** screen.
2. Select your **Username** from the drop-down box. Enter your **PIN**.
3. Press **Sign in**.



3 Protocols

Protocols screen

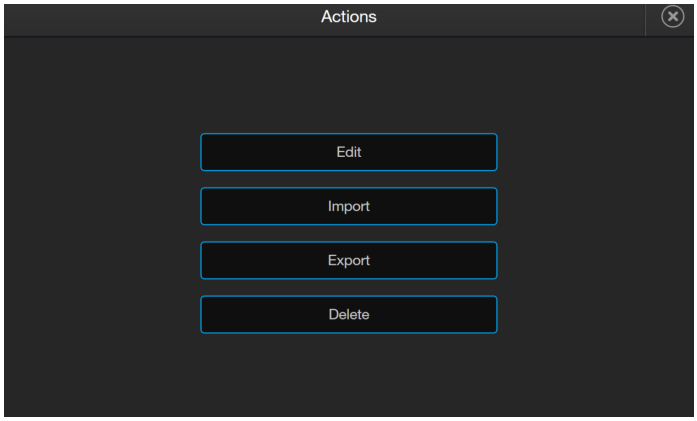
Protocols allows you to create and save customized protocols. Each custom protocol defines the count parameters (size, brightness, circularity, and fluorescence intensity) for a consistent and streamlined workflow.



- Protocols can be accessed from the **Home** and **Gating** screens.
- The currently selected protocol is displayed at the bottom of the **Home** screen.
- Preset protocols can be hidden from Protocols screen through **Settings/Instrumentsettings/preset protocols**.
- Automatic instrument functions and count parameters are defined in the **Edit protocol** screen. See “Create a protocol” on page 17 or “Edit a protocol” on page 17.
- The Default protocol and preset protocols contain 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/View** can be used to modify the currently selected protocol. Preset protocols can not be edited, only viewed, indicated by the lock icon. **Import**, **Export**, and **Delete** can be used on multiple protocols.



Preset protocols

The Countess™ 3 Automated Cell Counter includes several Preset protocols designed to streamline cell counting for users. These protocols are pre-configured with specific settings to aid users and provide a starting point to define cells of interest. The specific settings within the Preset protocols include the light cubes needed, count mode, reagent dilution, and gating on brightness, size, and/or circularity. The Preset protocols can be found in the protocols menu. Users can hide these protocols by navigating to **Settings** > **Instrument Settings** > **Preset protocols** and unchecking **Show**. Additionally, in this menu, users can find the kit SKU for the reagents needed for each protocol.

The following Preset protocols are available:

Table 1

Protocol name	Cat. No.	Light cube	Dilution	Count mode	Gating
Defaut		None	1:1	BF-based	None
Trypan Blue	T10282	NA	1:1	BF-based	None
SafeCount™	A40008024	NA	1:1	BF-based	None
PBMC Trypan Blue	T10282	NA	1:1	BF-based	Size minimum 4; circularity minimum 50

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.

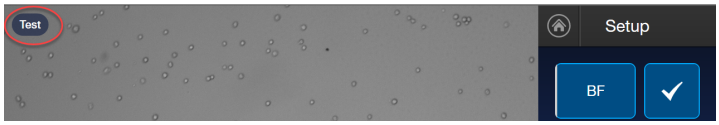
④ **Gating Boundary Sliders**

- **Setup** – Setup adjustment is not needed for the Countess™ 3 instrument, it is utilized in the Countess™ 3 FL instrument.
- **Calculators** – Press **Calculators** to access the Pre-Dilution and Cell Splitting Calculators and store default calculator values. For detailed protocols, see Chapter 6, “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 39.

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**.
   The instrument will keep the saved protocol, but will return to the previous screen without loading it.

Note: The **Protocol name** will be displayed in the upper left corner of the **Count** screen.

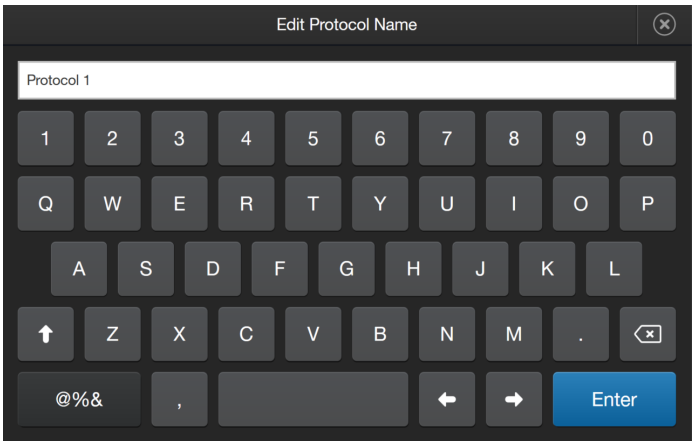


Note: The **Protocol name** will be displayed at the bottom of the **Home** screen.

If a saved protocol has been changed and there are unsaved changes, an asterisk (*) will follow the name.

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.



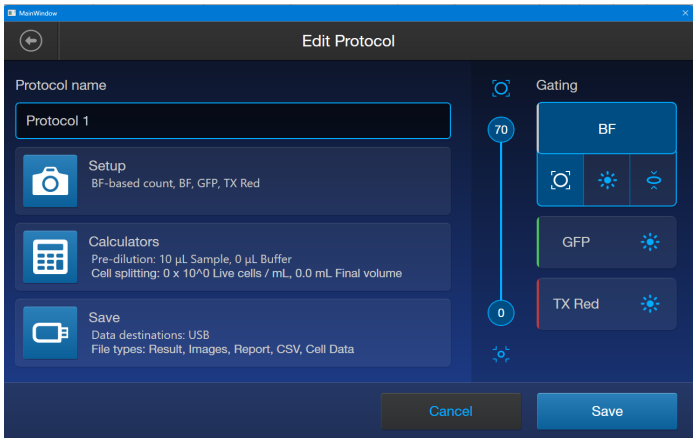
3. Type in the **Desired Protocol name**. To enter symbols, press the symbol (**@%&**) key. To return to the alpha-numeric keypad, press **ABC**.
4. Press **Enter** to save the name and return to the **New Protocol** screen. Press ✕ to return to the **New Protocol** screen without saving the name.
5. Press **Save** to save the new protocol.
6. 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 and Preset protocols cannot be edited.

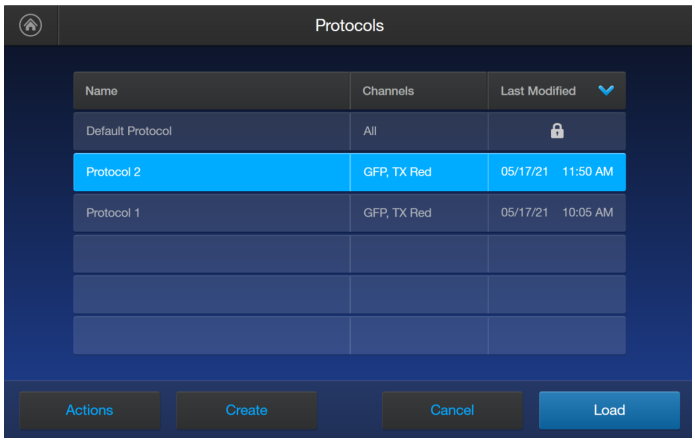
3. Press **Edit**.



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

4. 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

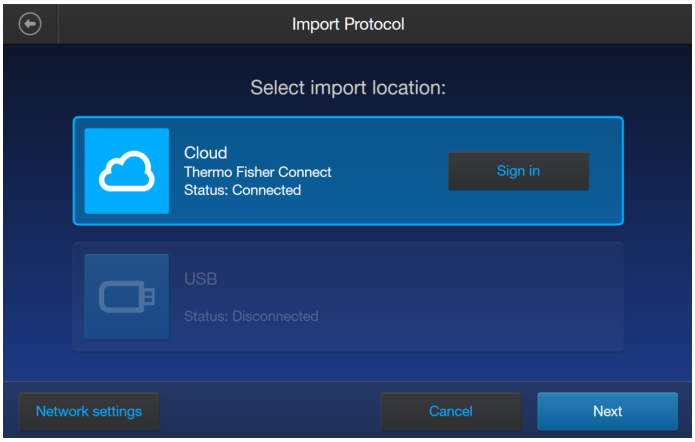
1. Press **(Protocols)**.

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.



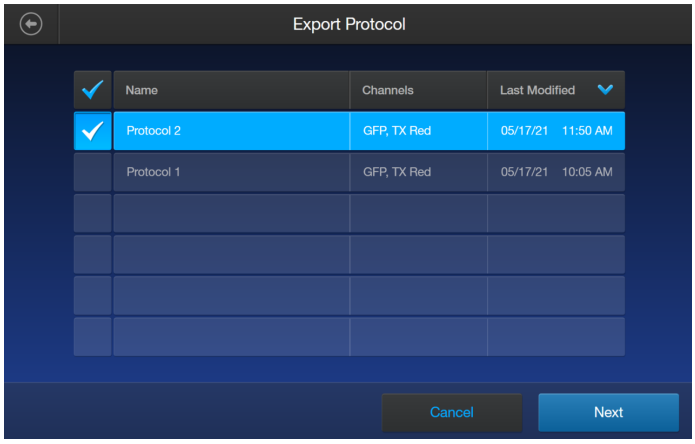
5. Press **Next**.
6. Select one or more protocols from the list.
7. Press **Import**.
8. Once complete, press **Done**.

Export a protocol

1. Press **(Protocols)**.
2. Press **Actions**.
3. Press **Export**.

4. Select one or more protocols from the list.

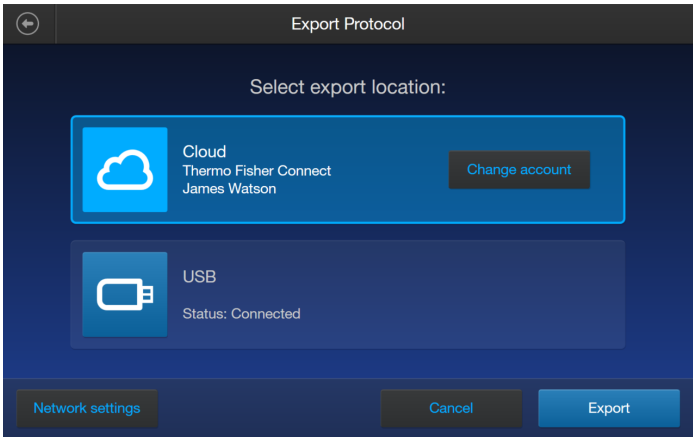
Note: The Default and Preset protocols cannot be exported.



5. Press **Next**.

6. Press the export location **Cloud** or **USB**.
   a. For **Cloud**, press **Sign in** to log in to your Thermo Fisher Scientific 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.



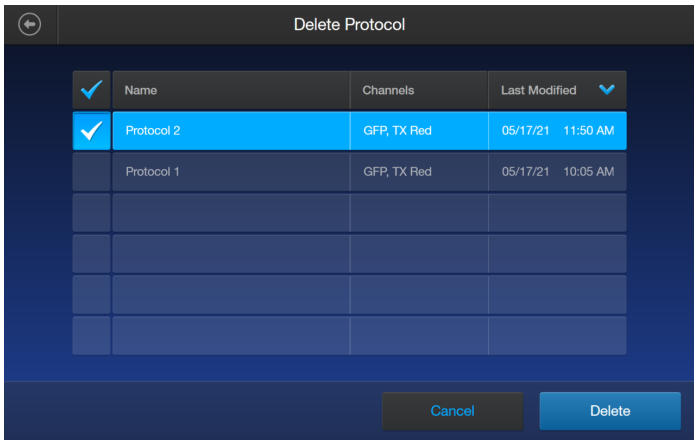
7. Press **Export**.

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.

Note: The Default and Preset protocols cannot be deleted.



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.

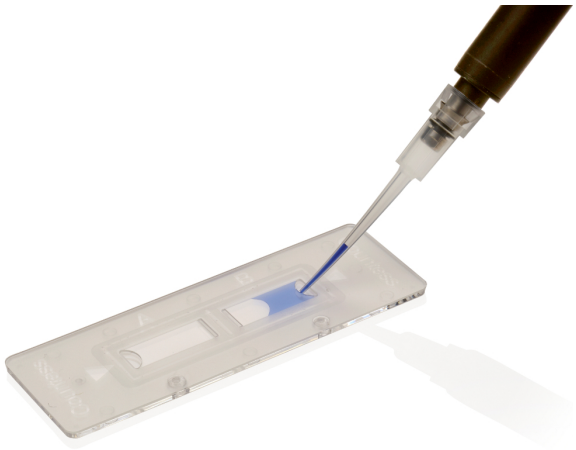
  IMPORTANT! A warning will appear on the Results screen when concentrations are out of optimal range.

- 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.
- Be careful to avoid forming bubbles in the sample.

Load Countess™ Cell Counting Chamber Slide

1. Prepare the sample: If using colorimetric viability stains, Trypan Blue or SafeCount™, add 10 µL of your cell suspension to 10 µL of stain. Mix the sample mixture well by pipetting it up and down a few times and follow protocol as indicated.

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 counting in brightfield” for more details.

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

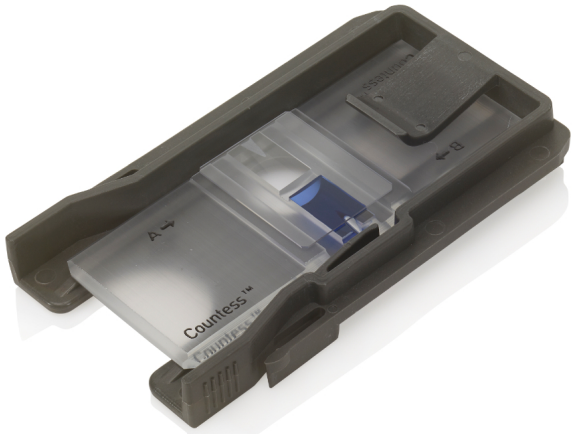
1. Before loading your sample into the Countess™ Reusable Slide, place a cover slip on the counting chamber, making sure the cover slip is clean and free of grease.

2. Prepare the sample: If using colorimetric viability stains Trypan Blue or SafeCount™, add 10 µL of your cell suspension to 10 µL of stain. Mix the sample mixture well by pipetting it up and down a few times and follow protocol as indicated.

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 has a 10-µL sample capacity. Do not overfill the slide chambers.

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



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

6. Use the touchscreen to run the sample count. See Chapter 5, “Cell counting in brightfield” for more details.

7. 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.

8. *(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.

9. 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.

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

5 Cell counting in brightfield

Overview

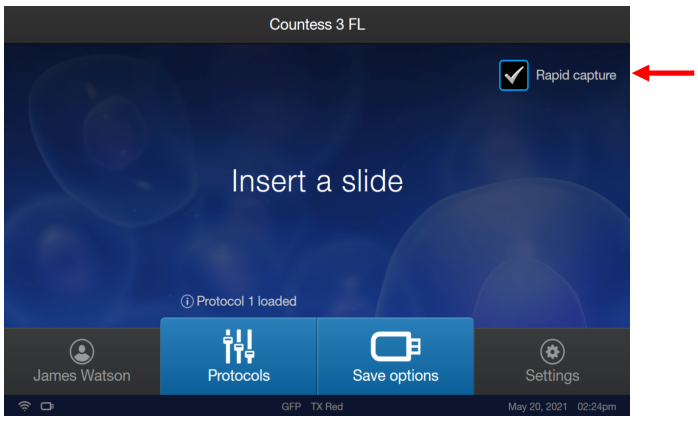
Countess™ 3 Automated Cell Counters count colorimetric viability stained samples using a brightfield image and two key colorimetric viability stains, Trypan Blue and SafeCount™. Both stains are used at a 1:1 dilution with the cell suspension. These viability stains are effective because they make dead cells appear dark, allowing for accurate differentiation between live and dead cells using the brightfield image. The Countess™ 3 Automated Cell Counters, leverages image analysis algorithms to provide detailed results on starting cell concentration (prior to staining), viability, and aggregate formation.

Count cells in brightfield

Capture and count

Rapid capture

To bypass adjusting individual parameters before the 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.



1. Load the samples into the instrument as described in Chapter 4, “Slide preparation and operation”.
2. See “View results” on page 29.

Customize capture

To view the image and adjust parameters prior to the count, follow the procedure below:

1. Load the sample into the sample slide as described in "Load Countess™ Cell Counting Chamber Slide" on page 23.

2. *(Optional)*: Press **(Protocols)** and load the desired gating parameter settings as described in "Load a protocol" on page 15.

3. Insert the sample slide into the instrument. Make 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.

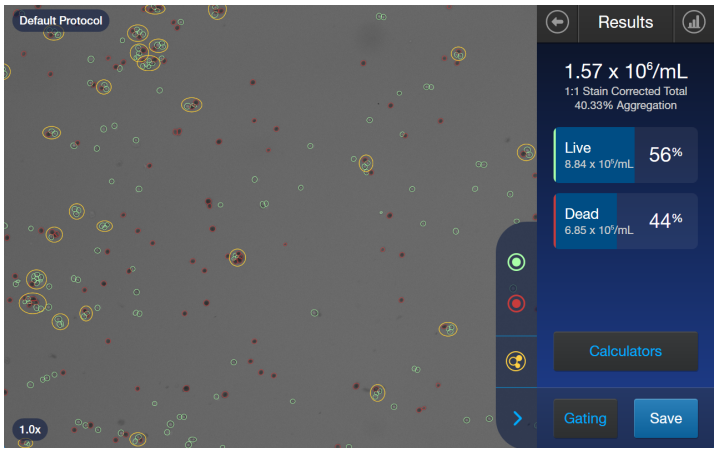
   Note: Auto lighting is optimal for producing accurate results, but if needed, can be disabled by adjusting the lighting slider.

5. *(Optional)*: Set light intensity and/or focus. The light source slider controls the LED intensity and allows you to adjust the image brightness. The auto-focus function searches the nominal focus, or Z-point (depth) to provide fine focus to the sample. If the focus is modified and applied, the new focus value will become the nominal focus value for subsequent counts in the session.

6. Press **Count**.

   The instrument captures the image and displays the following results: total concentration, percentage and concentration of Live and Dead cells, and percentage of Aggregates (clusters of three or more cells with touching membranes). For more information, see "View results" on page 29.

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



Next steps

- To identify the objects (for example, cells) counted as live, press . To identify objects counted as dead, press . To identify aggregates, press . See “Identify cells counted in cell count and cell viability assays” on page 30.

  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 31.
- To gate the results by object size, brightness, or circularity, press **Gating** to go to the **Gating** screen. See “Gate count results” on page 32.

  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 33.

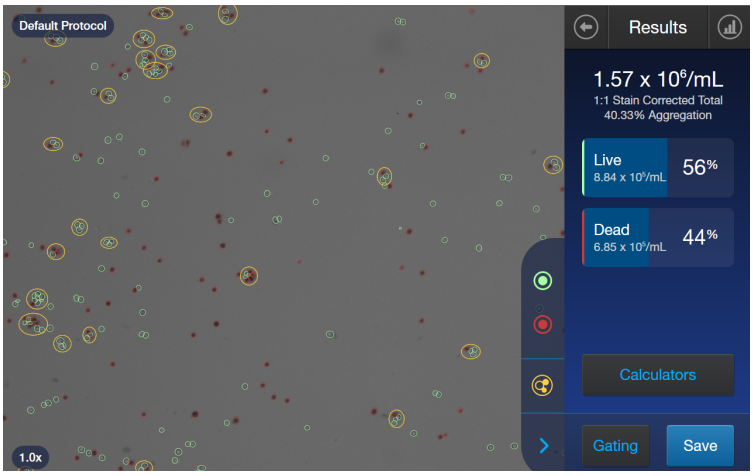
- To calculate the volume of cell sample and buffer needed to reach a desired concentration based on the count results, press **Calculators** to open the Calculator applications. See Chapter 6, “Calculators”.
- To permanently save the results, press **Save**. See Chapter 7, “Save results”.
- To perform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

View results

Results screen for colorimetric viability assays

The **Results** screen for cell count and cell viability assays performed using colorimetric viability stains displays a composite image of the objects counted and the results of the cell count and viability calculations. The results shown include:

- Total Concentration: The overall concentration of cells in the sample.
- Percentage and concentration of live cells: The number and percentage of live cells in the sample.
- Percentage and concentration of dead cells: The number and percentage of dead cells in the sample.
- Percentage of aggregates: The percentage of cell clusters (aggregates) consisting of three or more cells with touching membranes.



Note: When performing cell counts and viability assays with colorimetric stains, the counting algorithm assumes a 1:1 dilution of cells in the stain and takes this dilution into account when calculating the total cell concentration. The cell concentration displayed on the **Results** screen reflects the original cell concentration before dilution.

Identify objects counted

Results screen

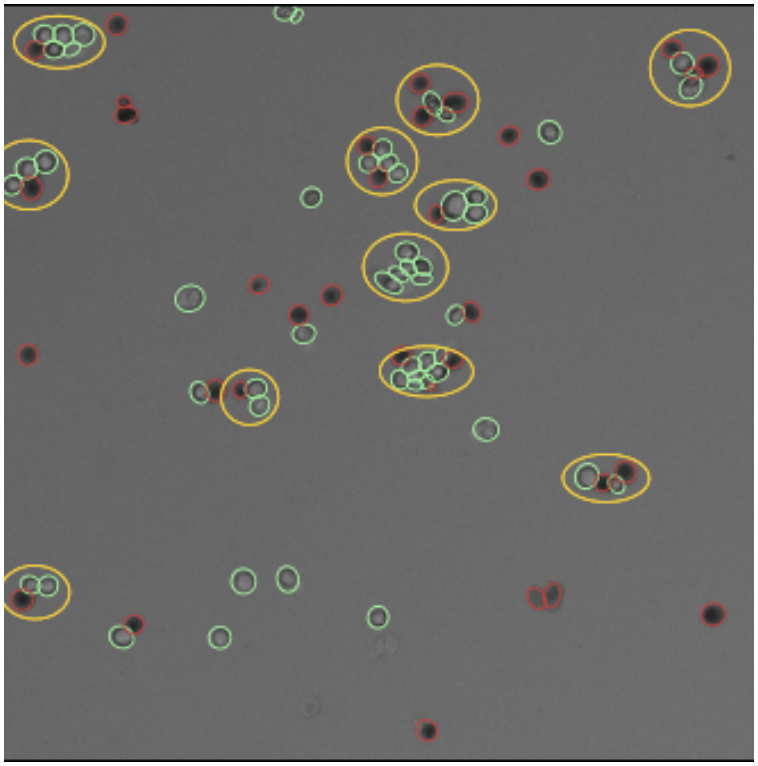
The **Results** screen allows you to identify the objects (for example, cells) counted in each channel and included in the count results for further review. After reviewing the marked objects, you can adjust the threshold for size, brightness, and/or circularity as desired for your application. See “Gate count results” on page 33.

Identify cells counted in cell count and cell viability assays

On the **Results** screen, live, dead, or aggregates (clusters) of cells can be selected.

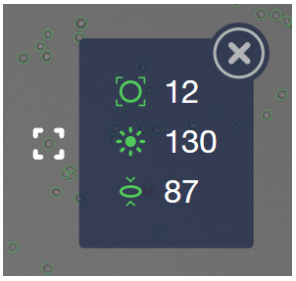
1. To view **Live cells**, press on the right panel. This marks all Live cells in green.
2. To view **Dead cells**, press on the right panel. This marks all Dead cells in red.
3. To view **Aggregates of cells**, press on the right panel. This marks all the aggregated cells with a yellow circle.

Note: You can select one, two, or all three options. The detailed image below shows Live (green), Dead (red) cells, and Aggregates (yellow) are selected.



4. 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 the desired cell and a pop-up displays the parameters. Press ✕ to close the pop-up.



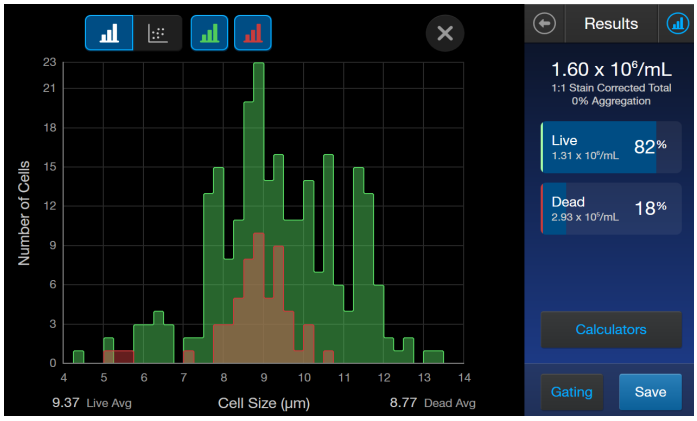
Histogram count results

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)*: On the **Gating** screen, use the **(Size)**, **(Brightness)**, and **(Circularity)** sliders, to adjust the count parameters. As you adjust the count parameters, the count results and the graph automatically update.

4. To close the histogram, press or .

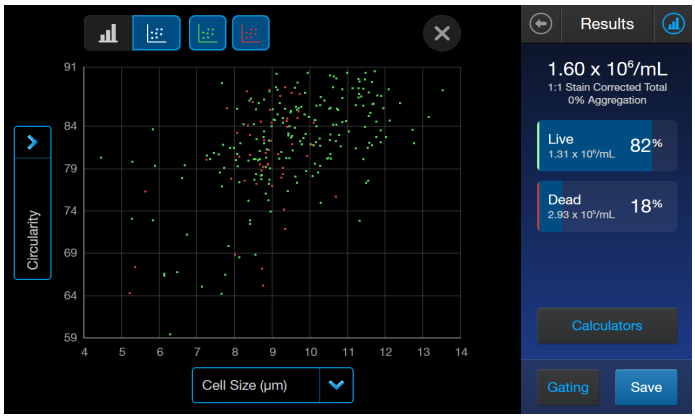
View scatter plot

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 scatter plot.

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

From the histogram view:

1. Tap (white).



2. *(Optional)*: Edit the x- or y-axis variables by tapping the variable arrow on the axis. You can choose between **Circularity** and **Cell Size (µm)** on either axis. Tap ✕ to return to the scatter plot.

3. To view the distribution of only the live or dead cells, press the corresponding **(Live)** (green) or **(Dead)** (red) button.

   The plot will automatically update and display the distribution of cells based on size only in the selected population.

4. *(Optional)*: On the **Gating screen**, use the **(Size)**, **(Brightness)**, and **(Circularity)** sliders, adjust the count parameters. As you adjust the count parameters, the count results and the graph automatically update.

5. To close the scatter plot, press 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 33).

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 33.
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 (for example, 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 17.

   Note: You cannot change parameters of preset protocols. If gating is adjusted on Preset protocols, you must save as a new protocol by pressing **Protocols** on the **Gating screen**, and then **Create**.

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 or Preset Protocols.

   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.

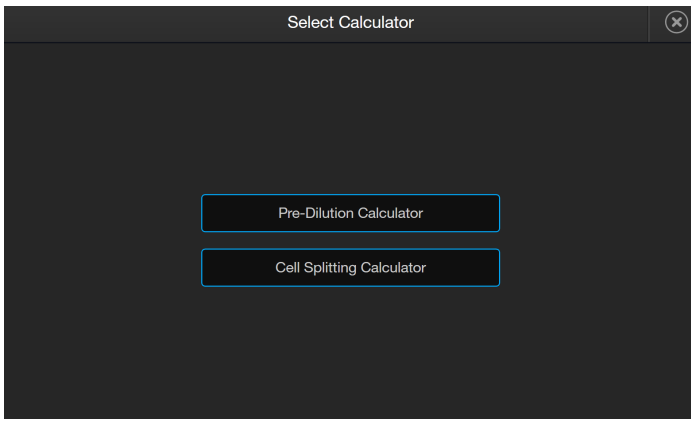
6 Calculators

Pre-Dilution Calculator

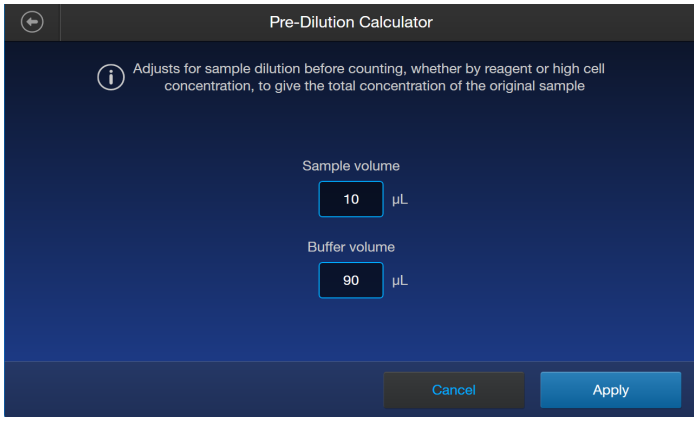
The **Pre-Dilution Calculator** is used to account for diluting a very concentrated sample before loading it into a sample slide for counting.

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

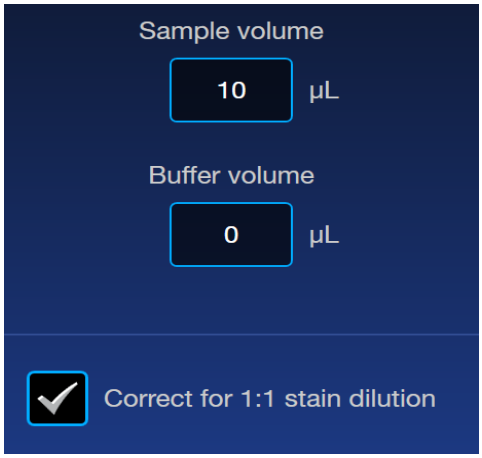
1. On the **Results** screen, press **Calculators** then select **Pre-Dilution Calculator**.



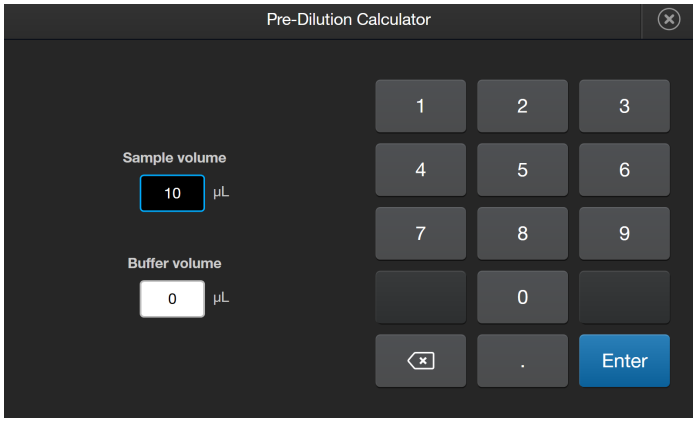
2. Click-in one of the text fields.



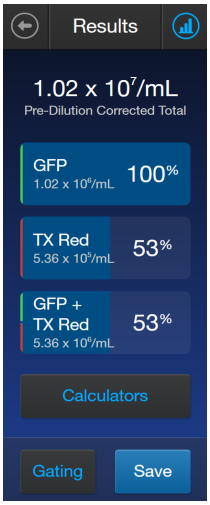
Note: You can select the checkbox at the bottom of the screen to correct for a 1:1 stain dilution.



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



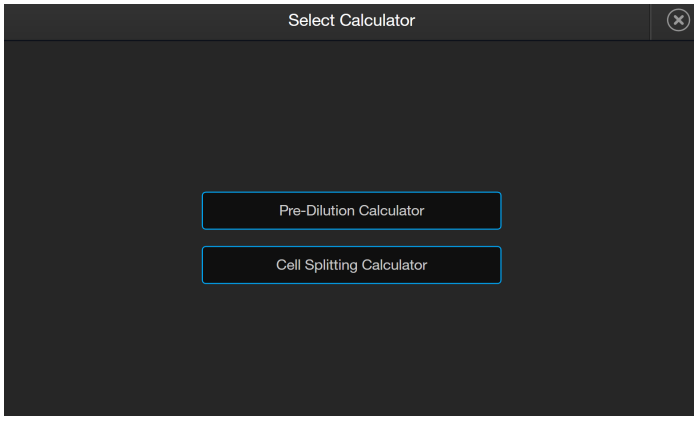
4. Press **Calculate**. The result will appear at the top of the **Results** screen.



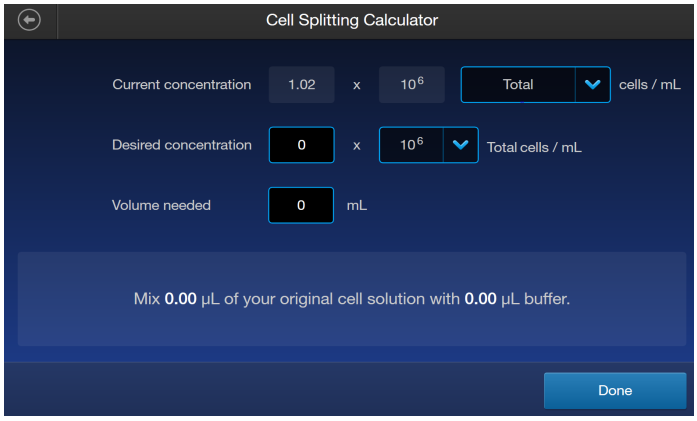
Cell Splitting Calculator

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

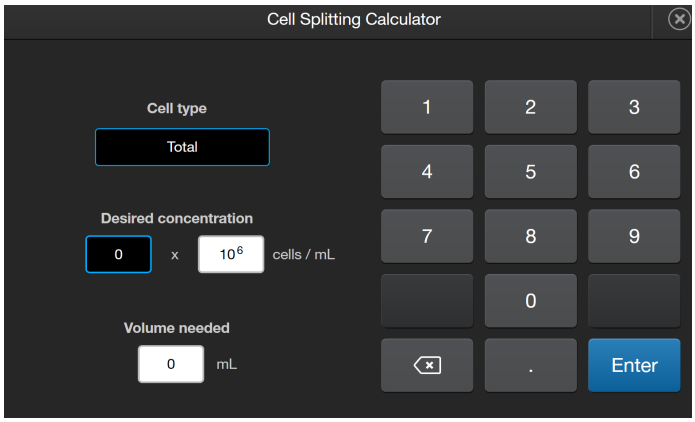
1. On the **Results** screen, press **Calculators** then select **Cell Splitting Calculator**.



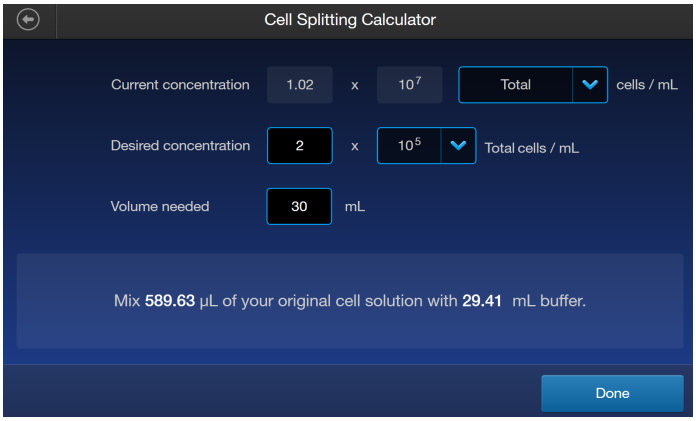
2. Click-in one of the text fields.



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**.



7 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, a link to a Thermo Fisher Connect account, or a link to a network drive.

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 42.
- **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 D, “CSV file format definition” Appendix E: CSV file format.

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

- **Cell Data:** Saves single cell data from the count in either a FCS or CSV format. FCS is a Flow Cytometry Data File Standard and allows for processing or re-displaying results. The CSV format allows for processing or re-displaying results with any third party software or spreadsheet program, providing each cells data on a separate row.

  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.

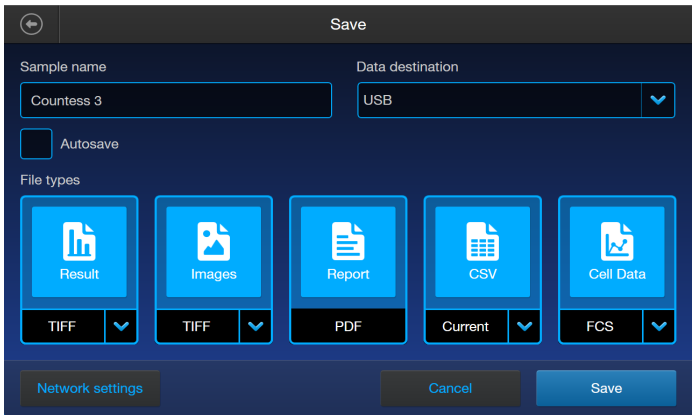
Save procedure

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 or network drive.

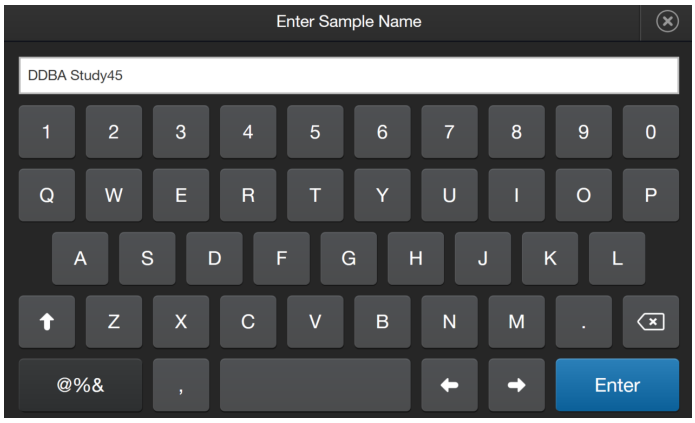
   Note: If the instrument is not connected to a network, then use **Network settings** at the bottom of the screen to reconnect.

   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.

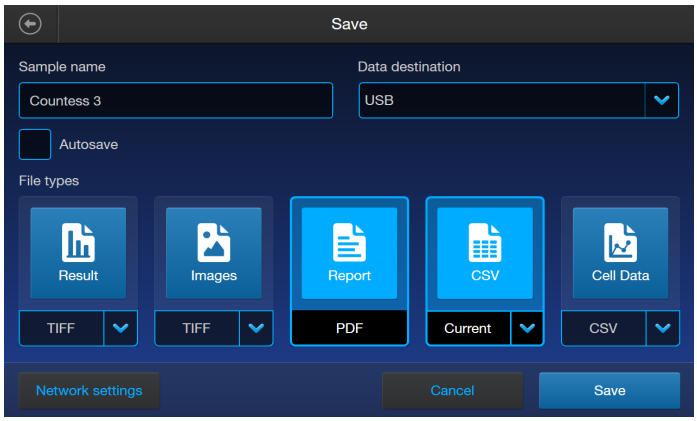


3. To assign a name to your count, press the **Sample name** text field and enter the name.
   To enter symbols, press the **@%&** key. To return to the keypad, tap the **ABC** key.



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 Count**, **Session**, **Today**, **Yesterday**, **Previous 7 days**, **Previous 30 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 ✕ **(Close)**.

8. Press **Save** to save your experiment to the selected mode(s) in the USB drive, Connect account, or network drive.

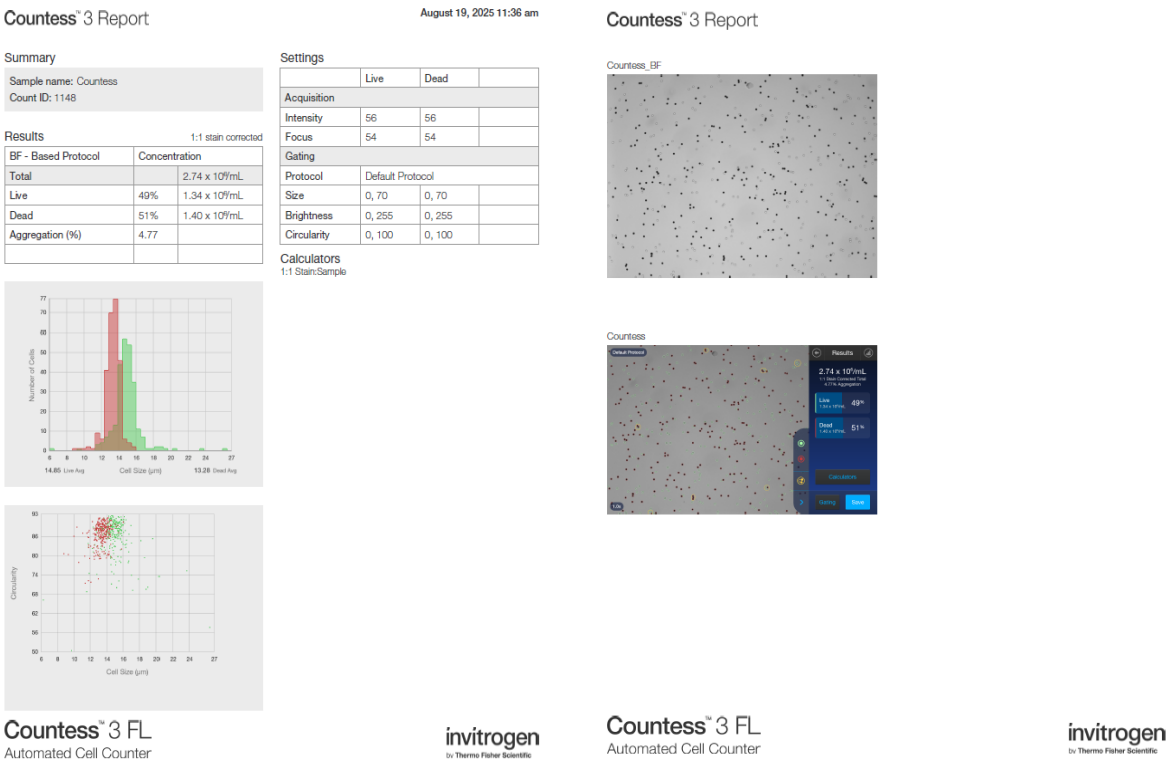
9. Press **Done** and then transfer the USB drive to the desired location.

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



- 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, along with the percentage of Aggregates. The report also displays the template information used to gate these images.
- Below the results table, the report contains the “Number of Cells (µm) vs. Cell Size” graph and "Cell Size (µm) vs. Circularity" scatter plot.
- Under the graph, the report contains the brightfield count image, with the live and dead cells identified by the green and red circles, respectively.

8 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 44)
- Set the date and time (see “Set the date and time” on page 45)

Connect to a wireless network

Network connecting options	
1. From the **Home** screen, press **(Settings)**. 2. Press **Instrument settings**. 3. Press **Wireless network**. 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.	

Connect to a network drive

1. From the **Home** screen, press **(Settings)**.
2. Press **Instrument settings**.
3. Press **Network drive**.
4. Press **Drive location**. Enter the IP address for your shared folder.
5. *(Optional)*: Enter any additional information, including a **Domain name**, **Username**, or **Password**.

   Note: Consult with your local IT representative for questions regarding drive location and domain name.

6. Press **Connect**.

The network drive is now connected. If needed, you can edit the **Drive location**, **Domain name**, or **Username** by pressing **Edit**.

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.

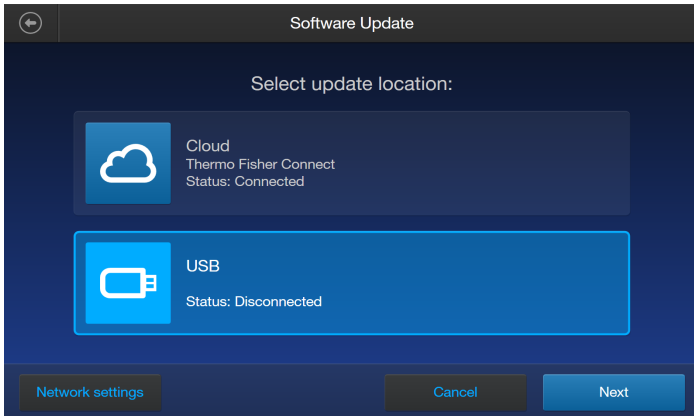
Update the Countess™ 3/3 FL Cell Counter software

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 (For example, Countess_xxxx for software version 2.0.2).

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**.

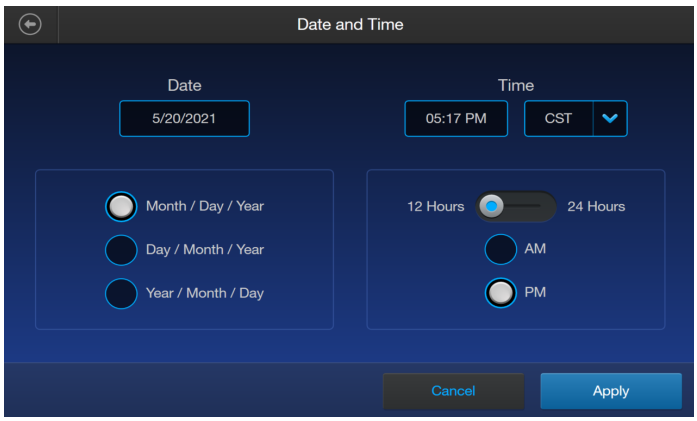
   If the **Cloud** option is disconnected, press **Network settings** to proceed to reconnect to your network.

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

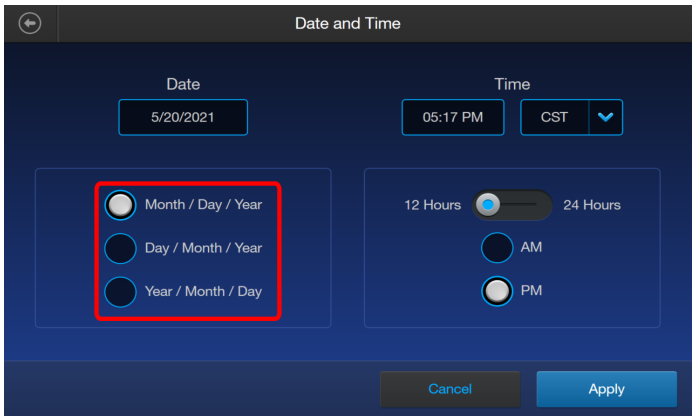
7. If an update is available, press **Update**. Once the update has completed, the **Home** screen will appear to resume normal operation.

Set the date and time

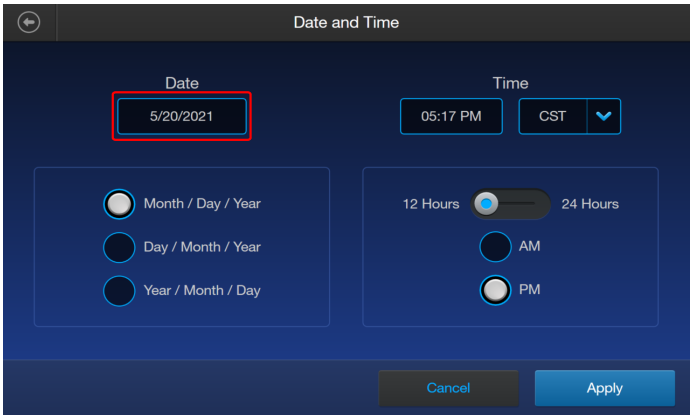
1. Press ⚙ **(Settings)** on the **Home** page.
2. Press **Instrument Settings**.
3. Press **Date and Time**.



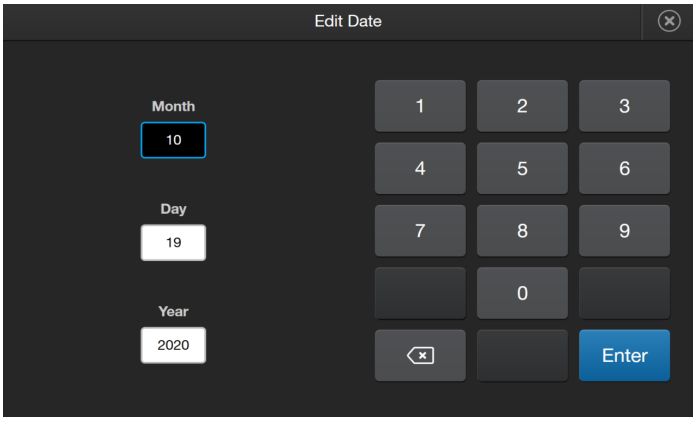
4. Select the date format (**Month/Day/Year**, **Day/Month/Year**, or **Year/Month/Day**).



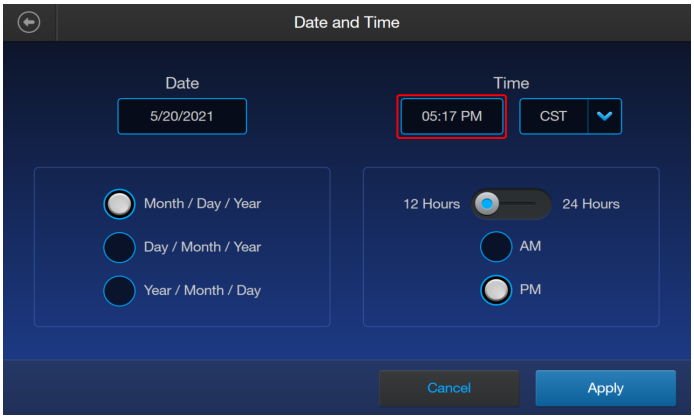
5. Press inside the **Date** text box to open the **Edit Date** keypad.



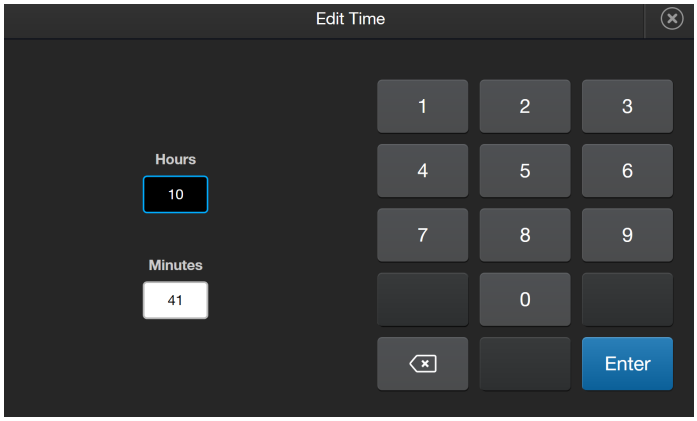
6. Using the keypad, enter the date into the **Month**, **Day**, and **Year** text boxes. Press **Enter**.



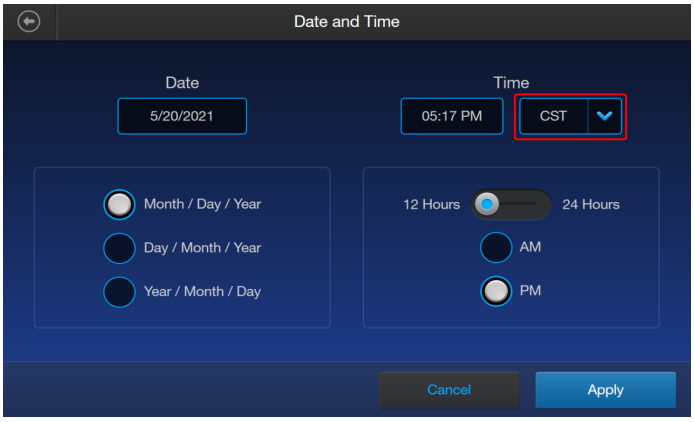
7. Press the **Time** text box to open the **Edit Time** keypad.

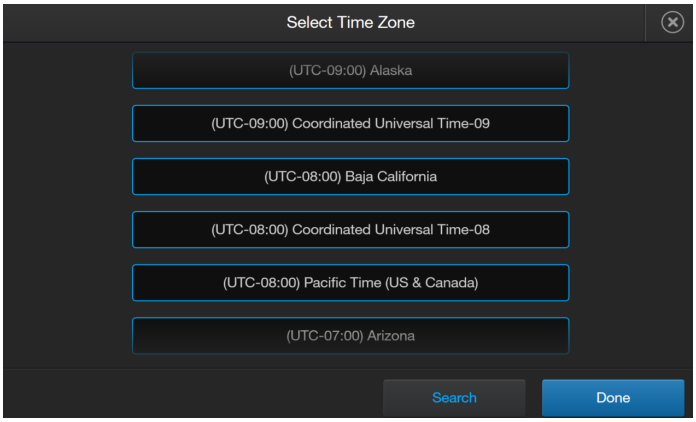


8. Using the keypad, enter the time into the **Hours** and **Minutes** text boxes.

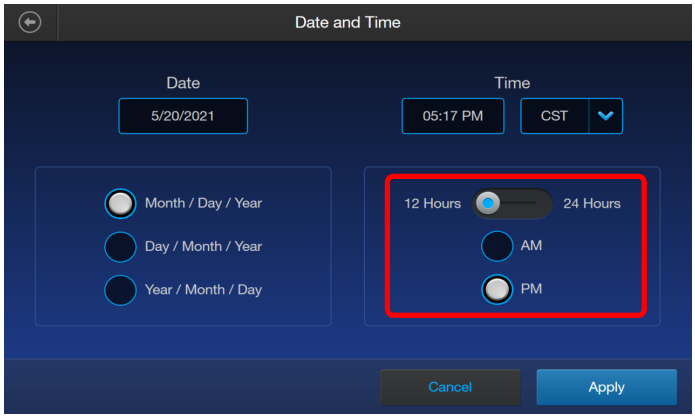


9. Press the drop-down menu under Time to select your time zone from the list. If needed, press **Search** to use a keyboard to type in your search term (e.g., pst, cst, etc.).





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



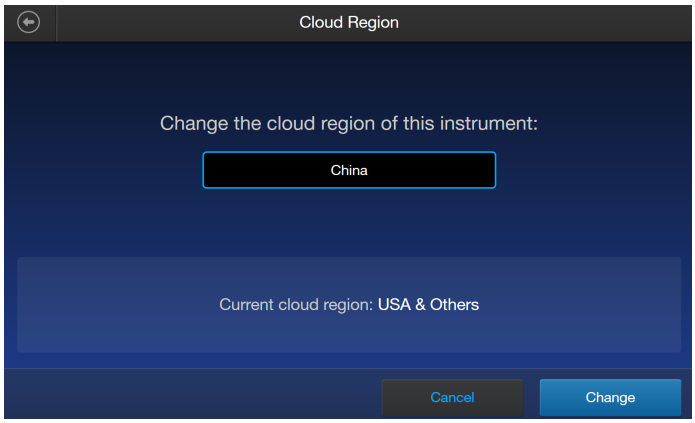
11. Press **Apply** to set the Date and Time and return to the **Instrument Settings** screen.

    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.

9 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 51.
- 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 69). 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.

10 Installation/Operational Qualification

The installation and/or operational qualification ensures that the instrument is installed and performing within the manufacturer specifications. IQ/OQ requires a thumb drive and the Countess™ 3 Standard Slide (Cat. no. A51876) shipped with user executed IQ/OQ (Cat. No. **A59080**) and user executed OQ (Cat. No. **A54115**) purchase.

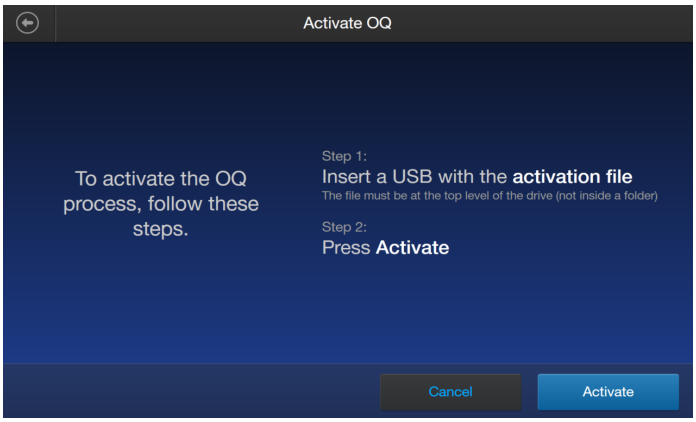
Generate and activate a license

1. To generate the license file, go to **apps.thermofisher.com**.
2. Sign in to to your Thermo Fisher™ Connect Platform account.
3. Open the **Countess™ Image Analysis** application.

   Note: If the Countess™ application is not displayed on the **Dashboard** page, click **View all apps** to see a full list.

4. On the **File Gallery** screen, click on **Execute IQOQ** in the top right corner. Then, select **IQ&OQ service** if completing both IQ and OQ or **OQ service** if completing just OQ. See image on page 54.
5. Enter the **Order ID (sales order number)** and **Countess 3 Serial Number** (found on your instrument). If completing IQ, please navigate to page 54 to **Execute the Installation Qualification**. If only completing OQ, continue to follow the instructions below.
6. Click **Get activation file**.
   A **Request Submitted** message will display when the order information is validated.
7. Once the file is ready, click on the **Download is Ready** window and select **Download.zip** to begin the license key file download.
8. Save the license file to a USB drive. Do not change the license name "Prod_xxxx.OQ-license" where xxxx is the instrument serial number.
9. Insert the USB drive into the instrument.
10. On the instrument, press **Settings** on the home screen.
11. Press **Execute OQ**.

12. Press **Activate**.

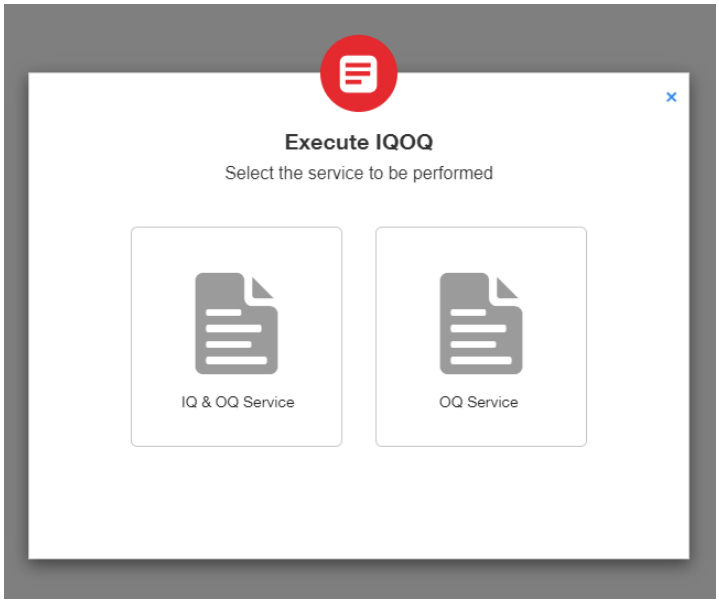


The license is now activated and will remain valid for 2 months on the instrument. Press **Next** to advance to the OQ testing. Follow the OQ instructions under Execute the Operational Qualification (OQ) on page 59.

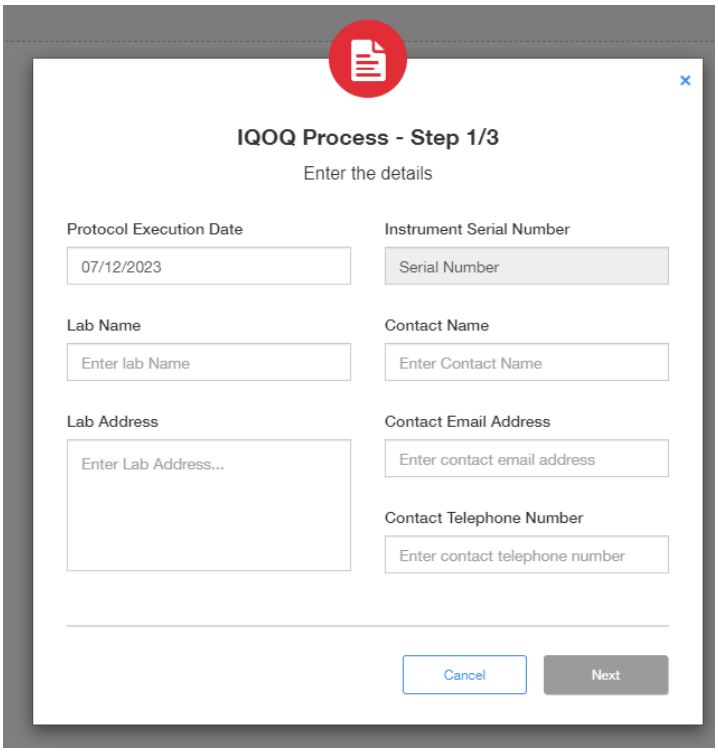
Execute the Installation Qualification (IQ)

Each entry throughout the IQ process is recorded in a final PDF report which is then saved to a USB drive at the end of the IQ procedure. During the IQ workflow, a license will be saved to the USB drive to allow execution of the on-instrument OQ workflow. After completion of the OQ on-instrument, the instrument will allow export of a final IQOQ report. If needed at any step, press **Cancel** to return to the last saved location.

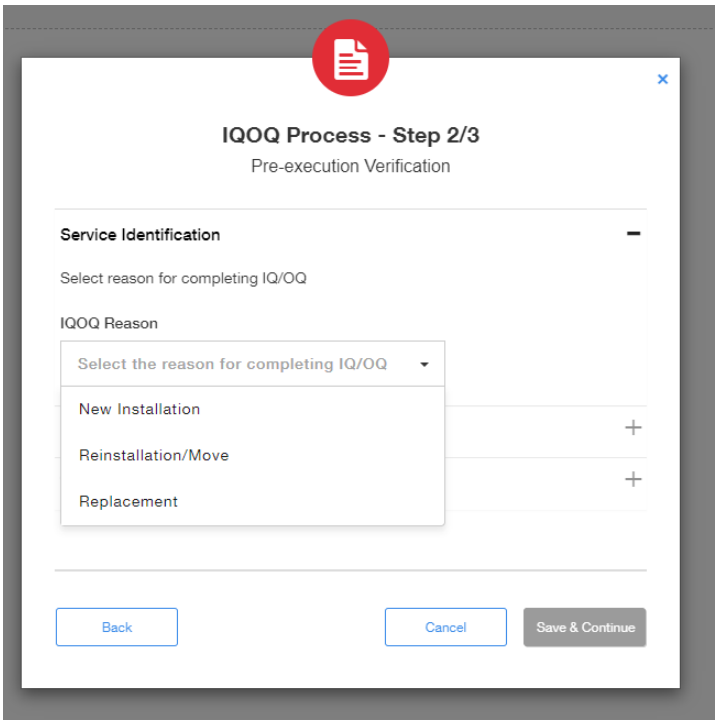
1. Select **IQ&OQ Service**.



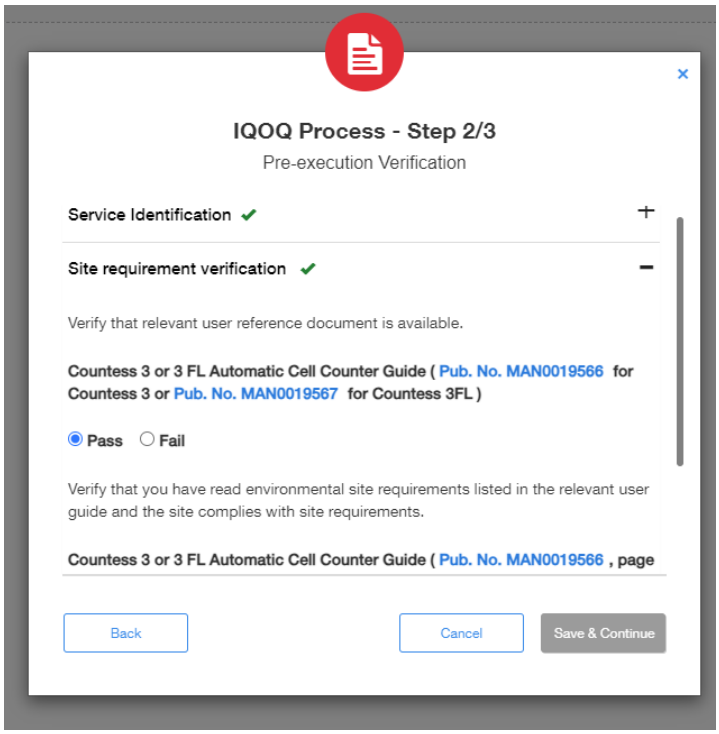
2. Enter the **Protocol Execution Date**, **Instrument Serial Number**, **Lab Name**, **Lab Address**, **Contact Name**, **Email**, and **Telephone Number**.



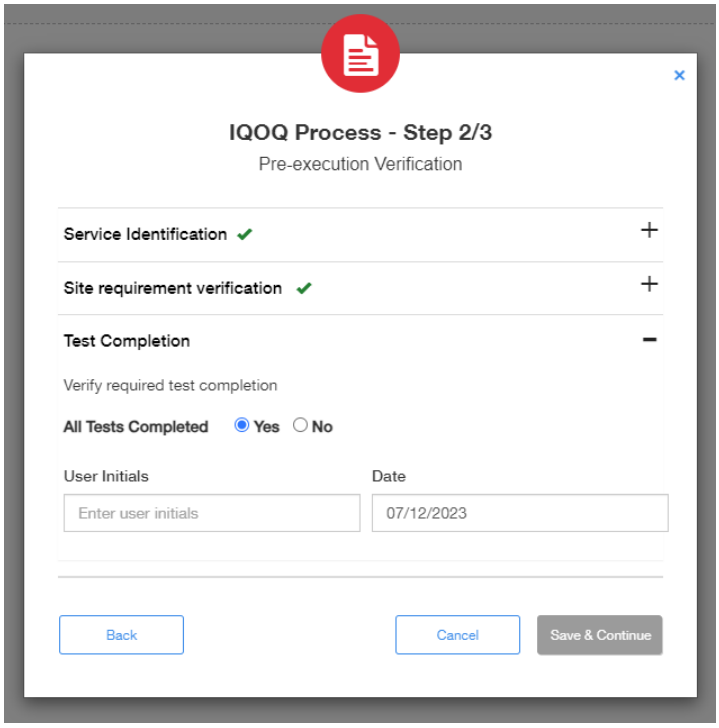
3. Select the reason (from the drop-down menu) for completing the IQ/OQ service .



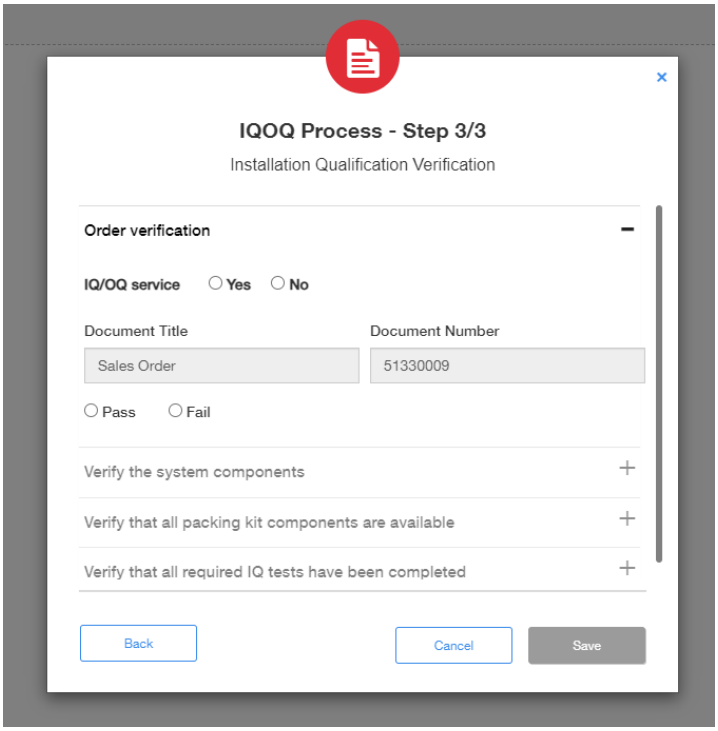
4. Ensure that you have access to the appropriate user guide and verify that you have read the site requirements.



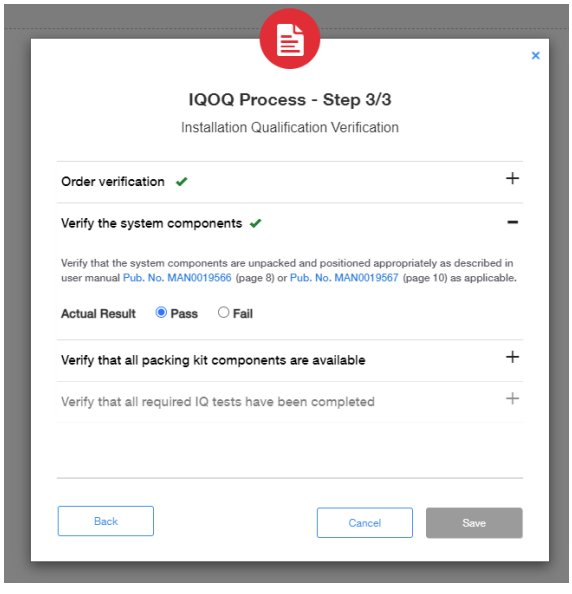
5. Verify that the **Service identification** and **Site requirement verification** sections have been completed. Enter **User name**, **User Initials**, and **Date**.

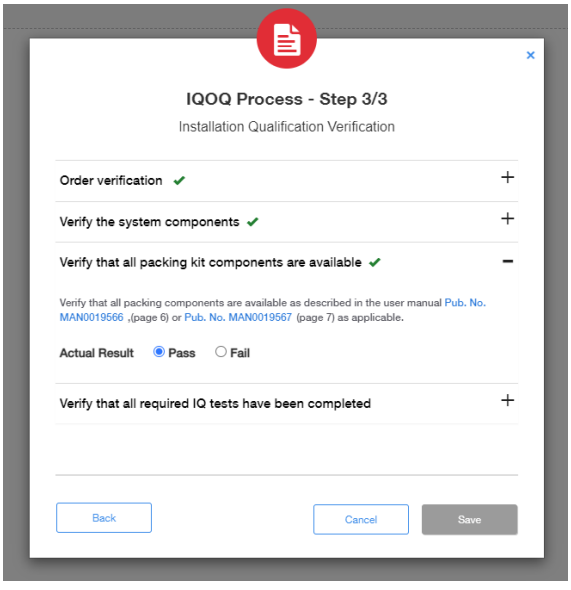


6. Confirm that the automatically-filled sales order number (Document Number) is correct.

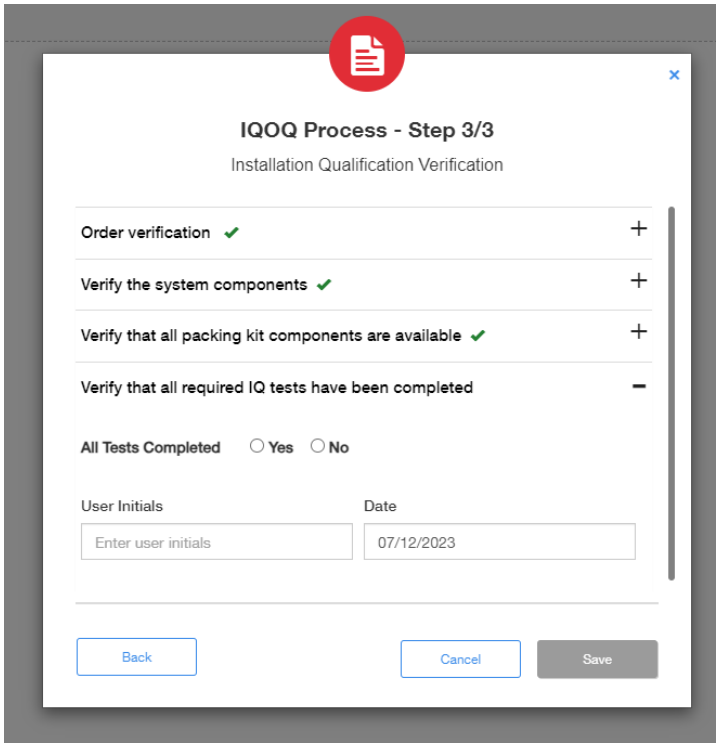


7. Verify that all of the system components are unpacked and available. Confirm that packing components are as described in the user guide.

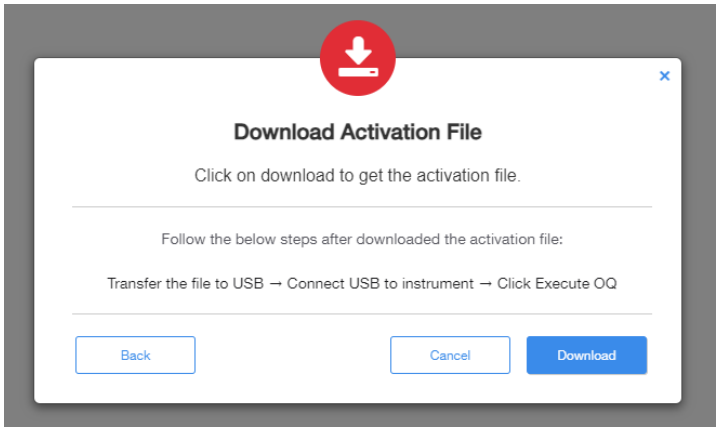




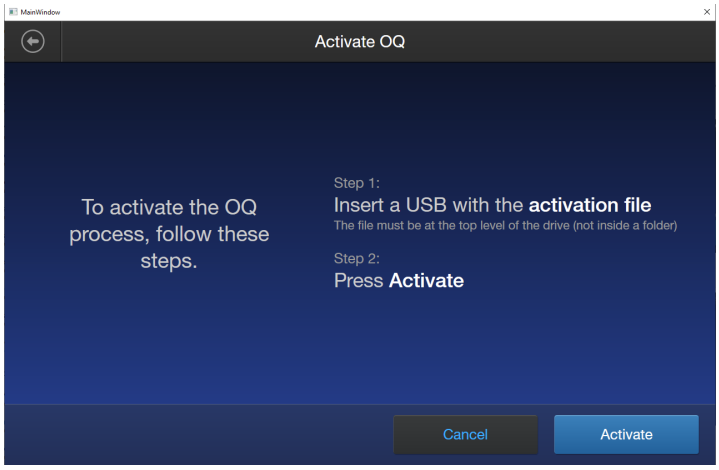
8. Verify that all required IQ tests are complete. Enter **Initials** and **Date**, then press **Save**.



9. Press **Download** to download the license to execute OQ on the instrument. Save the license to a USB drive. Do not change the license file name "Prod_xxxx.OQ-license" where xxxx is the instrument serial number. Next insert the drive into the instrument and navigate to **Settings-Execute OQ** on the Countess™ 3 / 3 FL touchscreen.



10. **Activate** the OQ workflow.



Note: The license is now activated and will remain valid for 2 months on the instrument.

11. Follow the OQ instructions in the next section, Execute the Operational Qualification (OQ)

Execute the Operational Qualification (OQ)

Each entry throughout the OQ process is recorded in a final PDF report saved at the end of the OQ protocol. If needed at any step, press **Cancel** to return to the last screen. The qualification can only be restarted by completing the full workflow and saving the report.

Before completing the OQ testing, ensure the checkbox for **Rapid Capture** on the main screen does not have a check mark. If the box is checked, please press the box to remove the checkmark. The OQ procedure must be completed with **Rapid Capture** turned off.

1. Enter the **Protocol Execution Date**, **Lab Name**, and **Lab Address**. The instrument serial number will automatically display. Press **Next**.

2. Enter your **Contact Name**, **Contact Email Address**, and **Contact Telephone Number**. Press **Next**.

3. Select an OQ reason of **Annual requalification**, **Software update/upgrade**, or **After repair**. Press **Next**.

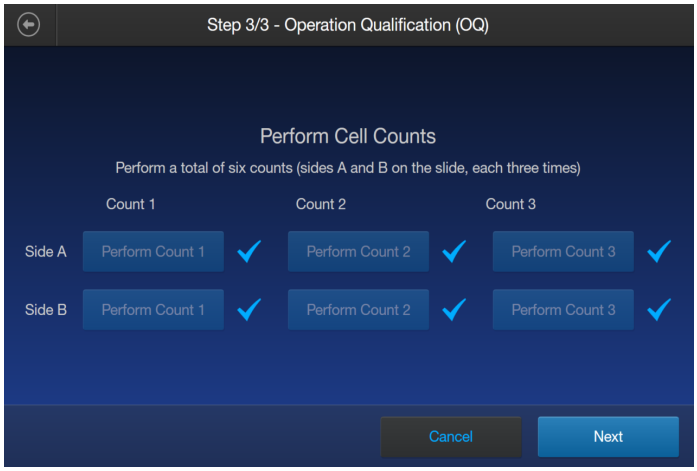
4. Verify pre-execution is completed by selecting **Yes** in **All Tests Completed** and entering your **User Initials** and the **Date**. Press **Next**.

5. Verify the instrument has successfully booted by selecting **Pass** or **Fail** and confirm the **Date & Time Format** are correct. Press **Next**.

6. Record if the software has been updated prior to OQ by selecting **Yes** or **No**. The instrument software version will automatically display. Press **Next**.

7. Enter the Part Number and Lot Number from the Countess™ 3/3 FL standard slide packaging. Press **Next**.

8. Perform a series of 6 counts of the standard slide (3 counts on each side). Insert side A of the standard slide and press the appropriate count number (**Perform Count 1**, **Perform Count 2**, or **Perform Count 3**). The instrument will automatically autofocus and take images in the background. After completing the 3 counts of side A, remove the slide and insert side B. Side B will only illuminate after removing the slide and insert side B. Insert side B of the standard slide and press the appropriate count number (**Perform Count 1**, **Perform Count 2**, or **Perform Count 3**). Press **Next**.



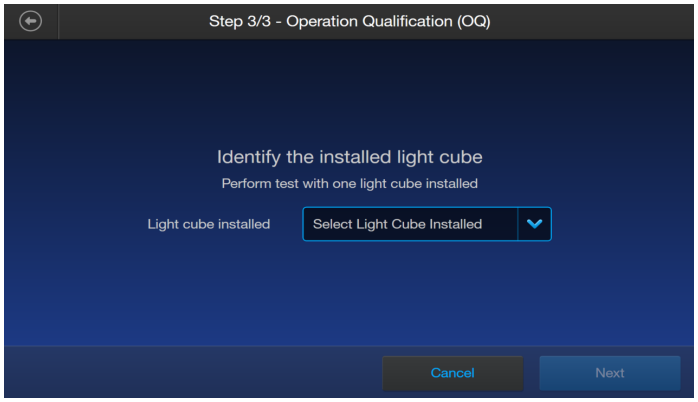
9. Total concentration results are displayed (not editable). **Pass** or **Fail** is automatically selected based on the recorded count concentrations shown above the counts. Press **Next**.

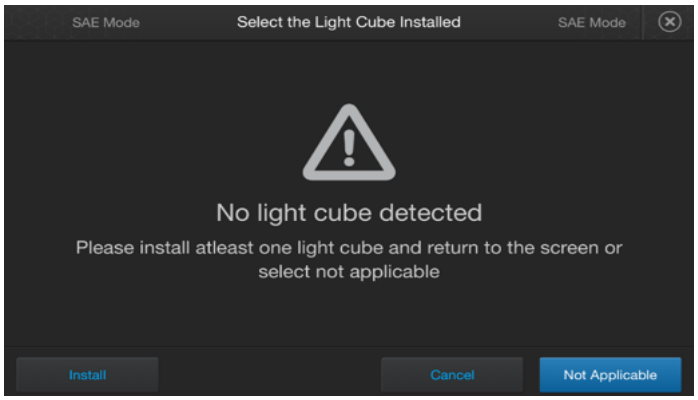
10. The mean, standard deviation, and coefficient of variation (Cv) are displayed (not editable). **Pass** or **Fail** is automatically selected based on the number shown under Cv. Press **Next**.

11. Total viability results are displayed (not editable). **Pass** or **Fail** is automatically selected based on the recorded % live results shown above the counts. Press **Next**.

12. The mean, standard deviation, and coefficient of variation (Cv) are displayed (not editable). **Pass** or **Fail** is automatically selected based on the number shown under Cv. Press **Next**.

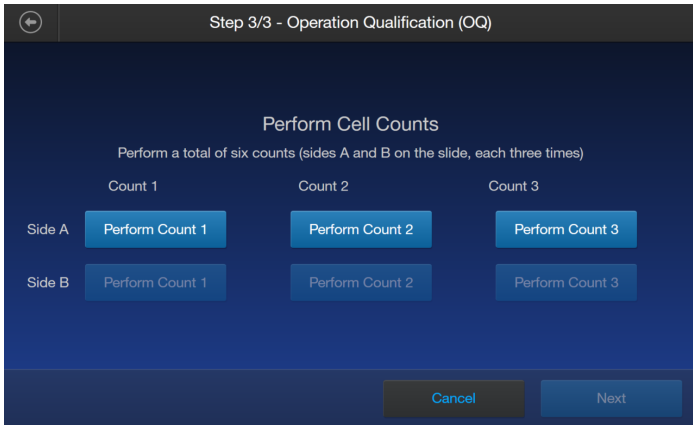
13. **(For the Countess™ 3 FL instrument)** Choose the appropriate light cube for testing. The OQ procedure tests fluorescent counts with a single light cube. If no cube is installed, press **Install**. If not required, press **Not Applicable**. If you have a Countess™ 3 instrument, proceed to step step 18.

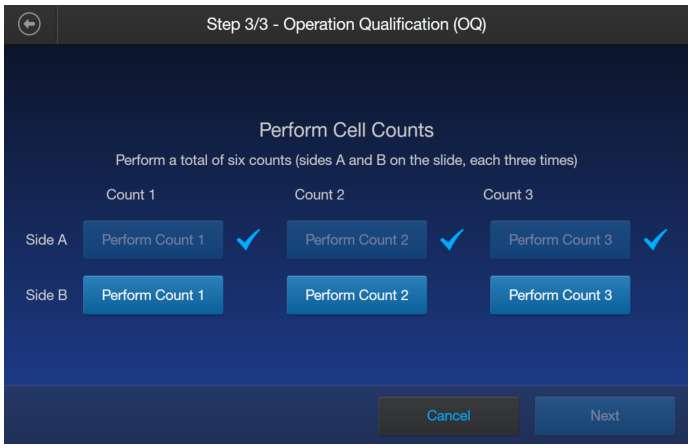


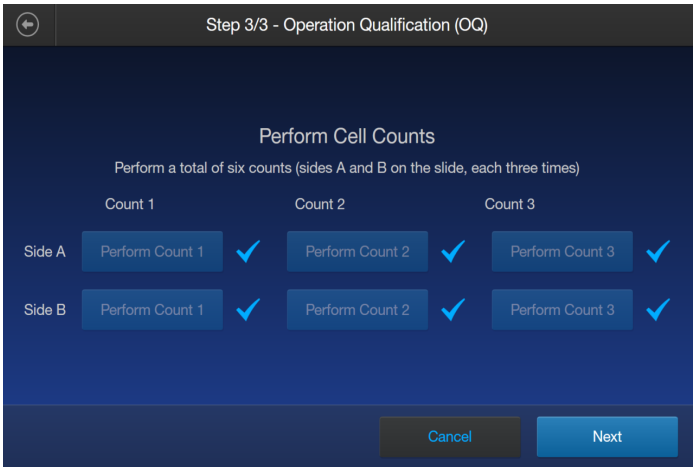


14. Select the appropriate light cube from the list of available cubes. Press **Next**.

15. Perform a series of 6 counts of the standard slide (3 counts on each side). Insert side A of the standard slide and press the appropriate count number (**Perform Count 1**, **Perform Count 2**, or **Perform Count 3**). The instrument will automatically autofocus and take images in the background. After completing the 3 counts of side A, remove the slide and insert side B. Side B will only illuminate after removing the slide and insert side B. Insert side B of the standard slide and press the appropriate count number (**Perform Count 1**, **Perform Count 2**, or **Perform Count 3**). Press **Next**.







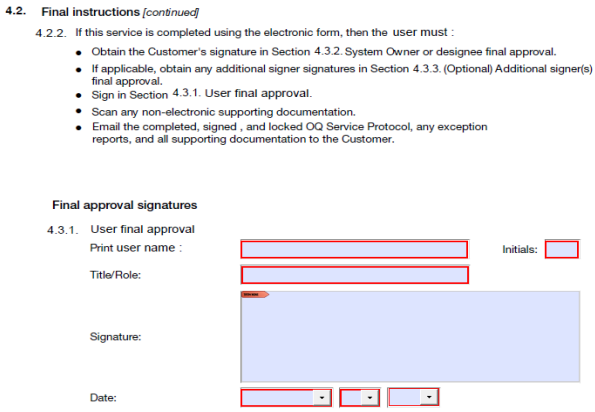
16. Total concentration results are displayed (not editable). **Pass** or **Fail** is automatically selected based on the recorded count concentrations shown above the counts. Press **Next**.

17. The mean, standard deviation, and coefficient of variation (Cv) are displayed (not editable). **Pass** or **Fail** is automatically selected based on the number shown under Cv. Press **Next**.

18. Confirm the tests are successfully completed by pressing **Yes** and entering your **User Initials** and the **Date**. Press **Save**.

19. Enter the name for the Qualification report and select the save location for the report and attachments. Press **Save**.

Note: Only the comment and signature sections of the exported PDF report are editable for verifying the report.



4.2. Final instructions *[continued]*

4.2.2. If this service is completed using the electronic form, then the user must :

- Obtain the Customer's signature in Section 4.3.2. System Owner or designee final approval.
- If applicable, obtain any additional signer signatures in Section 4.3.3. (Optional) Additional signer(s) final approval.
- Sign in Section 4.3.1. User final approval.
- Scan any non-electronic supporting documentation.
- Email the completed, signed , and locked OQ Service Protocol, any exception reports, and all supporting documentation to the Customer.

Final approval signatures

4.3.1. User final approval

Print user name : Initials:

Title/Role:

Signature:

Date:

Note: Ensure **Rapid Capture** is turned off before completing the OQ procedure.

Note: If any of the tests fail during the procedure, we recommend repeating the OQ workflow. Ensure the Countess™ 3 Standard Slide is free of debris by wiping with a lint-free wipe. If the OQ tests continue to fail, contact technical support at **thermofisher.com/support**.

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.
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 (only in the Countess™ 3 FL Automated Cell Counter) settings were not optimal.	BF-based counts rely on the BF image to be in focus. Adjust the focus and recount with BF-based count.
		FL-based counts are more sensitive and detect objects with low fluorescent intensity. Check the FL-based box to perform fluorescence counts to be sure they are included in the count.
		For cell count and cell viability assays performed in brightfield with BF-based counts, 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 with both BF-based and FL-based counts, 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 modes.

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.
	Capture settings were not optimal.	Gate out the debris using the size, brightness, and circularity sliders.

Observation	Possible cause	Recommended action
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°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 µm
Required sample volume	10 µL
Firmware	Countess™ Automated Cell Counting Platform Software
USB drive	32 Gigabytes (FAT32-, exFAT-, or NTSF-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 µL

Networking requirements

Configure the system behind a firewall. If outbound traffic is limited, the following firewall exceptions are required to support system features:

Firewall exception requirements			
URL	**Port**	**Purpose**	**Applies to…**
*.instrumentconnect.com	outbound 443	To support instrument management and identify	Thermo Fisher™ Connect Platform only
*.thermofisher.com	outbound 443	To support instrument management and identify	Thermo Fisher™ Connect Platform LAN connection
*.s3-us-east-1.amazonaws.com	outbound 443	To allow connection to the Thermo Fisher™ Connect Platform	Thermo Fisher™ Connect Platform only
*.iot-us-east-1.amazonaws.com	outbound 443	To allow connection to the Thermo Fisher™ Connect Platform	Thermo Fisher™ Connect Platform only
Allowed port requirements			
—	7443	To support instrument discovery Uses multicast address 224.0.0.251	LAN connection Direct connection
—	TCP 445 (SMB v3 or higher)	To support file sharing	LAN connection Direct connection
—	8443 (default)	Connection to SAE Administrator Console[1]	LAN connection Direct connection

[1] The SAE Administrator Console should be installed in a computer with a static IP address. The console opens in a browser (recommended Google Chrome) and communicates with the instrument. Communication between the SAE Administrator Console and the instrument uses the encrypted HTTPS protocol.

C

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	**AMQAX2000**
Countess™ 3 Power Adapter with four adaptor cords	1 each	A48207
Countess™ 3 USB Drive	1 each	A26774
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
Countess™ Test Beads (1×10^6 beads/mL)	1 mL	C10284
Trypan Blue Stain (0.4%)	2 × 1 mL	T10282
Countess™ Cell Counting Slander Slide	1 each	A51876
SafeCount™	1 each	A40008024

[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.

D

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	Count mode	BF-based or FL-based
Sample	F	Type	BF-Brightfield or FL-Fluorescence
	G	1:1 Stain corrected	Sample with 1:1 stain correction applied
	H	Pre-Dilution corrected	Sample with pre-dilution correction applied
	I	Total concentration	Concentration of the entire sample
	J	Total cells counted	Total number of cells counted in the sample
Brightfield/Live Dead	K	Live concentration	Concetration of just the "live" portion of the sample
	L	Live cells counted	"Live" cells counted in sample
	M	Dead concentration	Concentration of jus the "dead" portion of the sample
	N	Dead cells counted	"Dead" cells counted in sample
	O	Viability (%)	Percent viability of the sample based on trypan blue staining
	P	Live average size (µm)	Average size of "live" cells in microns
	Q	Dead average size (µm)	Average size of "dead" cells in microns
General	AE	Focus value	Focal position number

(continued)

Category	Column	Name	Description
General	AF	Focus motor value	Motor position in relation to focal position number
Brightfield/Live Dead	AG	BF light intensity	Brightfield light intensity value from 0-100%
	AH	BF LED intensity	LED intensity in brightfield mode
	AI	Live size min	Minimum size of "live" cells in microns
	AJ	LIve size max	Maximum size of "live" cells in microns
	AK	Live brightness min	"Live" adjustment slider value for minimum brightness
	AL	Live brightness max	"Live" adjustment slider value for maximum brightness
	AM	Live circularity min	"Live" adjustment slider value for minimum circularity
	AN	Live circularity max	"Live" adjustment slider value for minimum circularity
	AO	Dead size min	Minimum size of "dead" cells in microns
	AP	Dead size max	Maximum size of "dead" cells in microns
	AQ	Dead brightness min	"Dead" adjustment slider value for minimum brightness
	AR	Dead brightness max	"Dead" adjustment slider value for maximum brightness
	AS	Dead circularity min	"Dead" adjustment slider value for minimum circularity
	AT	Dead circularity max	"Dead" adjustment slider value for maximum circularity
Brightfield	AY	BF size min	Minimum size of "brightfield" cells in microns
	AZ	BF size max	Maximum size of "brightfield" cells in microns
	BA	BF brightness min	"Brightfield" adjustment slider value for minimum brightness
	BB	BF brightness max	"Brightfield" adjustment slider value for maximum brightness
	BC	BF circularity min	"Brightfield" adjustment slider value for minimum circularity
	BD	BF circularity max	"Brightfield" adjustment slider value for maximum circularity

(continued)

Category	Column	Name	Description
General	BQ	Protocol name	Current protocol used for the count
	BR	Software revision	Current software version used for count
	BS	Aggregation(%)	Total number of cells involved in an aggregate or aggregates vs. total cell count

E

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, visit **thermofisher.com/support**.

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/SF__19_308133-A_BMBL6_00-BOOK-WEB-final-3.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

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  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - 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 its affiliates 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 questions, contact Life Technologies at **www.thermofisher.com/support**.