High-Speed and High-Precision FL-Based Cell Count and Viability Assays Using The Cellaca™ MX High-Throughput Cell Counter

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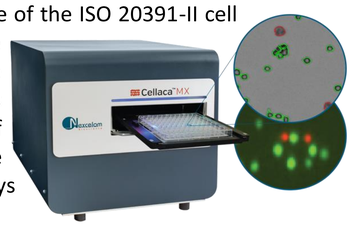
1. INTRODUCTION

With multiple FDA-approved cell therapy products available and others in pre-clinical and clinical trials, now, more than ever, it is critical to provide fast and accurate measurements of cell concentration and viability. For certain clinical products, cell concentration is synonymous with dosage, and accurate cell viability is crucial for avoidance of potential harmful side effects. The complex nature of patient-derived samples makes legacy cell analysis methods such as the trypan blue dye exclusion assay difficult or impossible to perform. Nonspecific objects such as RBCs and cellular debris can significantly increase cell counting variation. Meanwhile, the need for cell-based products that are custom-tailored to each patient has multiplied the number of samples requiring analysis, leading to cell counting bottlenecks.

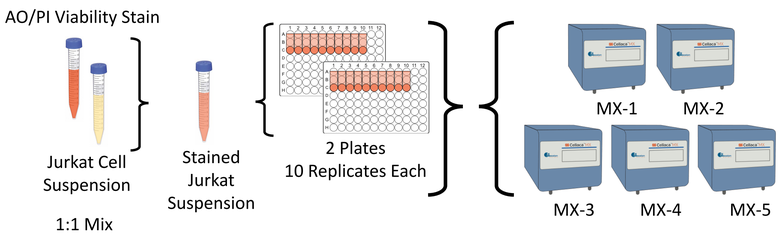
With the challenges of messy samples and counting bottlenecks in mind, we investigate the use of the Cellaca™ MX, a cell counter that can increase throughput and employ fluorescence-based cell counting methods. This high-throughput cell counter captures images in up to 5 fluorescence colors for cell count and viability analysis in less than 3 min for 24 samples. In addition, fluorescent cell-based assays such as apoptosis (Annexin V or Caspase 3/7), cell cycle, reactive oxygen species measurement can be performed to better characterize the target cell samples.

In this work, we demonstrate the capabilities of Cellaca™ MX for high-throughput fluorescence-based cell counting. Comparison among multiple instruments using Jurkat cells and fluorescent beads reveals high consistency between replicate counts, between Cellaca consumable plates, and between instruments. We also compare cell counts conducted on the Cellaca™ MX with counts performed using other fluorescence-based automated cell counting methods, as well as manual counts performed using a hemocytometer. In addition, we illustrate the use of the ISO 20391-II cell counting standard to evaluate and compare the performance of cell counting methods. Finally, we include real-world primary T cell linearity results over a 4-log range of concentration. The results of the experiments confirm the suitability of the Cellaca™ MX for general fluorescence-based assays applicable to cell therapy-related workflows.



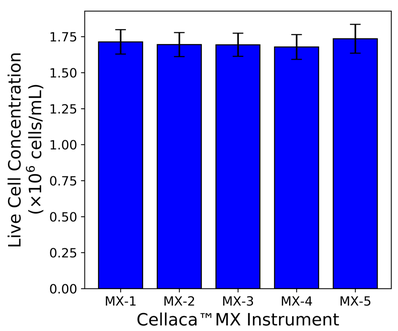
2. HIGH MULTI-INSTRUMENT CONSISTENCY AND PRECISION FOR JURKAT CELLS

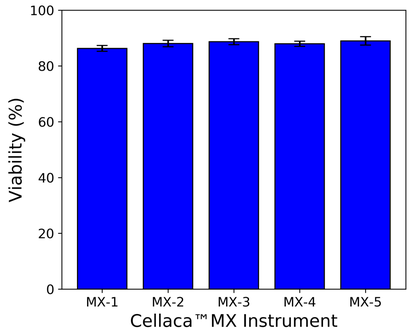
Experimental Protocol



- A sample of healthy Jurkat cells was gently mixed and stained 1:1 with ViaStain™ AOPI (Acridine Orange/Propidium Iodide) viability stain.
- The stained Jurkat cells were pipetted into 20 Cellaca™ MX counting chambers (10 on each of the 2 plates) .
- Both plates were then scanned on 5 Cellaca™ MX instruments using the same default settings for cell viability with AOPI.

Results





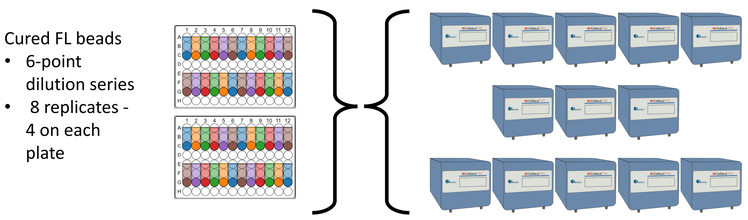
- The largest difference observed between any two of the instruments was 4% for live cell concentration and 3% for viability.
- The experiment was repeated an additional 8 times on different days, with 2-4 instruments used in each experiment (15 unique instruments). Jurkat concentration ranged from $1.8 * 10^6$ to $5.6 * 10^6$ cells/mL. The aggregated precision results are summarized below.

Cellaca™ MX Precision	Jurkat Total Conc. (CV)	Jurkat Live Conc. (CV)	Jurkat Viability (CV)
*Well-to-Well	5.8%	5.9%	3.8%
Plate-to-Plate	1.7%	1.7%	0.9%
Instrument-to-Instrument	3.4%	2.2%	1.8%
System-wide Precision	7.0%	6.6%	4.4%

* Well-to-well variation includes random sampling variation and any pipetting error.

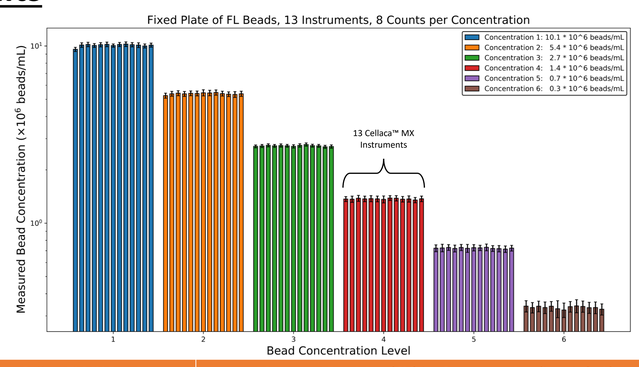
3. HIGH MULTI-INSTRUMENT CONSISTENCY AND PRECISION FOR FLUORESCENT BEADS

Experimental Protocol



- Prepared 2 plates of 7-µm fluorescent beads locked in clear UV-cured polymer (dilution series from 3.4e5 - 1e7 beads/mL).
- Counted the beads in the plates on 13 Cellaca™ MX units over the course of 6 months, using the same fluorescent counting settings.

Results



Cellaca™ MX Precision	Measured Precision (CV) by Concentration (beads/mL)					
	1.0 e7	5.4 e6	2.7 e6	1.4 e6	7.2 e5	3.4 e5
*Well-to-Well	3.1%	3.6%	2.3%	4.3%	4.9%	8.3%
Plate-to-Plate	0.8%	1.8%	0.3%	0.1%	0.3%	4.2%
Instrument-to-Instrument	1.7%	1.0%	0.8%	0.7%	0.7%	1.7%
System-Wide Precision	3.2%	3.5%	2.2%	3.8%	4.3%	7.8%

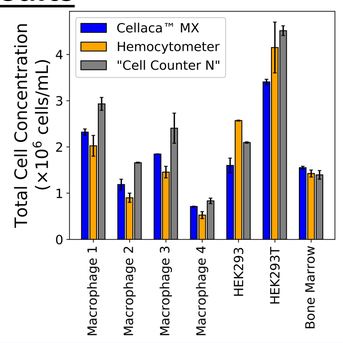
* Well-to-well variation includes random sampling variation and any pipetting error.

4. COMPARISON OF CELLACA™ MX TO OTHER CELL COUNTING METHODS

Experimental Protocol

- Cellaca™ MX, Hemocytometer, and fluorescence-based "Cell Counter N" were compared using 4 macrophage samples, 2 HEK 293 samples, and 1 bone marrow sample.
- Duplicate measurements were made for all counting methods and samples.
- Cellaca™ MX counts utilized Acridine Orange/Propidium Iodide.
- "Cell Counter N" imaged the cells after staining with Acridine Orange and DAPI.

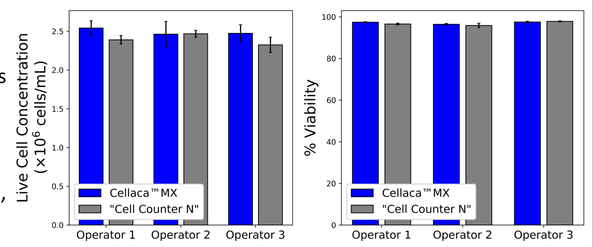
Results



Experimental Protocol

- Cellaca™ MX with AO/PI and "Cell Counter N" with AO/DAPI were compared by 3 operators using a single culture of HEK 293 cells (n=3 each count).
- Dead cells were counted, then total cells were counted after lysing.

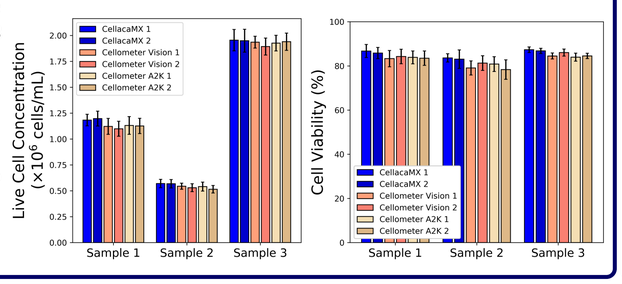
Results



Experimental Protocol

- A single Jurkat culture was used to prepare 3 samples at different concentrations.
- Cellaca™ MX, Cellometer® Vision, and Cellometer® Auto 2000 were used for counting and viability analysis (n=12).

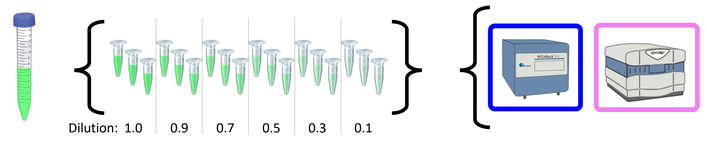
Results



5. CELLACA™ MX METHOD EVALUATION FOLLOWING THE ISO 20391-II STANDARDS

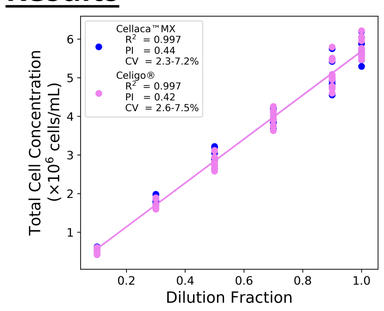
- FDA, NIST, ISO, and other industry partners published the ISO Cell Counting Standards Part I and II for guiding the selection and evaluation of cell counting methods
- Nexcelom Bioscience is a contributing member to the ISO standards
- We utilized the ISO 20391-II protocol to evaluate the performance of Cellaca™ MX for proportionality index, precision, and linearity

Experimental Protocol

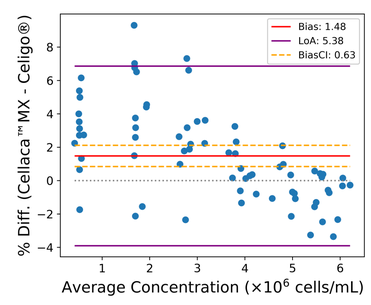


- A single tube of Jurkat cells was used to create 12 independent dilutions in 6 concentrations.
- The 12 samples were each mixed with Acridine Orange and counted in fluorescence mode on both the Cellaca™ MX and the Celigo® imaging cytometer (4 measurements per sample) .

Results



- Both methods show similar proportionality. We measure a proportionality index of 0.44 for Cellaca™ MX, and 0.42 for Celigo®.



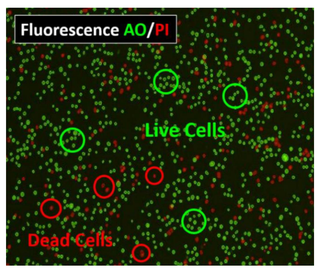
- A Bland-Altman plot comparing the two methods reveals a bias of 1.48%, with Cellaca™ MX counting higher.

6. LINEAR RANGE OF CELLACA™ MX INVESTIGATED WITH T CELLS

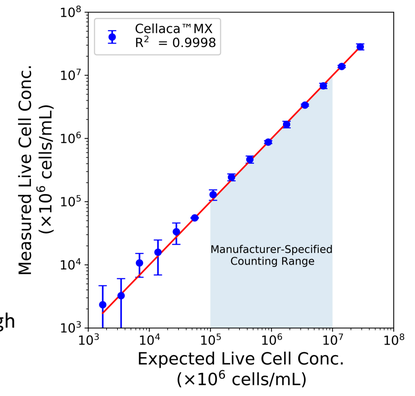
Experimental Protocol

- T cells were prepared in a 15-point 2x dilution series, with n=4 measurements for each concentration. Concentration ranged from $2.3 * 10^3$ to $3.4 * 10^7$ cells/mL.
- The cells were stained with Acridine Orange and Propidium Iodide.

Results



- Cell counts by Cellaca™ MX showed high linearity well beyond the instrument's recommended concentration range.



7. CONCLUSIONS

- The Cellaca™ MX demonstrates high consistency in fluorescent cell counts for a wide variety of cell types.
- Counts performed using the instrument are typically comparable to other fluorescence imaging methods and manual counting.
- The instrument exhibits linear counting of T cells over a large range exceeding the manufacturer's specifications.