



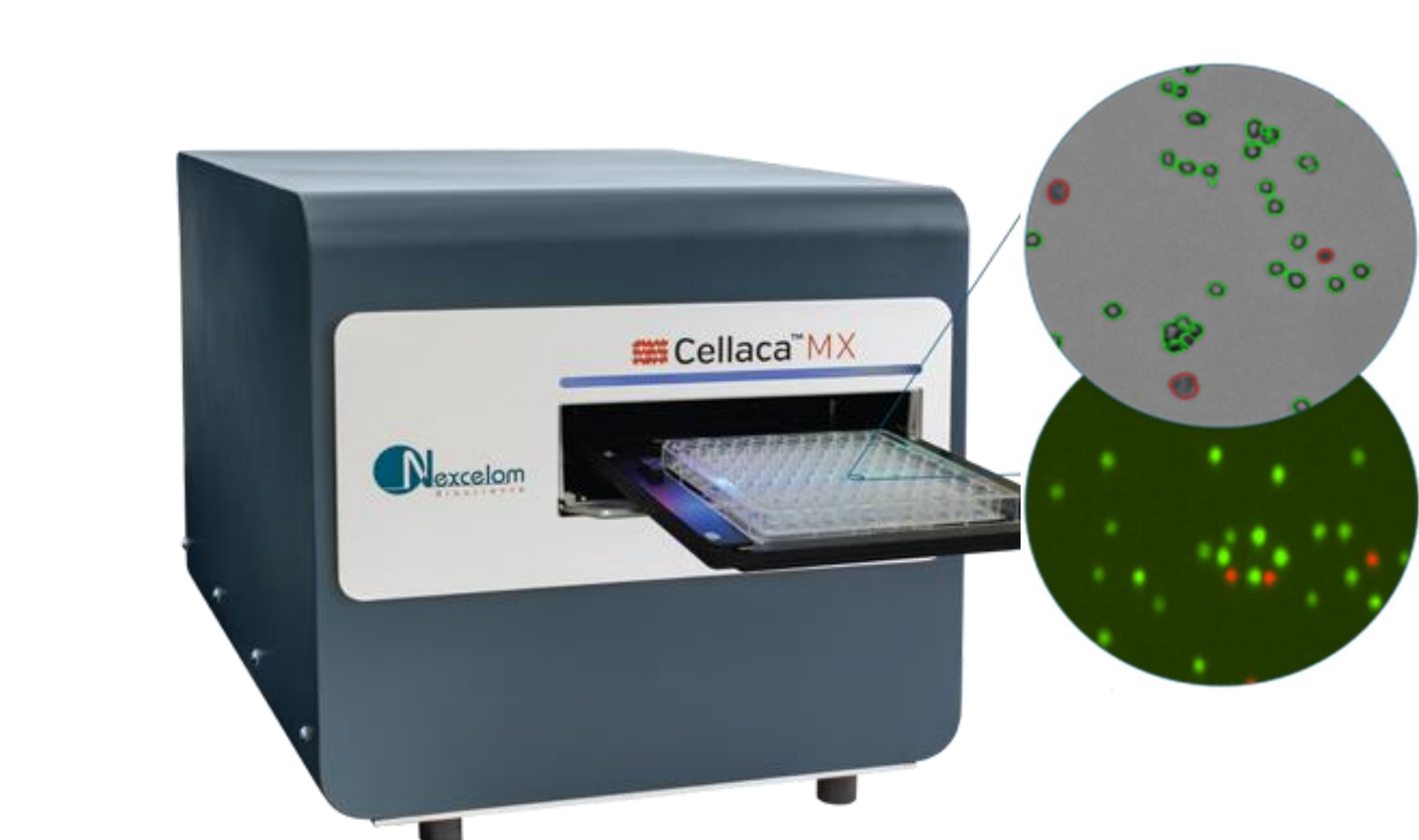
Cellaca™ MX: A Novel Instrument for High-throughput, High-speed Cell Counting, Concentration, and Viability

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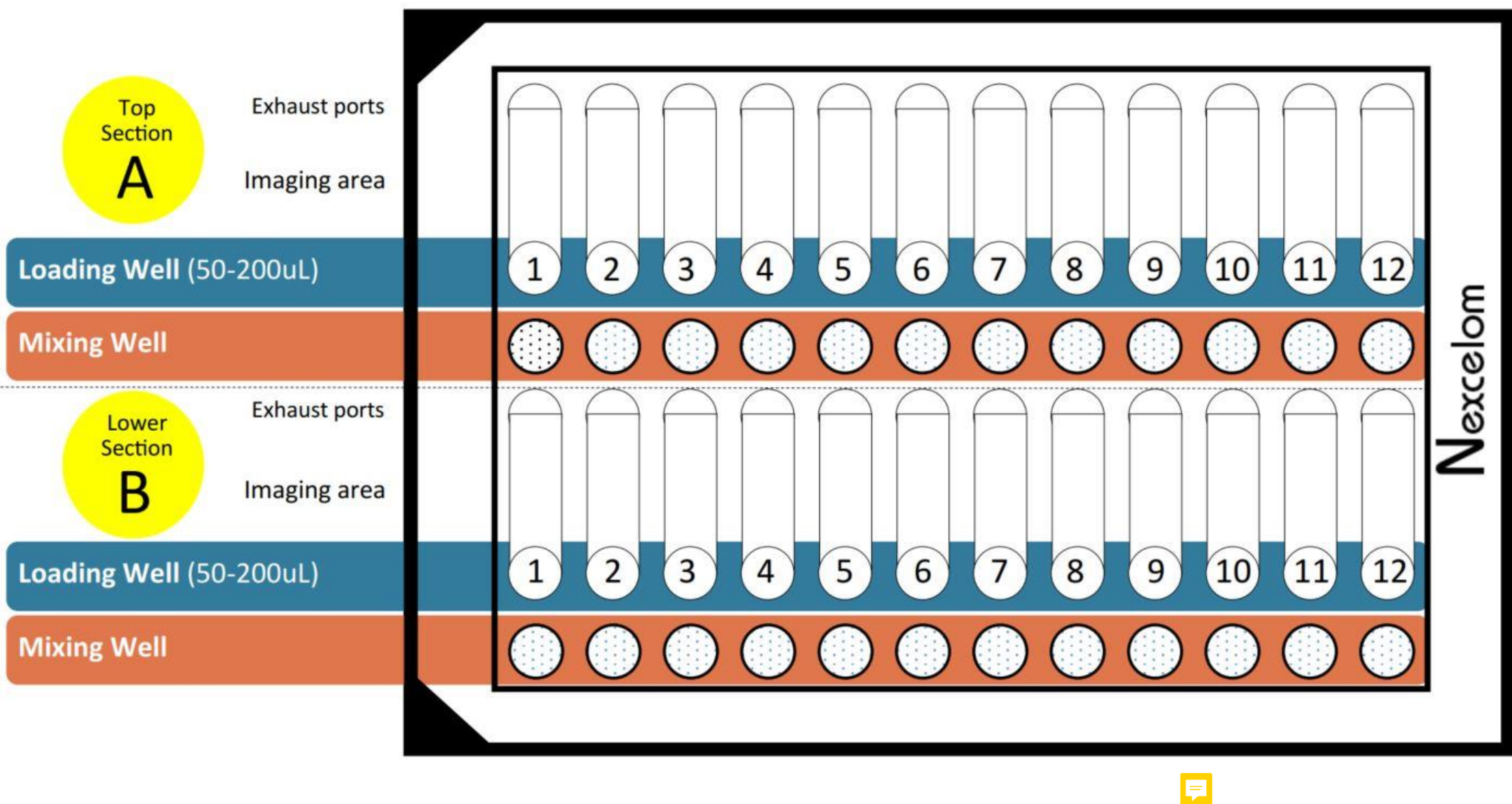
1. Abstract

The tedious and time-consuming process of manually counting cells on a hemocytometer has been greatly alleviated in recent years with the advent of various automated cell-counting instruments. Even with these advancements, the measurement of cell concentration for many samples is a common bottleneck in large scale experiments and many industrial processes. For example, single-sample automated cell counters may not be appropriate for counting multiple primary murine samples after a large take down and during cell line development and manufacturing. To address these challenges, we have developed a rapid and high-throughput cell counting system. The system can image, analyze, and report cell concentration and viability. The Cellaca MX accomplishes all of this in less than 1 minute using bright field (trypan blue) and in less than 3 minutes using multiple fluorescent imaging channels for 24 samples. The instrumentation was developed to operate in both a manual mode and as a fully automated, plate-based system, thus providing the user greater set-up flexibility depending on the project and sample quantity. In addition, small loading volume (50 – 200 µL per sample) assures that precious samples are conserved for more critical downstream assays. We present a comparison between counts obtained on the Cellaca instrument and those obtained using the laborious gold-standard hemocytometer method. We further tested the platform using CHO cells stained with trypan blue, routinely used in bioprocessing. Finally, we share results of an AOPI cell viability assay on Jurkat cells. Our experiments demonstrate a cell-counting system capable of increasing both consistency and throughput in biological workflows. This advancement is of significant value to the cell line development and bioprocessing communities. It provides an efficient method of counting and analysis of multiple samples where one previously did not exist.

2. Streamlined Work Flow – Achieves Fast Results



- High-throughput Automated Cell Counter
- Automation Ready: API compatible instrument
- Small footprint, perfect for lab bench



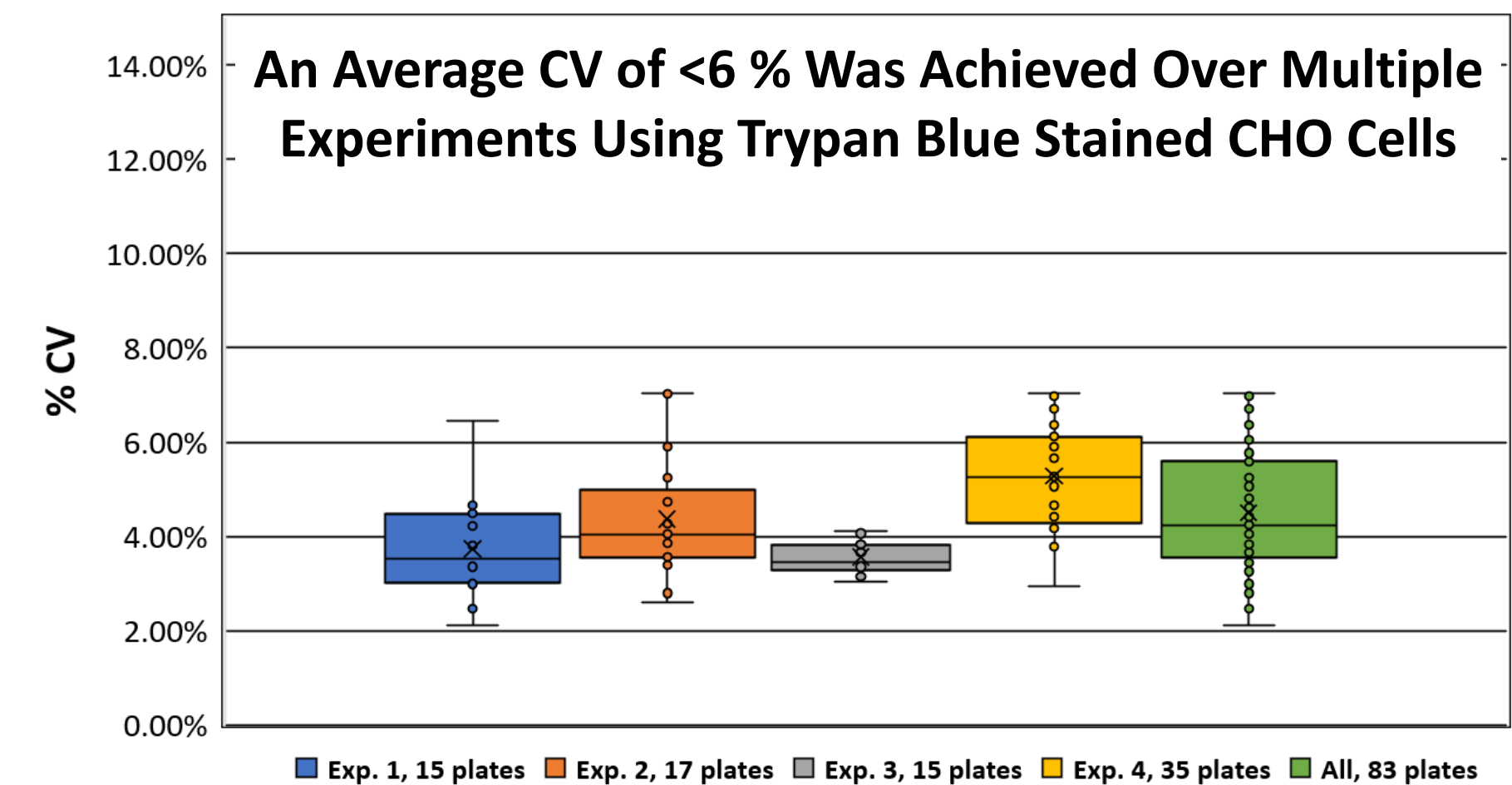
- Load and analyze 24 samples simultaneously
- Save precious samples, only 25 µL of cell sample needed
- Multiple plate configuration are available for ease of use

	Cellaca MX BF	Cellaca MX FL2
Channels	Brightfield	Brightfield, Green, Red
Number of Channels	1	4
Excitation LED	N/A	470, 527
Emissions Filters	N/A	534, 655
Commonly Used Compatible Dyes	Trypan Blue	Trypan Blue, AO/PI
Counting Speed Per 24 Samples	48 seconds	2.5 minutes

- Trypan Blue and/or Fluorescence imaging and counting
- Fast cell counting and analysis: 480 samples in 30 mins
- Reports viability, concentration, cell number and size

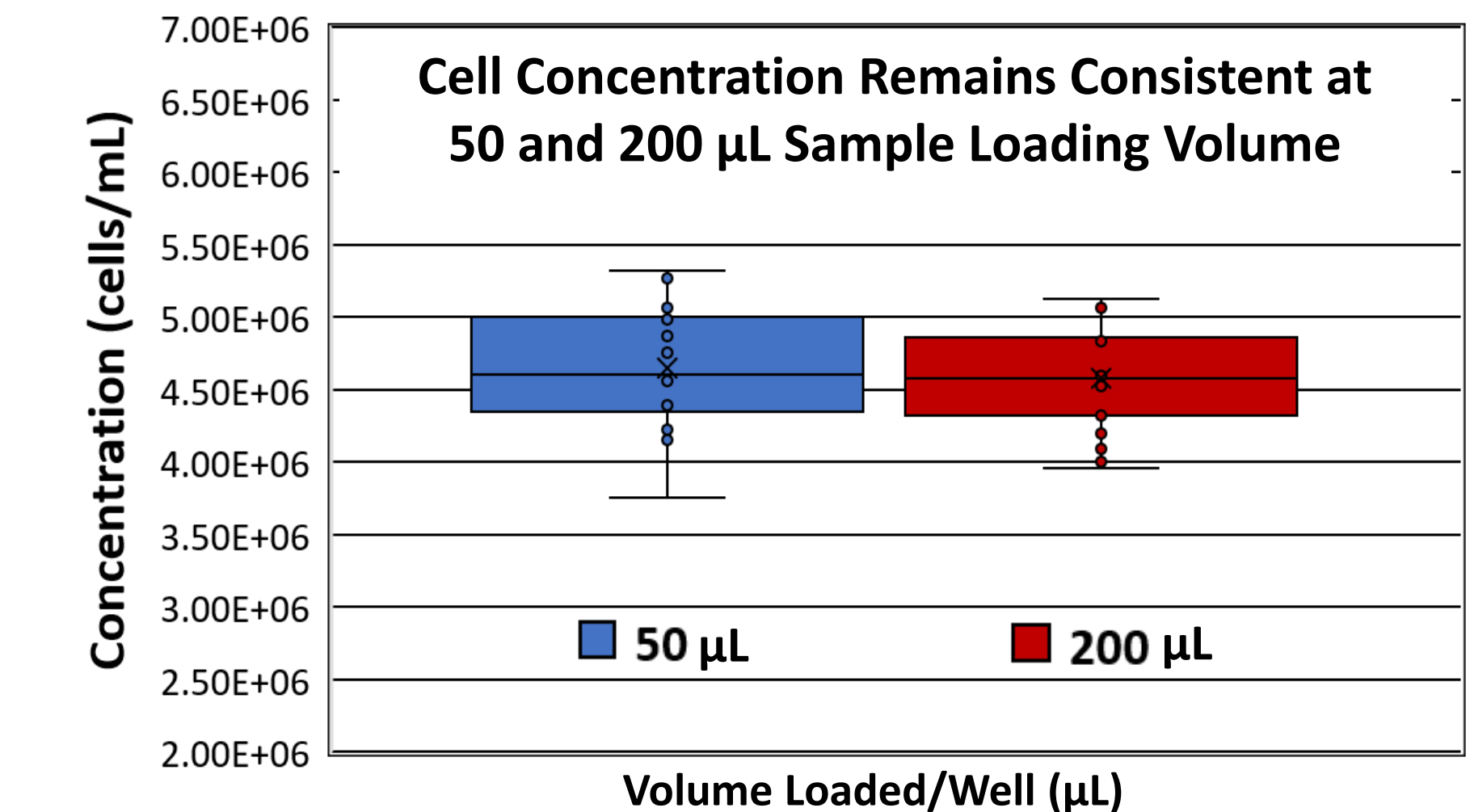
3. Reliable High-throughput CHO-S Cell Counting

CHO-S were continuously cultured over multiple weeks in order to complete the set of 4 experiments shown below. The cell concentration was standardized to approximately 2×10^6 cells/mL. For each experiment 50 µL of Trypan Blue (0.1 % final) stained CHO-S cells were loaded into the Cellaca consumable, imaged and analyzed using the Cellaca MX cell counter.

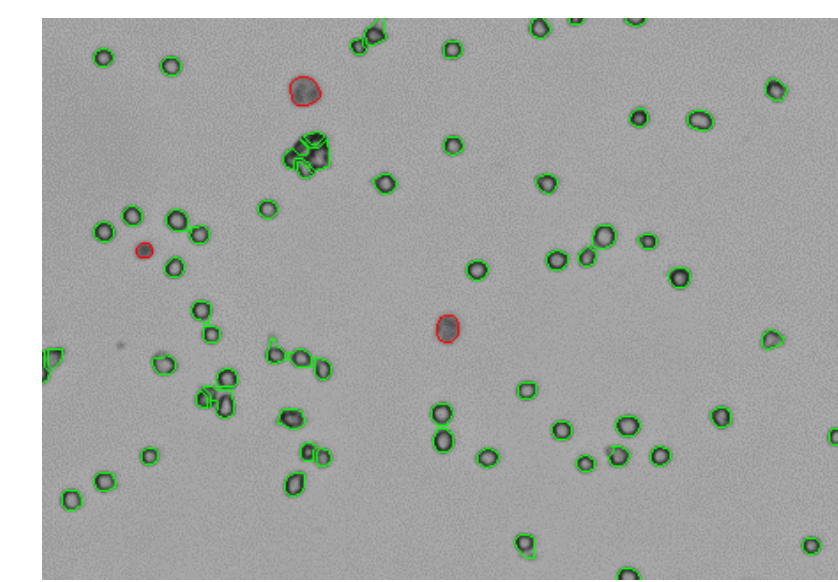


- Multiple users were able to yield an average plate to plate variability for Trypan Blue stained CHO-S cells of less than 6% CV
- A total of 1,992 individually loaded samples (83 plates) were counted and analyzed in 70 minutes

Trypan Blue (TB) stained CHO-S cells at $\sim 4.5 \times 10^6$ cells/mL were loaded at 50 and 200 µL well into the 24-well consumable plates, imaged, and analyzed on the Cellaca MX.



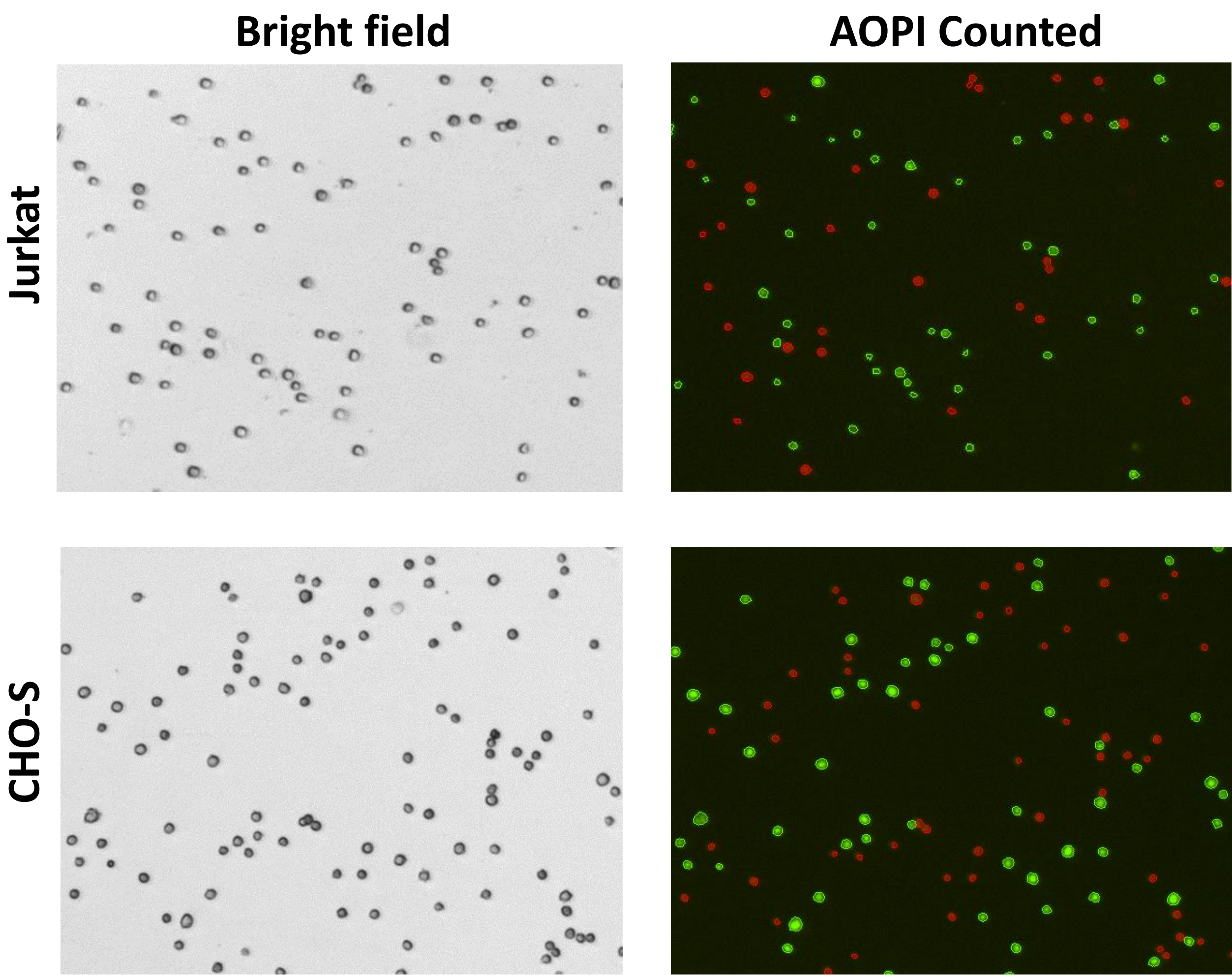
- A total of 18 plates (432 wells) per volume condition were run
- Results show that the difference in average concentrations between 50 and 200 µL loading volumes is 1.5 %



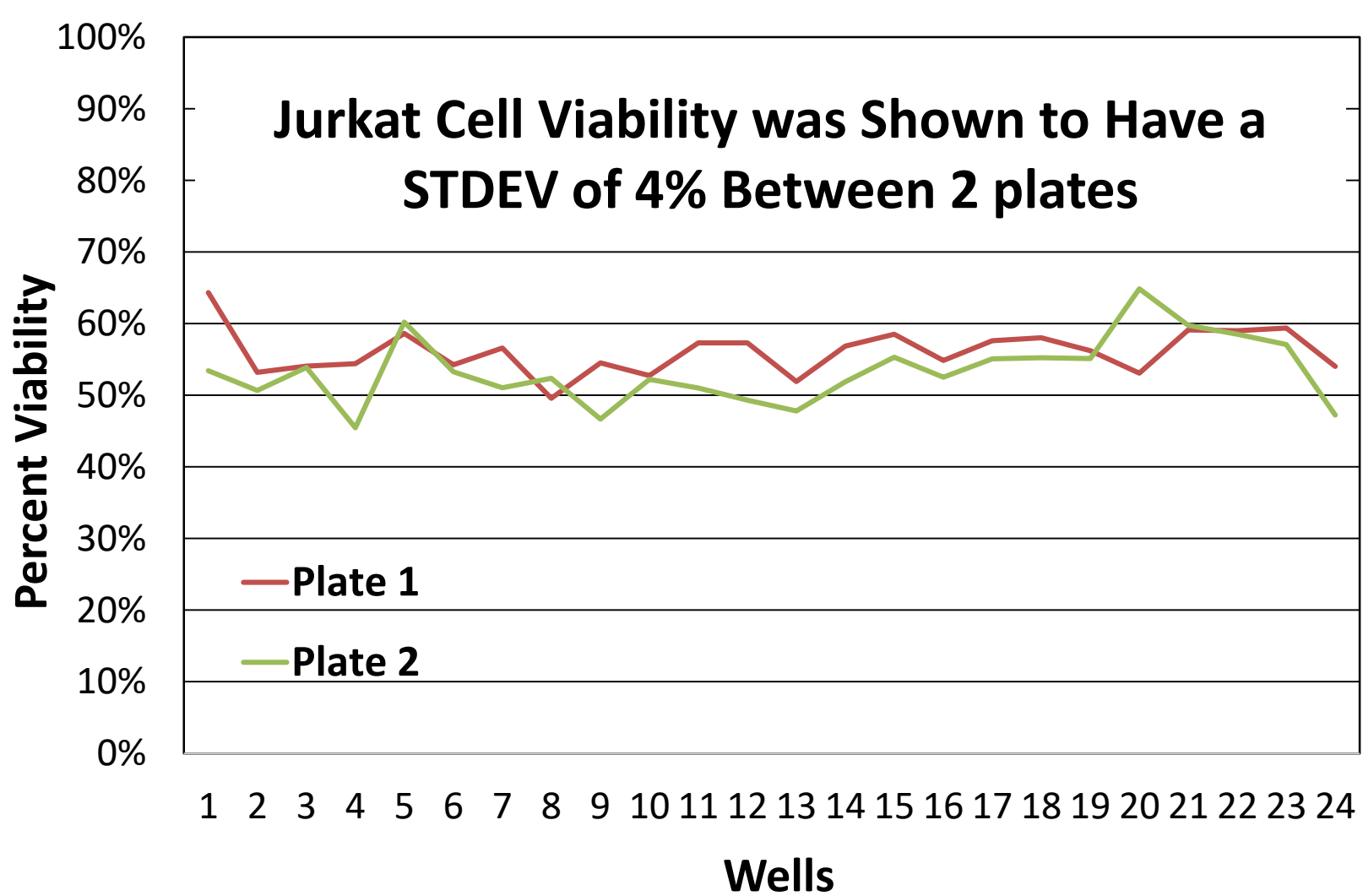
- Bright field image of Trypan Blue Stained CHO-S cells captured on Cellaca MX
- Red out lined cells are identified as dead TB positive cells, while cells in green are live TB negative cells

4. Consistent Viability Measurement Using AOPI

Three-day old cultures of Jurkat and CHO-S cells were standardized to approximately 2×10^6 cells/mL. They were then both stained with Acridine Orange and Propidium iodide (AOPI) and analyzed on the Cellaca MX.

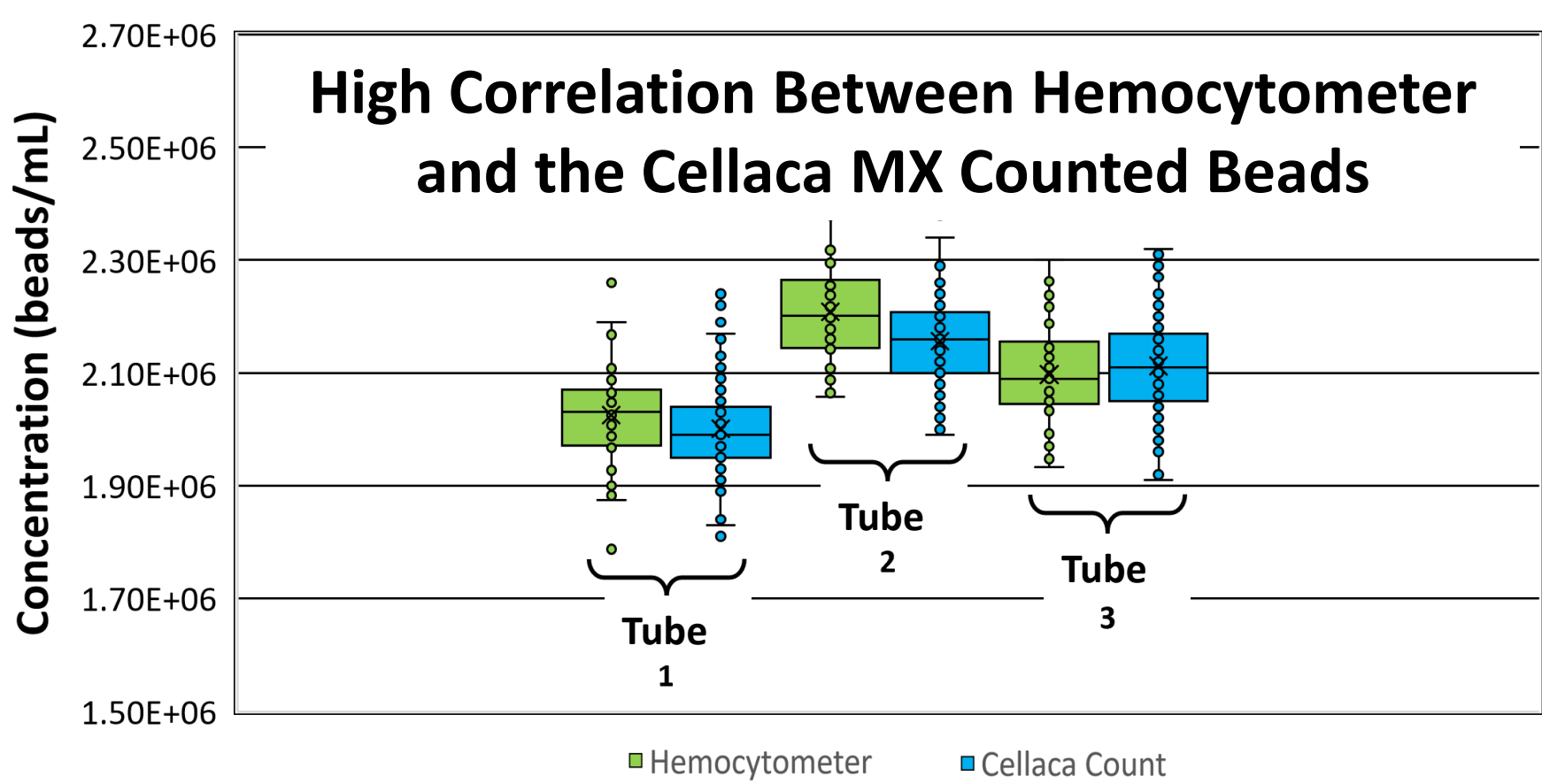


- Bright field and fluorescent images are acquired for each channel and are automatically saved. Shows above are representative BF and AOPI counted images
- Cellular counting and analysis is performed in the fluorescent channel while the bright field image is captured for documentation
- Viability is automatically calculated and reported for each well. The average viability for 24 samples in plate 1 was 56% while the average viability for plate 2 was 53% (shown below)
- The standard measured deviation between the combined 2 plates was 4%.
- This data shows excellent and consistent viability readings for low viability samples



5. Close Cellaca to Hemocytometer Correlation

Three tubes of 5 micron polystyrene beads were diluted to a concentration of $\sim 2 \times 10^6$ beads/mL. To determine certain concentration within each tube, 40 individual hemocytometer counts were performed for each tube. The same set of tubes was then used to measure the concentration on the Cellaca MX.



	Hemo CV	Cellaca CV	Hemo Ave	Cellaca Ave	[] % Diff
Tube 1	4.46%	3.69%	2.03E+06	2.00E+06	1.20%
Tube 2	4.33%	3.64%	2.21E+06	2.16E+06	2.35%
Tube 3	4.28%	3.94%	2.10E+06	2.11E+06	0.71%

- The results show a very close correlation between the hemocytometer counted beads and the Cellaca counts
- Notably, a total of 18 Cellaca plates (432 wells) over 3 individually counted Hemocytometer tubes all had an average CV values of < 4%.
- The measured concentration difference between the Cellaca and the Hemocytometer bead counts were between 0.7 – 2.3%

6. Conclusions

- Cellaca MX is a versatile instrument, capable of performing both bright field Trypan Blue and fluorescent concentration and viability measurements
- We showed consistent counts of Trypan Blue stained CHO-S by completing nearly 2000 samples with an average CV of <6%
- Samples may be precious, therefore sample volume may be critical. We showed that there is almost no difference (1.5%) between loading 50 and 200 µL
- For samples at high and low viability we recommend using fluorescent-based, nuclear dye AOPI to measure viability. Even at low viability (55%), the Cellaca MX produced consistent viability results
- Finally, we performed extensive comparison studies looking at counting accuracy and consistency between hemocytometer and Cellaca counted beads. Data shows that Cellaca and hemocytometer counts show excellent correlation (1.2, 2.3, and 0.7% difference for multiple samples)