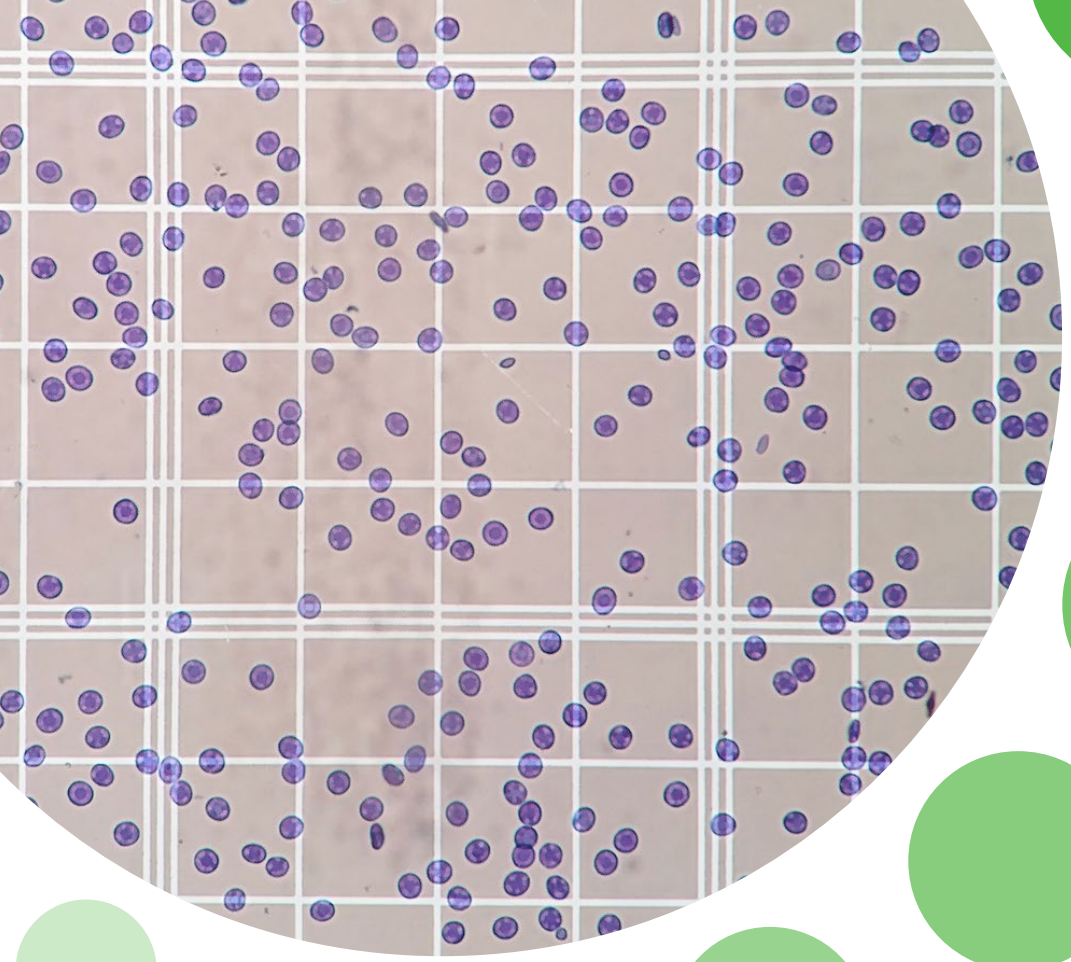

Picking the perfect automated cell counter:

Methods and instrumentation

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Contents

1. Welcome to automated cell counting

To improve throughput, accuracy and standardisation when counting cells, many researchers are choosing to replace their haemocytometer with an automated cell counter.

But with a variety of cell counting methodologies available, not to mention a bewildering array of instruments, selecting an automated cell counter to meet the exacting requirements of your busy laboratory can be challenging.

Before making a purchase, it is worth taking some time to consider which cell counting method best fits your needs. This will help you to identify the perfect instrument to support your research.

As a leading independent supplier of automated cell counting systems, Cambridge Bioscience is here to guide you through cell counter selection. Based on our experience with multiple systems and demos in many labs across the UK, we can offer our insights, advice and streamline your switch from manual to automated cell counting.

Whether you require a straightforward cell count before sub-culturing, wish to identify only nucleated cells within a heterogeneous population, or you want your new automated cell counter to function as an essential quality control component within a defined process, we have an instrument to suit you.

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2. Choose the right method

There are a number of different cell counting methodologies available and it is important to consider which method is most appropriate to your downstream application.

Image-based counting using Trypan Blue is an established method to distinguish live from dead cells. This method is simple and sufficient for counting cell culture samples, but it is unsuitable for complex, clumpy or low viability samples.

Fluorescent image-based counting provides greater accuracy by clearly differentiating nucleated cells from non-nucleated objects. Suited for counting more complex samples such as primary cells or PBMC samples, the overall number of cells counted is comparable to Trypan Blue-based methods.

Non-image-based methodologies enhance reproducibility by counting many thousands of cells, significantly increasing the overall quality of the cell counting process and providing far greater insight. Not being reliant on optics and camera resolutions, non-image-based methods are often suitable for counting very small objects (such as bacteria) and large objects such as cell aggregates, yeast, algae and micro-organisms.

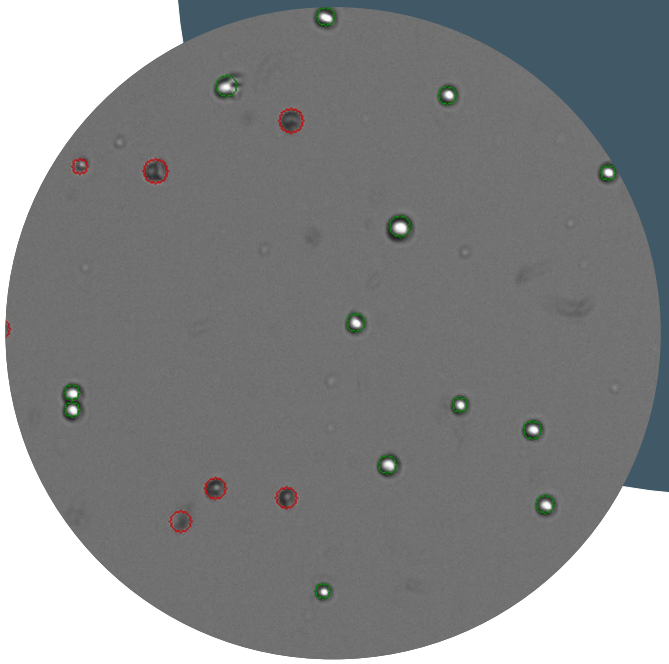
3. Automated cell counting using Trypan Blue

Manual, haemocytometer-based cell counting typically relies on the use of Trypan Blue, a dye which stains dead cells a bright blue colour following its diffusion across a leaky cell membrane. Using a brightfield microscope to visualise the cell population, researchers count cells within defined areas marked on a haemocytometer slide to determine cell concentration and viability.

Haemocytometer-based cell counts often show low levels of reproducibility. User bias leads to variability between counts performed by different researchers, while a tendency to count too few cells means that standard errors can be high.

Although statistical accuracy can be improved by counting multiple samples, this approach adds considerable time to workflows and can compromise cell health, for example as a result of leaving detached cells in suspension for an extended period during counting before they are transferred to fresh growth medium.

Moreover, Trypan Blue has the potential for toxicity if used incorrectly, causing cells to be destroyed during counting. Trypan Blue can also be incorporated into live cells following longer incubations and both these eventualities can compromise results.



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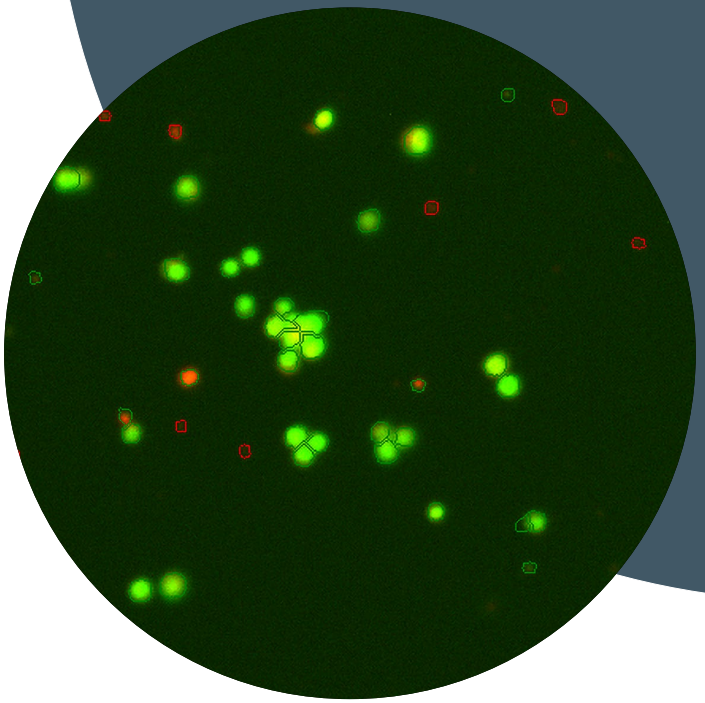
4. Automated cell counting using fluorescence

The main advantage of fluorescence-based cell counting over methods which use Trypan Blue is its capacity to eliminate debris and misleading objects to deliver a more accurate cell count. This is achieved by using fluorescent dyes to bind key nuclear components, generating a count of nucleated live and nucleated dead cells.

Acridine orange/propidium iodide (AO/PI) staining is widely used for fluorescence-based cell counting. AO is membrane permeable and stains the DNA of viable cells green, whereas PI enters only dead cells and generates a red signal. Using a fluorescence microscope or fluorescence-capable automated cell counter, it is possible to identify and count all nucleated objects within a sample for a more accurate cell viability measurement.



Since AO/PI staining is specific to nuclei, it is a popular choice for researchers working with complex samples such as peripheral blood mononuclear cells (PBMC), where it is often necessary to exclude non-nucleated red blood cells (RBC) from counts.

Fluorescence-based cell counting is also better suited to notoriously clumpy cell populations such as primary hepatocytes, allowing researchers to achieve much higher accuracy counting than that which is possible with Trypan Blue.

The CellDrop™ FL developed by DeNovix® is an automated cell counter with AO/PI and Trypan Blue capabilities. The combination of a high-quality camera and advanced de-clumping algorithms enables fast and accurate cell counts.

CellDrop™ is the first automated cell counter to be totally consumable free. Through its unique DirectPipette™ technology, the CellDrop™ series of instruments brings the familiar load, measure and wipe clean functionality of microvolume spectrophotometers to cell counting. Should the need arise, the CellDrop™ FL can also be used with common cell counting plastic slides.

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5. Non-image-based cell counting

In addition to image-based cell counting methods there are also non-image-based methods that can be used to accurately count cells.

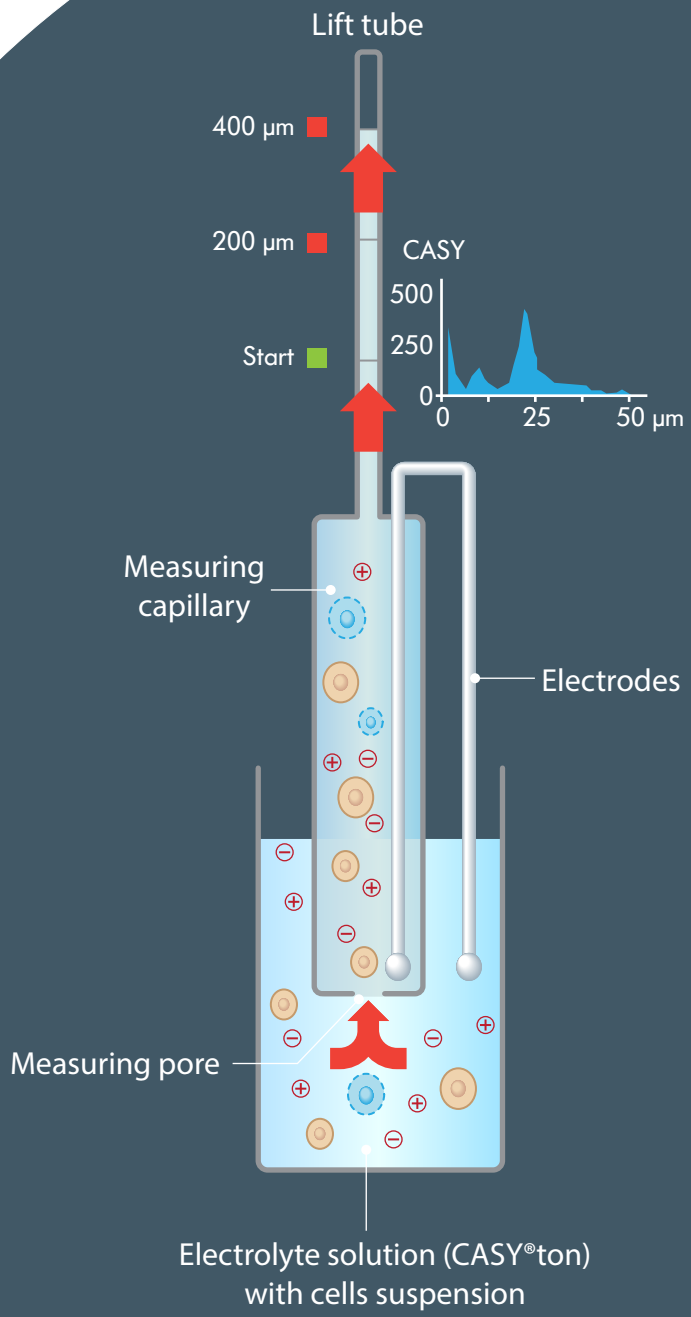
Electrical current exclusion (ECE) is a non-image-based cell counting method which delivers enhanced statistical accuracy over image-based counting methodologies reliant on Trypan Blue or fluorescence detection.

ECE counting works by drawing cells through an electrical field aperture, counting each passing object and recording the volume of each.

Because there are no limitations imposed by a field of view or camera resolution, many thousands of cells can be counted extremely rapidly using this method providing a very high level of statistical confidence and reproducibility.

Dead cells, with their ruptured membranes, 'leak' current when passing through the aperture and are recorded as being smaller than live counterparts. This phenomena provides an accurate method for defining cell viability. Size differences can be used to profile sub-populations of cells and track changes in mean size.

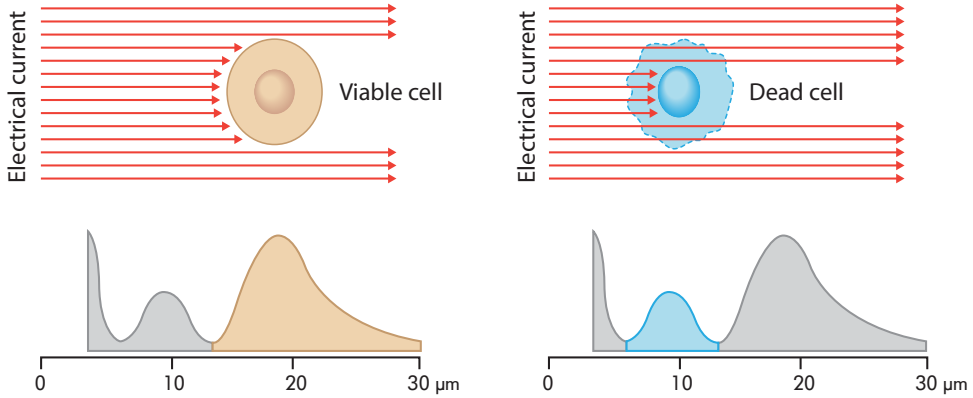
Furthermore, by measuring the size of each object, ECE is one of the few technologies to readily measure clumps of cells and aggregated biomass.

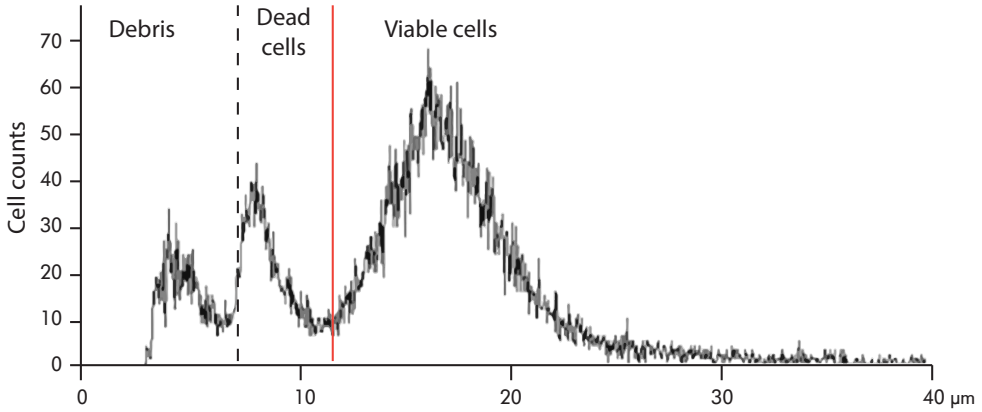


Researchers who require a detailed understanding of sample quality before proceeding with a downstream process often choose to use non-image-based cell counting methods such as ECE. For example, those working with blood samples may wish to investigate the distribution of specific populations before performing a time-consuming multiplex flow cytometry experiment.

Non-image-based cell counting is also ideal for counting both very small objects such as bacteria, and very large objects such as cell clumps, yeast, or micro-organisms.

The CASY Cell Counter is one of the few non-image-based solutions commercially available for automated cell counting, delivering accurate, multi-parametric data within seconds to improve the quality of workflows.





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6. Methodology selection guide

Assay	Type	Instruments	# cells counted	Cell culture	Primary cells	PBMC	Yeast	Bacteria
Trypan Blue	Image based / brightfield	C-Chip Haemocytometer NanoEnTek EVE DeNovix CellDrop BF	Few hundred	***	-	-	-	-
AO/PI	Image based / fluorescence	DeNovix CellDrop FL	Few hundred	**	**	**	*	-
Stain-free, image-free	Electrical current exclusion (ECE)	CASY Counter	Thousands	**	***	***	***	***

7. Choose the right cell counter for your research

Once you have identified the cell counting methodology which best meets your requirements, it's time to consider instrumentation. If your aim is simply to improve on manual, haemocytometer-based counting using Trypan Blue, either the NanoEnTek EVE™ or the DeNovix® CellDrop™ BF could be the model for you.

Alternatively, you may be working with complex or clumpy samples, meaning you need to distinguish nucleated objects from particles and debris. In this situation, you might choose the DeNovix® CellDrop™ FL, a slide-free automated cell counter which also has Trypan Blue capabilities.

If your process depends on high levels of accuracy, you should consider the CASY Cell Counter. Providing rapid, multi-parametric analysis for any cell type, the CASY Cell Counter enhances data quality by counting thousands of cells within just seconds.

At Cambridge Bioscience, we understand the importance of making the right choice. Acting as a neutral vendor of automated cell counting systems to meet diverse research requirements, we provide unbiased advice to guide you in your purchasing decision. Whether you have a question regarding the functionality of one of the cell counters in our portfolio, or you would like us to carry out an in-house evaluation of your current cell counting workflow, we're here to help.

Why not consider trialling multiple automated cell counters in parallel, supported by our friendly knowledgeable application specialist? We have a range of options to provide you with enhanced cell counting accuracy and reproducibility.

Contact:

instruments@bioscience.co.uk

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8. CASY Cell Counter

The most advanced model within our range of automated cell counting systems, the CASY Cell Counter uses electrical current exclusion to determine cell number and viability. This allows researchers to achieve exceptional statistical accuracy while benefiting from the largest size measurement range (0.7-120µm) of any method of cell size analysis.

Easily able to distinguish cell debris from live/dead cells and to accurately calculate the number of cells within aggregates, the CASY Cell Counter provides a non-invasive method suitable for analysing all cell types (including cell lines, primary cells, bacteria, yeast, algae, and parasites).

To generate a cell count using the CASY Cell Counter, all you need to do is suspend your cells in CASYton (a conductive isotonic buffer solution) and immerse the instrument's sensor in the sample solution. With no requirement for dyes, the CASY Cell Counter rapidly provides information on all aspects of the current status of your cell culture, backing this data with an integrated quality assurance (QA) system.

Using the CASY Cell Counter to evaluate your cells immediately prior to seeding greatly increases your confidence in experimental success and can provide valuable insight when you are about to start a long-term experiment with an enhanced requirement for resource.

- Utility across many applications
- Precise counting and seeding control for mammalian cells
- Monitor bacterial cell number, debris and aggregates
- Simultaneous measurement of cell proliferation and biovolume
- Yeast cell quality monitoring and QC
- Cytotoxicity assays
- T-cell monitoring

Download the CASY brochure

9. DeNovix® CellDrop™ BF and CellDrop™ FL

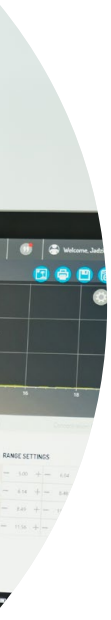
Featuring patented DirectPipette™ Technology to eliminate the need for disposable plastic slides, the DeNovix® CellDrop™ is incredibly easy to use. Just add 10 µl of your cell suspension into the counting chamber, allow the CellDrop™ to analyse the sample, then wipe the chamber clean with a dry laboratory wipe.

Powerful live-view imaging allows instant verification that cleaning has been successful, while for applications that require the cells to be contained when reading, the CellDrop™ is compatible with most common slides without the need for an adaptor.

Available as a brightfield model (CellDrop™ BF) and as a model which incorporates both brightfield and dual fluorescence optics (CellDrop™ FL), the CellDrop™ meets a diversity of user requirements.

Pre-installed applications allow for intuitive analysis of different assays, while the variable height sample chamber adjusts automatically to deliver accurate counting over a wide range of cell densities.

- Fast, accurate cell count and viability measurements
- Pre-installed applications for a wide range of assays
  - brightfield, Trypan Blue, AO/PI, GFP, yeast
- Eliminates slide costs and reduces plastic use
- Effective reporting software
- Fits inside most flow hoods for hazardous sample processing
- Option for slide use when counting cells that require containment



Download the CellDrop™ brochure

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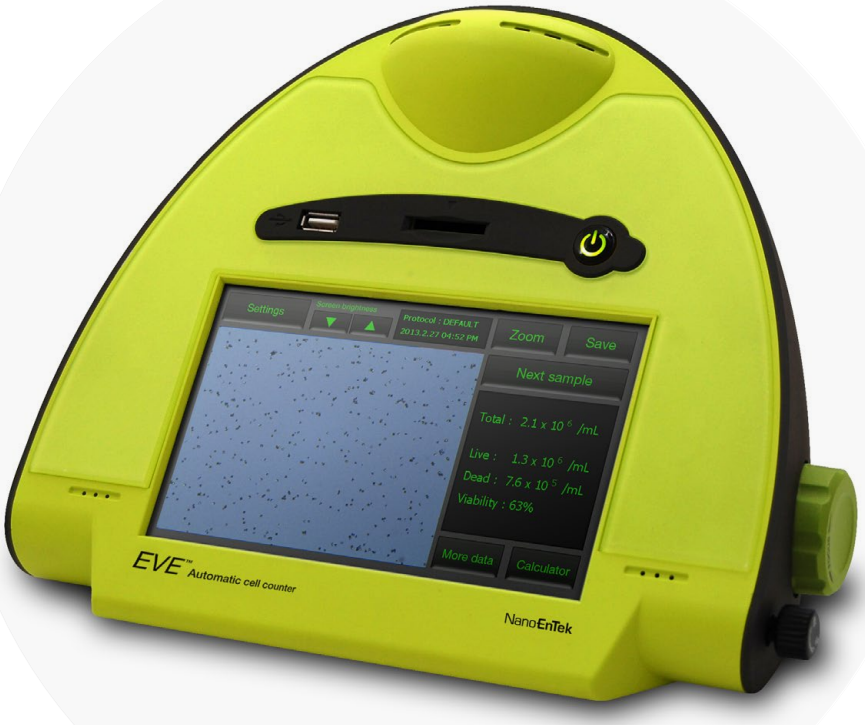
10. NanoEnTek EVE™

Widely recognised as a faster, more precise alternative to manual cell counting, the NanoEnTek EVE™ has a very small footprint, allowing it to fit easily into even the busiest laboratory. Using optics and image analysis to measure cell count and viability based on Trypan Blue staining, the EVE™ relies on an advanced analysis algorithm to evaluate individual cells within clumps.

To perform a cell count using the EVE™, simply load your sample into a disposable cell counting slide, adjust the focus, then allow the instrument to count live, dead and total cells in less than 20 seconds. With applicability for a broad range of cell sizes and types (including primary cell lines and stem cells), and with the inclusion of a cell size gating function, the EVE™ is ideal for researchers wishing to improve on haemocytometer-based methods of cell counting.

- Automated counting - samples counted in <20 seconds
- Compatible with a cell types from 5-60 µm
- User-friendly interface with the capacity to calculate dilutions
- Straightforward image (JPEG) and data (CSV) export via USB – stores 500 test results



Download the EVE™ brochure

11. Instrument selection guide

	Sample range	Size range	Speed	Measurement technique	Required consumables	Viability measurements	Subpopulation analysis
EVE	1 x 10^4 - 1 x 10^7 cells/ml	5 - 60 µm	~20 seconds	Image based (brightfield)	Disposable Slides	By Trypan Blue	No
CellDrop Bf	7 x 10^2 to 2.5 x 10^7 cells/ml	4—400 µm	~3 seconds	Image based (brightfield)	None	By Trypan Blue	No
CellDrop Fl	7 x 10^2 to 2.5 x 10^7 cells/ml	4—400 µm	~8.5 seconds	Image based (fluorescence)	None	By AO/PI	No
CASY	in volume > 1:70,000 in diameter > 1:40	0.7–120 µm	30 seconds (for triplicate measurement)	Electrical Current Exclusion	CasyCups	Stain free (size gating)	Yes

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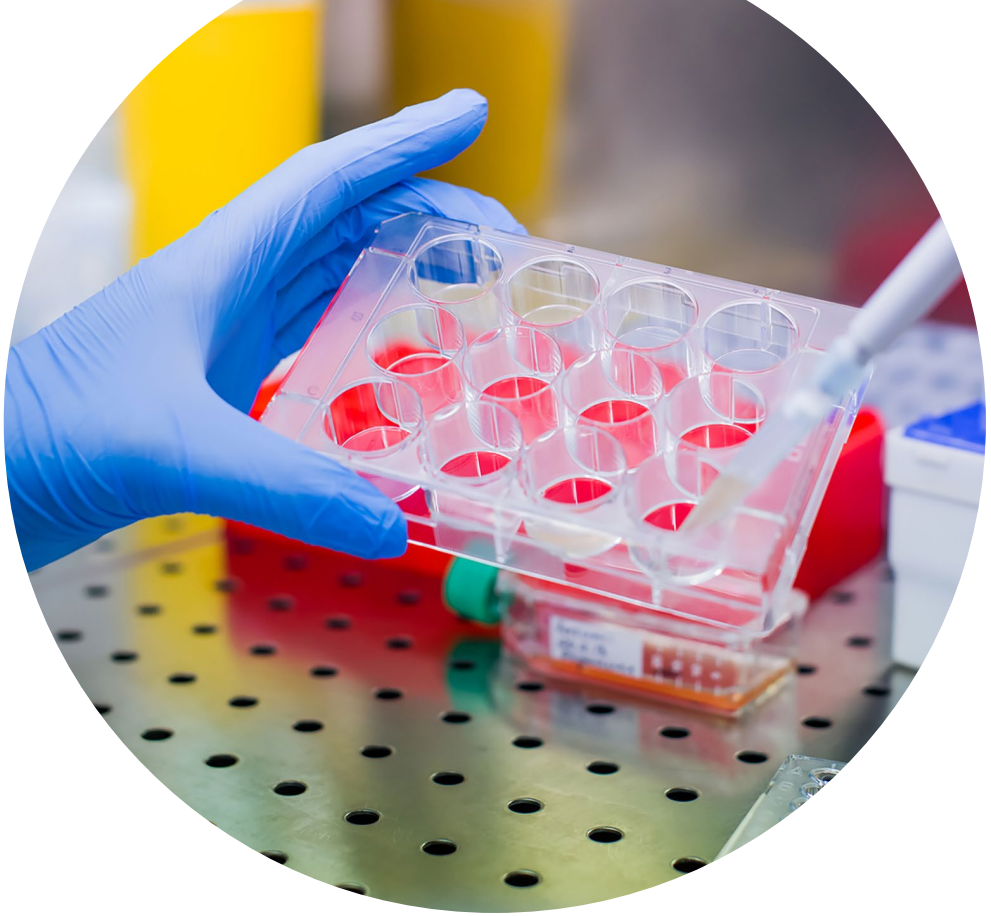
12. Next steps...



Having considered the different cell counting methodologies available and learnt how the automated cell counters within our product portfolio rely on one or more of these methods, you may already have identified an instrument to suit your needs. On the other hand, you may require some additional information to identify the methodology or instrumentation that's right for you.

With a wealth of experience in counting cells, we're here to help. Our dedicated specialists are on hand to answer your questions, and we also offer a variety of informative online resources to guide your decision.

Perhaps you would like to arrange a demonstration of one or more of our automated cell counters within your laboratory – we often find that comparing all three systems in parallel can help to clarify that which works best for you.

Whatever your approach, you can rely on Cambridge Bioscience for unbiased, friendly advice to streamline your transition from manual to automated cell counting.

To learn more about our automated cell counters, contact us at instruments@bioscience.co.uk

13. References

References

Trypan vs AO/PI: https://www.ncbi.nlm.nih.gov/pubmed/10864990 and https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1537-2995.2000.40060693.x and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4371569/#CR15

Historical reviews Davis, J.D., THE HEMOCYTOMETER AND ITS IMPACT ON PROGRESSIVE-ERA MEDICINE, in Department of History 1995, University of Illinois at Urbana-Champaign: Urbana. p. 268. and Verso, M.L., Some Nineteenth-Century Pioneers of Haematology. Medical History, 1971. 15(1): p. 5567.

Example of use of CASY, overlay data http://www.breach-hiv.be/media/docs/SymposiumLeuven/Poster22PerrineTriqueneaux(UCL).pdf and CASY analysis of cytotoxicities https://www.ncbi.nlm.nih.gov/pubmed/16372834 and CASY counting of PBMCs: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4956601/

Guide to using a haemocytometer: http://www.vivo.colostate.edu/hbooks/pathphys/reprod/semeneval/hemacytometer.html

Product websites & brochures

NanoEnTek EVE™

bioscience.co.uk/eve

DeNovix® CellDrop™

bioscience.co.uk/celldrop

CASY Counter

bioscience.co.uk/CASY

Website resource

bioscience.co.uk/products/cell-counting

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14. Authors

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He has worked for a range of instrumentation and supply companies and has extensive image analysis and cell counting experience.

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Henri is an Instrument Application Specialist at at Cambridge Bioscience with a speciality in cell imaging. Henri completed his PhD through the University of Manchester and A*Star, Singapore.

He has a strong technical background in optics, microscopy and imaging techniques and hardware.

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