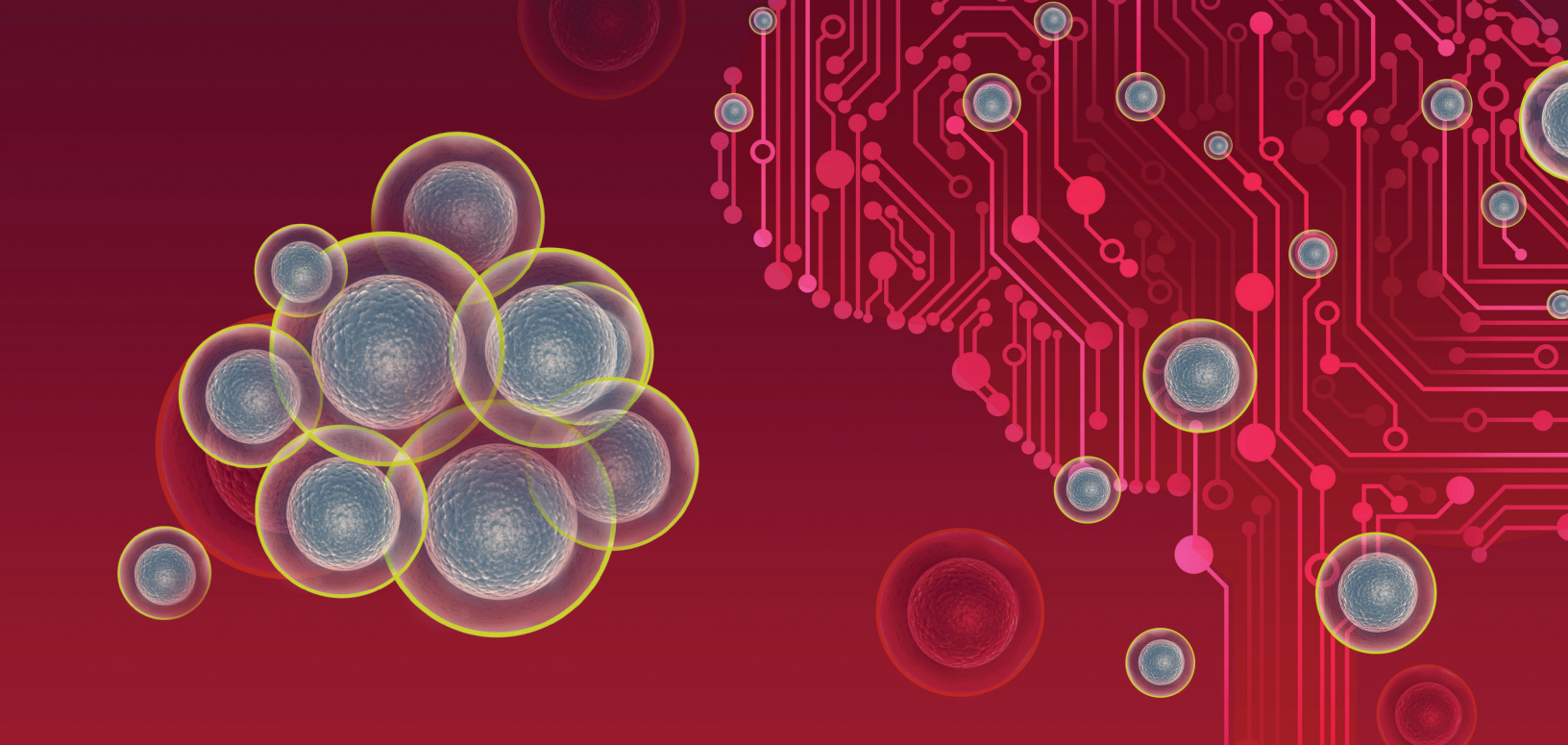
Thermo Fisher SCIENTIFIC

A counter that thinks like a cell biologist

Countess 3 Automated Cell Counters

Accuracy | Precision | Time savings | Convenience

invitrogen



Countess 3 Automated Cell Counters

Artificial intelligence takes accuracy to a higher level

Thermo Fisher Scientific offers two high-performance cell counters designed to meet the needs of any lab. The Invitrogen™ Countess™ 3 and Countess™ 3 FL Automated Cell Counters utilized artificial intelligence during development, applying a deep-learning neural network algorithm to generate highly accurate cell counts even for challenging cell sample types. The advanced algorithms in the Countess 3 and 3 FL instruments stem from the machine-learning algorithm that was taught by our scientists. Our advanced algorithms help ensure accuracy and precise cell counting, and avoid the user variation associated with manual hemocytometer cell counting.

The Countess 3 Automated Cell Counters come equipped with automated features like autolighting, autofocus, and autosave to provide convenience and time savings (Table 1).

- **Accuracy**—highly accurate machine-learning algorithm applied to cell counting
- **Precision**—autolighting and autofocus minimize user inputs and variability
- **Time savings**—rapid capture and autosave features help generate and save results fast
- **Convenience**—built-in pre-dilution and cell splitting calculators, histograms and gating, and a PDF report of results
- **Affordability**—both Countess 3 instrument models are compatible with the Invitrogen™ Countess™ Reusable Slide

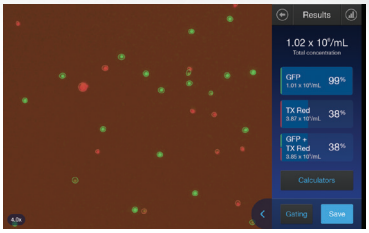


Table 1. Features of Countess 3 Automated Cell Counters.

Features	Countess 3 Automated Cell Counter	Countess 3 FL Automated Cell Counter
Machine-learning algorithm	✓*	✓*
1,280 x 800 pixel LCD screen	✓*	✓*
Wi-Fi–enabled cloud connectivity	✓*	✓*
Brightfield image and count	✓	✓
Brightfield autolighting	✓	✓
Brightfield rapid capture	✓*	✓*
Fluorescence images and counts	N/A	Two interchangeable fluorescence channels (user's choice)
Fluorescence autolighting	N/A	✓*
Saturated pixel indicator	N/A	✓*
Autofocus	✓	✓
Autosave	✓*	✓*
Countess chamber counting slides	✓	✓
Countess reusable slide	✓*	✓
Pre-dilution calculator	✓*	✓*
Cell splitting calculator	✓	✓
Single-cell data	✓*	✓*
FCS file	✓*	✓*
21 CFR Part 11 software support	✓*	✓*

* Featured upgrades of the Countess 3 Automated Cell Counters.

Features of the Countess 3 instruments

Accuracy and precision

Countess 3 Automated Cell Counters utilize machine-learning algorithms to provide fast and accurate focus determination as well as cell and viability counts. Countess instruments are compatible with a broad range of cell types and have been verified for use with several commonly used cell lines and primary cell types (Figure 1 and Table 4, pg. 14).

Upon insertion of the slide, the Countess instrument will automatically provide optimized illumination and focus on the cells, which helps to minimize the user variation associated with manual focusing and lighting adjustments. Cells ranging in size from ~4 µm to 60 µm and cell suspension density ranging from 1×10^4 to 1×10^7 cells/mL are optimal for counting.

Accuracy of the Countess 3 FL Automated Cell Counter compared to the Attune NxT Flow Cytometer and a manual hemocytometer

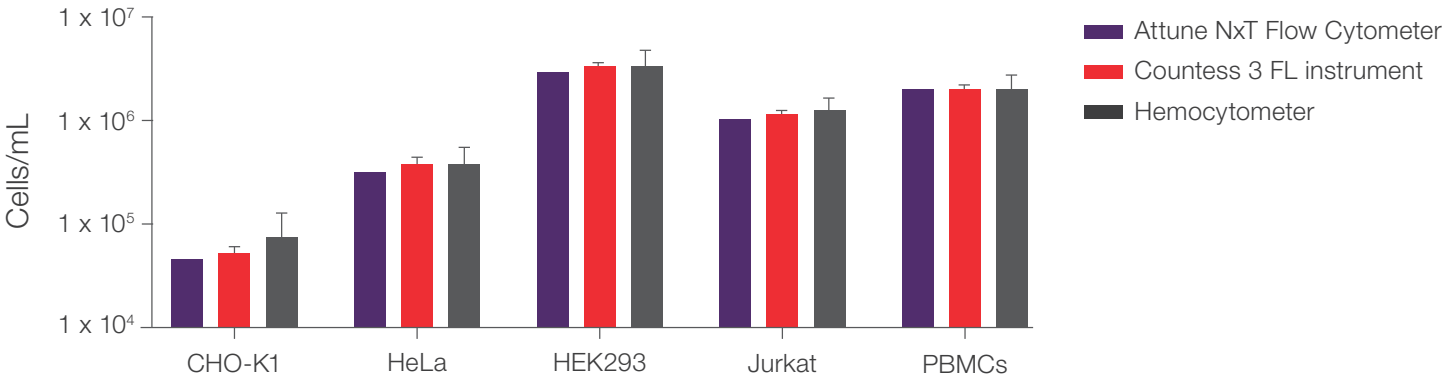


Figure 1. Machine-learning algorithms result in highly accurate cell counts, comparable with flow cytometer counts. CHO-K1, HeLa, HEK293, Jurkat, and human peripheral blood mononuclear cells (PBMCs) were counted using the Invitrogen™ Attune™ NxT Flow Cytometer (purple bar), a Countess 3 FL Automated Cell Counter (red bar), and manual counting with a hemocytometer and microscope (gray bar). The Countess 3 FL Automated Cell Counter and hemocytometer bars represent an average of 6 counts. Error bars indicate standard deviation from 6 independent counts.

Counting clumps, not debris

The machine-learning algorithm of the Countess 3 and Countess 3 FL Automated Cell Counters can clearly identify cell boundaries within clumps of cells and ignore debris, resulting in accurate cell counts (Figure 2).

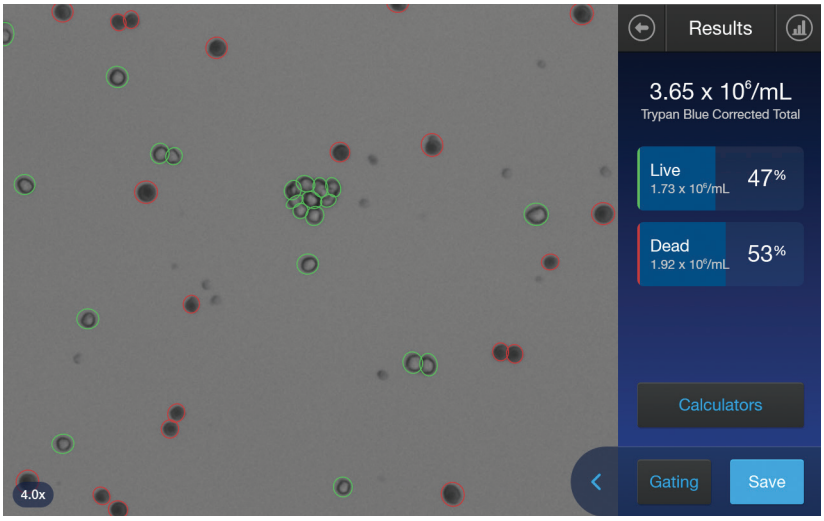
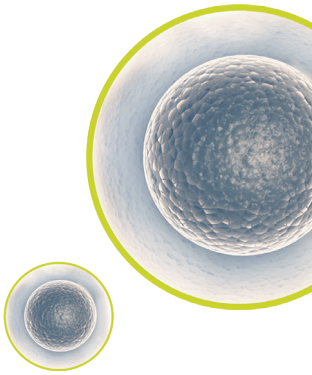


Figure 2. Machine-learning algorithms generate accurate counts with clumpy cells and sample debris. The Countess 3 FL Automated Cell Counter was used to count RAW cells, which are both small and have a propensity to clump. The counting algorithm can resolve the cells in the clump, and properly segment and count the cells. Debris is properly omitted from counts.



Counting PBMCs

Rodent PBMCs are small cells around 5–7 microns and can be challenging to count whether manually on a hemocytometer or with an automated cell counter. The machine-learning algorithm used by the Countess 3 Automated Cell Counters is robust and accurately counts these cells in both brightfield and fluorescence modes (Figure 3).

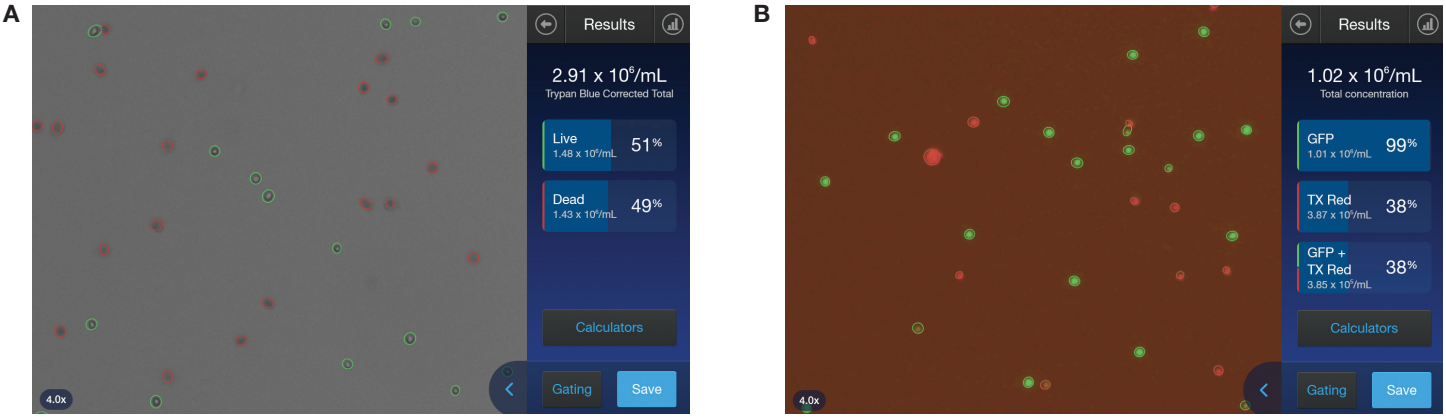


Figure 3. Rodent PBMCs accurately counted with machine-learning algorithm. The Countess 3 FL Automated Cell Counter was used to count small rodent PBMCs. Counts were performed with **(A)** brightfield lighting and **(B)** fluorescence lighting.

Time savings

The Countess 3 and Countess 3 FL Automated Cell Counters streamline the process of cell counting, eliminating many of the tedious steps associated with manual cell counting (Figure 4). This additional time can be applied to completing cell culture processing and other activities in the lab, resulting in a significant advantage when switching from manual to automated cell counting.

Comparison of cell counting processes

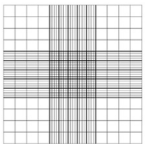
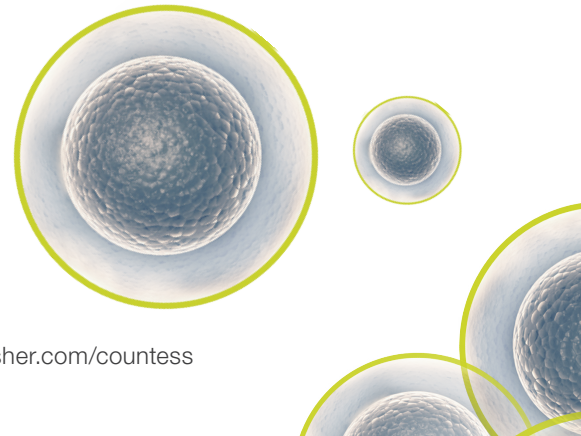
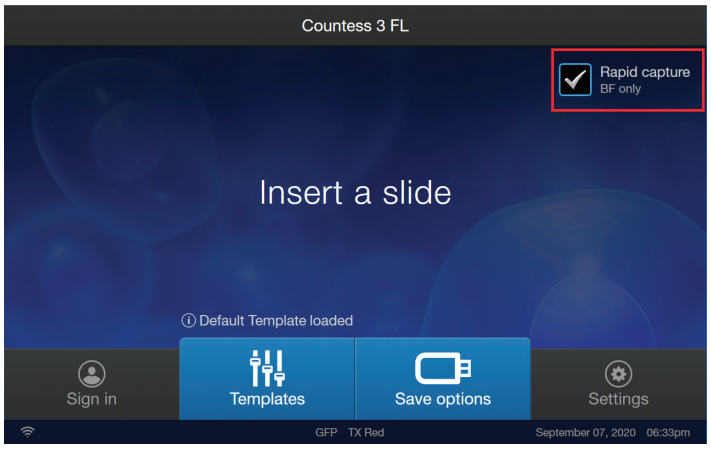
Manual counting with a hemocytometer and microscope	Countess 3 Automated Cell Counter with Countess Reusable Slide	Countess 3 Automated Cell Counter with Countess Cell Counting Chamber Slides
		
• Counting 4 quadrants	• Using rapid capture • Built-in autolighting and autofocus • Using autosave feature	• Using rapid capture • Built-in autolighting and autofocus • Using autosave feature
1. Clean and dry hemocytometer 2. Clean and dry coverslip 3. Load sample into hemocytometer 4. Mount on microscope 5. Adjust lighting 6. Adjust focus 7. Count cells 8. Write down counts 9. Calculate cell concentration 10. Calculate volumes for next steps 11. Clean hemocytometer 12. Clean coverslip	1. Clean reusable slide 2. Clean coverslip 3. Load sample into reusable slide 4. Insert slide into Countess 3 counter 5. Use built-in calculator to calculate volumes for next steps 6. Clean reusable slide 7. Clean coverslip	1. Load sample into chamber slide 2. Insert slide into Countess 3 counter 3. Use built-in calculator to calculate volumes for next steps
• Time to count 2 samples (A and B sides) • Counting 4 quadrants • **10–12 minutes**	• Time to count 2 samples (A and B sides) • **2–3 minutes**	• Time to count 2 samples (A and B sides) • **~1 minute**

Figure 4. Comparison of hands-on steps between manual hemocytometer cell counting and cell counting using the Countess 3 Automated Cell Counter.



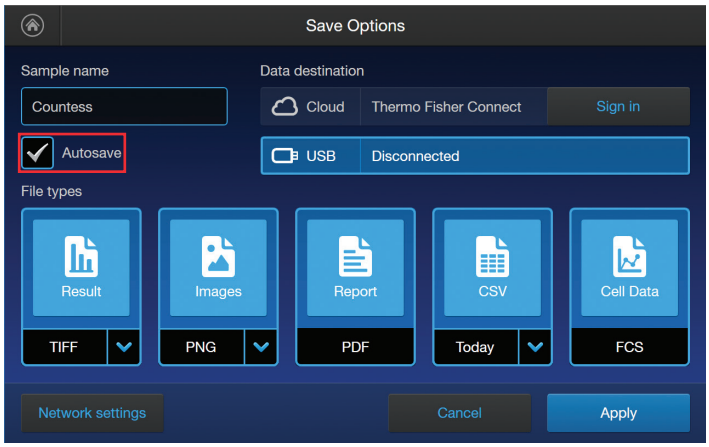
Rapid capture

Using the brightfield mode, once the slide is inserted, there is nothing further to do. The Countess 3 counter will automatically illuminate, focus, and count. This saves time by eliminating the need for additional menu selections to help get you quickly to results.



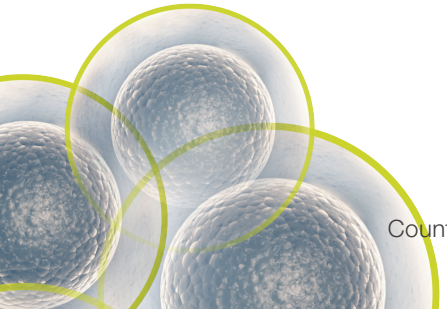
Autosave

By selecting the autosave feature, cell counts and data are automatically saved. Couple this with the rapid capture mode, and the cell counting and data retention process becomes fully automated.



Invitrogen™ Countess™ Cell Counting Chamber Slides

These disposable slides are convenient for quickly processing cell samples when there are many to process. There is no need to clean the reusable slide and wait for it to dry between cell samples.

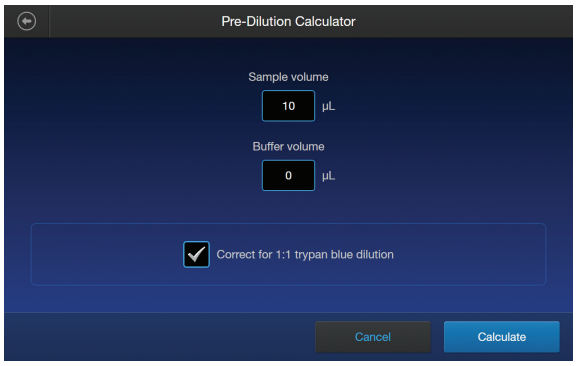


Convenience

On-board pre-dilution and cell splitting calculators make any necessary calculations seamless and integrated into the cell counting process. Viewing histograms and any additional desired gating of cell counts are easy to do on the Countess 3 cell counters, and a PDF report of your results can be saved and printed.

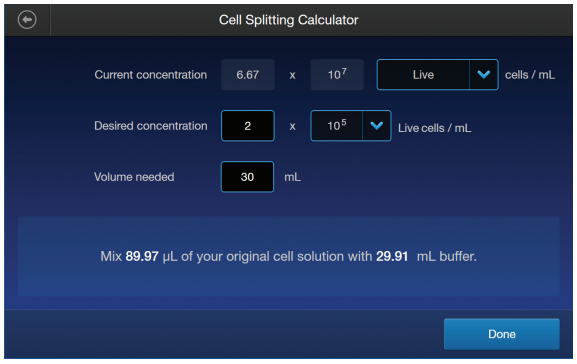
Pre-dilution calculator

If the cell sample is concentrated and a dilution was required prior to cell counting, the Countess 3 cell counter can quickly and easily calculate the sample concentration. If Invitrogen™ Trypan Blue Stain (0.4%) was omitted from the count, the Countess 3 counter can adjust the dilution accordingly.



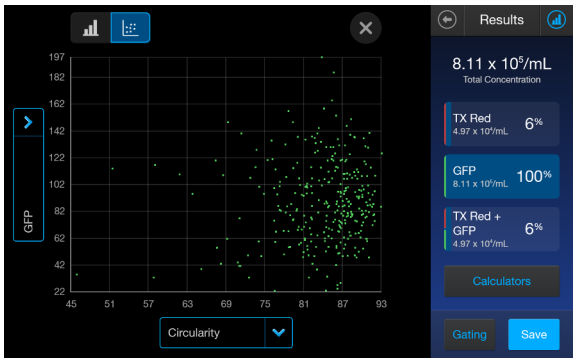
Cell splitting calculator

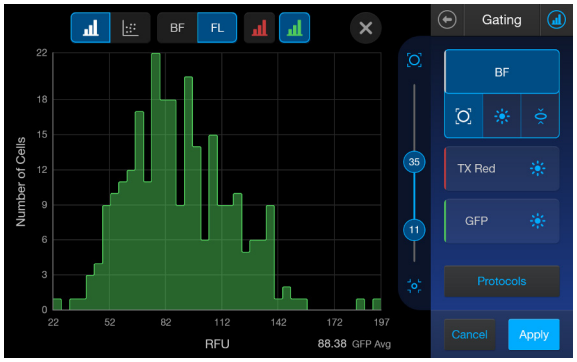
Easily determine the amount of cell sample and cell culture media/buffer needed for your experiment with the on-board cell splitting calculator. The cell count results are automatically used in the calculation; simply input the desired concentration and the volume needed.



Easily gate cells and view histograms

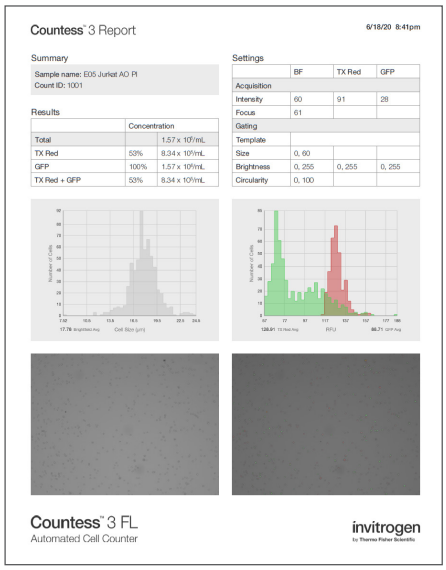
Cells can be gated based on cell size, brightness, and circularity to fine-tune what is included or excluded for specific applications. Observe scatter plots and histograms, and choose gating features, from the on-instrument display. Select the graph icon, and watch the changes in real time as you use the gating features. The average cell size is also displayed on the histogram.





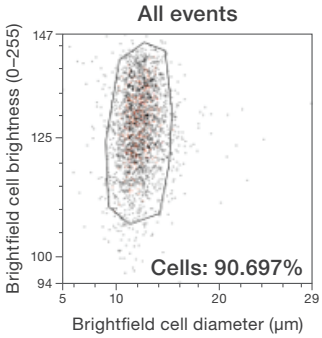
Printable report with cell counting results

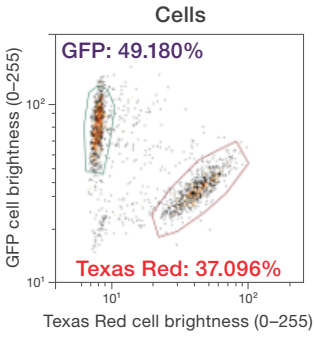
Using a USB drive or Wi-Fi to establish cloud connectivity with the Countess 3 and 3 FL Automated Cell Counters, you can save your cell count results and images, and transfer them to your own computer. Images can be saved as TIFF, PNG, or JPG files, and the results are saved as a CSV file. A printable PDF report with results, images, and settings used can also be saved.



Save FCS files

Generate an FCS file, which can be saved and transferred to an FCS analysis software for additional insights. The brightfield count can be used to gate out noncellular debris based on object size and intensity. Similarly, data from a fluorescent count can reveal cellular populations based on fluorescence visualized in a scatter plot. Plots demonstrate Jurkat cell viability using acridine orange and propidium iodide detected with Invitrogen™ EVOS™ GFP 2.0 and EVOS™ Texas Red™ 2.0 LED light cubes, respectively.





Responsive touchscreen and intuitive interface

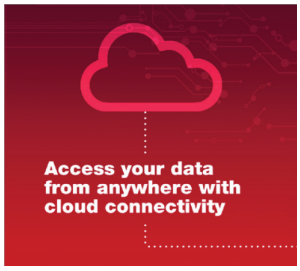
Screen selections on the Countess 3 and Countess 3 FL Automated Cell Counters are intuitive and easy to navigate. The LCD screen is a responsive touchscreen and does not require an external computer. The 1,280 x 800 pixel screen provides high-quality cell images. The display is flush across the instrument, making it easy to keep clean.

Countess 3 Reusable Slide

The Countess 3 Reusable Slide is an economical and sustainable choice. Both the Countess 3 and Countess 3 FL Automated Cell Counters can use the reusable slide with the reusable slide holder.

Wi-Fi–enabled cloud connection

The Countess 3 Automated Cell Counter can save files directly to the cloud-based Thermo Fisher™ Connect Platform. By connecting to a local Wi-Fi network with the Wi-Fi dongle, files can be saved to the cloud. Access these files at any time, inside the lab or out—for data that is available when and where you need it.

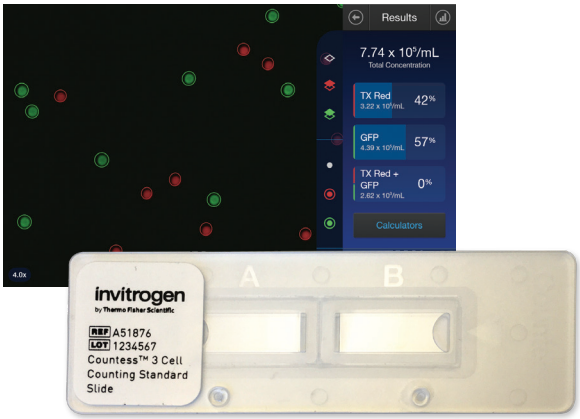


Software to support 21 CFR Part 11 compliance

Both the Countess 3 and Countess 3 FL Automated Cell Counters are available to support compliance with 21 CFR Part 11 FDA guidelines. The Countess 3 and 3 FL instruments use a security, audit, and electronic-signature (SAE) software.

Countess 3 Cell Counting Standard Slide

The Countess 3 Cell Counting Standard Slide is a reference slide. The standard slide has fixed microspheres loaded with fluorescent dyes that mimic cells. Time and again, the Countess 3 Cell Counting Standard Slide will provide reproducible cell counts and viability counts in both brightfield and fluorescence mode and between instruments.



Countess 3 FL Automated Cell Counter

Fluorescence detection beyond GFP and RFP

The Countess 3 FL Automated Cell Counter offers one brightfield and two interchangeable fluorescence channels. The fluorescence colors detected are determined by the insertion of individual light cubes. The wide variety of light cubes available provides flexibility for multiple research applications from UV-excitable DAPI to far-red.

Interchangeable LED light cubes

At the heart of the Countess system's fluorescence technology are the unique Invitrogen™ EVOS™ Light Cubes. Each cube contains an LED, illuminating optics, and filters. Light cubes are user-interchangeable and automatically detected by the Countess 3 FL system for plug-and-play ease. There are ample light cubes to choose from (Tables 2 and 3).

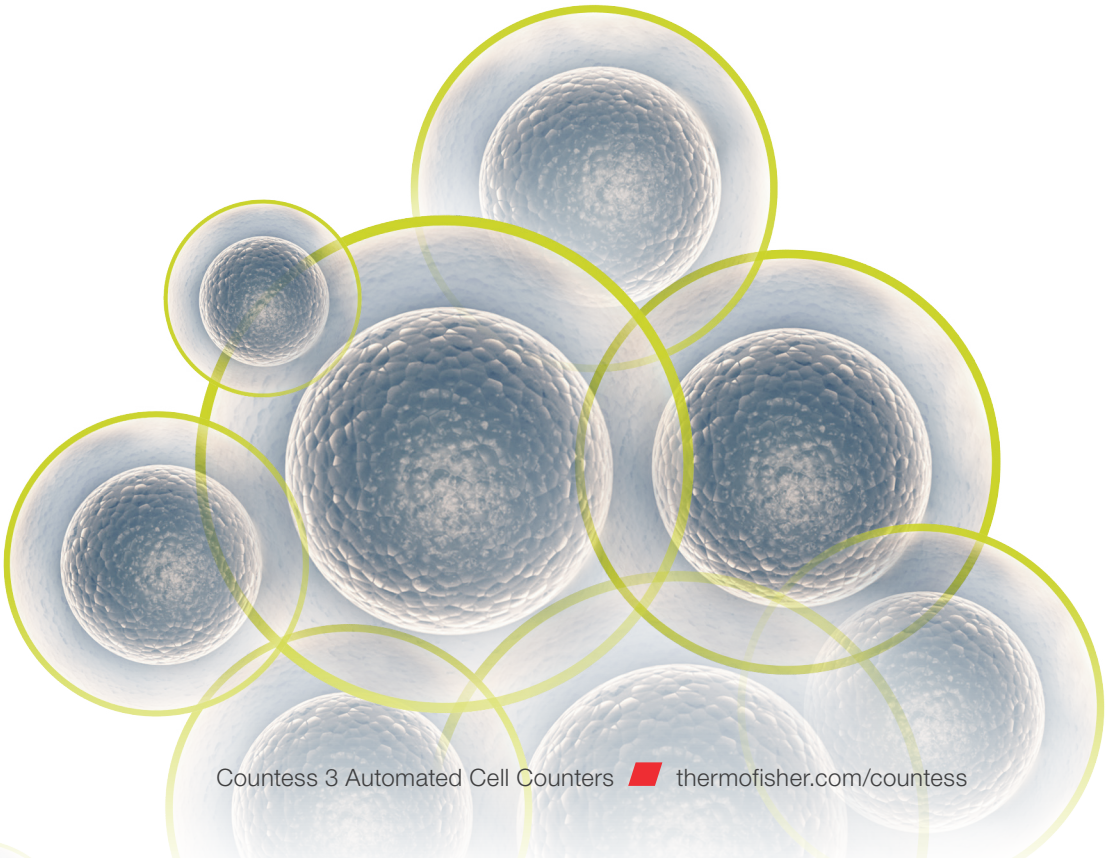
Table 2. List of commonly used light cubes.

Light cube	Excitation (nm)	Emission (nm)	Common compatible dyes/fluorescent proteins	Cat. No.
DAPI 2.0	357/44	447/60	DAPI, Hoechst, BFP	AMEP4950
GFP 2.0	470/22	525/50	GFP, Alexa Fluor 488, SYBR Green, FITC, acridine orange (AO) (when paired with secondary stain)	AMEP4951
YFP 2.0	500/24	542/27	EYFP, AO (+DNA)	AMEP4954
RFP 2.0	531/40	593/40	RFP, Alexa Fluor 546, Alexa Fluor 555, Cy3, DsRed, Rhodamine Red, dTomato	AMEP4952
Texas Red 2.0	585/29	628/32	Texas Red, Alexa Fluor 568, Alexa Fluor 594, MitoTracker Red, mCherry	AMEP4955
Cy5 2.0	628/40	692/40	Cy5, Alexa Fluor 647, Alexa Fluor 660, DRAQ5	AMEP4956
Cy7	710/40	809/81	Cy7, IRDye 800CW, DyLight 750	AMEP4667

See a complete list of available common and specialty light cubes at **thermofisher.com/evoslightcubes**

Table 3. Commonly used dyes and light cubes for use in the Countess 3 FL Automated Cell Counter for viability and apoptosis determination.

Dye	Light cube	Cat. No.
Viability dyes		
ReadyCount Blue Nuclear Stain	DAPI 2.0	A49904
ReadyCount Green/Red Cell Viability Stain	GFP 2.0 and Texas Red 2.0	A49905
ReadyCount Red Dead Cell Stain	RFP 2.0 or Texas Red 2.0	A49903
ReadyProbes Cell Viability Imaging Kit, Blue/Green	DAPI 2.0 and GFP 2.0	R37609
ReadyProbes Cell Viability Imaging Kit, Blue/Red	DAPI 2.0 and RFP 2.0 or Texas Red 2.0	R37610
LIVE/DEAD Viability/Cytotoxicity Kit	GFP 2.0 and RFP 2.0 or Texas Red 2.0	L3224
Propidium Iodide ReadyProbes Reagent	RFP 2.0 or Texas Red 2.0	R37108
SYTOX Green Nucleic Acid Stain	GFP 2.0	S7020
SYTOX Deep Red Dead Cell Stain	Cy5 2.0	S11381
7-Aminoactinomycin D (7-AAD)	Texas Red 2.0 or Cy5 2.0	A1310
Apoptosis dye combinations		
CellEvent Caspase-3/7 Green Detection Reagent	GFP 2.0	C10423



ReadyCount stains

Counting difficult cell samples is one reason to move beyond brightfield cell counting. Invitrogen™ ReadyCount™ stains provide cell-selective staining despite the presence of debris in primary cell samples (Figures 5–7). Further, they offer discrimination of nucleated cells from non-nucleated cells like RBC and platelets when in a heterogeneous sample. Coupling the Countess 3 FL Automated Cell Counter with room temperature–stable ReadyCount stains keeps the process simple with a 1:1 ratio of cell sample to stain.

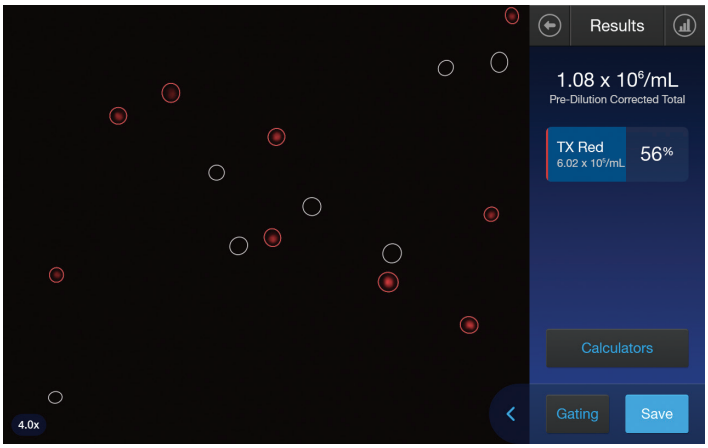


Figure 5. Jurkat cells treated with Invitrogen™ ReadyCount™ Green/Red Viability Stain.

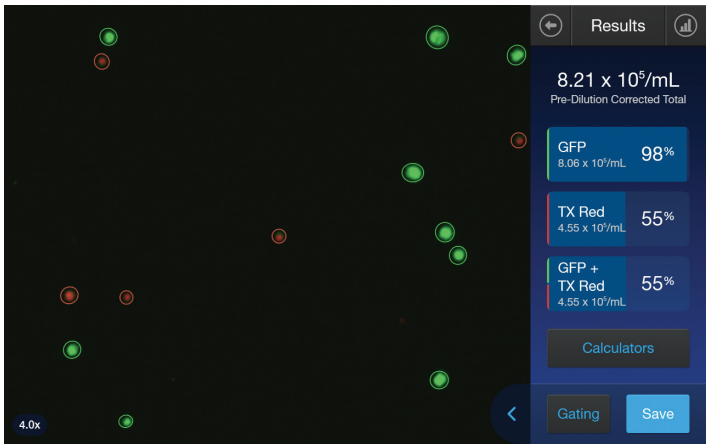


Figure 6. Jurkat cells treated with Invitrogen™ ReadyCount™ Red Dead Cell Stain.

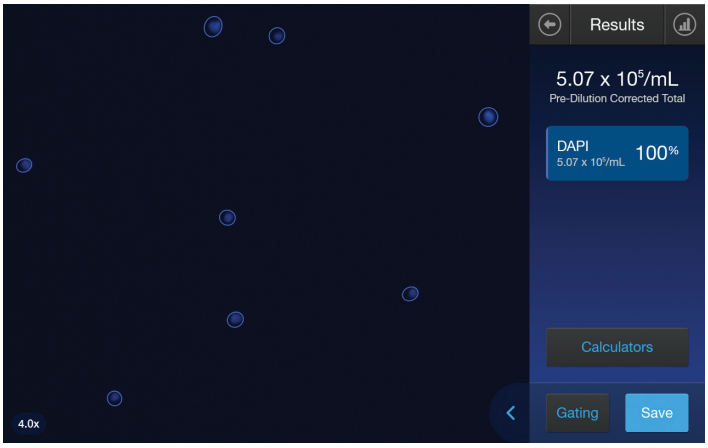


Figure 7. Jurkat cells treated with Invitrogen™ ReadyCount™ Blue Nuclear Stain.

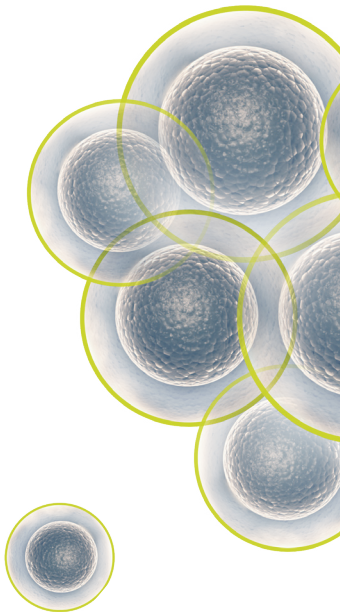
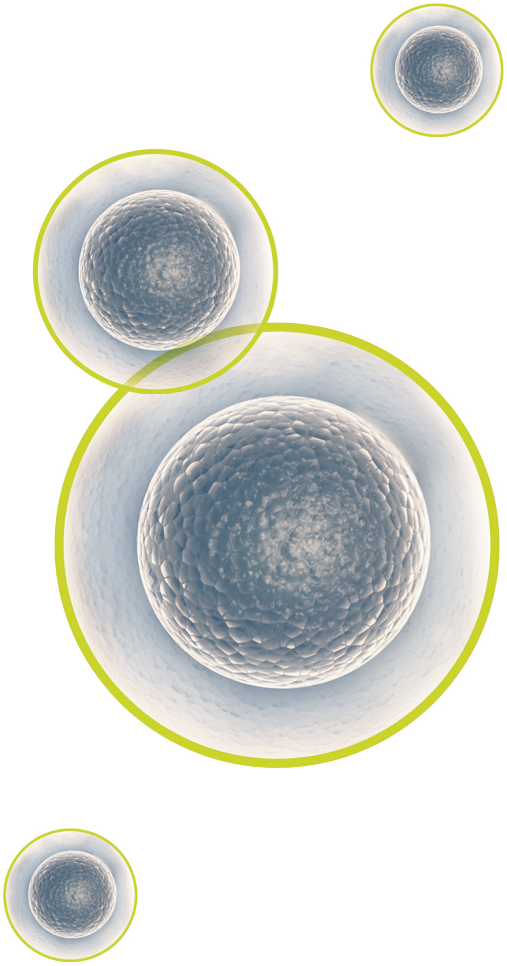


Table 4. Cell lines verified on the Countess 3 Automated Cell Counters.

Cell line	Organism	Cell type
HeLa	Human	Cervix
U2OS	Human	Bone
A549	Human	Lung
CHO-K1	Hamster	Ovary
HEK293	Human	Kidney
SKBR-3	Human	Breast
BPAE	Bovine	Smooth muscle
HCASM	Human	Smooth muscle
Jurkat	Human	Blood
Ramos	Human	Blood
THP-1	Human	Macrophage
RAW	Mouse	Macrophage
MMM	Mouse	Macrophage
U937	Human	Blood
HepG2	Human	Liver
MCF-7	Human	Breast
Human PBMC	Human	Blood
Mouse PBMC	Mouse	Blood
Mouse hepatocyte	Mouse	Liver
CAR T	Human	Blood
Human hepatocyte	Human	Liver
Rat hepatocyte	Rat	Liver
Human splenocyte	Human	Spleen
Mouse spleen/T cell mix	Mouse	Spleen and blood
AsPC-1	Human	Pancreas
HROC542TF fibroblast	Human	Colorectal
NSCLC-146	Human	Lung



Frequently asked questions

Q: What range of cell sizes do the Countess 3 and Countess 3 FL Automated Cell Counters count?

A: Accurate counts for cells between 4 and 60 µm can be obtained.

Q: What cell or bead concentration range will the Countess 3 and Countess 3 FL Automated Cell Counters count?

A: The core confidence range that will yield the best accuracy and precision is 1×10^5 to 1×10^7 cells/mL. Counting samples from 1×10^4 to 1×10^8 cell/mL has been performed, but with greater count-to-count variability.

Q: What count-to-count precision should be expected with the Countess 3 and Countess 3 FL Automated Cell Counters?

A: The count-to-count specification is <10%. However, CV values of <5% are routine due to the advanced focus and analysis algorithms. When considering differences between samples, be careful to control other variables such as ensuring a homogeneous sample by mixing it prior to pipetting.

Q: Do the Countess 3 and Countess 3 FL Automated Cell Counters provide count results that account for the 1:1 dilution of trypan blue to sample?

A: Yes, the 1:1 dilution is automatically accounted for by the Countess 3 and Countess 3 FL Automated Cell Counters. The concentration noted on the results screen reflects that of the original sample, not the trypan blue–diluted sample. If additional pre- or post-dilution calculations are needed, simply select the built-in dilution calculator option.

Q: Will the artificial intelligence (AI) algorithms continue to learn with new cell samples?

A: No, the algorithms in the Countess 3 and Countess 3 FL Automated Cell Counters are static. They were developed using AI and were taught by our experienced cell biologists to optimize focus and lighting, and to distinguish individual cells as live, dead, and in clumps.

Q: Can PBMCs be counted in brightfield with Countess 3 and Countess 3 FL Automated Cell Counters?

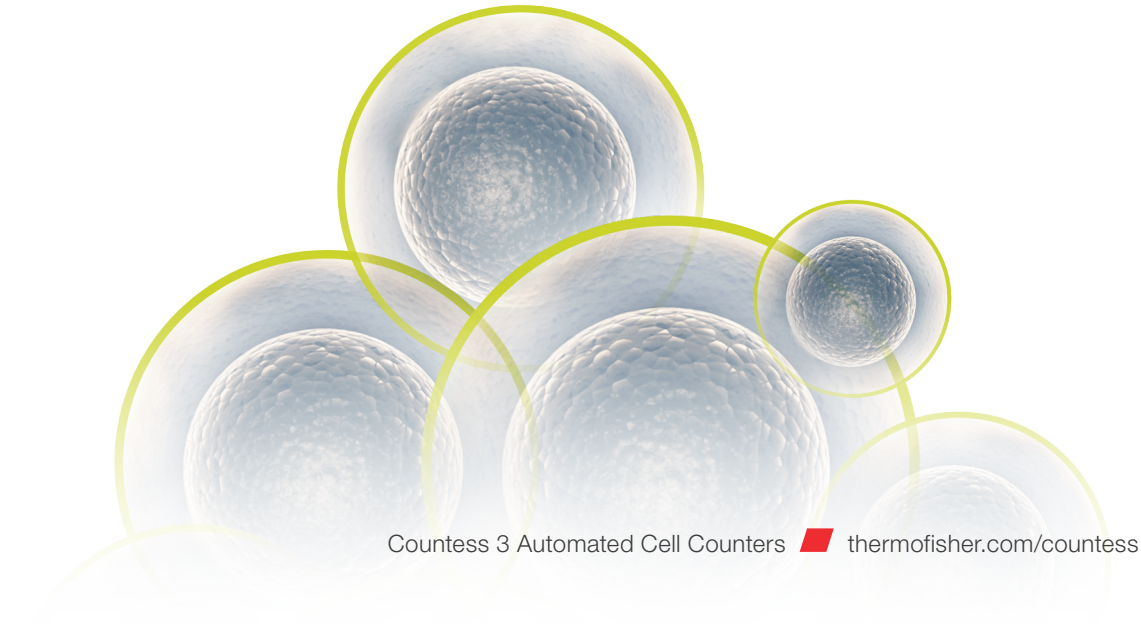
A: Yes, counting PBMCs can be performed in both brightfield and fluorescence modes. As primary samples can be very complex, we do recommend using fluorescence staining for the most accurate viability counting with these small cells.

Q. How long do the instruments take to count?

A: Less than 30 seconds from sample insertion to saving the result. The counting is only one element included in this timeframe, as the Countess 3 and Countess 3 FL Automated Cell Counters also perform autofocus, autolighting, autocapture, and autosaving during this time. The time is dependent on the concentration: more concentrated samples take longer, but still less than 30 seconds total.

Q. Can I buy the Countess 3 FL instrument without light cubes?

A: Yes, the Countess 3 FL instrument without light cubes installed will function exactly like the Countess 3 instrument—as a brightfield cell counter. Light cubes can easily be added to the Countess 3 FL instrument as application needs evolve. However, future addition of light cubes to a Countess 3 instrument is not possible.



Thermo Fisher SCIENTIFIC

Ordering information

Name	Quantity	Cat. No.
Countess 3 Automated Cell Counter		
Countess 3 Automated Cell Counter	1 instrument	AMQAX2000
Countess 3 Automated Cell Counter Starter Pkg	1 package	A49865
Countess 3 Automated Cell Counter w/Rapid Exchange	Instrument plus 3 yr REX ext warranty	A49862
Countess 3 Starter Pkg w/Rapid Exchange	1 package plus 3 yr REX ext warranty	A50298
Countess 3 Automated Cell Counter w/ABRC* support	Instrument plus 3 yr ABRC ext warranty	A49891
Countess 3 FL Automated Cell Counter		
Countess 3 FL Automated Cell Counter	1 instrument	AMQAF2000
Countess 3 FL Automated Cell Counter Starter Pkg	1 package	A49866
Countess 3 FL Automated Cell Counter w/Rapid Exchange	Instrument plus 3 yr REX ext warranty	A49892
Countess 3 FL Starter Pkg w/Rapid Exchange	1 package plus 3 yr REX ext warranty	A50299
Countess 3 FL Automated Cell Counter w/ABRC support	Instrument plus 3 yr ABRC ext warranty	A49893
Countess 3 FL instrument w/21 CFR Part 11 Software Module	1 instrument plus 1 license	AMQAF2001
Accessories		
Countess 3 Cell Counting Chamber Slides	50 slides	C10228
	500 slides	C10312
Countess Reusable Slide	1 slide	A25750
Countess 3 and 3 FL Reusable Slide Holder	1 slide holder	A48208
High-Power USB Wi-Fi Module (dongle)	1 module	A26774
ReadyCount Red Dead Cell Stain	100 tests	A49903
ReadyCount Blue Nuclear Stain	100 tests	A49904
ReadyCount Green/Red Viability Stain	100 tests	A49905
Countess 3 21 CFR Part 11 Software Module	1 license	A51025
Countess 3 Cell Counting Standard Slide	1 slide	A51876
Trypan Blue Stain (0.4%)	2 x 1 mL tubes	T10282

* ABRC = AB Repair Center.

Learn more at **thermofisher.com/countess**

invitrogen

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