

# Countess™ Automated Cell Counter

QUICK  
REFERENCE  
CARD

Catalog no. C10227

Revised: 23 September 2008

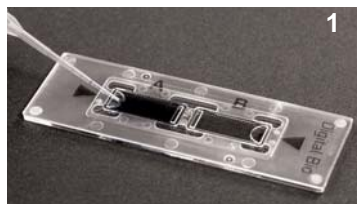
Instructions for using the Countess™ automated cell counter for cell counting with trypan blue stain are described below.

For detailed instructions, refer to the manual supplied with the counter or download the manual from [www.invitrogen.com](http://www.invitrogen.com). To receive notifications about countess updates and for faster service, register your instrument at [www.invitrogen.com/countess](http://www.invitrogen.com/countess).

## Using Countess™ Automated Cell Counter

### 1. Load Sample

- Add 10  $\mu$ L of your sample to 10  $\mu$ L of 0.4% trypan blue stain.
- Add 10  $\mu$ L of the sample mixture into the half-moon shaped chamber port (marked A or B) on the Countess™ cell counting chamber slide. You may load one chamber or both chambers.



### 2. Insert Slide

- Insert slide, sample side first into the slide port of the instrument until you hear a soft click.



### 3. Optimize Image

- Press **Zoom** button and use the focus knob to adjust the image for analysis such that:
  - Live cells have bright centers and dark edges (see images on next page).
  - Dead cells have uniform blue color throughout the cell with no bright centers (see images on next page).
- Use the grid to navigate the slide and check the image in several fields.
- If desired, lock focusing using Focus lock knob.



### 4. Count Cells

- Press **Count cells** button to obtain results.
- Record cell count.
- Press **More Data** button to see data details as well as graphical representation of data.
- To read the second chamber, remove and turn around the slide, and reinsert sample side first into the instrument slide port.



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 **invitrogen™**

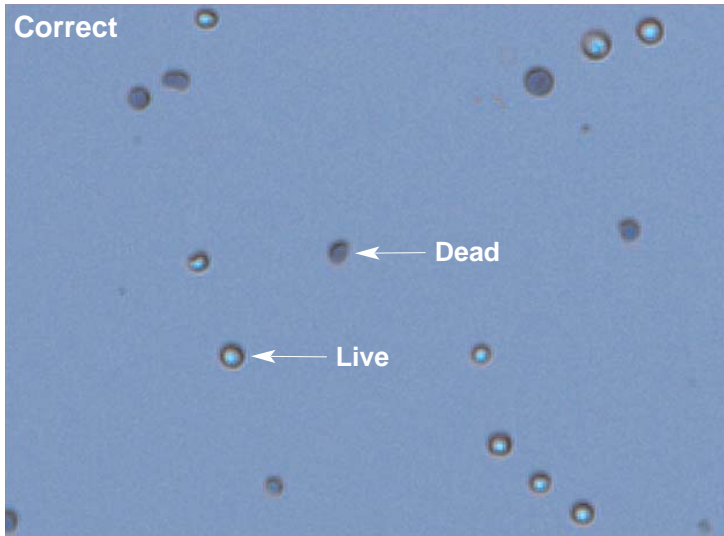
Contact Information for Other Countries:  
See our website: [www.invitrogen.com](http://www.invitrogen.com)

# Optimizing Image Analysis

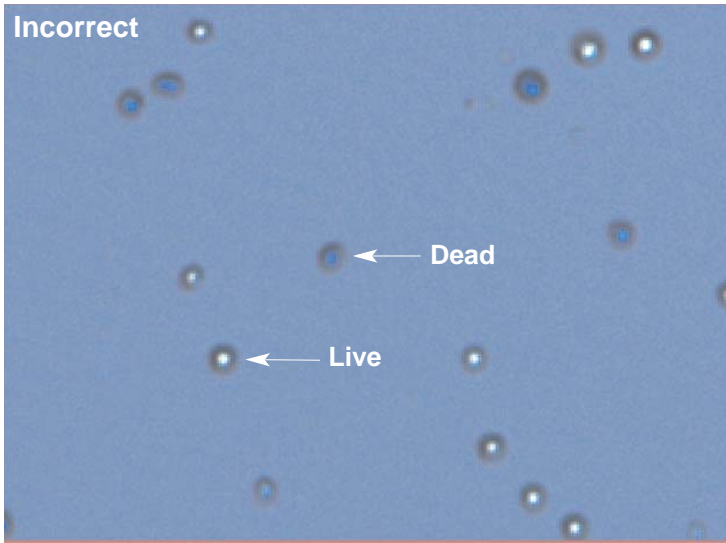
The images below show the correct image to obtain accurate cell count.



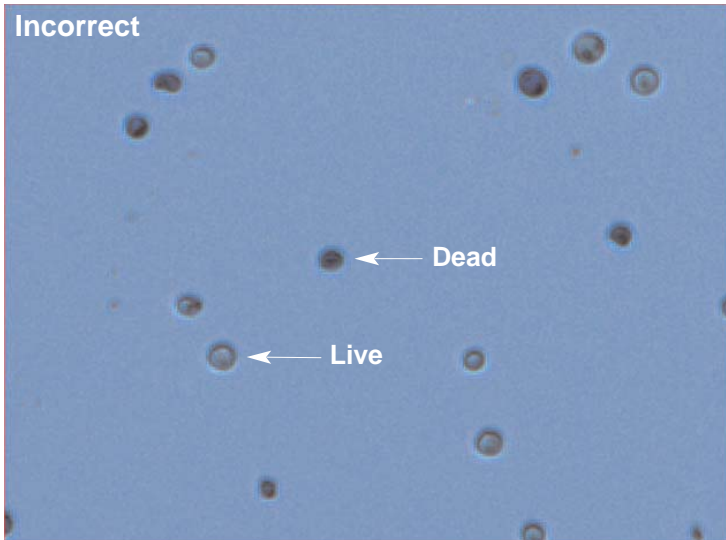
Use the focus knob to optimize the image for analysis.



**Correct Image** (Live cells have bright centers and dark edges)



**Incorrect Image** (Dead cells have bright, blue centers and are counted as live)



**Incorrect Image** (Live cells have dark centers and are counted as dead)

# General Guidelines

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- The Countess™ counter contains delicate optics. Place the instrument on a flat, level, dry surface that is free from excessive vibration. Avoid spilling any liquids on the surface or introduce any liquids into the interior.
- Wear gloves during sample handling. Do not touch the optical surfaces of the Countess™ cell counting chamber slides. Hold the chamber slides by the edges.
- The Countess™ cell counting chamber slides supplied with the counter are specially designed for use exclusively with the Countess™ automated cell counter. Use of other slides result in inaccurate cell counts and can damage the Countess™ counter.
- The instrument counts all cells in an entire field you see when the image is in **Zoom out** mode, even if you press **Count cells** in **Zoom** mode. This volume is equivalent to four (1 mm x 1 mm) squares in a standard hemocytometer (0.4 µL).
- For accurate viability count, ensure the counting area is covered with cell suspension and count cells within 3 minutes of mixing cells with trypan blue stain as trypan blue is toxic to cells.
- For best data with biological samples, count at least two samples and take an average.
- The Countess™ automated cell counter can accurately count cells from 5 µm–60 µm in size.
- The Countess™ automated cell counter accurately determines concentration range from  $1 \times 10^4$  to  $1 \times 10^7$  cells/mL, with the highest accuracy in the range from  $1 \times 10^5$  cells/mL to  $4 \times 10^6$  cells/mL.
- The instrument is supplied pre-calibrated. If you update the Countess™ firmware or use a different formulation of trypan blue that is not supplied with the instrument, recalibrate the instrument using calibration instructions on the instrument or in the manual. The calibration function sets the background of the instrument to a solution of trypan blue in the chamber slide.
- The Countess™ automated cell counter memory holds one set of data. To save your data after reading, transfer the data to the Countess™ USB drive and to your PC immediately as described in the manual.
- The numerical data is automatically saved as a .CSV file that can be opened with any spreadsheet program. To delete all data from the .CSV file and start with a blank file, press **Start .CSV file** button.
- After using Countess™ cell counting chamber slides, appropriately dispose slides as biohazardous waste. Do not reuse the chamber slides.
- We recommend that you turn off the instrument at the end of the day.

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