invitrogen

Countess™ II FL automated cell counter

USER GUIDE

For fluorescence and brightfield applications

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Publication Number MAN0010644

Revision E.0

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Life Technologies Corporation | 22025 20th Ave SE Ste. 100 | Bothell, WA 98021
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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Revision history: Pub. No. MAN0010644

Revision	Date	Description
E.0	07 June 2019	Updated trypan blue handling instructions. Document converted to XML.
D.0	30 June 2017	Add info about edited profile indicator, save profile from results screen, dilution calculator, and reports.
C.0	01 September 2015	Remove instructions for Countess II, update UI for the new SW version, rebrand
B.0	12 December 2014	Correct technical specification for cell size
A.0	08 September 2014	New user guide

Contents

1 About this guide

Audience

This user guide is for laboratory staff operating, maintaining, and analyzing data using the Countess™ II FL Automated Cell Counter.

User documentation

The guides listed below are available for the Countess™ II FL Automated Cell Counter.

Guide	Pub. No.
Countess™ II FL Automated Cell Counters User Guide	MAN0010644
Countess™ II and Countess™ II FL Automated Cell Counters Quick Reference Card (QRC)	MAN0010826

Additional resources are available on the Countess™ Technical Resources page. Go to **www.thermofisher.com/countess** to access protocols, application notes, and tutorials.

Text and keyboard conventions

Text and keyboard conventions used in this user guide are listed below. For safety alert words and symbols used in this document, see "Safety alert words" on page 8.

Convention	Use
Bold	**Bold** text indicates user action. For example: Press **More**.
▶	Right arrow symbol (▶) indicates a menu choice, and separates successive commands you execute or select from a drop-down or shortcut menu. For example: Select **More** ▶ **Adjust**.

User attention words

Two user attention words appear this document. Each word implies a particular level of observation or action as described below.

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

Safety alert words

Four safety alert words appear in this document at points where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

CAUTION! Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for **IMPORTANT!** safety alerts, each safety alert word in this document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instruments (see **Safety symbols** in Appendix E).

2 Product information

Product contents

The Countess™ II FL Automated Cell Counter is shipped with the following components.

Component	Quantity
Countess™ II FL Automated Cell Counter (Cat. No. AMQAF1000)	1 each
Power Cord with 4 adaptor cords (for U.S./Canada/Taiwan/Japan, Europe, or UK)	1 each
Countess™ Cell Counting Chamber Slides (50 slides/box)	1 box
Countess™ II FL Disposable Slide Holder	1 each
Countess™ II FL Reusable Slide Holder	1 each
Countess™ II FL Light Cube Removal Tool	1 each
Countess™ II USB drive	1 each
Countess™ II FL Automated Cell Counter Quick Reference Card	1 each

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 “Installation“ on page 12 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™ II FL Automated Cell Counter.

Product description

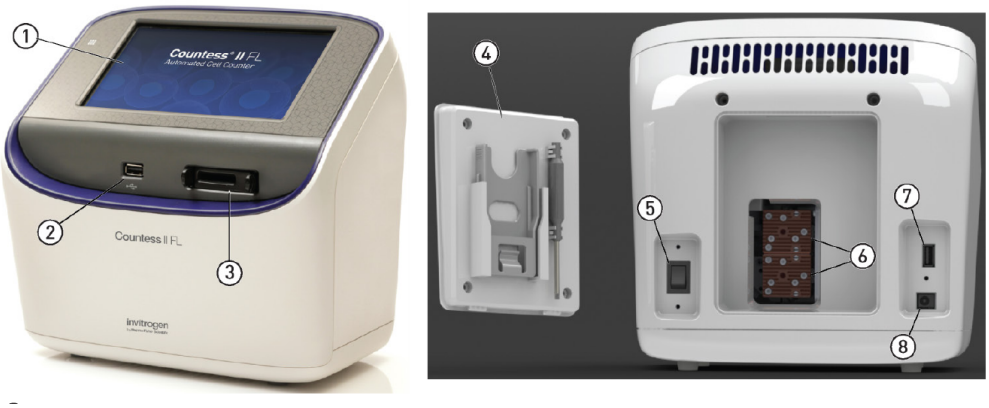
Countess™ II FL automated cell counter

The Countess™ II 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™ II FL Automated Cell Counter offers an intuitive user interface, and provides 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™ II FL Reusable Slides ("Prepare sample" on 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 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™ II FL Automated Cell Counter also provides information on cell size.
- In addition to the brightfield channel, the Countess™ II FL Automated Cell Counter can accommodate two interchangeable EVOS™ fluorescent light cubes ("EVOS™ light cubes" on page 64), enabling it to be used for multiple-fluorescence research applications.
- When equipped with EVOS™ light cubes, the Countess™ II 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). These assays are compatible with a wide variety of eukaryotic cells.

Instrument exterior components

Instrument exterior components



1. **Touch-screen display:** The 7-inch capacitive touch-screen display is the main user interface of the Countess™ II FL Automated Cell Counter. It contains the buttons for all instrument functions and displays data from the cell count.
2. **USB ports:** The USB ports allow 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-formatted USB drive for data transfer. If desired, you can plug in a USB mouse into the rear USB port for instrument control.

   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 and both USB drives cannot be accessed at the same time.
3. **Slide port:** The slide port is used to insert the analysis slide containing the sample into the counter.

   The Countess™ II FL instrument accepts both the disposable Countess™ Cell Counting Chamber Slides and the glass Countess™ II FL Reusable Slides via interchangeable, slide-specific carriers. For more information, see “Slide operation“ on page 20.
4. **Back panel:** The back panel of the Countess™ II FL Automated Cell Counter 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:** The 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:** The EVOS™ light cubes allow the Countess™ II FL Automated Cell Counter to analyze fluorescently labeled samples. The Countess™ II FL Automated Cell Counter can accommodate two fluorescent light cubes. For more information, see “EVOS™ light cubes“ on page 64.
7. **USB ports:** See 2 above.
8. **Power input jack:** The 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.

3 Getting started

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℃ (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 ("Instrument exterior components" on page 11).

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

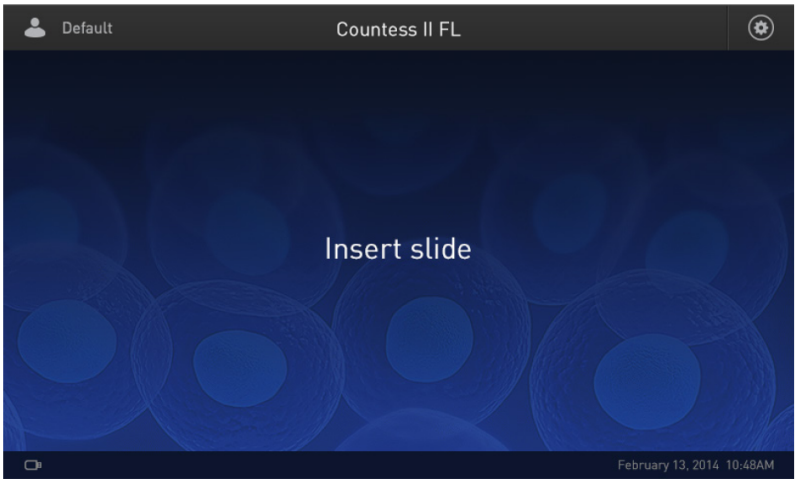
Install the instrument

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

1. Turn on the instrument by flipping the **power switch** on the back of the instrument ("Instrument exterior components" on page 11) to the **ON** position.

   The instrument initializes and displays the Home screen.



2. From the Home screen, you can proceed immediately to the assays by inserting a slide (Chapter 4, "Cell count and cell viability assays").

   Alternatively, you can change or add a profile (Step 3 on page 13) or change instrument settings (Step 4 on page 13).

3. To change the current profile or to add a new profile to the instrument, press the **Profiles** button in the upper left corner.

   Profiles allow you to create customized count preferences (i.e., gate counts based on cell size, brightness, circularity, and/or relative fluorescence intensity) ("Load profile" on page 14).

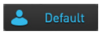
4. To change instruments settings, press the **Instrument Settings** button in the upper right corner.

   Instrument settings allow you to update the Countess™ II software, change the date and time, and install or change up to two EVOS™ light cubes (Chapter 8, "Instrument settings").

Load profile

Profiles screen

Profiles screen allows you to create and save up to 9 customized profiles. Each custom profile defines the count parameters (size, brightness, circularity, and fluorescence intensity) and automatic instrument functions (Auto Lighting and Auto FL Threshold) for a consistent and streamlined workflow.

- You can access the Profiles screen from the Home, Capture, Results, Advanced™, or Adjust screens.
- The current profile is displayed on the upper left corner of the Home, Capture, Results, Advanced™, or Adjust screens. 

- Automatic instrument functions (below) and count parameters ("Count parameters" on page 15) are defined in the Edit profile screen (see "Add/edit a profile" on page 16).
- The Default profile contains default count settings and cannot be edited.
- The count parameters specified in the selected profile are applied to all new cell counts.
- If you have already performed a count, loading a new profile from the Results screen applies the count preferences to the current counts results (total cells, viability etc.) and to all new counts.
- If you change any setting that is saved as part of the protocol (size, brightness, or circularity) on the Results screen, the profile name is appended with the (*) symbol. 

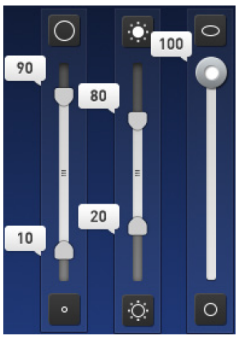
Automatic instrument functions

You can turn the Auto FL Threshold and Auto Lighting functions ON and OFF using the **Auto FL Threshold** and **Auto Lighting** checkboxes in the Edit profile screen ("Add/edit a profile" on page 16).

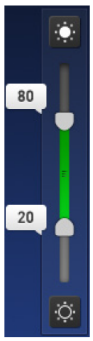
- **Auto FL Threshold:** Automatically applies threshold in fluorescence channels to subtract background fluorescence for improved analysis despite variable background levels between samples. This function is available only for instruments equipped with the optional EVOS™ light cubes.
- **Auto Lighting:** Automatically illuminates the sample in the brightfield for increased sample-to-sample consistency and decreased user-to-user variability.

Count parameters

Count parameters are adjusted in the Edit profile screen using the **parameter sliders**. Parameter sliders correspond to a single channel, which is selected using the **channel selection** radio buttons located above the sliders.



Size, brightness, and circularity sliders



Fluorescence intensity slider

- **Size:** As you move the slider up, the algorithm includes larger objects in the count. As you move the slider down, only the smaller objects are counted.
  = larger objects = smaller objects
- **Brightness:** As you move the slider up, the algorithm includes the brighter objects in the count. As you move the slider down, only the dimmest of objects are counted.
  = brighter objects = dimmer objects
- **Circularity:** As you move the slider up, the algorithm includes more objects with shapes other than circular in the count. As you move the slider down, only the objects that are perfect circles are counted.
  = less circular = more circular
- **Fluorescence intensity:** As you move the slider up, the algorithm includes the objects that fluoresce more brightly in the count. As you move the slider down, only the dimmest of objects are counted.
  = brightly fluorescent objects = dim or less fluorescent objects
- **Size, brightness, and fluorescence intensity** sliders are range sliders.

  To adjust the upper and lower boundaries without changing the data range, drag the slider by its middle section (i.e., the slider bar).

  To adjust only the upper or the lower boundary, move the upper or the lower handle in the desired direction. This will also change the range of values within which the cells are counted.
- The **circularity** slider only sets a single threshold value; cells that fall below the set value are counted, and cells that are beyond this range are excluded.

  To adjust the threshold for circularity, drag the slider in the desired direction.

Load a profile

1. Press the **Profiles** button located on the upper left corner of the screen to open the Profiles screen.
2. Press the desired profile to select, and then press **Load**.
   The instrument will load the count parameters specified in the selected profile and return to the **previous** screen.
3. To return to the previous screen without loading the new profile, press the **previous** button.
   The instrument will keep the saved profile, but return to the previous screen without loading it.

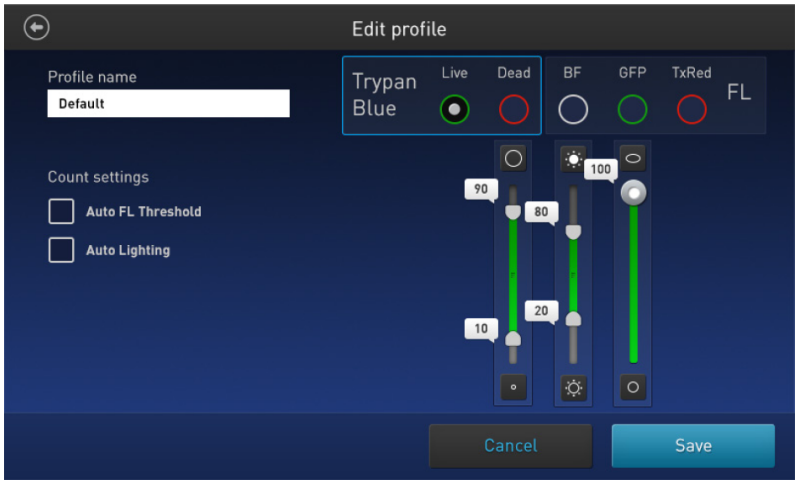
Add/edit a profile

1. Press the **Profiles** button located on the upper left corner of the screen to go to the Profiles screen.
2. To add or edit a new profile, select an empty or an existing profile, and then press **Edit**. The Edit screen for the selected profile opens.
   Note: The Default profile contains default count settings and cannot be edited.
3. Select or deselect the **Auto FL Threshold** checkbox to turn the Auto FL Threshold function **ON** or **OFF** ("Automatic instrument functions" on page 14).
   Note: This function is available only for instruments equipped with the optional EVOS™ light cubes.
4. Select or deselect the **Auto Lighting** checkbox to turn the Auto Lighting function **ON** or **OFF** ("Automatic instrument functions" on page 14).

Define count parameters for the brightfield channel:

To define the new count parameters in the brightfield channel:

1. From the **Trypan Blue** selection box, select the **Live** or **Dead** radio button.
2. Adjust the size, brightness, and circularity thresholds using the corresponding **parameter slider** ("Count parameters" on page 15).



Define count parameters for the fluorescent channels

1. To define the new count parameters for fluorescence assays (available only for instruments equipped with the optional EVOS™ light cubes):
   a. From the **FL** selection box, select the desired channel using the corresponding radio button.

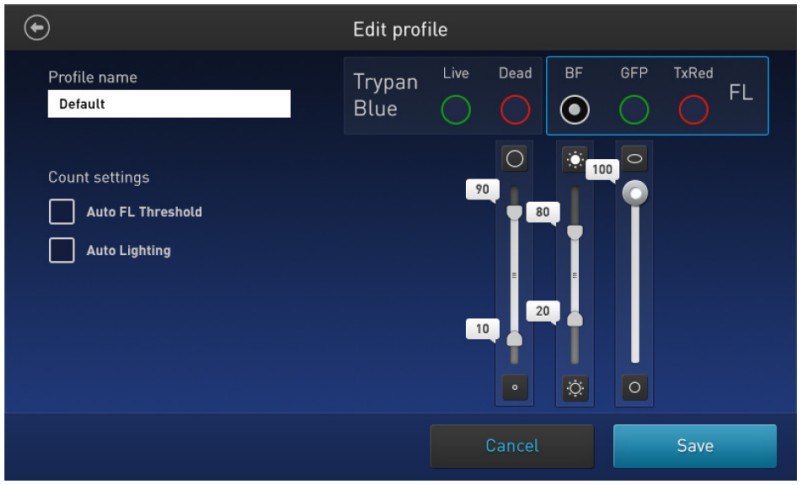
      The available options are **BF** (brightfield) and up to two fluorescence channels, depending on the light cubes installed (**GFP** and **TxRed** in the following example).

   b. Define the new count parameters for the selected channel using the corresponding **parameter slider** ("Count parameters" on page 15).

      Parameter sliders in the BF channel allow you to gate count results based on size, brightness, and circularity.

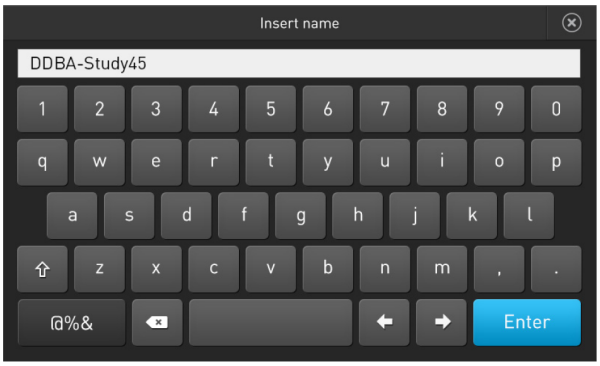
      The parameter slider in the selected fluorescence channel allows you to gate count results based on relative fluorescence intensity in that channel.

   c. Repeat for the remaining channels, as necessary.



Note: You can further adjust the size, brightness, or circularity parameters for the selected profile before or after performing a cell count, as needed. You can then save these additional changes to the count parameters to the current profile or as a separate profile directly from the Advanced™ screen (see "Save as new protocol" on page 29)

2. To assign a name to the new profile or to change the name of the existing profile, press the **Profile name** text box. The **alpha-numeric keypad** opens.



3. Type in the desired profile name using the alpha-numeric keypad.
   To enter symbols, press the **symbol (@%&)** key. To return to the alpha-numeric keypad, press **ABC**.

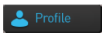
4. Press **Enter** to save the name and return to the Edit profile screen.
   To return to the Edit profile screen without saving the name, press the **close** button.

5. Press **Save** to save the new profile, and then press **Close** in the confirmation screen to return to the Profiles screen.
   To return to the Profiles screen without saving, press **Cancel**.

6. On the Profiles screen, press **Load**. The instrument will load the count parameters specified in the selected profile and return to the previous screen.

7. To return to the previous screen without loading the new profile, press the **previous** button. The instrument will keep the saved profile, but return to the previous screen without loading it.

Display of profile names

- The maximum display length for profile names on the screen is seven characters. 

- If the profile name is over seven display characters, the name is shortened to the first seven characters with "**…**" at the end. For example, "MyProfile" is shortened to "MyPro…". 

- If you change any setting that is saved as part of the protocol (size, brightness, or circularity) on the Results screen, the profile name is appended with the "**(*)**" symbol. 

  For example, "Count" becomes "Count (*)".
- When a profile with name of over four or five display characters is edited, only the first four or five characters is displayed and the name is appended with "**… (*)**". 

  For example, "MyProfile" becomes "MyPro…(*)".

Prepare sample

Recommendations

To obtain the best results, follow these recommendations:

- Ensure that the cell sample is homogeneously mixed.
- The measurement range extends from 1×10^4–1×10^7 cells/mL, but the optimal range is 1×10^5–4×10^6 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.
- Sterile filtering and centrifugation can be used to remove precipitates common within trypan blue solutions. Alternatively, avoid mixing and vortexing trypan blue stock solutions to allow precipitates to remain at the bottom of tube, thereby promoting more accurate cell counts. Also, precipitates can be reduced by gentle heating at 37°C for 10 minutes.

Load Countess™ chamber slide

1. Prepare the sample by adding 10 µL of your cell suspension to 10 µL of 0.4% trypan blue stain. 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.

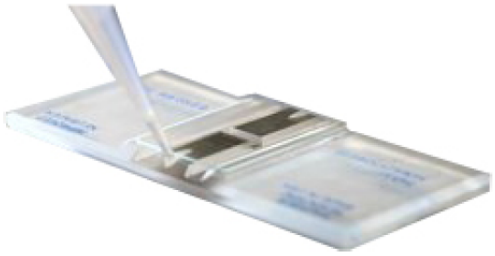
3. Let the sample mixture settle in the chamber for 30 seconds, and then insert the slide into the slide port (“Instrument exterior components“ on page 11). You will hear a soft click, if the slide is pushed in correctly.

4. To remove the slide, 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.

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

Load Countess™ II FL reusable slide

1. Before loading your sample into the Countess™ II FL Reusable Slide, place a cover slip on the counting chamber, making sure the cover slip is clean and free of grease.
2. Gently pipet 10 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.



3. After using the Countess™ II FL 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.

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

Slide operation

The Countess™ II FL instrument accepts both disposable Countess™ Cell Counting Chamber Slides and glass Countess™ II FL Reusable Slides on interchangeable, slide-specific carriers.

Countess™ cell counting chamber slide

1. To use the plastic, disposable Countess™ Cell Counting Chamber Slide with the Countess™ II FL Automated Cell Counter, insert the slide carrier (black, see image) into the slide port of the instrument until it clicks into place.

   Note: The Countess™ II FL Automated Cell Counter is shipped with the disposable slide carrier already installed

2. Load the chamber slide with your sample as described in "Load Countess™ chamber slide" on page 19, and then insert the slide into the slide carrier in the slide port until it clicks into place.

3. To remove the slide, 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.

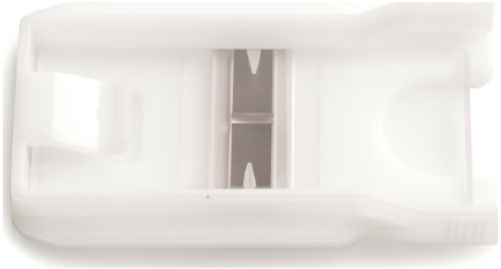
4. *Optional*: To remove the slide carrier, gently squeeze the tabs and pull the carrier completely out of the instrument.

   Note: You can store the slide carrier behind the access panel on the back of the instrument ("Instrument exterior components" on page 11).

Countess™ II FL reusable slide

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

2. Remove the reusable slide carrier (white) from inside of the back panel.



3. Load the reusable glass slide with the sample as described in "Load Countess™ II FL reusable slide" on page 20, and place the loaded slide into the white slide carrier.

4. Insert the carrier and reusable slide assembly into the slide port, and gently push into the instrument until it clicks into place.

5. To remove the slide, 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.

6. *Optional*: To count the second sample present on the reusable slide, simply remove the slide from the carrier, rotate, and reinsert the slide into the carrier so that the second sample is aligned with the sample viewing hole.

   Note: You can store the slide carrier behind the access panel on the back of the instrument ("Instrument exterior components" on page 11).

4 Cell count and cell viability assays

Count cells in brightfield

Capture and count

1. Prepare the sample by adding 10 µL of your cell suspension to 10 µL of 0.4% trypan blue stain. Mix the sample mixture well by pipetting up and down a few times.

2. Load 10 µL of the sample mixture per chamber into the sample slide as described in "Load Countess™ chamber slide" on page 19. Let the sample mixture settle for 30 seconds.

3. *Optional*: Press the **Profiles** Default button and load the desired profile as described in "Load a profile" on page 16.

4. Insert the sample slide into the slide port ("Instrument exterior components" on page 11), 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: To turn off the Auto Lighting function, see "Define count parameters for the fluorescent channels" on page 17.

6. *Optional*: To manually adjust the focus, press the **Focus™** button, and then use the **Focus™ slider** to bring your sample into focus as described in "Set nominal focus" on page 57.

7. Press the **Set** Set button to set the focus and collapse the focus controls.

   Once the focus has been set, the Set button on the focus slider becomes inactive, confirming that the focus setting has been stored.

   Note: If needed, **Zoom** in on the image to adjust focus or lighting.

8. *Optional*: Set exposure using the **light source slider**.

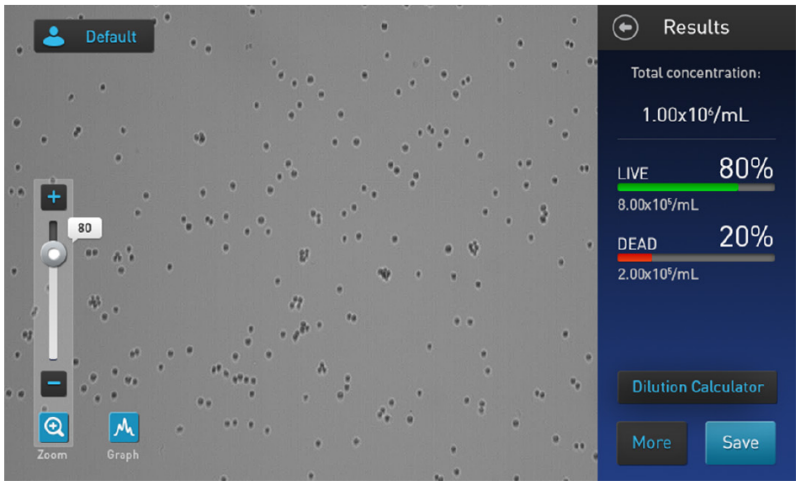
   The light source slider controls the LED intensity, camera gain, and exposure time and allows you to adjust the image brightness.

   Note: If your instrument is equipped with an EVOS™ light cube, first press **Adjust**, and then select **brightfield** (white circle) as the light source. Set the exposure, then press **Done** to return to the Capture screen.

9. Press **Capture**.

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

   The instrument temporarily 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 25.



Next steps

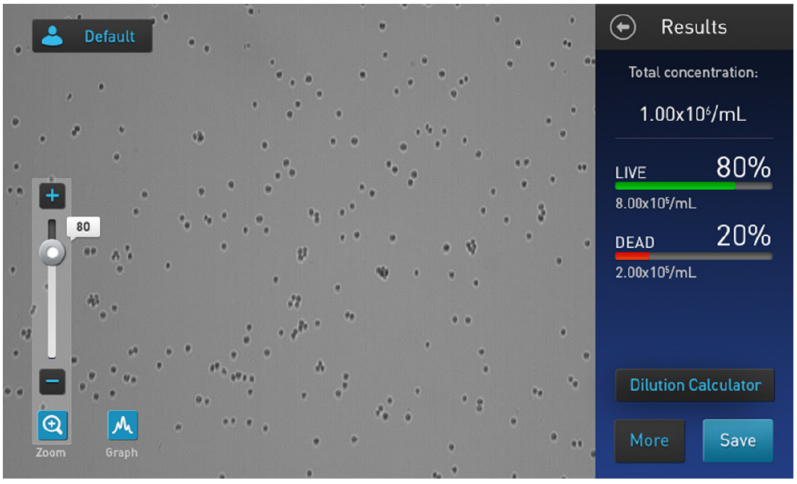
- To identify the objects (i.e., cells) counted as Live or Dead, press **More** to go to the Advanced™ screen ("Identify objects counted" on page 26).
- To see the distribution of live and dead cells in a graphical format, press the **Graph** button ("Graph count results" on page 27).
- To gate the results by object size, brightness, or circularity, first press **More** to open the Advanced™ screen, then press **Adjust** to go to the Adjust screen ("Gate count results" on page 27).

  Note: You can save the changes you make to the size, brightness, or circularity parameters in the Adjust screen to the current profile or as a separate profile directly from the Advanced™ screen (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 **Dilution Calculator** to open the Dilution Calculator application (Chapter 6, "Dilution calculator")
- To permanently save the results, press **Save** (Chapter 7, "Save results").
- To perform a new count, remove the slide and reinsert it second chamber first into the instrument, or 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.

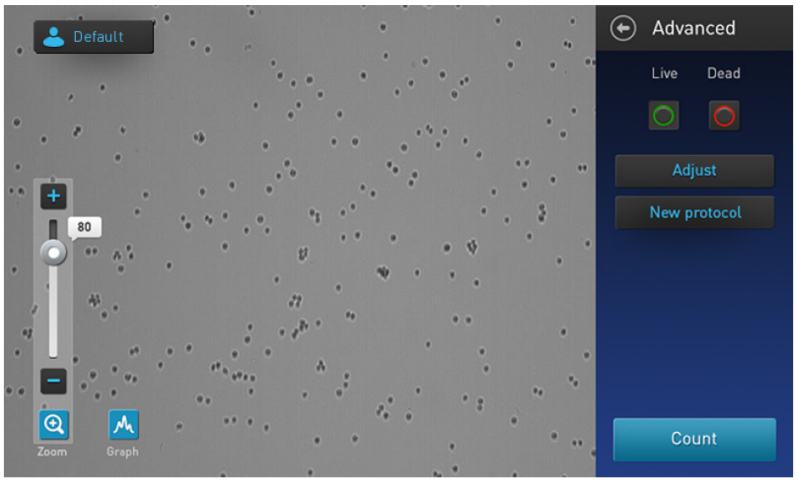
Identify objects counted

Advanced™ screen

The Advanced™ 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 ("Gate count results" on page 27).

Identify live and dead cells

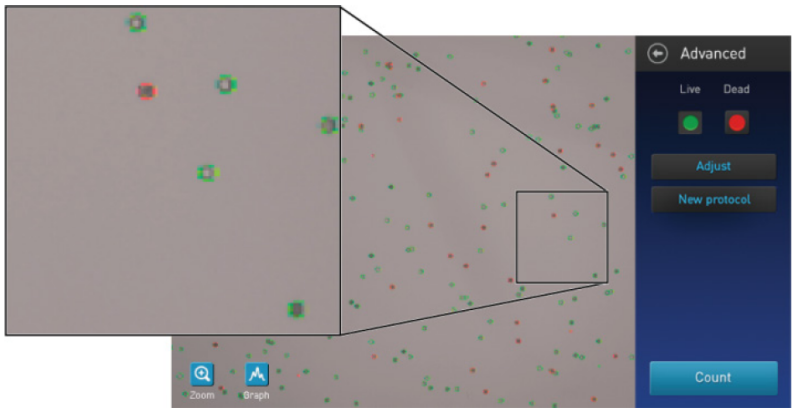
1. On the Results screen, click **More.** The Advanced™ screen opens.



2. To identify the cells that are included in the count as live, press the **Live** toggle button. Live cells will be circled in green on the screen.

   To identify the cells that are included in the count as dead, press the **Dead** toggle button. Dead cells will be circled in red on the screen.

   Note: You may select either or both options. In the following example, both **Live** and **Dead** buttons are pressed and live and dead cells are marked with green and red circles, respectively.



3. To unmark the cells identified as live (green) or dead (red) on the screen, press the **Live** or the **Dead** toggle button again, respectively.

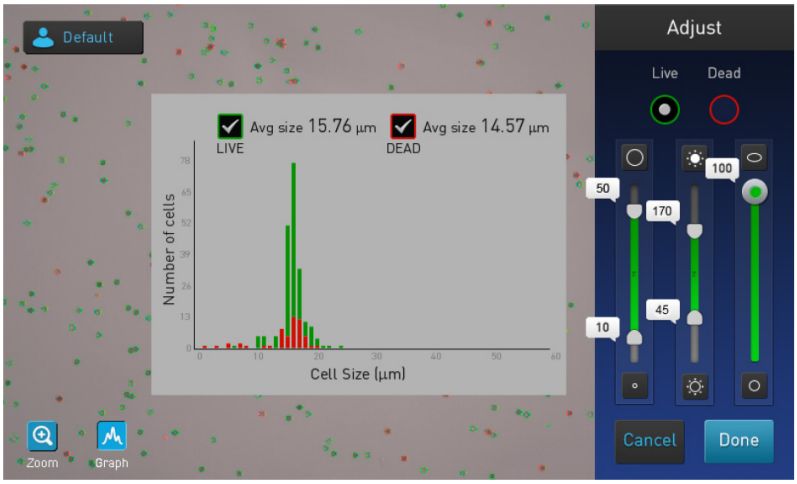
Graph count results

View graph

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 Graph on Results, Advanced™, and Adjust screens.

1. To view the graph showing the distribution live and/or dead cells based on cell size, press the **Graph** button.



2. To view the distribution of only the live or dead cells, check the corresponding **Live** or **Dead** check box on the graph.

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

3. *Optional*: 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 graph, press the **Graph** button again.

Gate count results

Adjust screen

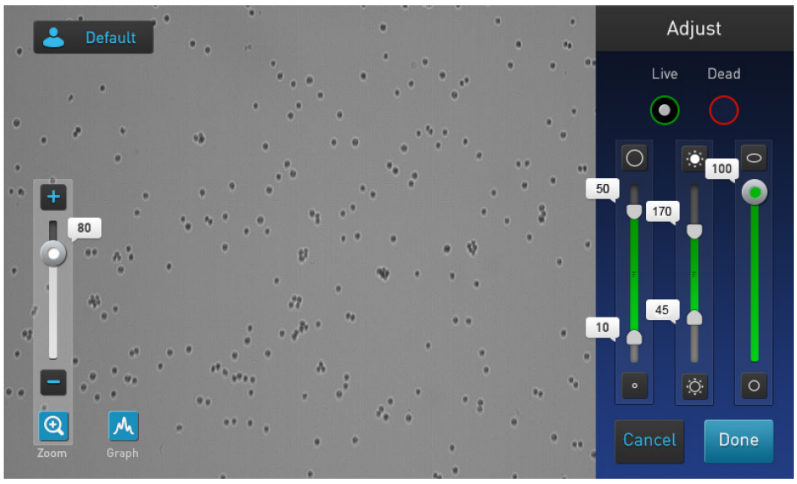
The Adjust screen for cell count and cell viability assays in the brightfield channel contains the controls for gating results based on size, brightness, and circularity. You can adjust the count parameters before or after performing a count, and save these changes to the current profile or as a separate profile ("Save as new protocol" on page 29).

Gate count results

1. On the Results screen, press **More** to open the Advanced™ screen.

2. *Optional*: Press the **Live** and/or the **Dead** button to identify the cells in the selected population ("Identify objects counted" on page 26).

3. On the Advanced™ screen, press **Adjust** to open the Adjust screen.



4. *Optional*: Press the **Graph** button to view the distribution of cells (live and/or dead) based on size as you gate the count results ("Save as new protocol" on page 29).

5. Select the channel **(Live** or **Dead)** you wish to gate.

6. Using the **size**, **brightness**, and **circularity 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.

7. When finished, press **Done** to save the changes to count parameters and return to the Advanced™ screen.

   Press **Cancel** to return to the Results screen without saving the changes.

8. On the Advanced™ screen, press **Count** to recalculate your results with the new count parameters.

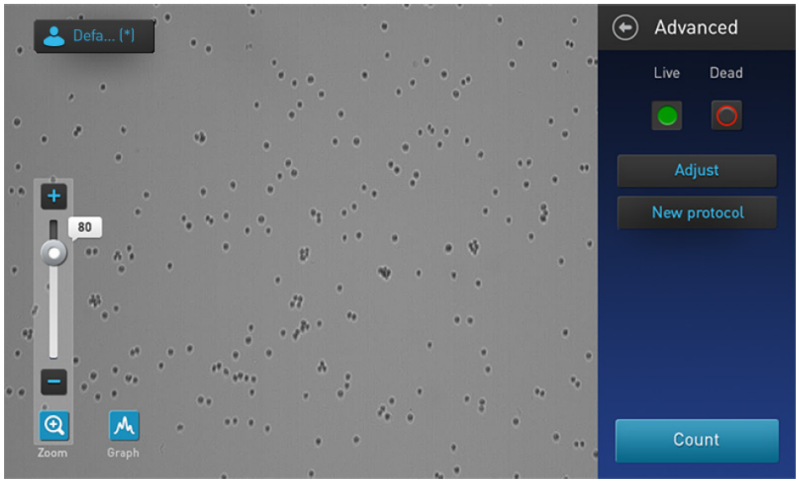
9. To save the changes to size, brightness, or circularity parameters to the current profile or to create a profile with the new count parameters, see "Save as new protocol" on page 29.

10. To permanently save your results, see Chapter 7, "Save results".

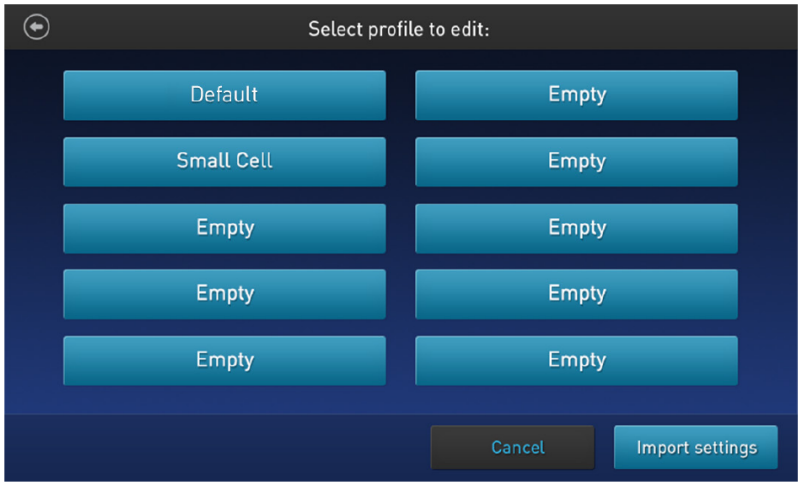
Save as new protocol

Edit and save profile as new protocol

1. If you have made any changes to the count parameters before or after performing a count, the displayed profile name is appended with the (*) symbol and the Advanced™ results screen displays the **New protocol** button, which allows you to save the changes to the current profile or as a separate protocol.



2. To save the changes to the count parameters to the current profile or to create a new profile with the edited parameters, press the **New protocol** button. The Select profile to edit screen opens.



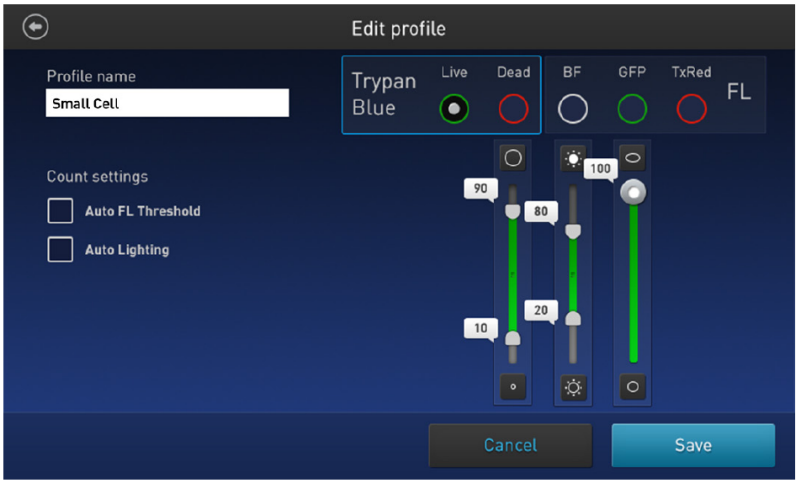
Note: By default, the current profile button is selected on the Select profile screen. If you are using the Default profile for the count, no profile button is selected on this screen.

3. Select the profile you wish to edit, then press **Import settings**.

Note: You can select only a previously saved or an empty profile. The Default profile cannot be edited.

4. The Edit profile screen opens and displays the edited count parameters from the Adjust screen (“Gate count results“ on page 27).

   Note: If you have selected a profile that had been previously saved, the name of that profile populates the Profile name text box by default. Otherwise, the textbox remains empty.



5. To change the name of the selected profile, press the **Profile name** text box and enter the desired name using the **alpha-numeric keypad** as described in “Display of profile names“ on page 18.

6. *Optional*: If desired, make additional changes to the profile and the count parameters as described in “Add/edit a profile“ on page 16.

7. Click **Save** to save the new profile settings and return to the Results page for the last count. The profile name will be displayed without the (*) symbols.

   Click **Cancel** to return to the Results page for the last count without saving the changes to the profile. The profile name will be displayed with the (*) symbol, indicating that the count parameters for the selected profile had been altered, but not yet saved.

5 Fluorescence assays

Count cell fluorescence

Overview

Countess™ II 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™ II FL Cell Counter, see “Change light cube“ on page 54.

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™ chamber slide“ on page 19. Let the sample mixture settle for 30 seconds.
3. *Optional*: Press the **Profiles** Default button located on the upper left corner of the screen to open the Profiles screen and load the desired profile as described in “Load profile“ on page 14.
4. Insert the sample slide into the slide port (“Instrument exterior components“ on page 11), 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.

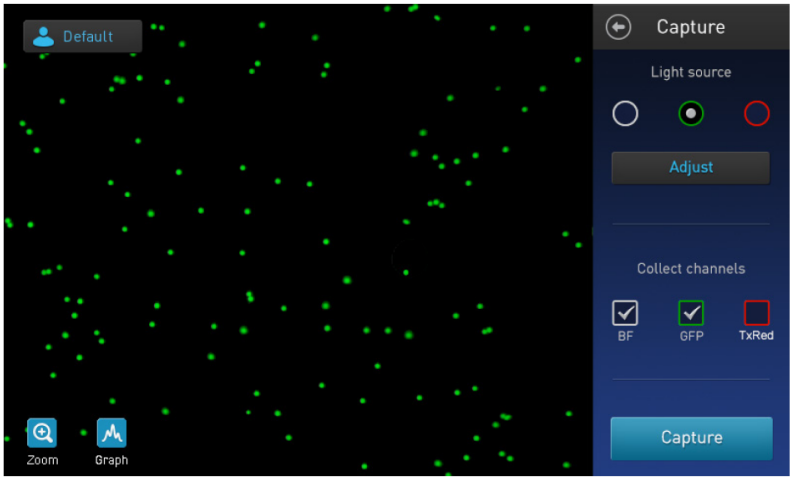
   Note: To turn off the Auto Lighting function, see “Define count parameters for the fluorescent channels“ on page 17.

6. *Optional*: To manually adjust the focus, press the **Focus™** button and use the **Focus™ slider** to bring your sample into focus as described in "Set nominal focus" on page 57.

7. Press the **Set** button to set the focus and collapse the focus controls. Once the focus has been set, the Set button on the focus slider becomes inactive, confirming that the focus setting has been stored.

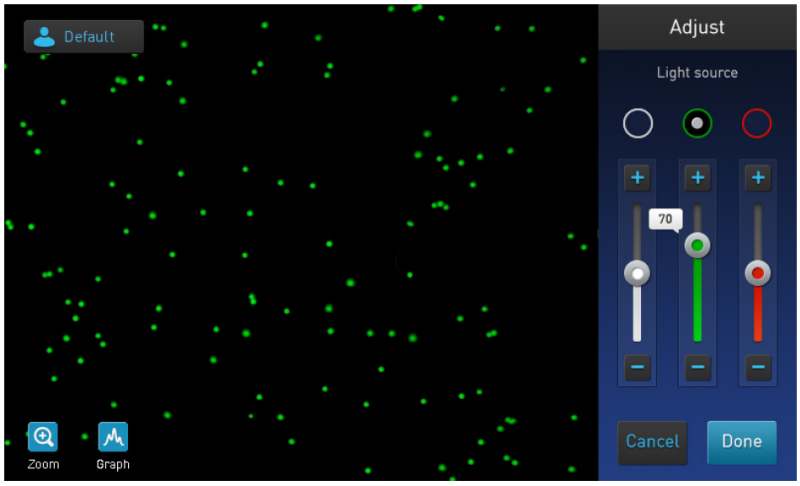
8. To view your sample under a different light source, press the desired **Light source** button. The instrument displays the sample in the selected channel (brightfield or fluorescent).

   In the example below, the sample is displayed in the GFP 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 on page 32–11 on page 33); they do not determine which channels are used for capturing the image.

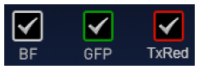
9. To set exposure, press **Adjust** to go to the Adjust screen.



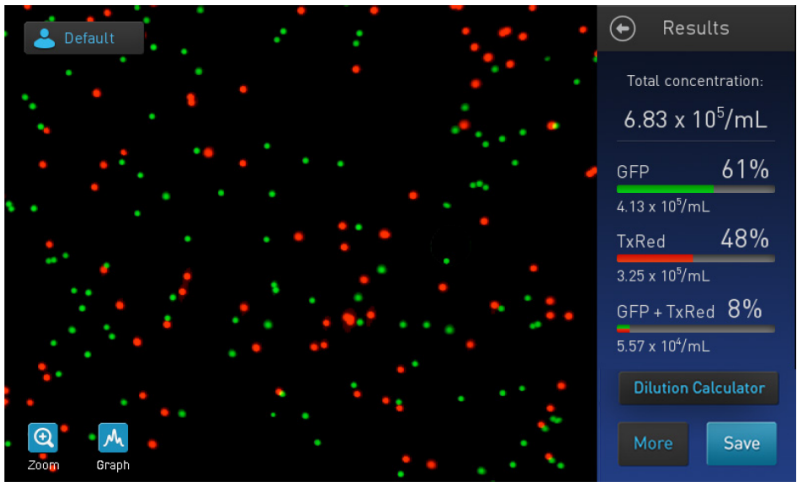
10. Press the **light source** button for the channel you wish to set exposure and adjust the exposure using the **light source slider**. Repeat the procedure for the remaining channels, if desired.

11. After setting the exposure, press **Done** to return to the Capture screen.
    To return to the Capture screen without changing the exposure, press **Cancel**.

12. On the Capture screen, select the **Collect channels** check boxes for the channels you wish to capture.



13. Press **Capture**.
    The instrument temporarily captures the image and displays the results (total concentration, percentage and concentration of cells counted in each fluorescence channel). For more information, see "View results" on page 34.



Next steps

- To identify the objects (i.e., cells) counted in each channel, press **More** to go to the Advanced™ screen ("Identify objects counted" on page 34).
- To see the distribution of cells counted through each channel in a graphical format, press the **Graph** button ("Graph count results" on page 36).
- To gate the results by object size, brightness, circularity, or relative fluorescence intensity, first press **More** to open the Advanced™ screen, then press **Adjust** to go to the Adjust screen ("Gate count results" on page 37).

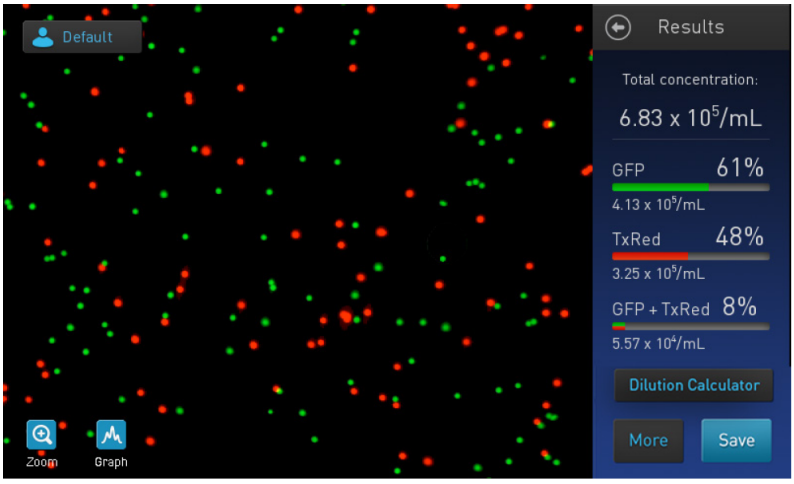
  Note: You can save the changes you make to the size, brightness, circularity, and relative fluorescence intensity parameters in the Adjust screen to the current profile or as a separate profile directly from the Advanced™ screen (see "Save as new protocol" on page 39).
- To calculate the volume of cell sample and buffer needed to reach a desired concentration based on the count results, press **Dilution Calculator** to open the Dilution Calculator application (Chapter 6, "Dilution calculator")

- To permanently save the results, press **Save** (Chapter 7, "Save results").
- To perform a new count, remove the slide and reinsert it second chamber first into the instrument, or 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.
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

Advanced™ screen

The Advanced™ 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.

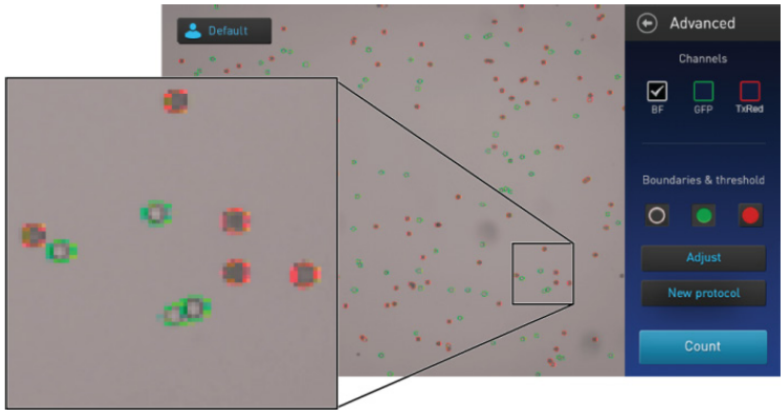
Identify cells counted in fluorescence assays

1. On the Results screen, click **More** to open the Advanced™ screen.

2. *Optional*: To view your sample under a specific light source (brightfield and/or fluorescent), select the desired **Channels** checkbox (brightfield in the example above). You may display your sample in any or all of the available channels.

3. To identify the cells that are counted in a specific channel, press the corresponding **boundaries** button. Cells counted in the selected channel will be circled on the screen with the same color as the selected channel.

   In the example below, both the **GFP** and **TxRed boundaries** buttons are pressed and the cells counted in the GFP and TxRed channels are marked with green and red circles, respectively.



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

Graph count results

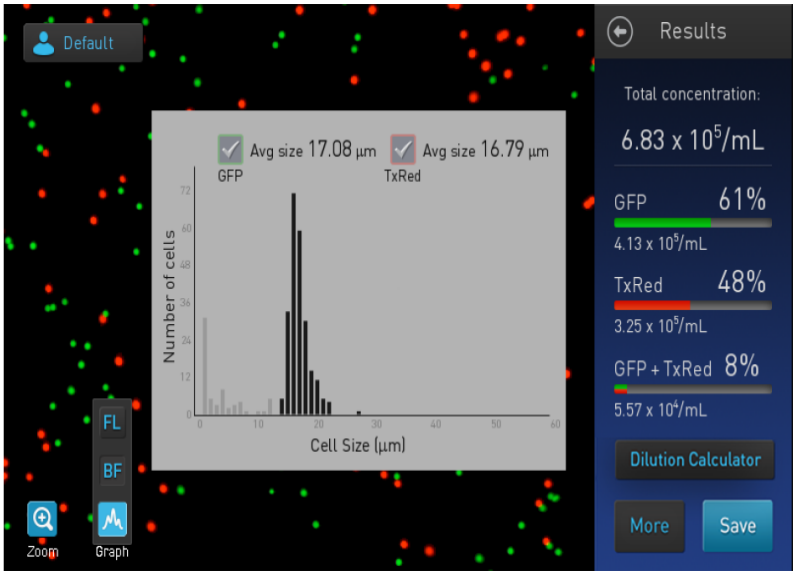
View graph for cell fluorescence assays

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 Graph on Results, Advanced™, and Adjust screens.

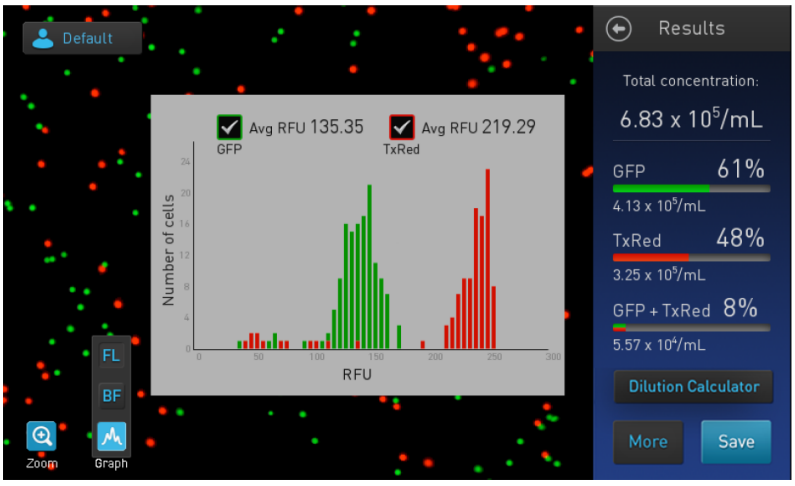
1. To view the graph showing the distribution of cells based on size, press the **Graph** button, and then select **BF** (brightfield).

   The graph 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.



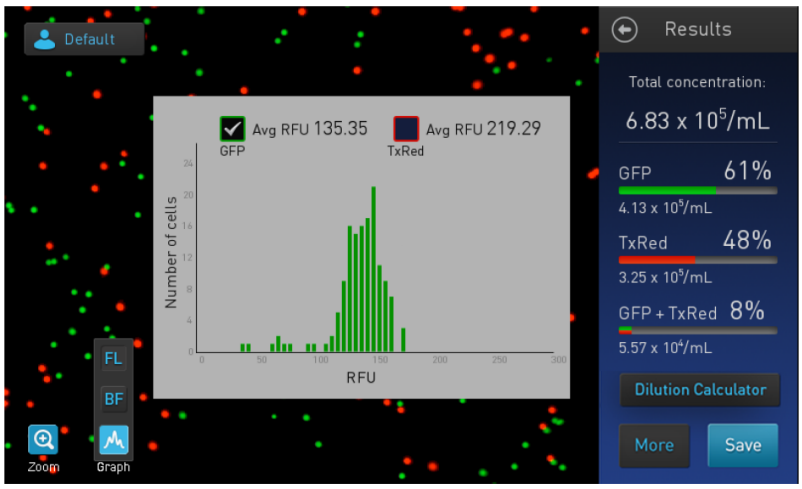
2. To view the distribution of cells based on relative fluorescence intensity, press the **Graph** button, and then select **FL** (fluorescence).

   The graph displays the distribution of cells based on fluorescence intensity.



3. To remove the cells counted in a specific channel from the graph, uncheck the corresponding **channel** check box on the graph.

The graph 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 graph, re-check the corresponding **channel** check box.

5. To close the graph, press the **Graph** button again.

Gate count results

Adjust screen

The Adjust 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 profile or as a separate profile (“Save as new protocol“ on page 39).

Gate count results

1. On the Results screen, press **More** to open the Advanced™ screen.

2. *Optional*: To view your sample under a specific light source (brightfield and/or fluorescent), select the desired **Channels** checkbox on the Advanced™ screen (“Identify cells counted in fluorescence assays“ on page 35).

3. *Optional*: Press the desired **boundaries** buttons on the Advanced™ screen to identify the cells counted in the corresponding channel (“Identify cells counted in fluorescence assays“ on page 35).

4. Press **Adjust** to open the Adjust count parameters screen, which contains 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.

5. *Optional*: Press the **Graph** button to view the distribution of cells based on size or fluorescence intensity ("Graph count results" on page 36).

6. Select the **brightfield channel** (white circle) to adjust the thresholds for size, brightness, and circularity using the **size**, **brightness**, and **circularity** sliders.

7. Select the desired **fluorescence channel** (colored circles) 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.

8. When finished, press **Done** to save the changes to count parameters and return to the Advanced™ screen.

   Press **Cancel** to return to the Results screen without saving the changes.

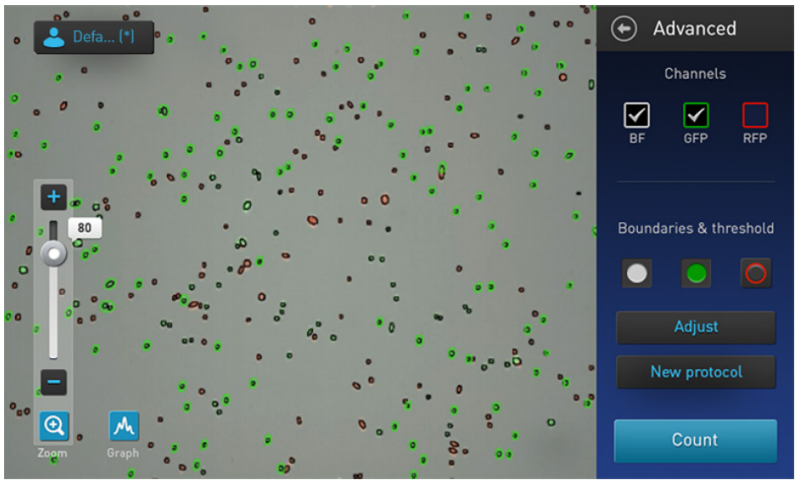
9. On the Advanced™ screen, press **Count** to recalculate your results with the new count parameters.

10. To save the changes to size, brightness, or circularity parameters to the current profile or to create a profile with the new count parameters, see "Save as new protocol" on page 39.

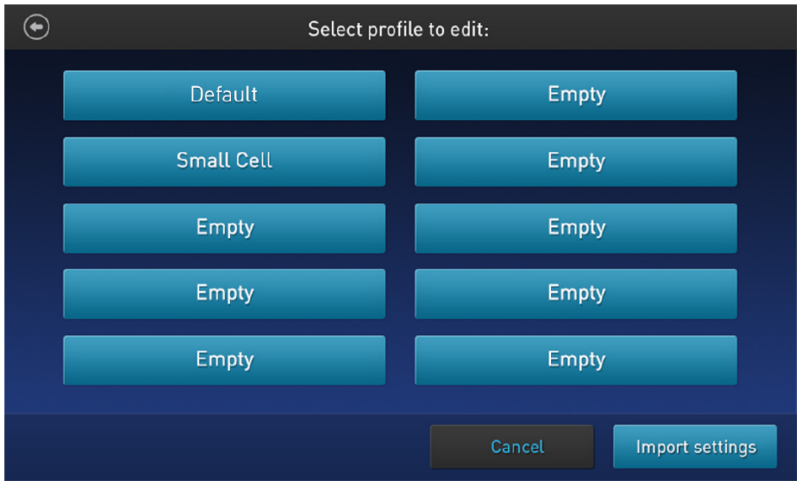
Save as new protocol

Edit and save profile as new protocol

1. If you have made any changes to the count parameters, the displayed profile name is appended with the (*) symbol and the Advanced™ results screen displays the **New protocol** button, which allows you to save the changes to the current profile or as a separate protocol.



2. To save the changes to the count parameters to the current profile or to create a new profile with the edited parameters, press the **New protocol** button. The Select profile to edit screen opens.



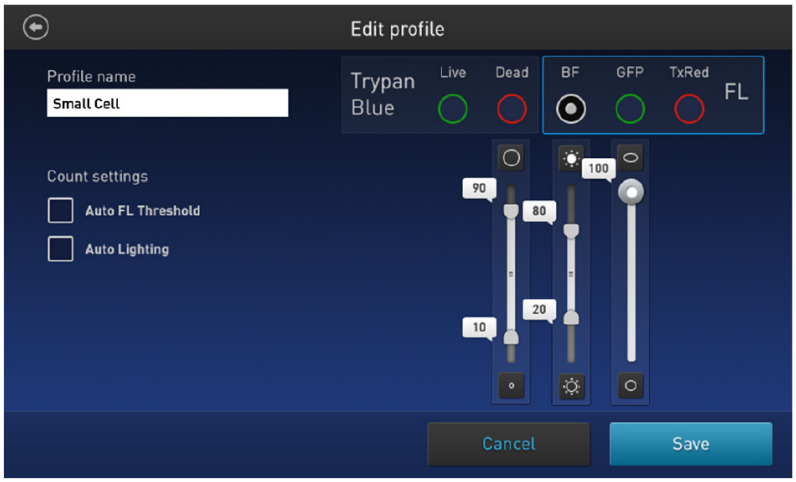
Note: By default, the current profile button is selected on the Select profile screen. If you are using the Default profile for the count, no profile button is selected on this screen, because the Default profile cannot be edited.

3. Select the profile you wish to edit, then press **Import settings**.

Note: You can select only a previously saved or an empty profile. The Default profile cannot be edited.

4. The Edit profile screen opens and displays the edited count parameters from the Adjust screen ("Gate count results" on page 37).

   Note: If you have selected a profile that had been previously saved, the name of that profile populates the Profile name text box by default. Otherwise, the textbox remains empty.



5. To change the name of the selected profile, press the **Profile name** text box and enter the desired name using the **alpha-numeric keypad** as described in "Display of profile names" on page 18.

6. *Optional*: If desired, make additional changes to the profile and the count parameters as described in "Define count parameters for the fluorescent channels" on page 17.

7. Click **Save** to save the new profile settings and return to the Results page for the last count. The profile name will be displayed without the (*) symbols.

   Click **Cancel** to return to the Results page for the last count without saving the changes to the profile. The profile name will be displayed with the (*) symbol, indicating that the count parameters for the selected profile had been altered, but not yet saved.

6 Dilution calculator

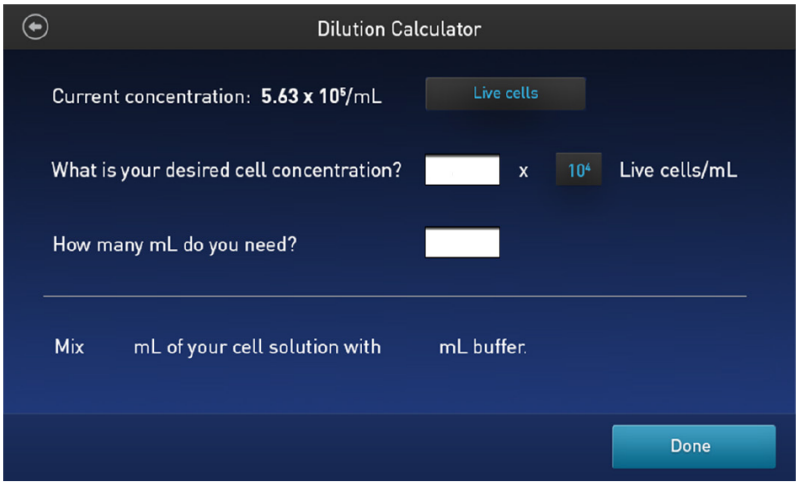
Calculate dilution

Dilution calculator

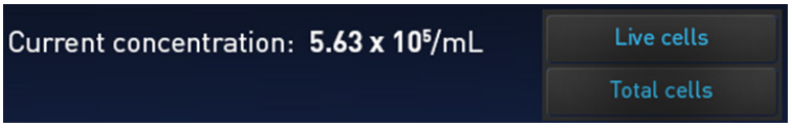
Dilution calculator function allows you to calculate the volume of cell sample and buffer needed to reach a desired concentration using the count results.

Calculate dilution

1. On the Results screen, press **Dilution Calculator** to open the Dilution calculator screen.



2. Press the **cell type** button located to the right of the current concentration, then select the count result you wish to use for the dilution calculation from the dropdown. The **current concentration** changes to reflect the results for the cell type selected.



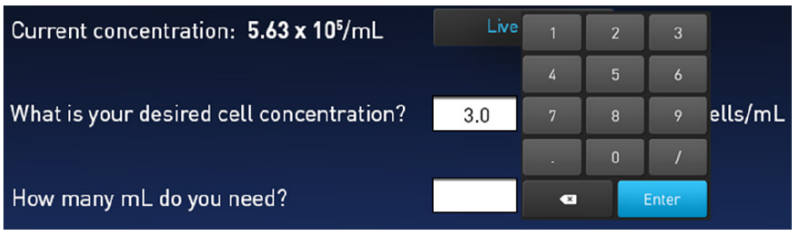
Available **Cell type** options are:

- For cell count and viability assays in brightfield:
  Live cells or **Total cells**.
  By default, **Live cells** is selected.

- For fluorescence assays: **Channel 1, Channel 2**, or **Total cells** (Channel 1 + Channel 2).
  By default, **Total cells** is selected.

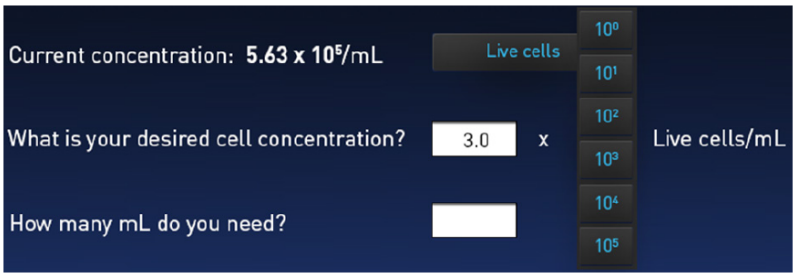
Note: Channel 1 and Channel 2 name displayed in the dropdown menu depends on the EVOS™ light cube installed. In the following example, GFP and TxRed cubes have been installed and the dropdown displays GFP and TxR.

3. Enter the **desired cell concentration** (What is your desired cell concentration?):
   a. Press the **value** text box, then enter the value using the number pad.



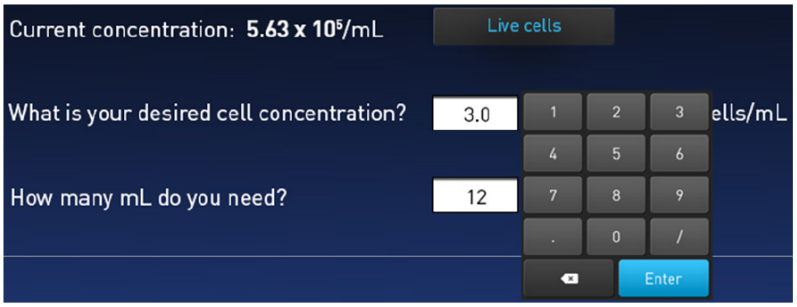
   You can enter a one digit to the left of the decimal separator (integer part) and one to the right (fractional part). If you do not enter the fractional part, the software enters a 0 by default.
   Press **Enter** or touch anywhere outside the number pad to close it.

   b. Press the **exponent** button, then select the exponent value for the desired concentration.



   By default, the exponent is (n–1), where n = the exponent value of the current count. The maximum selectable exponent is same exponent value as the current count.
   Pressing an exponent button selects that exponent and closes the window. Pressing anywhere outside the window keeps the previously selected exponent and closes the window

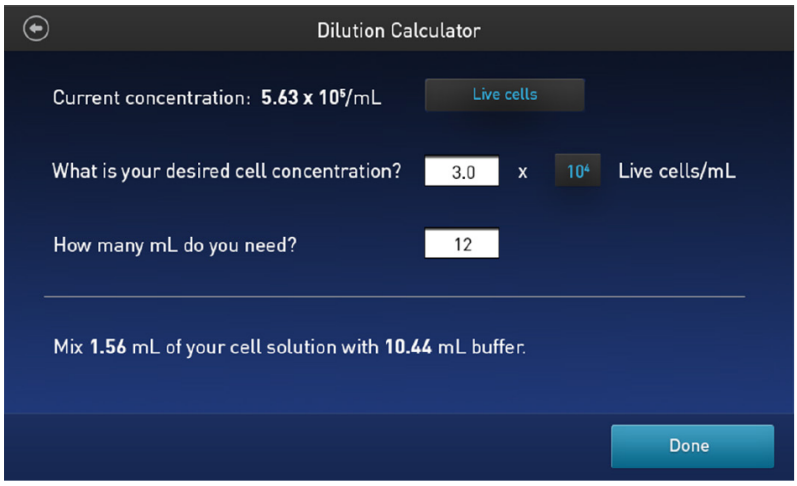
4. Press the **total volume** text box (How many mL do you need?), then enter the total volume of the sample you wish to have at the new concentration using the number pad.



By default, the total volume box is blank. The maximum volume you can enter is 999.9 mL and you can use only a single decimal.

Press **Enter** or touch anywhere outside the number pad to close the number pad.

5. When you have made valid entries for cell type, desired cell concentration, and total volume, and closed the last popup window, the bottom line of the dilution calculator displays the volumes of cell solution and buffer needed.



If you enter a combination of values that is not valid (e.g., desired concentration is greater than the current concentration), the results line remains on (or return to) the blank state, and screen displays a warning message.

If you make any changes to any of the input areas above, the results are recalculated automatically upon closing the popup window.

6. Press **Done** or the **Back** button to return to the main Results screen.

7 Save results

Save count results

Save screen

The Countess™ II FL Automated Cell Counter allows you to save your data and images using a USB flash 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, BMP, PNG, or TIFF).
- **Images:** Saves only the raw captured image in the selected image format (JPEG, BMP, PNG, or TIFF).
- **Data:** Saves the data from the experiment 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".
- **Report:** Saves a printer-friendly report of the results, graph(s), and image in the selected format (PDF, PNG, or JPEG). For more information, see "Report" on page 47.

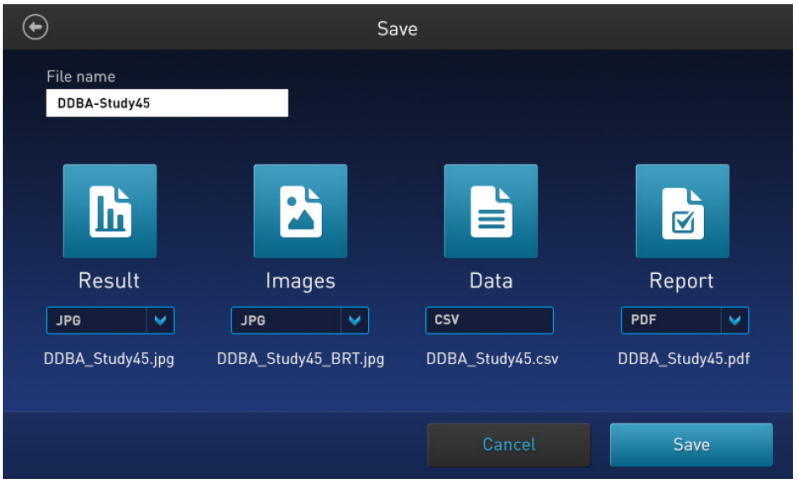
  Note: If you wish to save your results with the Graph showing the distribution of cells based on cell size or fluorescence intensity, make sure that the desired graph is displayed on the Results screen.

Save procedure

1. To save your data, insert the Countess™ II USB drive (or equivalent) into an available USB port on the instrument ("Instrument exterior components" on page 11).

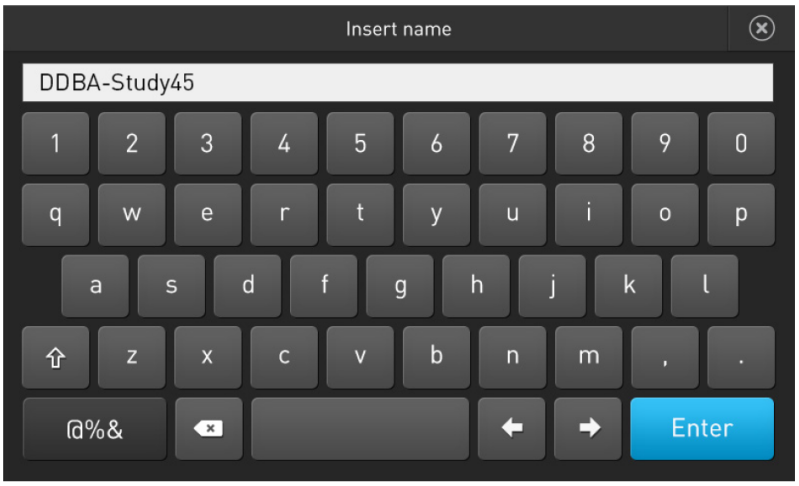
   Note: The USB ports located in the front and the back of the instrument function the same. However, the first USB drive connected will be the preferred saving location.

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



3. To assign a name to your experiment, press the **File name** text field. The alpha-numeric **keypad** opens.

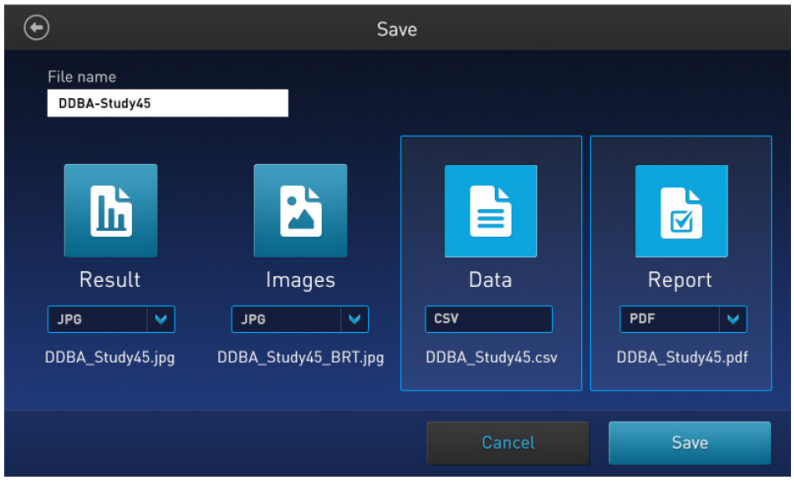
4. Enter the file name using the alpha-numeric **keypad**.
   To enter symbols, press the **symbol (@%&)** key. To return to the alpha-numeric keypad, press **ABC**.



5. Press **Enter** to save the name and return to the Save screen.
   To return to the Save screen without saving the name, press the **close** button.

6. Select the desired mode to save your experiment (**Result**, **Images**, **Data, Report**). 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, **Data** and **Report** are selected.

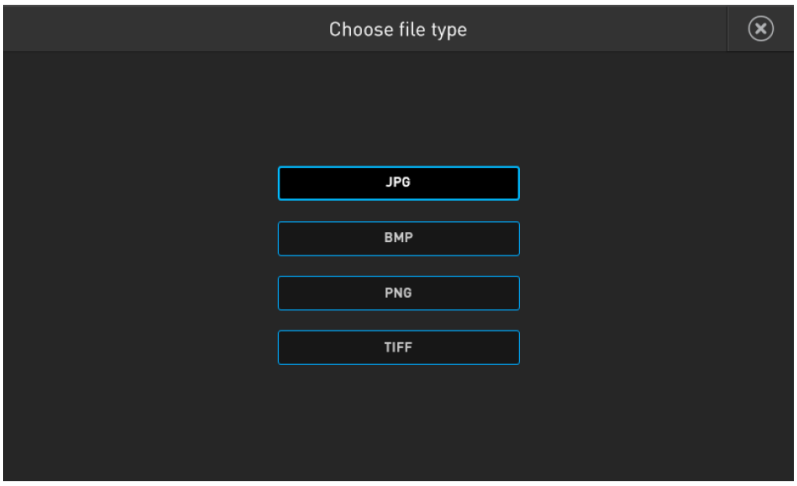


7. By default, Result and Images are saved as JPEG files, and Report is saved as PDF.

   To choose a different file format, press the **file type** button**.** The Choose file type screen opens.

   Note: Data can only be saved as a CSV file.

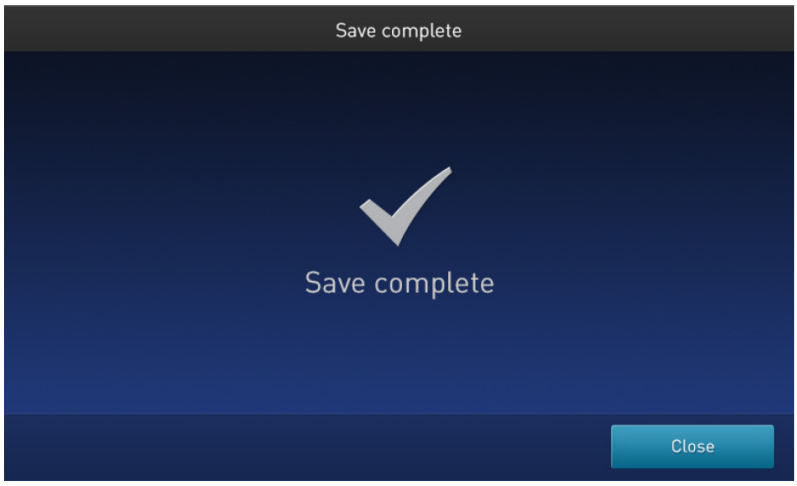
8. Press to select the desired **file type**. Available options are **JPEG**, **BMP**, **PNG**, and **TIFF**.



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

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

9. Press **Save** to save your experiment in the selected mode(s) in the USB drive.



10. Press **Close** and then transfer the USB drive to the desired location.

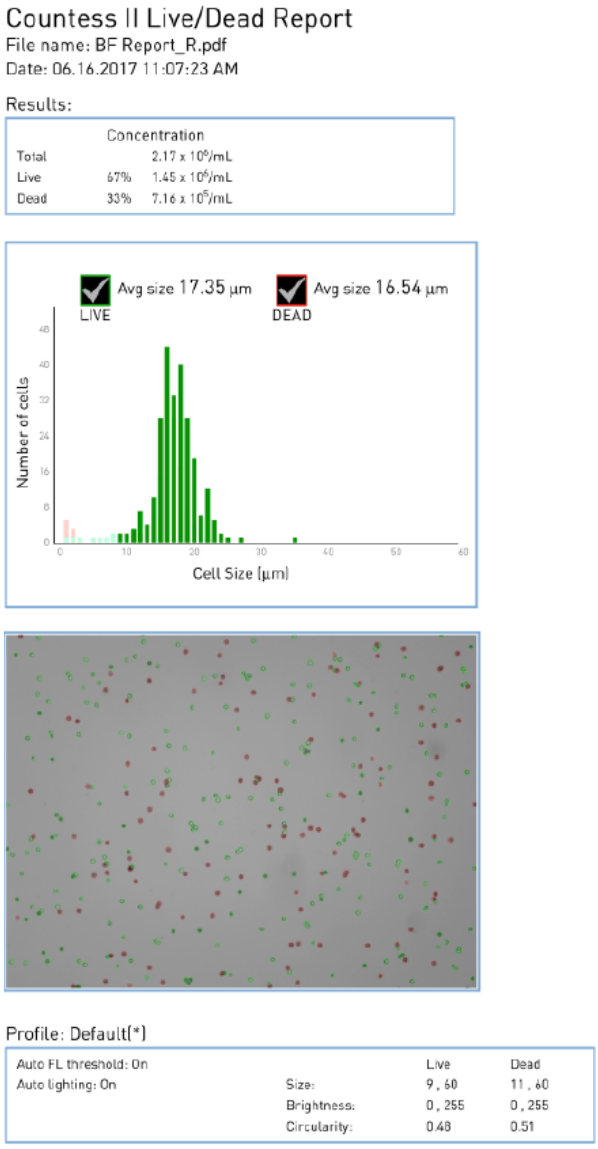
Report

Report file

The Report function allows you to save a printer-friendly report of the results, graphs, and images in the selected format (PDF, PNG, or JPEG).

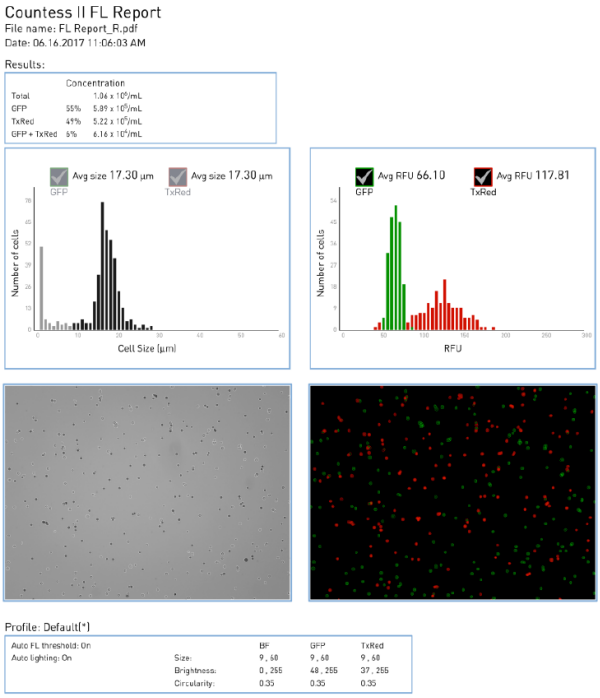
You can create reports using the **Report** dropdown as described in “Save count results“ on page 44.

Report from brightfield count



- The top section of the Report contains a table with the results as displayed on the Countess™ 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 bottom, the report displays the profile information used to gate these images.

Report from fluorescence count



- The top section of the Report contains a table with the results as displayed on the Countess™ 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.

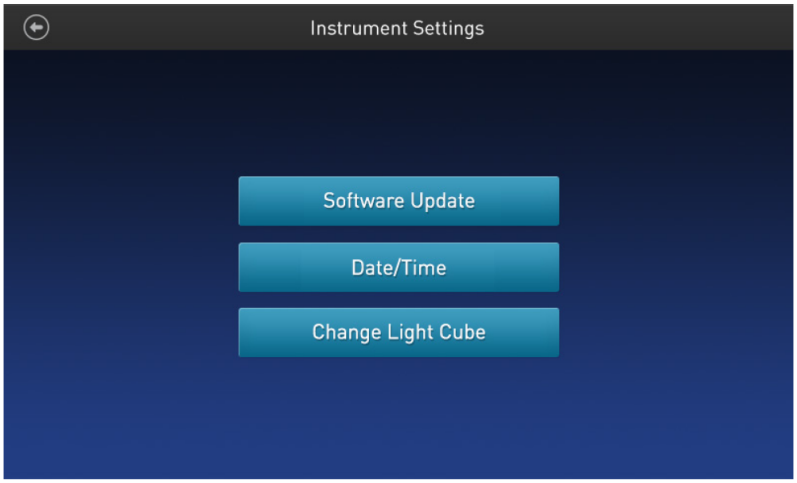
  In the fluorescence image (overlaid, if there are two channels), the cells counted are identified by the circles with the same color as the fluorescence channel in which they were counted (in two colors, if there are two cubes installed).
- At the bottom, the report displays the profile information used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

8 Instrument settings

Overview

Instrument settings screen

To access the Instrument Settings screen, press the **Instrument Settings** button on the Home page ("Turn ON the instrument" on page 13).



In the Instrument Settings screen, you can:

- perform software update ("Software update" on page 50)
- set the date and time ("Date/Time" on page 52)
- change or install EVOS™ light cube ("Change light cube" on page 54)

Software update

Guidelines for software update

- 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™ II/II FL software

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

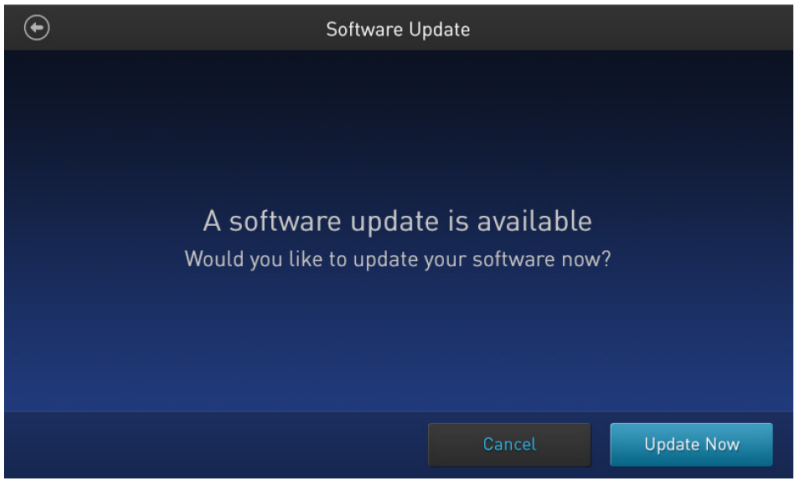
   Note: The software update file has a version-specific name followed by the extension .lft (e.g., Countess™_II_v_1_0_202.lft for software version 1.0.202).

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 ("Instrument exterior components" on page 11).

4. Press the **Instrument Settings** button on the Home page ("Turn ON the instrument" on page 13) to open the Instrument Settings screen (Chapter 8, "Instrument settings").

5. Select **Software Update** from the Instrument Settings menu. The instrument scans the USB drive for the latest software version.



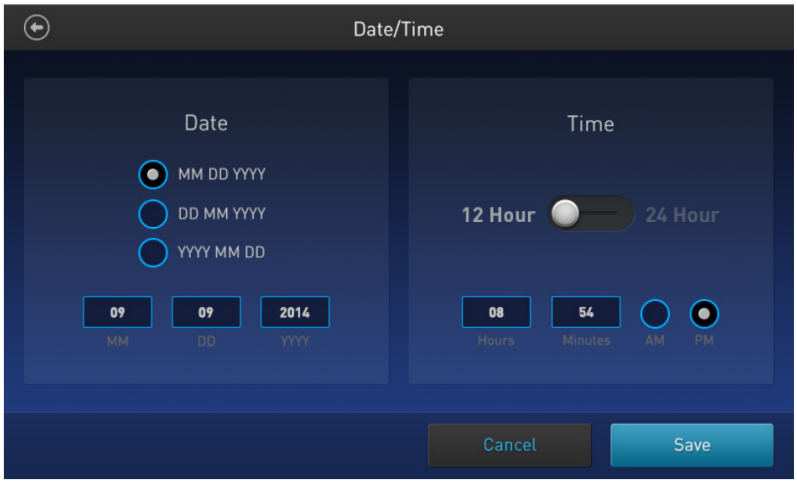
6. When prompted, select **Update Now**.

7. Once the update has completed, restart the instrument.

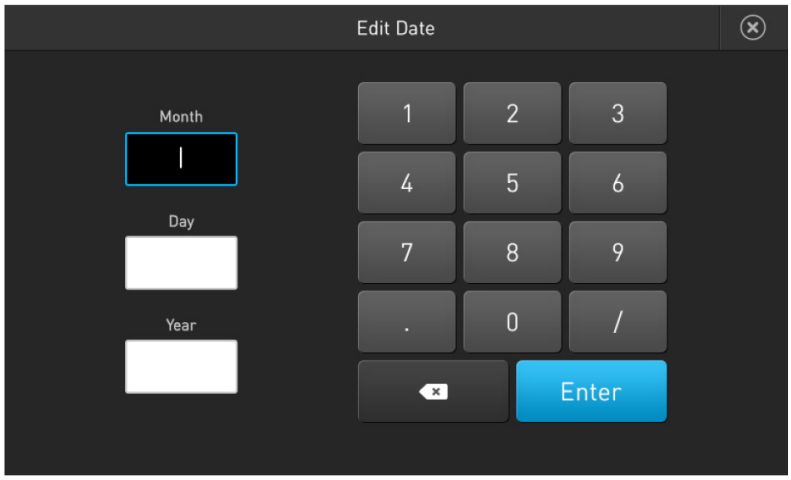
Date/Time

Set the date and time

1. Press the **Instrument Settings** button on the Home page ("Count parameters" on page 15) to open the Instrument Settings screen.
2. Press **Date/Time** on the Instrument Settings menu to open the Date/Time screen.

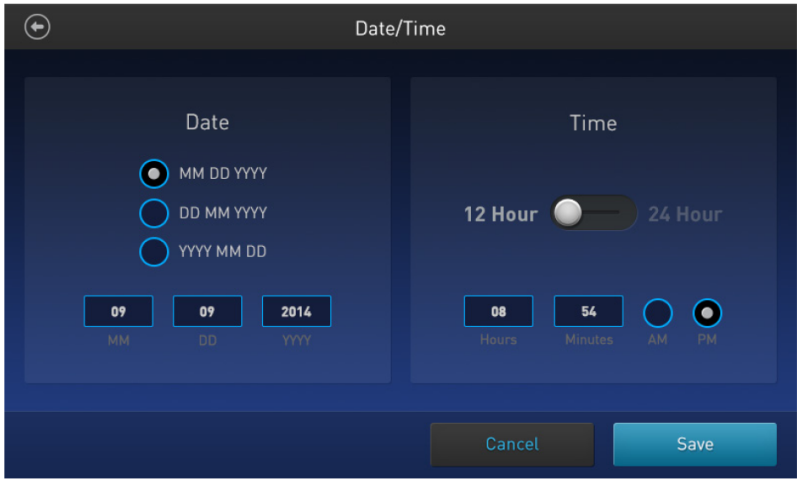


3. Select the **Date format** you wish to use.
4. Press any **Date** text box (**MM**, **DD**, or **YYYY**) to open the **Edit Date** keypad.

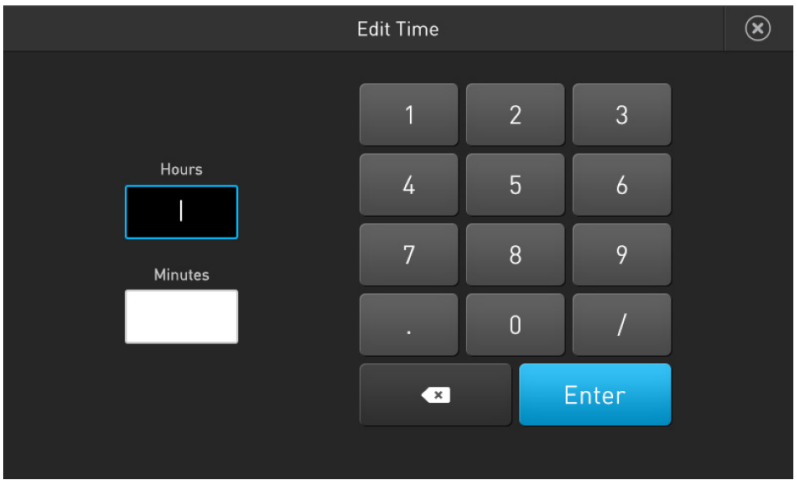


5. Using the keypad, enter the date into **Month**, **Day**, and **Year** text boxes, pressing **Enter** after each entry.
6. After you are finished entering the date, press the **close** button to return to the Date/Time screen.

7. Select the **Time format** you wish to use. Available options are **12 Hour** and **24 Hour formats**.



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



9. Using the keypad, enter the time into **Hours** and **Minutes** text boxes, pressing **Enter** after each entry.

10. After you are finished entering the time, press the **close** button to return to the Date/Time screen.

11. If you have selected the **12 Hour format**, select **AM** or **PM**.

12. Press **Save** to set the Time and Date and return to the Instrument Settings screen.
    Press **Cancel** to return to the Instrument Settings screen without saving your changes.

Change light cube

Install or change EVOS™ light cube

The Countess™ II 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 64). To install or change a light cube:

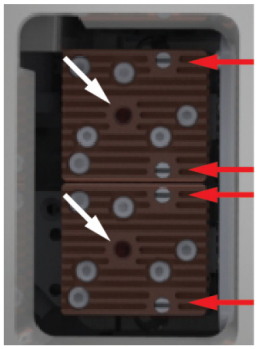
1. Press the **Instrument Settings button** on the Home page ("Turn ON the instrument" on page 13) to open the Instrument Settings screen.
2. Press **Change Light Cube**. The instrument positions the light cube tray to enable light cube installation.
3. When prompted, power off the Countess™ II FL Automated Cell Counter using the **power switch** on the back of the instrument ("Instrument exterior components" on page 11).
4. Unplug the power cord from the Countess™ II FL Automated Cell Counter.
5. Unlatch the back panel with the two captive ¼-turn fasteners (indicated by red arrows) that secure the back panel on the rear of the Countess™ II FL Automated Cell Counter and remove the back panel.

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

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

A

B



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

9. 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.

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

11. Plug the power cord back into the Countess™ II FL Automated Cell Counter.

12. Turn off the Countess™ II FL Automated Cell Counter by flipping the **power switch** on the back of the instrument to the ON position.

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 Countess™ II FL automated cell counter“ on page 56.
- If liquid spills on the instrument, turn off the power immediately and wipe dry.

Power supply

Always use the correct power supply. The power adaptor 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 63). Damage due to an incompatible power adaptor 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 Countess™ II FL automated cell counter

Introduction

Clean the Countess™ II FL 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™ II FL 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 touch-screen

- Wipe the touch-screen of the Countess™ II FL Automated Cell Counter using a soft, lint-free cloth moistened with an LCD cleaning solution. Do not apply excessive force during cleaning. Wipe the touch-screen 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™ II FL 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

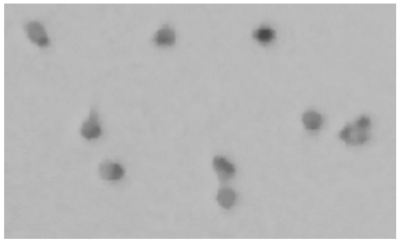
- Wipe the instrument case of the Countess™ II FL Automated Cell Counter using a soft, lint-free cloth moistened with 70% alcohol. Wipe the instrument dry immediately after cleaning.
- Avoid using a bleach solution, because it may leave a residue of bleach crystals on the instrument.
- Ensure that water 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.

Set nominal focus

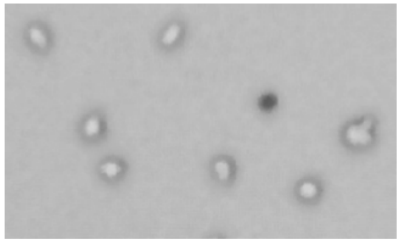
Overview

Nominal focus is the Z-point (i.e., depth) around which the auto focus function searches to provide fine focus to the sample.

The auto focus algorithm of the Countess™ II FL Automated Cell Counter is designed to highlight the differences between live and dead cells in the brightfield channel. The optimal focus level is where the live cells have a light colored center and the dead cells are dark throughout (see examples below).



Focus is not optimal

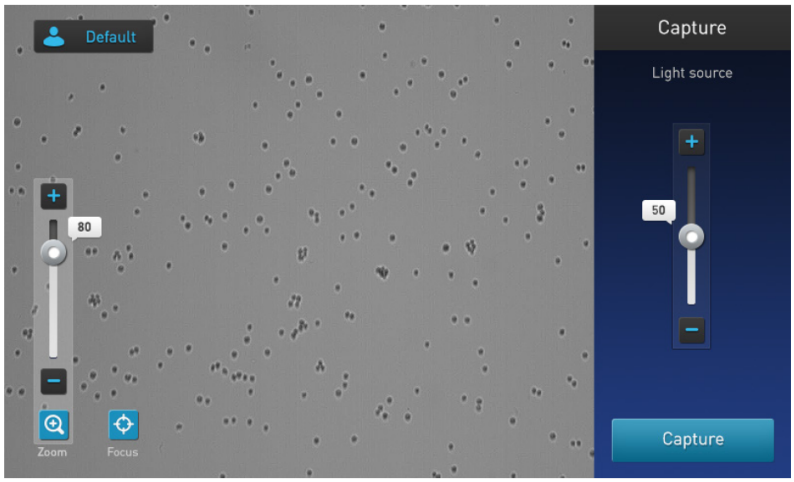


Focus is optimal

To enable optimal auto focus functionality, you may need to initially refine the brightfield focus by adjusting it manually and then setting the nominal focus. This allows the auto focus function to have a set point from which to focus on the cells in subsequent samples.

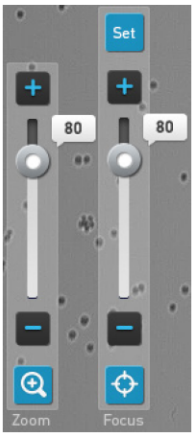
Set nominal focus

1. Prepare the sample by adding 10 µL of cell suspension to 10 µL of 0.4% trypan blue stain. Mix the sample mixture well by pipetting up and down a few times.
2. Load 10 µL of the sample mixture into the Countess™ Cell Counting Chamber Slide ("Load Countess™ chamber slide" on page 19) or the Countess™ II FL Reusable Slide ("Countess™ II FL reusable slide" on page 21). Let the sample mixture settle for 30 seconds to ensure a uniform focal plane.
3. Insert the sample slide into the slide port of the instrument ("Instrument exterior components" on page 11), making sure that the side containing the sample is inserted completely.
4. When the slide is inserted, the instrument automatically illuminates the sample, sets the intensity of the brightfield light source, and auto focuses on the cells.



5. To manually adjust the focus, press the **Focus™** button.

6. Use the **Focus™ slider** or the **plus** and **minus** buttons to refine the brightfield focus.



Note: If needed, **Zoom** in on the image to adjust focus or lighting.

7. After finding the optimal focus, press **Set** to set the nominal focus.
   Once the focus has been set, the **Set** button on the focus slider becomes inactive, confirming that the focus setting has been stored.

Troubleshooting

Note: The software for the Countess™ II FL is updated regularly. If you are having any issues with your experiments, first check the website to see if a new software version is available. You can download the most recent version of the software from **www.thermofisher.com/countessupdate**. You can also register your Countess™ II FL instrument at **www.thermofisher.com/registercountess** to be informed of any future software updates.

Problem	Possible solutions
Uneven screen illumination (screen is dark on one side, but brighter on the other)	Reset the light cube tray by selecting **Change Light Cube** on the Instrument Settings screen (Chapter 8, "Instrument settings").
Autofocus does not seem to focus on the cells very well	• 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. • Ideally, the live cells should have bright centers compared to the dead cells, which are dark throughout ("Set nominal focus" on page 57). • Setting the nominal focus will improve autofocus consistency with future slides. To set the nominal focus, see "Set nominal focus" on page 57.
Some cells appear on the image but are not included in the count	• For cell count and cell viability assays performed in the brightfield, adjust the size, brightness, and circularity gates for both live and dead cells to include all of the cells in the count ("Gate count results" on page 27). • 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 ("Gate count results" on page 37). • 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 0–60 for cell size and 0–255 for brightness.
Images are very bright and washed out	Enable Auto Lighting from the Profiles menu, or decrease the bright field light intensity before counting the cells.
Fluorescence is extremely bright and bleeding through into other filters	Decrease the fluorescence light intensity before counting the cells.

Problem	Possible solutions
Getting incorrect concentration for the Countess™ test beads	• 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	• 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. • Pipet the bead and trypan blue mixture up and down several times to make sure it is well mixed, and load 10 µL into the slide without delay.
Variable counts when performing replicate counts of the same slide	• 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 that you do not shake or agitate the slide between counts.

Problem	Possible solutions
Abnormally high percentage of dead cells or live cells counted as dead	• Ensure that the cells are focused correctly so that live cells have bright centers and dead cells are dark throughout (see "Set nominal focus" on page 57). If the cells are not well focused and look dark on the screen, the Countess™ II FL will count them as dead cells. • 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. • 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. • Gate out the debris using the size, brightness, and circularity sliders.
USB drive not recognized by the instrument	• The USB drive must be FAT32 formatted to be recognized by the instrument. If it is not, reformat the USB drive to FAT32 ("Software update" on page 50). • Try another correctly formatted USB drive.
Unable to update the Countess™ software	• 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. • The update file must sit on the top level of the USB drive, not within a folder or a 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

Product specifications

Technical specifications

Physical characteristics

Instrument type:	Benchtop cell counter and suspension cell-based assay platform
Instrument dimensions:	9 (W) × 5½ (D) × 9 inches (H)
Weight:	8 lbs
Operating power:	100–240 VAC, 0.58 A MAX
Frequency:	50/60 Hz
Electrical input:	12 VDC, 2 A
Installation site:	Indoor use only, Class A Environments (i.e., non-residential or light industrial); Pollution degree 2.
Operating temperature:	4°–40℃ (39°–104°F)
Operating humidity:	<80% (non-condensing)

Technical specifications

Processing time:	~15 seconds
Sample concentration range:	1×10^4–1×10^7 cells/mL
Particle/cell diameter range:	4–60 µm (particles); 7–60 µm (cells)
Required sample volume:	10 µL
Firmware:	Countess™ Automated Cell Counting Platform Software
USB Drive :	4 Gigabytes

Optics

Optics:	3 channels (brightfield and 2 slots for EVOS™ LED light cubes)
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

EVOS™ light cubes

LED illumination

The Countess™ II 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 64). 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™ II 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 "Change light cube" on page 54.

Light cube	Dye
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
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, Cy™3.5
Cy5	Cy5™, Alexa Fluor™ 647, Alexa Fluor™ 660, DRAQ5™
Cy5.5	Cy™ 5.5, Alexa Fluor™ 660, Alexa Fluor™ 680, Alexa Fluor™ 700
Cy7	Cy™ 7, IRDye™ 800CW

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

C Ordering information

Countess™ II FL automated cell counter and accessories

The following Countess™ II 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™ II FL Automated Cell Counter	1 each	AMQAF1000
Countess™ II power cord with four adapter cords	1 each	AMEP4716
Countess™ II USB drive	1 each	A25751
Countess™ II FL Light Cube Removal Tool	1 each	AMEP4747
Countess™ II FL Disposable Slide Holder	1 each	AMEP4745
Countess™ II FL Reusable Slide Holder	1 each	AMEP4746

Accessory products

The following products can be used with the Countess™ II FL Automated Cell Counter 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™ II FL Reusable Slide	1 each	A25750

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.

D

CSV file format

CSV file format, explained

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™ II data saved as a CSV file and opened with a spreadsheet program.

Category	Column	Name	Description
General	A	Number	Sequential sample run number
	B	File Name	Name of file
	C	Date & Time	Date and time of sample run
	D	Mode	BF-Brightfield or FL-Fluorescence
Trypan Blue/Brightfield	E	Total Concentration	Concentration of the entire sample
	F	Total cells counted	Total number of cells counted in the sample
	G	Live concentration	Concentration of just the "live" portion of the sample
	H	Live cells counted	Total number of "live" cells counted
	I	Dead concentration	Concentration of just the "dead" portion of the sample
	J	Dead cells counted	Total number of "dead" cells counted
	K	Viability (%)	Percent viability of the sample based on trypan blue staining
	L	Average size (um)	Average cell size in microns
Fluorescence	M	Cube 1 name	EVOS™ light cube name in the first (top) position
	N	Cube 1 concentration	Concentration of cells showing fluorescence in the first cube position
	O	Cube 1 (%)	Percentage of the total cells in brightfield that show fluorescence in the first cube position
	P	Cube 1 cells counted	Total number of cells counted in the first cube position
	Q	Cube 2 name	EVOS™ light cube name in the second (bottom) position

Category	Column	Name	Description
Fluorescence	R	Cube 2 concentration	Concentration of cells showing fluorescence in the second cube position
	S	Cube 2 (%)	Percentage of the total cells in brightfield that show fluorescence in the second cube position
	T	Cube 2 cells counted	Total number of cells counted in the second cube position
	U	Cube 1+2 concentration	Concentration of cells showing fluorescence in the first and second cube positions combined
	V	Cube 1+2 (%)	Percentage of the total cells in brightfield that show fluorescence in the first and second cube position combined
	W	Cube 1+2 cells counted	Total number of cells counted in the first and second cube position combined
General Details	X	Focus™ value	Focal position number
	Y	BF Light intensity	Brightfield light intensity value from 0-100%
Trypan Blue/Brightfield Count Parameters	Z	Live Size min	Minimum size of "live" cells in microns
	AA	Live Size max	Maximum size of "live" cells in microns
	AB™	Live Brightness min	"Live" adjustment slider value for minimum brightness
	AC	Live Brightness max	"Live" adjustment slider value for maximum brightness
	AD	Live Circularity	"Live" adjustment slider value for circularity
	AE	Dead Size min	Minimum size of "dead" cells in microns
	AF	Dead Size max	Maximum size of "dead" cells in microns
	AG	Dead Bright min	"Dead" adjustment slider value for minimum brightness
	AH	Dead Bright max	"Dead" adjustment slider value for maximum brightness
	AI	Dead Circ	"Dead" adjustment slider value for circularity
Fluorescence Count Parameters	AJ	Cube 1 Light intensity	First (top) light cube light intensity value from 0-100%
	AK	Cube 2 Light intensity	Second (bottom) light cube light intensity value from 0-100%
	AL	BF Size min	Minimum size of "Brightfield" cells in microns
	AM	BF Size max	Maximum size of "Brightfield" cells in microns

Category	Column	Name	Description
Fluorescence Count Parameters	AN	BF Brightness min	"Brightfield" adjustment slider value for minimum brightness
	AO	BF Brightness max	"Brightfield" adjustment slider value for maximum brightness
	AP	BF Circularity	"Brightfield" adjustment slider value for circularity
	AQ	Cube 1 Brightness min	First (top) light cube adjustment slider value for minimum brightness
	AR	Cube 1 Brightness max	First (top) light cube adjustment slider value for maximum brightness
	AS	Cube 2 Brightness min	Second (bottom) light cube adjustment slider value for minimum brightness
	AT	Cube 2 Brightness max	Second (bottom) light cube adjustment slider value for maximum brightness

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, see the "Documentation and Support" section in this document.

Safety alert words

Four safety alert words appear in this document at points where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below:

IMPORTANT! – Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

CAUTION! – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING! – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER! – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Except for **IMPORTANT!** safety alerts, each safety alert word in this document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard symbols that are affixed to the instruments (see **Safety symbols** in Appendix E).

Electrical symbols

The following table describes the electrical symbols that may be displayed.

Symbol	Description
	Indicates the **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.

Safety symbols

The following table describes the safety symbols that may be displayed. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see "Safety labels on instrumentsâ€). These safety symbols may also appear next to DANGERS, WARNINGS, and CAUTIONS that occur in the text of this and other product-support documents.

Symbol	Description
	Caution, risk of danger. Consult the manual for further safety information.
	Caution, risk of electrical shock.

Symbol	Description
	Caution, hot surface or other high-temperature hazard.
	Caution, laser.
	Caution, moving parts.
	Caution, potential biohazard.
	Caution, ultraviolet light.

Environmental symbols

The following symbol applies to all Thermo Fisher Scientific electrical and electronic products placed on the European market after August 13, 2005.

Symbol	Description
	Do not dispose of this product as unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your Customer Service representative for equipment pick-up and recycling. See www.thermofisher.com for a list of customer service offices in the European Union.

Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed Thermo Fisher Scientific instruments in combination with the safety symbols described in the preceding section.

Symbol	English	Français
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! Hazardous waste. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Thermo Fisher Scientific qualified service personnel.	**AVERTISSEMENT!** Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de Thermo Fisher Scientific.
	**DANGER!** Class 3B visible and/or invisible laser radiation present when open. Avoid exposure to beam.	**DANGER!** Rayonnement visible ou invisible d'un faisceau laser de Classe 3B en cas d'ouverture. Evitez toute exposition au faisceau.
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

General instrument safety

WARNING! PHYSICAL INJURY HAZARD. Use this product only as specified in this document. Using this instrument in a manner not specified by Thermo Fisher Scientific may result in personal injury or damage to the instrument.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs).

Safety precautions

- Do not install the instrument in heavy humidity, such as a greenhouse or an incubator, to avoid a danger of electric shock. If water or other material enters the instrument, the adaptor, or power inlet, disconnect the power cord and contact a service person.
- Do not press the main plug or power cord with wet hands.
- Always ensure that the power supply input voltage matches the voltage available in your location.
- Do not install the instrument on a slant or a place prone to vibrations, which induces the risk of instrument malfunction or damage of the instrument.
- Never insert any objects into the air vents of the instrument as this could result in electrical shock, personal injury, and equipment damage.
- Plug the power cord firmly into the wall outlet and the instrument.
- To avoid potential shock hazard, make sure that the power cord is properly grounded.
- Be sure to position the equipment such that it is easy to disconnect the instrument.
- Turn off the instrument before unplugging the power cord and/or moving the instrument.
- If the instrument is broken or dropped, disconnect the power cord and contact a service person. Do not disassemble the instrument.
- Use only authorized accessories (adaptor, power cord, and USB drive).
- If the instrument emits smoke, disconnect the power cord from the wall outlet and contact a service person.

Cleaning or decontaminating the instrument

CAUTION! Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.

Removing covers or parts of the instrument

CAUTION! PHYSICAL INJURY HAZARD The instrument is to be serviced only by trained personnel or vendor specified in the user guide.

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.

Chemical waste safety

Chemical waste hazard

CAUTION! HAZARDOUS WASTE. Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposal.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument 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.

Electrical safety

Part	Symbol	Description
General		**DANGER! ELECTRICAL SHOCK HAZARD.** Severe electrical shock can result from operating the Countess™ II Automated Cell Counter or Countess™ II FL Automated Cell Counter without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.
Fuses		**WARNING! FIRE HAZARD.** For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Part	Symbol	Description
Power		**DANGER! ELECTRICAL HAZARD.** Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.
		**DANGER! ELECTRICAL HAZARD.** Use properly configured and approved line cords for the voltage supply in your facility.
		**DANGER! ELECTRICAL HAZARD.** Plug the system into a properly grounded receptacle with adequate current capacity.
Overvoltage rating		The Countess™ II Automated Cell Counter and Countess™ II FL Automated Cell Counter have an installation (overvoltage) category of II, and are classified as portable equipment.

Biological hazard safety

WARNING! Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications.

ATTENTION! BIOHAZARD. Les échantillons biologiques tels que les tissus, les fluides corporels et le sang des humains et d'autres animaux ont la possibilité de transmettre des maladies infectieuses. Suivre tous les règlements municipaux, provinciaux/provincial et / ou nationales en vigueur. Porter des lunettes de protection approprié, des vêtements et des gants.

In the U.S.:

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at: **www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html**)
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Safety and electromagnetic compatibility (EMC) standards

Symbol	Description
C US	**U.S. and Canadian safety standards.** The **CSA C/US Mark** signifies that the product meets applicable U.S. and Canadian standards, including those from CSA, CSA America, ANSI, ASME, ASSE, ASTM, NSF and UL.
CE	**European safety and EMC standards.** The **CE Mark** symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. Operation of the instrument is subject to the conditions described in this manual. The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by Thermo Fisher Scientific.
	Australian EMC standards The **C-Tick Mark** indicates conformity with Australian and New Zealand standards for electromagnetic compatibility.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - 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/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**.