

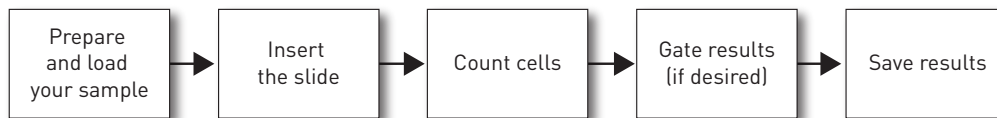
Countess™ II FL Automated Cell Counter

Catalog Number AMQAF1000

Pub. no. MAN0014631 Rev. A.0

Note: For safety and biohazard guidelines, refer to the “Safety” appendix in the *Countess™ II FL Automated Cell Counter User Guide* (Pub. no. MAN0010644). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Workflow



For detailed instructions, maintenance information, and troubleshooting, refer to the *Countess™ II FL Automated Cell Counter User Guide* on the USB drive supplied with the instrument or download it from www.thermofisher.com/countess.

Count cells in brightfield

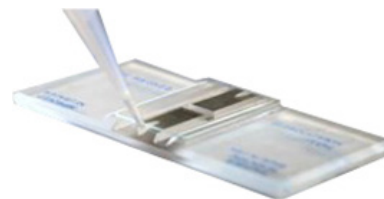
Prepare and load your sample

1. Add 10 μL of your cell suspension to 10 μL of 0.4% trypan blue stain. Mix well.
2. Load 10 μL of the sample mixture per chamber into the sample slide. Let the sample mixture settle for 30 seconds.

Note: The Countess™ II FL Automated Cell Counter accepts both disposable Countess™ Cell Counting Chamber Slides and glass Countess™ II FL Reusable Slides on interchangeable, slide specific carriers.



Countess™ Cell Counting Chamber Slide (disposable)



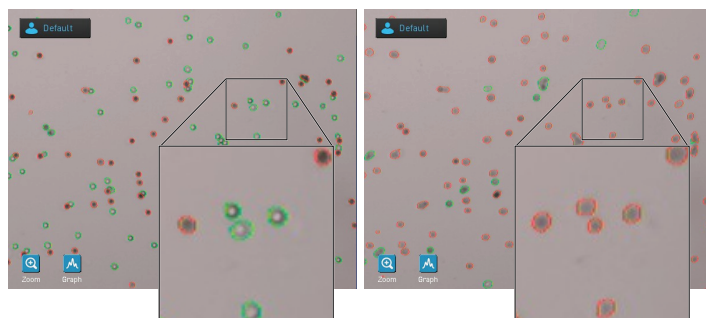
Countess™ FL Reusable Slide

Insert the slide

1. Insert the slide completely into the slide port until you hear a soft click.

The instrument will automatically focus and set brightfield illumination intensity.

Note: In new instruments, you may initially need to set the focus manually using the **Focus** button. Subsequent uses will then focus correctly using the autofocus feature.



Correct focus

Incorrect focus

Count cells

1. On the Capture screen, select **Capture**. The instrument captures the image and displays the results (total concentration, percentage and concentration of live and dead cells).

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

IMPORTANT! When performing cell counts and cell viability assays in brightfield, the cell concentration displayed in the Results screen is the original cell concentration before dilution into trypan blue.

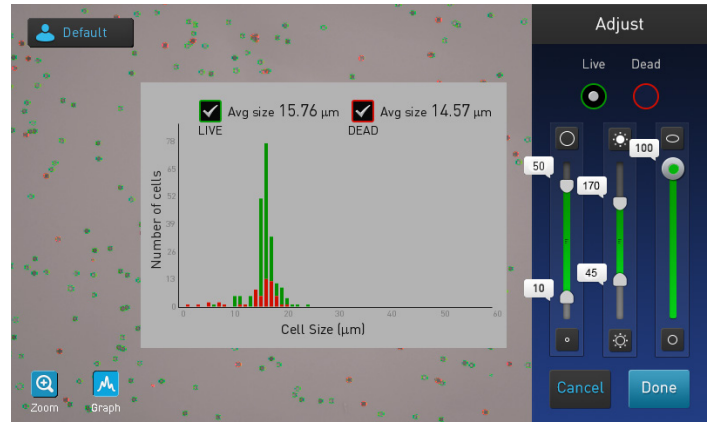
2. To view the histogram showing the distribution of live and/or dead cells based on cell size, press the **Graph** button. The histogram includes the average cell size for live and dead cells.



Gate results

1. On the Results screen, press **More** to open the Advanced screen.
 2. Press **Adjust** to open the Adjust screen and then select the channel (**Live** or **Dead**) you wish to gate.
 3. Select the **Graph** button to show the histogram.
- Note:** When using the gating feature, this helps to identify the population of cells of interest.
4. Using the **size**, **brightness**, and **circularity sliders**, adjust the count parameters. The graph and image display are updated in real time.

Note: This is especially helpful with samples that have a significant amount of debris.



Before gating – debris counted as dead cells result in falsely high dead cell count



After gating – accurate results when debris is excluded from cell count

5. Press **Done** to save the changes and return to the Advanced screen, or press **Cancel** to return to the Results screen without saving the changes.
6. On the Advanced screen, press **Count** to recalculate your results with the new count parameters. The results are updated automatically without the need to perform another count.
7. To permanently save your results, see “Save results” on page 4.

Count cell fluorescence

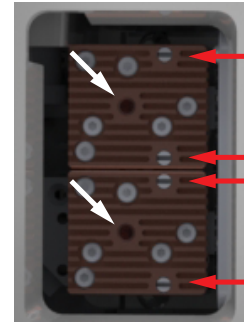
Note: To analyze fluorescently labeled samples, the Countess™ II FL Automated Cell Counter must be equipped with EVOS™ light cubes, which must be purchased separately.

Install EVOS™ light cube

1. On the Instrument Settings screen, press **Change Light Cube**.
2. When prompted, flip the power switch to the **OFF** position and unplug the power cord from the instrument.
3. Unlatch the back panel of the instrument and place the light cube into one of the two empty slots.
4. Using the light cube tool, tighten the two screws (red arrows) to securely attach the cube to the instrument.

Note: To remove a light cube, first unscrew the screws that secure it to the instrument, thread the light cube removal tool into the central hole in the cube (white arrow), and then gently pull the light cube out of the device.

5. Reinstall the back panel, plug the power cord back into the instrument, and flip the power switch to the **ON** position.



Count fluorescent cells

1. Insert the prepared slide completely into the slide port until you hear a soft click.

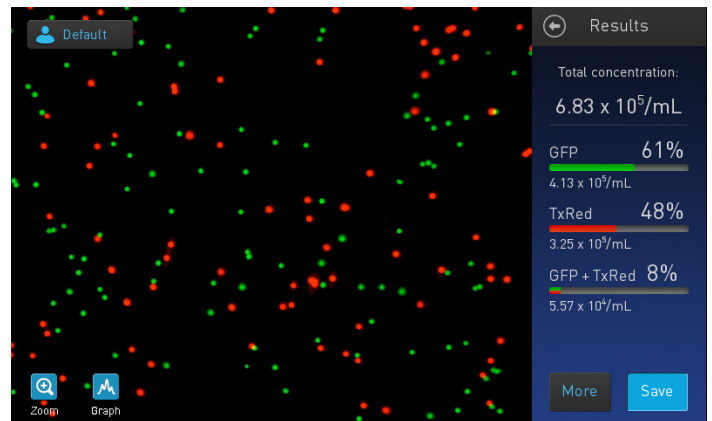
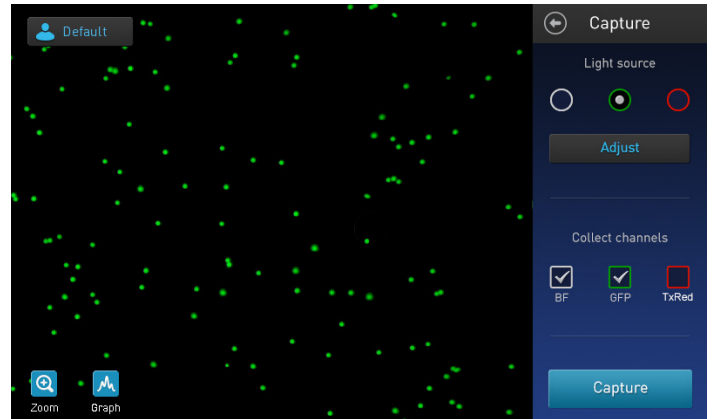
The instrument will automatically focus and set brightfield illumination intensity.

2. If needed, set the focus manually using the **Focus** button.
3. To view your sample in a different channel, select the desired **Light source**. The instrument will display the sample in the selected channel (brightfield or fluorescent).
4. If needed, press **Adjust** to modify the illumination/fluorescence excitation intensity.
5. Select the channels you wish to capture using the **Collect channels** check boxes.
6. Press **Capture**. The instrument collects each selected fluorescence channel along with the brightfield and displays the results (total concentration, percentage and concentration of cells counted in each fluorescence channel as well as those counted in both channels).

IMPORTANT! The total cell concentration displayed after a fluorescent count does not take any dilution into account. Therefore, the cell concentration displayed in the Results screen must be multiplied by the dilution factor to calculate the original cell concentration in the sample.

This is in contrast to the cell counts in brightfield, where the cell concentration displayed is the sample concentration before dilution into trypan blue.

7. To view the histogram, press the **Graph** button and then select:
 - **BF** (brightfield) to show the distribution of cells based on cell size. The histogram includes the average cell size.
 - **FL** (fluorescence) to show the distribution of cells based on relative fluorescence intensity (RFU). The histogram includes the average RFU in each selected fluorescence channel.

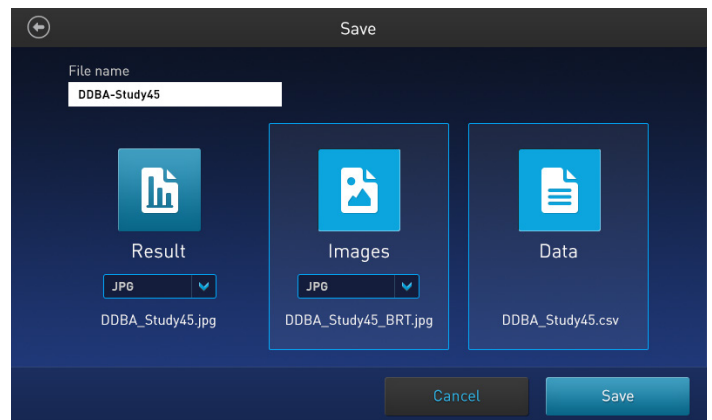
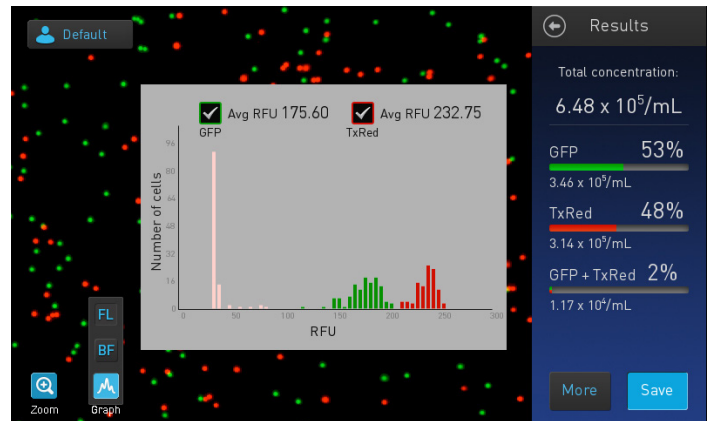
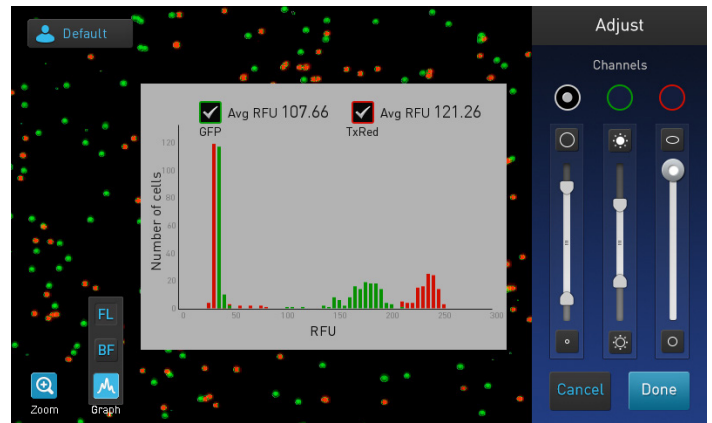


Gate results

1. On the Results screen, press **More** to open the Advanced screen.
2. Press **Adjust** to open the Adjust screen.
3. Press the **Graph** button and then select **BF** (brightfield) or **FL** (fluorescence).
Note: When using the gating feature, the graph function helps to identify the population of cells of interest.
4. Select the **brightfield channel** (white circle) to gate the results based on **size, brightness, and/or circularity**.
5. Select the desired **fluorescence channel** (colored circles) to gate the results by **fluorescence intensity**.
6. When satisfied with the adjusted gates, press **Done** to save the changes and return to the Advanced screen.
Note: As you adjust the gating parameters, the graph and image display are updated in real time.
7. On the Advanced screen, press **Count** to recalculate your results with the new count parameters. The results are updated automatically without the need to perform another count.

Save results

1. Insert the Countess™ II USB drive (or equivalent) into an available USB port on the instrument.
2. On the Results screen, press **Save** to go to the Save screen.
3. Press the **File name** text field and use the alpha-numeric keypad to name your files.
4. Select the desired files to save your experiment (**Result, Images, Data**). You can select any combination of result, image, and data files to save.
Note: Result and image files can be saved as **JPEG, BMP, PNG, or TIFF** files.
5. Press **Save** to save your experiment to the USB drive.



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

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