invitrogen

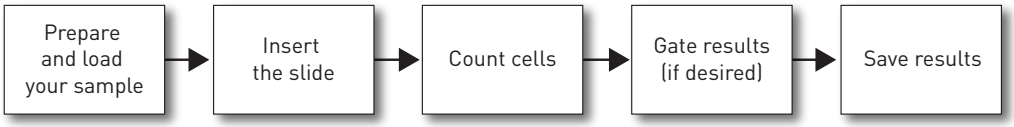
Countess™ II Automated Cell Counter

Catalog Number AMQAX1000

Pub. no. MAN0010826 **Rev.** C.0

Note: For safety and biohazard guidelines, refer to the "Safety" appendix in the *Countess™ II Automated Cell Counter User Guide* (Pub. no. MAN0014293). 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 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

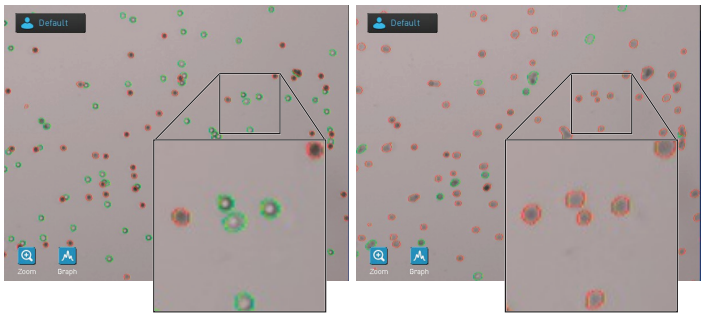
Prepare and load your sample

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.

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).
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.

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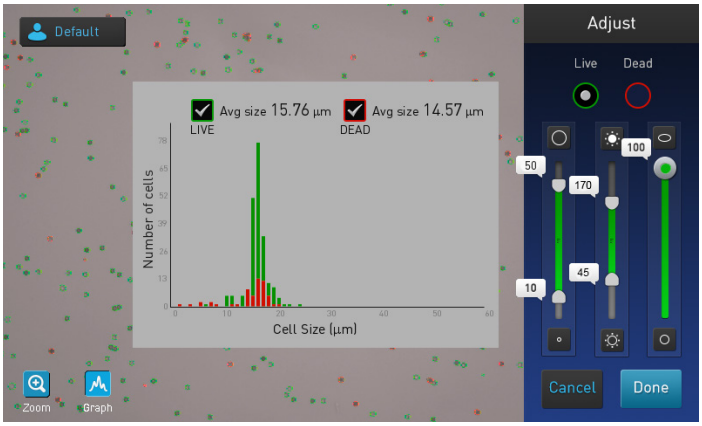
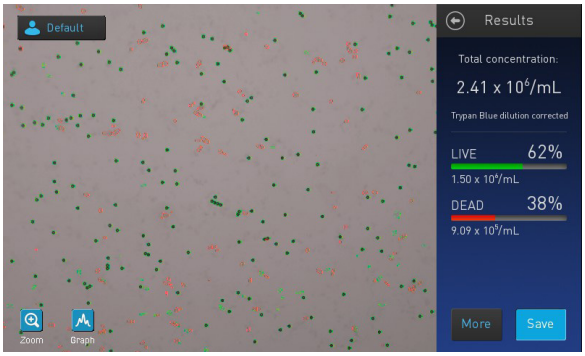
Gate results

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

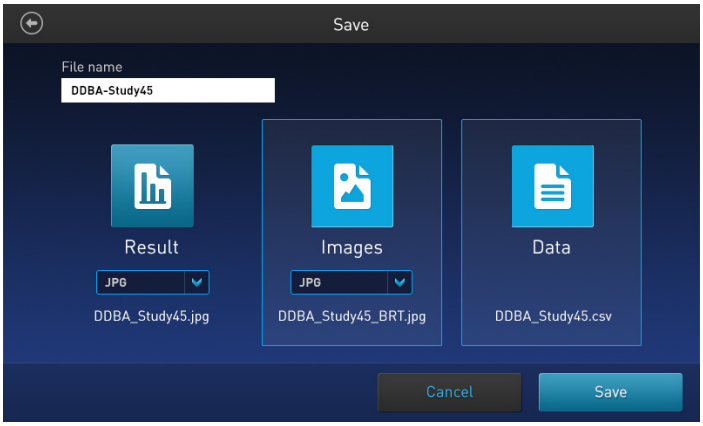
After gating – accurate results when debris is excluded from cell count

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.

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.



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