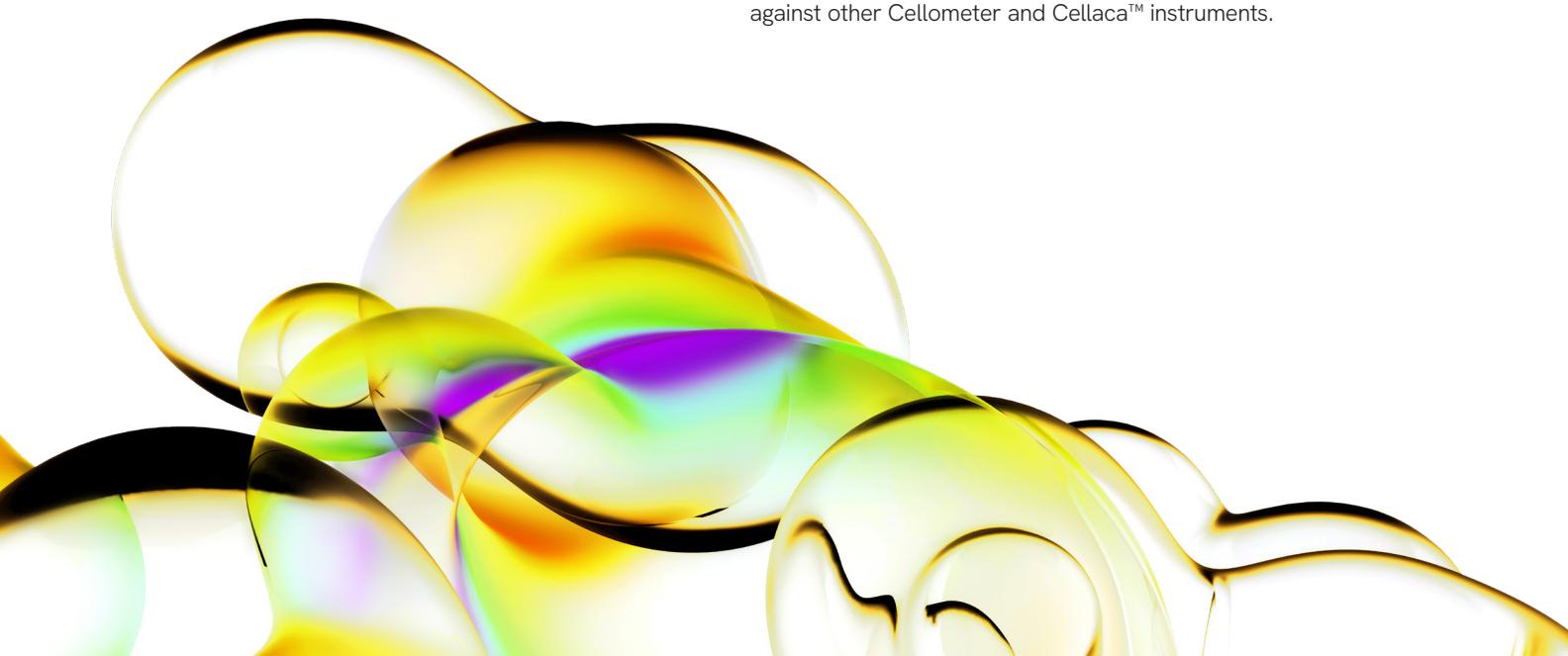
Cellometer Ascend advanced cell counting technology.

Introduction

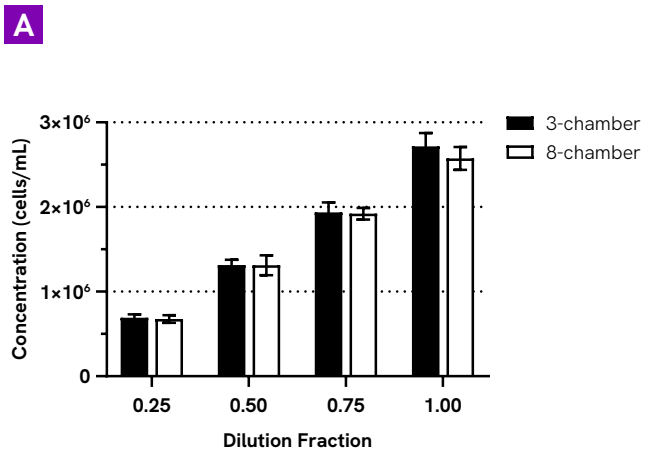
Reliable cell counting is a fundamental requirement across numerous biological research disciplines, including cell and gene therapy, regenerative medicine, bioprocessing, immuno-oncology, and virology/infectious disease. The accuracy and reproducibility of cell quantification directly impacts experimental outcomes, therapeutic efficacy, and manufacturing consistency. The Cellometer™ Ascend™ automated cell counter represents a significant advancement in automated cell counting technology, addressing the growing demands for higher precision, increased throughput, and enhanced operational efficiency in modern laboratory settings.

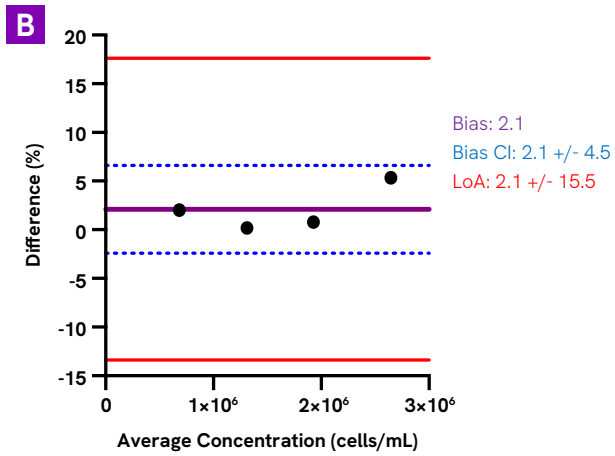
The Cellometer Ascend automated cell counter delivers reliable cell counting performance while reducing the laboratory footprint through an integrated computer system. This next-generation platform incorporates several key technological innovations that collectively enhance data quality and streamline workflows. The system's ability to analyze more cells while maintaining a minimal sample volume requirement (10-20 µL) substantially improves measurement precision and reduces acquisition time. Furthermore, the platform's higher throughput capabilities enable simultaneous loading of multiple chambers with automated sequential analysis, significantly accelerating experimental workflows. The incorporation of advanced autofocusing algorithms represents a fundamental improvement in the platform's ability to detect and quantify cells across diverse sample types. Additionally, the introduction of novel slide autofocus technology facilitates efficient cell segmentation and analysis, particularly for challenging low-concentration samples. In this technical note, we evaluate the performance of Cellometer Ascend consumables and compare the Cellometer Ascend's capabilities against other Cellometer and Cellaca™ instruments.



Cellometer Ascend consumable comparison

The Cellometer Ascend automated cell counter demonstrates multiple improvements over previous Cellometer instruments, notably in its enhanced automated capabilities and increased sample throughput. The system features a newly designed slide available in 3-chamber (20 µL sample volume) or 8-chamber (10 µL sample volume) formats. To evaluate performance consistency between these slide formats, Jurkat cell suspensions at four different dilution fractions (DF, at 1, 0.75, 0.5, and 0.25) were prepared and stained with acridine orange/propidium iodide (AOPI). Following a truncated version of the ISO Cell Counting Part 2 method[1], triplicate samples were prepared for each DF, and each replicate imaged/sampled three times (Figure 1A). Results demonstrated consistent measurement across all samples within their DF, independent of slide format, with coefficient of variation (CV) values for mean concentration measurements below 6.06%, while maintaining comparable high viability across all samples (Figure 1A, table). Bland-Altman analysis, commonly used to compare two techniques or devices in laboratory settings, revealed a small systematic bias between the two slide formats (Figure 1B), with 3-chamber slides reporting concentrations 2.1% higher than 8-chamber slides. These results confirm the consistency between the two slide formats, supporting their interchangeable use in cell counting applications.





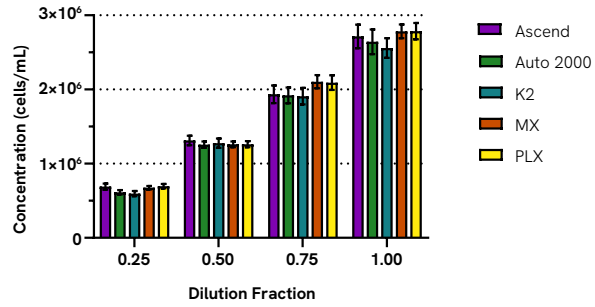
	Cellometer Ascend 3-chamber			Cellometer Ascend 8-chamber		
DF	**Mean (cells/mL)**	**CV (%)**	**Viability (%)**	**Mean (cells/mL)**	**CV (%)**	**Viability (%)**
0.25	6.90E+05	4.46	91.56	6.76E+05	4.78	91.78
0.5	1.61E+06	1.95	91.67	1.31E+06	6.06	90.11
0.75	1.94E+06	5.13	91.56	1.92E+06	4.19	92.56
1	2.72E+06	5.11	90.22	2.57E+06	5.80	87.22

Figure 1: **Cellometer Ascend Consumable Comparison. (A)** Mean total cell concentration plotted against different dilution fractions (DF) (n=9, error bars are s.d.). Table shows mean total cell concentration, CV, and viability for each DF. **(B)** Bland-Altman plot comparing 3-chamber and 8-chamber slides. The bias is the average difference between two measurements. The bias (purple line) of 2.1% indicates that 3-chamber slides yield mean total cell concentrations 2.1% higher than 8-chamber slides. Blue dotted lines represent the confidence interval (CI) or the uncertainty of the bias, while red lines indicate the limits of agreement (LoA), which is the coverage range of the bias based on the number of samples analyzed (n=9 per data point) and represents ±2 times the standard deviation from the bias.

Performance evaluation of Cellometer Ascend against established cell counting platforms

The Cellometer Ascend automated cell counter represents the latest advancement in the Cellometer product line, incorporating technological features previously available only in instruments such as the Cellaca MX high-throughput cell counter and Cellaca PLX high-throughput image cytometer. Following a truncated version of the ISO cell counting standard protocol[1], we conducted a comparative analysis of the Cellometer Ascend alongside the Cellometer Auto 2000, Cellometer K2, Cellaca MX, and Cellaca PLX instruments. Jurkat cell suspensions were prepared at four distinct DFs (range: 5.0×10^5 to 2.5×10^6 cells/mL), with triplicate samples at each dilution. Each replicate underwent three independent measurements per instrument (n=9 per dilution) to assess total cell concentration (Figure 2A) and viability (Figure 2B). Concentration measurements across all instruments demonstrate consistency, with CV values <6.7% throughout the tested range (Figure 2A, table). Viability assessments showed even greater consistency, with CV values <2.56% across all platforms and DFs. Linear regression analysis yielded R^2 values ≥0.998 for all instruments, confirming linearity across the concentration range tested (Figure 2C). Bland-Altman analysis revealed minimal systematic bias between the Cellometer Ascend cell counter and other platforms (Cellometer Auto 2000, Cellometer K2, Cellaca MX, and Cellaca PLX), with all bias measurements within ±3.4% (Figure 2D). This comparison data demonstrates that the Cellometer Ascend delivers performance equivalent to established high-end cell counting platforms while maintaining the accessibility and ease of use characteristic of the Cellometer product line.

A



DF	Ascend	Auto 2000	K2	MX	PLX
0.25	4.46	5.26	5.87	1.60	4.18
0.5	2.40	3.71	5.59	2.74	2.55
0.75	5.13	6.15	6.70	3.04	3.82
1	5.11	5.98	4.53	3.37	4.01

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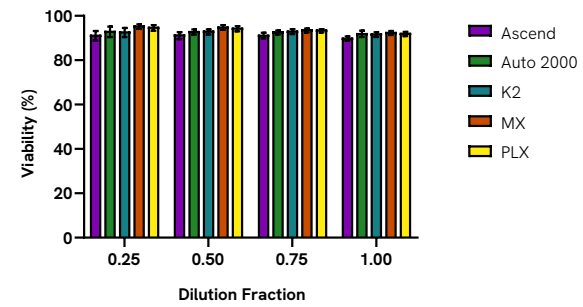

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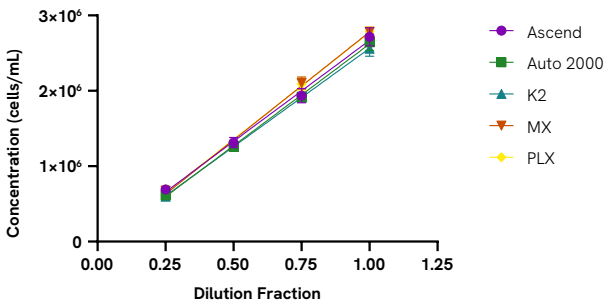

D

Bland-Altman Comparison	Bias (%)
Ascend - Auto 2000	2.6
Ascend - K2	3.4
Ascend - MX	-1.2
Ascend - PLX	-1.9

Figure 2: **Performance Evaluation of Cellometer Ascend Against Established Cell Counting Platforms. (A)** Mean total cell concentration plotted against different dilution fractions (DF) (n=9, error bars are s.d.). Table shows CV for each DF. **(B)** Mean viability plotted against different DF (n=9, error bars are s.d.). **(C)** Linear regression analysis of cell concentrations across different DF (n=9). **(D)** Bland-Altman comparison results between Cellometer Ascend and other systems (Cellometer Auto 2000, Cellometer K2, Cellaca MX, and Cellaca PLX), with reported percent biases for each direct comparison.

Low concentration cell detection with Cellometer Ascend

A primary development goal for the Cellometer Ascend automated cell counter was to extend the lower limit of cell counting through improved autofocus capabilities for low concentration samples. To demonstrate the novel autofocus mechanism, mouse kidney cell nuclei were extracted and diluted to a low concentration (5 x 10^4 nuclei/mL). They were subsequently stained with AOPI and loaded onto a Cellometer Ascend 3-chamber slide for imaging (Figure 3A). At times, conventional brightfield image-based autofocus methods can fail to detect the optimal focal plane for sparse nuclei/cells, especially in the presence of sample debris (Figure 3A, left). However, slide autofocus successfully identified the correct focal plane at the same imaging location containing debris, which enabled proper fluorescent imaging of the nuclei sample (Figure 3A, right).

The Cellometer Ascend performance was compared against Cellometer Auto 2000 and Cellometer K2 platforms across four low-concentration dilutions (range: 3.0 × 10^4 to 1.3 × 10^5 cells/mL) of Jurkat cells stained with AOPI. The enhanced optical system and detection algorithms of the Cellometer Ascend allow for higher sampling (more cell counts) compared to the other systems for improved cell detection at low concentrations (Figure 3B). For example, at the 3.0 × 10^4 concentration, the Cellometer Ascend counts on average 107 cells, compared to 8 and 17 from the Cellometer Auto 2000 and Cellometer K2, respectively (Figure 3B).

Mean total cell concentrations for low range DFs were similar between all three platforms (Figure 3C, graph). However, further statistical analysis demonstrated heightened precision with the Cellometer Ascend, which maintained a maximum CV of 10.06% across all low-range dilutions, compared to CV values reaching 29.74% for the Cellometer Auto 2000 and Cellometer K2 platforms (Figure 3C, table). Linear regression analysis yielded the highest R^2 value for the Cellometer Ascend (R^2 = 0.998), confirming maintenance of linearity at low concentration ranges (Figure 3D). The increased detection sensitivity and overall higher cell counts in the Cellometer Ascend resulted in more comprehensive cell counting, thereby enhancing the accuracy of viability measurements with increased precision (lower CVs; Figure 3E). Bland-Altman analysis revealed systematic biases of 4.7% and 5.4% when comparing the Cellometer Ascend to Cellometer Auto 2000 and Cellometer K2, respectively, reflecting the Cellometer Ascend's enhanced detection capabilities (Figure 3F). The Cellometer Ascend represents a significant advancement over previous Cellometer generations, demonstrating reliability and precision in low concentration sample analysis through improved autofocus technology and enhanced optical detection.

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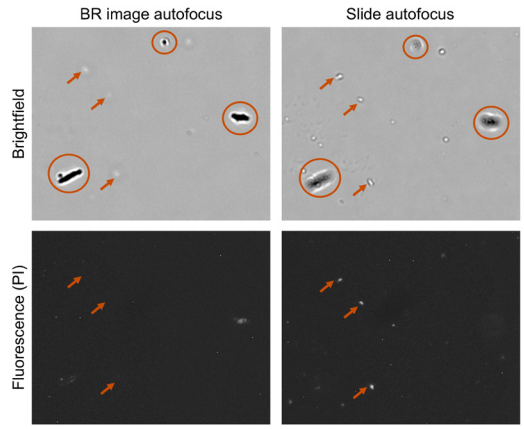

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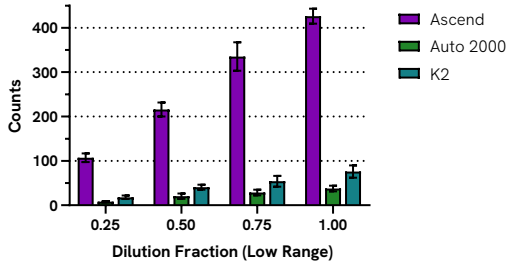

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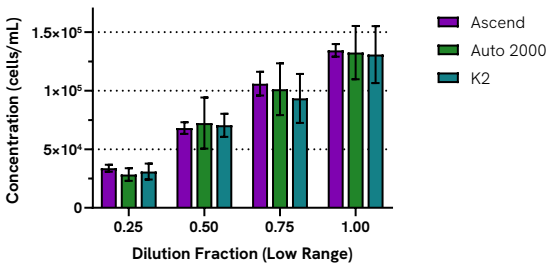

DF	Ascend	Auto 2000	K2
0.25	10.06	21.41	18.94
0.5	8.35	29.74	13.04
0.75	8.58	20.40	25.48
1	3.96	19.09	21.37

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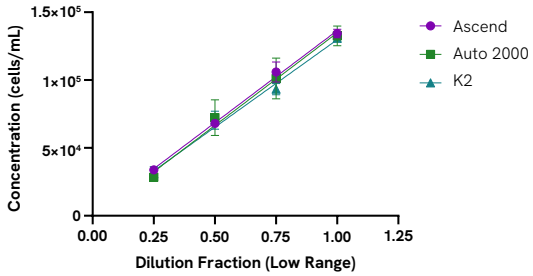

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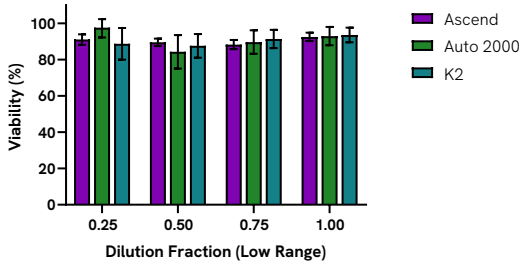

DF	Ascend	Auto 2000	K2
0.25	3.03	5.04	9.85
0.5	2.31	10.97	7.45
0.75	2.88	7.39	5.64
1	2.36	5.55	4.44

F

Bland-Altman Low Range Comparison	Bias (%)
Ascend - Auto 2000	4.7
Ascend - K2	5.4

Figure 3: **Low Concentration Cell Detection with Cellometer Ascend. (A)** Representative cropped brightfield and fluorescence images of mouse kidney cell nuclei comparing image-based autofocus (left) and Cellometer Ascend slide autofocus (right). Circles indicate debris in the sample and arrows, isolated nuclei. **(B)** Total counts across different dilution fractions (DF) from each instrument (n=9, error bars are s.d.). **(C)** Mean total cell concentration plotted against different DF (n=9, error bars are s.d.). Table presents CV for each DF. **(D)** Linear regression analysis of cell concentrations across DF. The R^2 value for the Cellometer Ascend, Cellometer Auto 2000, and Cellometer K2 are 0.998, 0.986, and 0.994, respectively (n=9). **(E)** Mean viability plotted against different DF (n=9, error bars are s.d.). Table includes CV for each DF. **(F)** Bland-Altman comparison results between Cellometer Ascend and two other systems (Cellometer Auto 2000 and Cellometer K2), with reported percent biases for each direct comparison.

Viability range assessment

Viability of cell samples is a critical metric in biological applications, notably in cell and gene therapy fields. Peripheral blood mononuclear cell (PBMC) samples were prepared at three target viabilities, designated as Low (45%), Intermediate (75%), and High (95%). Three independent replicates were prepared from each viability, stained with AOPI, and loaded into three separate Cellometer Ascend 3-chamber slides (n=9 per viability) and nine wells of a cell counting plate for analysis on the Cellometer Ascend and Cellaca MX, respectively. Live PBMCs imaged on the Cellometer Ascend are counted and outlined in green by the Matrix™ software and dead cells in red (Figure 4A). Both Cellometer Ascend and Cellaca MX reported comparable target viabilities (Figure 4B) and total cell concentrations across the different samples (Figure 4C).

In addition to the fluorescence-based AOPI viability assay, the Cellometer Ascend is equipped with a brightfield module that enables viability assessment using trypan blue. Here, we compare it to the Cellometer Auto T4, a well-established brightfield-based cell counter, specifically designed for trypan blue. Similarly, CHO suspension cells were prepared at Low (40%), Intermediate (70%), and High (95%) viabilities. Three independent CHO cell samples were prepared from each viability, stained with 0.2% trypan blue, and loaded into three Cellometer Ascend 3-chamber slides (n=9 per viability) and five CHT4 Cellometer counting slides (n=9 chambers analyzed per sample). Both the Cellometer Ascend and the Cellometer Auto T4 reported consistent viability across the different samples, with the Cellometer Ascend providing a tighter CV of up to 3% compared to Cellometer Auto T4's 7% CV (Figure 4D). Total cell concentration measurements were also consistent within each instrument, although the Cellometer Ascend reported higher concentrations in Low and Intermediate viability samples (Figure 4E). These data establish the Cellometer Ascend as an exceptional instrument for viability assays using either fluorescence-based AOPI or brightfield-based trypan blue.

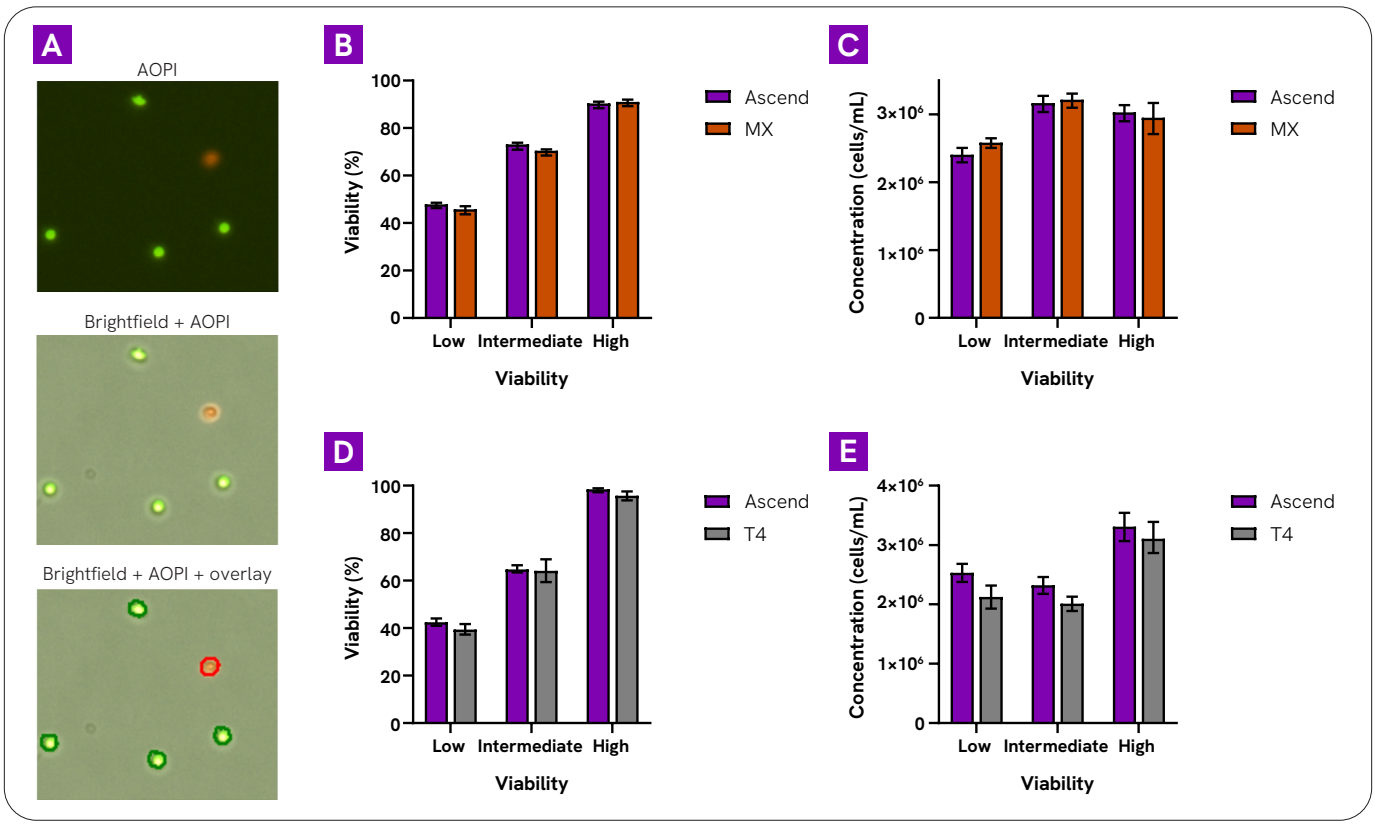

Figure 4: **Viability Range Assessment. (A)** Representative cropped images of PBMCs stained with AOPI acquired on the Cellometer Ascend. Green outline in the overlay indicates a live cell and red outline, a dead cell. **(B)** Mean cell viability of PBMCs stained with AOPI plotted against Low, Intermediate, and High viability samples (n=9, error bars are s.d.). **(C)** Mean total cell concentration of PBMCs stained with AOPI plotted against Low, Intermediate, and High viability samples (n=9, error bars are s.d.). **(D)** Mean cell viability of CHO cells stained with trypan blue plotted against Low, Intermediate, and High viability samples (n=9, error bars are s.d.). **(E)** Mean total cell concentration of CHO cells stained with trypan blue plotted against Low, Intermediate, and High viability samples (n=9, error bars are s.d.).

Evaluating image capture modalities for sample concentration range

The Cellometer Ascend 3-chamber slide format offers three imaging options (2, 4, or 8 images captured per chamber) to accommodate a wide concentration range. While higher image counts (4 or 8) provide improved accuracy across the broadest concentration spectrum, they significantly increase data acquisition and processing time, potentially impacting workflow efficiency. To evaluate this trade-off, Jurkat cell suspensions spanning a concentration range of 2.6×10^4 to 2.08×10^7 cells/mL were analyzed using each imaging mode, with CV and processing time evaluated for each condition. Here, we provide recommendations regarding these performance metrics (acquisition time and CV) as a simplified table (Figure 5A). Imaging modalities highlighted in green indicate a recommended imaging mode for that cell concentration. In contrast, combinations highlighted in red exhibit a CV >10%, which is considered suboptimal precision, and are not recommended.

Combinations highlighted in yellow, orange, and dark orange are not recommended but can be used with discretion as they exhibit increased analysis time, with time per sample of more than 45 seconds, 1 minute, and 3 minutes, respectively, (Figure 5A). These categorizations provide practical guidelines for selecting the appropriate imaging mode based on sample concentration and workflow requirements. Linear regression analysis of the 8-image capture mode demonstrated excellent linearity (R^2 = 0.994) across the entire concentration range (Figure 5B, top), with consistent performance maintained even at lower concentrations (Figure 5B, dotted box). These data demonstrate the Cellometer Ascend's flexibility in accommodating diverse sample concentrations while maintaining measurement linearity, allowing users to optimize the balance between analytical precision and processing time based on specific experimental requirements and sample characteristics.

A

Concentration	2 images	4 images	8 images
2.60E+04	27.85%	12.86%	6.97%
5.20E+04	11.32%	2.23%	2.05%
1.04E+05	14.75%	1.94%	2.07%
2.60E+05	5.42%	7.69%	6.74%
5.20E+05	9.29%	4.03%	1.15%
1.04E+06	5.87%	0.70%	0.80%
2.60E+06	3.23%	2.33%	1.91%
5.20E+06	0.93%	1.78%	0.19%
1.04E+07	8.18%	5.32%	4.13%
2.08E+07	5.41%	4.11%	5.57%

Green	Recommended
Red	Not recommended; CV >10%
Yellow	Analysis time > 45 sec per sample
Orange	Analysis time > 1 min per sample
Dark orange	Analysis time > 3 min per sample

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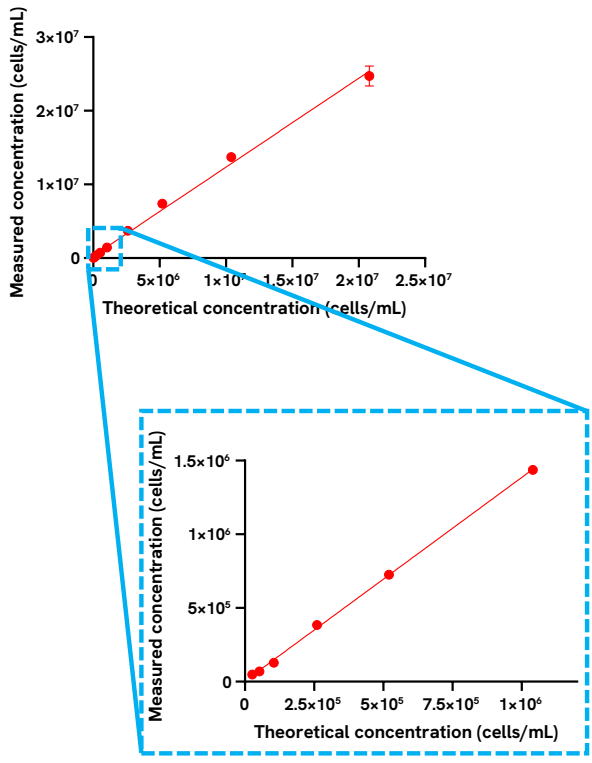

Figure 5: **Evaluating Image Capture Modalities for Sample Concentration Range. (A)** CV values for each concentration across different imaging modes (2, 4, and 8 images; n=3). Green highlight demonstrates recommended imaging mode for specific cell concentrations using AOPI. Red highlight indicates CV >10% and is therefore not recommended. Yellow, orange, and dark orange indicate high analysis times of >45 seconds, >1 minute, and >3 minutes per sample, and should be used with discretion. **(B)** Linear regression analysis across 12 distinct concentrations (top graph) and detailed analysis of the six lowest concentrations measured (dotted box).

Instrument-to-instrument variability assessment of Cellometer Ascend

To validate manufacturing consistency and ensure quality assurance of the Cellometer Ascend automated cell counter platform, we conducted a comprehensive instrument-to-instrument variability analysis across five independent units. Standardized bead suspensions were prepared at four concentration levels: two lower concentrations (2.5×10^5 and 5.0×10^5 beads/mL) loaded into eight 3-chamber slides each, and two higher concentrations (1.0×10^6 and 5.0×10^6 beads/mL) loaded into three 8-chamber slides each, yielding 24 measurements per concentration level.

Analysis demonstrated highly consistent performance across all five instruments, with minimal inter-instrument variability in both viability (maintained at 75%, Figure 6A) and concentration measurements (Figure 6B) across all tested ranges. These results confirm the manufacturing reproducibility of the Cellometer Ascend platform, ensuring reliable and consistent performance across multiple instruments for multi-site or longitudinal studies.

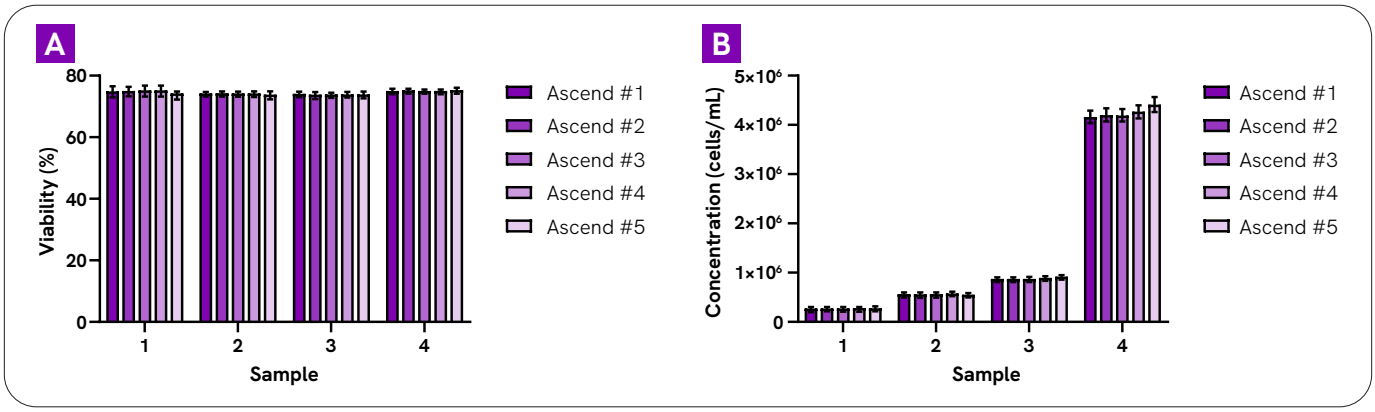

Figure 6: **Instrument-to-Instrument Variability Assessment of Cellometer Ascend. (A)** Mean bead viability comparison across different samples measured on five Cellometer Ascend instruments (n=24, error bars are s.d.). **(B)** Mean total bead concentration comparison across different samples measured on five Cellometer Ascend instruments (n=24, error bars are s.d.).

Conclusion

In summary, the Cellometer Ascend automated cell counter represents a significant advancement over previous Cellometer generations, achieving precision comparable to premium Cellaca instruments. Its key advantages include automated high-throughput capabilities via new slide designs, flexible imaging modes for counting across diverse concentration ranges, and innovative focal plane detection technology. These improvements, combined with a reduced laboratory footprint and integrated touchscreen, make the Cellometer Ascend an exceptional addition to laboratory cell counting solutions.

Abbreviations

CV: coefficient of variation
DF: dilution fraction

Authors

DongHo Kim
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Sopaul Hem
Allen Lin

Reference:

[1]ISO 20391-2:2019 Biotechnology — Cell counting — Part 2: Experimental design and statistical analysis to quantify counting method performance.