



Countess[™] Automated Cell Counter

Catalog no. C10227

Version B
26 November 2008
MP10227

User Manual

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Product Contents

Countess™ Automated Cell Counter

The contents of the Countess™ automated cell counter are listed below:

| Component | Quantity |
|--|-----------------|
| Countess™ automated cell counter | 1 |
| Power Cord with 4 adaptor cords (for U.S./Canada/Taiwan/Japan, Europe, or UK) | 1 |
| Countess™ cell counting chamber slides | 1 box of 50 |
| Trypan blue stain (0.4%) | 2 × 1 mL |
| Countess™ USB drive | 1 |
| Instruction manual | 1 |
| Quick reference card (QRC) | 1 |

See page vii for specifications and description of the Countess™ automated cell counter.

Upon Receiving the Instrument

Examine the instrument carefully for any damage incurred during transit. Ensure that all parts of the instrument including accessories listed above are included with the product. Any damage claims must be filed with the carrier. The warranty does not cover in-transit damage.

See page 3 to install the instrument.

Upon receipt, store Trypan blue stain at room temperature.

Safety Information

Safety Precautions

Review and follow the safety instructions below carefully.

- 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 **Product Specifications** (page vii).
- Do not touch the main plug or power cord with wet hands.
- Always ensure that the power supply input voltage matches the voltage available in your location.
- This instrument is air-cooled so its surfaces may become hot during operation. When installing the instrument, leave a space of more than 10 cm (4 inches) around it and do not place any objects between the instrument and the wall.
- 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 AC adapter.
- 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).

Continued on next page

Safety Information, Continued

Safety Precautions, continued

- Do not use metal objects when assembling or disassembling a battery.
 - Do not heat the battery or expose it to heat. For operating environment, see **Product Specifications** (page vii).
 - If the instrument emits smoke, disconnect the power cord from the wall outlet and contact a service person.
-

Symbols



The symbols used on Countess™ automated cell counter are explained below.

Used on the instrument to indicate a warning. Consult the manual to avoid possible personal injury or instrument damage.



Protective earth (Ground)



WEEE (Waste Electrical and Electronic Equipment) symbol

Product Specifications

Countess™ Automated Cell Counter Specifications

Environmental Conditions

| | |
|----------------------------|----------------------------|
| Operating Power | 100–240 VAC, 1.5 A |
| Frequency | 50/60 Hz |
| Electrical input | 12 VDC, 1.0 A |
| Installation site: | Indoor use only |
| Operating Temperature: | 10–35°C |
| Maximum Relative Humidity: | 20–80% |
| Altitude: | ≤2,000 m |
| Transient Category: | Installation categories II |
| Pollution Degree: | 2 |
| Degree of Protection: | IPX0 |

Instrument Specifications

| | |
|------------------------|--|
| Instrument Type: | Benchtop cell counter |
| Processing Time: | <1 minute |
| Cell Sample Range: | 1×10^4 – 1×10^7 |
| Software: | Countess™ Software www.invitrogen.com/countess |
| Instrument Dimensions: | 27 cm (w) × 20 cm (d) × 19 cm (h) |
| Weight: | 2.1 kg (4.6 lbs) |

Countess™ Cell Counting Chamber Slide Specifications

| | |
|-----------------------------|---------------------------------------|
| Material: (PMMA) | Polymethyl methacrylate |
| Dimensions: | 75 mm (w) × 25 mm (d) × 1.8 mm (h) |
| Chamber Depth: | 100 μm |
| Chamber Volume: | 10 μL |
| Countess™ USB Drive: | 1 Gigabyte |

Continued on next page

Product Specifications, Continued



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 Invitrogen.

Description of Countess™ Automated Cell Counter

Front View of Countess™ Automated Cell Counter

The Countess™ automated cell counter is a benchtop automated cell counter that performs cell count and viability measurements using trypan blue stain.

The front view showing various parts of the Countess™ automated cell counter is shown below.

Power button

The Power Button is used to turn the instrument on and off. The red status light indicates that the instrument is off; the green status light indicates that the instrument is on.

Touchscreen display located in the front of the instrument contains buttons for all the functions needed and displays data from the cell count.

Slide port

The Slide port is used to insert the Countess™ cell counting chamber slide containing the sample with trypan blue into the counter for analysis.

USB port

The USB port allows you to transfer and save the cell count data and image to your 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 15 for **Transferring Data to a PC**.



Continued on next page

Description of Countess™ Automated Cell Counter, Continued

Rear and Side View of Countess™ Automated Cell Counter

The rear and side view showing various parts of the Countess™ automated cell counter.

Power Inlet

Connect the counter to an electrical outlet using the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.

Image Adjustment (Focus) Knob

The Image Adjustment (Focus) knob is used to adjust the image quality to obtain better contrast between live (bright centers) and dead (dark blue centers) cells. This is important to obtain accurate cell counts and viability measurements. See page 13 for details.

Focus Lock Knob

The Focus lock knob is used to lock the Image Adjustment (Focus) knob once the image is optimized. There is no need to use the Focus lock knob, but is available for convenience, if you are measuring multiple samples of the same cell type.

Rear View



Power inlet

Side View



Focus knob

Focus lock

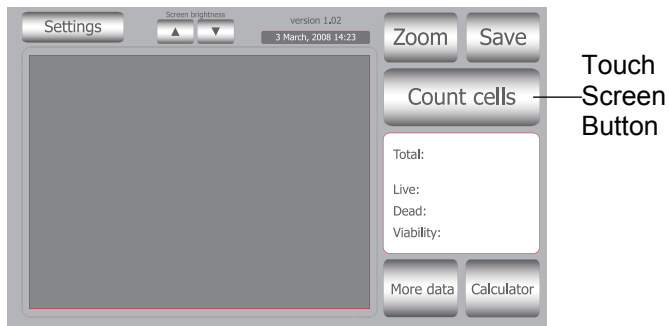
Continued on next page

Description of Countess™ Automated Cell Counter, Continued

User Interface

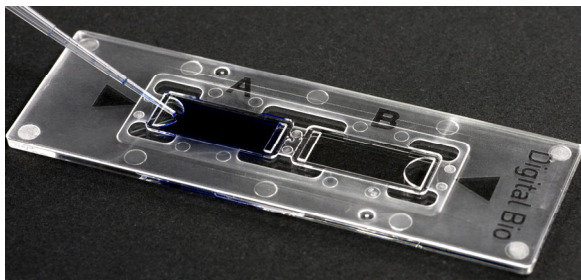
The touch screen user interface of the Countess™ automated cell counter is used to operate the instrument and consists of:

- The touch screen buttons to operate the instrument.
- The Digital Display shows the image of cells and sample data.



Countess™ Cell Counting Chamber Slide

The Countess™ cell counting chamber slides are plastic, disposable enclosed chambers that hold the sample in two separate chambers (A and B) for replicates. The cell counting occurs in the central location of the counting chamber. The entire volume of cells counted is 0.4 μL , the same as counting four (1 mm \times 1 mm) squares in a standard hemocytometer.



Accessory Products

Additional Products

The following products can be used with the Countess™ automated cell counter and are available separately from Invitrogen.

For more information, visit or contact Technical Support (page 24).

| Product | Quantity | Catalog no. |
|--|----------|-------------|
| Countess™ cell counting chamber slides *for Countess™ automated cell counter* *box of 50 with Trypan Blue* | 1 kit | C10228 |
| Countess™ power cord with four adapter cords | 1 each | C10285 |
| Countess™ USB drive | 1 each | C10286 |
| Trypan blue stain (0.4 %) | 2 × 1 mL | T10282 |
| Countess™ test beads | 1 mL | C10284 |

Introduction

Overview

Introduction

The Countess™ automated cell counter uses state-of-the-art optics and image analysis to automate cell counting. The Countess™ automated cell counter is a benchtop counter designed to measure cell count and viability (live, dead, and total cells) accurately and precisely, using the standard trypan blue technique.

Using the same amount of sample that you currently use with the hemocytometer, the Countess™ automated cell counter takes less than a minute per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells and provides information on cell size.

The Countess™ automated cell counter offers an intuitive user interface, and provides the option to save and print cell count data using the Countess™ software (download from www.invitrogen.com/countess) and USB drive supplied with the instrument or available separately.

The Countess™ automated cell counter is supplied with disposable Countess™ cell counting chamber slides that contain two enclosed chambers to hold the sample to allow you to measure two different samples or perform replicates of the same sample. The cell counting occurs in the central location of the counting chamber and the volume counted is 0.4 μL , the same as counting four (1 mm \times 1 mm) squares in a standard hemocytometer.

See page ix for details on various parts of the counter.

Continued on next page

Overview, Continued

System Overview

The Countess™ automated cell counter performs viability and cell counting measurements using the trypan blue method of dead-cell staining combined with advanced image analysis.

Cell sample is mixed with trypan blue and loaded into a Countess™ cell counting chamber slide. The camera acquires cell images from the sample on the slide and the image analysis software automatically analyzes acquired cell images, and measures cell count and viability using the trypan blue stain.

A single, sample measurement within a minute provides the following data:

- Live and dead cell concentration/mL
 - Total cell concentration/mL
 - Viability (% live cells to total cells)
 - Mean diameter
 - Cell images
 - Graphical data representation
-

Features

Important features of the Countess™ automatic cell counter are:

- User-friendly, benchtop design for simple, fast, automated cell count and viability measurements within a minute
 - Provides data on cell size and is compatible with a wide variety of eukaryotic cells without the need for any special changes between large or small sizes
 - Measures cell concentrations ranging from 1×10^4 to 1×10^7 cells/mL and cells with sizes ranging from 5 μm to 60 μm
 - Uses disposable counting chamber slides that eliminate washing steps and cross contamination between samples
 - Saves and print cell count data including images using the Countess™ software and Countess™ USB drive
 - Presents comprehensive data with graphical reports and as a .CSV (comma separated value) file for sample comparisons
-

Methods

Getting Started

Installing the Countess™ Automated Cell Counter

1. After unpacking the instrument, place the instrument on a flat, level, dry surface.
2. Plug one end of the supplied power cord into the Countess™ instrument. To the other end attach the appropriate plug adaptor, based on the electrical outlet configuration in your country.
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.
4. To **register** your Countess™ automated cell counter, visit www.invitrogen.com/countess. Enter the instrument serial number, your name, and your contact details. Registering your instrument ensures that you receive notifications of software upgrades and fast service.
5. When you are ready to use, start the Countess™ instrument by pressing the **Power** button.

Continued on next page

Getting Started, Continued

Optional: Installing the Countess™ Software

The Countess™ instrument is designed for stand-alone use and does not require the use of an external computer.

If you wish to archive data and generate reports, you must transfer data to your computer, and use the Countess™ software to generate and print reports (see instructions, below). Alternatively, data stored in the .CSV file may be transferred to your computer by the USB drive and imported into any spreadsheet program, without the need for Countess™ software.

Computer requirements (Countess™ is not compatible with Macintosh Operating systems)

- USB port (1.1 or later, 2.0 is recommended)
 - Windows XP/2000/Vista
1. Visit www.invitrogen.com/countess and follow instructions on the page to install the Countess™ software.
 2. When the software is installed, the Countess™ automated cell counting software icon appears on your desktop.
 3. To download data, use any USB drive to capture data from your experiment and transfer data to your computer as described in **Transferring Data to a PC** (page 15).
-

Start-Up Screen

When the instrument is turned on, the **Start Up** screen is displayed. Here you can proceed immediately to cell counting (page 8), set up the instrument for cell or bead count, or adjust the screen brightness.

Start Up Screen

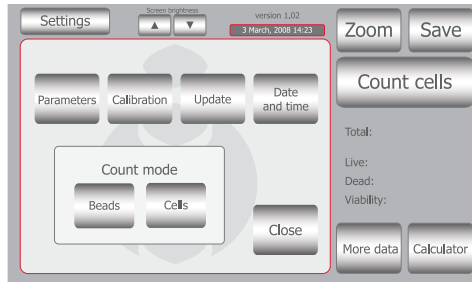


Continued on next page

Getting Started, Continued

Settings Screen

Press **Settings** from the Start-Up screen to display Settings.



The Settings menu allows you to set up the following:

- Parameters (see below and next page for details)
- Calibration to calibrate the instrument (page 19)
- Update to install new firmware versions as they become available
- Date and Time to set up date and time (page 7)
- Count mode to operate the instrument for cell counting (choose **Cells**) or bead counting (choose **Beads**)

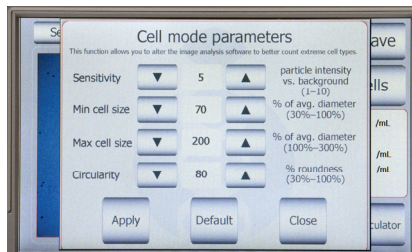
Screen Brightness

Use the scroll buttons to adjust the screen brightness.

Cell Mode Parameters Screen

Press **Parameters** from the Settings screen to display **Cell Mode Parameters** Screen.

The Countess™ instrument comes with pre-set parameters to match the majority of cultured cell types which can be accurately counted without changing any of the default parameters. The **Parameters** function allows you to change the image analysis algorithm for specific or mixed cell types, and the specific parameters must be determined empirically.



Continued on next page

Getting Started, Continued

Cell Mode Parameters Screen, continued

The Cell Mode Parameters are described below:

- **Sensitivity** (refers to the contrast of the objects from the background). Adjusting the sensitivity higher makes instrument more sensitive to objects; useful for cells that do not stain well with trypan blue while adjusting the sensitivity lower makes the instrument less sensitive and is useful if there is a lot of background.
- **Minimum cell size** is used to determine the low range of cell size to include in the measurement. The algorithm first identifies all objects, and calculates the average size (e.g., 15 μm). From the percent of average size setting, the algorithm calculates the smallest object size to include in the final measurement (e.g., 70% of 15 is 10.5 μm ; $15 - 10.5 = 4.5$ μm ; 4.5 μm would be the smallest particle included in the count). Adjusting the number up, increases inclusiveness thereby decreasing the lower cell size range (e.g., 50% of 15 is 7.5 μm ; $15 - 7.5 = 7.5$ μm).
- **Maximum cell size** is used to determine the high range of cell size to include in the measurement. The algorithm first identifies all objects, then calculates the average size (e.g., 15 μm). From the percent of average size setting, the algorithm calculates the largest object size to include in the final measurement (e.g., 200%; 200% of 15 $\mu\text{m} = 30$ μm ; 30 μm is the largest cell size included in the measurement).
- **Circularity** is used to determine the objects to include in the measurement based on roundness. Increasing the value from 80% requires objects to be more round for inclusion in the measurement. Decreasing the value from 80% allows objects to be less round. Adjusting this may be useful if the cell type is not particularly circular or perhaps oddly shaped.

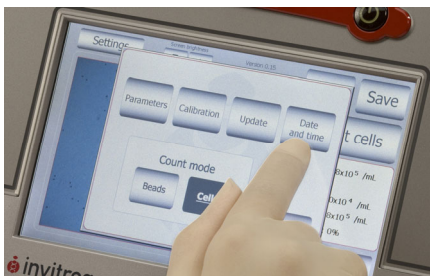
After modifying any parameters, press **Apply** to make the changes. To restore default parameters, press **Default**.

Getting Started, Continued

Date and Time Setup

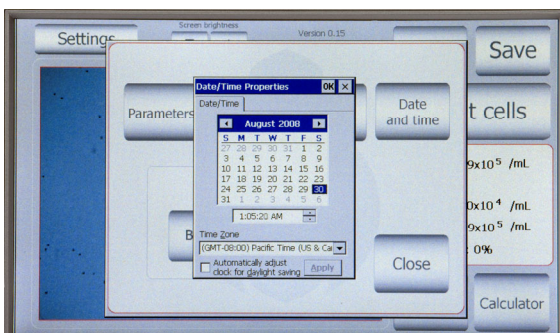
The date and time is already preset when you receive the instrument. To reset the date and time, follow these steps:

1. Turn on the Countess™ automated cell counter by pressing the **Power** button. The Start up screen is displayed after a few seconds.
2. Press **Settings** and then press **Date and Time**.



3. The **Date/Time Properties** screen is displayed. To select the month and day, scroll to the appropriate month using the arrow keys and then press the day on the calendar.

Note: Use a pointed object, like a stylus or pipette tip, to push the small buttons on the calendar.



4. To select the time, scroll to the appropriate time and select **Automatically adjust clock for daylight saving**, if needed. Press **Apply** or **OK** to make the date/time changes. The updated date/time is displayed on the top of the window. Once the date/time is set, there is no need to set it each time the instrument is turned on.
 5. Press **Close** to exit the screen.
-

General Guidelines

Introduction

General guidelines for using the Countess™ automated cell counter and disposable Countess™ cell counting chamber slides are discussed below.

Countess™ Cell Counting Chamber Slides

The Countess™ cell counting chamber slides supplied with the counter or available separately from Invitrogen (page xii) 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™ instrument.



To obtain the best results, follow these recommendations:

- 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.
 - Use the Countess™ automated cell counter at room temperature only (10–35°C).
 - For accurate viability count results, ensure the counting area is covered with cell suspension and count cells within 3 minutes of mixing the cells with trypan blue solution as trypan blue is toxic to cells. For best data with biological samples, we recommend counting at least two samples and taking an average.
 - The Countess™ automated cell counter is supplied pre-calibrated. To recalibrate your instrument, see page 19.
 - The Countess™ automated cell counter memory holds one set of data. Save your data to the USB drive after each reading. You may transfer the data to your PC, using the USB drive immediately as described in **Transferring Data to a PC** (page 15).
 - After using Countess™ cell counting chamber slides, appropriately dispose slides as biohazardous waste. **Do not reuse the chamber slides.**
-

Using the Countess™ Automated Cell Counter

Introduction

Instructions are provided in this section for preparing the cell sample with trypan blue dye for use with disposable Countess™ cell counting chamber slides for automated cell count using the Countess™ counter.



Note

The Countess™ automated cell counter is supplied precalibrated. However, if you wish to calibrate the Countess™ automated cell counter, see page 19.

Materials Needed

- Cell sample
- Countess™ cell counting chamber slides (supplied with the instrument or available separately, page xii)
- Trypan blue stain (0.4%); supplied with the instrument or available separately, page xii
- *Optional:* Countess™ USB drive for data transfer, supplied with the instrument or available separately (page xii)

Procedure

1. Push the **Power** button to start the instrument. The Start-up screen is displayed.

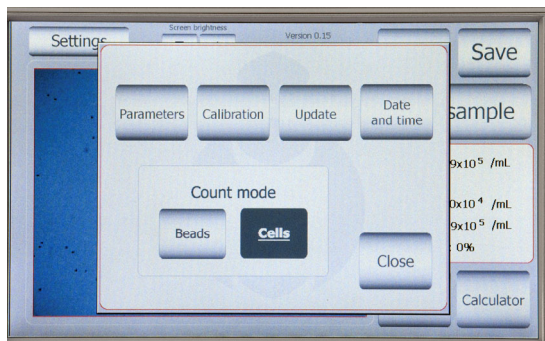


2. The Countess™ automated cell counter is preset to **Cell** mode. If you have changed the mode to bead for bead counting (page 16) or calibration (page 20), be sure to change the mode to **Cells** Mode prior to counting cells by pressing **Settings** and then **Cells**.

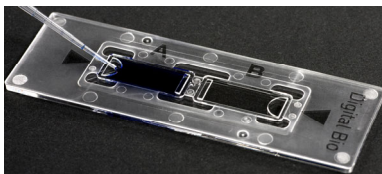
Continued on next page

Using the Countess™ Automated Cell Counter, Continued

Procedure, continued



3. Add 10 μL of your sample to 10 μL supplied trypan blue stain. Mix gently by pipetting up and down.
4. Add 10 μL of the sample mixture to the chamber ports on one side of the Countess™ cell counting chamber slide. The two chambers of the slide are labeled "A" and "B" for easy tracking of your samples. You can count the cells from one chamber or both chambers.



5. Insert the Countess™ cell counting chamber slide, sample side A first into the slide inlet on the instrument, 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. Each chamber is counted separately.



6. Press the **Count Cells** or **Next Sample** button.

Continued on next page

Using the Countess™ Automated Cell Counter, Continued

Procedure, continued

7. Adjust the image by pressing the **Zoom** button. Navigate by pressing the location you like to see on the grid.



8. While viewing cells in the Zoom mode, use the Focus knob to adjust the image.

Note: After you have counted the first sample, you may not have to use the Focus knob again. If measuring multiple samples of the same approximate size, you may lock the Focus knob. You are able to unlock the knob later, to adjust the image.



Optimize the image for analysis such that:

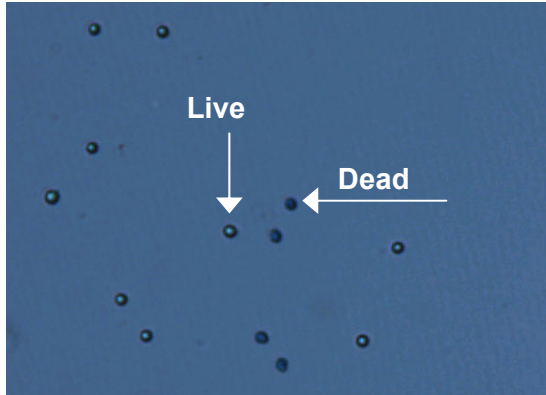
- **Live cells** have bright centers and dark edges (see image, next page)
- **Dead cells** have a uniform blue color throughout the cell with no bright centers, (see image, next page)

Continued on next page

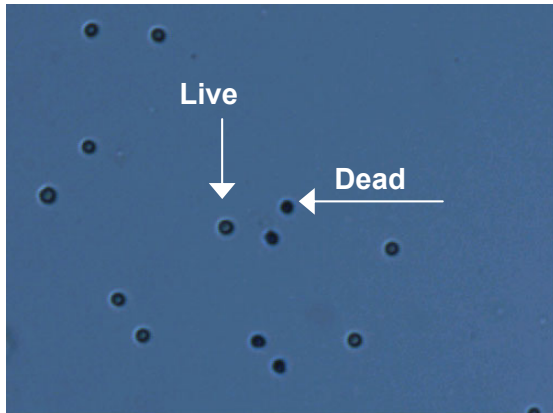
Using the Countess™ Automated Cell Counter, Continued

Procedure, continued

Correct Image



Incorrect Image



9. When you are satisfied with the image, press **Count Cells**.

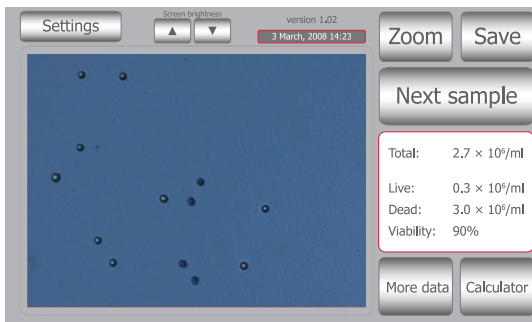


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Using the Countess™ Automated Cell Counter, Continued

Procedure, continued

10. The instrument takes approximately 30 seconds to count each sample, and the cell count for live, dead, and total cells as well as percentage viability is displayed on the screen. Record the cell count, or insert a USB drive and press **Save** (see page 15 for details on saving data).



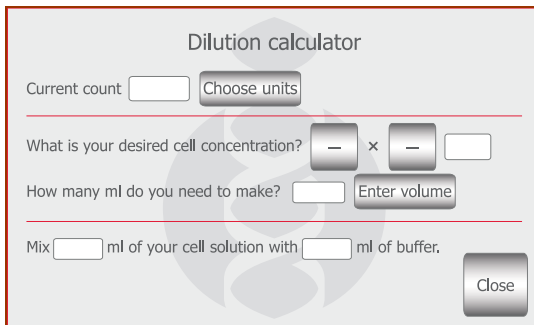
11. To see more details on the data as well as graphical representation of the data, press the **More Data** button. After reviewing the data, press the **Close** button to return to the main screen.

Continued on next page

Using the Countess™ Automated Cell Counter, Continued

Procedure, continued

The **Calculator** button allows you to quickly calculate adjustments to the cell suspension to obtain a desired concentration.



The screenshot shows a "Dilution calculator" interface with the following fields and buttons:

- Current count: Choose units
- What is your desired cell concentration?: ×
- How many ml do you need to make?: Enter volume
- Mix ml of your cell solution with ml of buffer.
- Close

- To count the cells in the other side of the slide chamber (side B), remove the Countess™ slide after side A is counted by pushing in the slide slightly and then pulling the slide out. Turn the slide around and reinsert into the slide inlet and repeat the procedure.



- The Countess™ automated cell counter memory holds one set of data. To save your data for future analysis or archiving, you must record the data or save after each reading. See page 15 for **Transferring Data to a PC**.
- After recording or saving the data, remove and discard the slide appropriately as biohazardous waste.
- At this point, the Countess™ cell counter instrument is ready for another sample. If you are not using the instrument, press the **Power** button to turn off the instrument.

Note: If the touch screen is not responding, you can turn off the instrument by pressing and holding the **Power** button for 4 seconds.

Continued on next page

Using the Countess™ Automated Cell Counter, Continued

Optional: Transferring Data to a Computer

1. To archive your data or generate a printed report, insert Countess™ USB drive into the USB port.

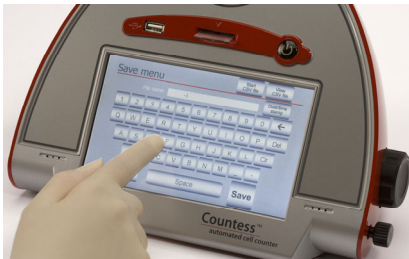
USB port



2. Save your data on the USB drive by pressing the **Save** button on the main screen. The image and the counting data are saved.

The numerical data is also 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.

3. Enter the file name using the keypad buttons displayed on the Save menu.



4. Transfer the Countess™ USB drive to the USB port on your PC. You may open the .CSV file using a spreadsheet program. To see the image and generate a report, use the Countess™ software.
5. Click on the Countess™ automated cell counting software icon on your PC (see **Installing the Countess™ Software**, page 4).
6. Open a file using the **Open** button. Select the saved file you wish to open.
7. Zoom in on the image obtained by clicking in the zoom grid, (this screen also allows you to examine which cells have been counted as live or dead).
8. Click on **Prepare Report** to obtain a printable version of the image and data.

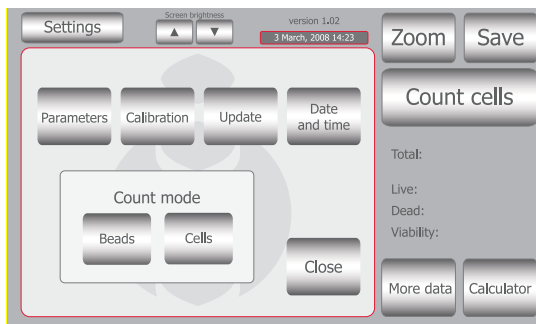
Continued on next page

Using the Countess™ Automated Cell Counter, Continued

Using the Beads Mode for Counting Beads

The Countess™ automated cell counter can also be used to count beads using the Bead mode of the instrument.

1. Push the **Power** button to start the instrument. The Start-up screen is displayed.
2. Press **Settings** and then press **Beads** to place the instrument into bead counting mode.



3. Add 10 μ L of beads to 10 μ L supplied trypan blue stain. Mix gently by pipetting up and down.
4. Add 10 μ L of the sample mixture to the chamber ports on one side of the Countess™ cell counting chamber slide as shown on page 9. You can count beads from one chamber or both chambers.
5. Insert the Countess™ cell counting chamber slide with beads into the slide inlet on the instrument as shown on page 10, making sure that the sample side is inserted completely into the instrument.
6. Press **Count Beads** button.
7. Adjust the bead image by pressing the **Zoom** button. Navigate the fields by pressing the location you like to see on the grid. Use the focus knob to adjust the image.

Continued on next page

Using the Countess™ Automated Cell Counter, Continued

Using the Beads Mode for Counting Beads, continued

- When you are satisfied with the image, press the **Count Beads** button.



- The instrument takes approximately 30 seconds to count each sample and the bead count is displayed on the screen. Record the bead count.
 - To count beads in the other side of the slide chamber (side B), remove the slide after side A is counted, turn the slide around, and reinsert into the slide inlet to repeat the counting procedure.
 - After counting beads, place instrument into **Cell Count** mode for counting cells by pressing **Settings** button and then pressing the **Cells** button.
-

Cleaning and Maintenance

Cleaning and Maintenance

Clean the surface of the Countess™ instrument with a damp cloth. To clean the LCD screen, turn off the Countess™ instrument, disconnect the power cable, and clean the LCD screen with a soft cloth lightly moistened with LCD cleansing detergent. Cleaning the screen with excessive force can damage the LCD the screen. Wipe the screen dry immediately. **Do not** reuse the counting chambers.

The Countess™ automated cell counter does not need regular maintenance. To troubleshoot problems with Countess™ cell counter, contact Technical Support (page 24). **Do not** perform any repairs or service on the Countess™ instrument to avoid damaging the instrument.

Changing Battery

To change the battery:

1. Turn off the Countess™ automated cell counter and disconnect the power cord.
2. Open the battery cover at the bottom of the Countess™ automated cell counter.



3. Remove the used battery and insert a new battery (battery type CR2032).
 4. Close the battery cover. Reconnect the power cord.
 5. Reset the date and time on the counter as described on page 7.
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Calibrating the Countess™ Automated Cell Counter

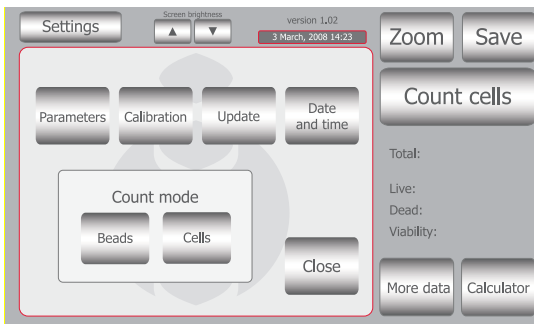
Introduction

Instructions are included below to calibrate the Countess™ automated cell counter to ensure its optimal performance. The calibration function sets the background of the instrument to a solution of trypan blue in the slide.

The Countess™ automated cell counter is supplied precalibrated. However, if you have updated the Countess™ firmware, or if you choose to use a different formulation of trypan blue that is not supplied with the instrument, you will need to calibrate the Countess™ automated cell counter.

Calibrating the Counter

1. Push the **Power** button to start the instrument. The Start-up screen is displayed.
2. Press **Settings** and then press **Calibration**.

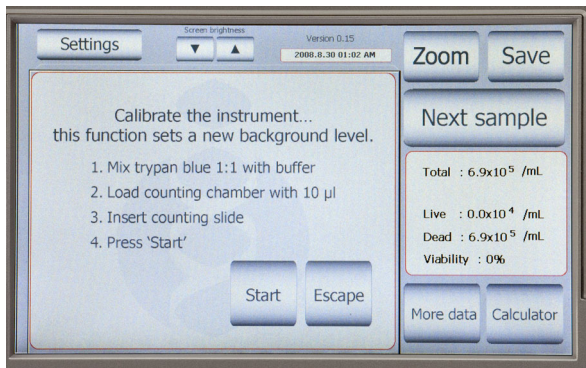


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Calibrating the Countess™ Automated Cell Counter, Continued

Calibrating the Counter, continued

3. To recalibrate the Countess™ Counter, mix 10 μL trypan blue solution with 10 μL of a standard buffer, such as phosphate buffered saline (PBS). Mix thoroughly.



4. Add 10 μL of the sample mixture to the chamber ports on one side of the Countess™ cell counting slide. Press **Start**.
5. The instrument takes approximately a minute to calibrate.
6. After calibration is complete, press the **Re-start** button to restart the instrument, and proceed to cell counting.

There is no need to recalibrate each time the instrument is turned on.

Troubleshooting

Introduction

Review the information below to troubleshoot your experiments using the Countess™ automated cell counter.

| Problem | Cause | Solution |
|-----------------------|-----------------------|---|
| Inaccurate cell count | Sample handling | <ul style="list-style-type: none">• Do not insert the Countess™ cell counting chamber slide upside-down as this may introduce liquid into the instrument that could damage it.• Do not reuse the Countess™ cell counting chamber slides, as leftover dye from the previous reading may affect the next reading.• Do not use any other counting chambers such as a glass hemocytometer with the Countess™ counter as it results in inaccurate cell count and may damage the instrument.• Ensure that the sample covers the entire counting area and the Countess™ cell counting chamber slide is inserted completely into the counter. |
| | Low and high readings | <p>The Countess™ automated cell counter is designed to read samples from 1×10^4 cells/mL to 1×10^7 cells/mL, with the highest accuracy between 1×10^5 cells/mL and 4×10^6 cells/mL.</p> <p>If your sample is not in this range, you may need to dilute the sample or add more cells and read the sample again.</p> |
| | Poor image quality | <p>While viewing cells in the Zoom mode, use the focus knob to adjust the image to ensure that live cells have bright centers, and dead cells have dark/blue centers.</p> |

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Troubleshooting, Continued

| Problem | Cause | Solution |
|----------------------------------|---|--|
| Inaccurate cell count | Cell clumping | Ensure the cells are not clumped. |
| | -- | To maintain instrument sensitivity, we recommend that you calibrate the counter each year as described on page 20. |
| Error codes | -- | See page 23 for a description of error codes. |
| Saving and printing problems | Incorrect USB drive | <ul style="list-style-type: none"> • Use the USB drive supplied with the counter or an USB 2.0 drive as some types of USB drive are not detected or recorded by the counter. • Do not save too many files in a USB drive as the counter may slow down to read the USB drive. |
| | Accidentally removed the USB drive | <ul style="list-style-type: none"> • Do not remove the USB drive or turn off the counter when updating. • Do not remove a USB drive when saving or reading data as it may damage the counter. |
| Instrument not updating firmware | May be using a corrupted firmware file or a damaged USB drive | Download a new version of the firmware from the website on a different USB drive and try updating the firmware on the Countess™ instrument. Contact Technical Support if the problem persists. |

Countess™ Automated Cell Counter Error Codes

Introduction

This section describes the error codes displayed by the Countess™ automated cell counter when it encounters a problem. Contact Technical Support (page 24) for details on error codes and if you need to send the instrument back for servicing.

| Position | Reason | Message | Error code |
|----------|-------------------------------------|--|------------|
| Driver | Camera driver loading fail | System Error (E0101) Reboot the device or refer to service personnel. | 0101 |
| | Camera driver open fail | | 0102 |
| | Camera driver initializing fail | | 0103 |
| | GPIO driver loading fail | | 0104 |
| Preview | Camera preview fail | | 0201 |
| Memory | Image buffer memory allocation fail | | 0301 |
| | Camera memory allocation fail | 0302 | |

Appendix

Technical Support

Web Resources



Visit the Invitrogen Web site at www.invitrogen.com for:

- Technical resources, including manuals, vector maps and sequences, application notes, MSDSs, FAQs, formulations, citations, handbooks, etc.
 - Complete technical support contact information
 - Access to the Invitrogen Online Catalog
 - Additional product information and special offers
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Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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Purchaser Notification, Continued

Instrument Warranty

Invitrogen warrants to the original purchaser ("Purchaser") that the Instrument ("Instrument") will be free from defects in materials and workmanship for a period of one (1) year from the date of delivery. Invitrogen agrees, as its sole responsibility under this limited warranty, and upon prompt notice of a defect, to repair, replace or refund purchase price, at its discretion, any Instrument discovered to be defective within the warranty period. This warranty does not include repair, replacement, or refund necessitated by accident, abuse, neglect, misuse, unauthorized repair, or modification of the Instrument.

In the event that Invitrogen determines that the Instrument is in need of repair and not replacement, this Standard Warranty includes replacement parts and labor for the Instrument. This Standard Warranty does not include shipment of the Instrument to and from service location or travel cost of service engineer, the costs of which shall be borne by the Purchaser.

This Warranty and the remedies set forth herein are exclusive and in lieu of all other express or implied warranties (including implied warranties of merchantability, fitness for a particular purpose and non-infringement), and no other warranties shall be binding upon Invitrogen. In no event shall Invitrogen be liable for any special, incidental or consequential damages resulting from the use or malfunction of this Instrument or the system With which it is used, even if such damages could be anticipated by Invitrogen.

To obtain service during the warranty period, contact Invitrogen Technical Support for further instruction.

OUT OF WARRANTY SERVICE

Contact Invitrogen Technical Support. We will be happy to assist you by phone at no charge. Repair service, if needed, will be billed depending on the parts replaced and labor hours needed to repair your instrument. You will be billed for shipment of the instrument to the recommended service facility.

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