

invitrogen

# Everything counts

But nothing counts like  
Countess II automated cell counters

Fast | Accurate | Affordable



**ThermoFisher**  
SCIENTIFIC

# Countess II automated cell counters

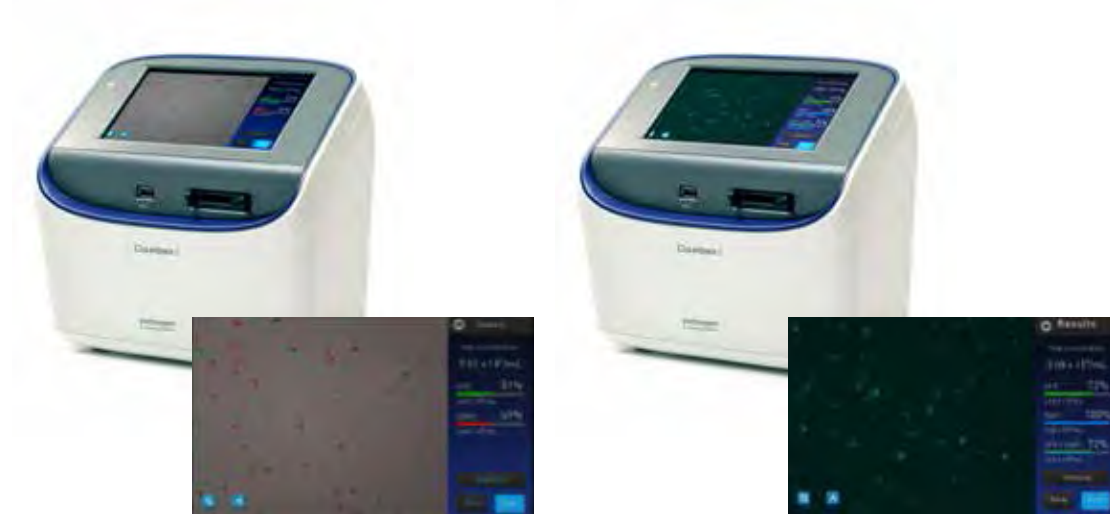
Advanced technology at an affordable price

## Precise, accurate counts in as little as 10 seconds

We offer two high performance automated cell counters designed to meet the needs of any lab. The Invitrogen™ Countess™ II and Countess™ II FL Automated Cell Counters contain advanced autofocus and counting algorithms to allow you to quickly and accurately identify and count cells within a population while avoiding user variation associated with manual focusing mechanisms.

Both automated cell counters offer the following features:

- Accurate—autofocus minimizes user-to-user variability
- Fast—results in as little as 10 seconds
- Convenient—save up to 10 user profiles



Feature	Countess II	Countess II FL
Counting mode	Bright-field	Bright-field channel plus two optional, user-changeable fluorescent channels
Slides	Disposable slides	Reusable and disposable slides
Counting time	As little as 10 seconds	
Focus	Auto focus with manual focus option	
User profiles	Customize and save up to 10 user profiles	
User interface	Intuitive touch screen with ability to use a mouse, if desired	

“It outperformed all other competitors' models that we tried.”

—Michael DaCosta, The Jackson Laboratory

# Features of the Countess II instruments

## Autofocus minimizes variability

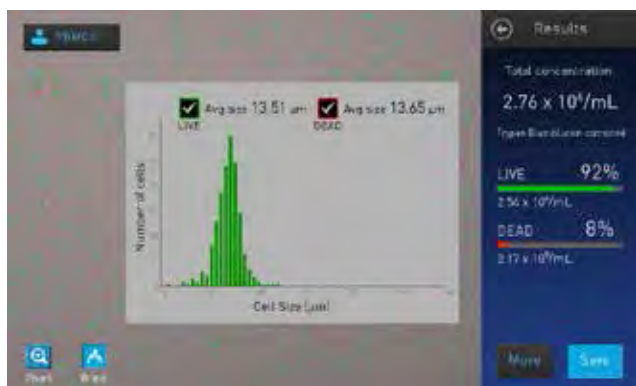
The autofocus feature of Countess II instruments works by analyzing more than 30 focal planes then selecting the plane with the best focal quality. This helps ensure that any variance from sample to sample, user to user, and slide to slide is addressed. Results are based on the optimal focal plane to enable highly accurate cell and viability counts. The results screen shown in Figure 1 allows for quick visual confirmation of cells being identified as live or dead.



**Figure 1. PBMCs counted using the Countess II Automated Cell Counter.** Counting and viability measurement can be done using standard trypan blue staining. The results screen displays total concentration and percentage of live and dead cells.

## Easily gate cells and view histograms

Cells can be easily gated based on cell size, brightness, and circularity to fine-tune precisely what is included or excluded for specific applications. View the histogram by selecting 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.



**Figure 3. Histogram is updated to reflect gating changes.** Multiple gating options can be selected separately for live cells and dead cells allowing easy inclusion or exclusion of debris or specific populations.

## Reusable counting slide

The mounting costs of disposable slides and tips cause many labs to still rely on manual cell counting. The Countess II FL instrument was designed to work with a reusable glass slide, to help significantly reduce the long-term consumable costs and waste associated with automated cell counting. For researchers who want the convenience of disposable slides, the Countess II FL instrument can also use the same PMMA plastic slides developed for the original Countess cell counter.

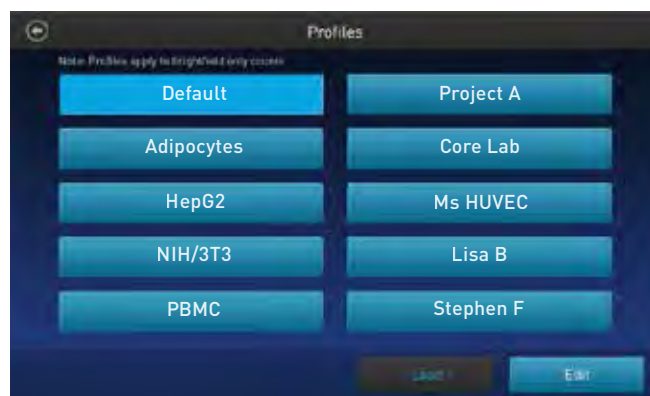
Note: Reusable slide option is only available with the Countess II FL model.



**Figure 2. Reusable slide for the Countess II FL Automated Cell Counter helps reduce consumable costs.**

## Save profiles for customized settings

Custom profiles can be saved and easily accessed from the capture and results screens. Settings for cell size, brightness, and circularity, as well as fluorescent thresholds can be customized and saved for easy retrieval.



**Figure 4. Profile names are user defined for easy identification.**



# Countess II FL Automated Cell Counter

## Fluorescence detection—not limited to GFP and RFP

The Countess II FL Automated Cell Counter offers bright-field and two optional, user-interchangeable fluorescent channels. The fluorescent colors collected are determined by the insertion of individual light cubes. The wide variety of light cubes available provides flexibility for multiple research applications.

## Interchangeable LED light cubes

At the heart of the Countess fluorescence technology lies the unique Invitrogen™ EVOS™ light cubes. Each cube contains an LED, illuminating optics, and filters. Light cubes are user interchangeable, auto-detected by the Countess system for plug-and-play capability.

Table 1 lists some of the most commonly used light cubes. There are more than 20 to choose from.



Table 1. Common fluorescent light cubes available for the Countess II FL Automated Cell Counter.

Light cube	Excitation (nm)	Emission (nm)	Common compatible dyes/fluorescent proteins
DAPI	357/44	447/60	DAPI, Hoechst, BFP
TagBFP	390/18	447/60	TagBFP
CFP	445/45	510/42	ECFP, Lucifer Yellow
GFP	470/22	510/42	GFP, Alexa Fluor™ 488, SYBR™ Green, FITC
YFP	500/24	524/27	EYFP, acridine orange
RFP	531/40	593/40	RFP, Alexa Fluor™ 546, Alexa Fluor™ 555, Cy®3, DsRed, Rhodamine Red, dTomato
Texas Red™	585/29	624/40	Texas Red, Alexa Fluor™ 568, Alexa Fluor™ 594, MitoTracker™ Red, mCherry
Cy®5	628/40	692/40	Cy®5, Alexa Fluor™ 647, Alexa Fluor™ 660, DRAQ5™
Cy®5.5	655/46	794/16	Cy®5.5

For a complete list of available common and specialty light cubes, go to [thermofisher.com/evoslightcubes](https://thermofisher.com/evoslightcubes)

# Applications

## Viability

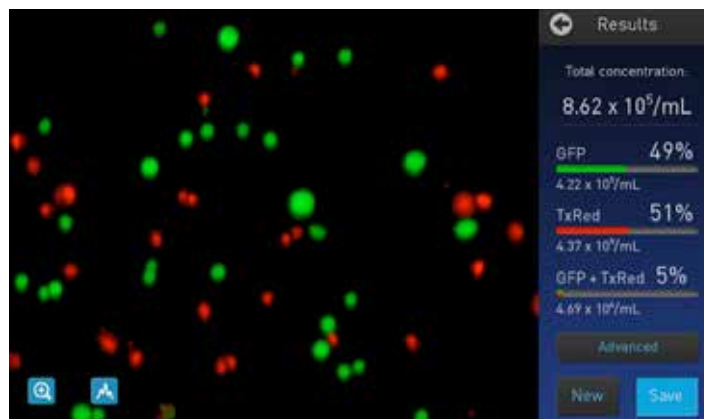
Assessing cell viability is a key step in daily cell manipulation and is required for accurate and efficient downstream processing. With the Countess II FL instrument there are many fluorescent options in addition to trypan blue staining to quickly and easily check viability prior to downstream sample analysis. Figure 5 shows results obtained using the Invitrogen™ Molecular Probes™ LIVE/DEAD™ Viability/Cytotoxicity Kit.

## Apoptosis

In addition to cell viability, knowing how many cells are dead or dying are key pieces of information that can be determined quickly with the Countess II FL instrument. Cells of interest can be stained with a viability dye, such as Invitrogen™ Molecular Probes™ SYTOX™ Red Dead Cell Stain, as well as an apoptosis indicator that measures caspase activation, such as Invitrogen™ Molecular Probes™ CellEvent™ Caspase-3/7 Green Detection Reagent. This staining combination, used together with GFP and Cy<sup>5</sup> light cubes, offers a fast and simple method to obtain apoptosis data.

## Transfection

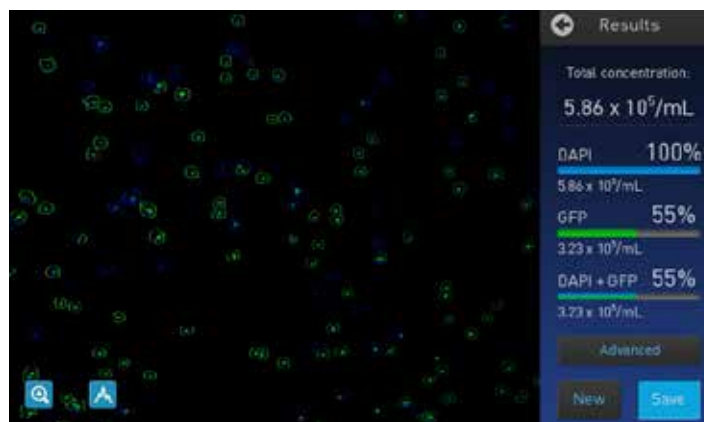
The ability to assess how many cells have been successfully transfected or transduced in a cell population is a basic and critical evaluation parameter. Commonly, the cells of interest are transfected or transduced with a construct resulting in the expression of a fluorescent protein reporter, such as GFP. The Countess II FL instrument loaded with your choice of light cubes offers a quick and simple method to easily obtain transfection efficiency data.



**Figure 5. Viability assay using the LIVE/DEAD Viability/Cytotoxicity Kit.** Live and heat-killed U2OS cells were mixed in an approximate 1:1 ratio, and then stained with calcein AM and EthD-1 supplied with the kit. The resultant suspension was then evaluated using the GFP and Texas Red light cubes with the Countess II FL Automated Cell Counter.



**Figure 6. Apoptotic and dead cells counted on a Countess II FL Automated Cell Counter.** After incubation with 0.5  $\mu$ m staurosporine, HeLa cells were labeled with 1:400 CellEvent Caspase-3/7 Green Detection Reagent to identify apoptotic cells, and then stained with 1:1,000 SYTOX Red Dead Cell Stain to denote all dead cells and incubated at room temperature for 30 minutes. The sample was evaluated using the Countess II FL Automated Cell Counter with EVOS light cubes for GFP and Cy<sup>5</sup> installed.



**Figure 7. Viability assay using the Invitrogen™ Molecular Probes™ ReadyProbes Cell Viability Imaging Kit, Blue/Green.** Live and heat-killed U2OS cells were mixed in an approximate 1:1 ratio, and then stained with NucBlue™ Live reagent and NucGreen™ Dead reagent supplied with the kit. The resultant sample was then evaluated using the Countess II FL Automated Cell Counter with DAPI and GFP light cubes installed.

**Table 2. Commonly used dyes and light cubes used with the Countess II FL cell counter for viability and apoptosis determination.**

Dye	Light cube(s)	Cat. No.
<b>Viability dyes</b>		
ReadyProbes Cell Viability Imaging Kit, Blue/Green	DAPI and GFP	R37609
ReadyProbes™ Cell Viability Imaging Kit, Blue/Red	DAPI and RFP or Texas Red	R37610
LIVE/DEAD Viability/Cytotoxicity Kit	GFP and RFP or Texas Red	L3224
Propidium Iodide ReadyProbes™ Reagent	RFP	R37108
SYTOX™ Green Nucleic Acid Stain	GFP	S7020
SYTOX™ Red Dead Cell Stain	Cy®5	S34859
7-Aminoactinomycin D (7-AAD)	Texas Red or Cy®5	A1310
<b>Apoptosis dyes</b>		
CellEvent Caspase-3/7 Green Detection Reagent	GFP	C10423
SYTOX Red Dead Cell Stain	Cy®5	S34859

**Table 3. Cell lines validated on the Countess II automated cell counters.**

Cell type	Animal	Organ	Cell Size (diameter)
A431	Human	Skin	15.5 µm
Adipocytes	Human	Adipose-derived stem cells	13 µm
Aortic smooth muscle	Human	Smooth muscle	20 µm
Blood, whole lysed	Human	Blood	NA
CHO-M1WT2	Chinese hamster	Ovary	NA
CHSE	Chinook salmon	Embryo	16–17 µm
COLO-205	Human	Colon	NA
COS 7	African monkey	Kidney	NA
HEK-293	Human	Kidney	13 µm
HeLa	Human	Cervix	NA
HepG2	Human	Liver	18 µm
HL 60	Human	Blood	NA
J774A.1	Mouse	Blood	13–14 µm
Jurkat	Human	Blood	12 µm
K562	Human	Bone marrow	NA
MCF7	Human	Breast	20–24 µm
MRC-5	Human	Lung	18 µm
NIH/3T3	Mouse	Embryo	18 µm
PBMC	Human	Blood	7–8 µm
PC-12	Rat	Adrenal gland	12–14 µm
Pulmonary artery endothelial cells	Human	Blood vessel	13 µm
Pulmonary artery smooth muscle	Human	Smooth muscle	20 µm
SF-21	Insect	Ovary	NA
U266	Human	Blood	12–13 µm
U2OS	Human	Bone	NA
Umbilical vein endothelial cells	Human	Blood vessel	17 µm

## Countess II Automated Cell Counter (AMQAX1000)

Technical specifications	
Optics:	Bright-field only
Slides:	Countess Cell Counting Chamber Slides (disposable)
Processing time:	As little as 10 seconds
Required sample volume:	10 $\mu$ L
Sample concentration range:	$1 \times 10^4$ – $1 \times 10^7$ cells/mL
Particle/cell diameter range:	Particles: $\sim 4$ – 60 $\mu$ m/Cells: $\sim 7$ – 60 $\mu$ m
Objective:	2.5x
Total magnification:	$\sim 55$ x
Illumination:	LED (50,000 hours)
Camera:	5 megapixels
Instrument dimensions (W x D x H):	9 x 5½ x 9 in; 228.6 x 139.7 x 228.6 mm
Weight:	8 lb
Operating power:	100–240 VAC, 0.58 A max.
Frequency:	50/60 Hz
Electrical input:	12 V DC, 2 A

## Countess II FL Automated Cell Counter (AMQAF1000)

Technical specifications	
Optics:	3 channels (bright-field and 2 slots for EVOS LED light cubes)
Slides:	Countess II FL Reusable Slide or Countess Cell Counting Chamber Slide (disposable)
Processing time:	As little as 10 seconds
Required sample volume:	10 $\mu$ L
Sample concentration range:	$1 \times 10^4$ – $1 \times 10^7$ cells/mL
Particle/cell diameter range:	Particles: $\sim 4$ – 60 $\mu$ m/Cells: $\sim 7$ – 60 $\mu$ m
Objective:	2.5x
Total magnification:	$\sim 55$ x
Illumination:	LED (50,000 hours)
Camera:	5 megapixels
Instrument dimensions (W x D x H):	9 x 5½ x 9 in; 228.6 x 139.7 x 228.6 mm
Weight:	8 lb
Operating power:	100–240 VAC, 0.58 A max.
Frequency:	50/60 Hz
Electrical input:	12 V DC, 2 A

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## See what users have to say:

"Fast count and nice instruments, easy to use."

—*Dr. Xin Xu, Baylor College of Medicine*

"The Countess II makes it very easy to count cells accurately and quickly—the fact that it tells you the dead cell and live cell count is icing on the cake."

—*Faryal Mir, MIT*

"We compared it to a hemocytometer and the Countess II always obtained very close results. The fluorescent measurements are a great tool for us to quantify the % transfection and transduction efficiency in our experiments. The light cubes used to detect fluorescence are very easy to switch out when more than two fluorescence sources are needing to be detected. The automatic focusing is very effective and accurate with the option to manually focus."

—*David Waynar, Santa Cruz Biotechnology*

"Great for a quick check of transfection/transduction efficiency."

—*Alex Peister, Morehouse College*

"Great device. Broad range of cell densities that it can handle. Good at distinguishing clumps and counting cells within clumps."

—*Joanna Rzepka, Veterinary Medical Research & Development*

"Low maintenance, fast easy accurate cell counts. Easy for students to use and comprehend."

—*Robert Dean, D'Youville College*

"A very accurate cell counter and you can use it to count fluorescent cells as well. Excellent product. I'm very happy with it. I highly recommend it."

—*Massimo Ammirante, University of California, San Diego*

"It's very easy to use and it also allows me to quickly update my lab notebook so that each experiment uses the same number of cells. Quick and efficient."

—*Lakeisha Tillery, Meharry Medical College*

"It's very consistent!"

—*Kim Sylvia, Sanford Burnham Medical Research Institute*

## Ordering information

Product	Quantity	Cat. No.
Countess™ II FL Automated Cell Counter	1	AMQAF1000
Countess™ II Automated Cell Counter	1	AMQAX1000
Countess™ Cell Counting Chamber Slides	50	C10228
Countess™ II FL Reusable Slide	1	A25750
Countess™ II FL Reusable Slide Holder	1	AMEP4746
<b>User-changeable light cubes</b>		
EVOS™ Light Cube, DAPI	1	AMEP4650
EVOS™ Light Cube, TagBFP	1	AMEP4668
EVOS™ Light Cube, YFP	1	AMEP4654
EVOS™ Light Cube, GFP	1	AMEP4651
EVOS™ Light Cube, RFP	1	AMEP4652
EVOS™ Light Cube, Texas Red	1	AMEP4655
EVOS™ Light Cube, Cy®5	1	AMEP4656
EVOS™ Light Cube, Cy®5.5	1	AMEP4673

Find out more at [thermofisher.com/countess](http://thermofisher.com/countess)

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