

BLOG
How to Count Cells

Flow Cytometry vs. Image-Based Analysis

Which One Should You Use for Cell Counting and Viability Assays?

logos biosystems
by ALIGNED GENETICS

© Aligned Genetics, Inc. All rights reserved.

Introduction

Before every passage, most researchers go through the same routine: lift the cells, dilute them, load them into a counter, and check the cell count and viability. If your lab also has a flow cytometer, you may have wondered, "Wouldn't this counting be more accurate on the flow cytometer?" Some of you have probably run the same sample on both instruments and gotten slightly different numbers, unsure which one to trust.
Both methods are used for a wide range of cell analyses, but this article focuses on the question researchers run into most often in day-to-day lab work: cell counting and viability measurement.

The Counting Principle Itself Is Different

Flow cytometry passes cells single-file through a laser beam and infers cell size and internal complexity indirectly from forward scatter (FSC) and side scatter (SSC). The same scatter pattern is also used to distinguish cells from debris. The catch is that if all you need is a cell count, this approach becomes unnecessarily involved. Getting an absolute count requires running calibration beads of known concentration alongside the sample, or applying a volumetric flow correction. In practice, small pipetting errors with the beads or concentration drift during storage can affect the result, and the setup and calibration steps themselves take real hands-on time — making it a somewhat cumbersome route for a simple count or viability check.

Image-based automated cell counters, by contrast, capture and count every cell directly. Because each counted particle can be checked in the image itself, you can visually confirm whether clumped cells or debris were miscounted. Absolute count is also immediate, since the slide's chamber volume is fixed — no bead calibration required.

This difference becomes more apparent with real samples. In cultures that are older or show aggregation, scatter signal alone makes it hard to distinguish cells, debris, and clumped cell masses. When debris of a similar size is present, errors accumulate no matter how carefully the gates are drawn. Image-based counting is more intuitive here: a declumping algorithm separates clustered cells by shape, and right after counting, you can check in the image exactly which particles were identified as cells — making the result easier to trust.

How Viability Is Determined Differs Too

In flow cytometry, viability is typically determined by gating on the fluorescence intensity of a dye such as PI or 7-AAD. Where you set the gate can shift the result, and pre-run setup such as compensation is also required.

Image-based methods read viability directly from the image — a color change with trypan blue under brightfield, or the fluorescence signal (green/red) with AO/PI. Because the classification criteria are captured in the image itself, if a result looks off later, you can open the image and immediately see which cells were called dead and why. In practice, that's a meaningful difference.

What the Data Shows

How well the two methods actually agree can be seen in several internal validation studies.

- In a somatic cell counting validation using milk samples, the LUNA-FX7™'s counts showed a strong correlation with a flow-cytometry-based somatic cell counter, the industry-standard method in dairy testing.
- In PBMC and leukocyte counting validation, serial 2-fold dilutions showed linearity with an R^2 of 0.98–0.99 or higher, confirming that counts tracked the expected concentration reliably.
- In a counting accuracy validation of the LUNA-FX7™, live/dead cell samples mixed at known ratios (0/25/50/75/100 %) showed measured viability correlating with expected viability at $R^2 \geq 0.999$, for both trypan blue and AO/PI methods.

In other words, for straightforward counting and viability applications, image-based automated cell counters deliver accuracy on par with flow cytometry, while sample preparation and result verification are considerably simpler.

Comparing the Two in Real Workflows

The difference between the two approaches becomes clearer in repeated, everyday workflows. Here are two examples.

The first is bioprocess batch monitoring using suspension cell lines. In an internal validation study, three cell lines — HL60, K562, and U937 — were seeded at 6 x 10^4 cells/mL and sampled twice a day for seven days, with 100 µL drawn for counting at each timepoint. The goal of this kind of repeated counting is straightforward: get a reliable cell count and viability reading, as consistently and effortlessly as possible. An image-based automated cell counter can use these counts to automatically calculate growth rate and doubling time and plot them as a trend chart. Repeating the same routine on a flow cytometer would mean recalibrating with beads at every single measurement — and over twice-daily sampling across seven days, that overhead adds up.

The second example is PBMC (peripheral blood mononuclear cell) counting. In an internal validation study, 1 mL of blood was processed by density-gradient centrifugation, stained with AO/PI fluorescent dye, and measured on an image-based counter; serial 2-fold dilutions showed linearity of $R^2 \geq 0.99$. PBMCs are a challenging sample: lymphocytes (7–10 µm) and monocytes (15–20 µm) differ substantially in size, and the isolation process often causes transient membrane damage that can make viability appear lower than it actually is. Flow cytometry can certainly count these samples too, but consistently distinguishing a size-heterogeneous population using scatter signal alone requires that much more careful gating.

A Practical Comparison

A couple of points are worth calling out before the table. Image-based counting typically needs only 10–20 µL of sample loaded per slide, which is a real advantage when working with precious primary cells or small-volume cultures. Flow cytometry, on the other hand, requires bead calibration and compensation setup for every run to get an absolute count — simply more steps to reach the same endpoint.

Criteria	Flow Cytometry	Image-Based Automated Cell Counter
Cell/debris discrimination	Indirect estimation via light scatter	Direct visual confirmation in images
Absolute count	Requires calibration beads or volumetric correction	Instant, from fixed chamber volume
Viability determination	Fluorescence gating (requires threshold setup)	Direct color/fluorescence reading on image
Result verification	Requires re-interpreting dot plots/histograms	Instant visual check via tagged images
Sample preparation	Requires compensation and other pre-run setup	Load immediately after staining
Turnaround time (for counting)	Relatively long once setup is included	Tens of seconds per sample
Instrument access	Often a shared/scheduled instrument	Ready to use at the benchtop

So Which One Should You Use, and When

For routine cell count and viability checks around passaging, image-based automated cell counters are clearly the more practical choice — no setup required, and results are verified instantly from the image.

The same holds for PBMCs and immune cells, or any primary cell that has undergone isolation stress and shows a wide size distribution. Image-based counting with nucleic acid dyes like AO/PI is less likely to misclassify transiently membrane-compromised cells as dead, which tends to make it the more reliable option for these samples.

For bioprocess batch monitoring — tracking cell count and viability twice daily to calculate growth rate and doubling time — image-based counting is also the more practical fit in practice. Instead of recalibrating and re-gating the flow cytometer each time, you simply load the sample into the automated counter.

On the other hand, if your lab already runs a flow cytometer continuously and you want to fold counting into the same run as other marker analyses, using flow cytometry for counting as well is a reasonable choice. Just keep in mind that the accuracy of the absolute count still depends on how well the beads are calibrated.

Bottom Line

The more useful question isn't "which one is more accurate," but "which one is more practical for this task." If the goal is simply an accurate cell count and viability reading, image-based automated cell counters deliver accuracy comparable to flow cytometry through a much simpler workflow. Flow cytometry's real strength lies elsewhere — in areas like multiplexed marker analysis that image-based methods can't replace.

Frequently Asked Questions (FAQ)

Q1. Can image-based counting be as accurate as flow cytometry?

A. For pure counting and viability applications, yes. In internal validation studies, image-based automated cell counters showed strong correlation with a flow-cytometry-based somatic cell counter in a milk-based somatic cell counting validation, and achieved $R^2 \geq 0.999$ accuracy in viability measurements. That said, detailed subset analysis based on cell-surface markers remains outside the scope of image-based methods.

Q2. Which method is more reliable for size-heterogeneous samples like PBMCs?

A. When image-based counting is paired with a fluorescent stain like AO/PI, viability is determined by fluorescence signal rather than cell size, which gives more consistent results in primary cells with wide size variation. Brightness- or morphology-based methods like trypan blue tend to lose accuracy on samples with broad size distributions.

Q3. If we already have a flow cytometer, is a dedicated cell counter worth adding?

A. For multiplexed marker analysis, flow cytometry remains the best choice. But routing every routine counting and viability check — which can happen several times a day — through the flow cytometer adds up in setup and calibration overhead. In most cases, splitting the work between the two instruments by purpose is the more efficient approach for overall workflow.

Logos Biosystems Image-Based Cell Analysis Solutions

- LUNA-FX7™ — Fluorescence + brightfield automated cell counter. Simultaneous 8-channel sample analysis, 21 CFR Part 11 support
- LUNA-III™ — Simple, accurate brightfield automated cell counter

Learn more: www.logosbio.com

Reference

1. Application Note: Somatic cell counting using the LUNA-FX7™ Automated Cell Counter.

2. Application Note: Rapid evaluation of PBMC counting and viability.

3. Application Note: The Counting Accuracy of the LUNA-FX7™ Automated Cell Counter.

4. Application Note: Bioprocess analysis with the LUNA-FX7™.