USER GUIDE

Countess® II and Countess® II FL Automated Cell Counters

Catalog Numbers AMQAX1000, AMQAF1000

Publication Number MAN0010644

Revision A.0

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

life technologies

Information in this document is subject to change without notice.

DISCLAIMER

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

IMPORTANT LICENSING INFORMATION

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

TRADEMARKS

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified.

Contents

About this guide

Audience

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

User documentation

The guides listed below are available with the Countess® II or the Countess® II FL Automated Cell Counter.

Guide	Pub. no.
Countess® II and 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.lifetechnologies.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 Life Technologies™ user documentation, see page 4.

Convention	Use
Bold	**Bold** text indicates user action. For example: Press **Capture**.
▶	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 **BRT ▶ Capture**.

User attention words

Two user attention words appear in Life Technologies™ user documentation. 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 Life Technologies™ user documentation at points in the document 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 a Life Technologies™ 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 Life Technologies™ instruments (see "**Safety symbols**" in Appendix D).

1. Product information

Product contents

The Countess® II Automated Cell Counter is shipped with the components listed below.

Component	Quantity
Countess® II Automated Cell Counter (Cat. no. AMQAX1000) or 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
Trypan blue stain (0.4%)	2 × 1 mL
Countess® II USB drive	1 each
Countess® II 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 page 11 for instructions on installing the Countess® II Automated Cell Counter.

Registering your instrument

Visit **www.lifetechnologies.com/countess** 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 Automated Cell Counter.

Note: In this user guide, Countess® II Automated Cell Counter refers to both the Countess® II and the Countess® II FL Automated Cell Counter when the instruments share the same function, feature, or experiment workflow.

Each instrument is explicitly is referred to when a function, feature, or workflow pertains to only to the Countess® II or the Countess® II FL Automated Cell Counter.

Product description

Countess® II Automated Cell Counter

The Countess® II Automated Cell Counter (Cat. no. AMQAX1000) is an automated benchtop cell counter that uses state-of-the-art optics and image analysis algorithms to perform cell count and cell viability assays for trypan blue-stained cells in suspension.

- The Countess® II 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 Countess® II Automated Cell Counter in Countess® Cell Counting Chamber Slides (page 11). 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 Countess® II Automated Cell Counter takes 15 seconds per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells.
- In addition to cell count and viability, the Countess® II Automated Cell Counter provides information on cell size.

Countess® II FL Automated Cell Counter

The Countess® II FL Automated Cell Counter (Cat. no. AMQAF1000) is a fully automated, 3-channel cell counter and assay platform that uses EVOS® light cube technology to analyze fluorescently labeled cells or trypan blue stained samples.

In addition to all the functions available on the Countess® II Automated Cell Counter, the Countess® II FL Automated Cell Counter offers the following features:

- The Countess® II FL Automated Cell Counter can counts cells from either disposable Countess® Cell Counting Chamber Slides or from glass Countess® II FL Reusable Chamber Slides (page 11).
- In addition to the bright field channel, the Countess® II FL Automated Cell Counter can accommodate two interchangeable EVOS® fluorescent or specialty light cubes (page 47), 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 assays for cells in suspension, including simultaneously counts of cells stained with two different fluorescent dyes, GFP and RFP expression, apoptosis, cell viability (live, dead, and total cells), and cell cycle assays. It is compatible with a wide variety of eukaryotic cells.

Note: In this user guide, Countess® II Automated Cell Counter refers to both the Countess® II and the Countess® II FL Automated Cell Counter when the instruments share the same function, feature, or experiment workflow.

Each instrument is explicitly is referred to when a function, feature, or workflow pertains to only to the Countess® II or the Countess® II FL Automated Cell Counter.

Instrument exterior components

Front view

The front view showing various parts of the Countess® II and the Countess® II FL Automated Cell Counter is shown below.

Press-screen display

The 7-inch capacitive press-screen display, located in the front of the instrument, contains the buttons for all the instrument functions and displays data from the cell count.

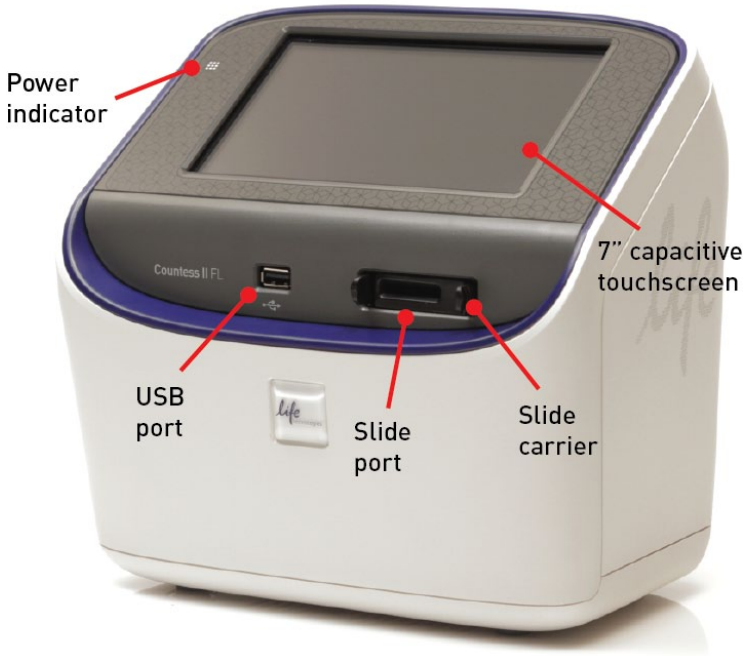
Slide port

The slide port is used to insert the analysis slide containing the sample into the counter.

The Countess® II instrument accepts the plastic, disposable Countess® Cell Counting Chamber Slides only, while the Countess® II FL instrument accepts both the Countess® Cell Counting Chamber Slides and the glass Countess® II FL Reusable Chamber Slides on interchangeable, slide-specific carriers (see "Slide operation", page 15).

USB port

The USB port allows you to transfer and save the cell count data and image to an external computer for record keeping and printing purposes. The USB drive supplied with the instrument or any other standard USB drive is inserted into the USB port for data transfer. See page 39 for instructions on exporting data files.



Rear view

The rear view of the Countess® II and Countess® II FL Automated Cell Counter illustrating the various parts of the instrument is shown below.

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.

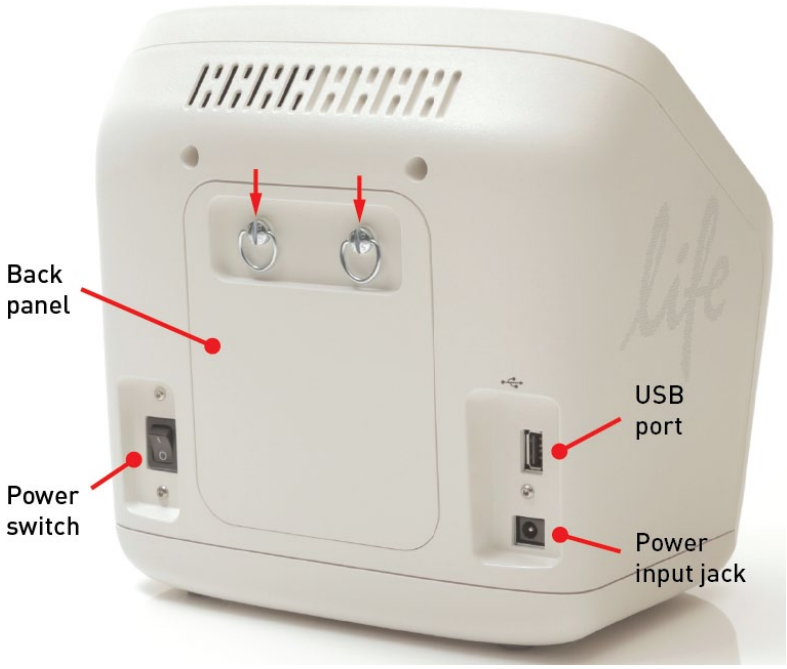
Power switch

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.

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 slide carrier (see next page). The back panel is secured to the instrument by two captive ¼-turn fasteners (indicated by red arrows).

The back panel of the Countess® II Automated Cell Counter is fixed to the instrument and cannot be opened.



Note: The EVOS® light cubes are available only for the Countess® II FL Automated Cell Counter. The Countess® II Automated Cell Counter uses brightfield illumination only and does not support the EVOS® light cubes. For more information on the EVOS® light cubes, see page 47.

Rear view (back panel open)

The rear view of the Countess® II FL Automated Cell Counter with the back panel open illustrates the location of the optional EVOS® light cubes and the storage for the light cube tool and the slide carrier.

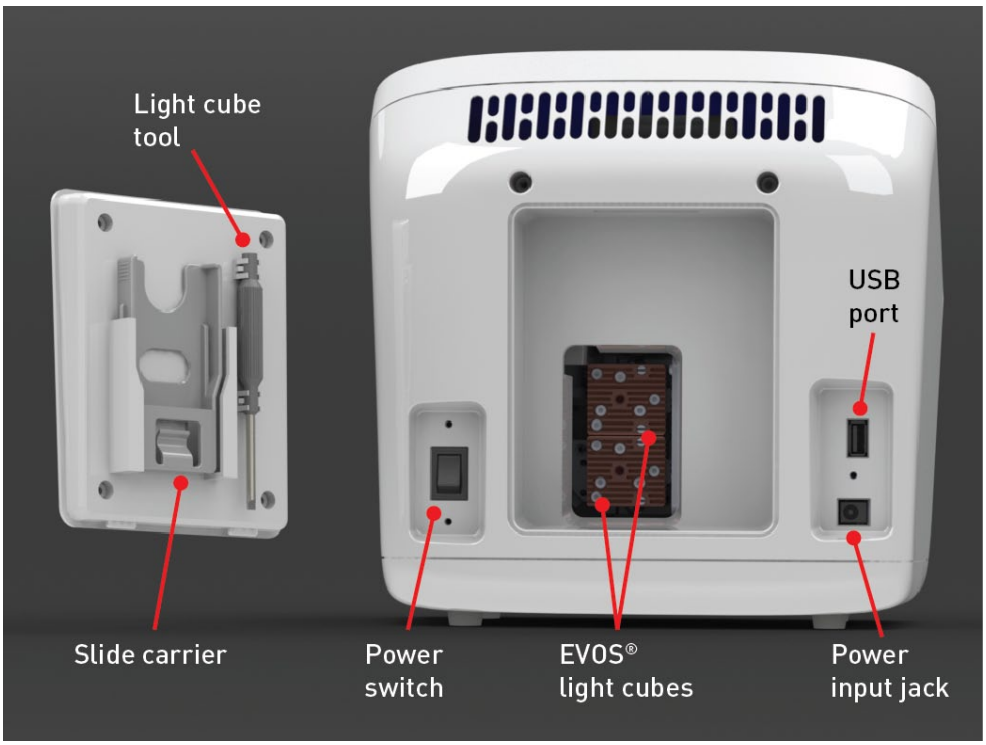
EVOS® light cubes

The EVOS® light cubes (page 47) allow the Countess® II FL Automated Cell Counter to analyze fluorescently labeled samples in addition to trypan blue-stained samples. The Countess® II FL Auto Imaging System can accommodate two fluorescent or specialty light cubes.

Slide carrier

The interchangeable slide carriers allow the Countess® II FL Automated Cell Counter to count cells from either plastic, disposable Countess® Cell Counting Chamber Slides or glass Countess® II FL Reusable Chamber Slides.

This option is not available for the Countess® II Automated Cell Counter, which comes with the slide carrier for the plastic, disposable Countess® Cell Counting Chamber Slides pre-installed.



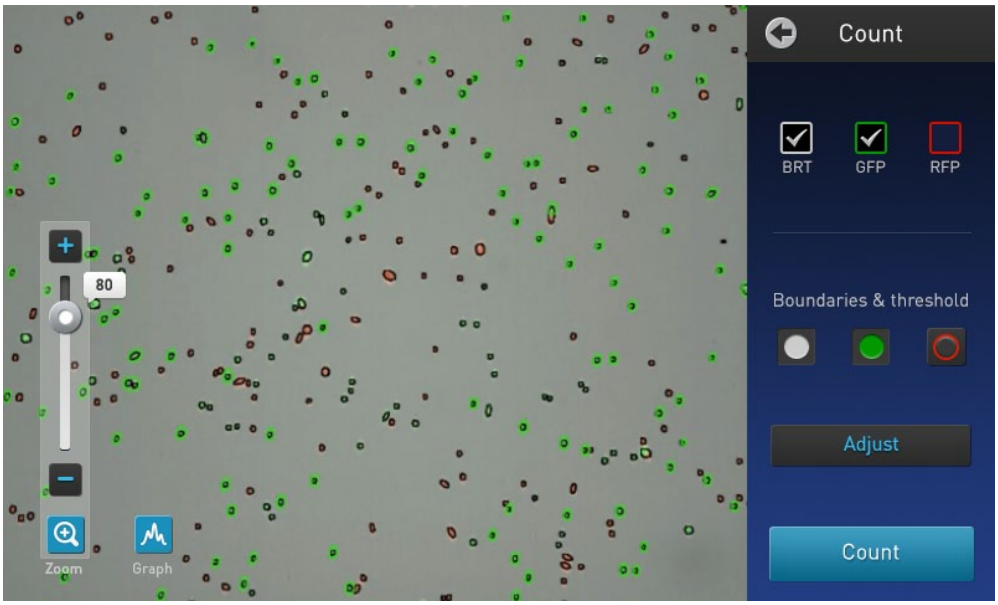
Note: The EVOS® light cubes are available only for the Countess® II FL Automated Cell Counter. The Countess® II Automated Cell Counter uses brightfield illumination only and does not support the EVOS® light cubes. For more information on the EVOS® light cubes, see page 47.

User interface

User interface

The 7-inch capacitive press-screen is the main user interface of the Countess® II and the Countess® II FL Automated Cell Counter.

The screens available on the user interface are contextual and depend on the instrument (Countess® II or Countess® II FL) and the EVOS® light cube installed (if applicable). The following example shows the Count screen of the Countess® II FL Automated Cell Counter that has the GFP and RFP light cubes installed.



Sample slides

Countess® Cell Counting Chamber Slides

The Countess® Cell Counting Chamber Slides are pre-sterilized, plastic, disposable sample slides designed for use with Countess® Automated Cell Counters.

- Each slide has two separate enclosed chambers (A and B) that allow the analysis of two different samples or replicates of the same sample. Each chamber has a 10-µL sample capacity.
- The Countess® Cell Counting Chamber Slide does not require a separate cover slip. Refer to page 14 for guidelines on loading the chamber slides.
- The Countess® II and the Countess® II FL Automated Cell Counter are supplied with one box of Countess® Cell Counting Chamber Slides, which are also available separately from Life Technologies™. Refer to page 48 for ordering information.

Countess® II FL Reusable Chamber Slides

The Countess® II FL Reusable Chamber Slides are reusable, glass sample slides designed for use with Countess® II FL Automated Cell Counters.

- Each slide has two separate enclosed chambers (A and B) that allow the analysis of two different samples or replicates of the same sample. Each chamber has a 10-µL sample capacity.
- The Countess® II FL Reusable Chamber Slide requires the use of glass cover slips for sample loading. Refer to page 14 for guidelines on loading the reusable chamber slides.
- The Countess® II FL Reusable Chamber Slides are available separately from Life Technologies™. Refer to page 48 for ordering information.

2. 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°C (40°–90°F).
- Relative humidity range: 30–90%.

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 (see page 8). 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. Plug one end of the power cord appropriate for your region into the instrument.
3. 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 (page 8) 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 (page 17) or you can adjust instrument settings (page 44) by pressing the **Settings** button in the upper right corner of the home screen.

Prepare sample

Recommendations

To obtain the best results, follow these recommendations:

- Wear gloves during sample handling.
- Use the Countess® II or the Countess® II FL Automated Cell Counter at room temperature only (10°C–35°C).
- For accurate viability count results, ensure the counting area is covered with cell suspension and count cells immediately after staining per the assay protocol.
- Do **not** press the optical surfaces of the chamber slides. Hold the slides by the edges.
- Take care to avoid forming bubbles in the sample.

Load Countess® Chamber Slide

1. Gently pipet the sample at an angle of approximately 80° into the half moon-shaped sample loading area. The sample is loaded into the chamber through capillary action.

2. After using the Countess® Cell Counting Chamber Slides, appropriately dispose of them as biohazardous waste. Do not reuse the chamber slides.

Load Countess® II FL Reusable Chamber Slide

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

3. After using the Countess® II FL Reusable Chamber Slide, rinse the glass slide and coverglass with water, and then sterilize with 70% ethanol. Use Kimwipes® laboratory tissues to clean and dry the slides, as needed.

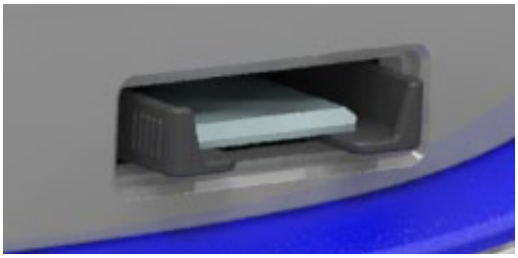
Note: Each chamber in a Countess® Cell Counting Chamber Slide or a Countess® II FL Reusable Chamber Slide has a 10-µL sample capacity. Do not overfill or underfill the slide chambers.

Slide operation

Slide operation: Countess® II Automated Cell Counter

The Countess® II Automated Cell Counter comes with the slide carrier (black) for the plastic, disposable Countess® Cell Counting Chamber Slides pre-installed. The disposable chamber slides are directly inserted into the slide carrier.

1. Load the Countess® Cell Counting Chamber Slide with your sample as described on page 14.
2. To insert a Countess® Cell Counting Chamber Slide into the instrument, push the slide into 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.

Slide operation: Countess® II FL Automated Cell Counter

The Countess® II FL instrument accepts both the disposable Countess® Cell Counting Chamber Slides (below) and the glass Countess® II FL Reusable Chamber Slides (page 16) 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) into the slide port of the instrument until it clicks into place.
2. Load the chamber slide with your sample as described on page 14, and then insert the slide into the slide carrier in the slide port.
2. 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.
3. 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 (see page 8).

Countess® II FL Reusable Chamber Slide

1. To use the Countess® II FL Reusable Chamber 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 and remove the reusable slide carrier (white) from inside of the back panel.

2. Load the reusable glass slide with the sample as described on page 14, and place the loaded slide into the white slide carrier.
3. Insert the carrier with the slide into the slide port, and gently push into the instrument until it "clicks" into place.
4. To remove, push inward to release the carrier and then pull the carrier the remainder of the way out.

3. Basic operation without EVOS® light cubes

Count cells in brightfield

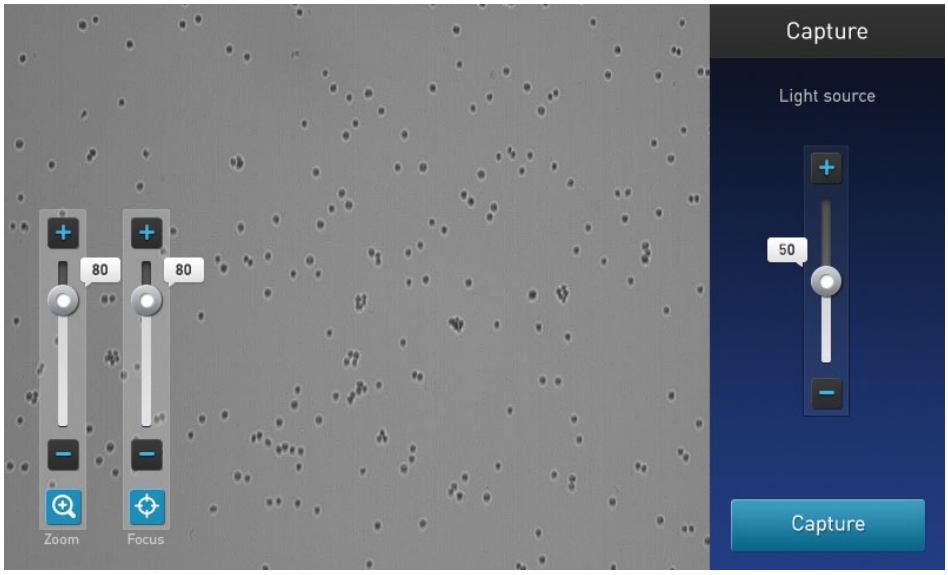
Overview

- The Countess® II and the Countess® II FL Automated Cell Counters capture images of cells in brightfield and use image analysis algorithms to perform cell count and cell viability assays for trypan blue-stained cells in suspension.
- The procedure and screens for counting cells in brightfield is identical for the Countess® II and the Countess® II FL Counters without the light cubes installed (see "Count procedure", below).
- For instructions on counting cells in brightfield using the Countess® II FL Automated Cell Counter with the EVOS® light cubes installed, see "Basic operation with EVOS® light cubes", page 19.
- The Countess® II instrument accepts only the disposable Countess® Cell Counting Chamber Slides, while the Countess® II FL instrument accepts both the Countess® Cell Counting Chamber Slides and the glass Countess® II FL Reusable Chamber Slides on interchangeable, slide-specific carriers.

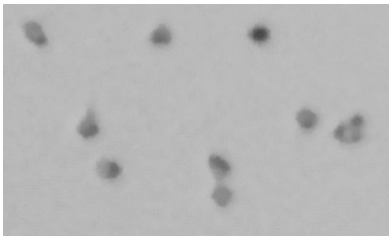
Count procedure

1. Prepare sample by adding 10 µL of your cell suspension to 10 µL of 0.4% trypan blue stain.
2. Load 10 µL of the sample mixture per chamber into the Countess® Cell Counting Chamber Slide as described on page 14.
3. Insert the Countess® Cell Counting Chamber Slide into the slide port (see page 7), making sure that the sample side A is inserted completely into the instrument. You will hear a soft click, if the slide is pushed in correctly.

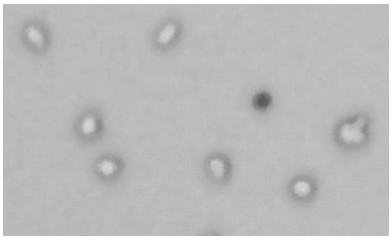
   Note: With the Countess® II FL Counter, you can also use the Countess® II FL Reusable Chamber Slide (see page 16).
4. When the slide is inserted into the instrument, the transmitted light source automatically illuminates the sample and the instrument auto focuses on the cells.



Note: The auto focus algorithm is designed to highlight the differences between live and dead cells. Therefore, the optimal focus level is slightly "under" focus, where optimally focused "live" cells have a light colored center and "dead" cells are dark throughout (see examples below). To enable the auto focus to function properly, manually set the optimal focus as described in Step 5, below.



Focus is not optimal

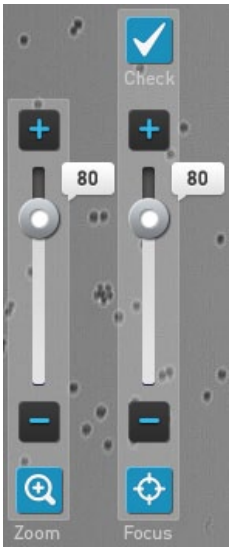


Focus is optimal

5. *Optional*: If you wish to manually adjust the focus, press the **Focus** button and use the **Focus slider** to bring your sample into focus.

   Alternatively, press the **plus** and **minus** buttons to focus.

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

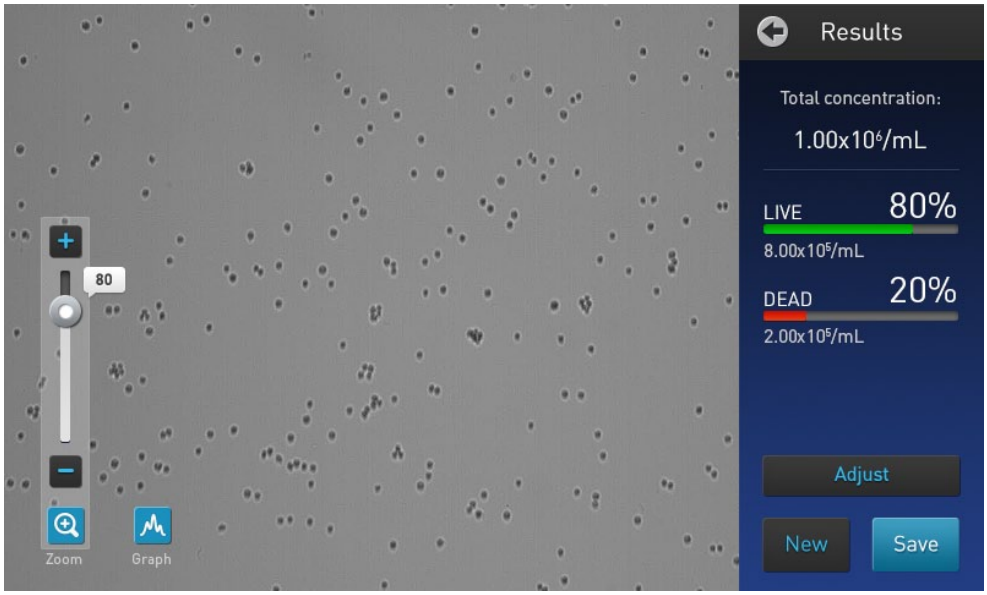


Note: If needed, **Zoom** in on the image to adjust focus or lighting. The Zoom function is also available on the Adjust screen.

6. Set exposure using the **light slider**.

Note: The light slider controls the LED intensity, camera gain, and exposure time and it is used for adjusting the image brightness and contrast.

7. Press **Capture**. The instrument temporarily captures the image and displays the results (total concentration, percentage and concentration of live and dead cells). For more information, see "Results", page 27.



8. To perform a new count, press **New**. Remove and turn around the slide, and reinsert it sample side first into the instrument to count the sample in the second chamber.

Next steps

- To see the distribution of live and dead cells in a graphical format, press the **Graph** button (see page 29).
- To gate the count results by object size, brightness, or circularity, press **Adjust** (see "Gate count results", page 31).
- To permanently save the results, press **Save** (see page 39).

4. Basic operation with EVOS® light cubes

Count cells in brightfield

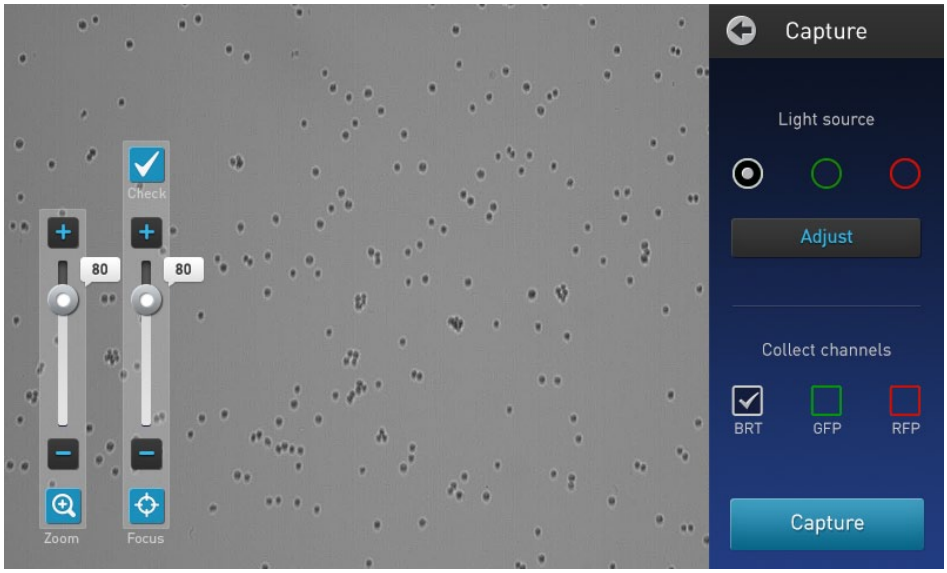
Overview

The Countess® II FL Automated Cell Counter with the optional EVOS® light cubes installed allows you to select the brightfield channel to perform cell count and cell viability assays for trypan blue-stained cells.

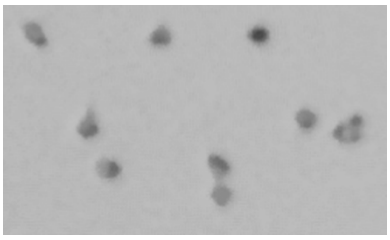
Count procedure

1. Prepare sample by adding 10 µL of your cell suspension to 10 µL of 0.4% trypan blue stain.
2. Load 10 µL of the sample mixture per chamber into the Countess® Cell Counting Chamber Slide as described on page 14.

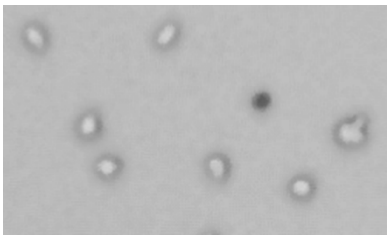
   Note: You can also capture your sample on a Countess® II FL Reusable Chamber Slide using the reusable slide carrier (white) as described on page 16.
3. Insert the Countess® Cell Counting Chamber Slide into the slide port of the Countess® II FL Automated Cell Counter (see page 7), making sure that the sample side A is inserted completely into the instrument. You will hear a soft click, if the slide is pushed in correctly.
4. When the slide is inserted into the instrument, the instrument automatically illuminates the sample and auto focuses on the cells.



Note: The auto focus algorithm is designed to highlight the differences between live and dead cells. Therefore, the optimal focus level is slightly "under" focus, where optimally focused "live" cells have a light colored center and "dead" cells are dark throughout (see examples on page 21). To enable the auto focus to function properly, manually set the optimal focus as described in Step 6, page 21.



Focus is not optimal



Focus is optimal

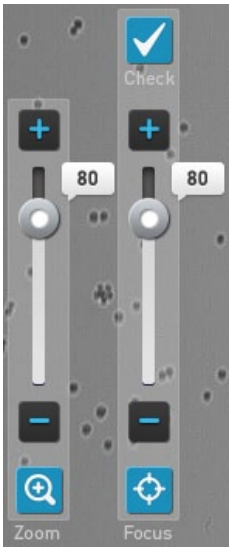
5. To select the light source, press the **brightfield** button. If needed, deselect the other light sources by pressing the appropriate light source buttons.

 Brightfield unselected  Brightfield selected

Note: The light source buttons select the light channel(s) (brightfield and/or fluorescence) used for illuminating the sample; they do not determine which channels are captured. Each channel can be turned ON and OFF independently and the exposure for each channel can be adjusted separately (see Steps 7–9).

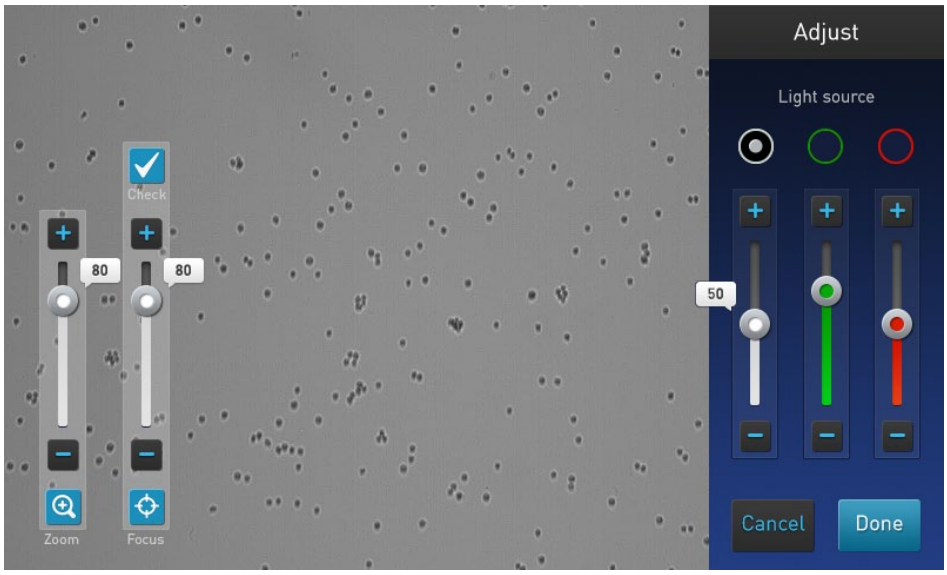
6. *Optional*: If you wish to manually adjust the focus, press the **Focus** button and use the **Focus slider** to bring your sample into focus. You can also use the **plus** and **minus** buttons to adjust the focus.

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



Note: If needed, **Zoom** in on the image to adjust focus or lighting. The Zoom function is also available on the Adjust screen.

7. To set exposure, press **Adjust**. The Adjust screen opens.



8. Using the **light slider** for the brightfield channel (white), adjust the exposure.

Note: The light sliders are used for adjusting the image brightness and contrast. Each slider controls the LED intensity, camera gain, and exposure time for the channel.

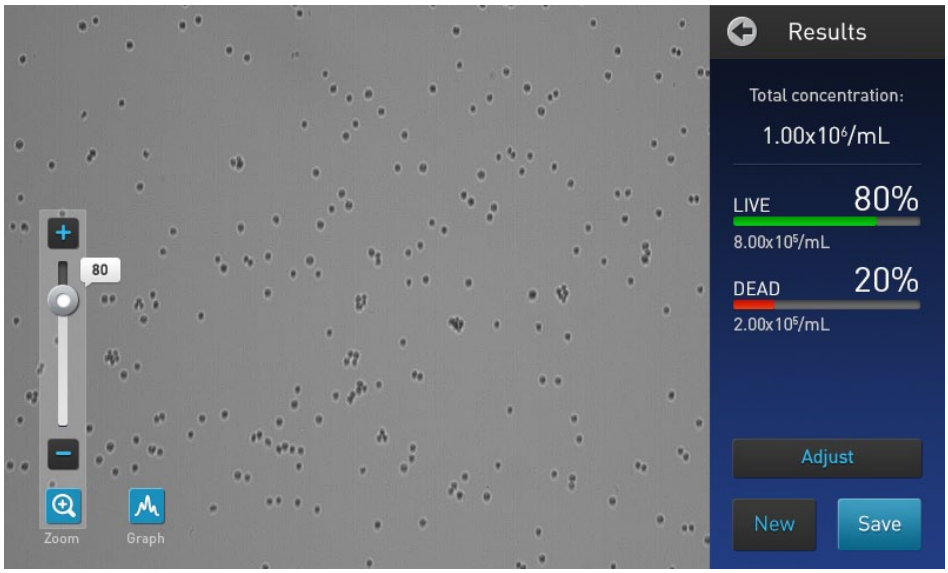
9. Press **Done** to return to the Capture screen.

   To return to the Capture screen without changing the exposure, press **Cancel**.

10. On the Capture screen under Collect channels, press **BRT** to select the brightfield channel.

If needed, deselect the other channels by pressing the appropriate channel selection buttons.

11. Press **Capture**. The instrument temporarily captures the image and displays the results (total concentration, percentage and concentration of live and dead cells). For more information, see “Results”, page 27.



12. To perform a new count, press **New**. Remove and turn around the slide, and reinsert it sample side first into the instrument to count the sample in the second chamber.

Next steps

- To see the distribution of live and dead cells in a graphical format, press the **Graph** button (see page 29).
- To gate the count results by object size, brightness, or circularity, press **Adjust** (see “Gate count results”, page 31).
- To permanently save the results, press **Save** (see page 39).

Count cell fluorescence

Overview

When equipped with the optional EVOS® light cubes, the Countess® II FL Automated Cell Counter can be used for a variety of fluorescent applications, including simultaneous counts of cells stained with two different fluorescent dyes, GFP and RFP expression, apoptosis, cell viability (live, dead, and total cells), and cell cycle assays.

For instructions on installing the optional EVOS® light cubes to your Countess® II FL Automated Cell Counter, see page 44.

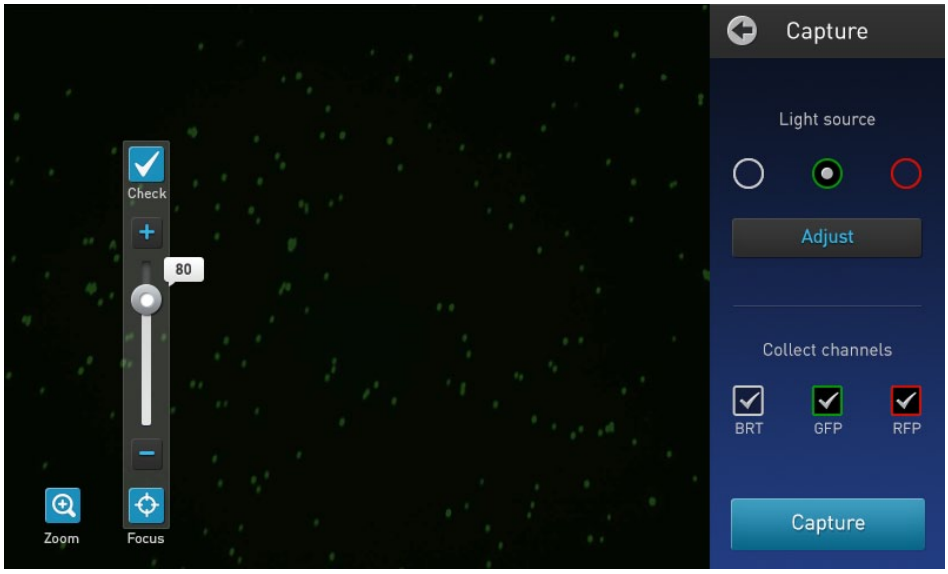
Count procedure

1. Load 10 µL of the fluorescent sample mixture per chamber into the Countess® Cell Counting Chamber Slide as described on page 14.

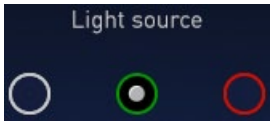
   Note: You can also capture your sample on a Countess® II FL Reusable Chamber Slide using the reusable slide carrier (white) as described on page 16.

2. Insert the Countess® Cell Counting Chamber Slide into the slide port of the Countess® II FL Automated Cell Counter (see page 7), making sure that the sample side A is inserted completely into the instrument. You will hear a soft click, if the slide is pushed in correctly.

3. When the slide is inserted into the instrument, the instrument automatically illuminates the sample and auto focuses on the cells.



4. To select the light source, press the desired **light source** button. The instrument displays the sample in the selected channel (brightfield or fluorescent)

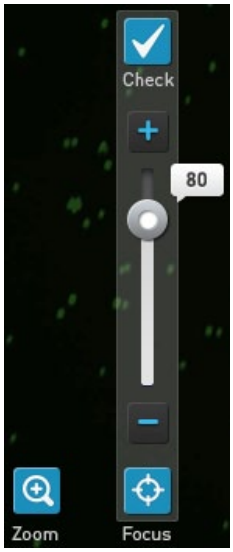


   In this example, the sample is displayed in the GFP channel.

   Note: The light source buttons select the light channel(s) (brightfield and/or the fluorescence) used for illuminating the sample; they do not determine which channels are captured. Each channel can be turned ON and OFF independently and the exposure for each channel can be adjusted separately (see Steps 6–8).

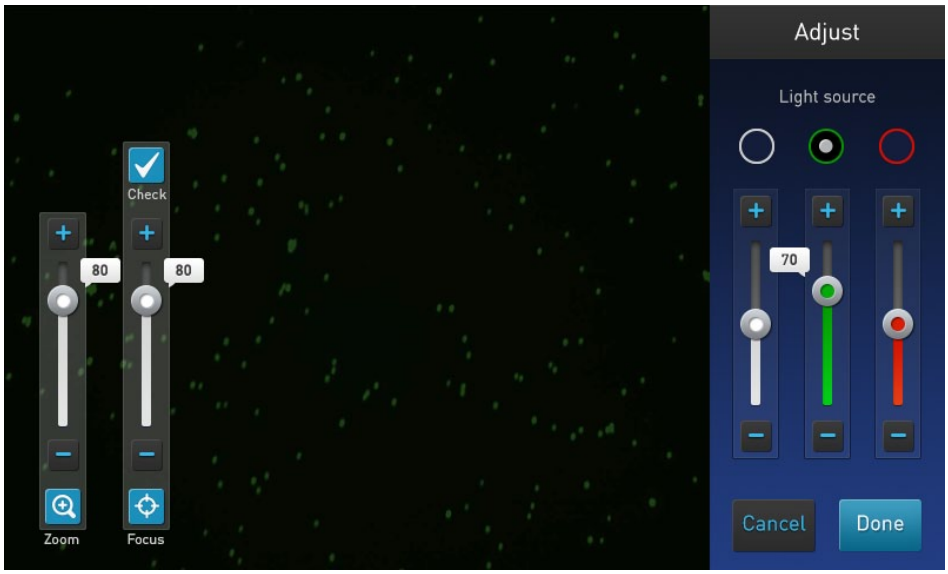
5. *Optional*: If you wish to manually adjust the focus, press the **Focus** button and use the **focus slider** to bring your sample into focus. You can also use the **plus** and **minus** buttons to adjust the focus.

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



   Note: If needed, **Zoom** in on the image to adjust focus or lighting. The Zoom function is also available on the Adjust screen.

6. To set exposure, press **Adjust**. The Adjust screen opens.



7. Using the **light** slider(s), adjust the exposure for the desired channel(s). You can turn each channel ON and OFF by pressing the **light source** buttons.

   Note: The light sliders are used for adjusting the image brightness and contrast. Each slider controls the LED intensity, camera gain, and exposure time for the channel.

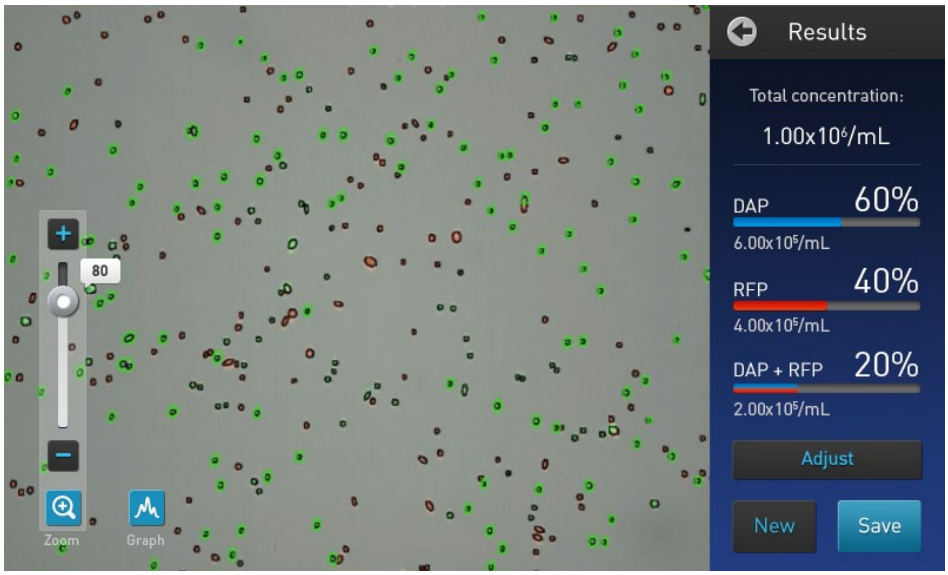
8. After setting the exposure, press **Done** to return to the Capture screen.

   To return to the Capture screen without changing the exposure, press **Cancel**.

9. On the Capture screen, select the channel(s) to capture by pressing the appropriate **collect channel** button(s).

10. Press **Capture**.

    The instrument temporarily captures the image and displays the results (total concentration, percentage and concentration of cells counted through each fluorescence channel individually and together). For more information, see "Results", page 27.



11. To perform a new count, press **New**. Remove and turn around the slide, and reinsert it sample side first into the instrument to count the sample in the second chamber.

Next steps

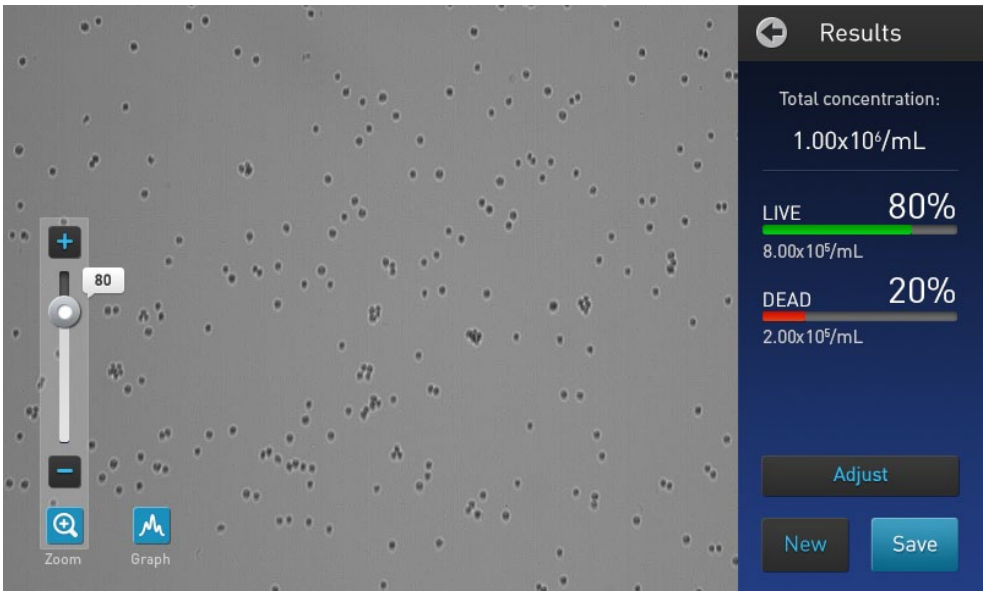
- To see the distribution of cells counted through each channel in a graphical format, press the **Graph** button (see page 29).
- To gate the count results by object size, brightness, or circularity, press **Adjust** (see "Gate count results", page 31).
- To permanently save the results, press **Save** (see page 39).

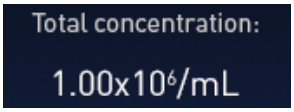
5. Results

Results screen

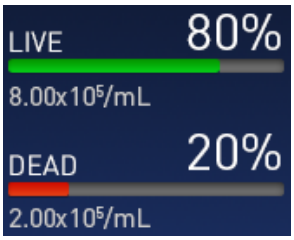
Results screen for cell count and cell viability assays

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).





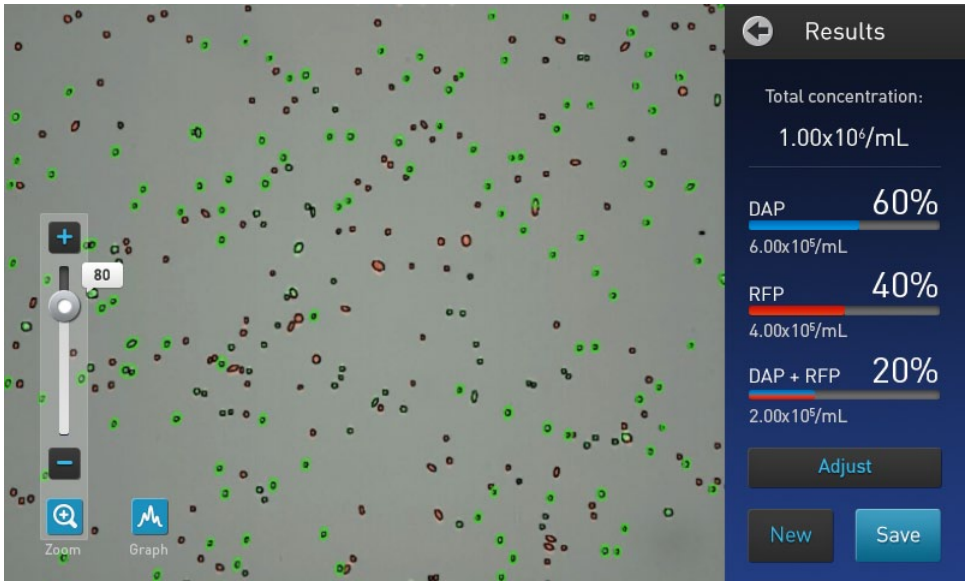
Total concentration (in cells per mL) of cells in the original sample

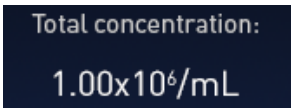


The percentage and concentration (in cells per mL) of "Live" and "Dead" cells in the original sample

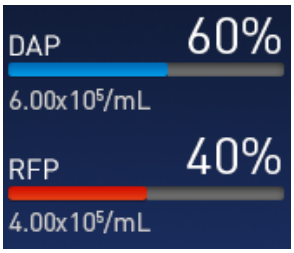
Results screen for cell fluorescence assays

The Results screen for cell fluorescence assays using the Countess® II FL Automated Cell Counter with the EVOS® light cubes installed 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 individually and together).

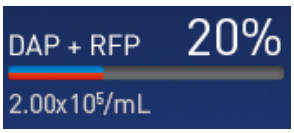




Total concentration (in cells per mL) of cells in the original sample



The percentage and concentration (in cells per mL) of cells in the original sample that are counted through each individual channel (in this example, expressing DAPI **or** RFP)



The percentage and concentration (in cells per mL) of cells in the original sample that are counted through both fluorescence channel (in this example, expressing both DAPI **and** RFP)

Next steps

- To view the objects (i.e., cells) counted in each selected channel, press **Adjust** to open the Count screen (page 29).

  In the Count screen, you can also gate the results by object size, brightness, or circularity (see "Gate count results", page 31).
- To see the distribution of cells counted through each channel in a graphical format, press the **Graph** button (see page 36).
- To permanently save the results, press **Save** (see page 39).

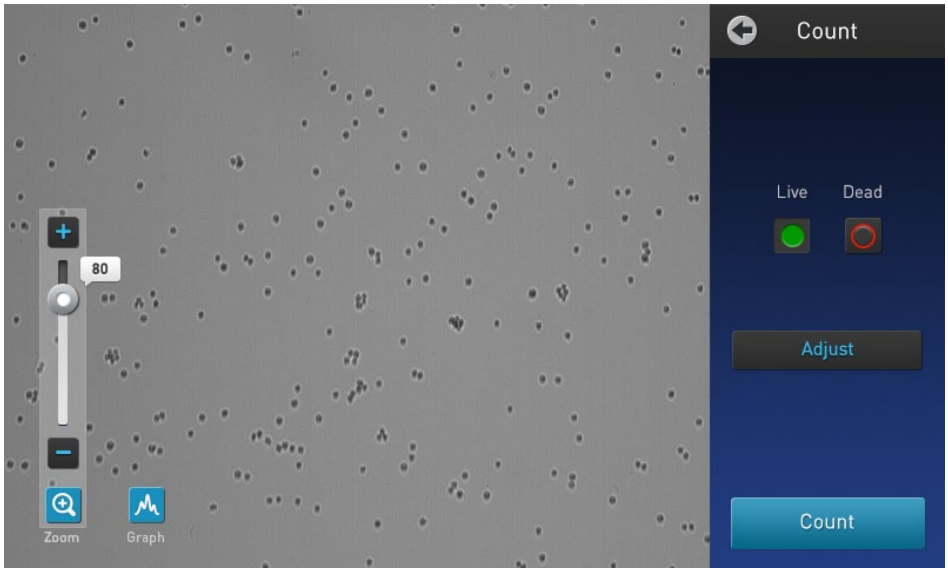
Count screen

Overview

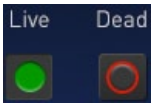
The Count screen allows you to identify the objects (i.e., cells) counted in each channel and included in the count results for further review. After reviewing the marked objects, you can adjust the threshold values for size, brightness, and/or circularity for more accurate count results.

Identify cells counted in cell count and cell viability assays

1. On the Results screen, click **Adjust.** The Count screen opens.



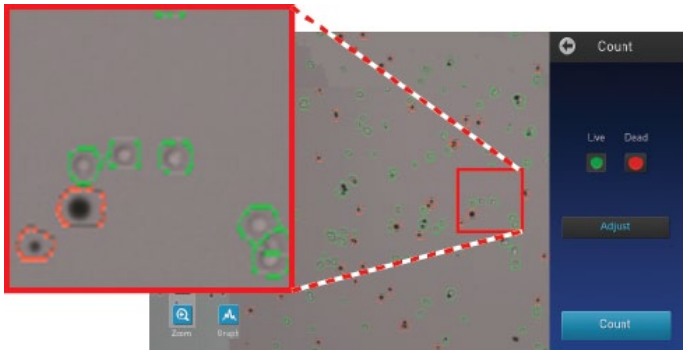
2. To identify the cells that are included in the count as “live”, press the **Live** button. “Live” cells will be circled in green on the screen.



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

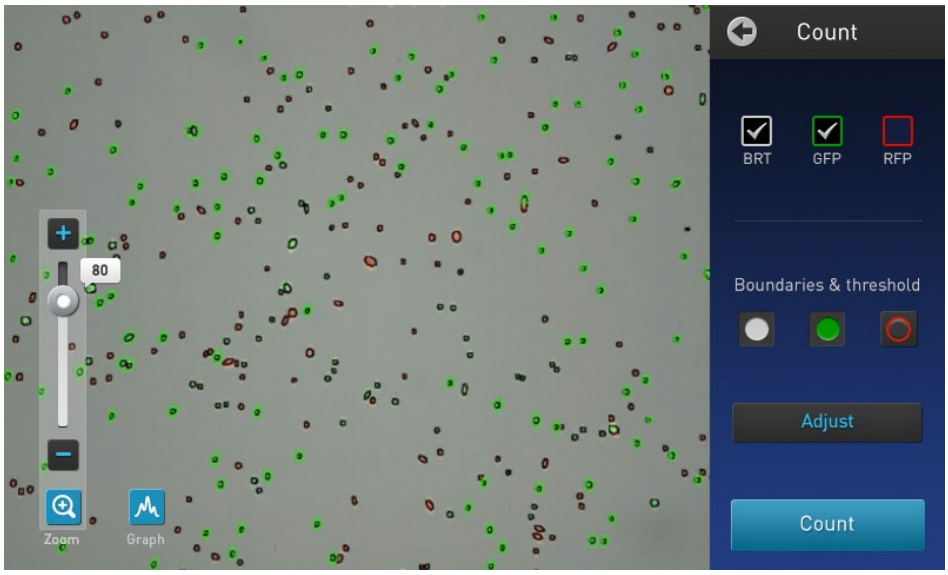
3. To unmark the cells identified as “live” (green) or “dead” (red) on the screen, press the **Live** or the **Dead** button again, respectively.

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

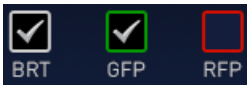


Identify cells counted in fluorescence assays

1. On the Results screen, click **Adjust.** The Count screen opens.

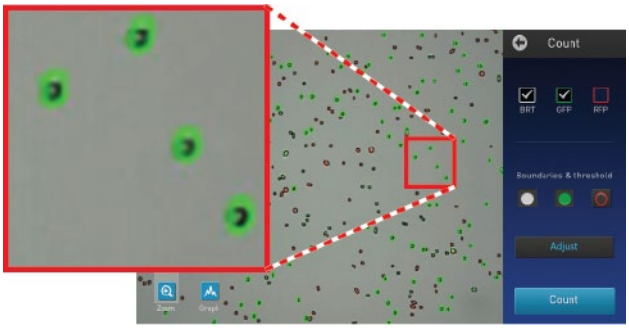


2. To identify the cells that are counted in a fluorescent channel, press the desired **identify channel** button. Cells counted in the selected channel will be circled on the screen with the same color as the channel.



For example, if **GFP** button is pressed, the cells counted in the GFP channel will be circled in green; if **RFP** button is pressed, the cells counted in the RFP channel will be circled in red.

Note: You may select either or both options. In the example below, the **GFP** button is pressed and the cells counted in that channel are marked with green circles around them.



3. To unmark the cells, press the appropriate **channel** button again.

Gate count results

Overview

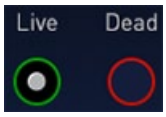
The Countess® II and Countess® II FL counters allow you to adjust the counting algorithm to include or exclude objects (i.e., cells) in the final count results based on the data range set for size, brightness, and/or circularity.

With the Countess® II FL counter, you can also adjust the counting algorithm based on relative fluorescence intensity in the selected fluorescence channel.

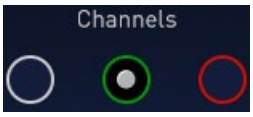
For more information on the parameters you can adjust in the counting algorithm, see "Count parameter controls", below.

Count parameter controls

- The data range for brightness, size, and circularity parameters can be set for both the brightfield channel and for fluorescence channels (if applicable). The data range for relative fluorescence intensity can be adjusted only of the instrument is equipped with the optional EVOS® light cubes.
- You can adjust the data range for the count parameters using the **parameter sliders**. Parameter sliders correspond to a single channel, which can be changed using the radio buttons above the sliders.



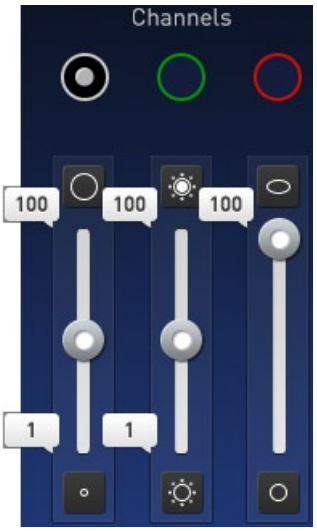
Channel selection (instruments without EVOS® light cubes)



Channel selection (instruments with EVOS® light cubes installed)

- **Brightness**, **size**, and **fluorescence intensity** sliders set the upper and lower boundaries within which the cells are counted. This range can be expanded using the **plus** and **minus** buttons that appear when the slider button is pressed.

  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.



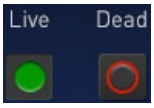
Parameter sliders for the brightfield channel (from l to r): size, brightness, and circularity

Parameter slider for fluorescence intensity (in this example, GFP)

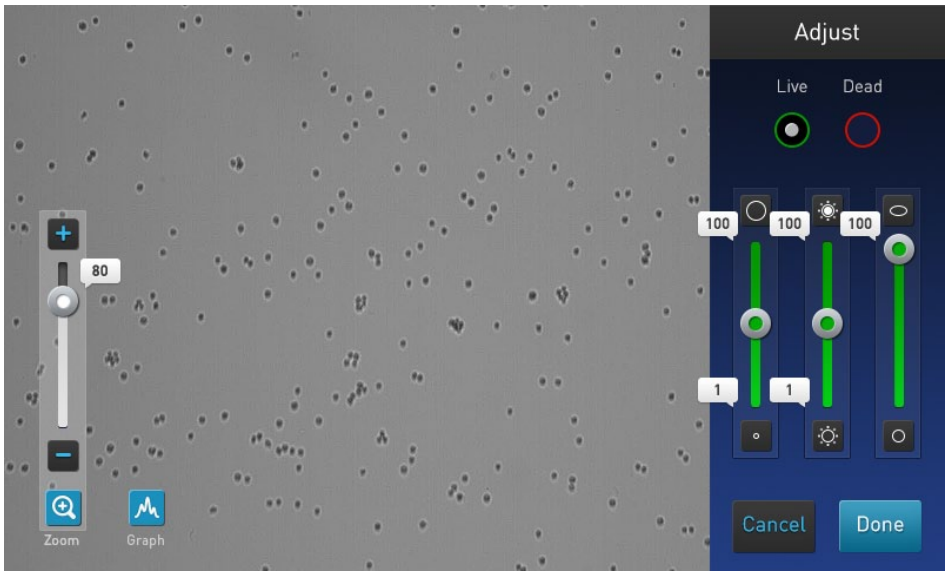
- **Brightness:** As you move the slider up, the algorithm will include the brighter objects in the count. As you move the slider down, only the dimmest of objects will be counted. You can expand the range of the slider using the plus and minus buttons.
- **Size:** As you move the slider up, the algorithm will include larger objects in the count. As you move the slider down, only the smallest of objects will be counted. You can expand the range of the slider using the plus and minus buttons.
- **Circularity:** As you move the slider up the algorithm will include more objects in the count that are different shapes other than circular. As you move the slider down, only the objects that are perfect circles will be counted. You can expand the range of the slider using the plus and minus buttons.
- **Fluorescence intensity:** As you move the slider up, the algorithm will include the objects that fluoresce more brightly in the count. As you move the slider down, only the dimmest of objects will be counted. You can expand the range of the slider using the plus and minus buttons.

Adjust counting algorithm for cell count and cell viability assays

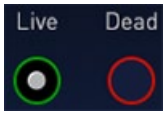
1. On the Results screen, press **Adjust** to open the Count screen.
2. *Optional*: If desired, press **Live** and/or **Dead** button(s) on the Count screen to identify the cells counted in each population (see page 29).



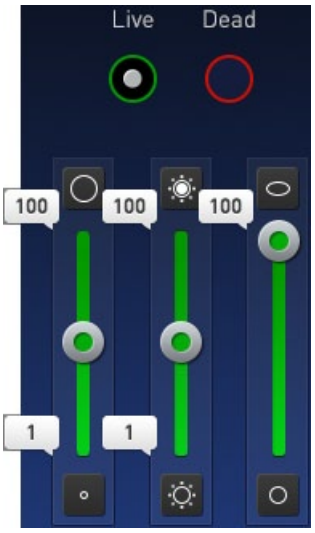
3. On the Count screen, press **Adjust**. The Adjust screen opens, which contains the controls for adjusting the data range for brightness, size, and circularity.



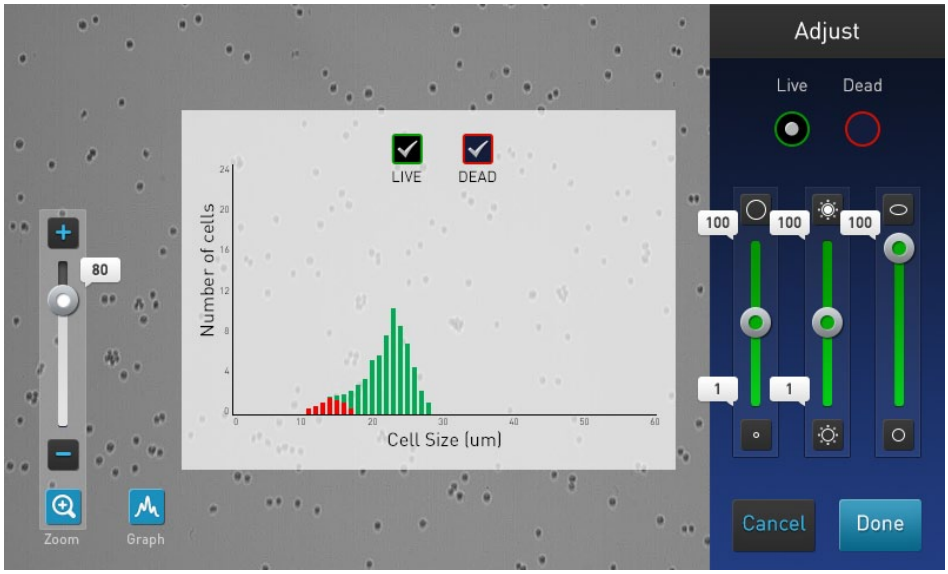
4. Press the **Live** or the **Dead** button to select the channel for which you wish to adjust counting parameters.



5. Using the **brightness**, **size**, and **circularity** sliders, adjust the data range for the count parameters of interest.



Note: Press the **Graph** button (see page 36) to view the distribution of the cells (live and/or dead) based on size as you adjust the count parameters, which changes the count results.

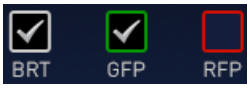


6. When finished, press **Done** to save the changes and return to the Results screen.

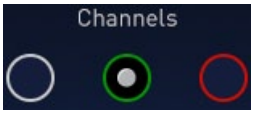
   Press **Cancel** to return to the Results without saving the changes to the count parameters.

Adjust counting algorithm for cell fluorescence assays

1. On the Results screen, press **Adjust** to open the Count screen.
2. *Optional*: If desired, press the desired **identify channel** button(s) to identify the cells counted in each selected population (see page 30).



3. On the Count screen, select the **channel** for which you wish to adjust the count parameters. You can also select the desired channel in the Adjust screen (see below)

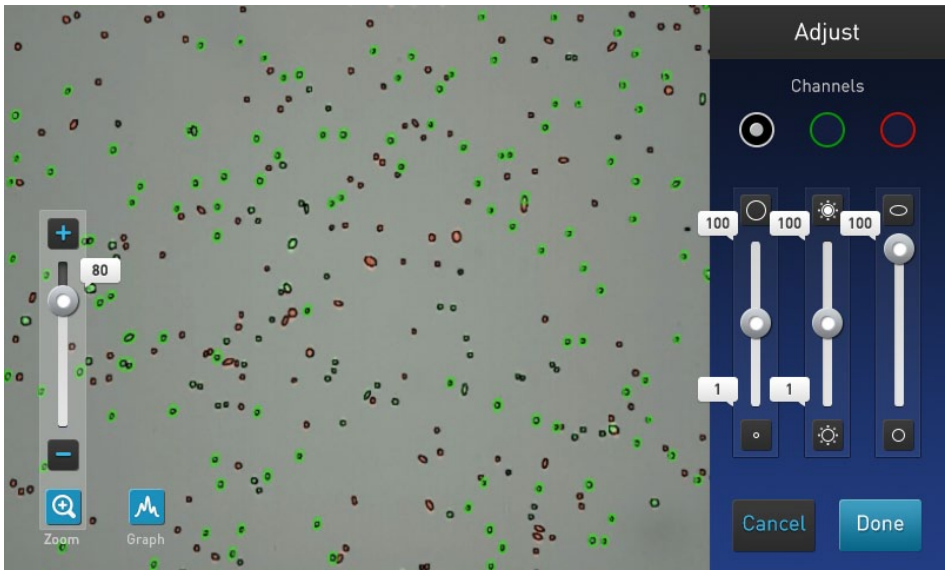


   Pressing the **select brightfield** button (white circle) allows you to adjust the data range for brightness, size, and circularity.

   Pressing a **select fluorescence channel** button (colored circles) allows you to adjust the data range for fluorescence intensity in the selected channel.

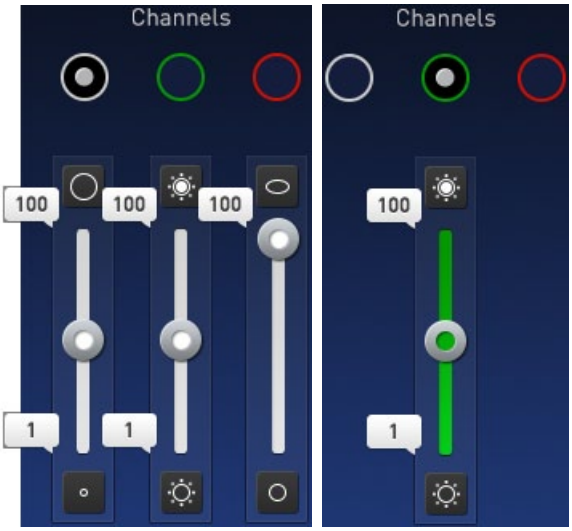
   Note: The fluorescence channels available depend on the EVOS® light cubes installed in the instrument.

4. Press **Adjust**. The Adjust screen opens, which contains the controls for adjusting the data range for parameter(s) in the selected channel.

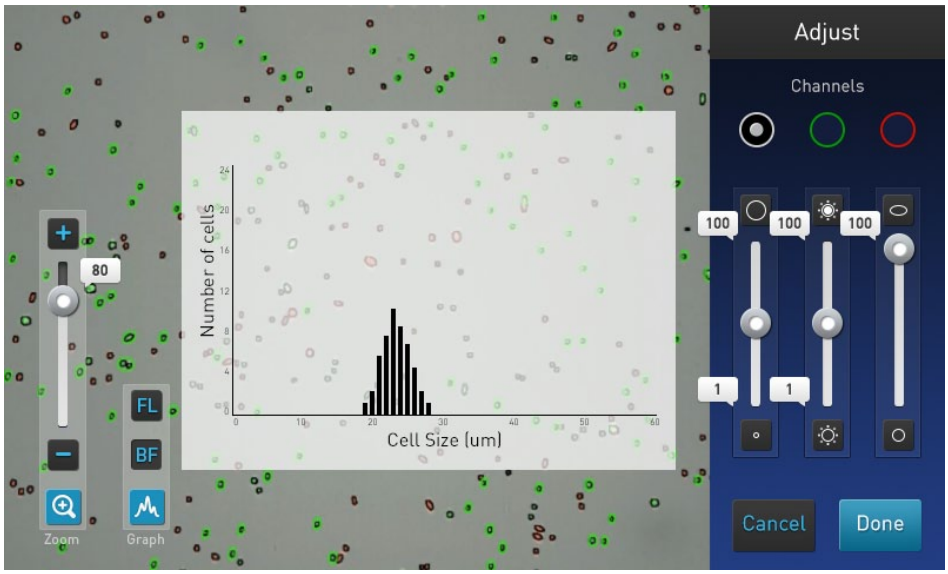


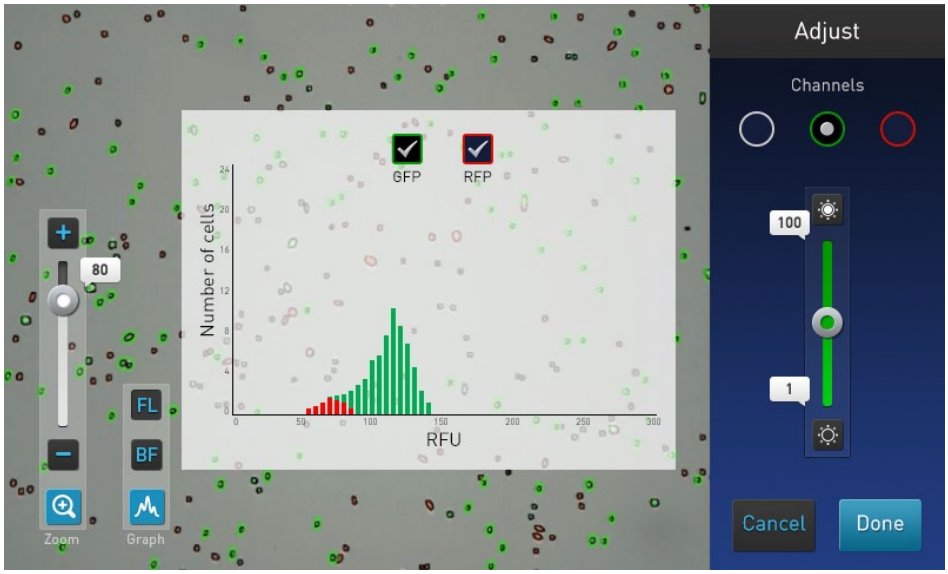
5. If the brightfield channel is selected on the Count screen (Step 3), adjust the data range for the count parameter of interest using the **brightness**, **size**, or **circularity** slider.

   If a fluorescence channel is selected on the Count screen (Step 3), adjust the range for the selected channel using the **fluorescence intensity** slider.

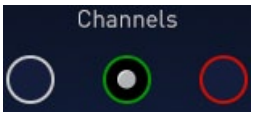


Note: Press the **Graph** button (see page 36) to view the distribution of the cells based on size (if brightfield channel is selected) or fluorescence intensity (if a fluorescence channel is selected) as you adjust the count parameters, which changes the count results.





6. To adjust the counting parameters in other channels, press the desired **select channel** button.



7. When finished, press **Done** to save the changes and return to the Results screen.

   Press **Cancel** to return to the Results without saving the changes to the count parameters.

Graph

Overview

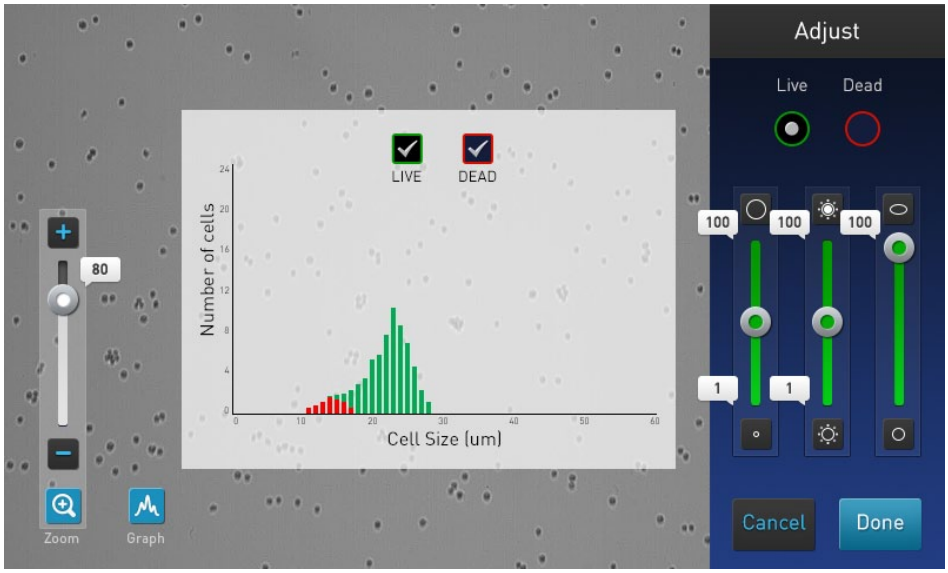
The Countess® II and Countess® II FL Automated Cell Counters allow you to view the distribution of cells counted through each channel in a graphical format.

- 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.
- For cell counts performed in fluorescence channels, you have the option of viewing the distribution of cells based on their size or based on their relative fluorescence intensity.

Graph for cell count and cell viability assays

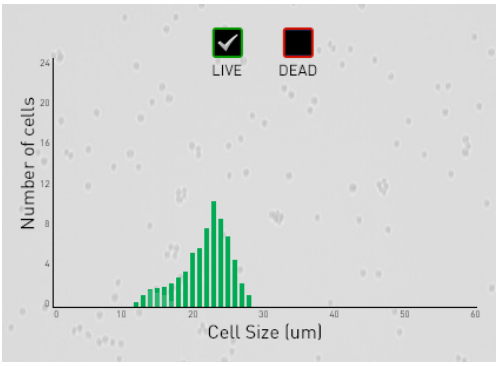
1. To view the graph showing the distribution live and/or dead cells based on cell size, press the **Graph** button. 

   Note: The Graph button is available on the Results, Count, and Adjust screens.



2. To view the distribution of only the live or dead cells, press the **Live** or **Dead** button to select or deselect the desired population. 

   The graph automatically updates and displays the distribution of cells based on size only in the selected population (Live cells in the example below).



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

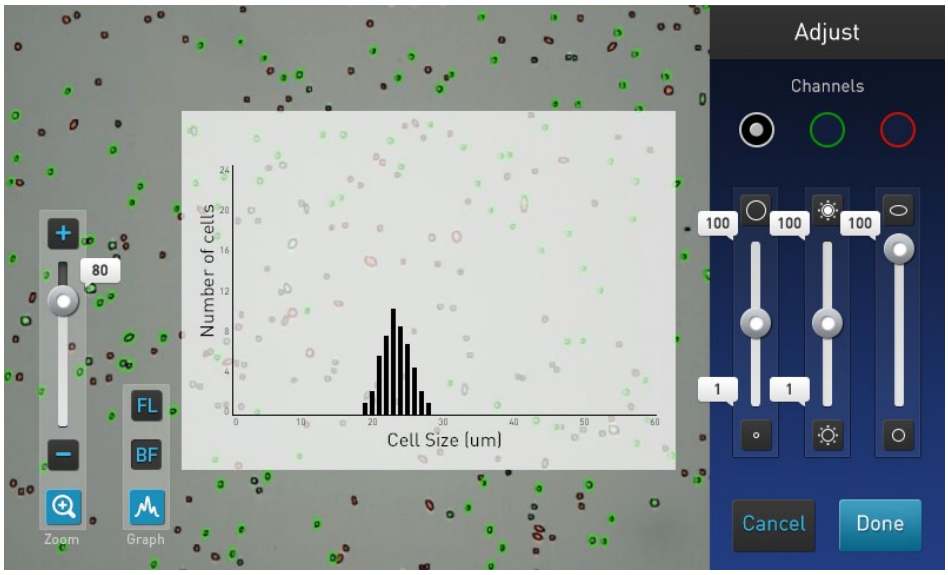
Graph for cell fluorescence assays

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

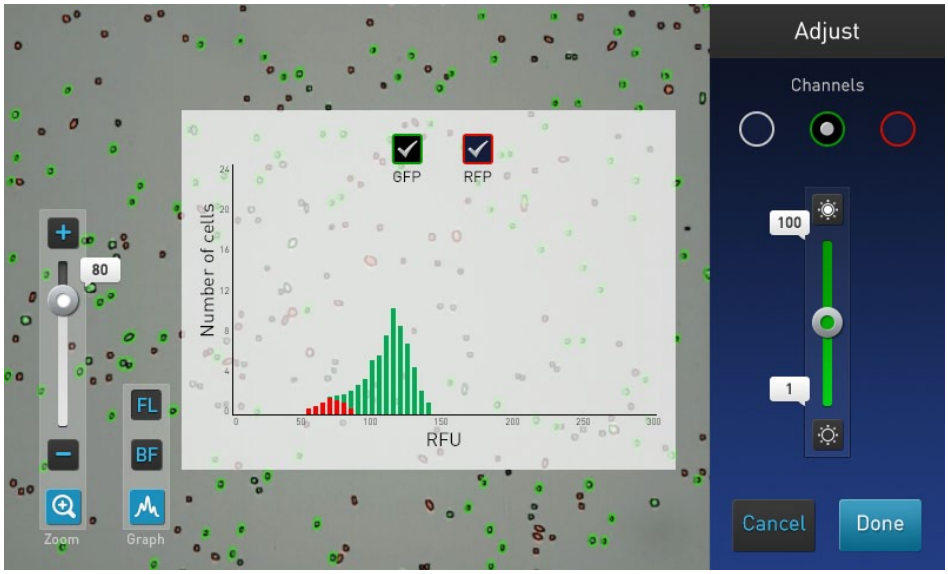
   The cell size graph opens and displays the size distribution of the total cell count (i.e., number of cells vs. cell size in µm).

   Note: The Graph button is available on the Results, Count, and Adjust screens.

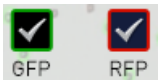


2. To view the distribution of cells captured in either or both fluorescence channels based on their relative fluorescence intensity, press the **FL Graph** (fluorescent graph) button.

   The cell fluorescence graph opens and displays the distribution of cells based on their relative fluorescence intensity (i.e., number of cells vs. relative fluorescence intensity).

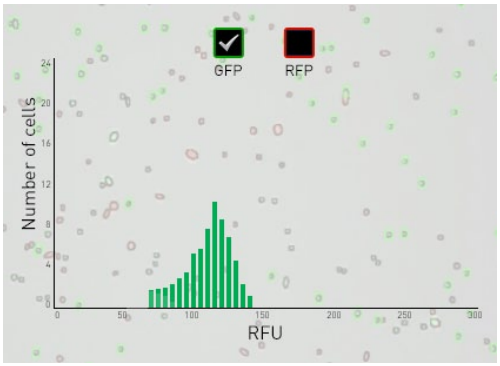


3. To view the distribution of cells only in a selected fluorescence channel, press the appropriate **channel** button to select or deselect the desired population.



The graph automatically updates and displays the distribution of cells based on their relative fluorescence intensity only in the selected channel.

In the example below, the **GFP** button is pressed, and the graph displays the distribution of only GFP-expressing cells based on their fluorescence intensity.



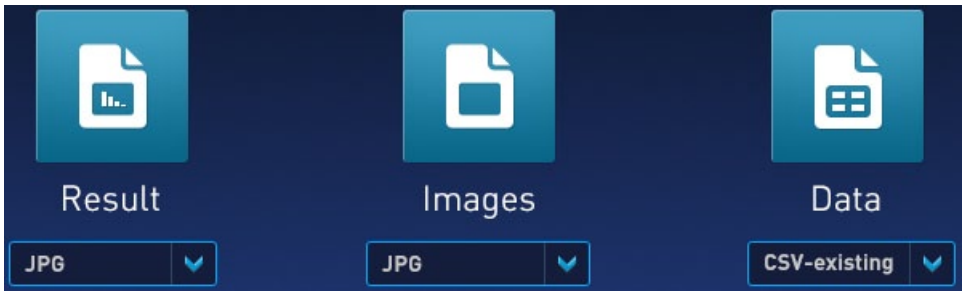
4. To close the graph, press the **Graph** button again.

Save results

Overview

The Countess® II and Countess® II FL Automated Cell Counters allow you to save your data and images on an external computer using a USB flash drive.

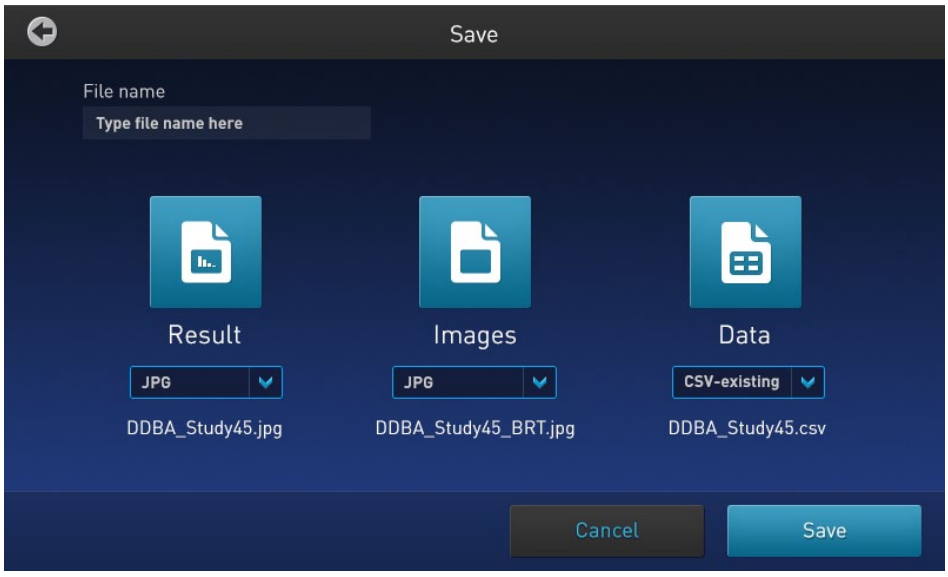
You can choose to save your experiment in the following formats, individually or in any combination:



- **Result:** Saves the Results screen as it is displayed, with or without Graph (page 36), in the selected image format (JPEG, PNG, TIFF, or BMP).
- **Images:** Saves the captured image in the selected image format (JPEG, PNG, TIFF, or BMP).
- **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 C: CSV file format", page 49.

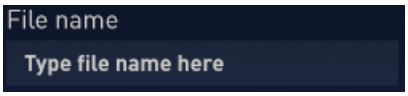
Save procedure

1. To archive your data or generate a printed report, insert the Countess® II USB drive (or equivalent) into the USB port of the instrument (see page 7).
2. On the Results screen, press **Save**. The Save screen opens.

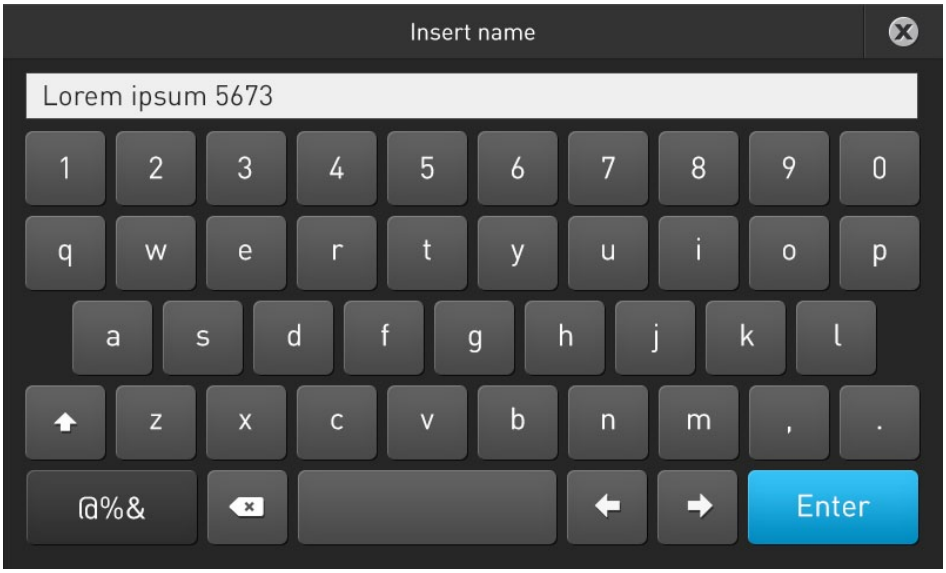


Note: If you wish to save your results with the Graph showing the distribution of cells based on cell size or fluorescence intensity, make sure that the desired graph is displayed on the Results screen (see page 36).

3. To assign a name to your experiment, press the **File name** text field.



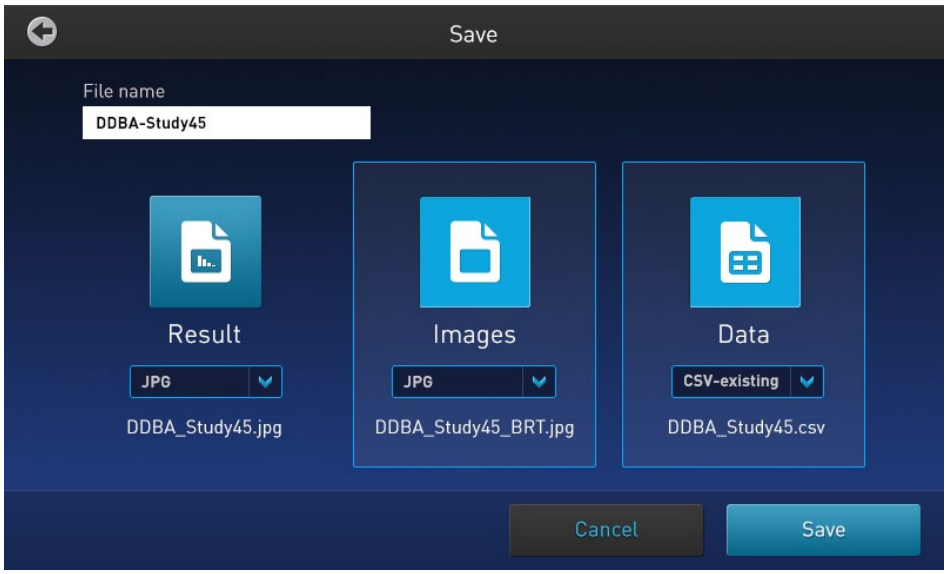
4. Enter the file name using the **keypad** buttons that are displayed. To enter symbols, press the **symbol (@%&)** key.



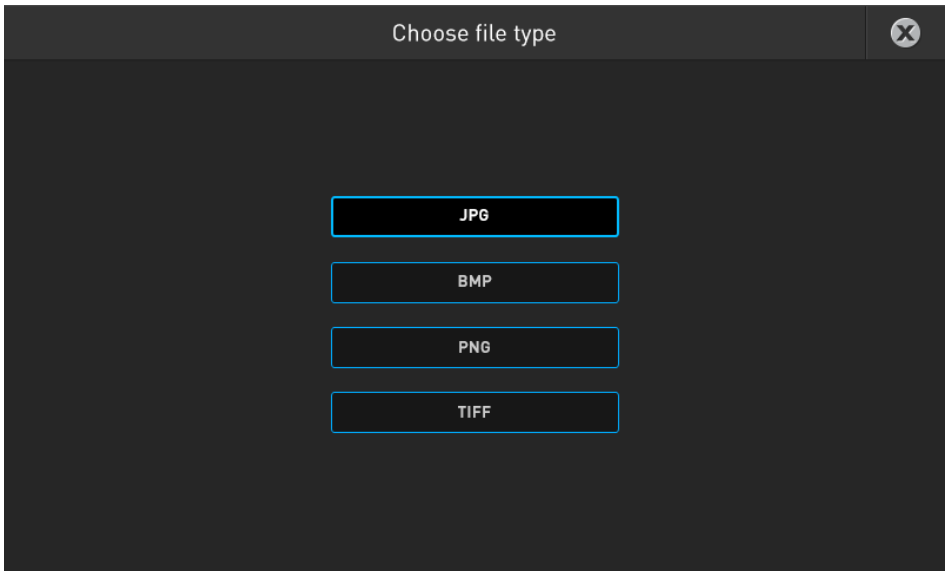
5. Press **Enter** to save the name and return to the Save screen.

6. Select the desired format(s) to save your experiment (**Result**, **Images**, **Data**). You may select an individual format (e.g., Result only) or any combination of formats (e.g., Result, Images, and Data).

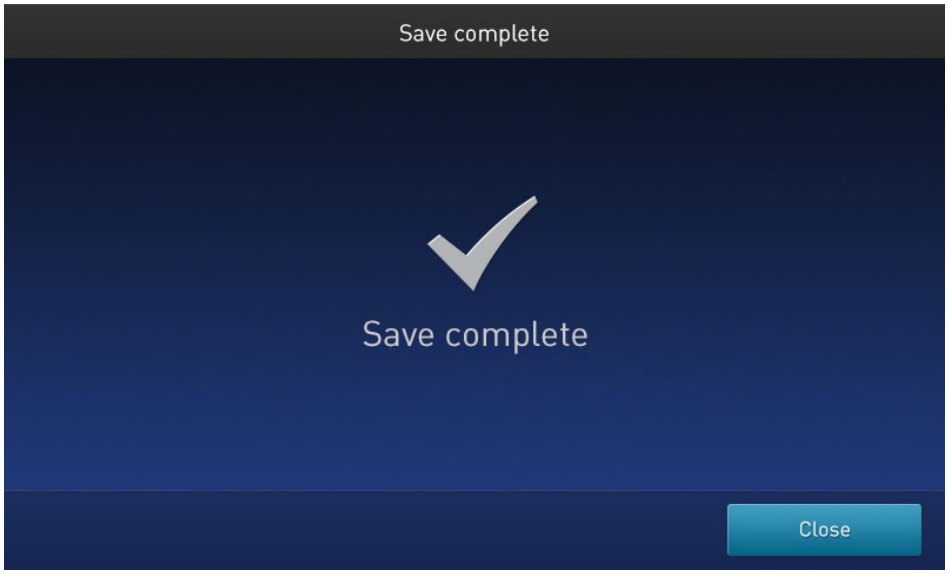
   In the example below, **Images** and **Data** formats are selected.



7. Select the file format you wish to save your Results and Images. Available options are JPEG, PNG, TIFF, and BMP. You can save your Data only as a CSV file.



8. Press **Save** to save your image and the counting data in the USB drive.



9. Press **Close** and then transfer the USB drive to the USB port on your computer. Copy the files on the USB drive to the desired location on your computer or network.

6. Maintenance and Troubleshooting

Care and maintenance

General care

- When cleaning optical elements, use only optical-grade materials to avoid scratching soft lens coatings.
- Use the appropriate cleaning solutions for each component, as indicated in the Sterilization Procedures below.
- If liquid spills on the instrument, turn off the power immediately and wipe dry.
- Do not exchange objectives between instruments unless you know that the components have been approved and recommended by Life Technologies™.
- After using, cover the instrument with the supplied dust cover.

Power supply

Always use the correct power supply. The power adaptor specifications appear on the serial number label (front of LCD hinge) and in the Specifications. 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 (page 61) or your local distributor for information.

Cleaning the Countess® II Automated Cell Counter

Introduction

We recommend cleaning the Countess® II Automated Cell Counter periodically to prevent the buildup of dust and dirt that might reduce its performance and cause contamination.

CAUTION! To avoid electrical shock, always turn off the Countess® II 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 manufacturer may result in damage to the instrument.

Clean the press-screen

- Wipe the press-screen of the Countess® II Automated Cell Counter using a soft, lint-free cloth moistened with an LCD cleaning solution. Do not apply excessive force during cleaning. Wipe the press-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 press-screen from getting scratched.

Clean the instrument case

- Wipe the instrument case of the Countess® II 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 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. Avoid cleaning the press-screen.
- 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.

EVOS® light cube installation

Install light cubes

The Countess® II FL Automated Cell Counter can hold up to two EVOS® light cubes. Light cubes do not come standard with the device and must be purchased separately (see page 47).

To install a light cube:

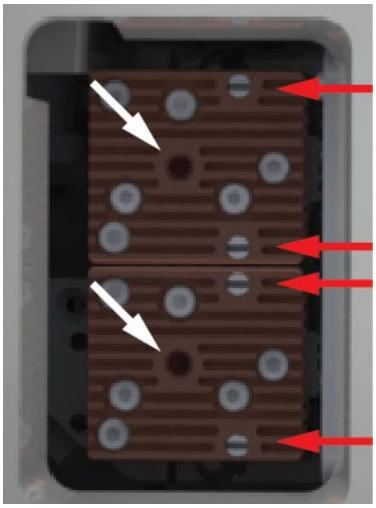
2. Turn off the Countess® II Automated Cell Counter by flipping the **power switch** (i.e., On/Off switch) on the back of the instrument to the OFF position (see image on page 8).
3. Unplug the power cord from the Countess® II Automated Cell Counter.
4. Unlatch the back panel with the two captive ¼-turn fasteners (indicated by red arrows) that secure the back panel on the rear of the Countess® II Automated Cell Counter and remove the back panel.

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

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

A

B



7. To remove a light cube, unscrew both screws that secure it to the instrument.
8. Thread the light cube removal tool into the central hole in the cube (white arrows in Figure B, above) and gently pull the light cube out of the device.

   Note: Always store the cube removal tool in the back panel for easy access.
9. Install the back panel and secure it in its place with both ¼-turn fasterners.
10. Plug the power cord back into the Countess® II Automated Cell Counter.
11. Turn off the Countess® II Automated Cell Counter by flipping the **power switch** (i.e., On/Off switch) on the back of the instrument to the ON position.

Appendix A: Product Specifications

Technical Specifications

Physical characteristics	**Instrument type:**	Benchtop cell counter and suspension cell-based assay platform
	Instrument dimensions:	9″ (W) × 5½″ (D) × 9″ (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:	10–40°C
	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:	5–60 µm
	Required sample volume:	10 µL
	Firmware:	Countess Automated Cell Counting Platform Software
	USB Drive :	4 Gigabyte
Optics	**Optics:**	Countess® II Automated Cell Counter: single bright field channel Countess® II FL Automated Cell Counter: 3 channels (bright field 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 below). 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 channel dedicated to the transmitted light from the condenser for brightfield contrast applications, the Countess® II FL Auto Imaging System can accommodate two fluorescent or specialty light cubes for multiple-fluorescence research applications.

The table below lists some of the common fluorescent and specialty light cubes available from Light Technologies™. For a complete list of available light cubes and to inquire about custom light cubes, go to **www.lifetechnologies.com/evosflauto** or contact Technical Support (see page 61). For instructions on changing the LED light cubes, see page 44.

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, Cy®3, MitoTracker® Orange, Rhodamine Red, DsRed
Texas Red	Texas Red®, Alexa Fluor® 568, Alexa Fluor® 594, MitoTracker® Red, mCherry, Cy®3.5
Cy5	Cy®5, 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.

Appendix B: Ordering information

Countess® II and Countess® II FL Automated Cell Counter

The following products can be used with the Countess® II and Countess® II FL Automated Cell Counters and are available separately from Life Technologies™. For more information, visit **www.lifetechnologies.com** or contact Technical Support (page 61).

Product	Quantity	Cat. no.
Countess® II Automated Cell Counter	1 each	AMQAX1000
Countess® II FL Automated Cell Counter	1 each	AMQAF1000
Countess® II power cord with four adapter cords	1 each	C10285
Countess® II USB drive	1 each	C10286

Accessory products

The following products can be used with the Countess® II and Countess® II FL Automated Cell Counters and are available separately from Life Technologies™. For more information, visit **www.lifetechnologies.com** or contact Technical Support (page 61).

Product	Quantity	Cat. no.
Countess® Cell Counting Chamber Slides, 50 Slides (100 counts)	1 box	C10228
Countess® Cell Counting Chamber Slides, 500 Slides (1000 Counts)	10 boxes	C10312
Countess® Cell Counting Chamber Slides, 1250 Slides (2500 Counts)	25 boxes	C10313
Countess® Cell Counting Chamber Slides, 2500 Slides (5000 Counts)	50 boxes	C10314
Countess® Cell Counting Chamber Slides, 5000 Slides (10,000 Counts)	100 boxes	C10315
Countess® II FL Reusable Chamber Slides		
Countess® II FL Reusable Chamber Slides		
Trypan blue stain (0.4 %)	2 × 1 mL	T10282

Appendix C: 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
	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

Category	Column	Name	Description
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
	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

Appendix D: Safety

Safety conventions used in this document

Safety alert words

Four safety alert words appear in Life Technologies™ user documentation at points in the document where you need to be aware of relevant hazards. Each alert word–**IMPORTANT, CAUTION, WARNING, DANGER**–implies a particular level of observation or action:

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 the document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard icons that are affixed to the instrument (see "Safety symbols").

Symbols on instruments

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 instruments"). 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
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.

Environmental symbols

The following symbol applies to all Life Technologies™,™ 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.lifetechnologies.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 on Life Technologies™,™ instruments in combination with the safety symbols described in the preceding section.

Hazard 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 Life Technologies™ 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 chez Life Technologies™.
	**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 Life Technologies™ 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). See "Safety Data Sheets (SDS)".

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. For operating environment, refer to "Operating environment" (page 12).
- 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

Chemical hazard warning

WARNING! CHEMICAL HAZARD. Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.

WARNING! CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

General safety guidelines

To minimize the hazards of chemicals:

- 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. (See "Safety Data Sheets (SDS)")
- 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.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

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

DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock can result from operating the Countess® II Automated Cell Counter or the 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.

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 Auto Imaging System has an installation (overvoltage) category of II, and is classified as portable equipment.

Biological hazard safety

WARNING! BIOHAZARD. 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 guidelines published in *Biosafety in Microbiological and Biomedical Laboratories*

  (stock no. 017-040-00547-4;
  www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm)
- 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

This section provides information on:

- U.S. and Canadian safety standards
- European safety and EMC standards
- Australian EMC standards

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.

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 Life Technologies™™.

Australian EMC standards

The **C-Tick Mark** indicates conformity with Australian and New Zealand standards for electromagnetic compatibility.

Documentation and support

Obtaining support

Technical support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies™ contact the chemical 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 found on Life Technologies™' website at **www.lifetechnologies.com/termsandconditions**.

If you have any questions, please contact Life Technologies™ at **www.lifetechnologies.com/support**.